

Environmental factors correlate with hybridization in stocked brook charr (*Salvelinus fontinalis*)

Amandine D. Marie, Louis Bernatchez, and Dany Garant

Abstract: Stocking is a common practice throughout the world that may increase hybridization between wild and domesticated populations. Stocking intensity alone does not always fully explain the observed patterns of hybridization, suggesting that the intensity of hybridization may be modulated by environmental factors. Using brook charr (i.e., brook trout, *Salvelinus fontinalis*) as a model, the objective of this study was to assess the relative effect of environmental factors and stocking intensity on the level of hybridization observed within brook charr from 15 lacustrine populations of two wildlife reserves in Quebec, Canada. The level of hybridization significantly increased with (i) the number of stocking events, (ii) a reduction in both surface area and maximum depth of lakes, and (iii) a reduction in dissolved oxygen and an increase in temperature and pH. These results suggest that levels of hybridization were affected by the availability and quality of lacustrine habitats as well as by the extent of propagule pressure. Our study provides the first demonstration that knowledge of environmental features may help predict the effects of stocking on the genetic integrity of wild populations.

Résumé : L'ensemencement est une pratique répandue dans le monde entier qui pourrait accroître l'hybridation de populations sauvages et domestiquées. L'intensité des ensemencements à elle seule ne peut toujours expliquer les patrons d'hybridation observés, ce qui porte à croire que l'intensité de l'hybridation pourrait être modulée par des facteurs environnementaux. En utilisant l'omble de fontaine (*Salvelinus fontinalis*) comme modèle, l'étude avait pour but d'évaluer les incidences relatives de facteurs environnementaux et de l'intensité des ensemencements sur le degré d'hybridation observé chez des ombles de fontaine de 15 populations lacustres dans deux réserves fauniques du Québec (Canada). Une augmentation significative du degré d'hybridation est corrélée (i) au nombre d'ensemencements, (ii) à une diminution de la superficie et de la profondeur maximum des lacs et (iii) à une diminution de la teneur en oxygène dissout et une augmentation de la température et du pH. Ces résultats suggèrent que la disponibilité et la qualité des habitats lacustres ainsi que l'ampleur de la pression de propagule avaient une incidence sur les degrés d'hybridation. L'étude démontre pour la première fois qu'une connaissance des caractéristiques environnementales pourrait aider à prédire les effets de l'ensemencement sur l'intégrité génétique de populations sauvages.

[Traduit par la Rédaction]

Introduction

Major threats to biodiversity come from human activities through habitat fragmentation, pollution, land cover change, overexploitation, and the introduction of invasive species (International Union for Conservation of Nature 2009). The general outcomes of these different practices are the degradation of ecosystems (International Union for Conservation of Nature 2009) and the erosion of genetic diversity and evolutionary potential (Frankham et al. 2005; Smith and Bernatchez 2008), which often lead to loss of wild populations and ultimately species (Dulvy et al. 2003; Laurance and Useche 2009). Hybridization is one of the main processes impacting

the genetic makeup of wild species in both plants, mainly through physical disturbances (Levin et al. 1996; Lamont et al. 2003; Pansarin and Amaral 2007), and animals, mostly because of deliberate or accidental introductions of exogenous individuals (Hindar et al. 1991; Marie et al. 2010; Winkler et al. 2011). Yet, the consequences of hybridization are complex and controversial (Allendorf et al. 2001). Although introgressive hybridization may contribute to diversification and adaptability (Mallet 2005), it is more often associated with deleterious effects, ultimately leading to species extinction (Rhymer and Simberloff 1996).

In fish, and especially in salmonids, introductions of domesticated individuals into wild populations are common practices throughout the world. Since salmonids have a considerable economical and recreational value, stocking of populations is often required to sustain sport fisheries. Hybridization, which is particularly frequent in fishes (Hubbs 1955), is the greatest genetic risk associated with these practices and represents a major issue for the management of such populations (Allendorf et al. 2001). The outcomes of stocking reported in salmonid taxa range from hybridization without introgression to complete admixture of native populations (Allendorf et al. 2001; Hansen 2002). The extent of hybridization partly depends on the level of the genetic differences between wild and domestic individuals (Naish et al.

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A.D. Marie and D. Garant. Département de biologie, Université Sherbrooke, Sherbrooke, QC J1K 2R1, Canada.

L. Bernatchez. IBIS (Institut de Biologie Intégrative et des Systèmes), Pavillon Charles-Eugène-Marchand, Université Laval, Québec, QC G1V 0A6, Canada.

Corresponding author: Dany Garant (e-mail: Dany.Garant@USherbrooke.ca).

