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ARTICLE

Anthropogenic and habitat correlates of hybridization between hatchery and wild brook trout

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Abstract: Human activities are breaking down barriers to interpopulation hybridization with results that range from populations that resist introgression to populations at serious risk of genetic extinction, particularly between wild and hatchery fish because of the routine stocking of lakes and streams containing wild populations. We investigated whether specific human actions and (or) habitat characteristics were associated with the extent of hybridization between hatchery and wild brook trout (*Salvelinus fontinalis*) occupying lakes in Algonquin Park, Ontario, Canada. Hatchery and wild trout tended to hybridize more in lakes (*i*) supporting smaller populations (i.e., having lower pH and situated at higher elevations), (*ii*) stocked earlier in the season, and (*iii*) with greater exposure to other human activities (fishing). Our results highlight how particular human activities may interact with specific habitat conditions to elicit hybridization in wild populations. Hence, the use of readily available habitat and land use data may provide an economical means of anticipating hybridization or identifying indigenous populations with minimal introgression.

Résumé : Les activités humaines abaissent les barrières à l'hybridation entre populations avec des résultats qui vont de populations résistantes à l'introgression à des populations à haut risque d'extinction génétique, particulièrement dans les cas d'hybridation entre poissons sauvages et poissons issus d'écloserie découlant de l'empoissonnement régulier de lacs et cours d'eau contenant des populations sauvages. Nous avons tenté de déterminer si certaines actions humaines ou caractéristiques de l'habitat étaient associées à l'ampleur de l'hybridation d'ombles de fontaine (*Salvelinus fontinalis*) sauvages et issus d'écloserie dans des lacs du parc Algonquin (Ontario, Canada). L'hybridation d'ombles sauvages et d'écloserie avait tendance à être plus importante dans les lacs (*i*) supportant de plus petites populations (c.-à-d. de plus faible pH et à plus haute altitude), (*ii*) empoissonnés plus tôt dans la saison et (*iii*) plus exposés à d'autres activités humaines (la pêche). Nos résultats soulignent comment l'interaction possible de certaines activités humaines avec des conditions précises de l'habitat peut favoriser l'hybridation dans des populations sauvages. L'utilisation de données facilement accessibles sur l'habitat et l'utilisation du territoire pourrait constituer un moyen économique de prédire l'hybridation ou d'identifier les populations indigènes qui présentent une introgression minimale. [Traduit par la Rédaction]

Introduction

Human-induced hybridization within species is an increasingly common occurrence across diverse taxa (Pierpaoli et al. 2003; Hails and Morley 2005) that can have both positive and negative ecological and genetic effects. On one hand, it may facilitate the genetic rescue of small populations by adding new alleles necessary to stave off inbreeding depression (Hogg et al. 2006) or enhance a population's adaptive potential in new or changing environments (Arnold 1992). On the other hand, hybridization can reduce fitness through outbreeding depression, potentially resulting in the loss of local adaptations and ultimately reduced population viability (Rhymer and Simberloff 1996; Tallmon et al. 2004).

Introgressive transfer of genetic material between two groups can result in novel, untested genes being introduced into a gene pool that subsequently lower the fitness of all individuals possessing such transgenes (Rhymer and Simberloff 1996; Burke and Arnold 2001). Following continued backcrossing, transgenes may spread throughout a population, eventually resulting in most individuals containing some portion of hybrid genotype. Such populations are referred to as being admixed (Allendorf et al. 2001). The occurrence and extent of admixture following hybridization between different populations can be highly variable (ranging from 0% to 100%; e.g., Hansen et al. 2009; Marie et al. 2010). The causes of such variation have not been studied thoroughly in most instances, but they likely include a number of ecological factors that affect both the frequency of hybridization and the fitness of hybrid individuals relative to the parents (Utter 2000; Hails and Morley 2005).

In fishes, escapes from aquaculture facilities and the intentional release of domestic or hatchery fish to supplement exploited wild populations have resulted in a growing number of cases of intraspecific hybridization (Rhymer and Simberloff 1996; Hutchings and Fraser 2008). Such hybridization often has had negative effects on wild populations, most commonly in the form of reduced fitness in hatchery–wild hybrids relative to wild conspecifics (Webster and Flick 1981; McGinnity et al. 2003; Araki et al. 2007; Fraser et al. 2010; but see Fraser et al. 2008) and homogenization of genetic

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differentiation among wild populations (Matala et al. 2008; Marie et al. 2010).

Recent studies have found that the extent of admixture from stocking can be correlated with a species' life history (Utter 2000), stocking intensity (Almodovar et al. 2006), fishing pressure (García-Marín et al. 1998; Mezzera and Largiadèr 2001), population size (Hansen et al. 2009), abiotic factors (Rasmussen et al. 2010; Marie et al. 2012), and human activities (Heath et al. 2010). Few of these studies have rigorously examined the relative influence of multiple habitat and anthropogenic factors in driving variation in hatchery–wild admixture among populations (but see Marie et al. 2012), though such investigations may bear practical results. First, they may shed light on which indigenous populations or species are more at risk of hybridizing. Second, they may provide an efficient and a cost-effective means of identifying indigenous populations that have resisted admixture following stocking using readily available data, an increasingly common situation for restoration and biodiversity conservation (see Meraner et al. 2008; Baric et al. 2010).

The brook trout (*Salvelinus fontinalis*) of Algonquin Provincial Park (Ontario, Canada) provides an opportunity to investigate what habitat and anthropogenic variables may affect hatchery–wild admixture. This is a species known to express negative side effects as a consequence of hybridization with domestic conspecifics (Mason et al. 1967; Lachance and Magnan 1990). In Algonquin Park, the long stocking history of lakes since the 1930s (Danzmann and Ihssen 1995) is well documented as is habitat and ecological data on stocked lakes.

