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NINA Report

Knowledge gained from evaluating 16 Norwegian stocking programs for Atlantic salmon (*Salmo salar*)

Ingerid Julie Hagen and Sten Karlsson

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Abstract

Hagen, I.J. & Karlsson, S. 2023. Knowledge gained from evaluating 16 Norwegian stocking programs for Atlantic salmon (*Salmo salar*). NINA Report 2347. Norwegian Institute for Nature Research.

The Norwegian Institute for Nature Research (NINA) and collaborators have evaluated the effects of stocking in 16 supplementation programs for Atlantic salmon in Norway. Of these, eight release eyed eggs or alevins, four release smolts and four release smolts as well as earlier life history stages such as parr, eyed eggs and alevins. Using molecular genetic methods, we have analyzed a total of 67 cohorts in the 16 stocking programs and genotyped around 6000 individuals in addition to the broodfish. For 39 cohorts the data was suitable to estimate the effective number of broodfish. The outcomes of these studies indicate:

1. Norwegian stocking programs typically use around 20 – 30 broodfish each brood year.
2. In 20 of the analyzed cohorts, a Ryman-Laikre effect was observed and in 17 of these cohorts, the effect was substantial. This means that the number of broodfish used has not been balanced against the proportion stocked fish and the number of wild breeders in the population. Particularly when smolts have been released, the proportion stocked fish has been too large considering the number wild spawners and the effective number of broodfish.
3. A severe Ryman-Laikre effect - as observed in some of the stocking programs - is expected to lead to reduced genetic variation over time, thus compromising the population's ability to adapt to environmental changes. In three stocking programs with severe Ryman-Laikre effect, the data allowed temporal comparisons and showed that the effective population size indeed had been reduced over time.
4. In stocking programs releasing smolts, the proportion stocked fish for 15 out of 21 analyzed cohorts was over 40% (found in five stocking programs) and around 80% (found in three stocking programs) for five of these cohorts.
5. Out of nine stocking programs where the size of the broodfish was compared to the population average, the broodfish was found to be significantly larger in seven stocking programs. This means that large individuals are often chosen as broodfish. As such, the broodstock generally do not represent the donor population. This practice introduces an artificial selection and may disrupt local genetic adaptation.
6. For some stocking programs where eyed eggs and alevins have been released, there was a low proportion stocked fish observed. For 11 cohorts, the proportion stocked fish was 5% or less (observed in seven stocking programs) and for two cohorts in two different stocking programs, no stocked individuals were observed. When none or few stocked individuals were observed, the removal of broodfish from the spawning population may have led to fewer juveniles being produced than if the broodfish were allowed to spawn naturally.
7. Molecular genetic tools have proven useful in avoiding some of the negative impacts of stocking: The mandatory broodfish control program is important to avoid amplification of domesticated genotypes (farmed salmon) in hatcheries. Some stocking programs have implemented analyses of relatedness between broodfish to avoid crossing closely related individuals. From genetic profiling of broodfish, all stocked fish can be traced to parental pairs to evaluate and improve stocking practice.

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Sammendrag

Hagen, I.J. & Karlsson, S. 2023. Knowledge gained from evaluating 16 Norwegian stocking programs for Atlantic salmon. NINA Rapport 2347. Norsk institutt for naturforskning.

Norsk institutt for naturforskning (NINA) og samarbeidspartnere har evaluert kultivering i 16 laksebestander i Norge. Det settes ut øyerogn eller yngel i åtte av disse bestandene, i fire bestander settes det ut smolt, og i fire bestander settes det ut både smolt og yngre stadier som øyerogn, yngel eller parr. Til sammen har vi studert 67 årsklasser ved hjelp av molekylærgenetiske metoder. Totalt har vi genotypet rundt 6000 individer i tillegg til stamfisken. For 39 årsklasser var det mulig å beregne effektivt antall stamfisk. Følgene punkter har utkrystallisert seg:

1. I norske kultiveringsprogram benyttes rundt 20 – 30 stamfisk per gyteår.
2. I 20 av de analyserte årsklassene ble det observert Ryman-Laikre effekt, og i 17 av disse årsklassene var effekten betydelig. Dette betyr at antallet stamfisk ikke har vært balansert mot andelen utsatt fisk og størrelsen på den ville gytebestanden. Særlig der det har vært utsettinger av smolt har andelen utsatt fisk i mange tilfeller vært for høy i forhold til den ville gytebestanden og det effektive antallet stamfisk.
3. En sterk Ryman-Laikre effekt forventes å medføre redusert effektiv bestandsstørrelse over tid, hvilket vil redusere bestandens evne til å tilpasse seg miljøforandringer. I tre kultiveringsprogram med sterk Ryman-Laikre effekt var det mulig å gjøre sammenlikninger med prøver samlet inn i forskjellige tidsperioder. Disse analysene viste at effektiv bestandsstørrelse har blitt redusert over tid.
4. I kultiveringsprogram der det settes ut smolt, har andelen kultivert fisk for 15 av 21 analyserte årsklasser vært over 40 % (observert i fem kultiveringsprogram) og rundt 80 % for fem av disse årsklassene (observert i tre kultiveringsprogram). Flere internasjonale fagfellevurderte publikasjoner viser at individer utsatt som smolt har lavere reproduktiv suksess enn naturlig produserte individer. Smoltutsettinger har derfor potensiale for sterk negativ påvirkning i og med at dette kan medføre høy andel kultivert fisk med dårligere tilpasset fenotype.
5. For ni kultiveringsprogram ble stamfiskens størrelse sammenlignet med gjennomsnittet i bestanden. I sju av disse kultiveringsprogrammene var stamfisken signifikant større enn gjennomsnittet i elvebestanden. Dette betyr at store individer er oftere brukt som stamfisk og stamfisken representerer ikke størrelsesfordelingen i gytebestandene. Denne praksisen introduserer kunstig seleksjon og kan forstyrre lokal genetisk tilpasning.
6. For noen kultiveringsprogram der øyerogn og yngel settes ut ble det observert ingen kultivert fisk eller en svært lav andel kultivert fisk. For 11 årsklasser var andel utsatt fisk 5 % eller mindre og for to av disse årsklassene ble det ikke observert kultivert fisk. For årsklasser der ingen eller få kultiverte individer ble observert kan uttak av stamfisk ha medført færre avkom i elven enn om stamfisken fikk gyte naturlig.
7. Molekylærgenetiske metoder er viktige for å unngå noen av de negative effektene av kultivering. Stamlakskontrollen er viktig for å unngå at individer med oppdrettsopphav oppformeres i anlegg. Enkelte kultiveringsprogram har innført slektskapsanalyser innen stamfisken for å unngå å krysse nært beslektede individer. At stamfisken er genotypet betyr at all utsatt fisk kan spores tilbake til deres stamfiskforeldre slik at kultiveringen kan evalueres og forbedres i henhold til evalueringen.

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Contents

Foreword

The Norwegian Institute for Nature Research (NINA) has evaluated stocking of Atlantic salmon in 16 populations. This work has been published in several NINA reports and peer-reviewed publications and has increased our understanding of the effects of stocking in Atlantic salmon. In order to make this information more easily available to conservation management, managers of stocking programs and the general public, the Norwegian Environment Agency asked NINA to compile the accumulated knowledge around the effects of stocking on salmon populations into one report. This summary of observed effects of stocking in Norway is supplemented by information derived from international peer-reviewed publications. We thank The Norwegian Environment Agency for this assignment.

Trondheim, November 2023

Ingerid Julie Hagen

1 Introduction

Supplementary stocking, here defined as the release of captive bred individuals into wild populations, is used as a measure to increase harvest opportunities, and as a conservation measure to avoid population declines. Worldwide, more than 180 aquatic species are stocked to varying degrees (Kitada 2018). While stocking in some cases may be necessary to maintain population sizes, many studies show that stocking can lead to an array of negative effects in recipient populations. With growing awareness of the potential negative effect of stocking, the motivation for stocking in Norway has increasingly turned away from harvest opportunities to becoming a conservation measure.

The first hatcheries for Atlantic salmon were established in Norway during the 1850s (MacCrimmon & Gots 1979). For over a century, there were few guidelines as to how stocking should be practised to avoid negative effects on the recipient populations. With increasing knowledge about the effect of introducing hatchery-produced individuals into natural populations, regulations concerning stocking have become increasingly more detailed: in 1988 came a recommendation to only use broodfish from the same watercourse (thus moving broodfish between watercourses was discouraged) and in 1992 the use of broodfish from the same watercourse became a formally introduced legislation (https://lov-data.no/dokument/NL/lov/1992-05-15-47). From 1995 onwards, all potential broodfish were submitted to scale reading, to identify and remove escaped farmed salmon. In 2014, with the advent of the genetic broodstock control program, all potential broodfish that are not identified as escaped farmed salmon from scale reading are genetically tested for farmed genetic introgression (Karlsson et al. 2011; 2014) to remove hybrids between farmed escapees and wild salmon and to identify farmed individuals that escaped at an early life history stage. Also, guidelines for stocking of anadromous populations have been developed by the Norwegian Environment Agency (Anonymous 2011; 2014).

