

## FRESHWATER ECOLOGY

# Effects of genetic origin on phenotypic divergence in Brook Trout populations stocked with domestic fish

PHILIPPINE GOSSIEAUX <sup>1,†</sup>, ÉMILIE LAVOIE,<sup>2</sup> PASCAL SIROIS,<sup>2</sup> ISABEL THIBAUT,<sup>3</sup> LOUIS BERNATCHEZ,<sup>4</sup> AND DANY GARANT<sup>1</sup>

<sup>1</sup>Département de Biologie, Faculté des Sciences, Université de Sherbrooke, 2500 Boul. de l'Université, Sherbrooke, Québec J1K 2R1 Canada

<sup>2</sup>Chaire de recherche sur les espèces aquatiques exploitées, Département des sciences fondamentales, Université du Québec à Chicoutimi, 555 Boul. de l'Université, Chicoutimi, Québec G7H 2B1 Canada

<sup>3</sup>Direction de l'expertise sur la faune aquatique, Direction générale de la gestion la faune et ses habitats, Ministère des Forêts, de la Faune et des Parcs, Québec, Québec, Canada

<sup>4</sup>Département de biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Pavillon Charles-Eugène-Marchand, 1030 Avenue de la Médecine, Québec, Québec G1V 0A6 Canada

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**Abstract.** Phenotypic changes due to human activities are occurring at a far greater speed than those originating from natural causes in animal populations. For instance, phenotypic divergence among individuals may arise in populations supplemented with farm-reared fish that are known to display different phenotypes from those of wild individuals because of domestication. Little is known about how these phenotypic differences are maintained when domestic and wild individuals face the same environment and hybridize, as it is the case after supplementation. In this study, we assessed the effect of genetic origin of individuals on phenotypic trait divergence (morphology, growth, and size-at-age) in stocked populations of Brook Trout (*Salvelinus fontinalis*). We also evaluated whether genetic origin influences habitat use by documenting trophic niche and/or level using stable isotope analyses. We found significant effects of genetic origin on phenotypic variables with domestic fish generally being more fusiform and larger than wild and hybrid individuals. Lake identity also explained most of the variation in phenotypic variables, meaning that population-specific attributes were important drivers of morphology and size. Our results also showed that domestic fish were feeding in more littoral niches and at a higher trophic level than wild and hybrid individuals, suggesting that differences in feeding habits could partially explain phenotypic differences. These results highlight the importance of accounting for the genetic composition of populations when assessing the causes of phenotypic divergence in the wild.

**Key words:** geometric morphometrics; otoliths; salmonids; stable isotopes; stocking.

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† **E-mail:** p.gossieaux@usherbrooke.ca

## INTRODUCTION

Different ecological contexts can generate phenotypic variability in natural populations, eventually leading to the coexistence of different ecotypes at various geographical scales (Taylor

1999, Marcil et al. 2006, Perreault-Payette et al. 2017). Differential use of resources or habitat among individuals within a local population can also lead to phenotypic divergence (Landry et al. 2007, Hendry et al. 2009, Baillie et al. 2016). While the determinants of phenotypic variation

and differentiation in a population can be natural (see Landry et al. 2007), it can also often result from human actions (Hendry et al. 2008). In fact, human activities have been shown to induce phenotypic changes at a far greater speed than natural causes in animal populations (Hendry et al. 2008, Alberti et al. 2017). A common modification induced by human actions is the intentional introduction of exogenous individuals in wild populations, which is performed for conservation or management purposes (Brown and Day 2002, Tallmon et al. 2004, Naish et al. 2008, Laikre et al. 2010). These exogenous individuals can be either transferred from other wild populations (Tallmon et al. 2004) or originate from farms where they are bred with the objective of being released in the wild (Laikre et al. 2010).

Farm-raised individuals are affected by domestication, a mostly unavoidable phenomenon that can occur as a result of active artificial selection, but that can also occur involuntarily (Hutchings and Fraser 2008, Wilke et al. 2015, Uusi-Heikkilä et al. 2017) and in as little as a single generation of captivity (Christie et al. 2012, Fraser et al. 2018). Major consequences of domestication often include a decrease of fitness for domestic individuals when compared to their wild counterparts in natural environments (Araki et al. 2008, Christie et al. 2012, 2014). The impacts of domestication could be potentially deleterious enough to strongly decrease the efficiency of animal introduction for conservation measures (Laikre et al. 2010).

Releasing domesticated individuals into wild populations is a widespread action in fisheries management (Brown and Day 2002, Araki and Schmid 2010). The consequences of domestication have been well studied in salmonids given that they have been massively farmed and stocked around the world for decades (Naish et al. 2008, Hutchings and Fraser 2008, Lorenzen et al. 2012). In these species, morphological traits seem particularly affected by domestication because of the difference between selective pressures in aquaculture and in the wild (Swain et al. 1991, Fleming et al. 1994, Jonsson and Jonsson 2006, Pulcini et al. 2013). For instance, environmental conditions such as water temperature (Beacham 1990), population density (Jonsson and Jonsson 2006), or water velocity (Samways et al. 2015) during early life have a crucial

importance for body shape and differ significantly between natural and artificial habitats (Thorpe 2004, Jonsson and Jonsson 2006). Even though morphological divergence between wild and domestic fish can be explained by phenotypic plasticity (Samways et al. 2015), it has also been showed to have a genetic basis (Taylor and McPhail 1985, Swain et al. 1991, Pulcini et al. 2013). Furthermore, morphological divergence from the wild phenotype is stronger for domestic strains that have been kept captive during multiple generations than for strains recently domesticated (Fleming et al. 1994).

