ORIGINAL ARTICLE

Making sense of the relationships between Ne, Nb and Nc towards defining conservation thresholds in Atlantic salmon (*Salmo salar*)

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Effective population size over a generation (Ne) or over a reproductive cycle (Nb) and the adult census size (Nc) are important parameters in both conservation and evolutionary biology. Ne provides information regarding the rate of loss of genetic diversity and can be tracked back in time to infer demographic history of populations, whereas Nb may often be more easily quantified than Nc for short-term abundance monitoring. In this study, we propose (1) an empirical context to Waples *et al.* (2014) who introduced a correction to bias due to overlapping generations, and (2) a mathematical relationship between Ne and Nb for direct application in Atlantic salmon populations in Québec, Canada. To achieve this, we investigate the relationships between Ne, Nb and Nc in 10 Atlantic salmon populations, Canada, for which we genotyped 100 randomly sampled young-of-the year individuals for 5 consecutive years. The results show a positive correlation between Ne, Nb and Nc, suggesting that Nb is an indicative parameter for tracking effective population size and abundance of Atlantic salmon. However, our model allows predicting Nc from Nb values at 27% that can be partly explained by high variance in Nb/Nc both among populations (37%) and among years (19%). This result illustrates the need for thorough calibration of Nb/Nc before using Nb in monitoring programs, as well as a full understanding of the limits of such an approach. Finally, we discuss the importance of these results for the management of wild populations.

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INTRODUCTION

In many ecological and evolutionary research contexts, two important variables to be estimated in natural populations are the effective population size (over a generation (Ne) or over a reproductive cycle (Nb or effective number of breeders)) and the adult census size (Nc) (Frankham, 1995; Waples, 2005; Waples *et al.*, 2014). In conservation biology, Ne is important chiefly because it determines the rate of loss of genetic variability and the rate of increase in inbreeding in a population. Ne is defined as the size of an ideal Wright–Fisher population exhibiting the same amount of genetic drift and inbreeding as the population under consideration (Wright, 1931). Ne may be complicated to estimate in most populations because of age structure, generation overlap and iteroparity (Waples *et al.*, 2014). Nb is a concept derived from Ne that refers to the effective number of breeders during a single breeding event (Waples, 1989). The two measures are directly connected because Nb times the generation time approximates Ne (Waples, 1989). For Nb, only one season of data collection is needed. This parameter is thus generally more easily quantifiable than Ne as it can be inferred from a single cohort (for taxa with easy distinguishable cohorts), and might be a more accessible parameter for managers dealing with yearly conservation decisions (Waples, 2005; Luikart *et al.*, 2010; Tallmon *et al.*, 2010). Nc, the adult census population size, is generally defined as the total number of

potential (sexually mature) breeders that can sometimes be assessed by directly counting individuals. However, this task is often problematic when Nc is large or because detecting all individuals is difficult and we thus often must estimate Nc. Therefore, estimates of Nb from genetic methods would be useful for tracking population trends if Nb reflects the number of adults in a population (that is, if a positive correlation is observed between Nc and Nb). In this case, Nb estimates would provide a complementary metric or could potentially be used in the place of abundance monitoring (Tallmon *et al.*, 2010).

Knowledge of the relative magnitudes of these three parameters (Ne, Nb and Nc), as expressed by the ratios Ne/Nc and Nb/Nc, is important for disentangling the relative risks that demographic, environmental and genetic factors might pose for population persistence in the short term (Frankham, 1995; Palstra and Ruzzante, 2008). Expressed simply, for a given Ne, a population with a small Ne relative to Nc (that is, small Ne/Nc ratio) will lose gene diversity more quickly than an equal-sized population with a greater Ne/Nc ratio. Factors that can maximize this ratio are (1) a maximum number of breeders in each subsequent generation (or year), (2) more equal family sizes, (3) more equal sex ratios of breeders and (4) a reduced fluctuation in population size. Temporal fluctuations in effective size and the Ne/Nc ratio are not uncommon in natural populations and can be larger than differences between populations (see, for example, Miller and

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Kapuscinski, 1997; Heath *et al.*, 2002, Waples, 2002, Shrimpton and Heath, 2003; Fraser *et al.*, 2007). Several studies have suggested that no simple relationship between *N*e and *N*c may exist, either because of habitat factors or because of population expansion and contraction (Ardren and Kapuscinski, 2003; Watts *et al.*, 2007; Fraser *et al.*, 2007; Belmar-Lucero *et al.,* 2012, and see review in Palstra and Fraser, 2012). Although these authors argued that more empirical studies are needed, they gave recommendations to accurately approximate such a relationship with a particular emphasis to better report the uncertainties in variables (Palstra and Fraser, 2012).

Genetic methods often represent the only feasible way to estimate effective sizes (*N*b and *N*e). The so-called temporal methods—which use genetic data in two or more temporally spaced samples taken from the same population (Waples, 1989) —exploit the temporal changes in allele frequencies and are in general more accurate and robust than other genetic methods. However, collecting temporal data separated by at least one generation could be expensive, especially for long-lived species that have large generation intervals and are usually more of conservation concern. Furthermore, such species usually have overlapping generations and a sampling interval much larger than a generation is necessary to yield accurate *N*e estimates. The most widely used single-sample method calculates *N*e from linkage disequilibrium (LD) among loci that are unliked (Waples and Do, 2008). The method relies on the fact that in a system where gametes are distributed at random among a small number of zygotes, there will be departures from expected genotype and gametic frequencies, both of which can be used to estimate *N*e. In the case of age-structured populations, genetic estimates using the LD methods are strongly affected by both *N*e per generation and *N*b per breeding season. Samples from single cohort are the most amenable to quantitative bias adjustments resulting from overlapping generations. In this way, Waples *et al.* (2014) showed that single-cohort samples can be used to estimate *N*e with little bias. They proposed formulas to adjust genetic estimates of effective size (*N*b and *N*e) to correct bias because of age structure using two or three simple life-history traits.