1.1 Effective population size

An important concept in the evaluation of stocking is the effective population size. This is a measure of how much each parent contribute to the next generation and is defined as the size of an ideal population where all Hardy-Weinberg assumptions are met, that lose heterozygosity at the same rate as the observed population. If some parents contribute many offspring and others contribute none, the effective population size will be lower than it would have been if all parents contributed equally. This means that a low variance in contribution is important for the effective size (N_e) to approach the census number (N_c) of parents. In natural populations, the contribution that parents make to the next generation is rarely equal and hence the N_e/N_c ratio in nature is generally lower than one. In hatcheries, conditions are much more controlled than in nature, and efforts can be made to ensure that the contribution from each broodfish is as equal as possible up to the point of release. However, after release, different families may respond differently to natural selection pressures and the true contribution by each family is not realised until the offspring has returned as adult individuals and the new year class is complete.

1.2 Theoretical framework for effective population size in stocking

The theoretical framework for balancing the effective wild and captive population sizes is thoroughly explained in the Norwegian guideline for stocking by Karlsson et al. (2016) and in Waples et al. (2016). Stocking should maintain genetic variation in the recipient populations such that the total effective population size is not negatively impacted by stocking, and preferably increased by stocking. To achieve a high total effective population size, the two components of the population – the naturally produced and the stocked – must be balanced against the proportion stocked fish in the population. If the effective population size of the stocked proportion of the population is low, then the proportion stocked fish must be kept low to avoid a negative response on the total effective population size. Similarly, in a large natural population with a high effective population size, the effective number of broodfish must be high if also a high proportion stocked fish in the population is desired. **Figure 1** illustrates how the total effective size of two populations with differing effective numbers of wild breeders respond to varying effective numbers of broodfish and proportions of stocked fish in the population. With an increasing proportion of stocked fish, the effective number of broodfish needs to increase in order to make a contribution to the total effective size or avoid a reduction in total effective size (Ryman-Laikre effect). Stocking of large populations will require facilities to maintain a large number of broodfish also for low or moderate proportions of stocked fish. In a population with 500 effective wild breeders and a $N_{eBroodstock}$ of 50 or less, the proportion stocked fish should not exceed 10 – 20% to avoid a negative effect on the total effective size.

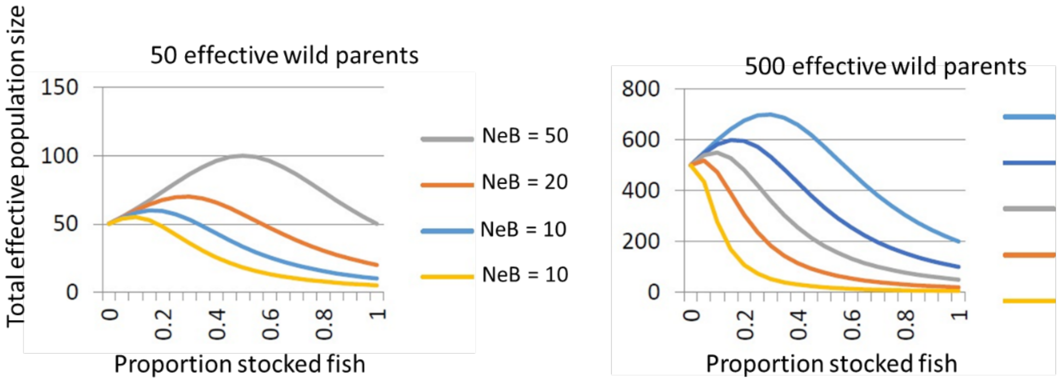

Figure 1. *The response in total effective populations (y-axis) at different proportions stocked fish (x-axis) in a population with 50 effective wild parents (left panel) and 500 effective wild parents (right panel). The figure was first presented in Karlsson et al. (2016).*

In the event of a negative response in total effective population size, the population is subject to a Ryman-Laikre effect (Ryman & Laikre 1991). This means that a small number of broodfish has produced a proportion of the recipient population that is too large to maintain genetic variation at the same level as without stocking, as illustrated in **figure 2**.

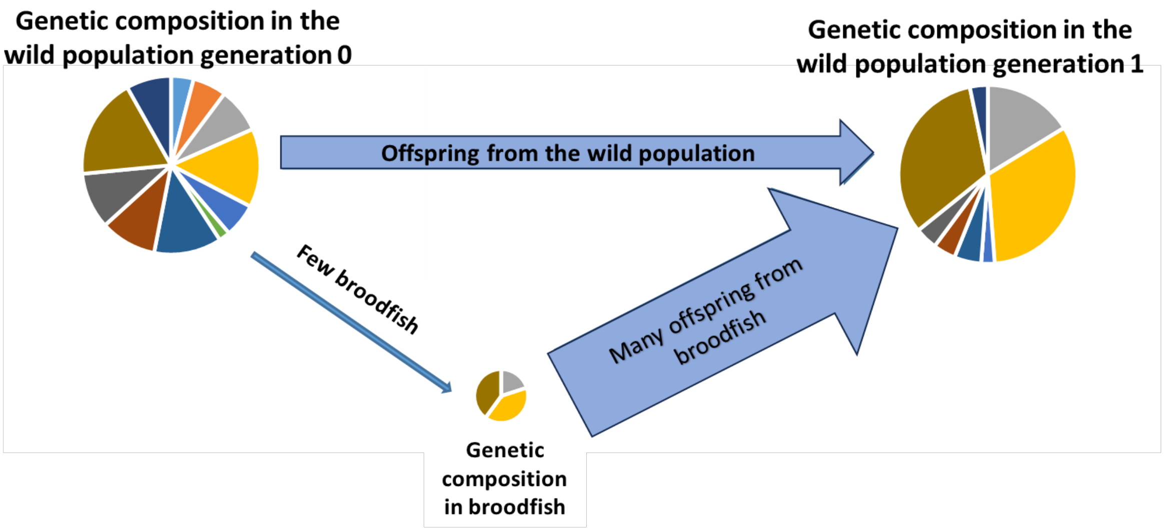

Figure 2. *Illustration of how stocking will lead to reduced genetic variation from one generation to the next in a population subject to a Ryman-Laikre effect. When this process is repeated over several generations, genetic variation may be depleted. The figure is modified from the one presented in Karlsson et al. (2016).*

1.3 The broodstock control program

Due to selection for traits favoured by the aquaculture industry, farmed salmon is less adapted to life in nature than wild salmon and introgression by domesticated genotypes into wild populations has the potential to negatively affect the viability of wild populations (Glover et al. 2017, Hindar et al. 1991, 2006, Skaala et al. 2012, Wacker et al. 2021) and affects the growth and sea age of wild individuals (Bolstad et al. 2017, Bolstad et al. 2021). Under hatchery conditions, however, farmed escapees and hybrids between farmed and wild salmon will outcompete wild individuals (Solberg et al. 2013; Hagen et al. 2019). Avoiding the use of farmed escapees and introgressed individuals as broodfish is therefore an important measure to prevent further introgression of domesticated genotypes in stocked wild populations. The broodstock control program is instrumental in ensuring the genetic integrity of individuals used as broodfish.

The broodstock control program involves both scale reading to remove farmed individuals that can be identified based on growth patterns in the scales, as well as genetic screening to remove individuals when scale reading fails to identify them as escaped farmed salmon. Genetic screening is also used to remove individuals that are genetically introgressed with farmed salmon.

As of 2022, broodfish from 50 stocking programs were screened in the genetic broodstock control programme, totalling 1550 individuals in 2021 (Karlsson et al. 2022) and more than 14 000 individuals since genetic screening started in 2014. Importantly, the genetic broodstock control programme ensures that genetic data for each potential broodfish is available and ready for use in evaluation of stocking. Moreover, the broodfish genotypes can be used to estimate rates of straying, as all stocked individuals recaptured and genotyped can be traced to their broodstock parents across stocking programs.

Figure 3. *Locations of the 50 populations where broodfish was caught in 2021 in Norway. The figure was first presented in Karlsson et al. (2022).*

2 Evaluation of stocking

2.1 Methodology

2.1.1 Molecular genetic approach

An evaluation of stocking that encompasses the effect on the total effective population size for the recipient population requires a pedigree-based approach where the contribution by each broodfish is quantified. This involves genetic assignment of fish caught in the river to either their broodstock parents or to the naturally produced proportion of the population, such that the contribution from stocking and natural production can be quantified and compared for each brood year (Christie et al., 2012b; Hagen et al. 2021a). Here, genetic samples from the adult population that include all phenotypes, smolt ages and sea ages (Hutchings & Jones 1998) for the evaluated cohorts are essential, as well as samples from all broodfish for each brood year. Samples of juveniles collected in the river may also be used. However, natural selection may alter the proportion stocked fish and relative contribution of parents during the time from sampling of juveniles to the return of all adult individuals from the given year class. When scale samples of adult individuals are limited, juvenile samples may provide adequate information on proportion stocked fish and relative contribution by parents. Juvenile samples were used in the evaluation of stocking in Rivers Bævra, Fortunelva and Osenelva (more information further down). For the 16 populations evaluated by NINA, individuals have been genotyped for 81 nuclear Single Nucleotide Polymorphism (SNP) markers and 15 mitochondrial markers. The latter serves as an extra control to verify mother-offspring pairs, as mitochondrial genotypes must be identical between mothers and their offspring. For more details on the genetic assignment of offspring to broodstock parents, see Supplementary Methods in Hagen et al. (2021a).