Another key trait strongly impacted by domestication is growth, because it is targeted by artificial selection. Fish farmers typically aim at producing fast-growing individuals by delaying the age of sexual maturation (Thorpe 2004, Jonsson and Jonsson 2006). Domestic fish thus generally have higher growth rates and size at a given age than their wild counterparts (McGinnity et al. 1997, Tymchuk et al. 2006, Solberg et al. 2013*a, b*). Again, these differences have a genetic basis (Tymchuk et al. 2006, Crespel et al. 2013*a*, Berejikian et al. 2017). However, the differences of growth rates between domestic and wild individuals could be context-dependent, as in natural conditions, wild individuals can display growth rates similar to those of domestic fish (Reisenbichler and McIntyre 1977, Solberg et al. 2013*a, b*). While hatchery-reared and wild fish often differ in their phenotypes (Jonsson and Jonsson 2006), it is less clear whether these differences are maintained when they both face the same environment after stocking. Moreover, when domestic and wild fish hybridize, it can be challenging to anticipate the potential impacts of genetic introgression on the morphology and growth of hybrids (Bougas et al. 2010, Granier et al. 2011).

Our goal in this study is to determine how the genetic origin of individuals influences morphology, growth, and size-at-age in stocked populations. Individuals with a domestic genetic background have hatched and spent their early life in aquaculture and are thus likely to present phenotypic differences compared to wild fish. The effect of these differences on the phenotype of hybrid individuals will then depend on the genetic basis of the measured traits. If phenotype has a strong genetic basis, phenotypic divergence

should be strong between individuals with different genetic origins and hybrids would be likely to display an intermediate phenotype. Alternatively, if phenotype is mostly shaped by environmental conditions, phenotypic divergence should be small and hybrids should resemble wild individuals since they shared the same environment since hatching. As a complementary question, we used stable isotope analyses to assess whether genetic origin influences habitat use and feeding habits, since these are two essential determinants of morphology (Bourke et al. 1997, Dynes et al. 1999, Bertrand et al. 2008, but see Samways et al. 2015, Andersson et al. 2017) and growth (Glaz et al. 2012, 2014, Morissette et al. 2018, 2019).

We investigated these questions in Brook Trout (*Salvelinus fontinalis*), a very popular salmonid for recreational angling, which has been massively stocked for decades in North America, and notably in Québec, Canada. Previous studies in this region showed that hybridization between domestic and wild fish is common and that stocked lakes present various levels of introgression of domestic genes (Marie et al. 2010, 2012, Gossieaux et al. 2018, 2019, Létourneau et al. 2018). We used data from 12 introgressed populations to determine the extent of phenotypic divergence between individuals that have different genetic origins. Since Brook Trout may display important plasticity for morphological traits (Kazyak et al. 2015, Samways et al. 2015, Zastavniouk et al. 2017), if an effect of genetic origin is detectable, we predict that it should be negligible compared to the effect of environmental variables. Also, since domestic individuals are actively selected for higher growth rates, we predict that domestic fish should outgrow wild individuals in the natural environment. This could be a consequence of genetic effects, early life conditions, or a combination of both. If rearing conditions are an important determinant of growth, we predict that we should observe differences due to genetic origin especially in young age classes (e.g., closer to the moment domestic fish were stocked and from their time spent in hatchery). Moreover, growth differences between domestic and wild fish have been shown to decrease as mortality increases (Solberg et al. 2013b) and we thus expect to see weaker effects of domestic origin in older age classes. We also

expect a strong effect of environmental conditions on growth and size (Solberg et al. 2013b, Fraser et al. 2018). Finally, we expect differences in trophic level or trophic niche between wild and domestic individuals, since domestication can affect feeding behavior (Huntingford 2004) and habitat use (Mittelbach et al. 2014).

## METHODS

### *Sampling and procedures*

We conducted sampling over two time periods (2007–2008 and 2014–2016) in three wildlife reserves (Portneuf 47°10'7.8" N, 72°20'32.7" W, Mastigouche 46°42'45.2" N, 73°25'37.7" W, and Saint-Maurice 47°04'00.0" N, 73°08'28.5" W) in Québec, Canada (see Gossieaux et al. 2018). Stocking practices are rigorously controlled in these reserves, and stocking history of lakes has been documented since 1964 (provided by the ministère des Forêts, de la Faune et des Parcs, Québec, Canada). Stocking intensity ranged from lakes that were massively stocked for decades to others that were not stocked for years or were never stocked. In order to stock its lakes, Portneuf reserve uses domestic fish from the Jacques-Cartier hatchery, a facility that kept fish in captivity for multiple generations. Mastigouche and Saint-Maurice reserves stock their lakes with hatchery-reared individuals from Lac-des-Écorces (a governmental facility) and Saint-Alexis-des-Monts hatcheries, which cross domestic and wild fish from Lake Bourassa (located in the Mastigouche reserve) to obtain hybrid strains. Fish are mostly stocked at very early life stages, such as fry.

For the phenotype analyses, we used samples from fish captured with gill nets in 12 lakes ( $n = 550$  fish; Appendix S1: Table S1) that are part of a larger study in the three wildlife reserves (see Gossieaux et al. 2019). For the stable isotopes analyses, we used data from four lakes in the same system ( $n = 438$ ; Appendix S1: Table S2). We sampled fish before the annual stocking events to avoid capturing recently stocked individuals. Therefore, all captured individuals spent at least between 10 and 12 months in the lakes. We euthanized fish with clove oil immediately after each capture. Each individual was then measured (total length,  $\pm 1$  mm). For 10 out of 12 populations used in phenotype

analyses, individuals were also sexed by observation of gonads during a dissection. Adipose fin of each fish was collected and preserved in 95% ethanol for later DNA extraction. Moreover, we obtained tissue samples from hatcheries (Jacques Cartier  $n = 53$ , Saint-Alexis des Monts  $n = 80$ , Lac des Écorces  $n = 40$ ) and from lake Bourassa ( $n = 40$ ). All protocols and procedures employed were reviewed and approved by the ministère des Forêts, de la Faune et des Parcs (Québec, Canada; see Gossieaux et al. 2018, 2019).