2008). Even with few genetic differences, deleterious effects have been reported in natural populations through, for example, loss of local adaptations and modification of population genetic integrity (Reisenbichler and Rubin 1999; Fraser 2008; Marie et al. 2010).

Most studies performed to date have been devoted to documenting the consequences of various stocking practices on resulting hybridization (Halbisen and Wilson 2009; Hansen and Mensberg 2009; Marie et al. 2010). For instance, the number of stocking events (Marie et al. 2010) or the number of fish released (Hansen and Mensberg 2009) have been shown to be positively correlated with the level of hybridization detected in stocked populations. In contrast, very few studies have attempted to decipher the role that environmental factors may play in promoting hybridization under wild conditions. Namely, it has long been argued that factors reducing habitat quality and availability may enhance hybridization (Hubbs 1955; Rhymer and Simberloff 1996). In the case of freshwater fishes, such factors could be either biotic (e.g., competition, Naylor et al. 2005) or abiotic (e.g., habitat reduction, DeHaan et al. 2010). For instance, Heath et al. (2010) showed that a combination of factors (e.g., logging activity, urban infrastructure development, stocking) influenced the level of hybridization observed among coastal cutthroat (*Oncorhynchus clarkii clarkii*) and rainbow trout (*Oncorhynchus mykiss*) populations on Vancouver Island (British Columbia, Canada). The paucity of studies that assessed the impact of environmental factors on hybridization contrasts with the increasing number of studies assessing the effects of environmental variables on population genetic structure by means of landscape genetics approaches (Sork and Waits 2010), namely in fishes (Angers et al. 1999; Leclerc et al. 2008; Launey et al. 2010). This is somewhat surprising given that the modelling approaches used in landscape genetics are directly applicable to the detection of the factors shaping hybridization.

The main objective of this study is to assess the relative effects of environmental factors on the level of hybridization using brook charr (i.e., brook trout, *Salvelinus fontinalis*) populations in 15 lakes of two wildlife reserves in Quebec, Canada, as a model. We have recently shown that intensive stocking greatly modifies both the extent of within- and inter-population genetic of those populations (Marie et al. 2010). Here, we revisit this data set to specifically model the relative influence of environmental factors (biotic, physicochemical, and morphometric), in addition to stocking intensity, on the level of hybridization observed in stocked lakes. This analysis represents, to our knowledge, the first demonstration that the effects of stocking on the genetic integrity of wild populations may be predictable based on knowledge of environmental features.

Materials and methods

Sampling procedures

Sampling was conducted in the Portneuf and the Mastigouche wildlife reserves in Quebec, Canada (47°09'N, 72°17'W and 46°40'N, 73°30'W, respectively; Fig. 1) in June 2007 and July 2008 (see Marie et al. 2010 for details of sampling procedures). Lakes were chosen in these reserves based on their well-documented stocking histories since 1971 (un-

stocked lakes described in Marie et al. 2010 were excluded from these analyses). The number of stocking events performed in those lakes since 1971 was used as a predictive variable in statistical models (see below). Domestic brook charr ($n = 51$) were obtained from a domestic strain maintained at the nearby Jacques-Cartier Hatchery (Quebec, Canada), which is used for stocking in the Portneuf Reserve. We also obtained domestic-wild F_1 hybrid brook charr ($n = 40$) from the three hatcheries (Alléghanys, Truites de Mauricie, and Lac-des-Écorces, Quebec, Canada) used for stocking in the Mastigouche Reserve (see Marie et al. 2010 for details). Tissue samples (adipose fins) were preserved in 95% ethanol until DNA extraction.

Environmental variables

Descriptors of the biotic, physicochemical, and morphometric environment to evaluate their relative effects on the level of hybridization observed in each lake were chosen based on current understanding of the factors important for brook charr (Power 1980; Venne and Magnan 1995; Warren et al. 2010). For each lake, the temperature (°C), dissolved oxygen (mg·L⁻¹), and pH were measured at 1 m below the water surface and at approximately 40 m from the shore. Two measurements of each parameter were done during the summer period, before the breeding season (June and July) in 2007 and 2008 for each lake. Data were then averaged for each parameter of each lake. The temperature and dissolved oxygen were obtained using a waterproof dissolved oxygen meter kit (OXI 330i WTW) and the pH using a pHTestr 20 (Eutech Instruments). Data for surface area (ha) and maximum depth (m) of each lake was obtained from the Ministère des Ressources Naturelles et de la Faune du Québec (MRNF). The presence of potential competitor species (presence or absence of white sucker (*Catostomus commersonii*) and (or) creek chub (*Semotilus atromaculatus*) was also taken into account (Magnan 1988; Bourke et al. 1999). All variables are detailed in Table 1.

