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A tale of two hatcheries: Assessing bias in the hatchery process for Atlantic salmon (*Salmo salar* L.)

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Abstract

Stock enhancement of Atlantic salmon (*Salmo salar* L.), a fish of considerable economic and social importance, is commonplace. Supportive-breeding is a well-recognised method of enhancement which, when compared with traditional hatchery practices, is thought to reduce the severity of selection pressures on broodstock fish. Critically, in supportive-breeding programmes, the eggs and sperm used in the breeding process are taken from wild adult fish originating from the same catchment that resulting juvenile fish are subsequently stocked into, thereby avoiding problems associated with a lack of local adaptation in the stocked fish. Previous studies have indicated that sex bias during the hatchery process may result in reduced genetic diversity of the offspring. Utilising 16 microsatellite loci and two expressed sequence tag (EST) loci, we examined progeny from two hatcheries located on the rivers Exe and Tamar in southwest England, assessing the genetic diversity and parental contribution at each. Two strains were assessed within each hatchery. Genetic diversity was found to be reduced in offspring compared with that of the parent fish. This is likely the result of utilising a small number of broodstock in combination with parental bias. In the four hatchery strains studied (Bar, LEx, Lyd and TXL), parental contribution ranged between 2.1–29.2%, 12.2–51.0%, 2.0–70.0% and 4.0–40.0%, respectively. If this practice is to be continued, efforts should be made to improve adherence to national rearing guidelines by increasing the number of broodstock fish utilised and ensuring a more balanced contribution of all adults during the crossing process. Ultimately, we suggest a need to review the suitability of current national Atlantic salmon hatchery guidelines, particularly with regard to their use and relevance in small European rearing systems.

Keywords

Salmo salar, supportive-breeding, genetic diversity, broodstock, parental bias

1. Introduction

Atlantic salmon, (*Salmo salar* L.) have declined in number across their range due to a variety of factors both in river and at sea (Parrish et al., 1998). In southwest England, potential causes include pollution, (Ellis et al., 2011; Hendry et al., 2007), historic exogenous stocking (Ellis et al., 2011 and references therein; Finnegan and Stevens, 2008; D. Solomon, *Pers. Comm*.), exploitation in river and at sea (Aprahamian et al., 2010, Consuegra et al., 2005, Klemetsen et al., 2003, Roberts, 2007) and disease (e.g. ulcerated dermal necrosis, UDN) (Roberts, 1993).

Due to the socio-economic importance of sport fish such as Atlantic salmon, stock enhancement activities, including exogenous stocking, captive and supportive-breeding, have long been commonplace (Horreo et al., 2011a; Kitada, 2009; Solomon et al., 2003). However, an increasing number of studies show exogenous stocking to have been largely ineffective in improving numbers of returning adult fish (Ciborowski et al., 2007; Griffiths et al., 2011; Young, 2013), whilst others have indicated natural re-colonisation from nearby catchments to be the dominant process in salmon population recovery in locations where the cause of decline has been removed (e.g. pollution, fishing pressure) (Griffiths et al., 2011; Ikediashi et al., 2012; Perrier et al. 2010). In some cases the genetic signature of stocked fish can persist in a new environment (e.g. Finnegan and Stevens, 2008; McGinnity et al., 2009), however, it is now deemed more appropriate to stock with 'local' fish that are genetically compatible and locally adapted (Solomon et al., 2003). For this reason, supportive-breeding using eggs and sperm stripped from native adult fish is a more logical option than utilising exogenous or captive-bred fish which are at an adaptive, reproductive and genetic disadvantage (Araki et al., 2007). In some hatcheries these broodstock are collected on one occasion and maintained for generations (e.g. Koljonen et al., 2002), whereas in other situations the broodstock are taken annually from the wild, so that the offspring to be released are all first generation hatchery stock (e.g. Horreo et al., 2008).

