

## Evaluation of genetic effects on wild salmon populations from stock enhancement

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Many salmonid populations are of conservation concern, and the release of hatchery-produced juveniles is a frequently used measure to alleviate declines and increase harvest opportunities. While such releases may be of conservation value for some populations, stocking may also decrease the effective population size and subsequently impose additional strain on already threatened populations. In this study, we assessed how the cohort-wise effective number of breeders in five populations of Atlantic salmon (*Salmo salar*) were affected by supplementation. Altogether, 19 cohorts were studied (2–7 cohorts per population) by estimating the proportion hatchery-released individuals and the effective number of wild and captive breeders in each cohort of the respective populations. We show that the effect of releasing captive-bred individuals varies both between populations and between years within the same population. A Ryman–Laikre effect—where the effective number of breeders has decreased as a consequence of supplementation—was observed for 11 cohorts. We discuss how supplementation can be adapted to optimize the effective population size, demonstrate that evaluation of supplementation can be reliably achieved, and show that supplementation programmes that lead to high proportions of hatchery-origin fish on spawning grounds are more likely to induce a Ryman–Laikre effect.

**Keywords:** Atlantic salmon, effective population size, population genetics, Ryman–Laikre effect, *Salmo salar*, stocking

### Introduction

Supplementary stocking—here defined as the release of captive bred individuals into wild populations—is a frequently used measure to alleviate population declines and increase harvest opportunities for around 180 marine and anadromous species worldwide (Kitada, 2018). While the release of hatchery-produced juveniles can serve as a necessary conservation measure for some endangered populations, there is a growing body of research showing that supplementation may also cause a range of negative effects on the gene pool of stocked wild populations. These include the loss of genetic integrity when non-local broodstock is used (Kohout *et al.*, 2012; Valiquette *et al.*, 2014), unintentional domestication selection (Christie *et al.*, 2012a), increased introgression from farmed escapees (Hagen *et al.*,

2019), epigenetic changes (Christie *et al.*, 2016; Le Luyer *et al.*, 2017; Rodriguez Barreto *et al.*, 2019), reduced genetic variation (Machado-Schiaffino *et al.*, 2007), and reduced effective population size despite increased census population size—the Ryman–Laikre effect (Ryman and Laikre, 1991; Christie *et al.*, 2012b). The latter is the result of differences in reproductive success between captive and wild spawners, a low number of captive parents compared to wild spawners, and high variance in reproductive success among captive broodstock that may further decrease the effective number of captive spawners (Ryman and Laikre, 1991). When a large proportion of a population is made up of individuals that originate from a low number of captive parents, the captive broodstock is allowed a disproportionate contribution to the population compared to wild spawners. This difference may

decrease the genetic diversity and increase genetic drift in recipient populations (Verspoor, 1988; Araki and Schmid, 2010; Christie et al., 2012b) and may place already vulnerable populations under additional strain.

Both Pacific (see e.g. Winton and Hilborn, 1994; Pitcher and Neff, 2007; Christie et al., 2012b) and Atlantic (see e.g. Salminen et al., 2007; Harrison et al., 2018; Almodóvar et al., 2020) populations of salmonids are frequently supplemented with hatchery-bred individuals. Stocking practices for salmonids vary and include the release of individuals at the eyed egg stage, alevins, parr and smolt (Kennedy, 1988). Growth in captivity may cause genetic (Hagen et al., 2019) and epigenetic (Christie et al., 2016; Le Luyer et al., 2017) domestication effects that make hatchery-released individuals less adapted to natural conditions. Individuals released as smolts therefore have lower fitness compared to naturally produced conspecifics (Araki et al., 2008; Araki and Schmid, 2010; Christie et al., 2012a; Milot et al., 2013), to the extent that the presence of captive reared individuals may reduce the total production of juveniles in the river (Jonsson et al., 2019). Increased awareness about genetic and epigenetic domestication effects has led to the recommendation that individuals should be released at the earliest possible life-history stage—such as planting of eyed eggs or alevins—and to not use hatchery-reared, but naturally produced fish as broodstock to avoid the accumulation of domestication effects over several generations (Willoughby and Christie, 2019; Rodriguez Barreto et al., 2019).

The Atlantic salmon (*Salmo salar*) is an economically and culturally important species with a complex life history. Adults typically enter the rivers during the summer months (May–August) and await the breeding season in October–December when the eggs are spawned into the riverbed. The eggs hatch the following spring and the juveniles typically spend 1–4 years in freshwater before they smoltify and migrate to sea, where they spend additional 1–4 years before returning to their natal river to spawn (Hutchings and Jones, 1998). Populations generally adapt to optimum smolt and sea ages that depend on the habitat of their respective rivers, but individual variation around these optima occurs (Heggberget et al., 1993) and individuals that originate from a given cohort (brood year) will therefore return as adults over multiple run years. The species has adapted to a wide range of different ecological conditions and the distribution covers both sides of the Atlantic (MacCrimmon and Gots, 1979; Thorstad et al., 2010). In Europe, the native range spans from Spain to Russia and supplementary stocking of Atlantic salmon populations is common and widespread across the entire distribution (Harrison et al., 2018). Of the near 400 waterbodies that support populations of Atlantic salmon in Norway (Forseth et al., 2017), around 60 populations are annually supplemented with hatchery-bred individuals (Karlsson et al., 2020), and for some of these populations, supplementation has been ongoing since mid-1800 (MacCrimmon and Gots, 1979; Berg, 1986). While there are large regional differences, hatchery practices are generally not static but are continuously adapting in parallel with increased knowledge; i.e. in Norway the use of non-local broodfish was discouraged in 1988 and outlawed in 1992 (Diserud et al., 2012); in 1995, supplementation programmes were encouraged to submit scale samples to authorised authorities to identify and remove 1st generation farmed escapees; after 2014 all broodfish of likely farmed ancestry have been identified through genetic analysis and excluded (Karlsson et al., 2011, 2014, 2016). The awareness of potential negative effects following supplementary release of captive-bred

