

Effective number of breeders in relation to census size as management tools for Atlantic salmon conservation in a context of stocked populations

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Abstract Monitoring short-term fluctuations in effective population sizes (N_e) and effective number of breeders (N_b), as well as their ratio to adult census population size (N_e/N_c and N_b/N_c), provide insight into population demography and inform conservation programs towards limiting long-term loss of evolutionary potential in wild populations. In this study, we monitored short-term variations of N_b and N_b/N_c over three consecutive years for nine Atlantic salmon populations from Quebec, Canada. We documented how these population genetic parameters were influenced by anadromous population size as well as yearly and long-term stocking intensity. Towards this end, 15 microsatellites were used to genotype about 100 one-year-old parrs for each of three consecutive years for nine genetically distinct populations (total $n = 2506$) from Québec, Canada. Yearly stocking intensity had a negative effect on N_b/N_c , possibly as a consequence of a reduced reproductive contribution of stocked relative to wild fish. However, the impact of long-term stocking intensity on N_b/N_c was not significant, which may indicate relatively weak carry-over effects of stocking on future generations. Also,

N_b/N_c was negatively correlated with N_c , suggesting compensatory mechanisms, as previously reported in other salmonids. Overall, this study provides evidence of relatively weak and short-term effect of stocking on N_b/N_c ratio in Atlantic salmon populations and suggests potential biological mechanisms leading to the significant negative relationship between N_b/N_c and N_c .

Keywords Effective population size · Effective number of breeders · Census size · Atlantic salmon · Linkage disequilibrium · Single sample estimator

Introduction

Effective population size over a generation (N_e) (Wright 1931) and effective number of breeders over a reproductive cycle (N_b), are among the most important parameters in conservation biology and are widely used for genetic monitoring. N_e influences the rate of loss of genetic variation due to evolutionary drift, the extent of inbreeding and inbreeding depression, the adaptive potential and the extinction risks of populations (Charlesworth 2009). N_b refers to the effective number of breeders during a single breeding event (Waples 2005). This parameter is generally more easily quantifiable than N_e since it can be inferred from a single cohort, and might thus be a more accessible parameter for managers dealing with yearly conservation decisions (Waples 2005; Luikart et al. 2010; Tallmon et al. 2010). Along with improved genotyping methods, many methods have been developed in order to infer N_e or N_b from individual multilocus genotypes (Luikart et al. 2010; Waples and Do 2010). N_e or N_b estimates can therefore be more readily used to formulate recommendations for the conservation of wild populations for mitigating loss of

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genetic diversity, preserving evolutionary potential and decreasing extinction risks (Frankham et al. 2010, 2014).

The ratio of effective population size and of effective number of breeders to census population size, N_e/N_c and N_b/N_c , are also important parameters for the understanding of population biology and management (Frankham 1995; Luikart et al. 2010; Palstra and Fraser 2012). From a management point of view, defining N_e/N_c and N_b/N_c could ultimately be useful to infer one parameter (e.g. N_c) from the other (e.g. N_e or N_b), which could be more cost effective or more appropriate for declining or endangered populations than demographic monitoring (Luikart et al. 2010). These genetic parameters can also be used to understand to what extent a variety of biological parameters and mechanisms cause N_e and N_b to be smaller than N_c . N_b/N_c and N_e/N_c considerably differ among species. For instance, small values are expected in species with high fecundity and fluctuation in N_c whereas high values are expected in monogamous species with low fecundity and variance in reproductive success (Frankham 1995; Vucetich et al. 1997; Hedrick 2005). Important differences in N_e/N_c among populations within species have also been documented, and smaller N_e/N_c values have notably been attributed to uneven sex-ratios, nonrandom mating, overlapping generations, variance in individual reproductive success and family size, and fluctuating N_c over generations (Crow and Kimura 1970; Nunney 1993; Frankham 1995; Kalinowski and Waples 2002; Hedrick 2005). In particular, negative relationships between N_e/N_c and N_c have been mainly attributed to the fact that larger populations are more likely to exhibit increased fluctuations in N_c , deviation in sex ratio, and variance in family size (Frankham 1995; Pray et al. 1996). Studies in salmonids suggest that an increase in N_e/N_c with a decrease of N_c may arise from mechanisms coined as “genetic compensation” through a reduction in the variance of reproductive success among individuals while N_c decreases, possibly mediated by variable reproductive interactions between anadromous and non-anadromous forms (Ardren and Kapuscinski 2002; Araki et al. 2007a; Palstra and Ruzzante 2008; Ficetola et al. 2010).

N_e/N_c and N_b/N_c may also be influenced by individual supplementation (i.e. stocking), although the genetic impacts of stocking on such parameters remain largely unmonitored (Laikre et al. 2010). While the central objective of stocking is to increase population size, N_c , the parameters N_b , N_e , N_b/N_c and N_e/N_c of the stocked population might actually decrease (Christie et al. 2012a). This is notably due to the relatively small number of captive breeders generally used, the increased variance in reproductive success in stocked populations, together with the large number of individuals being stocked (Ryman and Laikre 1991; Ryman et al. 1995). In addition, stocked

individuals generally have reduced reproductive success compared to their wild counterparts (Araki et al. 2007b; Thériault et al. 2011; Christie et al. 2012b; Milot et al. 2013), thus increasing N_c without a similar relative increase of N_b .

A major decline in abundance of Atlantic salmon (*Salmo salar*) has been observed worldwide and populations from Quebec, Canada, are not an exception (ICES 2014; Dionne et al. 2014). Despite the ban on commercial fishing of Atlantic salmon in many countries including Canada, as well as the application of strict conservation rules for recreational fishing, stocking, and dam construction, the majority of natural stocks remain low or in decline. An associated decrease of N_e and N_b may also increase inbreeding as well as local diversity loss, and ultimately may threaten the evolutionary potential of small populations. Moreover, stocking programs may possibly contribute to the erosion of genetic diversity of these populations (Ryman and Laikre 1991; Ryman et al. 1995). It thus appears essential to monitor trends in N_b and N_b/N_c in Atlantic salmon populations in order to understand the evolutionary mechanisms contributing to such declines, as well as to investigate potential effects of stocking on the genetic integrity of such threatened populations.

In this study, we applied a standardized sampling and genotyping protocol to monitor N_b and N_b/N_c over three consecutive breeding years, 2008–2010, in nine Atlantic salmon populations from rivers draining into the St Lawrence River, Québec, Canada. The specific aims were to (i) utilize a single sample estimator of N_b based on linkage disequilibrium signal (Waples and Do 2008, 2010; Luikart et al. 2010; Waples et al. 2014) to estimate N_b for the 27 samples considered in this study, (ii) test whether N_b and N_b/N_c varied among populations and among years, (iii) test whether stocking intensity, estimated as the expected ratio of hatchery origin adults, was negatively related to N_b/N_c , (iv) test whether the effects of stocking were exclusively immediate (i.e. only affecting the supplemented cohort) or if carry-over effects were observed on subsequent cohorts, and (v) investigate potential negative relationships between N_b/N_c and N_c that possibly suggest compensation mechanisms. We then discuss the practical value of DNA-based N_b and N_b/N_c estimates in relation to stocking for the management of wild Atlantic salmon populations.