Hatchery-reared fish are known to undergo selection to optimise survival in a hatchery environment, not survival in the wild (Blanchet et al., 2008; Christie et al., 2012a); thus, hatchery offspring often suffer reduced genetic diversity (Araki and Schmid, 2010; Horreo et al., 2008; Koljonen et al., 2002; Stahl, 1983; Verspoor, 1988), reduced Ne (Christie et al., 2012b; Ryman & Laikre, 1991; Verspoor, 1988) and reduced fitness (Ford, 2002; Fraser, 2008), though this is not always the case (Araki and Schmid, 2010). In some cases, little impact of hatchery processes has been found. For

example, Tessier et al. (1997) found a supportively-bred stock of landlocked *S. salar* to have decreased allele frequencies, however, heterozygosity was not altered. Similarly, Eldridge et al. (2008) found that the genetic diversity of a Chinook salmon (*Oncorynchus tshawutscha*) supportive-breeding stock did not contribute to a loss of genetic diversity in the captive offspring produced, nor the wild stocks that they were supplementing. Finally, Jeong et al. (2007) found no morphological difference (weight, length) between supportively-bred Black sea bream (*Sponyliosoma cantharus)* and their wild conspecifics, despite 69.3% of the offspring being assigned to one breeding pair of the 51 available broodstock. These examples illustrate that the matter is not clear-cut and that there may be ways to optimise practices and minimise negative effects.

Populations with reduced genetic diversity are known to be more vulnerable to stochastic changes and less able to adapt and survive (Koljonen et al., 2002). Supportive-breeding is known to minimise the effects of traditional hatchery practices on adaptation and selection (Araki, 2008), but this by no means eliminates the problems entirely (Blanchet et al, 2008; Christie et al., 2012 a). There is clearly a trade-off between supplementation and the genetic integrity of supplemented stocks (Fraser, 2008).

As evidenced above, a number of empirical and theoretical studies have been completed over the last 50 years to assess the impact of hatchery processes on both the F1 generation and wild fish from genetic and ecological viewpoints. Typically, however, these studies have focused on the Pacific Northwest (Waples, 1991) and/or at a global scale (Araki & Schmid, 2010, Fraser, 2008). These studies demonstrate an inconsistency in the effects on the F1 generation. The majority illustrate a negative effect on the fitness and genetic integrity of the stocks being produced and supported; however, with careful management these effects can be minimised. In England and Wales the Environment Agency (EA) have provided guidelines to minimise these effects (Environment Agency, 2011). These guidelines focus on the number of broodstock, sex ratios, mating strategies and maintaining environmental conditions similar to that in the wild. These strategies will later be discussed in terms of the results of this study.

Considering the inconsistent results, in addition to the calls for further empirical studies to clarify the effects of supportive-breeding practices (Fraser, 2008) and the uncertainties in associated theoretical analyses (Kalinowski et al., 2012), it would seem

prudent to explore the impacts of supportive-breeding practice at an approved hatchery following Environment Agency guidelines for the management of Atlantic salmon, to assess whether current UK guidelines are sufficient to preserve the genetic integrity of the F1 stock.

As such, in this study, the effectiveness of two hatcheries operating supportive-breeding programmes in southwest England were compared, with a view to informing management decisions on Atlantic salmon hatchery practice in future years. Using microsatellite-based genetic analysis we examined (i) the effectiveness of the hatchery at preserving genetic diversity, and (ii) the effectiveness of paired mating strategies in balancing parental contribution and maintaining effective population size. These results have been used to refine hatchery practices and will guide further fish-related management decisions within the catchments, whilst also highlighting potential issues and points of good practice for supportive-breeding programmes in general.

2. Methodology

2.1 Hatchery operations

This study analysed salmon from two hatcheries in Devon, England, one at Endsleigh on the river Tamar, the other at Exebridge at the confluence of the Rivers Barle and Exe (Figure 1). Supportive-breeding comprises the stripping of eggs and sperm from wild, sexually mature adult fish at a hatchery; typically, the adult fish are then returned to their native river. Whilst the stage at which juveniles are released can vary from ova to smolts (Aprahamian *et al.*, 2003; McGinnity *et al.*, 2004; Saltveit, 2006), both the hatcheries studied (Exe and Tamar, Figure 1) release the majority of their juveniles as 0+ parr; however, in the past some 1+ parr and smolts have also been released.