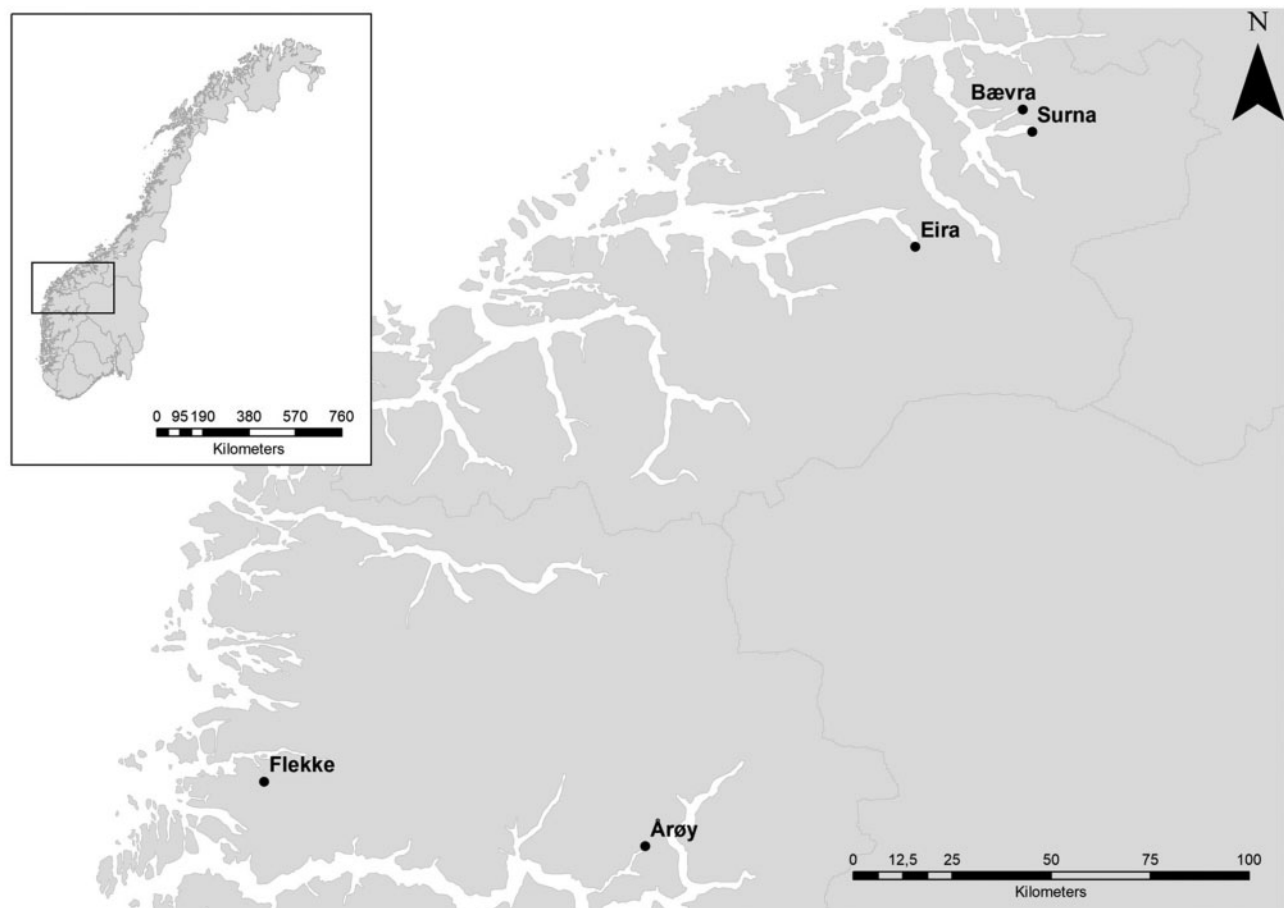
individuals is increasing, yet only a few supplementation programmes have been evaluated to assess the contribution of hatchery-released fish and subsequent genetic effects in the recipient populations (for review, see Kitada (2018) and references therein).

It is possible that the extensive sampling required to apply preferred pedigree approaches may deter conservation managers from evaluating the effects of supplementation. A pedigree-based method may involve genetic assignment of adult spawners to either their broodstock parents or to wild parents such that the relative contribution from hatchery-production and natural production can be compared (Christie et al., 2012b). Furthermore, this approach requires genetic samples from the adult population that include all phenotypes, smolt ages, and sea ages (Hutchings and Jones, 1998) for the evaluated cohorts. For genetic assignment of released fish, samples must also be collected from broodstock. However, as shown in our study, the relative contribution and effective number of breeders of hatchery-produced individuals compared to naturally produced individuals can also be estimated without samples from broodstock, given that a representative sample of the spawning population (both hatchery-released and wild proportions) is available and that the hatchery-released fish are tagged. In many Norwegian rivers, anglers are requested to collect scale samples of their catches of Atlantic salmon. For some rivers, a substantial number of scales covering long time series have been collected. These samples provide a valuable resource to study the effect of supplementation and constitute most of the samples used in this study.