The Atlantic salmon (*Salmo salar*, L.) is a well-known iteroparous species with overlapping generations and has a complex life history that includes spawning, juvenile rearing in rivers and extensive feeding migrations on the high seas. As a result, Atlantic salmon goes through several distinct phases that can be identified by specific changes in behavior, physiology and habitat requirements. It is an anadromous fish, typically spending 2–3 years in fresh water, migrating to the ocean where it also spends 2–3 years and then returning to its natal river to spawn. The abundance (*N*c) of Atlantic salmon has been declining worldwide over the past decades (Friedland *et al.*, 2003; Chaput, 2012; Dionne and Cauchon, 2014). This decline has been attributed to several causes including most ocean productivity decrease because of climate change (Mills, 2013), commercial fisheries (Friedland *et al.*, 2003) as well as habitat disconnection and deterioration (Hall *et al.*, 2012; Nieland *et al.*, 2015). As an indirect consequence of the drop of *N*c, *N*b and *N*e may also decrease, potentially resulting in increased inbreeding depression, loss of local diversity and of evolutionary potential in small populations. Although international management plans have been deployed to mitigate this worldwide decline (that is, cessation of commercial fishing, although with few exceptions), populations trends are mostly not reversing (Hermansen *et al.*, 2015). Meanwhile, based on descriptors of population health, fine-tuned management and conservation actions have been undertaken at the population level (Parrish *et al.*, 1998; Limburg and Waldman, 2009). In this context, monitoring trends in *N*e, *N*b and *N*c and understanding the links among these parameters may help to refine Atlantic salmon population health indices and ultimately conserve more adequately the genetic integrity of threatened populations. Monitoring these parameters and especially extrapolating a *N*c value from a *N*b or *N*e estimate could also possibly replace direct abundance monitoring in rivers where such classic operation can be logistically challenging.

The main goal of this study is to empirically explore the relationship between the three estimates, *N*c, *N*b and *N*e, taking into consideration different demographic factors and temporal fluctuations (sampling over a generation time) in 10 populations of Atlantic salmon. We took advantage of a large data set partly published in Perrier *et al.* (2016). To the data set of Perriers *et al.* (2016), we added one population and two additional years on all populations, thus comprising an entire generation time (that is, 5 years; Dionne and Cauchon, 2014). We genotyped ∼ 5000 1-year-old Atlantic salmon at 15 highly variable microsatellite markers in 10 different populations for 5 consecutive years (∼100 individuals per river and per year). Being an iteroparous and anadromous species, the Atlantic salmon appears a suitable species to propose an empirical context to Waples *et al.* (2014), who introduced a correction to bias because of overlapping generations on simulated data. Although fastidious, counts of spawn adult breeders returning to their natal river can be done to get an estimate of *N*c. Atlantic salmon has also been well studied and described in terms of variation among rivers and several key life-history traits. In this context, the large data set used in this study offers the opportunity to propose an empirical relationship between *N*e and *N*b for direct application in Atlantic salmon populations in Québec, Canada. Finally, we also aim to test how much *N*b reflects an *N*c estimate by examining the correlation between these two measures.

MATERIALS AND METHODS
Study sites, sampling and estimates of census size (*N*c)
A total of 4730 1-year-old parr (juveniles) were sampled using electrofishing in 10 rivers during summer in 2010, 2011, 2012, 2013 and 2014 corresponding to the breeding years 2008, 2009, 2010, 2011 and 2012 (Table 1 and Figure 1; Perrier *et al.*, 2016). These rivers were chosen according to the (1) representation of the different regional genetic groups identified in the studied area (Dionne *et al.*, 2008; Moore *et al.*, 2014), (2) variation in census size estimates, (3) levels of stocking and (4) logistical feasibility of sampling. Individuals were sampled with respect to the guidelines of the Canadian Council on animal care and details for the sampling within river are provided in Perrier *et al.* (2016). Whole fish were stored immediately in 50 ml tubes with 95% ethanol. The median number of juveniles effectively genotyped per river and per year was 98 individuals (Table 1).

The number of anadromous adults returning to a river for a given year has been assessed for each river allowing an estimate of *N*c for each sample. Basically, the number of spawners for each river is estimated using migratory pass, count barriers or snorkeling methods that are considered to provide the most reliable evaluation for a given river (Fontaine and Caron, 1999; see Perrier *et al.*, 2016 for details). Such estimates for the Laval River were not available for the corresponding 5 years. Instead, according to the standard protocol of the Ministère des Forêts, de la Faune et des Parcs du Québec (Fontaine and Caron, 1999), data for the Laval River were estimated assuming that interannual variations are approximately the same as those observed in the other rivers of the region. The census size within a river across a generation (hereafter named $N_{c_{pool}}$) was obtained by summing *N*c over years × (α/number of sampled years), with α = age at maturity (= 3.4 years).

Genotyping, genetic diversity and differentiation
All methodological steps from DNA extraction to genotyping through amplifications at 15 microsatellites are detailed in Perrier *et al.* (2016). Potential presence of null alleles and large allelic dropout were tested 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 heterozygotes excess or deficit. Using Genepop v 4.2.2 (Rousset,

Table 1 River name, sample abbreviation, breeding year, sample size and counted number of anadromous breeders (Nc) for Atlantic salmon populations from Québec

River	Sample abbreviation	Breeding year	Sample size	Nc
Laval	LAV08	2008	60	103
	LAV09	2009	95	131
	LAV10	2010	95	55
	LAV11	2011	91	34
	LAV12	2012	94	35
Grand-Pabos	GPA08	2008	64	150
	GPA09	2009	100	103
	GPA10	2010	97	284
	GPA11	2011	100	289
	GPA12	2012	98	137
Petite-Cascapedia	PCA08	2008	98	285
	PCA09	2009	94	245
	PCA10	2010	100	338
	PCA11	2011	100	384
	PCA12	2012	94	130
Trinite	TRI08	2008	98	838
	TRI09	2009	100	420
	TRI10	2010	100	763
	TRI11	2011	100	1060
	TRI12	2012	94	484
Sainte-Marguerite	SMA08	2008	96	743
	SMA09	2009	100	618
	SMA10	2010	99	744
	SMA11	2011	100	1400
	SMA12	2012	95	287
Malbaie	MAL08	2008	43	931
	MAL09	2009	99	625
	MAL10	2010	100	590
	MAL11	2011	100	687
	MAL12	2012	99	349
Godbout	GOD08	2008	99	615
	GOD09	2009	100	767
	GOD10	2010	97	842
	GOD11	2011	100	1160
	GOD12	2012	98	821
Aux-Rochers	ROC08	2008	54	615
	ROC09	2009	99	615
	ROC10	2010	97	1069
	ROC11	2011	100	1381
	ROC12	2012	97	850
Saint-Jean	SJE08	2008	87	947
	SJE09	2009	100	796
	SJE10	2010	94	917
	SJE11	2011	100	779
	SJE12	2012	96	617
Matane	MAT08	2008	92	1977
	MAT09	2009	99	1555
	MAT10	2010	100	1498
	MAT11	2011	100	2103
	MAT12	2012	97	1362
Average			98	622
Minimum			43	34
Maximum			100	2103