A small number of broodstock (≤15) were used at each hatchery. It should be noted that throughout this study broodstock collection and mating design followed standard hatchery protocols and guidance from the Environment Agency. While this may not constitute optimal experimental design, we opted to follow standard protocols to allow assessment of the success of current hatchery practices.

2.1.1 Exe catchment

Two separate strains are reared annually at the Exe hatchery. In 2010/2011, 13 adult fish were reported as having been collected by rod and line from two regions of the river Exe: (i) the main river, near the confluence with the Barle tributary (5 male, 4 female); (ii) the Little Exe (2 male, 2 female). In the subsequent analysis, 100 0+ parr

were sampled and analysed: 50 were of Barle heritage, and 50 were of Little Exe heritage (Table 1). Barle fish were sampled across three raceways whilst Little Exe fish were sampled from two raceways. The mating design at the hatchery was such that each raceway sampled contained the offspring from two females that had each been paired with two males. In some cases a third male was utilised by hatchery staff as a 'precaution'. No further information was recorded by hatchery staff regarding the parental crosses.

2.1.2 Tamar catchment

At the Tamar hatchery, 15 adult broodstock salmon (6 male, 9 female) from two regions of the river were used as founders for all strains at the Tamar hatchery; only 11 fish (6 male, 5 female) were recorded by hatchery staff as having contributed to the two raceways (TXL and Lyd) analysed in the current study (Table 1). Adult fish were net-caught or caught by rod and line in November and December 2009. Lyd fish (seven broodstock) were caught in the main channel of the Lyd (a separate sub-catchment of the Tamar), while Tamar fish (eight broodstock) were collected from throughout the Tamar catchment. Each female was crossed with two males (in case one was later shown to be infertile), and each male was crossed with more than one female; the scientific merits of this approach are open to debate, but this protocol represents standard hatchery practice and thus we chose to use it as the basis for our subsequent analysis of hatchery-reared genetic diversity. Eleven adults (three females and three males from the Tamar; two females and four males from the Lyd sub-catchment) were recorded in hatchery records as having contributed to these two raceways, with some adults contributing to both (Table A.3). Raceway Lyd was recorded as containing progeny of only Lyd fish (three females, four males), while raceway TXL contained progeny of Tamar fish and Tamar x Lyd crossed fish (three females, four males). Based on electrofishing surveys of juvenile salmon, the Lyd sub-catchment is described as performing well, with regard to the number of fish produced (Environment Agency, 2010; B. Stockley, *Pers. Comm*.), and as such would be expected to contribute naturally to the re-colonisation of the Tamar; for this reason, the Tamar x Lyd cross was specifically implemented by the hatchery manager.

2.2 Tissue collection

At both hatcheries, tissues for genetic analysis were taken by adipose fin-clipping accoring to UK Home Office guidelines. Shortly after spawning, fin tissue was clipped from anaesthetised fish and stored in absolute ethanol at approximately 4°C prior to DNA extraction. All fish were anaesthetised using MS222 (10mg/L). Adult salmon

were allowed to recover in aerated holding tanks prior to release back into their respective rivers. Juveniles were returned to their raceways prior to release into their respective catchments.

2.3 Microsatellite analysis

Genomic DNA for microsatellite analysis was extracted from adipose fin tissue using the HotSHOT protocol (Truett et al., 2000). A panel of 19 microsatellite loci, in five PCR multiplexes, was used to characterise each fish (Table A.1); this comprised two expressed sequence tag-related loci (ESTs) CAO48828, CAO6177 (Vasemagi et al., 2005), an MHC-linked microsatellite (Sasa-TAP2A; Grimholt et al., 2002) and 16 neutral microsatellite markers (Ikediashi et al., 2012): Ssosl417, Ssosl85 (Slettan et al., 1995), Ssa202, Ssa197, Ssa171 (O' Reilly et al., 1996), SSspG7, SSsp3016, SSsp2216, SSsp2210, SSsp1605, SSsp2201 (Paterson et al*.,* 2004), Ssa14, Ssa289 (McConnell et al*.,* 1995), SsaF43, (Sanchez et al*.,* 1996), SsaD144, SsaD157 (King et al*.,* 2005).