We have evaluated the contemporary effects of supplementation on the effective population size in five different populations of Atlantic salmon where supplementation programmes have been ongoing for several decades. The samples comprise a total of 2513 samples from adult individuals collected over 10 run years and 428 broodfish individuals representing 19 cohorts in total. We show how the use of a low number of broodstock that give rise to a large proportion of the individuals in the populations can reduce the total effective size in the recipient populations. We suggest how the supplementation programmes for these five populations can be adjusted to maximize the total effective population size. Moreover, our study shows how the proportion of hatchery-released fish in a population may influence the likelihood of negative population genetic effects; a response that is likely to be a concern for many salmonid supplementation programmes.

## Material and methods

Samples used in this study originate from five different populations in Western and Central Norway: River Eira, River Surna, River Bævra, River Flekke, and River Årøy (geographical locations, brood years analysed and run years sampled are described in Figure 1 and Table 1). The spawning target—a management benchmark that quantifies the female biomass that are needed for the river to produce its maximum number of smolts—varies greatly between the different populations (Table 1). Four of these are stocked to compensate for reduced natural production of juveniles following hydropower developments and subsequent altered flow of water (Rivers Eira, Bævra, Surna and Årøy). River Flekke is not impacted by hydropower development, but the salmon stock has suffered a population decline due to acidification of the river and supplementation has been ongoing since 1964. In Rivers Eira, Surna, and Bævra, hatchery-reared fish have



**Figure 1.** Map of Western Norway, where the five populations of Atlantic salmon included in the study are located as indicated.

been released since the 1960s and in River Årøy since the late 1800s. The supplementation programmes assessed in this study have released individuals as smolts (Eira, Surna, Bævra, and Årøy), parr (Bævra and Surna), alevins, and eyed eggs (Flekke). With the exception of one female caught in Surna and used as broodstock in Bævra in 2011 (which is located in the same fjord system as Surna), only locally caught spawners have been used as broodstock in the five supplementation programmes during the evaluated cohorts. Details about the number of released individuals, life-history stage of release and the number of samples available from each population are found in [Supplementary Table S1](#). The samples used in this study comprise (i) broodstock samples, which were scales sampled from adult spawners captured during broodstock collection by the hatcheries responsible for the supplementation programmes in the respective rivers (all populations—see [Supplementary Material](#) for more information), and (ii) samples of returning adult salmon in the evaluated rivers. The latter samples were scales collected from adult spawners captured by rod in the rivers during the annual summer harvest (all populations), and scales collected during seine net fishing in autumn for survey of escaped farmed fish (Årøy). The scale samples of returning adult salmon derived from the summer harvest and autumn surveys were analysed for growth patterns to determine the brood year (cohort) that the individuals originate from ([Fiske et al., 2005](#)). Due to a very low number of naturally produced adults in River Bævra, we also used scale samples of juveniles that

originated from the evaluated cohorts to estimate the effective number of wild spawners (see [Supplementary Material](#) for more information about these samples and methods). DNA was extracted from the scales ( $N$  adult spawners = 1396 wild and 1219 hatchery released, [Supplementary Table S1](#);  $N$  broodfish = 390, [Supplementary Table S3](#)) using the DNEASY tissue kit (QIAGEN) and genotyped at 81 nuclear and 15 mitochondrial SNPs ([Supplementary Table S2](#)) using a EP1<sup>TM</sup> 96.96 Dynamic array IFCs platform (Fluidigm). These markers are also used to identify and exclude broodfish that are likely to be of farmed origin ([Karlsson et al., 2011, 2014](#)).

### Estimates of effective number of breeders in captive broodstock

Upon genetically assigning hatchery offspring to their broodstock parent pair (for details, see [Supplementary Methods](#)), we calculated the mean number of offspring per broodfish ( $\mu$ ) and the variance ( $\sigma$ ) in reproductive success separately for male and female broodstock. These data were used to estimate the effective number of broodfish ( $N_{eb}$ ) for each sex, as adapted from [Falconer and Mackay \(1996\)](#):

$$N_{eb} = \frac{N\mu - 1}{\mu - 1 + \left(\frac{\sigma^2}{\mu}\right)}, \quad (1)$$

where  $N$  equals the number of male and female broodfish used in each brood year. The variance was scaled to two, which

**Table 1.** Overview of the different stocking programmes (denoted by the rivers): GPS locations for the different rivers; the spawning target as the number of spawning females averaged over the run years sampled in this study; the average reach of spawning target last four years (the Norwegian Scientific Advisory Committee for Atlantic Salmon, 2019); broodstock years analysed (cohorts); run years sampled for the different populations; and the approximate proportion individuals sampled in each population.

River	GPS location UTM 32	Spawning target	Average reach of spawning target last 4 years (%)	Brood years (cohorts) analysed	Run years sampled	Approx. proportion sampled (%)
Eira	455600–6951050	202	82	2005–2011	2006–2016	50
Surna	482200–6982700	829	100	2011–2013	2015–2018	10
Årøy	401850–6794300	20	100	2011–2012	2016–2018	40
Flekke	304900–6801700	51	100	2009–2011	2015–2017	15
Bævra	479319–6988098	250	<50 <sup>a</sup>	2010–2013	2013–2019	50

<sup>a</sup>The Norwegian Scientific Advisory Committee for Atlantic Salmon does not evaluate River Bævra. Average reach of spawning target is therefore derived from Ugedal et al. (2015, 2018).

corresponds to the number of offspring produced per pair that is required to maintain a stable population size. Following this, the estimates from each sex were combined to produce an estimate for the total effective number of broodfish per brood year as described in Wright (1931):

$$N_{\text{eb}} = \frac{4 (N_{\text{eb}} \times N_{\text{eb}})}{N_{\text{eb}} + N_{\text{eb}}}. \quad (2)$$