The purpose of this study was therefore to identify possible ecological correlates of hatchery–wild admixture from Algonquin lakes and thus shed light on potential determinants of the extent of admixture following stocking events. Given that the environmental parameters used in this study will in many cases represent a snapshot rather than a long-term trend and that stocking has not taken place since at least 1989 in most cases, this post hoc data analysis approach will therefore identify variables that represent potential candidates for further investigation along with possible interactions among any such variables. This method of data analysis can potentially provide ecologists, conservationists, and managers with useful tools for identifying populations that have resisted introgression and for further understanding the drivers of introgressive hybridization at the population level.

Methods

Study populations and their history of hatchery trout stocking

Algonquin Park covers an area of 7650 km² and contains approximately 250 naturally self-sustaining populations of brook trout (Quinn 1995) spanning six watersheds (Danzmann and Ihssen 1995). The genetic database used in this study consisted of 861 individuals over a range of size classes (cohorts) from two hatchery sources and 22 populations within Algonquin park, 14 of which were known to have been stocked with hatchery fish previously, while the remaining eight had no recorded stocking events (Fig. 1; Table 1). The original purpose of this database was to examine the effects of mixed ancestry on population genetic structure and diversity. It therefore contained sufficient data to generate accurate estimates of hatchery–wild admixture. Previous work and genetic analysis done for this project suggested that each lake sampled represented an isolated population with the exception of three lakes (Harry, Rence, and Welcome lakes (H-R-W)) that are connected and exchange high levels of gene flow (Addison and Wilson 2010). As such, for the purposes of this study, these three lakes were treated as one large lake. The resulting database therefore consisted of 12 previously stocked and eight nonstocked Algonquin populations as well as samples from two hatchery sources.

Stocking in Algonquin Park began in the 1930s and was variable in its frequency and intensity. Some lakes received regular stocking, while others were stocked only once or twice for research or remediation purposes (Ontario Ministry of Natural Resources (OMNR) fish stocking database). All stocking of Algonquin Park lakes (and the lake studied outside the park) occurred before 1989, with the exception of Charles Lake, which received 1500 hatchery fish in 1994. Three hatchery strains of brook trout were stocked within the park. The principal strain used, Hills Lake, originated in Pennsylvania, approximately 500 km south of Algonquin Park, and has been maintained as a brood stock in the Ontario hatchery system over the entire period that stocking in Algonquin Park took place (Kerr 2000). The second strain originated from Lake Nipigon (approximately 850 km west of the park) and was stocked into several lakes within the park, though to a much lesser extent than the Hills Lake strain. The third strain stocked was a first generation cross between the Hills Lake and Nipigon Lake strains and was only stocked in a few instances as part of a study on the fitness of F_1 hatchery–wild hybrids (Fraser 1981).

Habitat and stocking data

We collated habitat data and stocking records for each study lake stocked with hatchery trout from government records obtained from OMNR, local records kept by the provincial park authorities (Friends of Algonquin Park), the Algonquin Forestry Authority, and to a lesser extent published primary literature sources. Variables analyzed are outlined in Table 2 and include chemical, physical, and biological variables known to be important for the success of stocked brook trout (Godbout and Peters 1988; Kerr 2000; Cote et al. 2011). In cases where years in which the data collected differed from one lake to another, only variables recorded around the same time of year (midsummer) were used to minimize differences among lakes resulting from seasonal changes. Of the data available, only variables that offered complete data sets for each lake were used.

DNA analyses

Over six, nonconsecutive sampling years, fin clips were nonlethally collected from adult fish spanning multiple cohorts and a range of size classes (via angling and gill netting) in the study lakes and hatchery strains. Samples were stored in 95% ethanol until the genomic DNA was extracted using the extraction protocol of Wilson et al. (2007), which involved lysing tissue samples overnight in a mixture of 10 mmol·L⁻¹ Tris–HCl, 125 mmol·L⁻¹ NaCl, 5 mmol·L⁻¹ EDTA (ethylenediaminetetraacetic acid), 0.5% SDS (sodium dodecyl sulfate), 4 mol·L⁻¹ urea, and 0.2 mg·mL⁻¹ proteinase K. DNA was then precipitated using 10 μL 5 mol·L⁻¹ NaCl and 500 μL 80% isopropanol and centrifugation before being washed with ethanol and resuspended in TE (Tris–EDTA) buffer. Each individual was then genotyped at 14 microsatellite loci (*Sfo18*, *Sfo23*, *Sfo12*: Angers et al. 1995; *SfoC24*, *SfoD28*, *SfoC38*, *SfoD75*, *SfoC88*, *SfoC100*, *SfoC113*, *SfoC86*, *SfoC115*, *SfoC129*, and *SfoB52*: T. King and M. Burnham-Curtis, US Geological Survey, unpublished data). Four 10 μL multiplex polymerase chain reaction (PCR) reactions were used to amplify the DNA. Each multiplex consisted of 6.0 ng of DNA, 2× PCR buffer, 1× BSA (bovine serum albumin), 1.5 mmol·L⁻¹ MgCl₂, 0.2 mmol·L⁻¹ dNTPs, 0.025 units of Taq, and between 0.03 and 0.3 μmol·L⁻¹ of each primer (depending on the multiplex). PCR conditions were as follows: 94 °C for 5 min, 36 cycles of 94 °C for 1 min, 58 °C or 60 °C (depending on the multiplex) for 1 min, 72 °C for 1 min, and a final extension at 60 °C for 45 min. Amplified PCR product was visualized using capillary electrophoresis on an ABI 3730 automated DNA sequencer (Life Technologies Inc.) and scored using GeneMapper version 4.0 software (Applied Biosystems) and manual proofreading.