Methods

Study sites and sampling

A total of 2506 parr were sampled using electrofishing in nine rivers during summer in 2010, 2011 and 2012,

corresponding to the breeding seasons from autumns 2008, 2009 and 2010, respectively (Fig. 1). These nine rivers were chosen according to the (i) representativity of the different regional genetic groups identified in the studied area (Dionne et al. 2008; Moore et al. 2014), (ii) census size estimates, (iii) levels of stocking, and (iv) logistical feasibility of sampling. Each river was sampled each year for a targeted 100 parr. Sampling took place for each river in five distinct sections of 10–100 m, for approximately 14–25 parr per section in order to control for potential within-river genetic structure (Garant et al. 2000; Vaha et al. 2007) and to limit potential upward bias in relatedness due to spatial grouping of juveniles from similar parents (Garza et al. 2014). Sampling such a relatively large number of individuals per river was justified by the recommendations of previous studies (Tallmon et al. 2010; Whiteley et al. 2012; Frankham et al. 2014). Adipose fin clipped stocked fish were not sampled. For the SMA (Fig. 1; Table 1), only the principal branch (not the Northeast branch) was sampled since genetic structure has been documented among these branches (Garant et al. 2000). Fish length was used as a proxy of its age. Whole

fish were stored immediately in 150 ml tubes with 95 % ethanol. Individuals were sampled following the guidelines of the Canadian Council on animal care.

Estimates of census size (N_c)

We defined census size (N_c) as the number of anadromous adults returning to a river for a given year. The number of returning adults (Cauchon 2015) was determined by counts at a downstream fish ladder or by snorkelling when a fish ladder was not present, according to the protocol of the Québec government (Ministère des Forêts, de la Faune et des Parcs du Québec, MFFP). According to Caron et al. (1999), there is no bias associated with measures obtained through migratory pass or at a count barrier. According to the same study, visual counts through snorkelling may underestimate the real number of fish by 10 %. Because of logistical constraints and river size, mature male parr (non-anadromous maturing males) could not be taken into account in this estimate although they are likely to fertilize a significant proportion of the eggs spawned (Richard et al. 2012; Perrier et al. 2014).

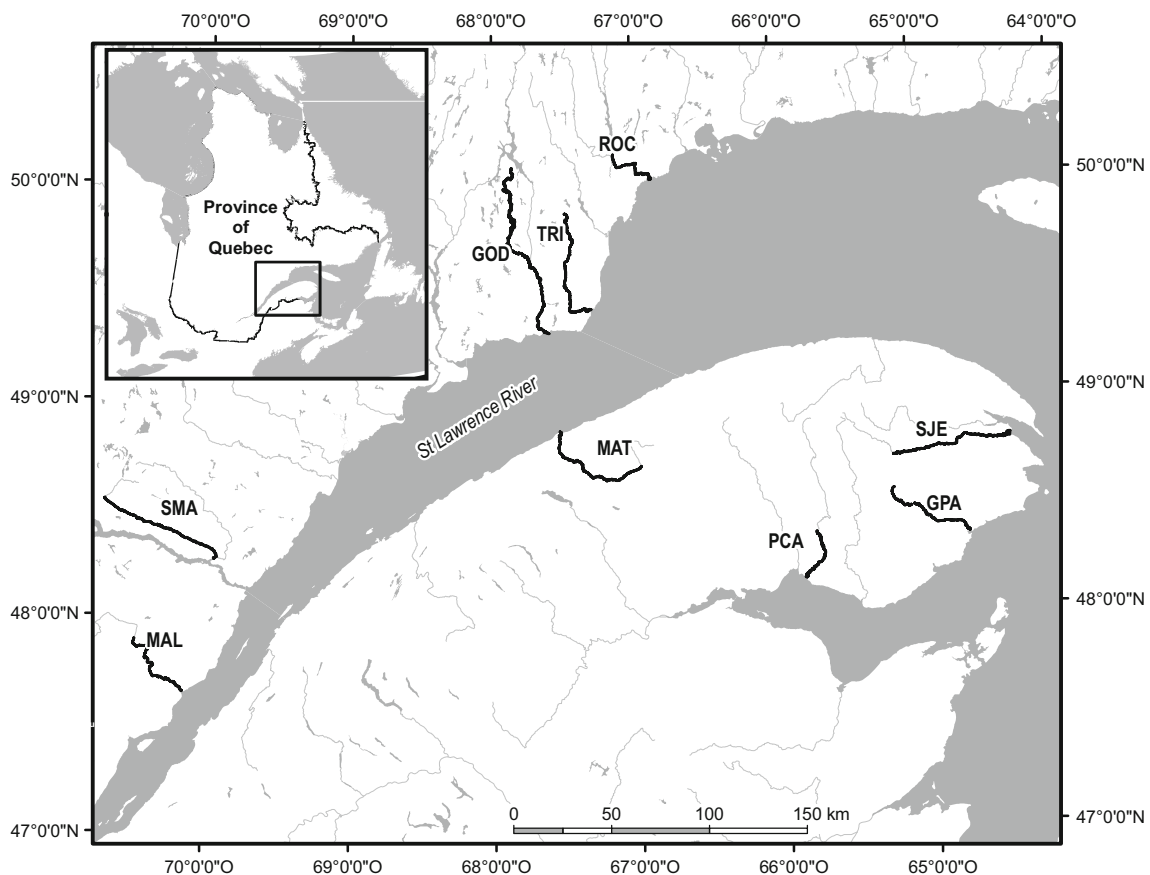


Fig. 1 Map showing the nine rivers sampled in the province of Québec, Canada. *GPA* Grand-Pabos, *PCA* Petite-Cascapedia, *TRI* Trinité, *SMA* Sainte-Marguerite, *MAL* Malbaie, *GOD* Godbout, *ROC* Aux-Rocher, *SJE* Saint-Jean, *MAT* Matane

Table 1 River name, river and sample abbreviation, sample size, number of anadromous breeders (N_c), expected ratio of hatchery-origin adults, expected ratio of hatchery-origin adults on average over 15 years