Genetic analyses

DNA was extracted from fin clips (25 mm²) using the salting out method of Aljanabi and Martinez (1997). A total of 549 and 647 brook charr originating from the Portneuf and the Mastigouche reserves, respectively, as well as all individuals from the hatcheries were genotyped using nine microsatellite loci: sfoC129, sfoC113, sfoC88, sfoB52, sfoD75, sfoC24, sfoC86, SCO218, sfoD100 as detailed in Marie et al. (2010). PCR products were visualized using an ABI 3130 capillary DNA sequencer (Applied Biosystems), and allele size was established using the software GeneMapper version 4.0 (Applied Biosystems). The individual admixture proportions (q values) and their posterior probability intervals were estimated for each individual in each lake using STRUCTURE v.2.2 (Pritchard et al. 2000). We have showed previously that the efficiency of the software STRUCTURE to estimate individual admixture proportions was sufficient given the levels of genetic differentiation present between wild and domestic fish in both reserves (mean F_{ST} : Mastigouche = 0.183 ± 0.033 , Portneuf = 0.220 ± 0.016 ; see Marie et al. 2010, 2011 for details). We thus constrained fish from stocked lake to be assigned to two distinct genetic groups ($K = 2$; i.e., wild and domestic individuals). An admixture model with correlated allele frequency was used for

Fig. 1. Geographical locations of lakes within the two wildlife reserves: (a) Portneuf and (b) Mastigouche in Quebec, Canada. See Table 1 for abbreviations of lake names.