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Fig. 1. Algonquin Provincial Park in Ontario, Canada, with the locations of the 22 study lakes. Asterisks indicate previously stocked populations. The following are abbreviations of the lakes sampled: ANI, Animoosh; CHR, Charles; CDS, Coldspring; DIK, Dickson; FARN, Farncomb; FRK, Frank; GUSK, Guskewau; HRY, Harry; HOG, Hogan; HND, Hound; LAV, Lavieille; LCRO, Little Crooked; LDIK, Little Dickson; OWE, Owenee; PHP, Philip; RED, Redrock; REN, Rence; SAL, Salvelinus; SCOT, Scott; STGR, Stringer; WELC, Welcome; WEST, Westward.
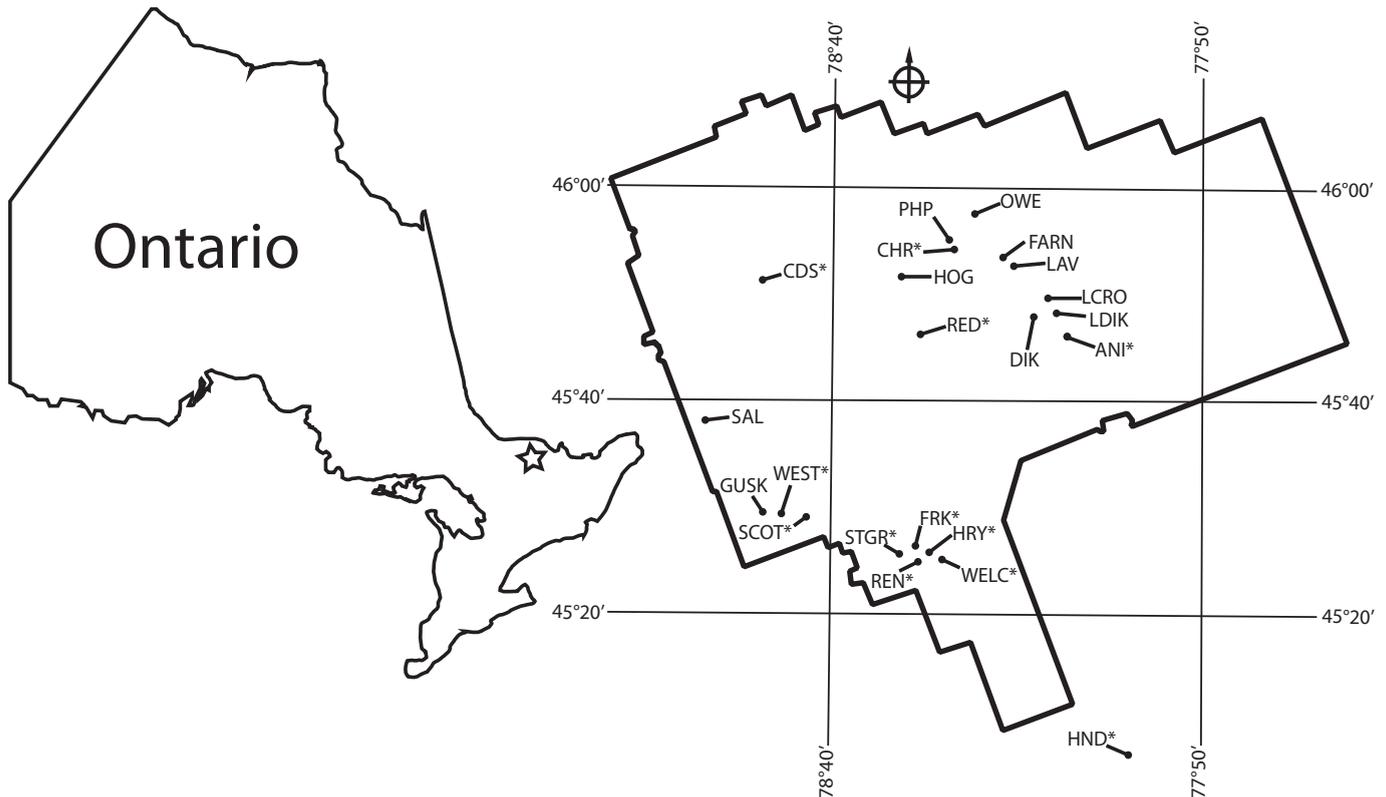
Table 1. Information on the (20) lakes and the two hatchery strains sampled.

Lake	Initial stocking	Final stocking	Genetic sampling	Sample size (n)	Hatchery strain	N_a	H_o	H_e	STRUCTURE (Q)	BAPS (Q)
Animoosh	1966	1970	2008	14	HL	3.50	0.46	0.43	0.96	0.99
Charles	1954	1994	2007	31	HL	2.71	0.34	0.34	0.82	0.88
Coldspring	1961	1961	2007	32	HL	3.35	0.36	0.37	0.93	0.96
Farncomb	1950	1950	2009	20	HL	3.64	0.48	0.46	0.98	0.99
Frank	1958	1981	2002	28	HL	6.71	0.67	0.70	0.37	0.36
Hound	1987	1989	2009	14	HL, LN	3.35	0.69	0.58	0.37	0.35
H-R-W*†	1940	1978	2002	93	HL	8.35	0.67	0.73	0.31	0.24
Philip*	1961	1963	2006	18	HL	3.28	0.36	0.45	0.95	0.99
Redrock*	1954	1970	2009	49	HL	5.85	0.51	0.54	0.88	0.93
Scott	1954	1977	2006	32	HL, LN	5.85	0.63	0.60	0.26	0.20
Stringer*	1954	1977	2007	40	HL	5.28	0.67	0.62	0.43	0.48
Westward*	1951	1968	2005	47	HL	3.78	0.38	0.44	0.78	0.87
Dickson*	—	—	2006	61	—	5.07	0.48	0.51	—	—
Guskewau	—	—	2008	35	—	4.21	0.40	0.42	—	—
Hogan	—	—	2009	29	—	3.78	0.39	0.44	—	—
L. Crooked*	—	—	2009	41	—	3.00	0.38	0.41	—	—
L. Dickson	—	—	2009	27	—	4.21	0.5	0.51	—	—
Lavieille	—	—	2009	29	—	4.14	0.45	0.45	—	—
Owenee	—	—	2008	31	—	4.64	0.52	0.51	—	—
Salvelinus*	—	—	2007	29	—	3.50	0.47	0.46	—	—
Hills*	—	—	2009	80	HL	8.28	0.71	0.73	—	—
Nipigon*	—	—	2006	81	LN	4.14	0.39	0.40	—	—

Note: Hatchery strain (Hills Lake (HL) and (or) Lake Nipigon (LN)) represents the strains stocked into each study lake-based stocking records, while the Q values represent mean admixture coefficients estimated using the program STRUCTURE or BAPS. Measures of genetic diversity include observed (H_o) and expected (H_e) heterozygosities and the mean number of alleles per locus (N_a).