River	Sample abbreviation	Breeding year	Sample size	N_c	Expected ratio of hatchery-origin adults	Expected ratio of hatchery-origin adults on average over 15 years
Grand-Pabos	GPA08	2008	64	150	0.12	0.23
	GPA09	2009	100	103	0.10	0.24
	GPA10	2010	97	284	0.01	0.24
Petite-Cascapedia	PCA08	2008	98	285	0.05	0.50
	PCA09	2009	94	245	0.01	0.43
	PCA10	2010	100	338	0	0.38
Trinité	TRI08	2008	98	838	0	0
	TRI09	2009	100	420	0	0
	TRI10	2010	100	763	0	0
Sainte-Marguerite	SMA08	2008	96	743	0.03	0.4
	SMA09	2009	100	618	0.03	0.39
	SMA10	2010	99	744	0.10	0.39
Malbaie	MAL08	2008	43	931	0.15	0.70
	MAL09	2009	99	625	0.23	0.71
	MAL10	2010	100	590	0.25	0.73
Godbout	GOD08	2008	99	615	0.08	0.16
	GOD09	2009	100	767	0.02	0.16
	GOD10	2010	97	842	0.06	0.17
Aux-Rochers	ROC08	2008	54	615	0.04	0.18
	ROC09	2009	99	615	0.08	0.15
	ROC10	2010	97	1069	0.04	0.13
Saint-Jean	SJE08	2008	87	947	0	0.03
	SJE09	2009	100	796	0	0.02
	SJE10	2010	94	917	0	0.02
Matane	MAT08	2008	92	1977	0	0.02
	MAT09	2009	99	1555	0	0.02
	MAT10	2010	100	1498	0	0.02
Average			93	737	0.05	0.24
Minimum			43	103	0	0
Maximum			100	1977	0.25	0.73

Stocking history and estimates of expected contributions of stocking

Stocking has been performed in eight of the nine rivers (Table 1). Governmental databases recorded the amount of stocked fish at various life stages since 1984 (Available upon request at <http://www.mffp.gouv.qc.ca/publications/enligne/faune/reglementation-peche/nous-joindre.asp>). While we had no traceability of the origin of fish stocked, the use of local progenitors has been recommended and applied in most rivers. We estimated the expected contribution of hatchery-born fish relative to wild-born fish for the three breeding years studied (Table 1). This estimate is a fairly rough estimate of the ratio of adults of hatchery-origin returning to the river. The governmental database was used to determine the number of

individuals stocked at various life stages in the river studied. We used available survival rates for stocked Atlantic salmon (Caron et al. 1999), probabilities of smelting/maturing measured from scale analyses and the proportions of single/two/three sea winter(s) (unpublished data from the MFFP) and reproductive success (Richard et al. 2012; Milot et al. 2013) to estimate the expected contribution of adult anadromous fish originating from stocking for each breeding year from 1994 to 2010. The contribution of stocked fish was first considered on a yearly basis (named expected ratio of hatchery-origin adults). This measure aimed at estimating the potential short-term effects of stocking on N_b and N_b/N_c . Stocking effect was then considered as an average over the 15 years since the considered breeding year (named expected ratio of hatchery-origin adults on average over 15 years). This second measure

aimed at estimating the potential long-term effects of stocking on N_b and N_b/N_c .

Genotyping

DNA was extracted from caudal and pelvic fins using a modified version of the Aljanabi and Martinez (1997) salt extraction method. In brief, DNA was precipitated using isopropanol for 30 min. After washing the pellets, samples were centrifuged at $10,000\times g$ for 10 min at 4 °C. Samples were then eluted in 100 μ L of sterile H₂O. DNA was amplified at 15 microsatellite loci (Supplementary material 1) using Qiagen Multiplex PCR Kit (Qiagen inc.). Amplifications were performed using a T1 Biometra thermocycler (Biometra, Kirkland, QC, Canada) using the following parameters: 15 min activation at 95 °C, 35 cycles with denaturation at 95 °C for 30 s, annealing for 3 min at 60 °C, and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Amplified fragments were electrophoresed using an ABI 3130xl capillary DNA sequencer (Applied Biosystems Inc.). Genescan 500 LIZ was used as a standard, and GENEMAPPER 4.0 was used for scoring (Applied Biosystems). Genotypes with more than 50 % of missing data were discarded. To ensure scoring reliability, 96 samples were scored independently by two laboratory members and problematic scores were examined.

Genetic diversity within and among rivers

Potential presence of null alleles and allelic dropout was verified using the software MICROCHECKER (Van Oosterhout et al. 2004). F_{is} , H_o and H_e for each marker and river were estimated using FSTAT 2.9.3 (Goudet 1995) and were mainly used to ensure the absence of technical problems that may have led to abnormal heterozygote excess or deficit. Using genodive2.0b23 (Meirmans and Van Tienderen 2004), we estimated F_{is} for each river in order to detect potential Wahlund effects (potentially linked with stocking or sub-structure) and F_{st} between rivers and between sampling site within rivers to assess the potential structure among and within rivers.

Estimates of effective number of breeders (N_b), effective population size (N_e) and their ratio to census size (N_c)

We estimated effective number of breeders (N_b) (Waples 2005) for each of the 27 samples using NeEstimator 2 (Do

et al. 2013) that implements the LDNe algorithm (Waples and Do 2008) with recent improvement for a better consideration of missing data (Peel et al. 2012). The LDNe program estimates effective population size from the extent of linkage disequilibrium in the sample. We used a threshold of 0.05 as the lowest allele frequency, which gives the least biased results according to Waples and Do (2010). We reported estimated N_b with 95 % (parametric) confidence intervals (CI). Because N_b estimates can be biased due to overlapping generations, we then used the method developed by Waples et al. (2014) to correct N_b using three simple life history traits, using the following equation:

$$N_{b(\text{adj})} = N_b / (0.991 - 0.206 \times \log(\text{Al}) + 0.256 \times \log(\alpha) + 0.137 \times \text{CVf})$$

where, Al, α , and CVf correspond to adult life span, age at maturity, and variation in age-specific fecundity, respectively. We used Al = 2, α = 3, and CVf = 0.88 (We computed parameters using the data published in Perrier et al. 2014 and according to unpublished data from the MFFP).

$N_{b(\text{adj})}$ and $N_{b(\text{adj})}/N_c$ were reported.

Then, in order to obtain a coarse estimate of N_e for each river, we used the following equation:

$$N_{e(\text{adj})} = N_{b(\text{adj})} / (0.833 + 0.637 \times \log(\text{Al}) - 0.793 \times \log(\alpha) - 0.423 \times \text{CVf})$$

Details concerning this method are available in Waples et al. 2014.