2008), we estimated F_{st} between rivers to assess the potential structure among rivers and F_{is} for each river. The stability across years of the genetic structure between rivers was tested computing Pearson's correlation coefficient between F_{st} estimates for each pairwise comparison year. Moreover, an analysis of molecular variance conducted with rivers nested within year was also performed with Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). The relationship between F_{is} values and Nb_{LDadj}/Nc ratio was subsequently tested by a linear regression model in order to test for a putative within-river Wahlund or Allendorf–Phelps effects (Allendorf and Phelps, 1981; Waples, 1998; Castric et al., 2002). Migrants across populations were identified using Geneclass2 (Piry et al., 2004) and then removed for subsequent analyses.

Estimates of effective number of breeders (Nb), effective population size (Ne) and their ratio to census size (Nc)

Our sampling design allowed assessing (1) Nb estimates from each consecutive cohort (from 2008 to 2012) of the 10 rivers and (2) Ne estimates over a generation by analyzing consecutive cohorts from a same river jointly. We estimated Nb for each of the 50 samples using two different single-sample estimator methods. The first was the Sibship assignment method (Wang, 2009) that uses sibship frequencies estimated from randomly sampled pairs of individuals as being sibs sharing one or two parents. The program Colony2 (Wang, 2009) was run under the full likelihood model to estimate Nb_{sib} by considering polygamous breeding systems for both sexes (Garant et al., 2001; Richard et al., 2013), and no prior information on candidate parents or sibship sizes. Second, we estimated Nb_{LD} by applying the LD (Waples and Do, 2008) method that uses the unbiased estimators of Burrow's Δ (Weir, 1979) to test for nonrandom associations between unlinked loci. For this, we used the program NeEstimator 2 (Do et al., 2014) that implements an improved version of the LDNe algorithm (Waples and Do, 2008) to better deal with missing data (Peel et al., 2013). We used a threshold of 0.05 as the lowest allele frequency that gives the least biased results according to Waples and Do (2010). We then used the method developed by Waples et al. (2014) that corrects bias due to overlapping generations using three simple life-history traits, and the following equation:

$$Nb_{LDadj} = \frac{Nb_{LD}}{(0.991 - 0.206 \times \text{Log}(\text{Al}) + 0.256 \times \text{Log}(\alpha) + 0.137 \times \text{CVf})} \quad (1)$$

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 = 3.4 and CVf = 0.88 (these parameters were computed using the data published in Perrier et al., 2014 and according to Dionne and Cauchon, 2014). Then, in order to obtain a Ne estimate for each river, we used the following equation from Waples et al. (2014) Table 3:

$$Ne_{Adj} = \frac{Nb_{LDadj}}{(0.833 + 0.637 \times \text{Log}(\text{Al}) - 0.793 \times \text{Log}(\alpha) - 0.423 \times \text{CVf})} \quad (2)$$

As these estimates (from each year and river) reflect temporal (across years) and demographic (across rivers) variation, they were subsequently used for modeling the prediction of Ne estimate from Nb estimate (see below). For each river, the ratio of number of breeders to census size was assessed dividing Ne_{LDadj} by the Nc values within each river (Waples, 2005).

Ne for each river was also estimated pooling all consecutive cohorts into a single analysis by river using the two methods previously employed. First, the program Colony2 (Wang, 2009) was run with the same setting used for Nb_{sib} estimates to get Ne estimates from pooled temporal samples (hereafter named $Ne_{poolSib}$). Second, the LDNe approach was performed to estimate the Ne_{pool}. Pearson's correlation was computed to test the congruence between Nb_{LDadj} and Nb_{sib} estimates and between Ne_{pool} and $Ne_{poolSib}$ estimates, as well as between the mean of Ne_{Adj} within a river and the corresponding Ne_{pool}. For each river, the ratio of effective size to census size was assessed dividing Ne_{pool} by the Nc_{pool} values (Waples, 2005). As the ratios Nb/Nc and Ne/Nc could differ among populations and hence reflect differences in sex ratios, nonrandom mating, variance in individual reproductive success and family size and fluctuating Nc over generations, we tested whether Nb_{LDadj}/Nc varied among rivers and among years. The results of this analysis directly reflect the variation of Ne_{Adj}/Nc as Ne_{Adj} was directly estimated from Nb_{LDadj}. We used the function LMER from the R package LME4 to fit linear mixed effects models accounting
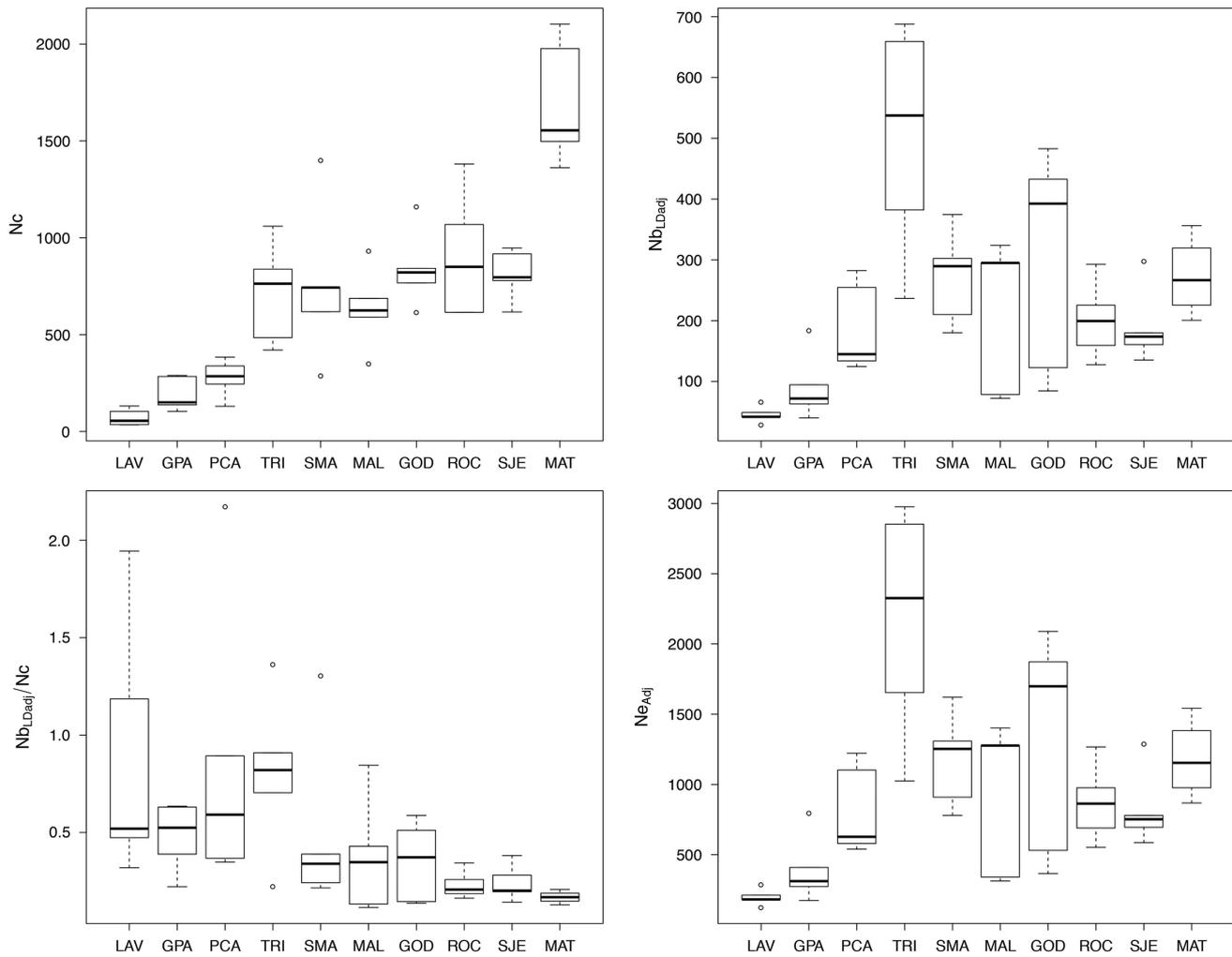
Figure 1 Boxplots displaying the variation in census size (Nc), adjusted effective number of breeders (Nb_{LDadj}) and the ratio of the adjusted effective number of breeders over census size (Nb_{LDadj}/Nc) across 5 years within each river. Rivers are ordered according to their census size.