#### Genetic analyses

We used adipose fins to extract DNA and genotype each fish at 20 microsatellite loci following the protocols described in Gossieaux et al. (2018). We then determined the genetic origin of each fish using the software Structure v. 2.3.4 (Pritchard et al. 2000) following the assignment method and parameters described in (Gossieaux et al. 2018, 2019). Each fish was attributed a  $q$ -value ranging from 0 to 1, respectively, designating pure wild and pure domestic individuals.

#### Morphometrics data

Quickly after capture, each individual was photographed on the left side on a soft surface to minimize deformation. We chose 18 landmarks (Appendix S1: Fig. S1) for every selected individual with the software tpsDig v.2.31 (Rohlf 2005). After digitization, we applied a generalized Procrustes analysis (Rohlf 1999) to superimpose landmark configurations ( $n = 457$ ). This step removes the variation in landmark configurations due to scale, orientation, and location. The resulting transformed landmark coordinates (Procrustes coordinates) can be used as response variable in further statistical analyses as their variation is only attributable to differences in shape between individuals (Webster and Sheets 2010).

To make sure that measurement error was negligible, we digitized a second time 50 randomly selected individuals and estimated the repeatability of each landmark placement using the rptR R package (Stoffel et al. 2017). We also used a Procrustes ANOVA to quantify measurement error, which is estimated by an interaction between digitization identity and individual identity (Klingenberg and McIntyre 1998).

#### Growth data

We estimated individual growth parameters using otoliths. Right and left sagittae were extracted for all individuals, and left sagittae were mounted on microscope slides in thermoplastic glue, polished, and photographed using a microscope (Panfili et al. 2002). We analyzed photographs using the software ImageJ v. 4.51 j8 (Abràmoff et al. 2004). We determined the age of each fish counting annuli and measured transversal width, dorsal radius (DR,  $\mu\text{m}$ ), and annual increments width along DR ( $\mu\text{m}$ ; Appendix S1: Fig. S2). Each photograph was read at least twice by the same reader with over three months between the two readings (Panfili et al. 2002). Depending on otolith quality and on the observer's confidence, a score of confidence was assigned to each reading (see Appendix S1: Fig. S3 for more details on readings and verifications). This score ranged from 1 to 4 with 1 being very unsure and 4 very confident in the reading (Stevenson and Campana 1992). We excluded individuals for which otoliths were too damaged and kept a total of 487 fish for further analyses (10.8% of rejection).

#### Trophic niche and trophic level

Stable isotopes, and more specifically carbon and nitrogen ratios, are widely used to evaluate both trophic niche and trophic level, notably in freshwater ecosystems (Post 2002). Nitrogen ratios ( $\delta^{15}\text{N}$ ) are representative of the trophic position with higher scores reflecting a higher trophic level (i.e., more predatory individuals, Minagawa and Wada 1984, Vander Zanden et al. 1997). Carbon ratios ( $\delta^{13}\text{C}$ ) are used to evaluate trophic niche with low ratios indicating that individuals feed in pelagic environments based on autochthonous production and high ratios being the sign that individuals feed in the littoral zone more enriched by allochthonous subsidies (France 1995, Glaz et al. 2012).

Three lakes of Portneuf reserve and one of Mastigouche reserve ( $n = 438$  fish) were selected for collection of stable isotopes samples between 2007 and 2014 (Appendix S1: Table S2). A sample of dorsal muscle without skin or bone was collected and frozen for each individual immediately after capture. We dried muscle samples at  $60^\circ\text{C}$  for 48 h and grinded them to obtain a fine powder that we then encapsulated to obtain

samples of  $1 \pm 0.5$  mg. Carbon and nitrogen stable isotopes ratios quantifications were conducted at the Ján Veizer Stable Isotope Laboratory (University of Ottawa, Ontario, Canada). Detailed procedure is available in Morissette et al. (2019). Results are expressed as part per thousand (‰) noted as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

#### Data and statistical analyses

Body length of each individual at each age was back-calculated using the body proportional hypothesis (BPH) with the following formula:

$$L_i = \left[ \frac{c + dS_i}{c + dS_c} \right] \times L_c \quad (1)$$

with  $L_i$  and  $S_i$  being, respectively, body length and otolith radius length at age  $i$ ,  $L_c$  and  $S_c$  being the same measures at the time of capture, and  $c$  and  $d$  being, respectively, the intercept and slope of the regression of body size on otolith DR (Francis 1990). We then considered growth as the difference of body length between age  $i$  and  $i + 1$ . Knowing the age of each individual and the sampling year, we were able to determine the hatching year (hereafter called “cohort”) of each fish and used it in further analyses. Cohort was used in our analyses as a control variable to account for unknown year effects.

All analyses were conducted in R v. 3.6.2 (R Core Team 2017). Both  $q$ -value and cohort were explanatory variables of interest for both morphometrics and growth analyses. Sex could also be a relevant explanatory variable in our analyses, but it was unknown for two of our populations. Furthermore, among our 12 chosen lakes, nine were in the Saint-Maurice wildlife reserve and three in the Portneuf reserve, including the two lakes for which sex was unknown. We thus performed our analyses on morphology and growth three times: (1) with all our 12 populations without sex in models, (2) with only nine populations, all belonging to the Saint-Maurice reserve without sex included in our models, and (3) with the nine populations of Saint-Maurice with sex included in models. We also checked for multicollinearity in all of our models using the variance inflation factor ( $\text{VIF} < 3$ , Graham 2003). We checked for residuals before and after each model selection and removed one outlier.