Estimated $N_{b(\text{adj})}$ and $N_{e(\text{adj})}$ values were compared to the latest proposed effective population size thresholds, if $N_e > 100$, that avoid inbreeding depression over the short term and maintain evolutionary potential, if $N_e > 1000$, over the long term (Frankham et al. 2014). In order to verify the ability of the studied populations to retain 90 % genetic diversity over 100 years (as a conservation target suggested by Frankham et al. 2010), these estimates were also compared to a conservation threshold calculated using the equation of loss of genetic diversity through time proposed by Wright (1969):

$$H_t/H_0 = (1 - [1/2N_e])^t$$

where H_t is heterozygosity after t generations, H_0 initial heterozygosity, N_e effective population size. Considering a generation time of 5 years for Atlantic salmon (Dionne and Cauchon 2012), the estimated threshold N_e value was 95 using this formula.

Effects of river, breeding year, stocking and N_c on the effective number of breeders (N_b)

We used the function LMER from the R package LME4 to fit linear mixed effects models accounting for the effects of the river and the year on $N_{b(\text{adj})}$ and $N_{b(\text{adj})}/N_c$. Rivers and breeding years were considered as random variables. The function BOXCOX from the library MASS in R (Venables and Ripley 2002) was used to determine the best normalization transformation to apply to $N_{b(\text{adj})}$ and to $N_{b(\text{adj})}/N_c$. $N_{b(\text{adj})}$ and $N_{b(\text{adj})}/N_c$ were thereafter log transformed. Chi square tests were used to compare the log likelihood of the models with only one variable with models including two variables. From the best models, we inferred the variance attributable to the river and to the year on $\log(N_{b(\text{adj})})$ and $\log(N_{b(\text{adj})}/N_c)$.

We then used a linear model in R to predict $\log(N_{b(\text{adj})}/N_c)$ from the following variables: (i) N_c , (ii) expected ratio of hatchery-origin adults and (iii) expected ratio of hatchery-origin adults on average over 15 years. We chose the best model according to the Akaike information criterion (AIC, Akaike 1973). We then reported the coefficients from the two best models, and their standardized coefficients, using the function lm.beta from the library QuantPsyz. We verified normality of the data using a Shapiro test and visual inspection of the distribution of residuals.

Results

A total of 2506 individual genotypes with less than 50 % missing data were generated. An average of 3 % of missing data was found among these 2506 genotyped. The median number of juveniles genotyped per river and per year was 98 individuals (Table 1). The number of alleles ranged from 10 to 41 alleles per locus. The results from MICROCHECKER suggested the absence of null alleles and allelic dropout. H_e per population estimated using FSTAT ranged from 0.81 to 0.86, with an average of 0.83. Over all populations and loci, only five out of 405 F_{is} were significant, ruling out the occurrence of systematic null alleles or other technical artefact. Average F_{is} per population per year ranged from -0.017 (MAL09) to 0.031 (MAL08) (average = 0.007) (Supplementary material 2), thus indicating the absence of within-river Wahlund effects. Average F_{st} among locations within rivers ranged from 0.002 (PCA, TRI, MAL) to 0.008 (SJE) (average = 0.005). In comparison, F_{st} among rivers ranged from 0.006 (MAT-PCA) to 0.049 (GOD-GPA) (average = 0.026). We found no statistical link between F_{is} and the expected ratio of hatchery-origin adults ($r^2 = 0.027$, $p = 0.27$) or N_c ($r^2 = 0.016$, $p = 0.57$), nor between intra-

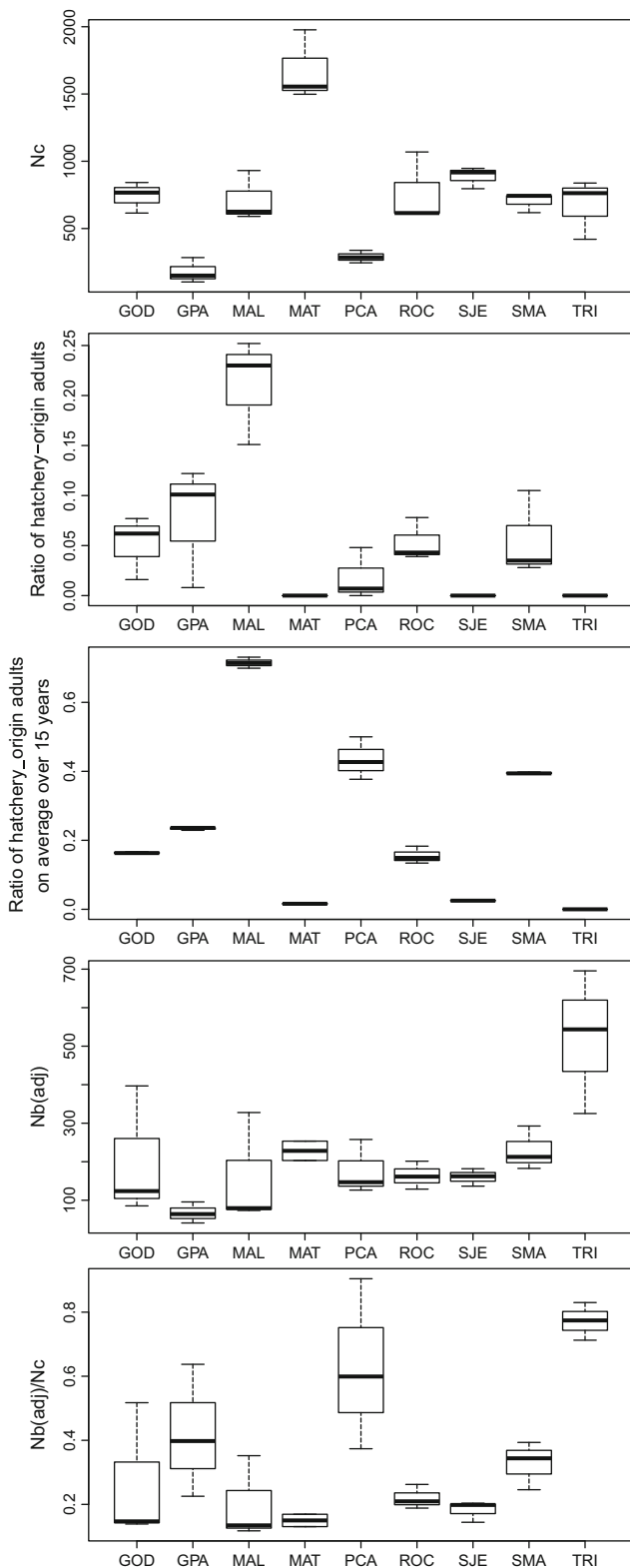
river F_{st} and expected ratio of hatchery-origin adults ($r^2 = 0.094$, $p = 0.11$) or N_c ($r^2 = 0.043$, $p = 0.71$) (Supplementary material 3).

