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**The co-evolution of adult body mass and excretion rate
between genetically size-divergent brook trout populations**

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Running title: Genetic/maternal divergence in excretion rates of salmonids

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21 **Abstract**

22 We tested if there was a difference in mass-specific excretion rate between two genetically size-
23 divergent brook trout populations that can be accounted for by genetic/maternal factors. We
24 conducted laboratory-based common garden experimentation using F₁ generation fish, with five
25 to seven families per population at two ages (0.5 y juveniles and 1.5 y adults). We found that
26 genetic/maternal differences in excretion rate on a per gram basis co-evolve with genetic
27 divergence in adult body mass between the populations. However, this co-evolution has also
28 resulted in no net difference in excretion rate between populations when differences in adult
29 body mass were accounted for. Uncertainty in census estimates and variation in body mass
30 distributions created substantial variation in extrapolated whole-population excretion estimates.
31 No other studies to our knowledge have tested for genetic/maternal divergence in excretion rate
32 between genetically size-divergent fish populations. Genetically-based population divergence in
33 body mass, energy allocation in reproduction, and mass-specific excretion rate in the brook trout
34 was likely a result of selection associated with differences in the availability of overwintering
35 habitat between streams.

36

Introduction

Mass-specific nutrient excretion rate in fishes (nutrients excreted per unit body mass per unit time) can be considered a potential 'ecosystem effect trait': it underlies an organism's direct or indirect effect on an ecosystem function (Matthews et al. 2011) and it can change the availability of limiting nutrients (nitrogen and phosphorus) at the base of aquatic food webs (Atkinson et al. 2016). Recent data compilations have shown that metabolic variables (e.g. body size and temperature) and certain ecological stoichiometric variables (e.g. trophic guild and vertebrate versus invertebrate classification, but not necessarily N:P ratios) are key for predicting nutrient excretion rates of aquatic invertebrates and vertebrates (Allgeier et al. 2015; Vanni and McIntyre 2016). A positive, concave down allometric relationship between animal body mass and nitrogen excretion rate ($\mu\text{g h}^{-1}$), with a power scaling coefficient of less than one, is well established. Larger-bodied fish and macroinvertebrates have higher absolute excretion rates because of their larger organismal biomass (Allgeier et al. 2015; Vanni and McIntyre 2016); however, on a mass-specific basis (per gram), smaller animals usually outpace larger animals in their rate of excretion (Vanni 2002).

For fish, recent research has focused on understanding factors that determine interspecific patterns in excretion rate (Villéger et al. 2012; Moody et al. 2015), and the overall role of excretion in aquatic biogeochemical cycling (reviewed in Atkinson et al. 2016). More recently, there is also growing interest in quantifying intraspecific variation in fish excretion rates and relating these to potential effects on nutrient recycling and trophic state in ecosystems (e.g., Tuckett et al. 2014; Bassar et al. 2017; El-Sabaawi et al. 2017). For example, the size structure of fish populations can have a strong influence on nutrient cycling in aquatic ecosystems if a population is dominated by smaller individuals with higher mass-specific excretion rates

60 compared to an equivalent biomass of larger-bodied individuals (Allgeier et al. 2016; Catalano
61 and Schaus 2016). However, despite a growing recognition and debate of the importance of
62 evolution in `ecosystem effect traits` in ecological and ecosystem processes (Schoener 2011), the
63 extent to which genetic/maternal differences can explain intraspecific variation in mass-specific
64 fish excretion rate is relatively unexplored (reviewed in El-Sabaawi 2017; but see El-Sabaawi et
65 al. 2016; Leal et al. 2017).

66 The objective of our study was to investigate intraspecific allometric relationships
67 between body mass and excretion rate among two, isolated fish populations that exhibit
68 genetically-based differences in body size and growth. We tested the following questions: 1) Is
69 there a difference in mass-specific excretion rate between genetically size-divergent populations
70 that can be accounted for by genetic/maternal factors? And, 2) which population potentially
71 contributes more nutrients to the streams in the wild, considering the combined effects of mass-
72 specific excretion rate, population body size structure, and population abundance?

73 Our study focused on two isolated, genetically size-divergent populations of a
74 widespread, socio-economically important salmonid, the brook trout (*Salvelinus fontinalis*
75 Mitchill 1814). The study populations inhabit freshwater streams at Cape Race, NL, Canada
76 (hereafter abbreviated CR) (Table S1; Fig. S1), where they share a common ancestor from which
77 they diverged during the last glaciation 10 000 to 12 000 years ago (Danzmann et al. 1998).
78 These streams are relatively pristine, having experienced negligible human influence such as fish
79 stocking and negligible fishing pressure due to the small size of adult trout (~3''-6'' in length;
80 Wood et al. 2014).