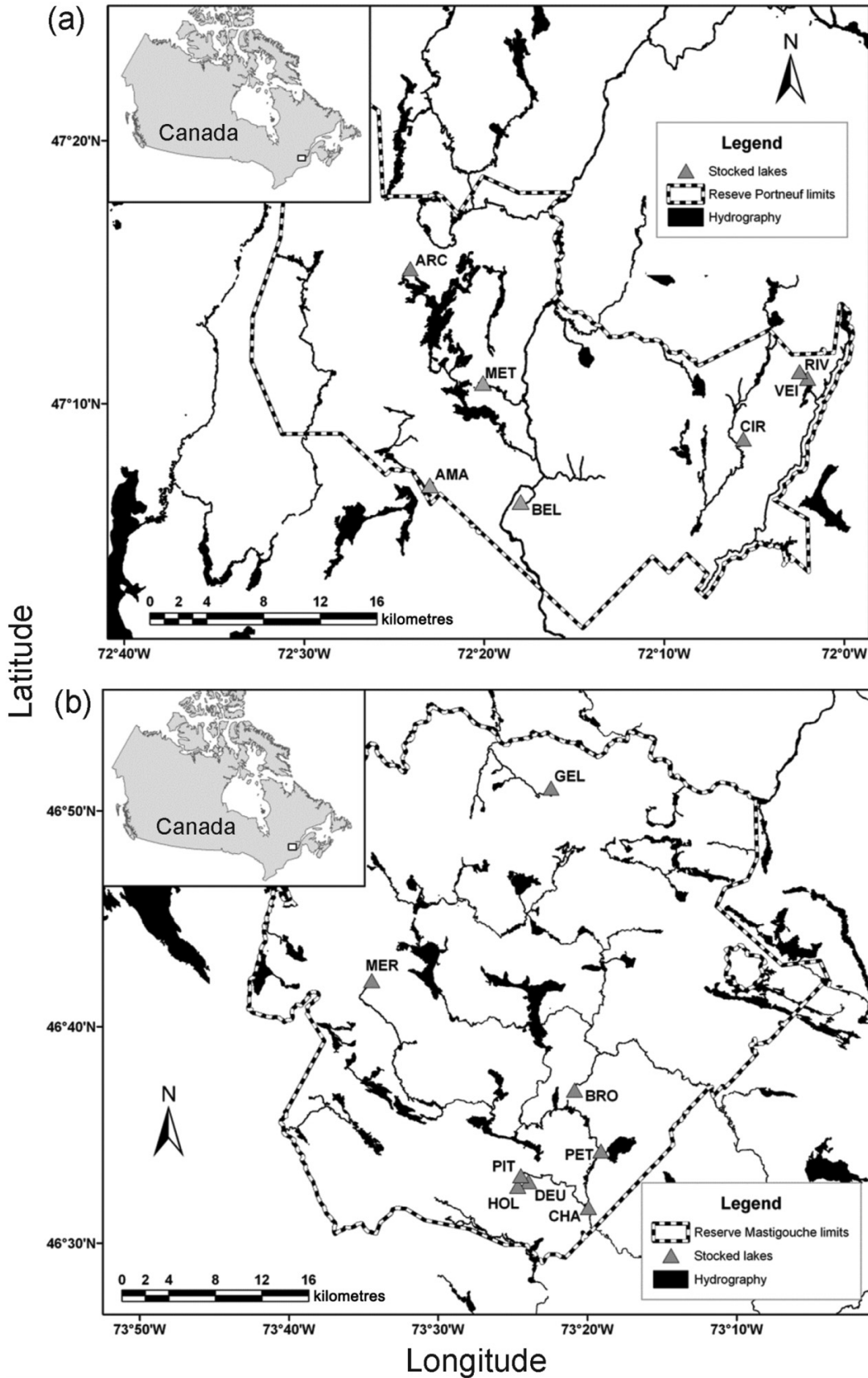


Table 1. Characteristics of the lakes in each reserve, including the number of fish genotyped (*N*) over 2 years of sampling, latitude and longitude, the mean level of hybridization (*q* value), number of stocking events since 1971 (NS), and the environmental factors: surface area (SA), maximum depth (MD), temperature (Temp.), dissolved oxygen (DO), pH, competition (Comp: 0 for absence of competitors, 1 for presence of competitors).

Lakes	<i>N</i>	Latitude (N)	Longitude (W)	<i>q</i> value ± SD	NS	SA (ha)	MD (m)	Temp. ± SD (°C)	DO ± SD (mg·L ⁻¹)	pH ± SD	Comp.
Portneuf											
Belles-de-Jour (BEL)	81	47°05'43"	72°17'41"	0.52±0.45	23	7.5	16.1	19.6±3.2	8.8±1.2	6.9±0.4	0
Amanites (AMA)	88	47°06'30"	72°22'43"	0.52±0.46	20	10.2	12.9	20.1±2.4	8.7±1.3	6.9±0.4	1
Methot (MET)	87	47°10'20"	72°19'33"	0.32±0.33	15	8.9	11.0	20.6±3.0	8.7±1.0	6.5±0.3	0
Rivard (RIV)	46	47°10'19"	72°01'56"	0.02±0.04	8	5.4	7.9	17.5±2.2	9.4±0.9	5.8±0.5	0
Veillette (VEI)	101	47°09'44"	72°01'24"	0.01±0.02	8	37.4	19.6	17.6±2.8	8.7±1.1	6.0±0.3	0
Arcand (ARC)	56	47°14'45"	72°23'33"	0.02±0.07	1	15.1	18.7	17.3±1.7	8.2±1.3	6.3±0.4	1
Circulaire (CIR)	77	47°07'50"	72°05'11"	0.03±0.07	1	1.4	1.0	17.3±3.5	8.9±1.0	5.7±0.4	1
Mastigouche											
Brochard (BRO)	67	46°36'41"	73°20'39"	0.74±0.22	17	15.5	12.6	21.9±1.2	8.1±1.1	6.1±0.3	0
Deux étapes (DEU)	85	46°32'33"	73°23'53"	0.35±0.27	19	12.3	22.0	21.5±1.2	8.3±1.3	6.1±0.5	0
Hollis (HOL)	82	46°32'16"	73°24'30"	0.32±0.24	18	16.7	15.8	21.6±0.6	8.2±1.3	6.5±0.4	0
Pitou (PIT)	100	46°32'48"	73°24'17"	0.38±0.23	19	8.0	16.9	20.7±1.1	7.6±1.0	5.9±0.4	1
Chamberlain (CHA)	103	46°31'13"	73°19'53"	0.39±0.24	9	18.4	5.0	21.2±0.8	8.2±1.1	6.4±0.2	1
Petit St-Bernard (PET)	54	46°33'58"	73°17'38"	0.18±0.30	4	14.3	13.7	21.3±1.6	8.3±1.4	6.5±0.5	1
Mercure (MER)	84	46°41'53"	73°34'08"	0.37±0.40	2	3.1	11.5	20.7±2.3	8.3±1.2	6.3±0.3	1
Gélinotte (GEL)	72	46°50'42"	73°21'47"	0.78±0.23	5	4.7	7.0	20.4±0.4	7.6±1.0	5.7±0.5	0

Note: SD, standard deviation.

each analysis with 250 000 steps of the Markov chain that were preceded by a burn-in period of 100 000 steps.

Statistical analyses

To facilitate the assessment of environmental effects on the level of hybridization, a principal component analysis (PCA) including all lakes from the two reserves was used for reducing and obtaining indexes resuming environmental data by category (e.g., morphometrical and physicochemical). First, data were standardized (zero mean, unit variance) to facilitate comparisons. Then, two PCA were performed separately, using a correlation matrix approach, to obtain (i) a morphometrical index (henceforth called SD), which includes surface area and maximum depth, as well as (ii) a physicochemical index (TOP), which describes temperature, dissolved oxygen, and pH. Although we retained the principal component loadings of the first axis for both analyses, which explained the majority of variance in data, the principal component loadings of the second axis of each index were also included in the model (see below). For the SD index, only the eigenvalue of the first axis was >1 , whereas the first two axes were >1 for the TOP index (Table 2).