2.1.2 Estimating effective population size in the hatchery-released proportion

After genetically assigning hatchery offspring to their broodstock parents, the mean number of offspring per broodfish (μ) and the variance (σ) in reproductive success were calculated separately for male and female broodfish. This information was used to estimate the effective number of broodfish ($N_{eBroodstock}$) for each sex, as adapted from Falconer & Mackay (1996):

$$\text{NeBroodstock per sex} = \frac{N\mu - 1}{\mu - 1 + (\frac{\sigma^2}{\mu})} \qquad (1)$$

N is the number broodfish of each sex separately used in each brood year. The variance was scaled to two, which is the number of offspring produced per pair that is required to maintain a stable population size. 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):

$$\text{NeBroodstock} = \frac{4\ (\text{Neb}♀ \cdot \text{Neb}♂)}{\text{Neb}♀ + \text{Neb}♂} \qquad (2)$$

2.1.3 Estimating the effective population size in the naturally produced proportion

Estimation of the wild effective population size can be done based on information from counts of adult spawners, or other information about the number of individuals that are expected to reside on the spawning grounds during a given brood year, for instance camera monitoring or snorkelling. However, because not all individuals contribute equally to the next generation (thus $N_e/N_c < 1$), the number of spawners estimated from counts of spawners cannot be directly applied as the effective number of spawners. In populations of Atlantic salmon, the proportion effective parents to census number of spawners on the spawning grounds vary between populations and between brood years within populations (Ferchaud et al. 2016, Wacker et al. 2022). In evaluation of stocking, we have used a proportion of effective number of spawners to observed number of spawners of $^1/_3$ to $^1/_2$ (Ferchaud et al. 2016; Wacker et al. 2022).

The effective number of spawners for a given brood year can also be estimated from genetic data using the program COLONY (Jones & Wang 2010). The individuals are assigned a brood year based on their age from scale reading and the effective numbers of parents are estimated for each cohort. However, this requires that a large proportion of the population is sampled and that the number of genetic markers is sufficient (Ackerman et al. 2017; Wacker et al. 2022).

2.1.4 Estimating the total effective population size

Effective number of breeders and the effective number of wild spawners make up the total effective population size according to Ryman and Laikre (1991):

$$\mathrm{NeTotal} = \frac{1}{\left(\frac{x^2}{\mathrm{NeBroodstock}}\right) + \frac{(1-x)^2}{\mathrm{NeWild}}} \qquad (3)$$

$N_{eBroodstock}$ is the effective number of broodfish, N_{eWild} is the effective number of wild breeders and *x* is proportion hatchery released salmon in the population each brood year. If N_{eTotal} is less than N_{eWild}, the total effective populations size is reduced by stocking, hence the population is subject to a Ryman-Laikre effect (Ryman and Laikre 1991).

2.2 Evaluated stocking programs

During the last six years, 16 stocking programs for Atlantic salmon in Norway have been evaluated (**table 1**). These populations differ from each other with respect to their population size, and how stocking is conducted. Stocking in these populations include release of eyed eggs and alevins, parr and one or two-year old smolts. The stocking programs that release early life-history stages generally have in common that the releases comprise 10% or less than the natural production, while for stocking programs that release smolts, the stocked individuals may comprise more than 50% of the natural production. The expected effects on the recipient populations are therefore highly variable and outcomes cannot be extrapolated from one stocking programme to another. Mostly, samples of adults were used in the evaluations, but for Rivers Bævra, Osenelva and Fortunselva, the data was partly supplemented with juvenile samples (for more information, see the respective published studies referred to in **table 1**).

Table 1. *The stocking programs for Atlantic salmon that have been evaluated by NINA, listed by population (name of river), the brood years that were studies and the where the results are published.*

Population (river)	Brood years studied	References
Eira	2005 – 2011	Hagen et al. 2020, Hagen et al. 2021a
Bævra	2010 – 2014	Hagen et al. 2020, Hagen et al. 2021a
Surna	2011 – 2013	Hagen et al. 2021a
Årøyelva	2011 – 2012	Hagen et al. 2021a, Skoglund et al. 2019
Flekkeelva	2009 – 2011	Hagen et al. 2021a
Ørstaelva	2010, 2012 – 2014	Hagen et al. 2021b
Fetvassdraget	2014 – 2015	Hagen et al. 2022
Korsbrekkelva	2013 – 2015	Hagen et al. 2021c
Fortunselva	2000 – 2018	Hagen et al. 2021d
Daleelva	2014 – 2016	Hagen et al. 2023a
Arnaelva	2014 – 2015	Hagen et al. 2023a
Loneelva	2014 – 2015	Hagen et al. 2023a
Osenelva	2014 – 2015, 2019	Hagen et al. 2023a
Suldalslågen	2014 – 2017	Hagen et al. 2023b
Gaula	2014 – 2015	Karlsson et al. 2023
Bondalselva	2014 – 2016	Hagen et al. 2023c

The locations of the different stocking programs are shown in **figure 4**. Indicated on the figure is also the life history stages that are released at the different locations.

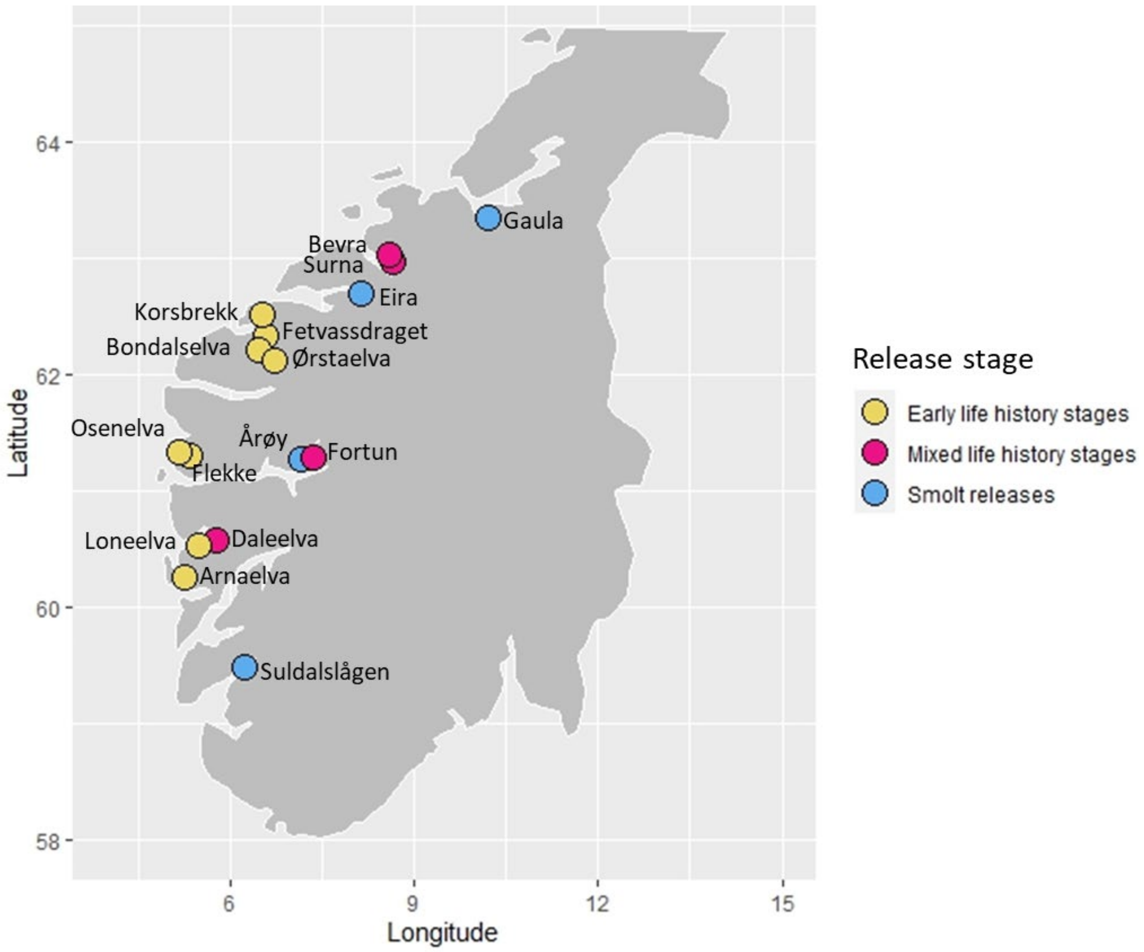

Figure 4. *Map of southern Norway showing where the evaluated stocking programs are located. Different colours indicate the life-history stages released in the different stocking programs.*

In **table 2** below, information about the number of genotyped samples for each study is presented, along with information about the scale of releases in the different rivers during the study periods, and estimated natural production based on Hindar et al. (2007) and Vollstet et al. (2022) where available.