81 Brook trout at CR represent a useful system for testing intraspecific allometric
82 relationships with excretion rate because local habitat differences have resulted in genetically-

based phenotypic divergence in body size and juvenile and adult growth rate between populations (Wood et al. 2015; Fraser et al. 2018). Research on CR streams has found that stream depth is an important determinant of fish body size: smaller-bodied fish with earlier age at maturity have higher fitness in shallow streams because they are less limited by deep-water habitat for breeding and overwintering survival (Hutchings 1993; Belmar-Lucero et al. 2012). Among our study populations, trout from Cripple Cove River (hereafter abbreviated CC) grow more quickly and are longer and heavier at maturity than trout from Freshwater River (hereafter abbreviated FW) both in the wild (Hutchings 1990, 1996) and under common conditions in a captive environment (Fraser et al. 2018).

We anticipated that excretion rate per gram of biomass would be lower for faster growing CC compared to FW trout at the juvenile stage. The quantity of nutrients in a consumer's excreted wastes is a balance between nutrient supply in the diet and nutrient demand for growth and reproduction in the body (Elser et al. 2003). CC trout may have a higher nutrient demand for growth compared to FW trout, and nutrient supply was controlled in our study because both CC and FW trout were fed to saturation on the same diet under the same laboratory environmental conditions during rearing. Given that animal body size is generally negatively correlated with excretion rate on a per gram basis (Vanni 2002), we also anticipated that the larger CC fish would have a lower mass-specific excretion rate than FW fish at the adult stage. For our second question, we expected that CC trout would have lower nutrient excretion at the whole population scale compared to FW trout. This was because we expected lower mass-specific excretion rates for the larger CC trout, and because CC population densities are lower compared to FW in the wild (Bernos and Fraser 2016).

Materials and Methods

We conducted controlled laboratory excretion experiments on captive-reared F_1 generation CC (46° 38.759' N, 53° 06.164' W) and FW (46° 38.760' N, 53° 13.304' W) brook trout. Parents (F_0) of the F_1 -generation individuals used for the experiments were wild-caught. Brook trout are the only species present in FW; in CC, this species co-exists with threespine stickleback (*Gasterosteus aculeatus* Linnaeus 1758) and American eels (*Anguilla rostrata* Lesueur 1817) (Hutchings 1990). We measured excretion rates at two age classes (age 0.5 y and 1.5 y) and modelled the allometric relationship between mass and excretion rate for each population using linear mixed-effects models. Our analyses were focused on differences between the two brook trout populations, and family was included as a random variable in the mixed models. We had 5 to 7 families per population and age category, and $n=3$ replicates per family (Appendix A). With an exception of two families that could not be re-measured for the older 1.5 y age class due to mortality, most of the same families were conserved between age categories for comparisons of excretion rate between life stages. We extrapolated individual-level measurements of within-population variation in nitrogen excretion rate to the whole-population level using body-size distributions and census population size (N_c) estimates for these populations in their natural stream habitat.

Fish origin and rearing

Brook trout gametes were collected in October 2014 from electrofished CC and FW adults, transported during the night to Montreal QC, and used to generate family crosses (one different female $\times$ one different male) at Concordia University. Methods for fish rearing and maintenance are provided in Appendix A and are also described in Fraser et al. 2018. Two sets

of excretion experiments were conducted with almost identical protocols but different age categories of the same families within each population: i) 0.5 y juvenile fish in summer 2015 and ii) 1.5 y adult fish in summer 2016. The majority (>96%) of the 2014 cohort of CR brook trout matured in the fall of 2016, consistent with the same age-at-maturation observed in a previous study involving captive trout from CR (Fraser et al. 2018). Sexually-mature individuals were determined from visual inspection of spawning “readiness”: a release of sperm for males and an elongated cloaca/soft belly for females with obvious egg bulges.

Excretion rate experiments

For the 2015 experiment involving juveniles, fish had been reared in tanks with primarily recirculated municipal water. Water used in the 2015 experiment was a 50:50 ratio of water from the fish rearing tanks: fresh dechlorinated municipal water to reduce initial quantities of ammonia and ammonium in the incubation water for the experiment. For the 2016 experiment involving adult fish, the water was 100% fresh dechlorinated municipal water because the water in the larger tanks for adult fish was flow through. The temperature of water was 14.7°C for the 2015 experiment (0.5 y juveniles) and 16.8°C for the 2016 experiment (1.5 y adults). Comparisons for this study were made within age classes, not between age classes, and so this difference in incubation temperature between experiments did not influence the interpretation of our results.

Immediately prior to experiments, the fish acclimated for 10 minutes in a 3.1 L acclimation container with oxygen diffusers that contained approximately 1 L of the same water as in the incubation containers for the experiments. A stress-related spike in ammonium excretion can occur immediately following handling and initial confinement (Whiles et al. 2009).