*Morphometric analyses.*—All morphometric analyses were performed using the package geomorph v.3.0.6 (Adams and Otárola-Castillo 2013). To determine which variables affected Procrustes coordinates (i.e., shape), we first performed Procrustes ANOVA (Klingenberg and McIntyre 1998) using the type III ANOVA to compare nested models in order to determine the significance of each variable with a randomized residual permutation procedure (10,000 iterations). The full model comprised the  $q$ -value, total body length, the identity of the population and cohort of each individual, as well as interactions between  $q$ -value and lake,  $q$ -value and cohort and lake and cohort (along with sex when only considering populations for which this variable was known) as explanatory variables. All variable removals were tested one by one (stepwise selection) to assess their respective importance for the predictive power of the full model.

To further characterize shape variation and its determinants, we performed principal component analysis (PCA) on Procrustes coordinates (e.g., relative warp analysis). We only kept the principal component axis (PCs, relative warps) that explained at least 5% of the variation in our further analyses as suggested in Zelditch et al. (2004). We then used each of these PCs as response variables in linear models, on which we performed backward stepwise selection. Full models included all the explanatory variables that remained significant after the Procrustes ANOVA.

*Growth analyses.*—We analyzed growth data using two types of response variables. First, we used size-at-age for each individual from 1 to 5 yr old; secondly, we used growth from 1 to 5 yr old. For both of these types of response variables, we developed linear mixed models with  $q$ -value, score of otolith reading confidence and cohort of each individual, as well as the interaction between  $q$ -value and cohort as explanatory variables. We included lake identity as a random variable. We then applied a backward stepwise model selection.

We also analyzed these data using lake identity as a fixed variable to include interactions between lake and cohort and lake and genetic origin in the models.

*Stable isotopes analyses.*—To determine how the genetic status influences feeding habits, we split individuals into three categories in accordance with their  $q$ -values. Several thresholds were possible to differentiate domestic, wild, and hybrid individuals. We considered that individuals with a  $q$ -value of 0.2 or lower were wild, 0.8 or higher were domestic, and values between 0.2 and 0.8 represented hybrids. We also conducted all of these analyses using a threshold of 0.1–0.9 to ensure that our results did not depend on the chosen threshold (Vähä and Primmer 2006).

For each lake, we ran ANOVAs using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as response variables and the genetic status assigned as explanatory variable. We then performed Tukey post hoc tests to characterize more precisely how isotopes ratios differed between groups. We applied a false discovery rate (FDR, Pike 2011) correction on these results.

## RESULTS

For both morphometrics and growth analyses, results were very similar when excluding lakes from the Portneuf reserve and/or when adding sex in our models. Thus, only results obtained with all lakes are presented (see Appendix S2 for detailed results of analyses on subsamples).

### Morphometrics analyses

All 18 landmarks were highly repeatable (lowest repeatability score was  $r = 0.98$ , 95% CI = 0.97–0.99,  $P < 0.001$ ), and measurement error quantified by Procrustes ANOVA was negligible (SS = 0.005,  $P = 0.27$ ).

All explanatory variables of the Procrustes ANOVA except the interaction between  $q$ -value and cohort remained significant (Appendix S1: Table S3) and were kept for the relative warp analyses. The first four PCs explained at least 5% of total variance of the PCA, with PC1 = 36.0%, PC2 = 15.6%, PC3 = 7.6%, and PC4 = 7.2%. Explanatory variables that remained significant after model selection were not the same in the four models (Table 1). The only variable present in all final models was lake, and  $q$ -value remained in two final models because of its interactions with lake. We determined that PC1 represented mostly body curvature and head orientation (Fig. 1A; Appendix S1: Fig. S4); PC2,

Table 1.  $F$ -values from backward stepwise selection of linear models on morphometric data (relative warp analysis,  $n = 457$ ).

PC axis	Lake: $q$ -value	Lake	$q$ -value	Total length	Adjusted $R^2$ (%)
PC1	1.03	<b>3.93</b>	0.55	2.05	6.6
PC2	<b>2.29</b>	<b>inter</b>	<b>inter</b>	<b>5.79</b> (–0.0005)	53.9
PC3	1.50	<b>inter</b>	0.05	3.43	31.1
PC4	<b>1.96</b>	<b>inter</b>	<b>inter</b>	0.03	21.7

Notes: Significant variables ( $p < 0.05$ ) are in bold. Estimates are provided in parenthesis for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested; thus, we provide no value in these cases and indicate them with the term “inter.”

3, and 4 were more reflective of body depth and eye size (Fig. 1B–D).

### Growth analyses

For growth and size-at-age mixed models analyses, significant variables were different depending on age (Table 2). Genetic origin significantly influenced growth for two of our five models because of its interaction with cohort. Size-at-age analyses showed an effect of genetic

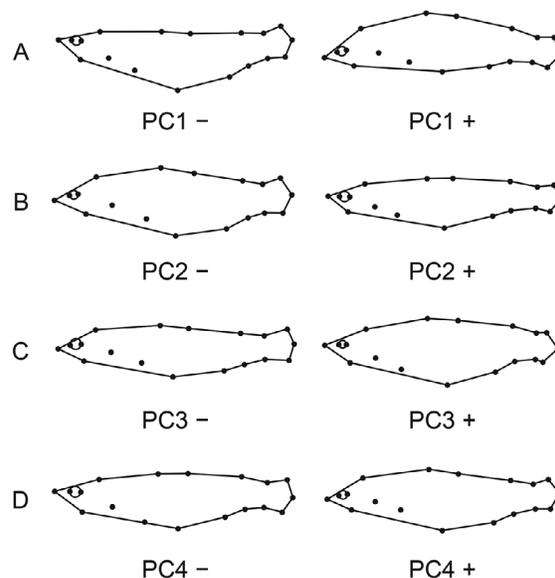


Fig. 1. Extreme shapes of each PC from PC1 to PC4. They, respectively, explain (A) 36.0%, (B) 15.6%, (C) 7.6%, and (D) 7.2% of total shape variation. PC1 mostly reflects body curvature, and PC2 to PC4 mostly characterize body depth.