Table 2. *The brood years studies, total number of adult samples genotyped in addition to the broodfish as well as any juveniles (j) genotyped, approximate number (in 1000) of individuals released during the study period, the spawning targets for the respective rivers, the natural production as estimated in Hindar et al. (2007) and Vollset et al. (2022) presented as either smolts or eggs (corresponding to the release stage), how much (in %) the releases constitute compared to natural production and the observed fraction stocked fish for each population during the respective study periods.*

Population (river)	Brood years studied	Samples genotyped	Approx. annual releases in study period	Spawning target (kg ♀)	Estimated natural prod.	% of natural prod.	Observed stocked %
Eira	2005 – 2011	1517	50K smolt	761	31 718 smolt	158%	25-79%
Bævra	2010 – 2014	117	30K parr + 8K smolt	1074	-	-	12-79%
Surna	2011 – 2013	473	35K parr + 20K smolt	4836	16 4786 smolt	< 33% **	14-23%
Årøyelva	2011 – 2012	210	13K smolt	128	5 145 smolt	253%	34-71%
Flekkeelva*	2009 – 2011	298	80K eggs/fry*	277	-	-	5-25%
Ørstaelva	2010, 2012 – 2014	447	50-100K eggs/fry	1353	191 600 eggs	2-5%	1-16%
Fetvassdraget	2014 – 2015	127	80K eggs/fry	484	-	-	0-8%
Korsbrekkelva*	2013 – 2015	423	100K fry*	161	-	-	3-4%
Fortunselva	2000 – 2018	429 + 275 j	15-20K smolt + eggs, fry, parr	-	-	-	50%
Daleelva	2014 – 2016	158	50K eggs + 10-25K smolt	195	282 320 eggs / 11 310 smolt	>88-221%	79-84%
Arnaelva	2014 – 2015	174	100K eggs/fry	168	-	-	7-14%
Loneelva	2014 – 2015	202	45K fry	153	221 460 eggs	20%	5-16%
Osenelva	2014 – 2015, 2019	26 + 104 j	225 K eggs/fry	1029	-	-	5-13%
Suldalslågen	2014 – 2017	461	40K smolt	2319	78 978 smolt	>50%***	40%
Gaula	2014 – 2015	186	15K smolt	26 000	533 571 smolt	3%	3%
Bondalselva	2014 – 2016	313	100K fry	582	844 520 eggs	12%	0-10%

* Stocking discontinued

** Stocked parr have lower survival to adulthood than released smolt in River Surna. The released individuals probably constitute less than 33% of natural smolt production

*** Negative effects of hydropower regulation may have decreased the natural production

3 Observed effects in 16 evaluated stocking programs

3.1 The number of broodfish used and the N_e of broodfish

In the 16 stocking programs that we have studied, the number of broodfish used in each brood year generally ranged around 20 – 30, with some variation, while the effective number of broodfish ranged from 0 – 35.7 (**table 3**). The highest possible number of broodfish used per brood year was 42, however crossings for this brood year in River Ørstaelva was not documented and it is likely that not all 42 individuals were used in crossings (Hagen et al. 2021b). While some brood years in some stocking programs achieved a high $N_{eBroodstock}/N_c$ ratio, the effective number of broodfish was generally around half of the number of broodfish that was crossed (average 0.52). In cases where few offspring were observed due to a low proportion stocked fish in the population, it is possible that the effective number of broodfish is underestimated, due to the low probability of recapturing stocked individuals. Moreover, we observed considerable variation in the ratio of effective number of broodfish to the number of broodfish crossed ($N_{eBroodstock}/N_C$) between brood years within stocking programs.

Table 3. *The lowest and highest number of broodfish crossed in the evaluated brood years (N_c - see table 2 for details) and average number of broodfish crossed, with the average number of broodfish in brackets, the observed range in effective number of broodfish ($N_{eBroodstock}$) and the average $N_{eBroodstock}/N_c$ ratio for each of the evaluated stocking programs. The average $N_{eBroodstock}/N_c$ ratio was calculated from brood years where $N_{eBroodstock}$ was >0.*

Population (river)	Number broodfish used (average)	$N_{eBroodstock}$	$N_{eBroodstock}/N_c$ ratio
Eira	24 – 33 (26)	16.0 – 32.3	0.81
Bævra	8 – 20 (15.8)	2.8 – 9.5	0.44
Surna	14 – 29 (23.5)	11.7 – 26.6	0.71
Årøyelva	10 – 12 (11.0)	9.5 – 10.9	0.93
Flekkeelva	18 – 20 (19.3)	4.4 – 18.5	0.62
Ørstaelva	16 – 42 (23.8)	1.8* – 12.6	0.23
Fetvassdraget	30 (30.0)	0 – 9.6	0.32
Korsbrekkelva	14 – 26 (21.3)	2.7* – 5.3*	0.20
Fortunselva	7 – 25 (15.0)	Not estimated	Not estimated
Daleelva	19 – 38 (28.6)	7.8 – 28.2	0.52
Arnaelva	28 – 30 (29.0)	2.9* – 12.5	0.26
Loneelva	32 – 37 (34.5)	6.0* – 12.8	0.28
Osenelva	30 – 35 (33.0)	Not estimated	Not estimated
Suldalslågen	27 – 28 (27.5)	16.5 – 35.7	0.94
Gaula	18 – 22 (20.0)	Not estimated	Not estimated
Bondalselva	27 – 30 (28.3)	0 – 14.7	0.54

* $N_{eBroodstock}$ uncertain and probably underestimated due to a low proportion stocked fish and thus a low probability of recapturing stocked fish.

3.2 Changes in effective population size

3.2.1 Observations of the Ryman-Laikre effect

Stocking is expected to affect the number of individuals in the population such that the number of individuals (the census N_c) in a stocked river is higher than if the river was not stocked. Preferably, this should not lead to a negative response in total effective population size of the stocked populations (Ryman-Laikre effect). In **figure 5** we have summarised the effect that stocking has had on 39 brood years in the evaluated populations expressed as N_{eTotal}/N_{eWild} and plotted this against the proportion stocked salmon in the populations. As shown in the figure, for more than half (N=20) of the analysed brood years N_{eTotal}/N_{eWild} was less than one (below the yellow line) and thus subject to a Ryman-Laikre effect. Also, there was a relationship between the strength of the Ryman-Laikre effect and the proportion stocked fish in the populations, such that with a higher proportion stocked fish, the Ryman-Laikre effect became more severe. Importantly, when the proportion stocked fish in the populations exceeded ~0.4, there has not been a high enough effective number of broodfish to avoid a Ryman-Laikre effect in any of the evaluated populations. Particularly for stocking programs releasing smolts (red and green dots in **figure 5**), there was strong Ryman-Laikre effect observed. In the stocking programs or brood years when a Ryman-Laikre effect has been observed, the number of broodfish (which generally range between 20 – 30 with effective number of broodfish generally half of the number that was crossed) has been too low for the proportion stocked fish in the stocked populations and the effective numbers of wild breeders.