for the effects of river and breeding year on Nb_{LDadj}/Nc, considered as random variables. The function BOXCOC from the library MASS in R (Venables and Ripley, 2002) was used to determine the best normalization transformation to apply to Nb_{LDadj}/Nc. Nb_{LDadj}/Nc values were thereafter log transformed. The χ^2 tests were used to compare the log likelihood of the models built using only one variable with models including two variables. From the best models, we inferred the variance of log (Nb_{LDadj}/Nc) attributable to the river and to the year. Finally, the relationship between $\log(Ne_{Adj}/Nc)$ and Nc on one hand and between $\log(Nb_{LDadj})$ and Nc on the other hand were explored using Pearson's correlation test.

Predicting *Ne* and *Nc* from *Nb*

Taking into account the variability across rivers and years, we used a hierarchical linear mixed effects model to predict (1) Ne_{Adj} from Nb_{LDadj} and (2) Nc from Nb_{LDadj}. This model includes the Nb_{Adj} and Ne_{Adj} variability (95% confidence interval) within each river by an extension of bootstrapped type II regression model with random effect of 1000 iterations (Sokal and Rohlf, 1995; Legendre and Legendre, 1998) using a custom R-script. Linear model were run using the function *lme()* of the NLME R-cran package (Pinheiro *et al.*, 2015). The function BOXCOC from the library MASS in R (Venables and Ripley, 2002) was used to determine the best normalization transformation to apply to Ne_{Adj}, Nb_{LDadj} and Nc. Ne_{Adj}, Nb_{LDadj} and Nc values were thereafter log transformed.

RESULTS

Genetic diversity within and among rivers

A total of 4730 juveniles were genotyped for the 15 microsatellites with an average of 3.03% of missing genotypes. The median number of juveniles genotyped per river and per year was 98 and ranged from 43 to 100 (Table 1). The mean number of alleles per locus was 27 and ranged from 10 to 47, whereas the number of alleles per locus per population varied from 4 to 31 with an average of 15. MICROCHECKER analysis was performed that confirmed the absence of null alleles and allele dropout. H_e estimates per population per year varied from 0.79 to 0.86 with an average of 0.81 (Supplementary Material 1). Average F_{is} per population per year ranged from − 0.02 to 0.04 with an average of 0.01 (Supplementary Material 1), and no significant relationship was found between F_{is} and $NbAdj/Nc$ ($r^2Adj = 0.016$, $P = 0.19$, F = 1.854), confirming the absence of significant within-river Wahlund or Allendorf–Phelps effects. F_{st} among rivers within a given year were significant ($P < 0.001$) and ranged from 0.005 (PCA10-MAT10) to 0.063 (LAV12-GOD12) with an average of 0.0288 (Supplementary Material 2). The genetic structure between rivers was stable across years as t Pearson's correlation coefficient between F_{st} estimates between years was highly significant ($P < 2.2e − 16$) for all comparisons and ranged between 0.83 (2008–2012) and 0.96 (2009–2010)

(Supplementary Material 2). The hierarchical analysis of molecular variance conducted with rivers nested within year revealed that the genetic variation is 2.75 times as important among rivers within a year as among years.

Estimates of census size (*Nc*), effective number of breeders (*Nb*), effective population size (*Ne*) and their ratio to census size

Census size varied from 34 (LAV11) to 2103 (MAT11), with an average of 691 and a median value of 622 (Table 1 and Figure 1). Average *Nc* values per river across the 5 years ranged from 72 (LAV) to 1699 (MAT), with an average of 691 and a median of 736.