A generalized linear mixed model was used with a quasibinomial error structure (controlling for under dispersion; logit link function) to assess the effects of reserve of origin (Portneuf or Mastigouche, factor), stocking intensity (continuous), and environmental variables (TOP and SD indexes as continuous variables, as well as the presence of competitors coded as a factor) on the individual level of admixture. Lakes were included as a random effect in the model, and the significance of fixed effects was assessed from their Wald statistic when dropping them from the full model. Only significant terms ($P < 0.05$) were retained in the final model. Analyses were performed with GenStat (version 12; VSN International Ltd., Hemel Hempstead, UK).

Results

The mean admixture levels (mean q values) were of 0.207 ± 0.239 (range from 0.012 ± 0.018 to 0.520 ± 0.463) and 0.438 ± 0.207 (range from 0.184 ± 0.295 to 0.777 ± 0.227) for the Portneuf and the Mastigouche reserves, respectively (Table 1). The average proportion of hybrid individuals (defined from q values >0.1 and <0.9) was 0.40 and ranged from 0.10 (Veillette) to 0.93 (Pitou), with a large variance observed in most lakes (Table 1). The distribution of individual q values was highly variable among lakes (see online Supplemental Fig. S1¹). For example, lakes in Portneuf Reserve with few stocking events are mainly composed of pure wild individuals (Supplemental Fig. S1a), whereas heavily stocked lakes in Mastigouche Reserve show patterns of extensive admixture (Supplemental Fig. S1b). Our study system is thus representative of a diversity of hybridization scenarios ranging from low admixtures to almost complete hybrid swarms. Loading coefficients, eigenvalues, and percentages of variation obtained from the PCA of the SD and TOP indexes are summarized in Table 2. The PCA first axis explained 74.4% and 53.4% of the total morphometric and physicochemical variation, respectively. Surface area and maximum depth of the SD index were positively correlated

Table 2. Loading coefficients, eigenvalues, and percentages of variation obtained from the PCA analyses of the SD and TOP indexes.

Environmental variables	PC1	PC2	PC3
SD index			
Maximum depth	0.707	0.707	—
Surface area	0.707	-0.707	—
Eigenvalue	1.488	0.512	—
Variation (%)	74.39	25.61	—
TOP index			
Temperature	0.733	0.092	0.674
Dissolved oxygen	-0.615	0.511	0.600
pH	0.289	0.854	-0.432
Eigenvalue	1.602	1.114	0.284
Variation (%)	53.41	37.12	9.47

(both having loadings coefficients of 0.71). For the TOP index, temperature and pH were positively correlated but were both negatively correlated with dissolved oxygen. The loading coefficient of temperature and dissolved oxygen (loading coefficients of 0.73 and -0.62, respectively) were much higher than that of pH (0.29), which suggests that the first axis of the PCA is mostly representative of the former two variables. Moreover, the percentage of variation of the second axis of the PCA (37.1%) was less important than the first axis, and this second axis was principally represented by pH (loading coefficient of 0.85 vs. 0.09 and 0.51, respectively, for temperature and dissolved oxygen).

The fixed effects retained in the final model explained 36.8% of the observed variance in hybridization. Part of this variance was explained by the number of stocking events that caused a significant increase in the level of hybridization (effect size of 0.506 ± 0.123 ; $P = 0.001$; Table 3; Fig. 2a). Also, both environmental indexes were significantly predictive of the level of hybridization detected in lakes. Thus, hybridization increased significantly with a smaller morphometric (SD) index, meaning that decreasing surface area and maximum depth both resulted in higher value of hybridization (effect size of -0.544 ± 0.117 ; $P < 0.001$; Table 3; Fig. 2b). The level of hybridization was also positively associated with the physicochemical (TOP) index (effect size of 0.840 ± 0.108 ; $P < 0.001$; Table 3). Hybridization thus increased with reduction in dissolved oxygen (negative loading for the first axis of PCA; see Table 2), as well as with higher temperature and pH values (positive loadings; Table 2; Fig. 2c). Reserve of origin ($P = 0.26$), the presence of competitors ($P = 0.20$), and the SD ($P = 0.79$) and TOP ($P = 0.71$) indexes measured from the second axis of the PCA did not have a significant effect on the level of hybridization.