### Estimates of effective number of breeders in wild spawners

For all wild cohorts, the effective number of breeders for each brood year was estimated using the sibship method (Wang, 2009) implemented in the programme COLONY 2.0.2.3 (Jones and Wang, 2010). COLONY was run with the full likelihood method, polygamous mating system, without inbreeding and no parental or sibship information. As input, we used all available naturally produced adult individuals assigned by scale reading to a given brood year and all brood years were run separately. In River Bævra, few adult wild individuals were available due to the small size of this population. However, a large number of naturally produced juveniles assigned to brood year was instead used to estimate sibship  $N_{\text{eWild}}$  for River Bævra (see Supplementary Methods for more information about these samples). To compare and verify the sibship method, we also estimated the effective number of breeders using COLONY for all hatchery-produced cohorts, for which  $N_{\text{eb}}$  is known from recapture data. COLONY was then run with the same parameters as described above, but with monogamous mating system for females and polygamous mating system for males, given that crossings are usually performed with one male to one female but males are sometimes crossed with more than one female. As input, we used only individuals that were assigned to broodstock parents in a given brood year and all brood years were analysed separately. The results from sibship analysis in COLONY were compared to the  $N_{\text{eb}}$  from parent–offspring assignment with linear regression in R version 3.5.3 (R Development Core Team, 2018).

### Ryman–Laikre effect of supplementary stocking

To assess a Ryman–Laikre effect in the respective populations, we calculated the total effective population size as presented in Ryman and Laikre (1991):

$$N_{\text{eTotal}} = \frac{1}{\left(\frac{x^2}{N_{\text{eb}}}\right) + \frac{(1-x)^2}{N_{\text{eWild}}}}, \quad (3)$$

where  $N_{\text{eb}}$  is the effective number of broodfish,  $N_{\text{eWild}}$  represents the effective number of wild breeders as estimated from sibship analysis, and  $x$  is the proportion hatchery fish in the river in each brood year. The estimation of  $x$  was derived from the proportion of adult individuals captured in the river that were assigned to broodstock parents and was estimated separately for each population and brood year (see Supplementary Methods for more detail). If  $N_{\text{eTotal}}$  is less than  $N_{\text{eWild}}$ , the total effective population size has been reduced because of supplementation; thus, the population has been subject to a Ryman–Laikre effect (Ryman and Laikre, 1991). Because the Ryman–Laikre effect is related to the proportion hatchery-released fish in the populations, we investigated the relationship between proportion hatchery-released fish and  $N_{\text{eTotal}}/N_{\text{eWild}}$  using linear regression in R version 3.5.3 (R Development Core Team, 2018).

### The captive and wild $N_{\text{eb}}/N$ ratios

In the supplemented proportion of a population, the ratio of the effective number of broodstock to number of broodfish used in crossings should preferably be as high as possible. In many wild populations, however, the ratio of effective number of breeders to the adult census size can be very low (Araki et al., 2007b). The two aforementioned  $N_{\text{e}}/N$  ratios have been denoted  $\alpha_{\text{hatchery}}$  and  $\alpha_{\text{wild}}$ , respectively. The proportion between the two  $\alpha$ -ratios is denoted the  $\beta$ -ratio ( $\beta = \alpha_{\text{hatchery}}/\alpha_{\text{wild}}$ ) and provides information about the skew in contribution from different broodfish compared to the skew in contribution from the different wild breeders under natural spawning (Waples et al., 2016). A  $\beta$ -value above 1 indicates that the reproductive success of the different broodfish is less skewed than is the case under natural spawning. In supplementation programmes, it is desirable to bring the  $\beta$ -ratio as high as possible, and in terms of total effective number of breeders a  $\beta$ -value below 1 means that the broodfish used for supplementation would have contributed to a higher number of effective breeders if spawned naturally. The estimation of adult census size for the different populations is described in Supplementary Methods.

### Relatedness analysis

In some supplementation programmes, the broodfish are collected at the same time and place every year. This procedure could lead to the broodfish being a non-random sample of the

population, where certain families, life histories, or habitat preferences may be over- or under-represented. Frequent use of hatchery-released fish as broodstock may also lead to a higher relatedness within the broodstock compared to the spawning population in the river. We estimated relatedness using the software COANCESTRY (Wang, 2011) and Wang's estimator of relatedness (Wang, 2002), which was chosen following simulations involving 100 unrelated individuals, 50 half siblings and 50 full-siblings that were all generated *in silico*. Wang's estimator was compared to other alternatives in COANCESTRY and found to perform well (data not shown). Relatedness was compared between the broodstock used in crossings for a given brood year and all adult spawners caught in the river the same year to assess if the broodstock is more or less related to each other than the average spawning population in the river. The mean relatedness estimates within each group was compared with bootstrapping (1000 iterations). If the difference in the mean values were outside of the 2.5 and 97.5% confidence intervals, the mean relatedness estimates of the two groups were regarded as significantly different. The broodstock samples used in relatedness analysis were collected in the same run years as described in Table 1.