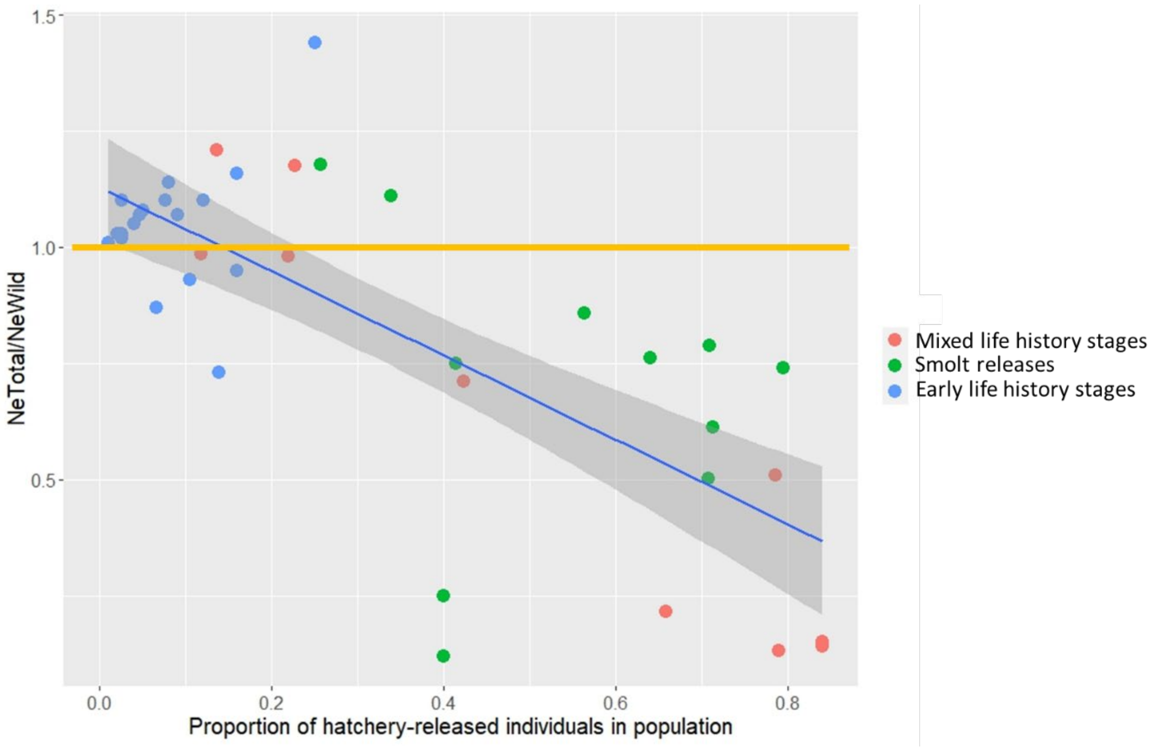

Figure 5. *Relationship between the proportion hatchery-produced individuals and the Ryman-Laikre effect presented as N_{eTotal}/N_{eWild} for 39 cohorts in the evaluated populations. The blue line is derived from simple least squares regression and the shaded area represents the standard error. The yellow line (N_{eTotal}/N_{eWild} = 1) represents combinations for which wild effective population sizes are equal to the total effective size, and stocking does not change the total effective population size. Above the yellow line stocking increased the total effective populations size and below the yellow line stocking leads to a decrease in total effective populations sizes. The colour of the dots indicates release stage.*

3.2.2 Reduced effective population size over time

When a population is subject to a Ryman-Laikre effect over several generations, which means that the process described in **figure 2** (section 1.2) is repeated several times, stocking has the potential to reduce the genetic variation in the recipient population. While the census numbers (N_c) of individuals in the populations may remain stable, the ratio of N_c/N_e may decrease. For given run years (which constitutes individuals from several brood years), the effective population size can be estimated using the 'linkage disequilibrium' approach. If samples from different time points are available, this method will inform us about changes in effective population size over time. For three of the evaluated populations (Rivers Eira, Årøy and Suldalslågen), historic samples were available, and serve as a comparison with the effective population size estimated using contemporary samples. These three populations were subject to a strong Ryman-Laikre effect, and a general decrease in effective population size over time was observed (**figure 6**).

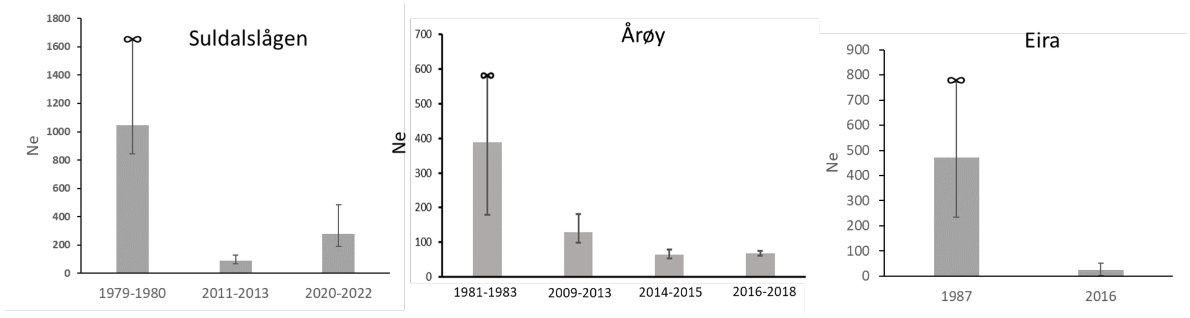

Figure 6. *A reduction in effective populations size (N_e) over time was observed in the salmon populations in the rivers Suldalslågen, Årøy and Eira. N_e was estimated using the linkage disequilibrium approach. It is highly probable that stocking contributed to the reduction in effective population size. For the earliest time points, the upper confidence interval was very high, or infinite for all three populations.*

The samples from the earliest time points in the three populations are from periods when stocking may have occurred, but with much lower survival of hatchery-produced individuals and subsequently lower proportions stocked fish in the populations. It is highly probable that stocking with subsequent strong Ryman-Laikre effect contributed to the reduced effective population size over time in these three populations. Bottlenecks occurring between the historic and the contemporary sampling points also have the potential to reduce the effective population size in populations. Stocking with a subsequent Ryman-Laikre effect in a population that has experienced a bottleneck will accentuate the negative effects of the bottleneck.

3.2.3 Adjusting the number of broodfish to avoid a Ryman-Laikre effect

To implement stocking such that a negative response on the total effective populations size is avoided, the effective number of broodfish must be balanced against the effective number of wild spawners and the proportion stocked fish in the population. Here, we present two examples illustrating the effective number of broodfish required to avoid a Ryman-Laikre effect in i) a population with a census of 300 wild spawners and ii) a population with a census of 2000 wild spawners for 25% and 50% stocked fish, respectively. As explained in section 2.1.3, the effective number of wild parents in Atlantic salmon has been estimated to be $^1/_3$ – $^1/_2$ of the census. For simplicity we here assume a N_c/N_e ratio of $^1/_2$, thus we estimate the required effective number of broodfish to avoid a Ryman-Laikre effect given 150 and 1000 effective wild parents and 25 and 50% stocked fish. As illustrated in **figure 7**, in a population with a census of 300 wild breeders, the effective number of broodfish must be 50 to avoid a Ryman-Laikre effect if there is 50% stocked fish in the population. Given that there is 25% stocked fish, the effective number of broodfish must be at least 21. In the evaluated stocking programs, the effective number of broodfish was around half of the number of broodfish used. This means that under the same average N_e/N_c ratio, the number of broodfish crossed must be 42 or 100, respectively, to achieve a sufficient effective size to avoid a Ryman-Laikre effect at 25 and 50% stocked fish. In a population with 150 effective wild parents and 25% stocked fish, an effective size of 50 broodfish will increase the total effective size of the recipient population. Simliarly, in a population with a census size of 2000 wild breeders, the effective number of broodfish must be 335 to avoid a Ryman-Laikre effect if there is 50% stocked fish in the population. Under the same N_e/N_c ratio as previously observed for broodfish, 670 broodfish would have to be taken into the hatchery and crossed to avoid a Ryman-Laikre effect. Reducing the proportion stocked fish to 25% subsequently reduces the required effective number of broodfish to 145, whilst an $N_{eBroodstock}$ of 300 will increase the total effective size of the recipient population. The effective numbers of broodfish required to avoid a Ryman-Laikre effect at 50% stocked fish are much higher than what has traditionally been used in Norwegian stocking programs.

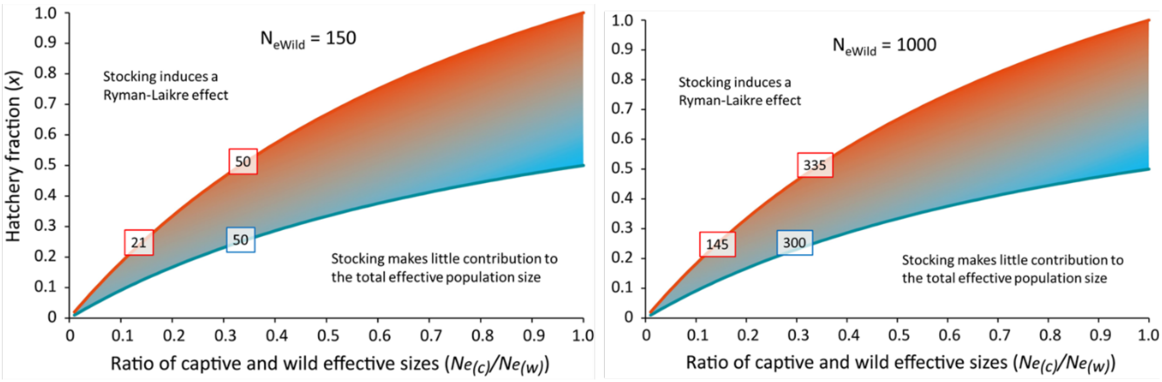

Figure 7. *The ratio of the stocked and wild effective populations sizes plotted against the proportion of stocked fish according to the model presented in Karlsson et al. (2016) and Waples et al. (2016). The red line represents combinations for which wild effective population sizes are equal to the total effective size, and stocking does not change the total effective population size. Above the red line, stocking reduces the total effective population size. The blue line represents the maximum total effective population size that can be achieved at different proportions stocked fish. Below the blue line, the contribution from stocking to the total effective population size is minor. The red boxes illustrate the effective number of broodfish that is required to avoid a Ryman-Laikre effect at 25 and 50% stocked fish. The blue boxes illustrate the effective number of broodfish that produce the highest total effective size given N_{eWild} of 150 or 1000 and 25% stocked fish.*

Using individuals as broodfish means they are not part of the wild spawning population. The broodfish are thus excluded from the census in the above examples. Achieving $N_{eBroodfish}$ of 50 given N_e/N_c ratio of 0.5 means that 100 broodfish must be taken out from the population. Consequently, the population must consist of 400 individuals, out of which 25% of the population must be used as broodfish if there is 50% stocked fish in the population. Similarly, for the larger population with a census of 2000 spawners, the population must consist of 2670 individuals before the broodfish are removed, given an N_e/N_c ratio of 0.5 and 50% stocked fish.