Table 2. Chi-squared values and their associated degrees of freedom from backward stepwise selection of mixed linear models on growth (cm/yr) and total length (cm) at each age.

Metric and age class	n	Cohort:q-value	Cohort	q-value	Otolith reading confidence	Marginal R <sup>2</sup> (%)	Conditional R <sup>2</sup> (%)
Growth							
0–1 YO	485	<b>16.78; 8</b>	<b>inter</b>	<b>inter</b>	6.08; 3	21.1	25.0
1–2 YO	463	<b>22.82; 7</b>	<b>inter</b>	<b>inter</b>	0.69; 3	9.5	13.0
2–3 YO	324	3.92; 6	1.98; 6	1.16; 1	5.68; 3	0	3.4
3–4 YO	196	3.44; 5	<b>11.89; 5</b>	1.19; 1	1.44; 3	5.6	14.6
4–5 YO	90	3.23; 4	8.05; 4	0.05; 1	2.03; 3	0	21.1
Length							
1 YO	485	<b>16.78; 8</b>	<b>inter</b>	<b>inter</b>	6.08; 3	21.1	25.0
2 YO	463	<b>21.80; 7</b>	<b>inter</b>	<b>inter</b>	6.84; 3	15.8	24.8
3 YO	324	<b>19.88; 6</b>	<b>inter</b>	<b>inter</b>	5.19; 3	14.6	24.2
4 YO	196	8.33; 5	4.14; 5	<b>3.90; 1 (1.24)</b>	2.52; 3	2.3	17.7
5 YO	90	4.73; 4	6.09; 4	0.61; 1	4.03; 3	0	44.4

Notes: Number of individuals used for each analysis is indicated in the *n* column. Significant variables ( $p < 0.05$ ) are in bold. Estimate is provided in parenthesis for *q*-value for length at 4 YO (years old) since it is a significant continuous variable not in an interaction. Removal of variables that are in an interaction was not tested; thus, we provide no value in these cases and indicate them with the term “inter.”

origin in interaction with cohort from one to three years old and an effect of genetic origin alone at four years old (Table 2). In the latter case, the effect of *q*-value was positive, meaning that fish with more domestic genetic background were larger (Appendix S1: Fig. S5). When *q*-value interacted with cohort, the direction of effect was variable (Fig. 2; Appendix S1: Fig. S6). The difference between marginal and conditional  $R^2$  for both analyses increased with age (Table 2), indicating that lake identity influenced more strongly growth and size in older age classes.

Results of analyses using lake as a fixed effect were similar and are presented in Appendix S1: Table S4.

#### Stable isotopes analyses

Results were similar with the 0.2–0.8 and 0.1–0.9 thresholds of genetic origin determination, and we thus only present below results obtained with the 0.2–0.8 threshold (see Appendix S1: Table S5 for the 0.1–0.9 threshold).

Genetic origin significantly influenced  $\delta^{13}\text{C}$  in all lakes and also  $\delta^{15}\text{N}$  in three lakes out of four (all  $P < 0.001$ , except for  $\delta^{15}\text{N}$  in lake MER where  $P = 0.18$ ). Tukey tests showed that there was no difference between wild and hybrid individuals for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Table 3, Fig. 3; Appendix S1: Fig. S7). However, domestic fish had significantly higher  $\delta^{13}\text{C}$  than wild

individuals in all lakes and also higher  $\delta^{15}\text{N}$  in three out of four populations (Table 3, Fig. 3; Appendix S1: Fig. S7). Domestic fish also displayed higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than hybrids in two out of four populations (Table 3, Fig. 3; Appendix S1: Fig. S7).

## DISCUSSION

We found an influence of genetic origin of Brook Trout on morphology, growth, and size-at-age, which varied with population-specific attributes. We also established that domestic fish differed from their wild and hybrid counterparts in terms of trophic niche and level, as their isotopic signatures indicated that they were feeding more often on higher trophic level preys found in littoral environments.

#### Morphology

The main driver of morphological variation was the lake identity, suggesting that shape of individuals was primarily determined by the characteristics of the population they belonged to. Local environmental differences among lakes, which vary in terms of abiotic (e.g., temperature, dissolved oxygen levels, depth, lake area; Marie et al. 2012, Létourneau et al. 2018) and biotic conditions (e.g., presence of competitors, parasitic fauna; Gossieaux et al. 2018), have been shown