*Populations that deviated significantly ($\alpha = 0.0036$, $k = 14$) from expected Hardy–Weinberg equilibrium after Bonferroni corrections.

†H-R-W represents the combination of Harry, Rence, and Welcome lakes.

Table 2. Habitat and anthropogenic variables considered for each of the previously stocked populations of brook trout from Algonquin Provincial Park, Ontario, used in this study.

Parameter	Description	Minimum	Mean	Maximum
SArea	Lake surface area in hectares (ha)	12.3	107.2	469.7
SDI	Shoreline Development Index	1.2	1.8	2.9
Elevation	Metres above sea level (m a.s.l.)	352	419	473
pH	Midsummer pH value	6	6.8	7.5
MEI	Morphoedaphic Index: total dissolved solids (ppm)/mean depth (m)	0.8	6.2	14.2
MDepth	Mean lake depth (m)	2	6.7	20.9
SPresent	Total number of fish species present in lake (n)	3	9.6	19
Distance	Shortest trail distance from nearest road (km)	0.5	24.1	49.0
StMonth	Mean month of year brook trout were stocked into lake	3.9	5.9	10.0
StEvents	Total number of years in which stocking occurred (n)	1.0	6.9	16.0
StAge	Mean age of fish at time of stocking (months)	6.0	11.9	15.6
StDensity	Mean stocking density ($n \cdot ha^{-1}$)	2.7	48.9	148.6
StTotal	Total number of fish stocked into lake (n)	1 000	21 158.1	137 052
AdmixPeriod	Time between initial stocking event and genetic sampling (years)	22	48.9	62

Note: Minimum, maximum, and mean values are lake values, taken in midsummer.

Within-lake measures of genetic diversity

Prior to conducting an analysis of admixture using STRUCTURE (Pritchard et al. 2000), it was important to insure that the subset of the genetic data met the assumptions of the program, namely that marker loci were unlinked and at linkage equilibrium within populations and that populations were at Hardy–Weinberg equilibrium (HWE). GENEPOP 4.0.10 (Raymond and Rousset 1995) was used to test for genetic linkage disequilibrium among loci as well as deviations from HWE within populations. To determine if hatchery stocking has had any effect on microsatellite allele frequencies, measures of allelic diversity were calculated for all sampled populations and hatchery strains using Genetic Analysis in Excel (Peakall and Smouse 2006). These measures included the observed (H_o) and expected (H_e) heterozygosity and the mean number of alleles per locus (N_a).

Estimation of the degree of hatchery–wild admixture

The extent of admixture between hatchery and wild trout in different lakes was assessed using the Bayesian clustering methods implemented in the program STRUCTURE. As historical samples from stocked lakes prior to stocking do not exist, an approach used in Pritchard et al. (2007) was implemented, whereby STRUCTURE was informed as to which populations represented Algonquin Park reference samples ($q1$–$q8$) and which represented hatchery reference samples ($q9$ and $q10$); no information was provided for the stocked lakes. STRUCTURE was then run using a burn-in length of 100 000 iterations followed by 500 000 Markov chain Monte Carlo (MCMC) steps, which preliminary trials indicated was sufficiently long to allow α, F, D, and the likelihood to reach convergence values as recommended (Pritchard et al. 2000). The number of genetic clusters among the samples was set to 10, and allele frequencies within populations were considered correlated and updated from reference samples alone. The Dirichlet parameter for the degree of admixture (α) was set to an initial value of 1 and was estimated separately for each stocked population. This analysis was performed three separate times to insure convergence had been reached and individual admixture coefficient (q) values were averaged across the three runs.

Previous studies on the accuracy of such Bayesian clustering methods have suggested the use of two separate methods when population differentiation may be low to compare results (Latch et al. 2006). To that end a parallel analysis was conducted using the admixture model in the program BAPS (version 6; Corander and Marttinen 2006). As in the analysis above, the program was provided with 10 reference samples and asked to assign admixture coefficients to the samples from stocked lakes based on the predefined clusters. As above, this analysis was repeated three times, and individual q values were averaged across the three runs.

In both cases, to arrive at a single q value representing the extent of hatchery introgression, the proportion of each individual's genotype assigned to clusters $q9$ and $q10$ was summed. This value represented alleles that did not cluster into the native Algonquin Park clusters. As suggested by Vähä and Primmer (2006), to account for uncertainty associated with highly variable microsatellite loci, individuals within each stocked population were then assigned as either pure wild ($q = 0$) or hatchery ($q = 1$) if their individual q values were ≤0.1 or ≥0.9 respectively. Admixed individuals whose q values fell within 0.1 and 0.9 were left as they were. Population admixture coefficients (Q) were then calculated by averaging the q values within each population.

Correlates of hatchery–wild admixture

Prior to testing for correlations between the potentially explanatory variables and mean lake admixture values, the habitat or anthropogenic variables were tested for correlations among each other. Very few habitat and anthropogenic variables showed any sign of significant correlation (Table 3), so all variables were included in further model selection analyses. In keeping with the post hoc exploratory nature of this study, an exhaustive model creation procedure was undertaken to determine the most explanatory variable or combination of variables. This procedure was conducted using beta regressions because of the nature of admixture values (between 0 and 1). All possible additive and interactive models were constructed over a range of model complexity (K), where K represents the number of parameters included in the model. Model fit was assessed using a second-order information criterion, AIC_c, because of the large number of explanatory variables and small sample size (Burnham and Anderson 2002). For this reason model size was also limited to a maximum of $K = 6$.