Therefore, the fish were acclimated for 10 min to reduce stress associated with handling and different water used in the incubation containers versus the tanks. Following 10 min acclimation period, the fish fed for 15 min in the acclimation container on the maximum amount of food pellets that the fish could consume in this period through observation. This was done to minimize differences in the level of feeding satiation between fish at the start of the experiment, which could have influenced excretion rate (Kajimura et al. 2004; Whiles et al. 2009). Differences in feeding satiation may have occurred among individuals in the larger rearing tanks where smaller and larger fish were together. By the end of the 15 minutes, all individuals within each container had stopped feeding, i.e. had stopped taking pellets from the surface of the water in the container, in the water column, or from the floor of the container. After 15 min of feeding, fish for each replicate were transferred into 3.1 L incubation containers with 565 ml of water for the onset of the experiment. Fish were hidden from exterior movements to reduce handling stress during acclimation and feeding.

Our response variable in experiments was excretion rate (nutrients excreted per unit time) in the form of excreted nitrogen (ammonia NH_3 + ammonium NH_4). We followed a modification of the protocol for measuring brook trout nutrient excretion described by Whiles et al. (2009). For the 2015 juvenile experiment, we had $n=3$ replicates per family, and each replicate contained a group of three 0.5-y fish in a 3.1 L container with 500-565 ml of water. The exception was one family for which we had only two replicates because of a limited availability of individuals (CC15). We included three 0.5-y fish per replicate for this age class because laboratory analytical detection limits were too low to reliably detect excretion from a single individual. For the 2016 adult experiment, each family-level replicate contained only one 1.5-y fish per 3.1 L container

with 500-565 ml of water as in 2015; the level of replication was maintained at $n=3$ within each family.

To determine the initial quantity of nitrogen present in the water prior to the experiment and/or associated with fish excretion response to handling, an initial water sample for $\text{NH}_3 + \text{NH}_4$ analyses were collected per family at five minutes following the introduction of fish into the incubation container. This reduced the water volume in incubation containers from 565 ml to 500 ml. The fish were incubated in the containers for a total of 30 min, at which time 65 ml sample of water was removed for the final measure of $\text{NH}_3 + \text{NH}_4$. Fish were then removed from the incubation container to a larger aquarium to recover. Each individual fish was subsequently weighed to 0.01 g for the juveniles (0.1 g for adults); each individual fish was photographed along with a reference scale to quantify length using the program ImageJ (Rasband 2011).

Standard length (cm) was measured from the tip of the nose to the beginning of the caudal fin.

Initial and final 65 mL water samples were 30- μm filtered to remove traces of feces and food, and then refrigerated with a 50:50 solution of incubation sample water: sulfuric acid to preserve the $\text{NH}_3 + \text{NH}_4$ (American Public Health Association et al. 2012). Experiments were conducted on two consecutive days; water samples were sent by courier in coolers to the St. Lawrence Institute of Environmental Sciences (Cornwall, ON) for laboratory analyses of excreted nitrogen (N): $\text{NH}_3 + \text{NH}_4$. Laboratory protocols for $\text{NH}_3 + \text{NH}_4$ analyses were based on the phenate method (4500- NH_3 F.) (American Public Health Association et al. 2012) that has been modified with substitution of sodium salicylate for phenol (Verdouw et al. 1978). The detection limits were $0.04 \pm 0.02 \text{ mg } \text{NH}_3 + \text{NH}_4 \text{ L}^{-1}$. To calculate the final quantity of excreted nitrogen in incubation water ($\mu\text{g } \text{NH}_3 + \text{NH}_4 \text{ L}^{-1}$), the initial measure of $\text{NH}_3 + \text{NH}_4$ was

subtracted from the final measure. We calculated excretion rate as the amount of nitrogen excreted per unit time ($\mu\text{g NH}_3 + \text{NH}_4 \text{ h}^{-1}$), with an incubation time of 0.5 h.

Statistical Analyses

Excretion -Excretion rates (Ex , $\mu\text{g}\cdot\text{h}^{-1}$) are often modelled as scaling non-linearly with body mass (M , g) according to an exponential function with the form $\text{Ex} = aM^b$ (El-Saabawi et al. 2015). Therefore, for both juveniles and adults, linear models in which excretion rate and body mass were natural-log transformed were compared to models in which the data were left untransformed, by examining adjusted R^2 values using R, version 3.3.3 (R Core Team 2017). R^2 values for adults indicated that excretion rates were better modelled using a simple linear regression rather than with natural-log transformed excretion rates and body mass ($R^2 = 0.733$ vs. 0.689, respectively); for juveniles, neither model explained significant variation ($R^2 = 0.045$ and -0.016, respectively). Similarly, generalized additive mixed models (including family as a random effect) with non-linear smoother functions did not significantly improve model fit above simple linear models (Supplementary Material, Table S2, Figure S2), and visual examination of residual QQplots from mixed-effects models (see below) indicated a better fit when data was untransformed (Supplementary Material, Figure S3). Untransformed excretion rate and body mass data were therefore presented herein, but see Supplementary Material, Table S3 for log-transformed body-mass excretion scaling coefficient estimates.

Between-population comparisons of excretion rates from the laboratory experiments were tested using general linear mixed effects models. Excretion rate was modeled with population included as a categorical fixed effect and mean-centered individual mass included as a continuous fixed covariate. Metabolic rates can vary in scaling with mass across age classes in

fish (Norin and Gamperl 2017). In our study, age class covaried strongly with mass, with no overlap between age-groups. As a result, we could not model age-dependent scaling factors by including an age-by-body mass interaction in our mixed models (variance inflation factor (VIF) for interaction term was > 300). The relationship between excretion and mass for juvenile and adult age classes was therefore analyzed separately.