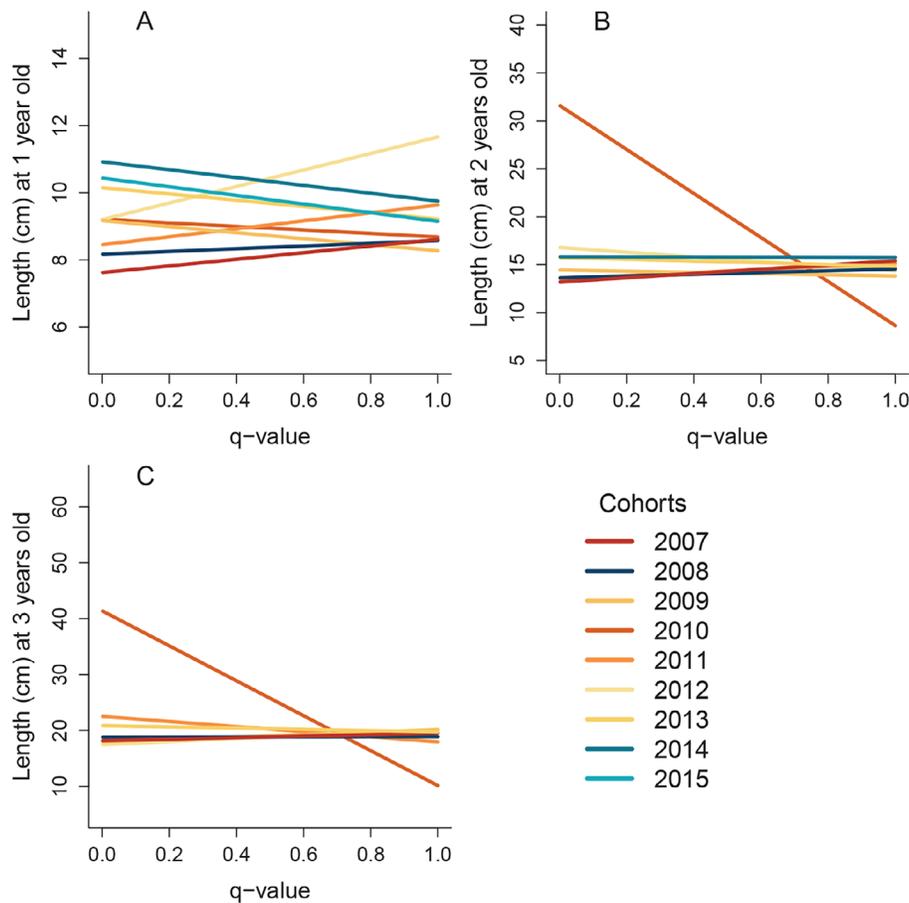


Fig. 2. Graphs of significant interactions between  $q$ -value and cohort at (A) 1 yr old, (B) 2 yr old, and (C) 3 yr old on total length (cm).

to influence morphology (Magnan 1988, Bertrand et al. 2008, Baillie et al. 2016, Zastavniouk et al. 2017). Brook Trout were previously shown to display highly variable morphologies among geographically close lakes (Kazyak et al. 2015) and are known to be phenotypically plastic (Kazyak et al. 2015, Samways et al. 2015, Zastavniouk et al. 2017). However, it should be noted that Brook Trout populations, including those in our study system, exhibit strong genetic differentiation among them (Marie et al. 2010, Lamaze et al. 2012). These genetic differences were linked to some phenotypical differences among populations (Bougas et al. 2010, Crespel et al. 2013b) and can even result in strain-specific genotype–environment interactions (Crespel et al. 2013a). Therefore, morphological variation observed here is probably widely influenced by

environmental conditions, but it could also be, at least partially, attributable to genetic differences among lakes.

Morphological characteristics that were particularly affected by local conditions in our study were body curvature, head orientation, eye size, and body depth (Fig. 1). Variation in head orientation leads to variation in oral gape axis, which is generally linked to selected food type, with fish that hunt fast prey having a more terminal orientation while fish that forage or feed on the bottom of lakes have a sub-terminal orientation (Diderich 2006). Eye size is linked to light condition and prey size and is a proxy of visual acuity (Diderich 2006). Body depth depends on swimming lifestyle and maneuverability (Diderich 2006). Similar variations in shape patterns among populations have been observed by Zastavniouk

Table 3. Results of the Tukey post hoc tests of the effects of genetic origin on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios.

Lake	Genetic status	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
		Lower	Upper	<i>P</i>	Lower	Upper	<i>P</i>
AMA	H-D	<b>-7.68</b>	<b>-3.12</b>	<b>&lt;0.001***</b>	<b>-1.01</b>	<b>-0.26</b>	<b>&lt;0.001***</b>
AMA	W-D	<b>-7.95</b>	<b>-4.84</b>	<b>&lt;0.001***</b>	<b>-1.07</b>	<b>-0.56</b>	<b>&lt;0.001***</b>
AMA	W-H	-3.14	1.15	0.67	-0.53	0.18	0.69
BEL	H-D	<b>-14.61</b>	<b>1.48</b>	<b>0.20</b>	<b>-2.16</b>	<b>0.85</b>	<b>0.73</b>
BEL	W-D	<b>-8.07</b>	<b>-2.04</b>	<b>&lt;0.001***</b>	<b>-1.58</b>	<b>-0.45</b>	<b>&lt;0.001***</b>
BEL	W-H	-6.55	9.57	0.97	-1.86	1.15	0.92
MER	H-D	-3.19	-0.20	<b>0.03*</b>	-1.28	0.19	0.33
MER	W-D	<b>-2.51</b>	<b>-0.71</b>	<b>&lt;0.001***</b>	-0.47	0.42	0.99
MER	W-H	-1.34	1.50	0.99	-0.18	1.22	0.33
MET	H-D	<b>-8.44</b>	<b>-4.60</b>	<b>&lt;0.001***</b>	<b>-1.92</b>	<b>-0.33</b>	<b>&lt;0.001***</b>
MET	W-D	<b>-8.63</b>	<b>-5.84</b>	<b>&lt;0.001***</b>	<b>-1.90</b>	<b>-0.75</b>	<b>&lt;0.001***</b>
MET	W-H	-2.36	0.93	0.67	-0.88	0.48	0.91

Notes: Genetic status (D, domestic; H, hybrid; W, wild) was determined with the 0.2–0.8 threshold of *q* values ( $q < 0.2 = W$ ;  $0.2 < q < 0.8 = H$ ;  $0.8 < q = D$ ). Names of the lakes are AMA, Amanites; BEL, Belles de Jour; MER, Mercure; and MET, Méthot. Intervals are based on the studentized range statistic with a 95% confidence level and are reported for the lower and upper intervals. *P* values are presented here after the application of the false discovery rate (FDR) correction. Significant differences between groups ( $P < 0.05$ , intervals do not overlap 0) are in bold.