A higher N_e/N_c ratio than the average observed here (0.5) can be achieved by ensuring equal contribution between different family groups and is very important to pursue because it will allow a higher proportion stocked fish in relation to the number of broodfish used. If the sex ratio is equal (e.g., the same number of males and females are crossed) and the variance in contribution is low, the N_e/N_c ratio may exceed 1. In an extreme case where all broodfish contribute equally, the N_e/N_c ratio will be close to 2 (Karlsson et al. 2016). For the 2014 brood year in River Suldalslågen the N_e/N_c ratio was 1.28, due to the relatively equal contribution of the different families. However, as the spawning population in River Suldalslågen is relatively large with more than 1000 spawners, the number of broodfish used was not sufficient to avoid a severe Ryman-Laikre effect despite the high N_e/N_c ratio. Moreover, for the brood year 2015, the N_e/N_c ratio in the same population was 0.61, mainly due to larger variation in contribution between families, thus illustrating how variance in contribution can differ between brood years.

3.2.4 Reusing stocked fish as broodfish

In some populations, hatchery-produced fish has been frequently used as broodfish. Of the broodfish used in River Eira in 2005 and 2006, 14 broodfish pairs were found to have on average 20 observed 'grandchildren' in the population (range 1-86) (Hagen et al. 2020). In River

Fortunselva, where a comprehensive pedigree for the broodfish was developed, some broodfish were found to have 5 generations of hatchery background, and more than 50% of the individuals used as broodfish were of hatchery origin (Hagen et al. 2021d). Also in River Daleelva, more than 50% of the broodfish were hatchery-produced (Hagen et al. 2023a). In Rivers Eira and Daleelva, a Ryman-Laikre effect was observed, and in River Fortunselva a Ryman-Laikre effect is probable due to the high proportion stocked fish. Reusing stocked fish as broodfish may mean that some families are allowed a disproportionately large number of descendants compared to other families, which again may lead to reduced genetic variation. Moreover, reusing stocked fish as broodfish will further accentuate the negative effects of a Ryman-Laikre effect. Stocked fish should therefore be avoided as broodfish, particularly in populations where the proportion stocked fish is large.

3.3 When the proportion stocked fish in the recipient population is low

Fertilising and keeping eggs in a hatchery over winter is presumably done under the expectation that survival of eggs and alevins will be higher in the hatchery and that these will have an overall higher probability of surviving until adults than if they were spawned naturally. However, for some stocking programs releasing young life history stages, a very low proportion stocked fish in the population has been observed. For some brood years in rivers where eyed eggs or alevins have been released, no or few hatchery-produced individuals have been recaptured, and hatchery released individuals may comprise 0-2% of the adult individuals that were produced a given brood year (Hagen et al. 2021b, Hagen et al. 2022, Hagen et al. 2023c). Yet, broodfish are removed from the population to be stripped in the hatchery and thus denied the opportunity to spawn naturally. For instance, in River Ørstaelva, around 40 genetically wild broodfish have been caught annually (Hagen et al. 2021b). In populations where the spawning target is not met, such as in River Ørstaelva, this may constitute a considerable part of the spawning population and when the proportion stocked fish is very low, it is possible that the broodfish would have produced more offspring if they spawned naturally in the river.

One can assume that the contribution by stocking should be at least equal to the proportion that the released eggs or fry make up compared to the spawning target. If survival of the released individuals is lower than the average survival among naturally spawned individuals, the catch of broodfish will lead to fewer individuals in the population compared to no stocking. If the objective of stocking is to increase the number of individuals in the river but a quantification of the proportion stocked fish suggests that stocking has little or no contribution or even reduce the number of individuals in the river, conservation managers and administrators of stocking programs should either:

- Try to find the cause for the relatively low survival and change stocking practice, for example by adjusting time, space, temperature regimes, etc, such that the broodstock produce more offspring to the next spawning generation.
- Discontinue stocking of the population in question.

The development from fertilization to hatching is dependent on water temperature (Crisp 1981; 1988) and a probable reason for poor survival of stocked eyed eggs and alevins is a mismatch between the temperature in the river, and thus food availability, and the developmental stage of the hatchery-produced individuals. Better monitoring and control of the development in the hatchery and informed decision about the appropriate release time according to river temperature is likely to improve survival rates of hatchery-released eyed eggs and alevins. Also, survival of hatchery-produced individuals can be increased by separating the release stage of each family into eyed eggs, unfed alevins and alevins that have had a short period of pellet feed.

3.4 Are the broodfish representative for the population?

The guidelines of The Norwegian Environment Agency state that the broodstock should be a random sample of the phenotypes of the donor population (Anonymous 2011). This means that the average length and weight of broodfish should not deviate from the distribution that is observed in the rivers from which the broodfish originate. In nine of the evaluated stocking programs, we investigated the size of the broodfish and how this compared with the average weight (for Gaula only length was available) of individuals in the population. In all cases we found the broodstock to be larger than the population average (**figure 8**), and in all but one case the difference in size was significant at an alpha level of 0.05. For River Bondalselva, the p-value was 0.06 for weight as shown in **figure 8**, but no difference was found for length. For River Gaula, the underlaying data is uncertain, as anglers must observe fishing regulations that limit the culling of females and fish over 80 cm during parts of the angling season (Robertsen et al. 2021).

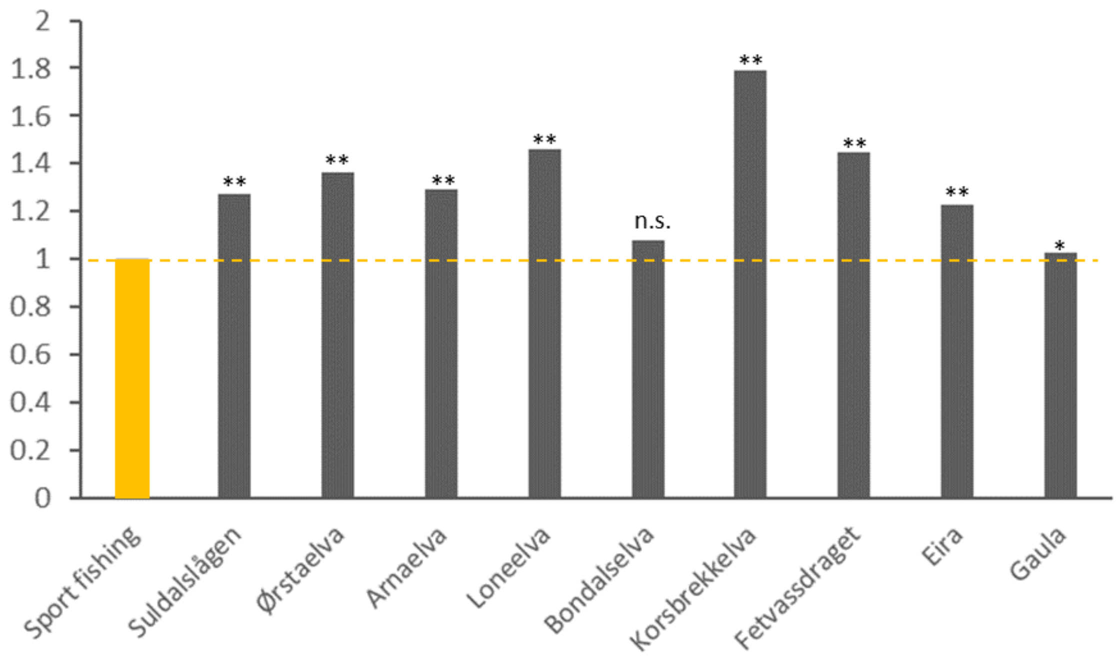

Figure 8. *The weight of broodfish from eight stocking programs and length for River Gaula scaled according to the size of individuals caught during sport fishing (yellow column) in the same populations. Asterisk above columns represent p-values for the respective comparisons with the wild populations. One asterisk indicated p-values < 0.01, whilst two indicate p-values < 0.001. The underlying data for River Gaula is uncertain, due to fishing regulations to protect females and large individuals in this population.*

These results show that for most stocking programs (with exception of Rivers Bondalselva and possibly Gaula) there has been a tradition for selecting the largest individuals as broodfish, a precedence that is not supported by the guidelines provided by the Norwegian Environment Agency.