Polymerase chain reaction (PCR) amplification was performed in a 10µl reaction mixture consisting of 1µl DNA (approximately 10 – 30ng), 1µl primer mix, 3µl water and 5µl 2x Mastermix from a Qiagen HotStarTaq mastermix kit. Three multiplexed and two single PCRs were performed. Multiplex PCRs conditions were as described in Ikediashi et al., 2012. Single reactions were subjected to a 5 minute denaturation step at 95˚C, 35 cycles of: 94˚C denaturation for 30 seconds, 55˚C annealing for 30s and 72˚C extension for 60 s, and a final 10 minute extension at 72˚C.

Allele size determination of fluorescently-labelled PCR products was performed using a Beckman-Coulter CEQ8000 automatic DNA sequencer and the associated software (Beckman-Coulter, Inc., Fullerton, California). All automatically assigned allele sizes were also checked by eye. One locus, Sasa-TAP2A, was discarded due to inconsistent amplification; experience in our laboratory suggests that the primers for this locus amplify more reliably in brown trout (*Salmo trutta* L.). All subsequent statistical analyses were conducted using 18 loci.

2.4 Statistical analysis

2.4.1 Quality control

Prior to statistical analysis, three individuals for which 14 or less loci had amplified were removed from the dataset, leaving 97 juveniles and twelve broodstock adults

genotyped at 15 or more loci at the Exe hatchery. All Tamar samples (broodstock and offspring) amplified at more than 15 of the 18 loci studied.

The data were screened for genetic irregularities, such as the presence of null alleles or the violation of Hardy-Weinberg equilibrium (described below), which could affect the results of further statistical analyses, e.g. measures of genetic diversity and assessment of effective population size.

Two strains were analysed at each hatchery (Table 1). At the Tamar hatchery two raceways were sampled, each containing fish from a separate strain. At the Exe hatchery two strains were maintained; one was sampled from across two raceways (Little Exe), whilst the other (Barle) was sampled from across three raceways. The four broodstock groups (Bar [Barle], LEx [Little Exe], TXL [Tamar x Lyd], Lyd [Lyd], Table A.2) used in all quality control and genetic diversity analyses, were based on a combination of hatchery records (Tables A.3 and A.4) and parentage analysis of genetic data (Section 3.3).

Microchecker v2.2.3 (Van Oosterhout et al., 2004) was utilised to identify allelic dropout, null alleles, and scoring errors in the adult broodstock (A) and juveniles (J) of each strain: Bar-A, Bar-J, LEx-A, LEx-J, TXL-A, TXL-J, Lyd-A, Lyd-J. Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for the adults and offspring of each strain were calculated in Genepop-on-the-Web v4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) using standard parameters (de-memorisation number 1000, 100 batches, 1000 iterations per batch), prior to sequential Bonferroni correction (Rice, 1989).

2.4.2 Genetic diversity of parents/offspring

The number of alleles (Na), allelic richness (Ar), gene diversity (He) and inbreeding coefficient (F_{IS}) were calculated in FSTAT v2.9.3.2 (Goudet, 1995) for the adults and juveniles of each of the four hatchery strains. These measures of diversity were compared between the adults and juveniles of each strain using the Shapiro-Wilk test for normality and the Wilcoxon signed-rank test or paired *t*-test as necessary; all tests were performed in SPSS v16 (Polar Engineering and Consulting, 2007). Sequential Bonferroni correction (Rice, 1989) was applied to all multiple tests. Pairwise F_{ST}s within hatchery were also calculated in FSTAT v2.9.3.2 (Goudet, 1995).

2.4.3 Parentage

Hatchery records of parental crosses in breeding programmes are notoriously inaccurate (Miño et al., 2009, Witzenberg and Hochkirch, 2013), so for each hatchery all broodstock were considered as possible parents of the offspring genotyped. As far as possible, subsequent analysis of data from both hatcheries was conducted in the same manner.