Nb_{sib} estimates varied from 46 (LAV10) to 186 (SMA12), with an average of 127 and a median of 139 (Table 2). Nb_{LD} estimates varied from 34 (LAV10) to 815 (TRI08) with an average of 269, and Nb_{LDadj} estimates varied from 29 (LAV10) to 688 (TRI08), with an average of 227 and a median of 199. The 95% confidence intervals ranged from ± 25 (LAV10) to infinite (MAT08, TRI08 and TRI12). Over the 50 samples, we obtained a single 'infinite' value, for MAT08, for which the lower limit of the confidence interval was 521 (Table 2). Overall, the mean coefficient of variation across rivers was 0.41 and varied from 0.24 (MAT) to 0.61 (GOD). Overall, Nb_{LDadj} and Nb_{sib} were positively correlated ($r^2 = 0.70$, P-value $= 2.33e - 08$). Nb_{LDadj}/Nc varied from 0.12 (MAL09) to 2.17 (PCA12). Average Nb_{LDadj}/Nc values per river over 5 years ranged from 0.17 (MAT) to 0.89 (LAV), with an average value of 0.50 and a median value of 0.35. The estimations of effective sizes across a generation (*Ne*) were first assessed from single-cohort samples estimates using the equation of Waples *et al.* (2014). Ne_{Adj} values estimated from Nb_{LDadj} varied from 124 (LAV10) to 2976 (TRI08), with an average of 980 and a median value of 863. Ne_{Adj} estimates averaged per river varied from 196 (LAV) to 2167 (TRI), with a mean of 984 and a median value of 895. The mean coefficient of variation across rivers for Ne_{Adj} was 0.41 and varied from 0.24 (MAT) to 0.61 (GOD). *Ne* estimates were also obtained by pooling all consecutive cohorts into a single analysis by river. Ne_{pool} estimated per river ranged from 132 (LAV) to 643 (MAT), with a mean of 352 and a median value of 375. A significant positive correlation was found between the mean of Ne_{Adj} within a river and the corresponding Ne_{pool} ($r^2 = 0.63$, P-value $= 0.04$). $Ne_{poolSib}$ estimates per river ranged from 246 (LAV) to 646 (MAT), with an average of 496 and a median value of 542. Ne_{pool} and $Ne_{poolSib}$ were also significantly and positively correlated ($r^2 = 0.89$, P-value $= 0.0006013$, Supplementary Material 3). The mean ratio Ne_{pool}/Nc_{pool} was 0.22 and ranged from 0.11 (MAT, GOD and MAL) to 0.54 (LAV) (Table 2).

The LMER model that best predicted log (Nb_{LDadj}/Nc) variation integrated both the effects of the river and that of year (log likelihood $= -48.78$). Overall, 37% of the variance in log (Nb_{LDadjj}/Nc) was explained by the river and 19% was explained by the year, leaving 44% of the variance unexplained (Table 3). A significant negative correlation was also found between log(Nb_{LDadj}/Nc) and *Nc* ($r^2 = -0.62$, P-value $= 1.58e - 06$), as well as between log(Ne_{Adj}/Nc) and *Nc* ($r^2 = -0.61$, P-value $= 3.77e - 06$; Figure 2).

Predictions of *Ne* and *Nc* from *Nb*

Because we applied a log transformation on *Nc*, Nb_{Adj} and Ne_{Adj} variables, the linear model that actually describe our data is:

$$\log (Nc) = b0 + b1 * \log \left(Nb_{LDadj}\right) + e$$

where b0 is the intercept, b1 corresponds to the slope and *e* is the error term.

We convert to raw data by taking the exponential of independent and dependent variables of this model, and thus we get:

$$Nc = \exp(5.75272301) * (Nb_{LDadj} {}^{\wedge}0.08286482), \text{ with a } r^2\text{adj} \\ = 0.27 \text{ and a } p\text{-value} = 2.98e - 05$$

Similarly, the equation obtained for the relationship between Ne_{Adj} and Nb_{LDadj} is

$$Ne = \exp(2.9199183) * (Nb_{LDadj} {}^{\wedge}0.7207817), \text{ with a } r^2\text{adj} \\ = 0.99 \text{ and a } p\text{-value} = 1.44e - 08.$$

Here, a quasi-perfect correlation is obviously expected as *Ne* was directly estimated from *Nb* estimates. However, the main point here was to use our empirical data set to propose an empirical equation directly applicable in our system. Figure 3 shows the fit between the observed data and the hierarchical linear model for the relation between Nb_{Adj} and *Nc*. The mean correlation between Nb_{LDadj} and *Nc* among years within a river is 0.68. For the relationship between Nb_{LDadj} and *Ne* the mean correlation between Nb_{LDadj} and *Nc* among years within river is 0.15.

DISCUSSION

The goal of this study was to empirically explore the relationship between the three parameters, *Nc*, *Nb* and *Ne*, taking into consideration different demography factors (including genetically distinct populations) and temporal fluctuations (sampling 5 consecutive years) in 10 populations of Atlantic salmon. To achieve this, we generated one of the most comprehensive microsatellite data sets generated to date in Atlantic salmon. Our results allow performing a detailed empirical assessment of the mathematical relationship between *Ne* and *Nb* developed by Waples *et al.* (2014). Precise estimates of cohort-specific *Nb* were quantified across a wide range of population sizes that allowed drawing the relationship with census size (*Nc*). In the following sections, we first discuss about factors potentially affecting *Nb*, *Ne* and their ratio to *Nc* in comparison with other studies conducted on the same species as well as on other taxa. Then, we consider the reliability of these relationships for wild populations and specifically for Atlantic salmon conservation.

Factors potentially affecting *Nb*, *Ne* and their ratios to *Nc*

In our study, Nb_{LDadj} (from 29 to 696), Ne_{Adj} (from 106 to 2538) and *Nc* (from 34 to 2103) estimates generally fall within the range of values reported in other northeast Atlantic salmon populations. *Nb* 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 (Newfounland, Johnstone *et al.*, 2013) and ranged from 42 to 3784 with an average of 1016 among 9 rivers in Newfounland (Palstra *et al.*, 2009). Moreover, when estimating *Ne* from pooled years, $Ne_{poolSib}$ estimates were downwardly biased for large populations in comparison with estimates from the LDNE approach ($Ne_{poolAdj}$). Comparison between Nb_{LD}, Nb_{LDadj} and Nb_{sib} estimates in our study also strengthens the point that Nb_{sib} is likely downwardly biased because of overlapping generations. A putative explanation that has already been inferred either in Atlantic salmon (Johnstone *et al.*, 2013) or in the threespine stickleback (DeFaveri and Merilä, 2015) is that in large populations, the Sibship method as implemented in Colony2 (Jones and Wang, 2010) would be more sensitive to sample size (that is, a bigger sample size would better reflect the entire Sibship network (Carrea, 2011, but see Wang, 2016). In contrast, Ne_{Adj} inferred from each year seems to better reflect the extent of LD within the cohort. Moreover, to overcome the bias due to overlapping generations using the LDNe method (which assumes