Estimates of census size, effective number of breeders and expected stocking contributions

Census size (N_c) varied from 103 (GPA09) to 1977 (MAT08), with an average of 737 and a median value of 743 (Table 1; Fig. 2). Average N_c values per river over the three years ranged from 179 (GPA) to 1677 (MAT), with an average value of 737 and a median value of 715. The expected ratio of hatchery-origin adults per river over the 3 years ranged from 0 (in several rivers) to 0.25 (MAL10), with an average value of 0.05 and a median value of 0.03. The expected ratio of hatchery-origin adults on average over 15 years per river ranged from 0 (TRI) to 0.73 (MAL10), with an average value of 0.24 and a median value of 0.17. N_b estimates varied from 48 (GPA09) to 815 (TRI08) with an average of 248 and a median of 202 across all samples (Table 2). Over the 27 samples, we obtained a single “infinite” value, for MAT08, for which the lower limit of the confidence interval was 618. Confidence intervals (CI) ranged from ± 13 (GPA09) to infinite (MAL08 and samples from TRI), with a median value of ± 162 (excluding infinite CI). On average, CI were the smallest for samples from MAL (31) and GPA (42) and were the highest for samples from SMA (307), GOD (530), and TRI (infinite). $N_{b(\text{adj})}$ estimates were approximately 15 % smaller than the non-adjusted values and varied from 41 (GPA09) to 696 (TRI08) with an average of 211 and a median of 172 across all samples (Table 2). Harmonic mean of $N_{b(\text{adj})}$ values per river over the 3 years ranged from 59 (GPA) to 472 (TRI), with an average value of 188 and a median value of 159. $N_{b(\text{adj})}/N_c$ varied from 0.12 (MAL09) to 0.90 (PCA08), with an average of 0.36 and a median value of 0.26. Average $N_{b(\text{adj})}/N_c$ values per river over the 3 years ranged from 0.15 (MAT) to 0.77 (TRI), with an average value of 0.35 and a median value of 0.27. $N_{e(\text{adj})}$ estimates varied from 149 (GPA09) to 2537 (TRI08), with an average of 771 and a median value of 627. $N_{e(\text{adj})}$ estimates average per river varied from 217 (GPA) to 1723 (TRI), with an average of 686 and a median value of 578.

Effects of river of origin, breeding year, stocking contribution, and abundance on N_b/N_c ratio

The LMER model best describing $\log(N_{b(\text{adj})})$ variation according to AIC included the river effect only (log likelihood = -24.09) (Table 3). This model was not significantly different from the model integrating both the effect of the river and of the breeding year (log



◀**Fig. 2** For the three breeding years considered, *boxplots* are shown for the nine rivers for census size (N_c), expected ratio of hatchery-origin adults over N_c for a given breeding year, expected ratio of hatchery-origin adults averaged over 15 years, adjusted effective number of breeders ($N_{b(adj)}$), and the ratio of the adjusted effective number of breeders over census size ($N_{b(adj)}/N_c$). Rivers are ordered alphabetically

$\log(N_{b(adj)})$ was explained by the river effect, and the other 50 % remained unexplained. The LMER model best describing $\log(N_{b(adj)}/N_c)$ variation integrated both the effects of the river and that of year (log likelihood = -22.22). However, this model was not significantly different from the model considering the effect of river only (log likelihood = -22.22 , χ^2 test $p = 0.90$). The model including only the year as the explanatory variable was significantly inferior (log likelihood = -26.21 , χ^2 test $p < 0.01$). Overall, 59 % of the variance in $\log(N_{b(adj)}/N_c)$ was explained by the river and 1 % was explained by the year, leaving 40 % of the variance unexplained.

The linear model with explanatory variables N_c and the expected ratio of hatchery-origin adults on $\log(N_{b(adj)}/N_c)$ obtained the lowest AIC (45.78) (Table 4 A) with an adjusted R-square of the model of 0.32 (Table 4 B). The standardized coefficients for N_c and expected ratio of hatchery-origin adults were -0.57 ($p = 0.005$) and -0.39 ($p = 0.047$), respectively (Table 4 B). Shapiro tests confirmed the normality of the data for the best model ($W = 0.95$, $p = 0.30$). Figure 3 illustrates (i) the negative linear correlation between $\log(N_{b(adj)}/N_c)$ and N_c , (ii) between $\log(N_{b(adj)}/N_c)$ and expected ratio of hatchery-origin adults and (iii) between $\log(N_{b(adj)}/N_c)$ and expected ratio of hatchery-origin adults on average over 15 years.

Discussion

N_b and N_e as genetic indices in monitoring programs

In this study, N_b values estimated using LDNe ranged from 48 to 815 with an average of 248, and are of the same order of magnitude as in the few other studies having measured N_b in Atlantic salmon (e.g. Palstra et al. 2009, Johnstone et al. 2013, Perrier et al. 2014). Correcting these N_b estimates following the method developed by Waples et al. (2014) to take into account probable overlapping generations in Atlantic salmon from Quebec, $N_{b(adj)}$ values were reduced by $\sim 15\%$ compared to N_b and ranged from 41 to 696 with an average of 211. The adjustment of N_b values was achieved using three single life history traits that were set identically for the nine rivers, and may therefore be refined for each river given that adult life span, age at maturity, and variation in age-specific fecundity may slightly differ among these rivers. In comparison to our N_b

likelihood = -24.09 , χ^2 test $p = 1.00$). The model including only the breeding year as the explanatory variable was significantly inferior (log likelihood = -26.91 , χ^2 test $p = 0.02$). Approximately 50 % of the variance in

Table 2 Effective number of breeders (N_b) with 95 % confidence interval, adjusted number of breeders ($N_{b(adj)}$), Harmonic mean of $N_{b(adj)}$ per river, $N_{b(adj)}/N_c$ ratio, average $N_{b(adj)}/N_c$ per river, adjusted effective size ($N_{e(adj)}$), and average $N_{e(adj)}$ per river