Genotypes of all adults and progeny were collated in Excel and manually assigned. Analysis was conducted by first identifying alleles unique to one parent. Where parentage was not resolved in this way, each locus was examined with consideration to the identified parental genotypes until parentage was resolved. Results were corroborated using Cervus v3.0.3 (Kalinowski et al., 2007, Kalinowski et al., 2010) and Colony v2.0.1.4 (Jones and Wang, 2010; Wang, 2004; Wang and Santure, 2009). Colony v2.0.1.4 was run assuming male and female polygamy, no inbreeding, dioecious and diploid individuals and no sibship prior. Runs were of medium length using the full likelihood method with a medium likelihood of precision. The output of both Cervus v3.0.3 and Colony v2.0.1.4 were compared and each parentage assignment was manually checked to verify or rectify the parentage inferences. Significantly, manual assignment at this stage indicated that not all broodstock that had contributed to the juveniles sampled for analysis were provided as tissue samples from the Exe hatchery. All genotyping errors –as evidenced by a single allelic discrepancy between assigned parents and offspring– were checked and resolved.

Specifically, genetic profiles of juveniles from the Exe hatchery strain Bar indicated that two additional adults (for which we had not been provided with tissue samples) had contributed to this particular strain. The genotypes were inferred following the analysis above and manual determination of the missing adults' individual genotypes. The analysis for the Exe hatchery was then re-run as above, this time assuming broodstock sexes to be unknown.

2.4.4 Effective population size

While the number of breeders (Nb) could be calculated manually and verified through Cervus v3.0.3 and Colony v2.0.1.4, LDNE (Waples and Do, 2008) and Colony v2.0.1.4 were employed to assess the effective population size ($Ne_{(LD)}$ and $Ne_{(SA)}$, respectively) of each hatchery juvenile sample. LDNE results were taken for minimum allele frequency 0.02, as recommended for sample sizes greater than 30 (Waples and Do, 2010). Measures of effective population size (Ne) and the number of breeders (Nb) were tested for correlation using Excel 2007.

3. Results

3.1 Quality control

Homozygote excess, indicative of null alleles, was found at three loci in three different groups: Ssa289 (Bar-J), SsaF43 (TXL-J) and Ssa14 (Lyd-J). Additionally, locus Ssa289 (Bar-J) was identified as possibly demonstrating scoring errors due to stuttering, as indicated by a dearth of heterozygote genotypes.

All four broodstock groups were in Hardy-Weinberg equilibrium (Bar-A, $p = 0.6707$; LEx-A, $p = 0.9675$; TXL-A, $p = 0.9817$; Lyd-A, $p = 0.6410$); however, three loci consistently demonstrated a deviation from HWE (SSsp2216, SsaD144, Ssa197) in the juvenile strains, suggesting the presence of related individuals within the sample. While 88 – 108 of the 153 pairs of loci analysed were found to be linked in each of the juvenile samples, no linkage disequilibrium was found in the adult broodstock fish. Given the lack of LD and conformity to HWE in the adult broodstock groups, LD and HWE were not deemed to be of concern.

3.2 Genetic diversity

All data, with the exception of gene diversity, were normally distributed. Wilcoxon signed-rank tests indicated significant declines in gene diversity (He) in all strains between adults and juveniles (Table 2). A significant decline in allelic richness was found between parents and offspring, while the number of alleles was significantly lower in the juveniles of each strain in all except for the Lyd strain at the Tamar hatchery. Contrastingly, F_{IS} (inbreeding coefficient) showed no significant difference between adults and juveniles in any of the strains (Table 3). Pairwise F_{ST}s within hatcheries indicated no significant difference between the offspring and broodstock of any strain, but highlighted the differentiation between offspring of different matings within each hatchery (Table 4).