Excretion rates estimated for the 0.5 y aged treatments represented the total excretion rate of the three individuals used in each experimental replicate; the total mass of all individuals in a replicate was therefore used. For both juvenile and adult models, all possible interactions between fixed effects were tested. A family-level term was included as a random effect in all models regardless of significance to account for non-independence of measurements across related individuals. A random family-by-mass term was also tested to account for potentially different mass-excretion slope relationships among families. When excretion rate scaled significantly with body mass for an age-class, we compared excretion rates for two scenarios: i) standardizing for size differences across populations and ii) comparing average-sized fish from each population.

Specific Growth Rates – Mixed effect models were used to test for population-level differences in mass and growth rates measured on individuals in the laboratory experiments. Individual mass measurements were *ln*-transformed and used as dependent variables in all models; this assumes a growth rate that is proportional to size and constant over time (i.e. specific growth rate). Population (CC vs. FW) was firstly included as a categorical fixed effect. A family-level term was also included as a random effect in all models regardless of significance to account for the non-independence of measurements across related individuals. Time between the two experimental periods (2015 and 2016) was included as a continuous fixed effect; the

slope of mass across time therefore represented specific growth rate. A population-by-time interaction term was included to represent the effect of population on specific growth rate along with a random effect of family-by-time term to account for repeated mass measurements within families across experimental time periods.

Statistical analysis was conducted in R using the *nlme* (Pinheiro et al. 2017) and *lme4* (Bates et al. 2015) packages. Separate variances for each experimental period were initially modelled using the ‘varIdent’ function in *nlme*. However, this term did not improve model fit (log-likelihood ratio test (LRT); $\chi^2_1 = 0.629$, $P = 0.428$) and was dropped; all subsequent analyses were performed in *lme4*. Backwards stepwise model selection was conducted to remove non-significant model terms ($P > 0.05$). Complex random-effects terms were tested first using LRTs for models estimated using restricted maximum likelihood (REML). Fixed effects terms were then tested for models estimated using maximum likelihood (ML); F -tests were used for fixed-effect term testing as implemented in *pbkrtest* (Halekoh and Højsgaard 2014), which estimates denominator degrees of freedom using the Kenward-Roger approximation (Kenward and Roger 2009). If an interaction term was found to be significant, all lower-order terms were automatically included in the final model. Pairwise contrasts between term levels were conducted using t -tests. Final parameter estimates were obtained from models estimated using REML.

Extrapolated population-level excretion

Excretion rates estimated from the adult brook trout in laboratory conditions were extrapolated to their natural populations, which included a mixture of size and age classes. Captive-reared fish were, on average, larger than similarly aged wild fish; the body size

distribution of the captive fish, overall, was therefore relatively similar to their (older) wild counterparts (Table 1). Adult census population size (N_c) population estimates and mass distributions were previously obtained for CC and FW in both natural populations (see Bernos and Fraser 2016 for methodology). Total absolute population excretion was estimated through simulations based on the natural size distributions observed for each population ($CC_n = 204$, $FW_n = 137$). To account for uncertainty associated with body size distribution estimates, n random samples (where $n = N_c$) for each population were drawn from a normal distribution with a mean and standard deviation equal to that of the $\ln$ -transformed observed population body-mass distribution. New excretion rate values for these samples were generated from back-transformed mass values using the `simulate.Mermod` command in *lme4* (with random effects marginalized). The simulated excretion rates were then summed to obtain a total population level excretion rate; this process was repeated 10000 times to generate a mean estimate and 95% confidence intervals for the total population level excretion rate. To account for error in N_c estimates, this process was repeated for lower and upper 95% confidence intervals for N_c estimates. Stream length and width were also quantified in habitat surveys for both populations, enabling an estimate of total stream area. Simulated whole-population excretion rates were divided by total stream area to obtain an approximate per-area excretion rate estimate – this controlled for differences in density observed across the two populations examined. Populations were considered significantly different when confidence intervals did not overlap.

Results

Excretion rates

Juveniles (age 0.5y) - Body mass of juvenile brook trout was uncorrelated with mass-specific excretion rate (Table 2, Fig. 1a). Neither the random effect family-by-mass term (LRT; $\chi^2_1 = >0.001$, $P = 1.00$) or the fixed-effect mass term (Table 2) were significant. The best-fit model describing excretion rates in the two populations included only an intercept term.

Adults (age 1.5y) - The best-fit model describing excretion rates in adult brook trout included the population and mass terms (Table 2). The random effect family-by-mass term was not significant (LRT; $\chi^2_1 = 2.002$, $P = 0.157$) suggesting that families did not exhibit different allometric relationships between mass and excretion rate. Body mass of adult brook trout was positively correlated with excretion rate (Table 2, Fig. 1b). The population-by-body mass interaction was not significant indicating that within the adult age-class, populations exhibited a consistent allometric scaling of absolute excretion rate with body mass (Table 2). Individuals from FW excreted, on average, significantly more than those from CC, when comparing fish of the same body size (Table 2, Fig. 1b; mass-specific excretion rate).