## Results

The different supplementation programmes report that 10–33 (mean 20.40) individuals were used as broodstock each brood year (see Table 2). The average effective number of breeders was 15.7 and thus lower than the numbers of broodfish individuals used. While there was some variation in the number of male and female broodfish used per cohort (see Supplementary Table S3), none of the supplementation programmes had a large skew in sex ratio, with an average of 47% males to 53% females. Moreover, there was a modest difference in male and female variance in number of recaptured offspring across the different supplementation programmes, with the largest difference in variance found in River Flekke (Supplementary Table S3). The number of offspring per broodfish ranged from 0 to 43 (average 7.95). Together with the slight difference in sex ratio, this skew in individual contribution will likely have contributed to bringing the effective number of breeders below the census. For wild breeders, the average effective size per cohort ranged from 37 to 94 (average 63). The effective number of captive breeders was therefore around one third of the effective number of wild breeders. Provided that the proportion stocked fish in the population balances the number of captive and wild breeders such that the total effective number of breeders is not compromised as a result of supplementation, this difference in captive and wild breeders need not diminish genetic diversity (Figure 2).

For the rivers Eira and Årøy, it is evident that the proportions of hatchery-released fish have been high (mean proportion hatchery for evaluated cohorts = 0.59 for Eira and 0.53 for Årøy) and not optimally balanced with the number of captive and wild breeders, thus resulting in a Ryman–Laikre effect in most cohorts. In River Flekke, planting of eyed eggs and release of alevins have given rise to few adult spawners (mean proportion hatchery for evaluated cohorts = 0.13) and supplementation therefore neither reduce nor add much to the total effective population size. The supplementation contributes moderately in River Surna (mean proportion hatchery for evaluated cohorts = 0.20) and supplementation has for two cohorts contributed to increase the total effective number of breeders. In River Bævra,

supplementation has a moderate to large effect (mean proportion hatchery for evaluated cohorts = 0.46) and has for all but one cohort decreased the effective number of breeders (Figure 2 and Table 2).

A linear regression model shows a negative relationship ( $r^2 = 0.61$ ;  $p < 0.000$ , intercept =  $1.02 \pm 0.12$ , estimate for  $N_{eTotal}/N_{eWild} = -0.673 \pm 0.13$ ) between the proportion hatchery-released fish in the populations and  $N_{eTotal}/N_{eWild}$  values for the different brood years in the five populations (Figure 3) thus indicating a close relationship between the amount of stocked fish in the populations and the potential for a Ryman–Laikre effect. Supplementation programmes that result in greater proportions of hatchery-origin fish on the spawning grounds will therefore likely result in greater reductions in the effective population size.

The  $\beta$ -ratio—which describes the skew in contribution from different broodfish compared to the skew in contribution from the different wild breeders under natural spawning ( $\alpha_{hatchery}/\alpha_{wild}$ )—varied considerably between cohorts but exceeded 1 for all populations and cohorts, with the exception being the 2005 cohort in River Eira (Table 2). This indicates that the contribution from different broodfish is more even than the contribution from the different wild breeders and that the broodfish contribute more to the effective population size as a result of being held in the hatchery, than they would have done if spawning naturally in the river. There was a close agreement between the effective number of captive breeders estimated from parentage assignment according to (1) and (2) and the sibship method implemented in COLONY ( $r^2 = 0.88$ ) (Supplementary Figure S2). The sibship method therefore appears to provide reliable results with respect to the effective number of parents based solely on a sample of offspring.

Within one generation of the River Eira broodstock a total of 33 parent–offspring pairs were found. In 2010, we observed that 16 (42%) broodfish were the progeny of broodfish used in 2005 and 2006, while in 2011 we found 13 (52%) broodfish that were progeny of the broodfish used in 2005 and 2006. The broodfish whose offspring are also used as broodstock may have a very high contribution to the population and the number of known “grandchildren” these individuals have produced ranged from 1 to 86 (average 16.9). Relatedness analysis of the Eira broodstock also indicated a high relatedness within broodfish used (Table 3), which is probably related to the high proportion of hatchery-released individuals used as broodfish. From the brood years 2005 to 2016, on average 57% of the broodfish were hatchery-released fish. Over the same time period, relatedness has overall been significantly higher within the broodstock compared to sports angling samples, which are expected to be a random sample of the river population (Table 3). The same pattern was not seen in the Rivers Årøy and Flekke, where relatedness in broodstock was either lower, or not significantly different from, relatedness within the spawning population. In these populations, few or no broodfish of hatchery origin have been used. In Rivers Surna and Bævra, a low number of hatchery-released fish has been used as broodstock. In River Bævra, the relatedness within broodstock was on average not higher than that within adult spawners caught the same year, while for three run years in River Surna, the broodstock is more related than the spawning population.

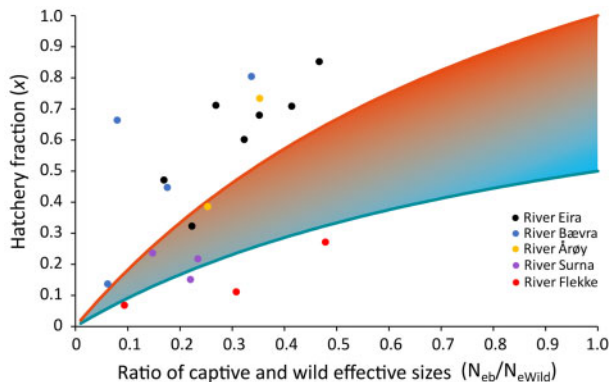
## Discussion

In this study, we have evaluated the Ryman–Laikre effect for five different supplementation programmes. The evaluation provides

**Table 2.** Effective number of wild spawners ( $N_{eWild}$ ), the estimated census of wild spawners ( $N_{Wild}$ ), the effective number of broodfish ( $N_{eb}$ ), number of broodfish used in crossings ( $N_b$ ), the total effective population size as derived from (3) ( $N_{eTotal}$ ), a measure of whether stocking has caused a decrease in the total effective population sizes ( $N_{eTotal}/N_{eWild}$ ), the proportion of hatchery-released individuals in the populations for each cohort, and the  $\beta$ -value for each cohort based on data from adult individuals.