Sample abbreviation	N_b	N_b 2.5 %	N_b 97.5 %	$N_{b(adj)}$	Harmonic mean $N_{b(adj)}$	$N_{b(adj)}/N_c$	$N_{b(adj)}/N_c$ 2.5 %	$N_{b(adj)}/N_c$ 97.5 %	Average $N_{b(adj)}/N_c$	$N_{e(adj)}$	Average $N_{e(adj)}$
GPA08	112	82	169	96	59	0.64	0.47	0.96	0.42	349	217
GPA09	48	42	55	41		0.40	0.35	0.46		149	
GPA10	75	64	89	64		0.23	0.19	0.27		233	
PCA08	302	201	565	258	161	0.90	0.60	1.69	0.63	940	588
PCA09	172	130	246	147		0.60	0.45	0.86		535	
PCA10	148	116	199	126		0.37	0.29	0.50		461	
TRI08	815	344	Inf	696	472	0.83	0.35	NA	0.77	2537	1723
TRI09	381	233	Inf	325		0.77	0.47	NA		1186	
TRI10	637	317	10,641	544		0.71	0.35	11.90		1983	
SMA08	214	153	339	183	221	0.25	0.18	0.39	0.33	666	805
SMA09	249	176	408	213		0.34	0.24	0.56		775	
SMA10	343	219	721	293		0.39	0.25	0.83		1068	
MAL08	384	155	Inf	328	102	0.35	0.14	NA	0.20	1195	374
MAL09	86	73	102	73		0.12	0.10	0.14		268	
MAL10	93	79	112	79		0.13	0.11	0.16		290	
GOD08	100	82	125	85	134	0.14	0.11	0.17	0.27	311	490
GOD09	465	260	1727	397		0.52	0.29	1.92		1448	
GOD10	145	114	195	124		0.15	0.12	0.20		451	
ROC08	151	101	279	129	159	0.21	0.14	0.39	0.22	470	578
ROC09	189	145	265	161		0.26	0.20	0.37		588	
ROC10	236	167	384	201		0.19	0.13	0.31		735	
SJE08	160	120	234	137	158	0.14	0.11	0.21	0.18	498	576
SJE09	190	140	285	162		0.20	0.15	0.31		591	
SJE10	213	153	336	182		0.20	0.14	0.31		663	
MAT08	NA	618	Inf	NA	226	NA	0.27	NA	0.15	NA	823
MAT09	238	170	377	203		0.13	0.09	0.21		741	
MAT10	297	200	539	253		0.17	0.11	0.31		925	
Average	248	172	800	211	188	0.36	0.24	1.02	0.35	771	686
Minimum	48	42	55	41	59	0.12	0.09	0.14	0.15	149	217
Maximum	815	618	10641	696	472	0.90	0.60	11.90	0.77	2537	1723

Table 3 Linear mixed effects models accounting for the effects of the river and of the year on $N_{b(adj)}$ and $N_{b(adj)}/N_c$

Models	Likelihood	Chi square test <i>p</i> value	Variance explained by the river	Variance explained by the year
lmer (log ($N_{b(adj)}$) ~ 1 + (1 River) + (1 Breeding Year))	-24.09		0.50	0.00
lmer (log ($N_{b(adj)}$) ~ 1 + (1 River))	-24.09	1.000		
lmer (log ($N_{b(adj)}$) ~ 1 + (1 Breeding Year))	-26.91	0.018		
lmer (log ($N_{b(adj)}/N_c$) ~ 1 + (1 River) + (1 Breeding Year))	-22.22		0.59	0.01
lmer (log ($N_{b(adj)}/N_c$) ~ 1 + (1 River))	-22.22	0.900		
lmer (log ($N_{b(adj)}/N_c$) ~ 1 + (1 Breeding Year))	-26.21	0.005		

Chi square tests were used to compare the models including only the river or the breeding year to the model including both terms. For both $N_{b(adj)}$ and $N_{b(adj)}/N_c$, the model including only the breeding year had a significantly lower likelihood than the model including both terms

Table 4 A) AIC for the seven linear models describing the variable $\log(N_{b(\text{adj})}/N_c)$ by the variables N_c , expected ratio of hatchery-origin adults and expected ratio of hatchery-origin adults on average over 15 years. B) Coefficients for the best model

A/Model (sorted by increasing AIC)		AIC			
lm ($\log(N_{b(\text{adj})}/N_c) \sim N_c + \text{expected ratio of hatchery-origin adults}$)		45.78			
lm ($\log(N_{b(\text{adj})}/N_c) \sim N_c + \text{expected ratio of hatchery-origin adults} + \text{expected ratio of hatchery-origin adults on average over 15 years}$)		47.55			
lm ($\log(N_{b(\text{adj})}/N_c) \sim N_c$)		49.10			
lm ($\log(N_{b(\text{adj})}/N_c) \sim N_c + \text{expected ratio of hatchery-origin adults on average over 15 years}$)		49.18			
lm ($\log(N_{b(\text{adj})}/N_c) \sim \text{expected ratio of hatchery-origin adults}$)		54.29			
lm ($\log(N_{b(\text{adj})}/N_c) \sim \text{expected ratio of hatchery-origin adults on average over 15 years}$)		56.06			
B/lm ($\log(N_{b(\text{adj})}/N_c) \sim N_c + \text{expected ratio of hatchery-origin adults}$)					
Term	Estimate	Standardized estimates	Std error	T	Pr(> t)
Intercept	-0.32		0.26	-1.22	0.23
N_c	0	-0.57	0	-3.39	0.003
Expected ratio of hatchery-origin adults	-3.55	-0.39	1.55	-2.29	0.032

Adjusted R-squared: 0.32

F statistic: 7.00, p value: 0.004

estimates, using the LDNe method in Atlantic salmon populations, N_b was estimated at 198 in the Escoumins River (Québec, Canada, Perrier et al. 2014), ranged from 70 to 352 over a 30 year study in the Northeast Brook (Newfoundland, Canada, Johnstone et al. 2013), and ranged from 42 to 3784 with an average of 1016 among nine rivers in Newfoundland (Palstra et al. 2009). The present study represents, alongside Palstra et al. (2009), one of the rare ongoing attempts to document N_b variation among rivers through time in Atlantic salmon populations. However, some other studies have focused on estimating N_b using the same single-sample estimator in other species such as brook charr (*Salvelinus fontinalis*, Whiteley et al. 2012) and steelhead (*Oncorhynchus mykiss*, Christie et al. 2012b). While estimating N_e over an entire generation can be challenging, N_b can be more easily quantifiable from a single cohort sample (Waples 2005) and might thus be a more accessible parameter for managers. Yet, N_b remains less exploited than N_e (Waples and Do 2010). Here, $N_{e(\text{adj})}$ estimated from $N_{b(\text{adj})}$ was of the same order of magnitude as other studied populations. Palstra et al. (2007) found that N_e ranged from 443 to 1697, with an average of 1067, among 20 Atlantic salmon populations from Newfoundland and Labrador. Ozerov et al. (2012) found that N_e varied from 26 to 8318, with an average of 888 among 26 Atlantic salmon populations from Russia. Overall there seems to be a trend that populations distributed more towards the southern part of the species' range have lower effective sizes, as shown by Perrier et al. (2013; N_e ranged from 48 to 1289, with an average of 304, among 34 populations from France), Horreo et al. (2011; N_e ranged from 37 to 96, with an average of 60, among five populations from Spain)

and Lage and Kornfield (2006; N_e of an endangered population from Maine was about 48). Our N_b and N_e estimates are also in the same order of magnitude as in other salmonid species (Palstra and Ruzzante 2008).