The average adult mass across both populations was 29.6 g. At this size, the smaller FW adults excreted significantly more compared to CC adults on a per gram basis ($t_{28.89} = 4.273$, $P < 0.001$; Fig. 2a). However, excretion rate comparisons between populations must additionally account for between-population average body mass differences; an average sized FW adult (at 26.8 g) did not exhibit significantly different absolute excretion rates relative to an average-sized CC adult (at 32.7 g) ($t_{27.05} = 1.691$, $P = 0.112$; Fig. 2b). While FW adults had greater per-gram excretion rates, CC individuals typically grew much larger, resulting in no difference in absolute excretion rates when comparing an average-sized FW adult to an average-sized CC adult.

Body mass and specific growth rates

The best-fit model describing growth rates in the two populations over time included the population and time terms (Table 1). At initial measurement, CC trout were larger than FW trout ($t_{11.6} = 2.476, P = 0.030$, Fig. 1). However, a lack of significance associated with the population-by-time interaction term (Table 1) indicated that both populations continued to grow at the same proportionate rate ($0.897\% \text{ day}^{-1} \pm 0.013$) during this experimental period. Consequently, CC trout were similarly proportionately larger than FW trout at the second experimental period as they were during initial measurement. The random effect family-by-time term was not significant (LRT; $\chi^2_1 < 0.001, P = 0.987$) suggesting that families did not exhibit different SGRs.

Extrapolated population-level excretion

Point-estimates for FW N_c varied between 4000 and 6500 individuals; CC N_c varied between 1500-2400. In the wild, CC fish were over twice as large as FW fish (mean of 16.7 g vs 8.1 g). Total sampled stream area was similar between streams (FW at 6537 m² and CC at 6706 m²). N_c estimate availability was incomplete for the sampled populations in some years – only CC N_c estimates were available in 2010 and 2012 and only FW estimates are available in 2014. For years in which N_c estimates were available for both populations (2011 and 2013), total population and per-m² excretion estimate confidence intervals did not overlap, and FW exhibited significantly greater total absolute excretion rates relative to CC (e.g. approximately a 2-fold and 4-fold higher per-m² N excretion in FW in 2011 and 2013, respectively) (Fig. 3; Supplementary Material: Table S4).

Discussion

Mass-specific and absolute excretion rates

Our study is among the first to provide evidence of genetic/maternal differences in mass-specific excretion rate between fish populations (Fig. 1b; Fig. 2a). In line with our first prediction, the smaller-sized FW adults had a higher gram-specific excretion rate across all body masses compared to the larger adult CC fish under common environmental conditions (different intercepts, Fig. 1b; Fig 2a). Smaller fish are generally known to have higher excretion rates than larger fish on a per gram basis (Vanni 2002); we found evidence that, regardless of size, fish from FW consistently excreted more nitrogen than CC fish. However, since the larger (on average) CC adults excreted more total nitrogen on an absolute basis compared to the smaller FW adults that excreted more nitrogen on a per gram basis, there was no difference in average absolute individual excretion rates between the two populations (Fig. 2b). While genetic/maternal differences in excretion rate on a per gram basis co-evolved with genetic divergence in body mass between these trout populations, this co-evolution has also resulted in no net difference in average absolute individual excretion rate between populations when average population adult body masses were taken into consideration. Other research on population-level differences in excretion rate in fish has not differentiated between environmental acclimation and genetic/maternal sources of intraspecific variation in excretion rates (e.g., Trinidadian guppies: Bassar et al. 2015; El Sabaawbi et al. 2015b; Bassar et al. 2017; three-spine stickleback: El Sabaawi et al. 2016). The only research that we are aware of to date that has addressed a genetic basis for population divergence in nutrient excretion was done on three-spine stickleback with divergent population morphology in plating armour and ecological stoichiometry (Leal et al. 2017). Our research provides a unique contribution because no other studies, to our knowledge, have tested for genetic/maternal divergence in mass-specific and absolute excretion rate between genetically size-divergent fish populations.

The body mass of adult brook trout individuals positively correlated with mass-specific excretion rate, as predicted by metabolic theory for interspecific differences across animal taxa (Allgeier et al. 2015; Vanni and McIntyre 2016). There was no difference in allometric scaling of mass-specific excretion rate with adult body mass between the two populations (similar slopes, Fig. 1b), which was also consistent with this literature. No relationship was detected between body mass and mass-specific excretion rate in the juvenile 0.5 y fish (Fig. 1a). However, this finding for the juvenile fish may be related to a low range in small body sizes combined with analytical limits for nitrogen detection in the lab ($0.04 \pm 0.02 \text{ mg NH}_3 + \text{NH}_4 \text{ L}^{-1}$).