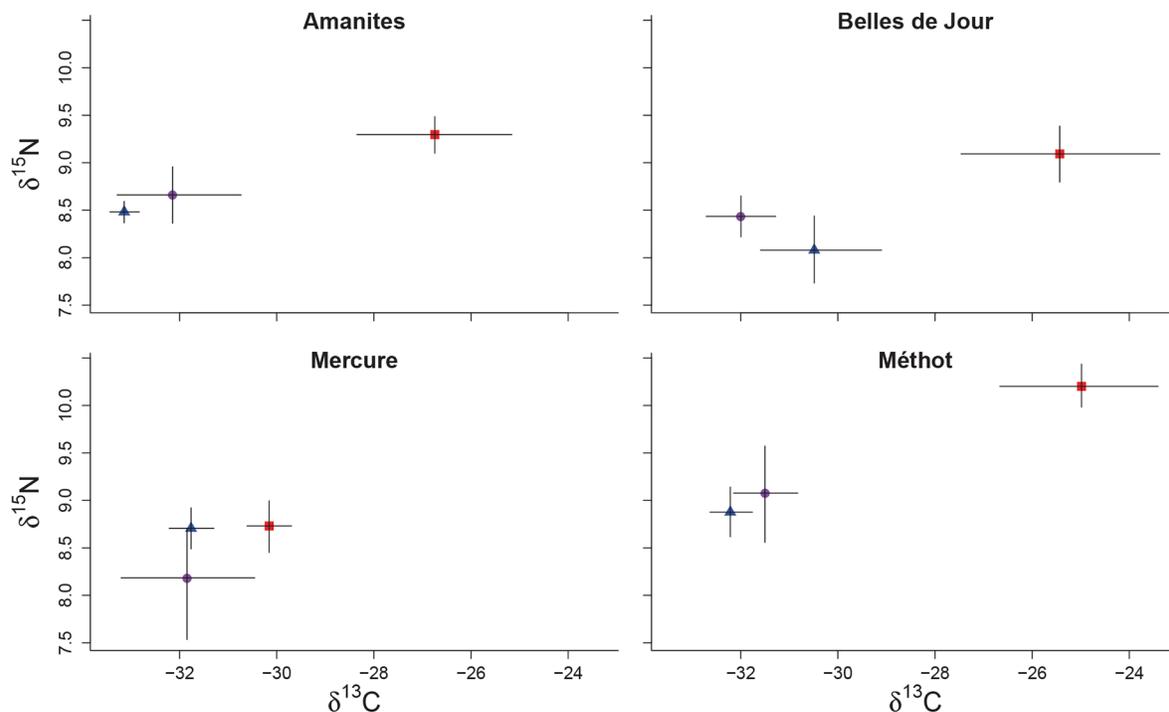


Fig. 3. Stable isotopes ratios of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for Brook Trout in four lakes. Red squares represent domestic trout, purple circles hybrids, and blue triangles wild trout. Genetic status was determined with the 0.2–0.8 threshold of *q*-values ( $q < 0.2 = W$ ;  $0.2 < q < 0.8 = H$ ;  $0.8 < q = D$ ). Error bars represent 95% confidence intervals.

et al. (2017) in a study of Brook Trout, which led the authors to conclude that selection acted differently among the various populations, leading to phenotypic divergence.

A portion of variance in shape was also explained by interactions between lake and genetic origin of fish. This result suggests that genetic background influenced body shape (here mostly body depth and eye size, Fig. 1B–D) in some environments more than others (e.g., genetic by environment effects, also shown in Harbicht et al. 2014). These morphological differences can be genetically based, as shown by previous studies (Taylor and McPhail 1985, Swain et al. 1991, Fleming et al. 1994, Pulcini et al. 2013). However, they can also be attributable to the long-lasting effect of early life rearing conditions (hatcheries vs. wild) which are strong determinants of body shape (Beacham 1990, Samways et al. 2015). Another possible explanation for the morphological differences between domestic and wild fish is that they reflect differences in terms of niche occupation and/or feeding habits as suggested by our results on stable isotopes (see the “Trophic niche and trophic level” section).

### Growth

Growth and size-at-age also varied depending on the population identity, as showed by the differences between marginal and conditional  $R^2$  in our analyses. Again, this is likely a consequence of either different environmental conditions among lakes, genetic differentiation among lakes, or a combination of both with variable genotype–environment interactions (Crespel et al. 2013a). This is in line with the fact that local adaptations are prominent in salmonids and, in several cases, crucial for populations’ persistence and productivity (Taylor 1991, Fraser et al. 2011). In our results, the importance of lake identity also increased with age for both size-at-age and growth. This could be due to domestic fish being stocked at early life stages, which could reduce phenotypic differentiation among stocked populations in young age classes. In older ages, the effect of lake identity increased, probably because domestic individuals are less represented and/or environmental effects are more prominent with time. Domestic fish could, for instance, incur a higher mortality rate (Solberg et al. 2013b) or have higher chances of being

caught by anglers (Härkönen et al. 2014, Uusi-Heikkilä et al. 2017).

Growth and size-at-age were influenced by the interaction between cohort and genetic origin in early age classes. It suggests that early environmental conditions may be an important determinant of size and growth (Jonsson and Jonsson 2014, but see Granier et al. 2011), but that the influence of this effect depends on the origin of individuals. After the first year, the interaction seems largely due to one particular cohort (2010, see Fig. 2 and Appendix S1: Fig. S6). Among the individuals born in 2010, the domestic ones are smaller and have a lower growth rates than wild fish. All the fish from the 2010 cohort belong to the same lake (Amanites; Portneuf reserve), which suggests a lake-specific effect. However, the interaction between cohort and genetic origin remains significant even when this lake is excluded from the analyses (Appendix S1: Table S5).