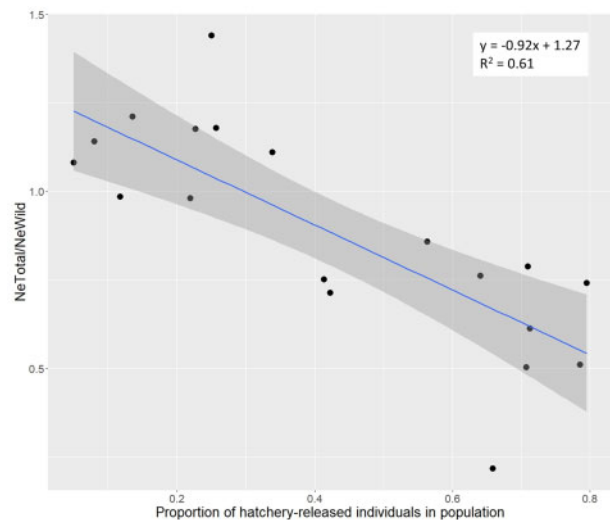
Pop	Cohort	$N_{eWild}$	$N_{Wild}$	$N_{eb}$	$N_b$	$N_{eTotal}$	$N_{eTotal}/N_{eWild}$	Proportion hatchery	$\beta$
Eira	2005	106	148	16.0	25	79.5	0.75	0.46	0.89
	2006	76	530	32.3	33	59.8	0.79	0.68	6.82
	2007	56	271	19.8	22	42.6	0.76	0.65	4.57
	2008	91	930	23.9	24	45.8	0.50	0.69	10.19
	2009	72	265	16.0	30	84.8	1.18	0.28	1.96
	2010	67	214	21.8	25	57.4	0.85	0.57	2.91
Bævra	2011	35	373	22.0	25	30.8	0.88	0.83	7.20
	2010	48	>108	2.8	10	47.3	0.98	0.11	NA
	2011	57	>43	9.5	12	40.6	0.71	0.42	NA
	2012	75	>45	7.2	20	16.2	0.22	0.66	NA
Surna	2013	29	>47	9.3	19	14.8	0.51	0.79	NA
	2011	109	1617	25.2	29	131.9	1.21	0.14	23.24
	2012	126	1886	14.7	29	123.3	0.98	0.22	6.51
Flekke	2013	48	1145	9.8	14	56.4	1.18	0.23	27.44
	2009	45	556	4.4	18	48.5	1.08	0.05	3.05
	2010	40	641	18.5	20	57.5	1.44	0.25	14.82
Årøy	2011	44	824	13.8	20	50.4	1.14	0.08	12.92
	2011	44	236	10.9	10	48.9	1.11	0.34	4.87
	2012	29	264	9.5	10	17.8	0.61	0.71	8.65

Confidence intervals for  $N_{eWild}$  are found in [Supplementary Table S4](#).



**Figure 2.** The ratio of the captive and wild effective sizes plotted against the proportion of hatchery-released individuals in the five different populations according to the model presented in [Waples et al. \(2016\)](#). The red line represents combinations for which wild effective sizes are equal to the total effective size and hatchery-reared individuals do not contribute to the total effective size. Above the red line, hatchery supplementation reduces the effective population size. The blue line represents the maximum effective size that can be achieved at different proportions of hatchery-released fish in the population. Below the blue line, hatchery supplementation contributes little to the effective population size. Each dot represents one cohort. Different populations are represented by different colours.

information about how the broodstock is utilized and how the proportion of hatchery-released fish in the population is balanced compared to naturally produced fish. If the effective number of broodfish is considerably lower than the census, adjustments to hatchery practices should be done to make the contribution from different broodfish less skewed and to increase the contribution



**Figure 3.** Relationship between the proportion of hatchery-released individuals and the strength of Ryman–Laikre effects presented as  $N_{eTotal}/N_{eWild}$  for all analysed brood years in the five populations. The blue line is derived from simple least squares regression and the shaded area represents the standard error.

from each captive breeder. A balanced reproductive success among captive broodstock can increase the total  $N_e$  of stocked populations ([de Mestral et al., 2013](#)).

Among the 19 evaluated cohorts, a clear Ryman–Laikre effect was detected in 11 cohorts, while in one cohort (the 2012 brood year in Surna), the total effective size was marginally reduced as a result of supplementation ( $N_{eTotal}/N_{eWild} = 0.98$ , [Table 2](#)). An overall modest skew in sex ratio and a large variation in family

**Table 3.** The mean relatedness estimates within broodstock and wild spawners and the proportion hatchery-released fish used in crossings presented for different populations and run years.