Discussion

The main objective of this study was to assess the relative influence of environmental factors and stocking intensity on the level of hybridization detected in brook charr populations. As expected, our analyses confirmed that hybridization

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/f2012-027>.

Table 3. Final model showing significant effects of the number of stocking events and environmental factors on the level of hybridization detected in brook charr populations.

	Wald statistic	df	P value	Estimate	SE
Fixed effects					
No. of stocking events	16.79	1	0.001	0.506	0.123
SD	21.85	1	<0.001	-0.544	0.117
TOP	61.02	1	<0.001	0.840	0.108
Random effects					
Lakes	—	—	—	0.138	0.067
Residual	—	—	—	0.215	0.009

Note: Results obtained from final general linear mixed models (with quasibinomial error structure) are presented with corresponding Wald statistic for each fixed term when fitted last in model. Significant random effect (lakes) is also presented with its standard error (SE). SD index includes maximum depth and surface area; TOP index includes temperature, dissolved oxygen, and pH.

increases with the number of stocking events in these populations. However, results revealed that the morphometry and physicochemistry of lakes also influence the level of hybridization observed in these populations. More specifically, the level of hybridization decreased with greater availability of habitats, whereas it increased with these physicochemical environmental conditions of the lakes that may be limiting for brook charr.

Stocking effects

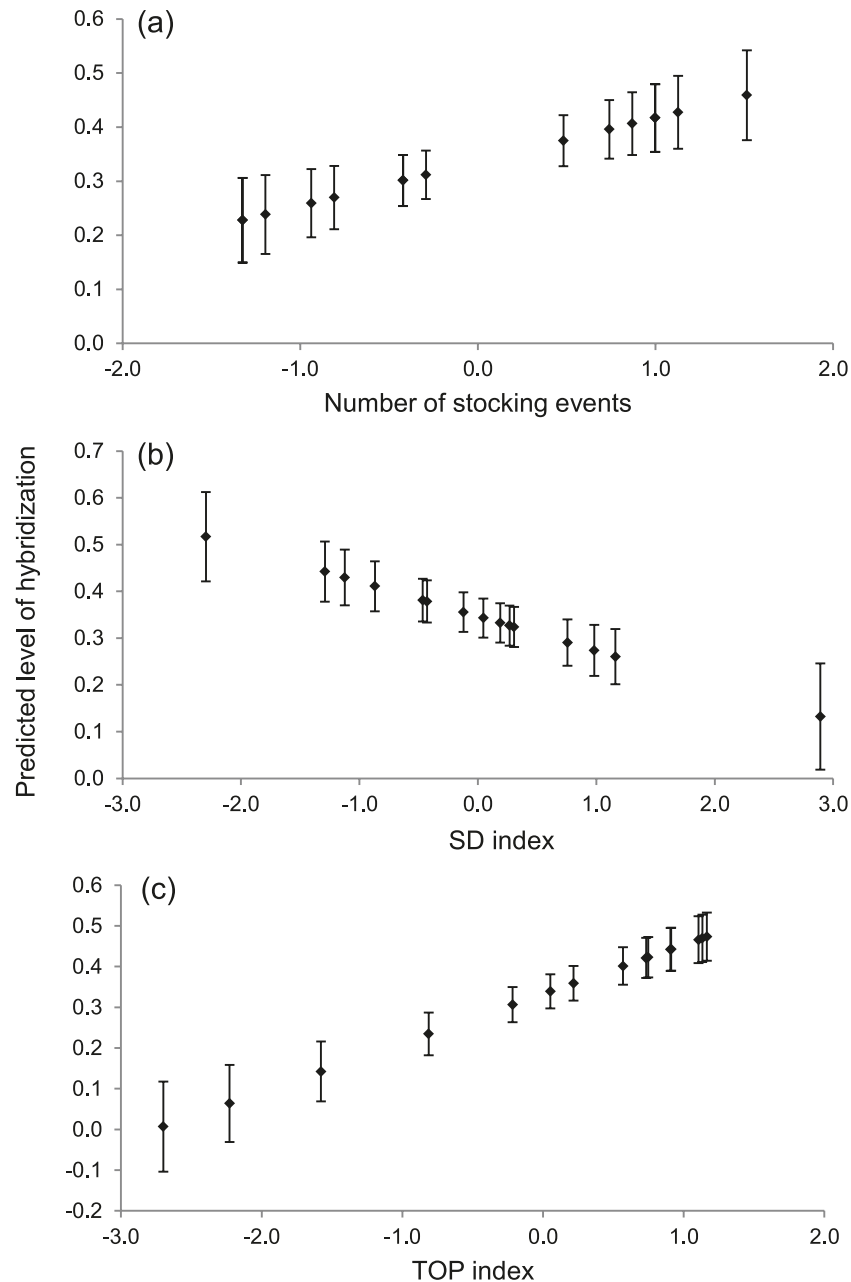
We previously showed that stocking intensity was an important determinant of the extent of admixture between wild and domestic brook charr (Marie et al. 2010). We also showed that date of last stocking event of a given lake was influencing the estimated level of hybridization (Marie et al. 2010). For example, the levels of hybridization found in the moderately stocked lakes of the Mastigouche Reserve are higher than in moderately stocked lakes of the Portneuf Reserve, partly because of more recent stocking (last stocking event performed on average 3.7 ± 3.3 and 18.1 ± 14.0 years ago, respectively, for the Mastigouche and Portneuf reserves; see Marie et al. 2010 for further details). Here, we showed that stocking intensity, and thus propagule pressure, remained a significant determinant of hybridization, even when accounting for other environmental effects. More specifically, hybridization increased linearly with the number of stocking events performed in lakes. The effect of stocking intensity on hybridization was also underlined in previous studies in salmonids. For example, Hansen and Mensberg (2009) showed that the levels of hybridization increased in rivers that were more intensively stocked with brown trout (*Salmo trutta*). Another study on the supplementation of lake trout (*Salvelinus namaycush*) populations in southern Ontario (Canada) with exogenous individuals also showed that stocking intensity of domestic individuals explained the pattern of hybridization observed in stocked lakes (Halbisen and Wilson 2009). Yet, these authors observed that trout populations from some stocked lakes had a genetic profile characteristic of wild populations, whereas others were more characteristic of hatchery fish (Halbisen and Wilson 2009). Halbisen and Wilson (2009) thus suggested that other unknown factors (e.g., ecological, anthropogenic), in addition to stocking intensity, probably played a role in the pattern of hybridization observed in their stocked lakes.