Table 2 Sample abbreviation, Nb_{sib}, Nb_{LD}, Nb_{LDadj}, Nb_{LDadj}/Nc, Ne_{Adj}, $Ne_{poolAdj}$ and $Ne_{poolAdj}/Nc_{pool}$ estimated using the LDNe method (Waples and Do, 2008) and $Ne_{poolSib}$ estimated using the Sibship method (Wang, 2009)

Sampling abbreviation	Nb_{sib}	Nb_{LD}	Nb_{LDadj}	Nb_{LDadj}/Nc	Ne_{Adj}	Ne_{sib}	Ne_{pool}	Ne_{pool}/Nc_{pool}
LAV08	51 (34–78)	58 (47–73)	49 (40–62)	0.47	211 (172–268)	246 (205 –299)	132 (121–145)	0.54
LAV09	81 (59–114)	50 (43–57)	42 (36–48)	0.32	181 (158–210)			
LAV10	47 (32–72)	34 (30–38)	29 (25–32)	0.52	124 (110–139)			
LAV11	72 (50–104)	78 (66–96)	66 (55–81)	1.94	286 (239–349)			
LAV12	69 (26–62)	49 (43–57)	41 (36–48)	1.19	180 (157–208)			
GPA08	77 (54–110)	112 (82–169)	95 (69–142)	0.63	409 (299–617)	304 (258 –360)	167 (152–184)	0.26
GPA09	66 (47–95)	48 (42–55)	40 (35–46)	0.39	173 (152–199)			
GPA10	90 (66–124)	75 (64–89)	63 (54–75)	0.22	272 (232–324)			
GPA11	128 (95–174)	218 (158–333)	184 (134–281)	0.64	794 (578–1217)			
GPA12	81 (67–105)	85 (72–102)	72 (61–86)	0.52	311 (264–373)			
PCA08	155 (116–206)	302 (201–565)	255 (170–477)	0.89	1102 (734–2063)	522 (453–599)	391 (339–457)	0.42
PCA09	107(80–142)	172 (130–246)	145 (109–207)	0.59	627 (474–897)			
PCA10	99 (73–135)	148 (116–199)	124 (97–168)	0.37	539 (422–726)			
PCA11	104 (76–141)	159 (124–216)	134 (104–182)	0.35	580 (451–788)			
PCA12	177 (154–208)	335 (213–715)	282 (180–603)	2.17	1222 (777–2608)			
TRI08	146 (111–194)	815 (344–Inf)	688 (290–Inf)	0.82	2976 (1256–10 951)	602 (526–683)	469 (395–568)	0.19
TRI09	147 (113–191)	453 (266–1312)	382 (224–1107)	0.91	1654 (971–4791)			
TRI10	158 (118–215)	637 (317–10 641)	537 (267–8975)	0.7	2326 (1156–38 844)			
TRI11	155 (114–207)	281 (186–527)	237 (157–444)	0.22	1024 (680–1922)			
TRI12	184 (165–214)	782 (346–Inf)	659 (292–Inf)	1.36	2853 (1262–10 951)			
SMA08	143 (109–192)	214 (153–339)	180 (129–286)	0.24	780 (557–1237)	643 (562 –738)	412 (356–483)	0.16
SMA09	154 (116–206)	249 (176–408)	210 (148–344)	0.34	909 (641–1488)			
SMA10	158 (121–208)	343 (219–721)	290 (185–608)	0.39	1253 (801–2632)			
SMA11	151 (116–201)	359 (235–708)	302 (198–598)	0.22	1309 (856–2586)			
SMA12	186 (168–215)	444 (259–1322)	374 (219–1115)	1.3	1621 (946–4826)			
MAL08	84 (55–135)	384 (155–3000)	324 (130–2530)	0.35	1401 (564–10 951)	401 (340–470)	234 (210–262)	0.11
MAL09	95 (69–129)	86 (73–102)	72 (61–86)	0.12	312 (265–373)			
MAL10	107 (80–145)	93 (79–112)	79 (67–94)	0.13	340 (289–407)			
MAL11	145 (109–193)	350 (231–675)	295 (195–570)	0.43	1277 (842–2466)			
MAL12	102 (86–126)	350 (112–180)	295 (94–152)	0.85	1277 (409–658)			
GOD08	96 (72–130)	100 (82–125)	84 (69–106)	0.14	365 (299–457)	562 (493–647)	303 (266–347)	0.11
GOD09	148 (113–197)	465 (260–1727)	392 (219–1457)	0.51	1699 (949–6305)			
GOD10	149 (115–178)	145 (114–195)	123 (96–165)	0.15	530 (416–713)			
GOD11	151 (117–203)	513 (287–1936)	433 (242–1633)	0.37	1872 (1047–7067)			
GOD12	165 (41–88)	573 (275–114 861)	483 (232–96 880)	0.59	2090 (1005–419 296)			

Table 2 (Continued)