3.3 Parentage

3.3.1 Exe hatchery

Having inferred the missing broodstock genotypes (see 2.3.3), use of parentage software (Cervus v3.0.3 and Colony v2.0.1.4) confirmed manual analysis of parentage on the Exe, with one exception. Cervus continuously identified BC5 as the paternal contributor, while all other analyses and manual checks indicated BC1 to be the father and suggested that BC5 had not contributed to any offspring at the hatchery. Each

female produced viable offspring with between one and three males and each male was mated with two to five females (Figure 2). One 'Barle caught' male (BC1) contributed to all five raceways, both the Little Exe and Barle strains. Whilst the individual sizes of adult broodstock were not recorded, one male was reported by hatchery staff to have been widely used because it was a 'particularly large individual'; parentage analysis suggests this individual may have been BC1, which contributed to 37.5% of the Barle strain and 36.7% of the Little Exe strain. Some pairings (e.g. BC1 x BH2A; BC3 x BH2B) were present in multiple raceways; however, no pairings were common to both the Little Exe and Barle strains (Figure 2). Whilst twelve different adult pairings were undertaken to produce the Barle hatchery strain, only four were undertaken to produce the Little Exe strain (Figure 3), of which one (LEC1 x LEH2) contributed to 51.0% of all Little Exe juveniles (Table A.5). Another example of the uneven contribution that can arise in hatcheries can be seen in raceway Bar3, where, despite four successful crosses occurring, one cross (BC2 x BH4) dominated, contributing to 80.0% of the offspring in the raceway. Raceway LEx1 highlights the potential variation in male contribution with two pairings (LEC1 x LEH2 and BC1 x LEH2) contributing 79.2% and 20.8% to the raceway respectively, though considering the use of BC1 across all five raceways, this may have more to do with hatchery process rather than the varied reproductive success of the fish caught.

3.3.2 Tamar hatchery

Manual analysis of parentage at the Tamar hatchery identified parent-pairs for all 100 offspring. The results were again confirmed using parentage programs (Cervus v3.0.3 and Colony v2.0.1.4), although Colony v2.0.1.4 was unable to infer a small number of fathers. Results indicated that twelve of the 15 adult broodstock sampled contributed to the two raceways sampled (TXL, Lyd; Figure 4), not eleven as suggested by the hatchery records (Table A.3). Raceway TXL was dominated by two crosses LC2 x TH4 (40%) and TC2 x TH3 (36%), while crosses TC2 x TH2 and TC1 x TH3 contributed 20% and 4% respectively (Figure 5; Table A.5). Progeny of two crosses (TC1 x TH2 and LC1 x TH4) recorded at the hatchery as being in raceway TXL were not represented in the sample of 50 offspring from the TXL raceway. This may be because the offspring were present in comparatively low proportions compared to the other crosses and, consequently, that the sample was not large enough to detect them. All four broodstock were detected as parents in other crosses, so it would seem infertility was not the issue. The raceway containing Lyd fish was dominated by one cross (Figure 5), LC1 x LH1 (70%). The next largest contributing cross, LC2 x LH3 (14%), was not recorded in hatchery records as being present in this raceway (Table

A.4). The remainder of the samples could be attributed to LC4 x LH2 (10%), LH1 x LC2 (4%) and LC3 x LH2 (2%).

3.4 Effective population size

For each strain, the number of parents inferred through manual and statistical parentage assignment based on genetic data closely resembled hatchery records. However, estimates of effective population size –as computed by both the linkage disequilibrium method ($Ne_{(LD)}$) and the sibship assignment method ($Ne_{(SA)}$)– were lower than the number of breeders (Nb, Table 5). Pairwise comparisons of Ne and Nb indicated strong correlations, particularly between $Ne_{(LD)}$ and $Ne_{(SA)}$ (Table 6). Ne for the hatchery was estimated by $Ne_{(SA)}$ in Colony v2.0.1.4, and was lower for the Tamar hatchery than the Exe, despite the use of an equal number of breeders at each hatchery; this may in part be due to the fact that progeny of only a limited number of parental crosses were sampled at the Tamar hatchery.