Results

Within-lake measures of genetic diversity

Of the 22 populations and hatchery strains sampled, 10 exhibited significant deviation from HWE after Bonferroni corrections

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Table 3. Pearson correlation matrix of pairwise correlation coefficients for the habitat and anthropogenic variables included in this study of correlates of hatchery–wild hybridization in brook trout populations in Algonquin Park, Ontario, Canada.

|	SArea	SDI	Elevation	pH	MEI	MDepth	SPresent	Distance	StMonth	StEvents	StAge	StDensity	StTotal	AdmixPeriod
SArea	1.00													
SDI	**0.56**	1.00												
Elevation	−0.04	0.10	1.00											
pH	−0.14	0.07	−0.47	1.00										
MEI	−0.24	0.16	**−0.18**	0.64	1.00									
MDepth	0.09	−0.33	0.16	−0.36	**−0.73**	1.00								
SPresent	0.29	0.16	−0.44	0.28	−0.25	0.15	1.00							
Distance	−0.13	0.18	−0.69	**0.68**	0.80	−0.70	0.02	1.00						
StMonth	−0.07	0.07	−0.19	**0.75***	0.61	−0.30	0.17	0.50	1.00					
StEvents	0.23	0.05	**0.59**	−0.43	−0.48	0.29	0.01	−0.70	−0.56	1.00				
StAge	0.09	0.34	0.34	−0.53	−0.44	0.19	−0.14	−0.41	**−0.79***	0.44	1.00			
StDensity	−0.31	−0.32	0.01	−0.08	**0.34**	−0.20	−0.14	0.17	−0.04	−0.08	0.00	1.00		
StTotal	**0.61**	0.07	0.06	−0.05	−0.23	0.17	0.44	−0.31	−0.22	**0.64**	0.02	−0.04	1.00	
AdmixPeriod	0.49	**0.70**	0.23	−0.21	**−0.18**	0.24	0.38	−0.25	−0.17	0.37	0.40	**−0.21**	0.36	1.00

Note: Bold values indicate significant (α = 0.05) correlations, while asterisks (*) indicate significance after Bonferroni correction. Descriptions of the variables contained in this table can be found in Table 2.

(α = 0.0036, k = 14; Table 1). Among these, eight displayed heterozygote deficiencies, but these were spread out over the 14 different loci and depended on the particular population (in total 60 loci deviated significantly from HWE out of 308 tests; the minimum and maximum number of loci that deviated significantly within a population were 0 and 7, respectively); this suggested biological explanations for these deviations. Two of the deficiencies were found in the hatchery strains: the Hills Lake strain, which has been maintained within the Ontario hatchery system for over 40 years with few genetic introductions (Fraser 1981), and the Lake Nipigon strain, which was established from wild spawn collections in the late 1970s (Kerr 2006). Of the remaining eight lakes displaying heterozygote deficiencies, three had large surface areas (>181 ha) and a fourth (H-R-W) was composed of three connected lakes shown to exchange genetic material and resemble one large population (Addison and Wilson 2010). It would not be unexpected to find subtle subpopulation structure present and hence a Wahlund effect (Wahlund 1928). Heterozygote deficiencies in smaller lakes have been detected in other brook trout populations and may be due to limited suitable breeding sites being available, a situation previously observed in Algonquin park (Ridgway and Blanchfield 1998), and that can reduce dispersal from spawning sites and increase occurrences of inbreeding compared with larger lakes (Castric et al. 2002). Of the 2002 tests of linkage disequilibrium across populations (91 tests per population), only 21 were significant after Bonferroni corrections (α = 0.000549, k = 91), and these were spread out over all 14 loci and in 11 different populations.

Degree of hatchery–wild admixture amongst study lakes

Though STRUCTURE produced smaller Q values for 8 of the 12 stocked lakes than BAPS, the mean difference between the Q estimates of both methods was only 0.04. The primary difference between the results of both admixture analyses was in the numbers of individuals found to be admixed versus pure wild or hatchery ancestry. In all 12 stocked lakes, STRUCTURE assigned more individuals to an admixed state (0.1 < q < 0.9), while BAPS tended to assigned individuals to either pure state (Fig. 2). The mean (±SE) admixture across all stocked lakes after correcting for microsatellite uncertainty (Vähä and Primmer 2006) was 0.38 ± 0.013 for STRUCTURE and 0.36 ± 0.015 for BAPS, yet few lakes displayed intermediate admixture; roughly half of the lakes had mean admixture above 0.57 or 0.52 for the STRUCTURE and BAPS methods, respectively, while the other half demonstrated admixture levels below 0.22 or 0.13, respectively (Table 1). Within-lake variance in admixture was generally low (0.0016 to 0.016 for STRUCTURE and 0.0006 to 0.050 for BAPS), which is to be expected because in most

Fig. 2. The number of individuals assigned to one of three categories (Wild: $q \leq 0.1$, Hatchery: $q \geq 0.9$, or Admixed: $0.1 < q < 0.9$) per stocked lake in Algonquin Provincial Park according to admixture analyses done in BAPS (left bars) or STRUCTURE (right bars).
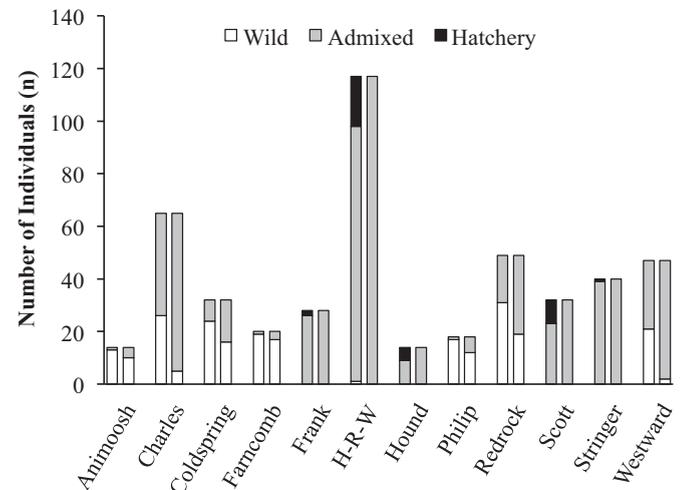
cases at least seven generations have passed (Liskauskas and Ferguson 1991) since stocking ceased.