| Sampling abbreviation	Nb_{sib}	Nb_{LD}	Nb_{LDadj}	Nb_{LDadj}/Nc	Ne_{Adj}	Ne_{sib}	Ne_{pool}	Ne_{pool}/Nc_{pool}
ROC08	88 (61–131)	151 (101–279)	127 (85–235)	0.21	551 (368–1018)	493 (428–569)	359 (310–421)	0.12
ROC09	128 (96–172)	189 (145–265)	159 (122–223)	0.26	689 (527–966)			
ROC10	136 (102–182)	236 (167–384)	199 (141–324)	0.19	863 (611–1401)			
ROC11	132 (97–178)	267 (189–438)	225 (159–369)	0.16	976 (688–1599)			
ROC12	183 (162–218)	347 (222–729)	293 (187–615)	0.34	1267 (811–2660)			
SJE08	123 (90–168)	160 (120–234)	135 (101–197)	0.14	585 (436–854)	543 (476–620)	416 (354–497)	0.15
SJE09	131 (98–176)	190 (140–285)	161 (118–240)	0.2	695 (512–1040)			
SJE10	116 (87–156)	213 (153–336)	180 (129–284)	0.2	779 (559–1227)			
SJE11	141 (107–187)	353 (223–767)	298 (188–647)	0.38	1289 (814–2799)			
SJE12	160 (141–189)	206 (152–307)	174 (128–259)	0.28	752 (556–1119)			
MAT08	146 (112–196)	Inf (618–Inf)	Inf (521–Inf)	Inf	Inf (2256–10 951)	646 (571–734)	643 (524–815)	0.11
MAT09	157 (118–208)	238 (170–377)	201 (144–318)	0.13	868 (622–1377)			
MAT10	159 (121–212)	297 (200–539)	250 (169–454)	0.17	1084 (731–1967)			
MAT11	157 (120–213)	422 (251–1154)	356 (212–974)	0.17	1542 (916–4214)			
MAT12	179 (152–198)	335 (220–654)	283 (186–552)	0.21	1224 (804–2387)			
Average	127 (96–164)	269 (173–3153)	227 (146–2659)	0.5	980 (633–11 510)	496 (431–572)	352 (303–418)	0.22
Minimum	47 (26–62)	34 (30–38)	29 (25–32)	0.12	124 (110–139)	246 (205–299)	132 (121–145)	0.11
Maximum	186 (168–218)	815 (618–114 861)	688 (521–96 880)	2.17	2976 (2256–419 296)	646 (571–738)	643 (524–815)	0.54

Values in brackets represent the 95% confidence interval.

discrete generations), we corrected Nb and Ne estimates according to Waples *et al.* (2014) using three life-history traits that were identically set for the 10 rivers because river-specific data were not available. These Nb_{LDadj} and Ne_{Adj} estimates could therefore be refined for each river in the future given that adult life span, age at maturity and age-specific fecundity probably differ among rivers. The age at maturity is probably the parameter that varies the most among Atlantic salmon populations and consequently could have the most considerable effect on effective size estimates. However, it is difficult to obtain a good estimate for such a parameter. In particular, this would require knowing the exact proportion of mature male parr (which are early sexually maturing male salmon that are not counted in Nc estimates) in a population that could also vary among years.

With a mean Ne_{pool}/Nc_{pool} ratio of 0.22 across sampled rivers, our results are in agreement with the review of Palstra and Ruzzante (2008) reporting a low median ratio (= 0.14) across studies. However, we observed a pronounced variability for Ne_{pool}/Nc_{pool} estimates across populations (from 0.11 for the largest population MAT to 0.54 for the smallest population LAV). Consistently, the median Ne_{pool}/Nc_{pool} ratio has previously been shown to be highly variable within species (Shrimpton and Heath, 2003; Palstra and Fraser, 2012) and generally larger in small than in large populations, suggesting changes in biological interactions with populations abundance (Frankham, 1995; Palstra and Fraser, 2012). Values of <1 in our populations could probably be attributed to common factors found in the wild and already reported in several studies, such as skewed sex ratios, high variance in reproductive success and fluctuations in population size through time (Nunney, 1993; Vucetich *et al.*, 1997; Hedrick, 2005). The inverse relationship between Ne_{Adj}/Nc and Nc found in this study and other previous studies (Pray *et al.*, 1996; Ardren and Kapuscinski, 2003; Araki *et al.*, 2007; Fraser *et al.*, 2007; Watts *et al.*, 2007) could potentially be explained by an increase of the standardized variance in family size as Nc increases, and conversely, that this variance decreases as Nc decreases (Hedrick, 2005). At low abundance, lower variance in reproductive success leads to relatively higher effective size, either per generation (Ne) or per cohort (Nb) (Ardren and Kapuscinski, 2003). Indeed, Palstra and Ruzzante (2008) argue that mechanisms of genetic compensation may counteract reductions of Ne (and hence increase Ne/Nc). Such genetic compensation can be viewed as resulting in a buffering effect against loss of genetic diversity at low Nc. For salmonid fishes, genetic compensation can be reconciled with aspects of their breeding ecology (Fleming, 1996, 1998). Specifically, it is hypothesized to be due to density dependence in competition for spawning territories, competition for mates, redd superimposition or embryo mortality caused by delayed spawning (Ardren and Kapuscinski, 2003). Contrarily, Ne/Nc ratios in large populations are generally lower because there is higher variance in breeding success.

Making sense of the relationships between Nb, Ne and Nc in the context of salmon conservation

Estimating Ne over an entire generation can be challenging, whereas Nb can more easily be quantified from a single-cohort sample
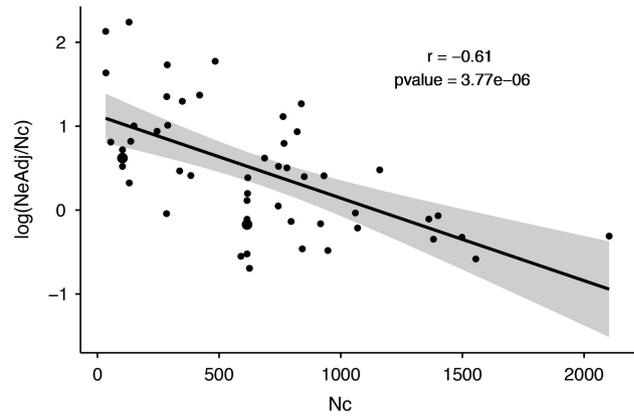
Figure 2 Relationship between log(Ne_{Adj}/Nc) and Nc. Each dot represents a single sample (one population for a specific year) and the line was fitted with a linear regression including the 95% confidence interval (display in gray area Pearson's correlation coefficient and the associated *P*-value are also reported.
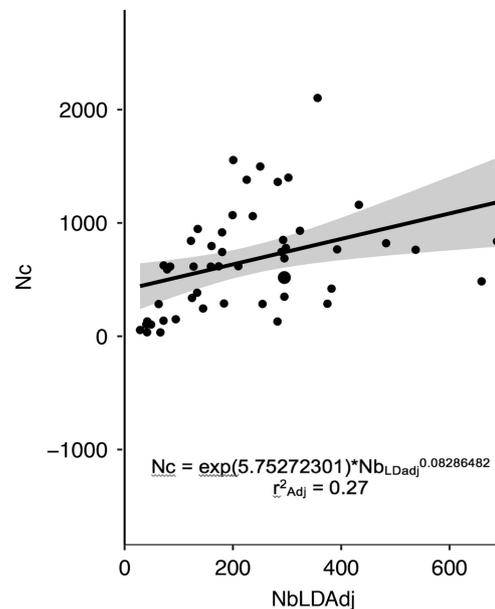
Figure 3 Relationship between Nb_{LDadj} and Nc variables including the 95% confidence interval (in gray area). Each dot represents a single sample (one population for a specific year) and curves were fitted based on the coefficients of the hierarchical linear mixed model.