Growth and size-at-age were influenced by genetic origin of individuals, but this effect was mostly dependent on environmental conditions (interactions with cohort). The genetic effect was significant independently of environment at 4 yr old for size-at-age only. At this stage, domestic genetic background resulted in larger fish. This is in line with previous findings (McGinnity et al. 1997, Tymchuk et al. 2006, Solberg et al. 2013a, b) and likely a result of artificial selection to produce fast-growing individuals in hatcheries (Petersson et al. 1996, Huntingford 2004). The observation that genetic background affected growth mainly at an early stage may suggest that growth advantage disappeared with age, perhaps due to a lower survival of domestic fish in the wild (Solberg et al. 2013b), and/or because they are more likely to be harvested during recreational fishing (Härkönen et al. 2014, Uusi-Heikkilä et al. 2017). It may also become harder to detect an effect of genetic origin in older age classes because of our smaller sample sizes, and thus more limited statistical power. Still, genetic origin impacted size at most ages in our analyses, as domestic fish were larger than wild individuals. This result can be explained by artificial selection leading to genetically based differences in growth between domestic and wild fish (McGinnity et al. 1997, Tymchuk et al. 2006, Solberg et al. 2013a). This could also be due to an

early boost since hatchery-reared fish were fed ad libitum before being released and are thus larger during early life stages (Pettersson et al. 1996). Body size has been shown to influence trophic levels in Brook Trout with larger individuals consuming larger prey (Glaz et al. 2012, 2014), and it is thus possible that domestics stay larger than wild fish because of early difference in body size, without maintaining higher growth rates.

#### *Trophic niche and trophic level*

Our results showed that domestic trout differed from their wild counterparts both in terms of trophic level and trophic niche. More specifically,  $\delta^{13}\text{C}$  ratios showed that domestic individuals were feeding consistently in more littoral habitats than wild and hybrid fish. This difference in trophic niche could be due to behavioral differences induced by domestication leading to different preferences in habitat selection (Mittelbach et al. 2014). In the same region as our study system, it has been shown that Brook Trout select preferentially littoral trophic niches and can shift their diet to forage in the pelagic zone when environment is disturbed (Glaz et al. 2014), or in presence of competitors (Magnan 1988, Tremblay and Magnan 1991). This may suggest that domestic fish displaced wild individuals from littoral niches. Domestication often increases boldness levels (Huntingford 2004, Mittelbach et al. 2014), and it is possible that bolder domestic fish outcompeted wild individuals and took over littoral habitats. This could be accentuated by the fact that domestic trout are larger, which could give them an advantage over wild fish in intraspecific competition (McGinnity et al. 1997). A closer look at the feeding niche distribution of each genetic category shows that wild and hybrid individuals almost strictly feed in pelagic niches, while domestics feed in both pelagic and littoral environments (Appendix S1: Fig. S7). This pattern could be explained by an age structure in trophic niche for domestics, with some age classes feeding in littoral zone and other age classes feeding in pelagic environment. However, supplementary analyses showed that this is unlikely (Appendix S1: Table S6, Fig. S8).

In three out of four populations,  $\delta^{15}\text{N}$  ratios showed that domestic trout displayed higher trophic levels than wild fish. The difference in trophic niche probably explains this pattern since

prey tend to be larger in littoral environments (Vander Zanden et al. 2006). Moreover, body size has been shown to positively correlate to  $\delta^{15}\text{N}$  in Brook Trout (Glaz et al. 2012, 2014). Thus, there is probably a link between our results on growth and on trophic niche and level. Larger size of domestic fish may provide a competitive advantage to take over littoral habitats and feed on larger prey, which in turn allow them to maintain their size advantage.

Interestingly, hybrids clustered either closer to wild trout or had an intermediate position in terms of trophic level or niche. Hybrids shared the same niche as wild individuals in two populations and were not different from either wild or domestic fish in two other populations. However, we note that the two populations in which hybrids were not different from wild or domestic individuals had very low numbers of hybrids (Appendix S1: Table S2). In these lakes, we thus had more limited statistical power to analyze this group, which is a possible explanation for the absence of difference between hybrids and other groups. The similarity between hybrids and wild individuals suggests that rearing conditions are more important than genetic origin in shaping feeding habits, since both wild and hybrid fish, unlike domestic trout, were reared in the same environment. An alternative explanation is that genetic differences between groups influence their trophic habitat use, but that niche occupation behavior is governed by genes that have a dominance-recessive pattern, with wild genes being dominant. For instance, mechanisms of dominance were shown to affect traits such as transcription regulation in a context of hybridization in Brook Trout (Bougas et al. 2010). Hybrid traits are difficult to predict in natural systems (Granier et al. 2011), and their heritability can vary according to environmental conditions (Crespel et al. 2013a). It is thus possible that in other contexts or populations, hybrids would cluster differently than what we observed here.

#### CONCLUSION

Our results showed an effect of genetic origin of individuals on phenotypes and feeding habits, which varied depending on population-specific attributes. Domestic trout seem to grow larger

than wild fish and to monopolize the best quality feeding niches. In other salmonids, stocked domestic fish have been showed to displace wild populations (Morissette et al. 2019), possibly because of their size advantage (McGinnity et al. 1997). This size advantage of domestic fish appears in early life stages in our results and likely leads to domestics outcompeting wild individuals. Thus, limiting stocking to fish that are already large enough to be caught by anglers (e.g., using put-and take rather than put-and-grow stocking practices) could reduce competition in early life stages for wild fish. In addition, assuming that Brook Trout spend most of their time in their feeding habitat, we can speculate that focusing angling pressure on littoral habitats could alleviate fishing pressure on wild individuals and limit the impacts of stocked fish by controlling their population. Reducing risks of wild population displacement from their preferential niches when stocking is an important step for an effective management of Brook Trout and salmonids in general. Since environment seems to strongly influence phenotype, but also the relationship between genetic background and phenotype, further research about environmental conditions would be needed to better identify the conditions in which such phenotypic divergence should be enhanced or inhibited. Finally, experiments in controlled environments, such as artificial lakes, could be conducted to better understand how genetic introgression acts on stocked population and interacts with environmental variables.

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