Observed differences in N_b and N_e among rivers could justify defining different status and adjusting conservation practices for some populations. For instance, two out of nine rivers monitored showed a relatively low effective number of breeders ($N_b < 100$: GPA, MAL), with GPA having a harmonic mean below 100 for the overall study period. Relative N_e values, although roughly estimated from N_b values, were higher than recently revised conservation thresholds above which inbreeding depression in the short term may be minimised ($N_e > 100$; Frankham et al. 2014). These N_e estimates were also above the specific minimum threshold we estimated for Atlantic salmon ($N_e = 95$), suggesting the potential for retaining 90 % of the populations' genetic diversity over 100 years. Moreover, these estimates are likely conservative since gene flow often occurs among Atlantic salmon anadromous populations (Fraser et al. 2004; Consuegra et al. 2005; Dionne et al. 2008), limiting the loss of diversity and inbreeding in small populations (Duchesne and Bernatchez 2002). These results suggest that negative inbreeding effects may not be a major threat to these nine populations. However, eight out of the nine populations had N_e estimates below the conservation threshold under which the long-term maintenance of the evolutionary potential could be uncertain ($N_e = 1000$; Frankham et al. 2014). It is also important to be cautious while interpreting N_e values calculated from N_b yearly estimates since N_b and thus extrapolated N_e vary between years. This underlines the

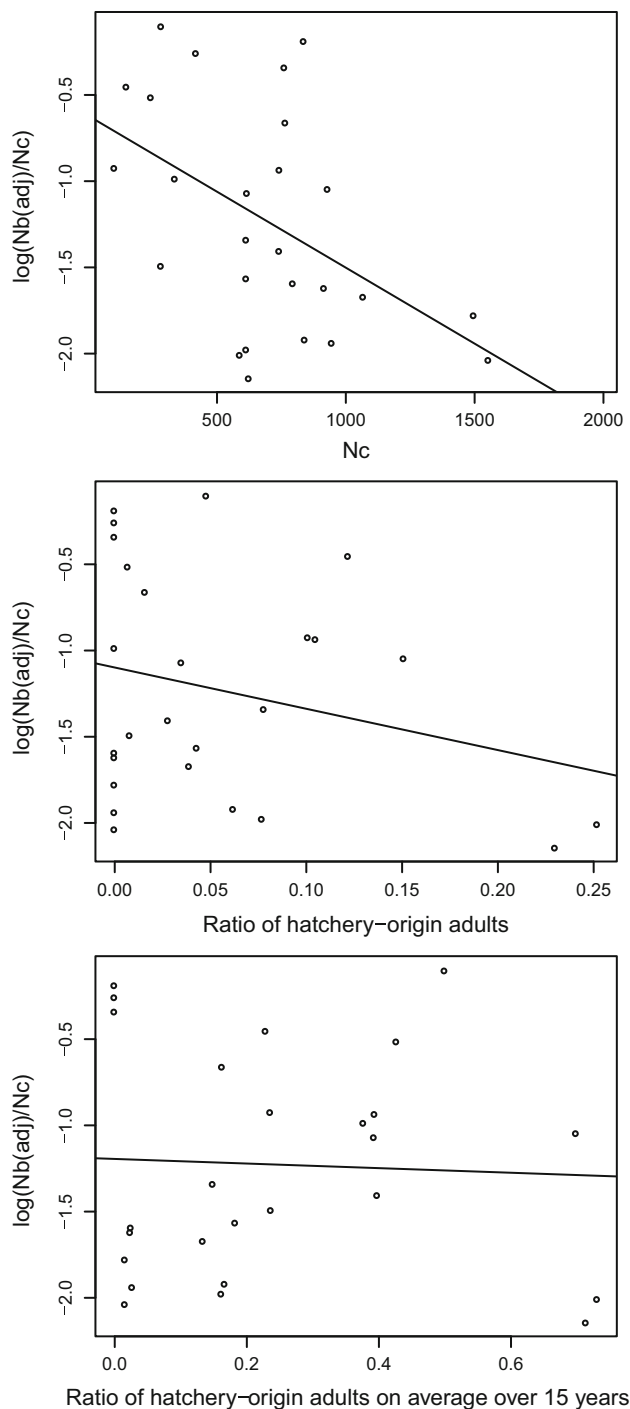


Fig. 3 Linear regressions of the of the ratio of the adjusted effective number of breeders over census size ($\log(N_{b(\text{adj})}/N_c)$) as a function of census size (N_c) (*upper panel*); $\log(N_{b(\text{adj})}/N_c)$ as a function of expected ratio of hatchery-origin adults (*middle panel*); and $\log(N_{b(\text{adj})}/N_c)$ as a function of expected ratio of hatchery-origin adults on average over 15 years (*lower panel*)

need to develop a more precise relationship between N_b and N_c for species with overlapping generations based on field data over at least a generation, in order to relate N_b to

N_c thresholds in conservation genetics. In sum, yearly measures of effective number of breeders N_b (and realted ratios derived from it) appears to be an appropriate and accessible management tool for the conservation of Atlantic salmon populations.

N_b/N_c estimates obtained in this study (ranging from 0.12 to 0.90 and averaging 0.36) were similar to N_e/N_c obtained in other studies in Atlantic salmon. For example, Consuegra et al. (2005) documented N_e/N_c from 0.18 to 0.54 in four Atlantic salmon populations from Spain. Similar estimates were found in other salmonid species. In five populations of Chinook salmon, Shrimpton and Heath (2003) found N_e/N_c values ranging from 0.02 to 0.57. Several authors reviewed N_e/N_c estimates in many species from various taxa and reported variable values from 10^{-6} to above 1, averaging 0.34 (Frankham 1995) and 0.19 (Palstra and Ruzzante 2008). However, for salmonids it is also important to consider that these estimates are calculated without considering mature male parr, which may substantially decrease N_c estimates and thus upwardly biasing N_e/N_c estimates (Saura et al. 2008; Johnstone et al. 2013; Perrier et al. 2014).

In the present study, N_b/N_c fluctuated temporally in several rivers (especially in TRI and GOD). This corroborates previous studies in Atlantic salmon (e.g. Palstra et al. 2009; Johnstone et al. 2013; Perrier et al. 2013). N_b/N_c temporal variation has also been reported in other salmonid species (Heath et al. 2002; Palm et al. 2003). Even though N_b/N_c temporally fluctuated for each population, the linear mixed effects models revealed that most of the variance in N_b/N_c was explained by the river rather than year, suggesting that N_b/N_c differ among rivers according to their biological characteristics and/or stocking status. Such variability in N_b/N_c could be attributed to various biological processes including fluctuations of the variance in reproductive success among individuals and fluctuations of sex ratio (Crow and Kimura 1970; Nunney 1993; Frankham 1995; Kalinowski and Waples 2002; Hedrick 2005), the temporally variable number of male parr that reproduce, as well as the impacts of stocking (see below).