Table 3 Linear mixed effects models accounting for the effects of the river and of the year on Nb_{Adj}/N_C

Explained variable	Models	Log likelihood	The χ^2 test *P*-value	Variance explained by the river	Variance explained by the year
Nb_{Adj}/N_C	lmer (log(Nb_{Adj}/N_C) ~ 1+(1 ∣ river)+(1 ∣ breeding year))	−48.78		0.37	0.19
	lmer (log(Nb_{Adj}/N_C) ~ 1+(1 ∣ river))	−52.52	0.006		
	lmer (log(Nb_{Adj}/N_C) ~ 1+(1 ∣ breeding year))	−54.55	0.000		

The χ^2 tests were used to compare the models including only the river or the breeding year to the model including both terms.

(Waples, 2005) and be a more practical parameter for managers. Here, we presented the first empirical application to the correction for overlapping generations estimating Nb_{LDadj} and Ne_{Adj} (Waples *et al.*, 2014). Our sampling design incorporated the temporal, environmental and demographic variability and thus allows proposing an accurate prediction for this model. Therefore, for future management of Québec Atlantic salmon populations (or elsewhere in the species range), our results suggest that a single-cohort sample would be enough to get an estimate of *Nb* and then an extrapolated estimate of *Ne*.

In the present study, *Nb/Nc* fluctuated temporally; this also corroborates previous studies in Atlantic salmon (see, for example, Palstra *et al.*, 2009; Johnstone *et al.*, 2013; Perrier *et al.*, 2013, 2016). *Nb/Nc* temporal variation has also been reported in other salmonid species (Heath *et al.*, 2002; Palm *et al.*, 2003). Frankham (1995) and Vucetich *et al.* (1997) suggested that fluctuation in census size was the largest contributor to the reduction of effective to census size ratios on both theoretical and empirical grounds, whereas Storz *et al.* (2001) suggested that variance in reproductive success has a pivotal role based on results of paternity analysis. If the former is correct, then genetic monitoring may be an efficient and independent means of estimating abundance of exploited populations of conservation and/or commercial interest (Ovenden *et al.*, 2007; Waples *et al.*, 2008). A significant positive correlation between *Nb* and *Nc* has also been reported in several long-term studies (Osborne *et al.*, 2010; Charlier *et al.*, 2012) but not in others (Ardren and Kapuscinski, 2003; Palstra *et al.*, 2009; Berry and Kirkwood, 2010; Serbezov *et al.*, 2012; Duong *et al.*, 2013; Johnstone *et al.*, 2013; Dowling *et al.*, 2014; Whiteley *et al.*, 2015). The positive correlation between *Nb* and *Nc* suggests that in the case of Québec Atlantic salmon populations, Nb_{Adj} is an indicative parameter for tracking temporal trends within populations and that genetic monitoring can be used for detecting fluctuations in abundance. However, it is noteworthy that our model allows explaining only 27% of the variance in *Nc* value from Nb_{Adj} estimates, and that 73% of the remaining variation could be explained by other factors. As reported in Perrier *et al.* (2016), most of the variance in Nb_{Adj}/Nc was explained by the river rather than year, suggesting that Nb_{Adj}/Nc differ among rivers according to their biological characteristics. Such variability in Nb_{Adj}/Nc could be attributed to various biological processes including differences in variance of individual reproductive success and in sex ratio (Crow and Kimura, 1970; Nunney, 1993; Frankham, 1995; Kalinowski and Waples, 2002; Hedrick, 2005). Moreover, estimates of *Nc* employed in this study correspond to adult run size, ignoring the reproductive potential of individuals that mature without going to the sea. In Atlantic salmon such individuals are primarily males (Klemetsen *et al.*, 2003) and known as mature male parr (Verspoor *et al.*, 2007). It has been recently shown that these mature male parr can contribute importantly to the effective population size via both their reproductive effort and the fact that they are generally maturing asynchronously from females they mate with. Thus, several studies have shown that mature male parr may fertilize relatively large proportions of eggs in the wild, ranging typically from 30 to 60% (Martinez *et al.*, 2000; Saura *et al.*, 2008; Grimardias *et al.*, 2010; Richard *et al.*, 2013). Thus, Ne_{Adj}/Nc and Nb_{Adj}/Nc reports using *Nc* inferred from anadromous run are expected to be upwardly biased as *Nc* does not include mature male parr (Perrier *et al.*, 2014). This bias could be especially problematic because anadromous adults often contain fewer males than females (Dalley *et al.*, 1983; Myers and Hutchings, 1987), potentially resulting in a high degree of polygamy that may cause more pronounced reductions in Ne_{Adj}/Nc ratios. Hence, the efficacy of *Ne* as a predictor of abundance may depend on

intrinsic population characteristics of the species, and in particular the proportion of mature male parr and skewed sex ratio.

CONCLUSION

Here, we showed the utility of using a comprehensive empirical data set to obtain robust census and effective population or breeder sizes and depict demographic and temporal fluctuations. *Nb* and *Ne* estimates from the LDNe method and the Sibship method were congruent overall. The results show a significant correlation between *Nb* and *Nc* and between *Nb* and *Ne*, suggesting that in the case of Atlantic salmon populations, *Nb* offers potential to be used for tracking population abundance and effective population size. Moreover, based on Waples *et al.* (2014) we proposed an empirical relationship between *Ne* and *Nb* for a direct application in the conservation of the Atlantic salmon. However, our model allows explaining only 27% of the variation in *Nc* from *Nb* estimates, indicating that a large proportion of the variance in *Nb/Nc* exists both among populations (37%) and among years for the same population (19%). This result illustrates the need for a thorough calibration of *Nb/Nc* before using *Nb* in monitoring programs, as well as a full awareness of the limits of such approach.

DATA ACCESSIBILITY

Empirical data (genepop file) available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.jh146.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

LB and MD conceived the study. MD supervised the sampling. CH performed lab analysis for the two last years. A-LF conducted analysis and writing and editing the manuscript with help from JA, CP, MD and LB.

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