Population	Year	Broodstock	Wild spawners	Significance	Fraction hatchery fish in broodstock	
Eira	2006	0.009	0.008	ns	0.52	
	2007	<b>0.035</b>	−0.005	Significant	0.62	
	2008	<b>0.018</b>	−0.013	Significant	0.67	
	2009	<b>0.002</b>	−0.037	Significant	0.60	
	2010	<b>0.026</b>	−0.022	Significant	0.79	
	2011	−0.005	−0.018	ns	0.54	
	2012	<b>0.040</b>	−0.003	Significant	0.62	
	2013	<b>0.050</b>	0.009	Significant	0.61	
	2014	<b>0.071</b>	0.010	Significant	0.50	
	2015	−0.022	0.007	ns	0.49	
	2016	<b>0.037</b>	−0.107	Significant	0.35	
	Bævra	2014	0.039	0.024	ns	0.15
		2015	<b>0.065</b>	−0.006	Significant	0.04
2016		0.001	0.005	ns	0.17	
2017		0.066	0.020	ns	None	
2019		0.009	0.042	ns	0.07	
Surna	2015	<b>0.003</b>	−0.008	Significant	0.04	
	2016	−0.008	<b>0.013</b>	Significant	0.11	
	2017	<b>0.030</b>	−0.013	Significant	0.14	
	2018	<b>0.053</b>	−0.007	Significant	0.05	
Flekke	2015	−0.021	<b>0.020</b>	Significant	0.05	
	2016	0.015	0.003	ns	0.20	
	2017	0.043	0.023	ns	0.05	
Årøy	2016	0.032	0.024	ns	None	
	2017	−0.023	<b>0.020</b>	Significant	None	
	2018	0.003	0.017	ns	None	

If the difference in relatedness means was outside of the 2.5 and 97.5% confidence interval, the means of the different groups were considered significantly different. Non-significant differences are denoted “ns”. Where the differences are significant, the highest value of relatedness is indicated with bold numbers. Only two individuals were crossed in 2018 in the Bævra stocking programme; analysis was omitted.

size were observed among broodstock compared to an ideal situation, while variance in reproductive success appeared lower among broodstock compared to wild spawners (Supplementary Table S3). A high variance will lower the effective number of breeders and may contribute to a Ryman–Laikre effect, as seen in River Bævra (Table 2). However, the potential for a Ryman–Laikre effect is in the present supplementation programmes more closely linked with the proportion of hatchery-released fish in a population (Figure 3) (Waples *et al.*, 2016). In Eira, the only cohort between 2005 and 2011 for which a Ryman–Laikre effect was not observed was 2009. This was also the cohort that produced the lowest proportion of hatchery-released fish. Similarly, in River Årøy, a Ryman–Laikre effect was observed for the brood year with the highest proportion of hatchery-released fish. In River Flekke, we observed a high variance and a somewhat skewed sex ratio, but given the low proportion of hatchery-released fish, the low effective number of breeders was of little consequence to the recipient population. In the evaluated cohorts, a Ryman–Laikre effect occurred when the proportion of hatchery-released fish in the populations exceeded 0.4 (Figure 3). Theoretically, it is possible to avoid a Ryman–Laikre effect also at higher proportions of hatchery-released fish, provided that the  $N_{eb}/N_{eWild}$  ratio is close to 1 (see Figure 2). However, for many supplementation programmes, it may not be practically feasible to increase the number of captive breeders, either because of difficulties in collecting them, or due to limited facilities in the hatcheries. Furthermore, it may not be desirable, nor advisable, depending on the level of threat and habitat degradation the

population is facing, to collect a large proportion of the wild spawners to be used as hatchery broodfish due to the potential for domestication selection or epigenetic effects (Araki *et al.*, 2007a; Araki *et al.*, 2008; Araki *et al.*, 2009; Christie *et al.*, 2012a; Wilke *et al.*, 2015; Le Luyer *et al.*, 2017). Increasing the number of captive breeders is therefore often not a realistic strategy to compensate for high proportions of hatchery-released fish.

In most cases, a motivation for supplementation is to increase the number of fish in the population without compromising genetic variation and decrease the effective population size. In this perspective, supplementation is only meaningful if the broodfish in the hatchery produce more offspring than the same individuals would have produced naturally (thus a  $\beta$ -value of  $>1$ ). The  $\beta$ -value is for most populations a challenging measure to estimate due to uncertain census of wild spawners. An accurate census may be possible in populations with migratory barriers or traps where spawners are recorded and passed over (Araki *et al.*, 2007b; Christie *et al.*, 2012b; Jonsson *et al.*, 2016), however, also in such closely monitored populations, there may be individuals such as precocious male spawners that are unaccounted for (Orton *et al.*, 1938); the presence of which is expected to lead to an underestimate of  $\beta$ .

In the River Eira supplementation programme, we observed a high number of hatchery-released fish used as broodstock, which has plausibly contributed to the high relatedness within the Eira broodstock (Table 3). Such use of hatchery-released individuals as broodfish is likely to accentuate skewed family sizes and thus further decrease the genetic variation in the offspring and

subsequently in the recipient population. This is further extenuated by adaptation to captivity leading to higher reproductive success in the hatchery for broodfish of hatchery origin compared to broodfish of wild origin (Christie *et al.*, 2012a). Also, in the Eira supplementation programme, it has been shown that female broodfish of hatchery origin gave rise to more returning adult offspring than female broodfish that were naturally produced (Hagen *et al.*, 2019). The estimated effective number of captive breeders using (1) and (2) does not take the “grandparent effect” into consideration. It is therefore likely that the realized Ryman–Laikre effect in the Eira population is larger than what the current estimates within cohorts suggest. From our results we advise that the recipient populations in the Rivers Eira and Årøy would benefit from a lower proportion hatchery-released fish in order to increase the total effective population size. In River Årøy, the spawning target in the recent years has been reached by fivefold (Table 1) and supplementation therefore appears obsolete and unnecessary to maintain this population. In River Eira, the spawning target is mostly reached, although possibly due to hatchery-released individuals. While a reduced number of released individuals may prevent a Ryman–Laikre effect and thereby increase the effective size of the population, a lower population census size may lead to a need for more stringent harvest restrictions. The salmon population in River Surna is large, and the spawning target is reached for most years. While no apparent negative population genetic effects following supplementation were observed in River Surna, the modest effect of the supplementation programme and the large size of the population indicate that conservation managers may consider the necessity of continued supplementation in River Surna. The salmon population in River Bævra is severely affected by hydropower development and the spawning target is not reached, despite supplementation. While hatchery-released individuals may constitute an important contribution to the small adult population, a Ryman–Laikre effect was observed in most cohorts. Supplementation may be warranted from conservation purposes, yet negative population genetic effects were observed. Hatchery practices should therefore be optimized to increase the effective number of captive breeders and thus also the total  $N_e$  in the population. Considering the high variance in number of recaptured offspring (Supplementary Table S3) following a large skew in contribution from different broodfish, the supplementation programme probably has the potential to increase captive  $N_e$  without increasing their number of broodfish. Supplementation has little effect in River Flekke and termination of the programme will therefore be of little consequence to the population in this river, where the spawning target is also reached (Tables 1 and 2).