4. Discussion

4.1 Genetic diversity and quality control

It is essential for stock enhancement programmes to consider the genetic integrity of the wild stock. Hatcheries have been shown to produce offspring with a reduced genetic diversity when compared with wild stocks in a variety of species, even after one generation (Allendorf and Phelps, 1980; Horreo et al., 2008; Koljonen et al., 2002). Critically, reduced genetic diversity can compromise the ability of a population to adapt to stochastic changes, thereby limiting its evolutionary potential (Koljonen et al., 2002).

Given the small number of adult broodstock used at both the Exe and Tamar hatcheries, it is perhaps not surprising that some loci were out of HWE in the resulting juvenile samples, with significant LD also detected. LD was also found at an increased number of loci in a study of steelhead trout (*Oncorynchus mykiss*; Christie et al., 2012b) and is a recognised issue with supportive-breeding programmes. Additionally, Van Doornik et al. (2011) suggested that HWE departures can result from non-random sampling or sampling of multiple populations as one. Given that one of the four strains of juveniles analysed here was the product of only four adult broodstock, juveniles from family groups were undoubtedly sampled. This is likely to have contributed to the departures from HWE observed and led potentially to alleles being inherited in such a way as would suggest linkage between loci which, based on previous studies (e.g. Ellis

et al., 2011; Horreo et al., 2011b; Ikediashi et al., 2012) and analysis of the broodstock, are not physically linked. Using a small number of broodstock is known to cause a likely reduction in genetic diversity (Verspoor, 1988; Wang and Ryman, 2001), for example, loss of alleles and increased inbreeding in a process resembling an artificial bottleneck and founder effects (Cross & King, 1983). Nonetheless, it is to some extent possible to mitigate these effects by using appropriate mating design (Environment Agency, 2011) and this will be discussed in due course.

The adult broodstock used at both the Exe and Tamar hatcheries showed gene diversity (He) approximating to that of wild fish (Ellis et al., 2011), however, the broodstock used had much lower values of allelic richness (Ar) than the wild populations, in some cases by a factor of four. Additionally, the study of wild Tamar salmon (Ellis et al., 2011) was conducted using a different panel of microsatellite loci, so results are not directly comparable and, while 15 adult broodstock were collected and used at the Tamar hatchery, by design not all of these fish contributed to the two (of six) raceways sampled. Finally, the wild populations studied previously were sampled between 2003 and 2005, at least four years and approximately one generation prior to the adult broodstock collection underpinning the current analysis.

Within each strain, both He and Ar were found to have undergone a significant reduction between the broodstock and offspring life-history stages (Tables 2 and 3). Previous studies have also detected declines in genetic diversity as a result of the hatchery process in trout (*Salmo trutta* L.; Aho et al., 2006; Griffiths et al., 2009). Aho et al. (2006) also found Ar and He to be correlated; however, the system they studied involved re-using the same broodstock or offspring of the broodstock, whereas at the Tamar and Exe hatcheries broodstock were wild-caught annually and released after spawning. Other hatchery studies have shown a greater reduction in Ar than He, e.g. in the Rivers Sella (Horreo et al., 2008, Machado-Schiaffino et al., 2007) and Cares (Horreo et al., 2008), though not all hatcheries are affected in this way, e.g. the Narcea (Horreo et al., 2008). The more significant declines in Ar may be due to the loss of rare alleles, which are known to have a lesser effect on heterozygosity (Kitada, 2009).

4.2 Parentage

Results in this study show no significant F_{ST} or F_{IS} results within strains, though there were differences in F_{ST}s between strains (Table 4), an encouraging result for the Exe hatchery, for which 2010/11 was the first year multiple strains were maintained. Unfortunately, this differentiation could also reflect simply the differentiation between