For two of the stocked populations, we studied the frequency of a gene that is tightly linked to age at maturity and body size; the *vgll3* gene (Barson et al. 2015). Females with at least one ‘late’ allele and males with two ‘late’ alleles are likely to stay longer at sea compared to females with two ‘early’ alleles and males with one ‘early’ allele. By comparing the frequency of the ‘late’ allele, we were able to relate the non-random selection of broodfish to selection for a specific genotype. Where the underlying data allowed for a comparison of *vgll3* allele frequency between broodfish and individuals caught during sport fishing, we found that the larger size of broodfish coincided with the broodfish having a higher proportion of the ‘late’ allele for the gene *vgll3* (**figure 9**). This means that there has been artificial selection for individuals with a different allele frequency compared to the frequency that has been selected during local adaptation in nature.

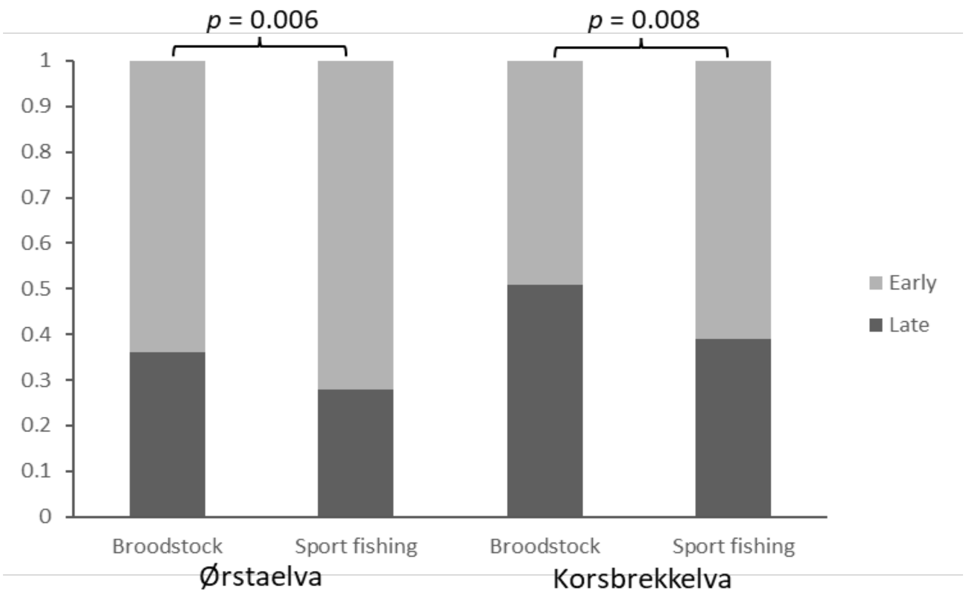

***Figure 9.** Frequency of the 'early' and 'late' alleles of vgll3 in broodstock and individuals caught during sport fishing in Rivers Ørstaelva and Korsbrekkelva.*

3.5 Domestication effects

When individuals are kept in captive environments, adaptation to captivity is inevitable and may occur in a single generation (Christie et al. 2012, Milot et al. 2013). Domestication selection can occur also when it is unintended and when the main objective for captive breeding is motivated by conservation purposes to maintain genetic integrity *ex situ* and to provide demographic support until threats to the wild populations can be resolved. A review of 70 studies where the fitness of hatchery produced individuals and naturally produced individuals was compared found that in 23 of these studies, the hatchery reared individuals had lower fitness. Moreover, 28 studies documented reduced genetic variation in stocked populations (Araki & Schmid 2010). Fitness of hatchery reared rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon and brown trout (*Salmo trutta*) have been found to cumulatively decrease with several generations in hatchery conditions (Araki et al. 2007). Hatchery produced coho salmon (*O. kisutch*) had lower lifetime reproductive success than wild fish, also when they were released as unfed fry, but not if they used a sneaker mating strategy, thus suggesting that absence of sexual selection in the hatchery could contribute to the observed fitness declines (Thériault et al. 2011). A review by (Christie et al. 2014) covering four salmonid species found reduced reproductive success in all species following hatchery rearing. Millions of hatchery-produced pink salmon (*O. gorbuscha*) are released annually into Price William Sound, Alaska. Hatchery-produced pink salmon in this region has been found to arrive freshwater later than the naturally produced pink salmon and to migrate further up the rivers than the non-hatchery pink salmon, which often spawn in the intertidal outlets (Knudsen et al. 2021; May et al. 2023). Such differences between naturally produced and hatchery released individuals may indicate that there is selection in the hatchery that impacts both temporal and spatial aspects of the return migration.

3.5.1 Genetic domestication effects

Because individuals that are already genetically adapted to a captive environment will have higher fitness in captivity than individuals that are not adapted to captivity (Christie et al. 2012, Hagen et al. 2019), individuals with hatchery background, farmed escapees and hybrids between farmed and wild salmon will survive better and produce more offspring in captivity than completely wild individuals. This means that the hatchery environment may accentuate domesticated genotypes in stocked populations. In rainbow trout it was found that individuals released as smolt produce more offspring as hatchery broodfish and fewer offspring when spawning naturally than naturally produced individuals (Christie et al. 2012). For Atlantic salmon in River Eira, we found that broodstock females with a high proportion farmed ancestry produced four times as many returning offspring in the river, compared to broodstock females with genetically wild ancestry (**figure 10,** left panel) (Hagen et al. 2019). This effect was likely due to a strong selection in the hatchery for individuals with farmed ancestry, as well as selection at sea, where large individuals have higher survival. The introgressed smolts released from the brood years 2005-2011 were 6.2% larger than genetically wild hatchery released smolt from the same brood years (Hagen et al. 2019). This effect led to overall increased farmed genetic introgression in River Eira during the following run years (**figure 10,** right panel). Additionally, we found that broodstock females with a high proportion farmed ancestry produced smaller eggs than genetically wild broodstock (**figure 10**, middle panel), most likely due to relaxed natural selection under hatchery conditions. The example from River Eira demonstrates the importance of the Norwegian broodstock control program, and that there is a high risk of unintentional selection towards individuals being adapted to an artificial hatchery environment. Similar screening programs should be in place in all regions where domesticated escapees or introgressed individuals may be used as broodstock.

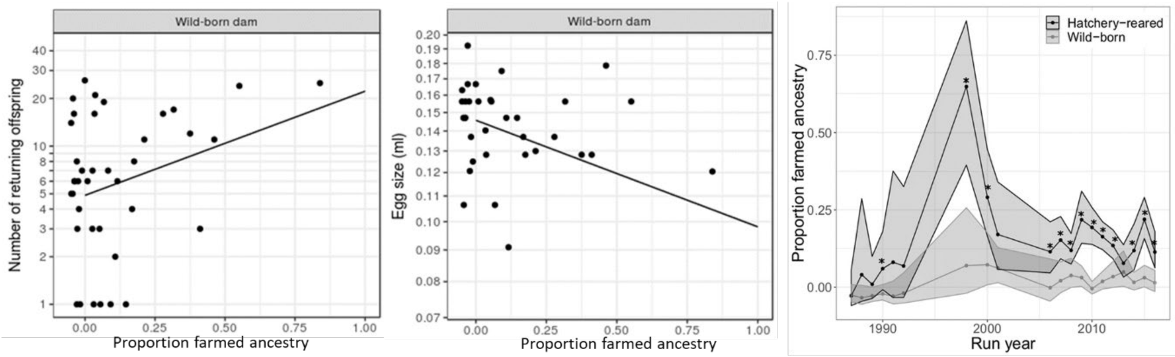

Figure 10. *The panel to the left shows the effect of farmed genetic introgression (here expressed as proportion farmed ancestry in broodfish females) on the number of returning offspring from smolt releases in River Eira. The middle panel shows the effect of farmed genetic introgression in broodfish females on egg size. The panel to the right shows the farmed genetic introgression in hatchery-reared and wild-born adult spawners caught in River Eira. During all run years, hatchery reared individuals were more introgressed than naturally produced individuals. Stocking therefore increased the farmed genetic introgression in River Eira during these run years. Figures are from Hagen et al. (2019).*

3.5.2 Epigenetic domestication effects

An important mechanism in rapid adaptation is physical changes to the genome that are functionally relevant but do not involve a change in the genetic code (DNA sequence) - that is - epigenetic modifications (Christie et al. 2016, Jablonka & Lamb 2002, Le Luyer et al. 2017). Environmentally induced epigenetic modifications can alter the developmental trajectory of an individual and lead to the expression of alternative phenotypes from a single genotype and represent a mechanism of phenotypic plasticity (Kucharski et al. 2008). It is therefore likely that the large phenotypic difference between captive and wild individuals is partially due to epigenetic differences that are induced in each generation due to nutrition and the captive environment (context-dependent) and heritable epigenetic changes that have accumulated over generations (transgenerational epigenetics).

In the River Eira stocking program, a probable epigenetic adaptation to the hatchery environment was observed: broodstock females that had been released as smolt and spent 1-3 years at sea prior to being caught as broodfish produced 1.75% more adult offspring compared to female broodfish that did not have a hatchery background. Also, the broodfish females with hatchery background produced eggs that were 0.86% smaller than broodfish females that did not have prior hatchery background (Hagen et al. 2019). It is likely that the hatchery reared females spawning naturally have eggs of comparable size as the hatchery reared broodfish and that this may decrease the fitness of hatchery reared offspring in the wild, as egg size is associated with fitness (Heath et al. 2003).