Negative effect of stocking on N_b/N_c

Our results showed that stocking (expected ratio of hatchery-origin adults) has a significant negative effect on N_b/N_c . This result could be partly mediated by the existence of Wahlund effects in stocked populations, in which native fish and differentiated hatchery-origin fish can be found, causing a downward bias in N_b estimates and thus in N_b/N_c . However, we confirmed the absence of detectable Wahlund effects that might have been associated with stocking (e.g. Taggart and Ferguson 1986). Alternatively, the observed negative effect of stocking on N_b/N_c may be

linked to Ryman-Laikre effects (Ryman and Laikre 1991; Ryman et al. 1995) occurring in stocked populations, whereby stocking increases N_c but may decrease N_b . Such effects have also been identified in steelhead (Araki et al. 2007a; Christie et al. 2012a). While N_c may increase as a consequence of the addition of hatchery-reared fish, N_b may decrease through increased variance in individual reproductive success (Araki et al. 2007a; Christie et al. 2012a), and through family-correlated survival (Moyer et al. 2007). Reduced genetic diversity among breeders of hatchery-origin (Machado-Schiaffino et al. 2007) and associated inbreeding (Blanchet et al. 2008) can lead to decreased N_b through higher LD signals (Christie et al. 2012b). In the specific case of Atlantic salmon, reduced reproductive success of stocked fish compared to wild fish has previously been documented (Fleming et al. 2000; Milot et al. 2013) and could also account for a reduced N_b/N_c ratio. Family-dependent survival may also be linked to a reduced N_b/N_c ratio if progeny of stocked fish had lower survival rates, as observed in other studies (e.g. Reisenbichler and McIntyre 1977). Sampling 1+ year old juveniles (instead of 0+) could have allowed enough time for higher mortality in progeny of stocked fish compared to wild fish and thus ultimately resulting in decreased N_b/N_c . However, this has rarely been shown in Atlantic salmon (but see McGinnity et al. 2003). This result therefore corroborates previous recommendations that stocking must be carefully considered since it can notably decrease N_b/N_c . Specifically, it is important to monitor stocking effects and better balance the aim of increasing N_c with preserving the genetic diversity of the wild population. For example, it has been proposed to increase the number of captive breeders as well as the frequency of their replacement (Duchesne and Bernatchez 2002), as well as to improve rearing conditions to limit both acclimation and selection in captivity (Christie et al. 2012a; Milot et al. 2013).

Despite a significant negative effect of stocking intensity identified on a yearly basis, our analyses did not reveal a significant effect of the expected ratio of hatchery-origin adults on average over 15 years on the effective number of breeders measured. There are several non-exclusive explanations in the context of this study for having detected effects of stocking on N_b/N_c only on a year-to-year basis. First, this could suggest that the negative relationship between N_b/N_c and stocking intensity mainly originates from reduced fitness of stocked fish due to early life acclimation in the hatchery environment, but that inbreeding, which could have long term effects, has relatively little impact on decreasing N_b . Secondly, this result could suggest family-dependent survival, with reduced survival for progeny of stocked fish. Both reduced reproductive success of breeders and/or pedigree-dependent survival of the progeny would limit the propagation of a

decreased N_b/N_c over generations. It is also possible that more recent effects of stocking intensity on N_b/N_c could mask the effects of past stocking events. Overall, even though we did not observe a significant cumulative effect of the expected ratio of hatchery-origin adults on average over 15 years preceding sampling on N_b/N_c ratio, we propose that this should be investigated further by quantifying N_b/N_c ratios in populations in which stocking ceased, and thus could not mask possible long term effects.

Negative relationship between N_b/N_c and N_c

The negative relationship between N_b/N_c and N_c most likely results from increased fluctuations in N_c , deviation in sex ratio, or variance in reproductive success and family size in years or populations with larger N_c . Alternatively, within-river genetic structure has been observed in large river systems (Garant et al. 2000; Vaha et al. 2007) and could have resulted in a downward bias in N_b with increasing N_c . However, the absence of significant within-river F_{st} and F_{is} argues against this possibility. Hedrick (2005) theoretically revisited the idea that very low N_e/N_c ratio can occur due to higher variance in reproductive success within a generation in populations comprising large number of adults. Hedgecock (1994) also investigated the limiting effect of the variance in reproductive success on effective population size in marine organisms with large N_e . In the case of salmonids, some studies suggested that a decrease of N_b/N_c with an increase of N_c might arise from mechanisms that have been coined as “genetic compensation” consisting of a reduction of the variance in reproductive success among individuals with a decrease in N_c , perhaps through relaxation of inter-individual competition during reproduction (Ardren and Kapuscinski 2002; Christie et al. 2012b). Moreover, reproductive contribution of individuals that may not have been considered while estimating N_c may lead to an artificially higher N_b/N_c while N_c decreases. For instance, Ardren and Kapuscinski (2002) and Araki et al. (2007a) showed in steelhead that an enhanced reproductive contribution of non-anadromous breeders occurred when the anadromous run size was small, thus suggesting a genetic compensation between anadromous and non-anadromous life-history forms. In the case of Atlantic salmon, mature male parr are known to contribute in variable proportions to breeding, through fertilizing a variable proportion of the spawned eggs (Fleming 1996; Jones and Hutchings 2001; Richard et al. 2012). It has been recently shown that these mature male parr can contribute importantly to N_b (Saura et al. 2008; Perrier et al. 2014). Since mature male parr contribute to N_b but are not included in N_c , N_b/N_c may often be overestimated (Perrier et al. 2014). Consequently, if the relative contribution of mature male parr to N_b rises in smaller populations or during years with

reduced numbers of anadromous fish returning to the river, the N_b/N_c ratio is expected to rise while N_c remains small or decreases (as discussed in Fraser et al. 2006). It is also possible that a potentially higher impact of gene flow on smaller populations may also contribute to the negative relationship between N_b/N_c and N_c , as discussed earlier (Palstra and Ruzzante 2008). For example, Baalsrud et al. (2014) showed that N_c/N_c increased with immigration rates in a house sparrow metapopulation. Lastly, such a negative relationship between N_b/N_c and N_c could result from an underestimation of N_b in large populations due to too small of a sample size relative to N_c (Tallmon et al. 2010; Whiteley et al. 2012; Frankham et al. 2014). Clearly, from a management perspective, factors influencing the link between N_b/N_c and N_c must be carefully measured and understood before estimating one parameter from the other. Moreover, compensation mechanisms by which N_b/N_c decreases less than would be expected during years with reduced N_c may be of prime importance in a conservation context.

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Authors' contributions Conceived the study: M.D. and L.B. supervised sampling: M.D. Performed lab analyses: G.C. Performed statistical analyses: C.P. Wrote the paper: C.P. Contributed to the paper improvement: J.A., M.D., L.B.

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