Body mass and specific growth rates

The body size differences that we observed between the study populations under common environmental conditions concur with previous research. CC adult trout were longer and heavier compared to FW trout in the wild (Hutchings 1990, 1993, 1996; Fraser et al. 2018) and under common environmental conditions (Fraser et al. 2018; this study). It is possible that CC and FW fish have similar efficiency in converting food to biomass, but they differ in how this biomass is allocated to growth and reproduction, as has been found in guppies (Reznick 1983). For instance, CC produce more eggs per female and have slightly smaller alevins (Hutchings 1996), yet in the wild reproduce at one year later and at a larger female body size compared to FW (Hutchings 1990, 1993, 1996).

Our common-garden experiment could not entirely control for maternal effects from the wild in mass-specific excretion rate since we examined F_1 rather than F_2 generation fish. However, it is also possible that had we reared the trout to the F_2 generation, this could have imparted maternal effects in excretion rate more related to rearing conditions in a tank than

maternal effects from the wild. This aside, in the wild, FW females invest proportionally more in their offspring in terms of egg size and number (Hutchings 1990) and alevin size (Wood et al. 2015), and so we might have expected that a strong maternal effect would have resulted in larger FW juveniles than CC juveniles at 0.5 y. Instead, at initial measurement, CC trout were larger than FW trout and there was no evidence for a between-population difference in specific growth rate (Table 1): both populations continued to grow at the same proportionate rate from juvenile to adults during the experimental period examined. Genetic divergence in body size, combined with a comparison of lab-reared F₁ offspring under common environmental conditions, supports a genetic component to the phenotypic difference in mass-specific excretion rate between the trout populations (Wood et al. 2015; Fraser et al. 2018). By using controlled laboratory experiments with F₁ generation fish that minimized environmental effects that could have influenced nitrogen excretion rates, such as water temperature, dietary quality, and water source, our study provides evidence to support a genetic basis for differences in body mass and in per gram excretion rate between the brook trout populations.

Extrapolated population-level excretion

Consistent with our second prediction, whole population-level estimates of absolute fish nitrogen contributions to streams through total excretion were lower in CC compared to FW (Fig. 3). These estimates were based on extrapolations from allometric relationships measured between average adult trout body mass and absolute excretion rate in the lab (Fig. 2b), and they took into consideration stream population abundance and body size distribution as well as stream area. We attributed this finding to similar average individual adult excretion rates between populations (Fig. 2b) but lower population abundances in CC compared to FW (Wood et al.

2014; Bernos and Fraser 2016). The population-level excretion estimates incorporated several sources of error – variability associated with body size distribution estimates in the wild, family-level variability in excretion rate, and uncertainty in N_c estimates, all of which were significant sources of error in simulated data (see Fig. 3). Our preliminary findings are suggestive of the potential importance for stream population body size distributions in relation to allometric relationships in determining total absolute excretion at the whole population-level, which would need to be confirmed with excretion studies conducted in the wild. In support of these findings, other research using field-measured excretion rates has also found that population density and biomass can have a stronger contribution to population-level excretion relative to organismal attributes such as body size and growth (mammals: Habeck and Meehan 2008; fish: El-Sabaawi et al. 2015; Tuckett et al. 2014).

Caveats

It is unknown if the genetic/maternal basis for the difference in mass-specific excretion rate between brook trout populations that we observed plays an important role in nature. In nature, local environmental conditions can differ between populations and can influence population excretion rates in aquatic ecosystems. For example, temperature effects on metabolism and body size have been identified as the primary drivers of excretion rate for many animal groups (Allgeier et al. 2015; Vanni and McIntyre 2016). Warmer temperatures within the thermal optima of species can increase metabolic rates and excretion rates of aquatic organisms (Vanni and McIntyre 2016). However, temperature unlikely has a strong effect on between-population differences in excretion rates in our study's streams. For example, average summer (main growth season) temperatures in CC and FW rivers were 14.9°C and 14.7°C, respectively

(four-year average: 2012-2015; M.C. Yates, Concordia University, unpublished data; 17°C in CC and 16.9°C in FW in 2015, Table S1). Body mass differences between trout populations, rather than temperature differences between the streams, are likely more important for determining mass-specific and absolute excretion rates of brook trout in CC and FW streams.

Other factors that can potentially influence fish excretion rates are diet (Moody et al. 2015) and body tissue stoichiometry (McIntyre and Flecker 2010). In our experiments, carry-over acclimation effects from source river conditions were reduced by controlling for dietary quality during rearing conditions and by using F_1 fish that were reared exclusively in the laboratory. However, in nature, there is often variation between streams in terms of the quantity and quality of dietary resources for brook trout: for instance, there is higher invertebrate biomass in CC compared to FW (Hutchings 1993). The body tissue stoichiometry of brook trout from these populations has not been measured, and it is possible that variation in N:P ratios of tissue content between populations and families within each of the populations could influence both phosphorus and nitrogen excretion rates in the wild because of local differences in resource quantity and quality. However, extensive data syntheses have indicated that tissue N:P is a relatively weak predictor of animal excretion rate compared to metabolic predictors such as body mass and water temperature for generalized carnivores such as salmonids (Allgeier et al. 2015; Vanni and McIntyre 2016).