Environmental effects

We first observed that hybridization was more pronounced in smaller and shallower lakes, independent of stocking intensity. Similarly, Heath et al. (2010) also observed that hybridization among coastal cutthroat (*O. clarkii clarkii*) and rainbow trout (*O. mykiss*) populations was higher in smaller watersheds. A possible explanation for such a result may relate to the extent of spatial isolation between domestic and wild fish during breeding. Smaller habitats are more likely to exhibit homogeneous environmental conditions compared with larger ones (Kohn and Walsh 1994). It is thus expected that the spatial distribution of reproductive habitats is more spatially restricted in small lakes, perhaps favouring encounter and mating among individuals. Population abundance could also potentially influence the level of hybridization. However, previous studies have shown that there is apparently no clear correspondence between fish population abundance and habitat size area (Grant et al. 1998; Imre et al. 2004), including in brook charr (Angers et al. 1999; Castric et al. 2001).

Physical and chemical characteristics appeared to be the most important factors explaining levels of hybridization in our model, as the TOP index had the largest effect size (0.84 ± 0.11). More specifically, our results suggest that hybridization increased with temperature. Brook charr is typically known as being a cold water species with a temperature preference around 16°C (Coutant 1977), although its typical habitat can range from 0 to 20°C depending on season (Power 1980). The average summer temperature of lakes we studied ($20 \pm 1.7^\circ\text{C}$) is thus in the upper limit of tolerance of brook charr. Consequently, as temperature increases, brook charr may become more constrained in their habitat use within lakes (e.g., behavioural thermoregulation above 20°C ; Biro 1998), which could promote the encounter among individuals. The same may be true for dissolved oxygen, as the negative relationship documented with the level of hybridization suggests that more limiting physiological conditions (lower dissolved oxygen) favoured hybridization. However, for brook charr, optimal oxygen levels range from $5\text{ mg}\cdot\text{L}^{-1}$ and above (Spoor 1990). The conditions found in our lakes were thus not particularly severe, with an average dissolved oxygen of lakes of $8.40 \pm 0.49\text{ mg}\cdot\text{L}^{-1}$. Finally, we found a positive relationship between pH and the level of hybridization. Although the pH

Fig. 2. Predicted hybridization values, obtained from the linear mixed model including all factors, for (a) the number of stocking events, (b) the morphometrical (SD) index, and (c) the physicochemical (TOP) index of each lake. Units of the x axis of each graph are values issued from principal component analyses (PCA). Units of the y axis represent the proportion of hybridization predicted according to one factor taking into account the effect of the other two factors.



values of the study lakes are not among the most severe (6.23 ± 0.39), all the studied lakes exhibit acidic pH levels (range from 5.7 to 6.9), which are typical of brook charr habitats in the Laurentian Shield (Baker et al. 1996; Warren et al. 2010). Thus, brook charr populations of these areas can be considered as tolerant to natural acidity. However, hatcheries in Quebec are almost all located in areas surrounding the plain of the St. Lawrence River and on the south bank of the St. Lawrence River, where water is typically alkaline (Laflamme 1995). It is thus possible that domestic individuals may perform better (e.g., survival, reproduction) when stocked in lakes exhibiting pH conditions that are similar to those found in hatcheries. This hypothesis, however, remains to be rigorously tested.