Moreover, a range of studies have documented epigenetic changes in individuals that experience hatchery environments. Both in Pacific salmon (Christie et al. 2016, Le Luyer et al. 2017) and Atlantic salmon (Rodriguez Barreto et al. 2019) were expression for several hundred genes altered by hatchery rearing compared to a natural environment. Large epigenetic differences were observed between juvenile rainbow trout held in tank environments that were either barren or contained natural substrate (Gavery et al. 2019, Reiser et al. 2021), and epigenetic changes have been observed in response to different food components (Saito et al. 2021). The duration of these environmentally induced epigenetic changes is generally not known.

4 Release of smolts versus younger life history stages

As illustrated in **figure 5**, the highest proportions stocked fish were observed in stocking programs where smolts are released. In stocking programs that release several life history stages, such as Rivers Daleelva (Hagen et al. 2023a), Fortunelva (Hagen et al. 2021d) and Bævra (Hagen et al. 2021a), individuals released as smolt constitute a large proportion of the stocked fish in the populations. In the Rivers Eira, Bævra, Årøy, Fortunelva, Suldalslågen and Daleelva (see **table 2** for details) the proportion stocked fish exceed 40% and for Daleelva the proportion stocked fish was 82%. The high proportion stocked fish in these rivers is the result of high numbers of smolts released, relative to the estimated natural production. In River Eira, 50 000 smolts are released annually, whilst the natural smolt production has been estimated to ~31 000 (Hindar et al. 2007). This means that that the releases in River Eira constitute 158% of the natural smolt production. Similarly, for River Årøy, the releases constitute 253% of natural smolt production, and probably more than 50% of the natural production in River Suldalslågen. Smolt production in River Daleelva was estimated to 11 310 in Vollset et al. (2022). In this river 10 000 – 25 000 smolt and around 50 000 eyed eggs are released annually. The smolt releases thus constitute 88 – 221% of the natural production in Daleelva.

In two different populations of Atlantic salmon (River Burrishoole in Ireland and River Imsa in Norway) O'Sullivan et al. (2020) and Jonsson et al. (2019) found that production of juveniles decreased with increasing proportion of spawners being released as smolts. As previously described, many studies have documented lower relative reproductive success of individuals released as smolt, and in some cases also individuals released as parr. This indicates that hatchery produced smolts are maladapted in natural populations.

Given the potential for high proportions of stocked fish in the populations when smolts are released, and the negative effects that hatchery produced smolts can have for production of juveniles when they return as adults, the release of smolts have the potential for severely negative effects in stocked populations. A high proportion of individuals released as smolt (as seen in Rivers Suldalslågen, Eira, Daleelva and Årøy) may therefore reduce the natural production more than if a lower proportion of the potential spawners were released as smolts. Lowering the numbers of released smolts and thus decreasing the proportion stocked fish may therefore both reduce the severe Ryman-Laikre effects documented in these populations and increase natural production. If available habitat exists within the rivers, releasing individuals at younger life history stages would allow more time for natural selection to act and produce individuals that are phenotypically and genetically better adapted to the local environment.

5 Conclusions

Each stocking programme is different with respect to how it is managed, the life history stages that are released, and the numbers of released individuals compared to the natural production. Information from one programme can therefore not uncritically be extrapolated to others. Nevertheless, the following general trends have emerged:

Proportion stocked fish and the effective number of broodfish can vary between brood years. Evaluating several brood years give a better representation of the variation and overall effects of stocking within each stocking programme.

In Norwegian stocking programs, the number of broodfish has been relatively stable over the years and ranged around 20 – 30 individuals regardless of the size of the wild population and the proportion stocked fish. When the proportion stocked fish has been low, the number of broodfish has in many cases been sufficient to avoid a Ryman-Laikre effect and for several cohorts the total effective size of the population has been increased by stocking. However, when the proportion stocked fish has reached 40% or higher, the effective number of broodfish has not been sufficient to avoid a Ryman-Laikre effect in any of the evaluated cohorts.

To maintain a high proportion stocked fish, a large proportion of the wild population must be taken into hatcheries as broodstock: 50% stocked fish in a population demands that 25% of the wild population is used as broodstock to avoid a Ryman-Laikre effect if the $N_{eBroodstock}/N_c$ ratio is 0.5, which is the average ratio observed in the 16 stocking programs.

A motivation for stocking is to maintain a higher number of adult fish compared to the un-supplemented population. To obtain this, the production from each broodfish in the hatchery must be higher than if the fish spawned in the river but at the same time not too high to avoid a Ryman-Laikre effect. Consequently, it is important to know the relative contribution of adults from the broodfish and from the naturally spawning fish. Furthermore, it is important to maximise the ratio between effective number of broodfish and the actual number of broodfish. This will allow a relatively larger contribution from the stocking programme into the wild populations, or alternatively, that fewer broodfish is needed.

A strong Ryman-Laikre effect over several generations may deplete the genetic variation in the focal populations over time and thus lead to the population being less able to adapt to environmental changes.

The highest proportions stocked fish and the most severe Ryman-Laikre effects were observed in the stocking programs that release smolts as compared to the release of younger life stages. This is probably due to the large number of individuals released and the relatively high survival of released smolts. Individuals released as smolt are subject to behavioural adaptation to artificial environments, genetic and epigenetic domestication selection and are less adapted to the natural environment than individuals that spend more of their life in the rivers. Negative relationships have been found between proportion adult individuals released as smolts and the natural production in the populations. This means that smolt releases have a potential for strong negative effects, also when there is no Ryman-Laikre effect because their phenotype is less adapted to the natural habitat.

A practice for selecting large individuals as broodfish is common. Size has a significant genetic component in Atlantic salmon and selection for large individuals means that hatcheries release individuals with gene frequencies that are different from the gene frequencies in the wild gene pool, which are a result of local adaptation in nature. Such practice in stocking will therefore counteract local genetic adaptation of the wild populations.

When the proportion stocked fish is lower than expected from the biomass of females used as broodfish compared to the biomass that spawned naturally, stocking should be discontinued, or efforts should be made to increase survival of stocked individuals.

In regions outside of Norway where stocking of Atlantic salmon occurs and farmed escapees or introgressed individuals are present, conservation managers should establish broodstock control programs to ensure that individuals with farmed ancestry are not used as broodstock and that stocked fish can be identified to enable evaluation of the stocking programmes.

With advancing research and more knowledge, the approach for estimating the effective number of wild breeders has altered from being based mainly on a genetic method, to being mainly based on counts of broodfish. This is due to a better understanding of the limitations of using only genetic samples to estimate N_{eWild}, as well as more data on the census numbers of spawners. This increasing knowledge base indicates that when N_{eWild} was estimated from genetic methods, the effective size of the wild population is in many cases underestimated. This also means that a documented Ryman-Laikre effect is more severe than estimated, and a Ryman-Laikre effect may also be the case in populations where this effect was previously not documented. **Table 3** summarises the observations with respect to the different stocking programmes, with corrected Ryman-Laikre effect where this is appropriate.

Table 3. *A summary of Ryman-Laikre effects (R-L effect), lowest and highest observed stocked fish with average in brackets, whether the broodfish were found to be representative of the donor population and comments pertain to the evaluation of the respective stocking programs. Strong Ryman-Laikre effect =* $N_{eTotal}/N_{eWild} < 0.5$*; Moderate Ryman-Laikre effect =* $N_{eTotal}/N_{eWild} < 0.8$*.*

Population (river)	R-L effect	% stocked (average)	Broodfish representative	Comments
Eira*	Strong	26-79 (58)	Not repr.	R-L effect probably further accentuated by use of stocked fish as broodfish
Bævra*	Strong	12-79 (50)	n.a.	
Surna*	Strong	14-28 (19)	n.a.	
Årøyelva*	Strong	34-71 (53)	n.a.	
Flekkeelva*	Probably strong	5-25 (13)	n.a.	Stocking discontinued
Ørstaelva	Moderate	1-16 (8)	Not repr.	2010: R-L effect. 2012 and 2014: the broodfish produced less than expected
Fetvassdraget	None	0-8 (4)	Not repr.	2014: broodfish produced less than expected
Korsbrekkelva	None	3-4 (3)	Not repr.	Stocking discontinued
Fortunselva	Not est.	50	n.a.	
Daleelva	Strong	79-84 (82)	n.a.	R-L effect probably further accentuated by use of stocked fish as broodfish
Arnaelva	Moderate	7-14 (10)	Not repr.	
Loneelva	None	5-16 (10)	n.a.	
Osenelva	None	5-13 (9)	Not repr.	
Suldalslågen	Strong	40 (40)	Not repr.	
Gaula	Not est.	3 (3)	Data uncertain	
Bondalselva	Minor	0-11 (4)	Mostly repr.	2015: the broodfish produced less than expected

* New analyses suggest that the spawning population was larger than previously estimated and reported. A Ryman-Laikre effect is therefore more severe than previously estimated.

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