Correlates of hatchery–wild admixture

Though not a formal test, the reappearance or absence of certain variables among the best-fitting models, and those within 2 ΔAIC_c units of the best-fit model, at each level of K, is suggestive as to which variables may be important and which may not be. The following variables were the most highly represented among the best-fit models in Table 4: lake elevation (15), stocking month (11), the admixture period (10), and the lake trail distance (8). Other variables such as the lake surface area, the mean stocking age, and the total number of fish stocked were present in one or fewer of the best-fitting models.

In the simplest model (K = 1), pH produced the lowest AIC_c value and a significant negative correlation with admixture; however, lake elevation and lake distance were within 2 ΔAIC_c units and also displayed significant positive and negative relationships, respectively, with hatchery admixture (Figs. 3a, 3b, 3c). The positive influence of elevation on hatchery admixture was maintained as model complexity increased until K exceeded 5, after which point lake elevation was no longer present among the best-fit models.

Table 4. All beta regression models within 2 ΔAIC_c units of the best-fit model for complete model sets at each level of K (the number of anthropogenic or habitat explanatory variables used).

Model	K	df	Log-likelihood	AIC_c	Within K ΔAIC_c	ΔAIC_c
pH	1	3	7.21	−5.42	0.00	16.53
Elevation	1	3	6.98	−4.96	0.46	16.99
Distance	1	3	6.64	−4.29	1.14	17.66
Elevation + StDensity	2	4	12.98	−12.24	0.00	9.71
Elevation + pH	2	4	12.44	−11.17	1.07	10.78
Elevation × pH	2	5	16.75	−13.49	0.00	8.46
Elevation × MEI	2	5	15.82	−11.64	1.86	10.32
Elevation + SPresent + StDensity	3	5	17.49	−14.99	0.00	6.96
Elevation + pH + StDensity	3	5	16.81	−13.63	1.36	8.32
Elevation + StDensity + StMonth	3	5	16.72	−13.45	1.54	8.50
AdmixPeriod × Elevation + StMonth	3	6	25.38	−21.95	0.00	0.00
AdmixPeriod + Elevation + StMonth + SDI	4	6	22.40	−16.00	0.00	5.95
AdmixPeriod × Elevation + StMonth + MEI	4	7	29.89	−17.79	0.00	4.16
AdmixPeriod × Elevation + StMonth + MDepth	4	7	29.14	−16.27	1.52	5.68
AdmixPeriod × Elevation + StMonth × Distance	4	8	35.76	−7.51	0.00	14.44
Distance + SDI + SPresent + StDensity + StEvents	5	7	26.56	−11.13	0.00	10.82
Distance + MDepth + SDI + SPresent + StEvents	5	7	26.07	−10.13	1.00	11.82
Elevation × SPresent + AdmixPeriod + SDI + StMonth	5	8	36.38	−8.75	0.00	13.20
AdmixPeriod × MEI + Distance + Elevation + StMonth	5	8	35.94	−7.87	0.88	14.08
Distance × MDepth + AdmixPeriod × StMonth + SDI	5	9	46.28	15.43	24.19	37.39
Distance + MDepth + SDI + SPresent + StDensity + StEvents	6	8	32.47	−0.95	0.00	21.00
AdmixPeriod + pH + SPresent + StAge + StEvents + StMonth	6	8	32.23	−0.46	0.48	21.49
Distance × AdmixPeriod + MEI × StMonth + MDepth + StTotal	6	10	68.74	102.52	0.00	124.47

Note: Mean hatchery–wild admixture levels are for brook trout in 12 Algonquin Park lakes.

The negative effect of lake pH was also present among the best-fitting models at lower model complexities $K < 3$, including an interaction with lake elevation (Fig. 3e); however, it was only present in one model above $K = 3$. Lake distance, although being among the best-fitting models at $K = 1$, was absent from other simpler models, occurring primarily in more complex models above $K = 5$. Admixture period was among the variables most represented in the best-fitting models and tended to display an interactive relationship with other variables, appearing primarily among more complex models ($K \geq 3$). When combined interactively with lake elevation, a common occurrence among more complex models, fitted admixture values were highest when lakes had a shorter admixture period or were found at higher elevations (Fig. 3f). In each case where the best-fitting models included an interaction between lake elevation and the admixture period, the stocking month was always present displaying a significant negative effect on hatchery admixture (Fig. 3d). In fact, a model with $K = 3$ containing an interaction between the admixture period and lake elevation, as well as an additive relationship with the positively correlated mean stocking month, displayed the lowest AIC_c value among all compared models at the varying levels of model complexity.

Discussion

Our results suggest that among Algonquin Park lakes, while the amount of admixture between hatchery and wild brook trout populations resulting from stocking is variable, the absence of lakes displaying intermediate admixture levels (0.57 < Q < 0.22, for the STRUCTURE estimates) suggests that hatchery fish either mix extensively or infrequently depending on multiple habitat or anthropogenic variables. Several variables (pH, lake distance, elevation) demonstrated a significant correlation with admixture, but extensive post hoc AIC_c model comparisons suggested more complex models containing multiple variables provided a better fit. Through exhaustive model formation over varying levels of model complexity, several environmental and anthropogenic variables demonstrated potential in modeling hatchery–wild admixture through their recurrence among the best-fit models. These factors appear to be associated with lake productivity and

brook trout density, specifically pH and elevation (see below). Certain anthropogenic variables were also important: the distance to the lake (from the nearest road), the mean month in which lakes were stocked, and the amount of time since the introduction of hatchery genotypes.

Habitat correlates of hatchery–wild admixture

Previous work on the correlates of hatchery–wild hybridization among brook trout populations found that admixture was greater when conditions constrained brook trout habitat, thereby increasing contact and mating opportunities between the two groups (Marie et al. 2012). The present study proposes an alternative relationship between environmental conditions and hatchery–wild admixture, one driven by negative density-dependent fitness effects. While information on the densities of brook trout is not available for most of the study lakes, we can hypothesize their effect on admixture by looking at the relationship between the particular environmental variables found to have higher relative importance and brook trout density.