Implications

Genetically-based population divergence in body mass and mass-specific excretion rate in brook trout was most likely a result of ongoing selection associated with differences in the availability of overwintering habitat between CC and FW streams, as has been found for other

streams at CR (Hutchings 1993; Belmar-Lucero et al. 2012). Energy allocation in reproduction rather than growth, as is the case for FW, can be an adaptive response to high post-reproductive mortality in the females (Reznick 1983; Hutchings 1993) that may result from reduced overwintering habitat availability in FW compared to CC, although this needs to be confirmed for these specific streams. The larger, faster-growing CC trout that reach reproductive maturity later may have a higher nutrient demand for growth compared to the smaller FW trout that reproduce commonly 1 y earlier (Hutchings 1993). Nutrient supply was controlled in our study because both CC and FW trout were fed to saturation on the same diet under the same laboratory environmental conditions during rearing. Since the quantity of nutrients in a consumer's excreted wastes is a balance between nutrient supply in the diet and nutrient demand for growth and reproduction in the body (Elser et al. 2003), lower metabolic rates could have resulted in a higher mass-specific excretion rate in FW trout because of a lower quantity of nitrogen assimilated from the food for growth. In FW trout, this could have enabled the re-allocation of energy to reproduction in terms of earlier reproductive maturity at a smaller adult body size and production of larger eggs (Hutchings 1990) and larger alevins (Wood et al. 2015). The consequences of intraspecific variation on basal metabolic rate, which is related with an organism's ability for maintenance, growth, and reproduction, on fitness is an emerging area of research (Burton et al. 2011; Pettersen et al. 2016). There is evidence that basal metabolic rate is often heritable (Pettersen et al. 2018), and our findings on excretion rates between genetically size-divergent brook trout support this view. Future research on brook trout at Cape Race NL could be used to inform an emerging field of research on the ecological and evolutionary consequences of basal metabolic rate on body size, reproductive investment, and organismal fitness, as well as on ecosystem processes related with nutrient cycling.

Salmonids have broad biogeographical distributions in north-temperate aquatic ecosystems (Scott and Crossman 1973) and high intraspecific trait variation (Fraser et al. 2011). There is a need to include salmonids in intraspecific studies of `ecosystem effect traits` because of the potential for these fish to impact the nutrient cycling of aquatic ecosystems through migratory behaviour (Wheeler et al. 2015), their presence as exotic species in certain ecosystems (Tronstad et al. 2015; Alexiades et al. 2017), or through differences in population size structure that may be a result of natural selection, as is the case in our study, or as a result of size-selective harvesting because of their socio-economic importance (Fraser 2013; Kendall et al. 2014).

Salmonids such as brook trout are often size-selectively harvested, and can undergo evolution of smaller body sizes and earlier maturation ages over time (Palkovacs 2011; Fraser 2013; Kendall et al. 2014). In our study of brook trout, which are not size-selectively harvested in the Cape Race NL system, we found evidence to support genetically-based co-evolution between body mass and mass-specific excretion rate in the adult fish. It is possible that fisheries-induced evolution in other brook trout populations that are size-selectively harvested could potentially result in increased mass-specific excretion rates. Indeed, other research has found that harvest-induced size structure of fish populations can induce changes in lake nutrient cycling (gizzard shad: Catalano and Schaus 2016) and nutrient contributions to coral reefs (many fish species: Allgeier et al. 2016). However, also consistent with other research (El-Sabaawi et al. 2015; Tuckett et al. 2014), our preliminary findings for extrapolated total population absolute excretion suggest that total population densities and biomass may be more important than allometry and population body size structure in determining total fish population nutrient contributions through excretion to aquatic ecosystems.

Future work in the CR system could measure the influence of genetically-based population divergence in brook trout excretion on nutrient contributions at the whole-ecosystem level. To do this, we would need to consider the water chemistry and nutrient export in the streams, excretion rates of F_0 fish, as well as nutrient cycling within the food web. This approach could contribute to a deeper understanding of the extent of reciprocal interactions between evolution and ecology (`eco-evolutionary interactions`) in nature (Schoener 2011).

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Table 1: Mean, minimum, and maximum sizes (g) of adult fish under laboratory conditions (age 1.5) and in the wild (age 1+ and older).

Population	Environment	Minimum	Maximum	Mean
FW	Laboratory	17.6	42.0	26.8
FW	Wild	3.0	47.8	8.1
CC	Laboratory	19.1	54.1	32.7
CC	Wild	2.4	51.6	16.7

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660 **Table 2: Test statistics for terms modelling excretion rate ($\mu\text{g h}^{-1}$) in two brook trout**
 661 **populations for juveniles and adults, with retained terms bolded.**

Age	Parameter	Df	<i>F</i> value	<i>P</i> -value
Juveniles	Population	1, 9.79	2.191	0.170
	Mass	1, 23.24	0.664	0.424
	Population $\times$ Mass	1, 28.23	0.202	0.656
Adults	Population	1, 32.38	85.369	<0.001
	Mass	1, 11.15	18.459	0.001
	Population $\times$ Mass	1, 31.73	0.176	0.677

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Table 3: Test statistics for terms modelling specific growth rate over time in two brook trout populations.