The absence of significant effect of competitors such as white sucker (*C. commersonii*) and creek chub (*Semotilus atromaculatus*) on the level of hybridization contrasts with results of other studies, which reported negative effects of competitive interactions between brook charr and these species (Magnan 1988; Bourke et al. 1999). However, these studies were not designed to assess the impact of the presence of these species on the reproductive success of brook charr but rather on the shift of food habits (Magnan 1988; Bourke et al. 1999) and associated phenotypic traits (Magnan 1988). Thus, it is possible that white sucker and creek chub do not interfere with the reproduction of brook charr. Moreover, domestic salmonids are typically more aggressive than

wild ones (Einum and Fleming 1997; Biro et al. 2004; Blanchet et al. 2008) and therefore perhaps less influenced by the presence of other species.

Admittedly, without further experimental investigation, we can only speculate at present on the causal links between variation in environmental parameters and the extent of hybridization we observed in each lake. Thus, the above interpretations should be taken as our best hypothetical explanations, given the current knowledge of the biology of brook charr and the lack of published information about ecological and physiological differences between wild and domestic brook charr. However, it is noteworthy that studies of gene expression in controlled environments revealed pronounced differences in patterns of gene regulation between wild and farm brook charr, thus revealing the existence of physiological differences between them (Bougas et al. 2010).

Potential limitations to our study should also be considered when interpreting these results (and the percentage of variation (36.8%) explained by our final model). First, although this study involved the analysis of a large number of fish, the number of lakes that could be included in the study may have limited the predictive power of our model. Second, a greater range of hybridization levels would probably strengthen the predictive power of our model, by allowing a better description of the effect of environmental factors and the number of stocking events on the level of hybridization. Even if the range of admixture patterns (as shown by the distribution of individual q values) in our lakes was variable, the mean level of hybridization (see Supplemental Fig. S1¹) observed in this study was relatively homogeneous, with half of the lakes (8 of 16) exhibiting a level of hybridization between 0.32 and 0.52 and four lakes around zero. Thus, a study maximizing at the same time variable scenarios of admixture and different average levels of hybridization would likely provide a more refined understanding of the consequences of stocking in the wild. For example, using a set of situations ranging from no admixture to hybrid swarms across a range of environmental conditions would lead to a better understanding of how environment affects hybridization as one of the consequences of stocking fish. However, this would require sampling a substantial number of lakes given that scenarios of admixture, as well as proportions and types of hybrids, cannot be known a priori. Using “experimental” lakes with known differences in environment and manipulating the number and kinds of stocking might also be feasible. Third, the interpretation of the TOP index effect must be done cautiously. Temperature and dissolved oxygen have been recorded in lakes twice per year (June and July) and for 2 years. Thus, although the dissolved oxygen values are high ($8.40 \pm 0.49 \text{ mg}\cdot\text{L}^{-1}$), they may not reflect the minimum values reached later during the summer. Finally, the model could have been improved by the inclusion of additional ecological and environmental variables (e.g., number, size, and distribution of spawning areas available; food production; age structure of the populations; etc.), would such data have been available.

Our results and those of Heath et al. (2010) suggest that it is important to consider environmental factors when assessing the determinants of hybridization in wild populations. Indeed, these studies indicate that genetic effects of stocking may be at least partially predictable based on the knowledge

of environmental factors. Results of such studies should improve stocking procedures in brook charr and other species alike. For example, these could allow managers to recommend (or not) stocking according to the probability of genetic contamination caused by stocking. Thus, stocking recommendations could vary according to different criteria as, for example, (i) no stocking in lakes having viable wild brook charr populations that have not been affected by stocking or (ii) stocking possible in lakes where current populations are likely to already be genetically perturbed, given the history of stocking and environmental characteristics. Moreover, knowledge of stocking history and environmental factors could allow to better predict if natural populations of given lakes are more or less likely to have been introgressed owing to past stocking, without performing genetic analyses for every single lake, which could be logistically difficult. Thus, the application of our findings should help better preserve the genetic integrity of wild brook charr populations and could also serve as a model that might be adjusted for the management of other salmonids species that are genetically impacted by stocking, both in North America and Europe (Hansen et al. 2009; Halbisen and Wilson 2009; Miller et al. 2004).

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