First, pH has been shown to be positively correlated to brook trout density (Godbout and Peters 1988; Nislow and Lowe 2003). This relationship is possibly the result of the effect that pH and alkalinity can have on macroinvertebrate densities (Krueger and Waters 1983; Baker et al. 1996). Second, elevation has also been linked to lake productivity in ways that can extend up the food web (Karlsson et al. 2005). For instance, in a neural net model of the relationship between brown trout (*Salmo trutta*) densities and environmental parameters, Lek et al. (1996) found that brown trout densities initially increased with increasing elevation, but beyond an elevation of 900 m above sea level (a.s.l.), this relationship became strongly negative. It is possible then that for brook trout a similar relationship exists, but in Algonquin Park, the critical elevation is lower for brown trout. The range in elevation of our study lakes (352–473 m a.s.l.) may therefore include or exceed the critical elevation, thereby resulting in a negative correlation between elevation and brook trout densities. A positive correlation between pH and brook trout density and a negative correlation with elevation, combined with their inverse correlations to hatchery–wild admixture, suggest that the density of

Fig. 3. Fitted relationships according to beta regression models between mean hatchery admixture levels and explanatory habitat and anthropogenic variables found to recur among the best-fit models at varying levels of model complexity based on AIC_c values. Points on panels *a* through *d* are lake values (*n* = 12); two points overlap in panel *b*.
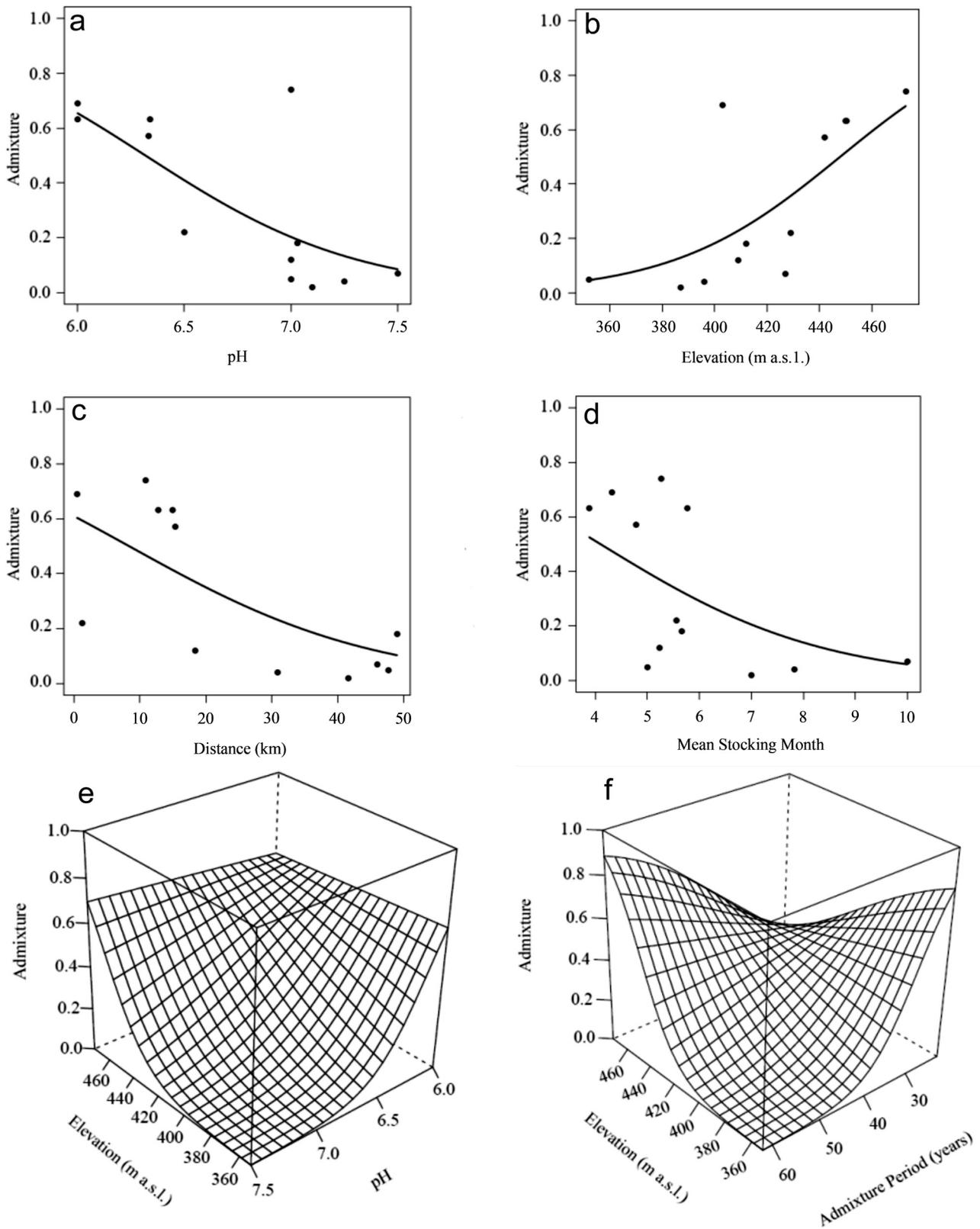
brook trout may have a negative association with the extent of hatchery hybridization in the wild. A similar correlation has been observed and commented on regarding hybridized populations of brown trout in Europe (Hansen et al. 2009).