Parameter	df	<i>F</i> value	<i>P</i> -value
Population	1, 11.6	6.129	0.030
Time	1, 136.4	4834.190	< 0.001
Population $\times$ Time	1, 136.0	0.475	0.492

Draft

List of Figures

Fig. 1. Allometric correlation between body mass (mass of individual fish, g) and excretion rate ($\mu\text{g h}^{-1}$) in two brook trout populations from Cape Race (CR) NL Canada (Cripple Cove River (CC) = circles and dotted line; Freshwater River (FW) = triangles and dashed line) for **a)** 0.5 y juvenile fish and **b)** 1.5 y adult fish.

Fig. 2. Mean population excretion rates of adult fish from two brook trout populations (mean $\pm$ standard error), CC and FW from CR NL Canada: **a)** mass-specific excretion rate at 29.6 g for both FW and CC adult fish, which is the average size of adult fish across both FW and CC populations; **b)** absolute excretion rate for each population at its population-specific average size: 26.8 g for FW adults and 32.7 g for CC adults. CC trout are represented by circle symbols and FW trout are represented by triangle symbols.

Fig. 3. Predicted whole population-level contribution of nitrogen from excretion to source streams for adult brook trout collected from CC (grey symbols) and FW (black symbols) at Cape Race, NL Canada between 2011 and 2014. Population-level total nitrogen excretion is expressed as the total amount of nitrogen excreted per m^2 of stream per unit of time: ($\mu\text{g (m}^2)^{-1} (\text{h})^{-1}$), after accounting for stream-area and body-size distributions of the two populations in the wild. Circles represent excretion estimates using mean abundance estimates, and triangles represent lower and upper 95% confidence interval abundance estimates.

690 **Appendix**

691 **Appendix A.** Methods for characterising physico-chemical conditions of FW and CC streams,
692 and lab-rearing of brook trout.

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Appendix A – Methods

Field - Physico-chemical and habitat characteristics of brook trout source streams

For temperature (Temp; °C), values were calculated from readings measured continuously (every 01h30min) between late June 2015 and early September in 2015 (n=511) in each of Cripple Cove River and Freshwater River. One temperature data logger was placed at the center of each stream from late June to early September 2015 to capture seasonal variation in the temperatures to which brook trout are naturally exposed over the duration of the pond experiment. pH, depth (cm) and specific conductivity (SPC; $\mu\text{S cm}^{-1}$) were measured during the month of June 2015 (Cripple n=35; Freshwater n=61). Dissolved oxygen (DO; mg L^{-1}) was only measured in Freshwater river during the month of June 2015 (Freshwater n=13). Vegetation of stream bed covered (Veg; %) and silt (Silt; %) were also measured for each river habitat. N.D. indicates where no data were collected. pH and dissolved oxygen were measured between 15 – 19 June 2015 along 34 transects in CC River (between 46° 38.854' N, 53° 06.269' W and 46° 39.733' N, 53° 06.756' W) and along 61 transects in FW River (between 46° 38.914' N, 53° 13.301' W and 46° 40.128' N, 53° 12.437' W).

Lab - Fish Rearing and Maintenance

Brook trout gametes were collected in October 2014 from electrofished CC and FW adults, transported during the night to Montreal QC, and used to generate family crosses (one different female $\times$ one different male) at Concordia University. Families were incubated in separate tubes within the same 1000 L recirculating tank at a controlled temperature of 7°C ($\pm$ 0.2). After hatching and yolk absorption, alevins from each family were transferred at the same density to separate 10L tanks for six months; fish were transferred again to larger (2200 L) tanks

from six months to 1.5 y of age and also maintained at the same densities. During these eighteen months, temperatures fluctuated seasonally as they do in nature (3-19°C) within tanks whilst minimizing among tank differences ($\pm 0.2^\circ\text{C}$). Dissolved oxygen and pH were also kept the same across tanks ($11.75 \pm 0.15 \text{ mg L}^{-1}$ standard deviation and $8.09 \pm 0.030 \text{ mg L}^{-1}$, respectively). Fish were fed multiple times daily ad libatum with a mix of live *Artemia salina* (Linnaeus 1758) and aquaculture feed until six months of age, and once daily ad libatum on aquaculture feed only from six months to 1.5 y.

Within these populations, juvenile growth rate and adult body size are more variable in CC than in FW in the wild (Hutchings 1996). Fish were fed standardized quantities of live feed depending on tank density. Higher variation in the Cripple Cove population was likely a result of aggression, coupled with greater intrinsic growth rate. Fish densities were standardized across tanks and populations.