As a first step to improve the supplementation practice, we therefore suggest to consider the necessity of supplementation according to hatchery contribution and the desired spawning target, to make adjustments to increase the  $N_{cb}/N_b$  ratio by attempting to equalize family sizes (such as standardizing egg numbers and use an even sex ratio), to adjust the number of fish being stocked and to avoid the use of hatchery-released individuals as broodstock. Increased awareness about hatchery-induced domestication effects (see e.g. Araki *et al.*, 2007a, 2008; Christie *et al.*, 2012a, 2014, 2016; Le Luyer *et al.*, 2017; Hagen *et al.*, 2019) has led to the general management advice (Karlsson *et al.*, 2015) that hatchery-produced individuals should be released at the earliest possible life-history stage. The recapture rate of individuals released as parr and smolt, respectively, in Rivers Bævra and Surna

(Supplementary Figure S1), suggests that the release of parr has the potential to be more successful (see Supplementary Results) than releasing individuals as smolts and may produce adult spawners that are better adapted to the natural environment. However, the release of hatchery-reared parr or younger life-history stages requires vacant habitat in the river to avoid competition with naturally produced conspecifics.

For many supplementation programmes, samples of broodstock for evaluation may not be available. In this study, we have shown that the effective number of broodfish can be reliably determined using sibship analysis (Supplementary Figure S2). This means that assessment of supplementation programmes can be done without samples from broodstock, provided that the hatchery-released offspring is marked—for instance by adipose fin clipping (smolt) or otolith marking (younger stages)—and can be assigned to cohort—for instance by scale reading (Fiske *et al.*, 2005). The estimated effective number of broodstock can then be inserted in (3), from which the Ryman–Laikre effect can be evaluated. Using this approach, the estimated values for  $N_{eTotal}/N_{eWild}$  based on sibship analysis deviated little from those based on parentage assignment (Supplementary Figure S2). This approach assumes that all hatchery-released fish are of local origin and not strays from other supplementation programmes, which would lead to an overestimate of the effective number of broodfish. In the current study, only hatchery-released individuals that were assigned to broodstock parents were included in the sibship analysis; an approach that excludes potential strays. Parentage assignment between the Surna and Bævra supplementation programmes (populations situated in the same fjord system) produced only two identified strays between the two rivers (data not shown), thus indicating that in the evaluated supplementation programmes, hatchery-released strays were not a significant factor.

Evaluation as performed in the current study provides brood year specific information about the contribution by the captive and wild components in the population and does not provide a quantitative estimate of the accumulated past and current effects of supplementation. Particularly for heavily impacted populations such as those in the Rivers Eira and Årøy, where individuals are released as smolts, it is possible that the total effect of supplementation leads to a general fitness decline in the populations, as described in Willoughby and Christie, 2017. However, it is challenging to quantify what the effective size would have been without supplementation because for most programmes it is not realistic to quantify the reproductive success of individuals on the spawning grounds, supplementation practices may be in continuous change (e.g. release of different life-history stages), and supplementation may impact gene flow (Jonsson *et al.*, 2003; Keefer and Caudill, 2014) as well as genetic introgression of farmed escapes (Hagen *et al.*, 2019; Karlsson *et al.*, 2020). However, the Ryman–Laikre effect is a parameter that can be quantified and a reoccurring Ryman–Laikre effect will negatively affect the total effective size of the population. Based on our results and previous studies, it is likely that any accumulated negative fitness effects will be most severe in populations with a strong Ryman–Laikre effect and a high proportion of hatchery-origin fish that have been released at a late life-history stage.

We have evaluated the effect of supplementation on the effective population size in five supplemented rivers in Norway. From these data, it is evident that supplementation affects various populations differently and that there is large between-cohort



variation, also within populations. The effect of supplementation can thus not be extrapolated between populations: each programme must be evaluated separately, preferably involving several cohorts to avoid biased outcomes due to stochasticity in the complex ecological systems. Nevertheless, the specific five cases analysed in the present study had in common that the most profound effect on the total effective number of breeders was the proportion of hatchery-released fish. If generalized, this implies that in a population of Atlantic salmon, the effective population size is more likely to respond negatively to supplementation if the proportion of hatchery-released fish is high compared to a low proportion. We have shown that supplementation may lead to both large and negligible population genetic effects and we advocate that supplementation programmes should be evaluated and subsequently adapted such that the effects on the recipient populations can be optimized.

### Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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### Author contributions

SK, IJH, and OU designed the study. OU, AJJ, BB, BF-L, HS, HSK, EH, and HL provided tissue samples and scale reading data from the salmon caught in recreational fishing and from broodstock, respectively. IJH and SK wrote the article with input from all authors. SK is the senior author and has been the project leader.

### Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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