Anthropogenic correlates of hatchery–wild admixture

One of the three anthropogenic variables that represents a potential determinant of hatchery–wild admixture was the distance to the lake (from the nearest road), which is negatively correlated to angling intensity (Kaufman et al. 2009) and which was found in this study to be negatively correlated to admixture, suggesting a positive correlation between angling intensity and admixture. Although this contradicts some previous findings in which fishing was negatively correlated with hatchery admixture (García-Marín et al. 1998), potentially because of anglers being more efficient at catching hatchery fish than wild fish (Mezzera and Largiadèr 2001; Champigneulle and Cachera 2003), it does support the hypothesis proposed by Evans and Willox (1991). These authors showed, through modeling, that fishing preferentially removes large, highly fecund wild and hatchery fish alike, but while wild fish have only one source of replacement (natural recruitment), hatchery fish are potentially replaced by both natural recruitment and further stocking events. The trend for higher levels of hatchery admixture in more southern lakes in Algonquin Park further supports the link between angling intensity and admixture, as the southern arm of the park was the most recent addition to the park and historically has maintained relaxed regulation and greater accessibility compared with more northern latitudes (Lambert and Pross 1967).

An alternative, but not mutually exclusive explanation for the relationship between the distance to the lake and admixture is the relationship among lake accessibility, fishing pressure, and stocking effort often observed among many sport fish species (Loomis and Fix 1998; Post et al. 2008). It seems likely that a similar situation is occurring in Algonquin Park, namely that more accessible lakes are being stocked more frequently, although this relationship among the study lakes is nonsignificant (Table 3). The presence of the number of stocking events among the more complex models containing lake distance suggests that these two variables likely work together to increase the incidence of hatchery–wild hybridization, a proposition previously theorized by Evans and Willox (1991).

The interactive relationship between the admixture period and lake elevation suggests that at lower elevations, the extent of hatchery genes present in a hybridized population decreases over time, whereas at higher elevations (where wild brook trout densities are hypothesized to be lower) it tends to increase over time. The mean stocking month was also a prominent feature of models that included the interaction between admixture period and lake elevation. The negative correlation between the mean stocking month and the extent of hatchery admixture suggests that lakes that tend to be stocked with brook trout earlier in the season are more heavily influenced, genetically, by those hatchery fish. Such a finding is inconsistent with the idea that brook trout stocked at an earlier age exhibit greater mortality in their first year (Fraser 1978) and may also relate back to density dependence (see below).

Density dependence

The postulated relationship between hatchery admixture and brook trout density might be the result of several negative density-dependent factors affecting the fitness of hatchery and domesticated strains. Density-dependent reproductive success due to a limited number of spawning sites is one possible explanation. Hatchery salmonids tend to experience greater reproductive success at lower densities, as has been observed in Pacific salmon (Fleming and Gross 1993), where hatchery fish are regularly outcompeted by wild fish at higher densities. In Ontario, lake surface area is the most common means of determining the num-

ber of fish stocked (Kerr 2000); however, in Algonquin Park the size of a lake is not correlated to the amount of spawning area (Ridgway and Blanchfield 1998). It is therefore foreseeable that a situation similar to that of the Pacific salmon mentioned above exists among the brook trout populations of Algonquin Park, as suggested by Blanchfield and Ridgway (2005). Higher overall densities of fish (hatchery and wild) relative to a limited resource (spawning sites) results in reduced hatchery–wild hybridization. A second possible explanation is density-dependent mortality. Hatchery rainbow trout have been shown to take greater risks in natural settings than wild fish to maintain their higher growth rates (Biro et al. 2004). They have also been found to venture further into riskier, but more productive, habitat at higher densities (Biro et al. 2003). Together, these two phenomena suggest that in natural settings domesticated hatchery salmonids with high growth rates and shorter life spans, such as the Hills Lake strain (OMNR 1999), likely experience greater mortality rates than wild trout at high densities. This would result in reduced survival to maturity and therefore reduce hatchery–wild admixture in lakes with higher densities.

Summary and implications

Our results suggest that populations found at low densities may be at greater risk of introgressive hybridization and admixture from conspecifics than those at high densities and that the intensity of angling pressure may have a positive effect on admixture, either through the dynamics of brook trout population growth and replacement or through more human-centric means of meeting the requirements of fishers with increased stocking frequencies. These findings may have implications for conservation efforts seeking to identify nonintrogressed individuals or populations or to control undesirable anthropogenic hybridization (e.g., Meraner et al 2008; Baric et al. 2010), as well as for risk assessment analyses of projects that may potentially result in unintentional hybridization (e.g., Hutchings and Fraser 2008). For this reason, we recommend that steps be taken to formally test some of the predictions that this study has proposed, as have other such recent studies (Heath et al. 2010; Marie et al. 2012; Yau and Taylor 2013).

Confirming the predictions made in this study would provide wildlife managers and conservation authorities with useful guides to aid them when dealing with hybridization situations. Confirmation of these predictions may simplify the identification of populations more resistant to introgressive hybridization with hatchery fish by focusing their efforts on environments that support relatively higher densities or promote competition for limited resources. Such environments may be ones in which the number of fish stocked relative to the spawning area was high or where the local environmental features promoted a high density of native individuals at the time of hybridization, effectively lowering the relative fitness of introduced domestic individuals (Naylor et al. 2005). An example of such a situation may exists in the eastern Rockies where westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) display reduced introgression from introduced rainbow trout in colder waters (Yau and Taylor 2013), potentially because of metabolic traits possessed by westslope cutthroat trout better suited to colder water (Rasmussen et al. 2012), thereby giving them a competitive advantage. Identifying such causative relationships would also assist in removing hybrids from a system (if this is deemed to be desirable). Under certain conditions it may be possible to artificially increase the density of both wild and hybridized individuals to the point where density-dependent natural selection can exhibit stronger selection against those individuals lacking local adaptations. Certainly, reducing the population density through the nondiscriminate removal of both wild and domesticated individuals from a population undergoing hybridization should be avoided, as such removal may potentially increase the relative success of foreign conspecifics (e.g., Fleming and Gross 1993; Biro et al. 2003, 2004). Finally, in situations where

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unintentional hybridization is a possibility, the number of accidental introductions should be minimized wherever possible, and the choice of locations for such activities as aquaculture and farming should focus on areas where environmental conditions are likely to greatly reduce the reproductive success of any potential escapees (e.g., Naylor et al. 2005; Hutchings and Fraser 2008; Thorstad et al. 2008).

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