family groups that constitute the strains, so the significance of this finding remains unclear. Horreo et al. (2008) found differences in genetic variability between adults and broodstock at one of three hatcheries and attributed this to the low number of contributing female broodstock at the River Sella hatchery. Conversely, Machado-Schiaffino *et al.* (2007) discerned a lack of males as the source of a sex bias at the same hatchery in a previous year. Sex bias, however, does not seem to be an issue at the Tamar or Exe hatcheries, where inferred contributing parents closely approximated hatchery records. In the current study, the bias in parental contribution and the small number of broodstock utilised are more likely to be contributing to the decline in genetic diversity of the offspring; between four and eight broodstock were used to produce each strain (i.e. 14 or 15 per hatchery). In comparison with Spanish hatcheries, which used a minimum of 37 broodstock on the Cares and a maximum of 112 on the Narcea (Horreo et al., 2008), numbers used in these English hatcheries are very low. However, pedigree analysis of the Spanish hatcheries highlighted that, despite the higher number of broodstock used, only a limited number of the adult fish used contributed to the resulting juveniles (Horreo et al., 2008). Specifically, at one of the three Spanish hatcheries, multi-sea-winter (MSW) females were found to contribute fewer offspring than female grilse; Horreo et al. (2008) suggested two possible explanations for this result: (i) over-maturity in the MSW females; and (ii) competition between different sized offspring. Clearly, while the hatcheries studied here (Exe and Tamar) utilised fewer broodstock than the Spanish hatcheries, a greater proportion of adult fish were found to have contributed to the next generation; this may also be indicative of broodstock fish having been caught closer to spawning time and in better condition. Horreo *et al.* (2008) state that their broodstock were ‘randomly caught’, whereas, the majority of Exe and Tamar broodstock were caught by rod and line, a few were caught by electrofishing and others were sampled from a nearby leat, thus providing a broad cross-section of returning adults.

In the current study, contributing adult broodstock pairs were found to produce between 2–70%, 4–40%, 2.1–29.2% and 12.2–51% of the offspring in the Lyd, TXL, Bar and LEx strains, respectively. This variability may be attributed to a number of factors including the fecundity of the broodstock and the genetically pre-determined competitive ability of the offspring at a particular life-history stage. Selection pressures acting on fish in a hatchery are somewhat different to those acting in the wild; therefore, a strong selection bias towards one family group for survival in a hatchery environment may have negative repercussions for a local wild population if/when the hatchery-reared offspring return to breed (McGinnity et al., 2009) or if they breed as

precocious parr soon after release, as ranched fish have been reported to do (McGinnity et al. 2004).

4.3 Effective population size

In England and Wales supportive-breeding programmes have to be approved by the Environment Agency (the relevant statutory body within these countries). General guidelines include: (1) broodstock should be chosen based on known population structure (through genetics or life-history analysis) and collected from the population to which the offspring will be returned; (2) hatchery offspring should not be used as broodstock; (3) mature wild adults should be used; (4) sperm competition should be prevented; (5) full factorial mating should be performed (all females with all males) or partial factorial (3 x 3) if this is not possible; and (6) as it is difficult to calculate the exact number of broodstock needed to maintain good genetic diversity and effective population size, expert advice was sought (NASCO, Finland); however, due to the variation in the requirements and status of each river, guidelines on the number of broodstock required are provided on a case-by-case basis (Environment Agency, 2011). Elsewhere in Europe, the process is more regulated. For example, the Finnish Game and Fisheries Research Institute suggest a minimum effective population size of 50 individuals (Aho et al., 2006), while Pante et al. (2001) suggest a 'moderate effective population size' to be between 25 and 94 individuals based on a study of inbreeding in rainbow trout (*Oncorhynchus mykiss* (Walbaum)); Meuwissen and Woolliams (1994) suggest 31 to 250 broodstock following mathematical modelling, and Blanchet et al. (2008) recommend more than 20 broodstock as a minimum. All of the above recommendations are for a greater number of productive broodstock than were used in the Exe and Tamar hatcheries studied here; however, the reasons for selecting so few broodstock were two-fold: (i) the desire not to over-sample from the resident wild populations; (ii) logistics.

Consideration should also be taken as to the impact of removing potential breeding adults from the wild; for example, Saltveit (2006) explained the value of using a surplus of adults to produce smolts when the river is already at carrying capacity due to habitat limitations. However, the Tamar and Exe rivers appear to not be achieving carrying capacity (Environment Agency, 1997, 2003), so consideration must be given to the negative impacts on resident populations when hatchery offspring are introduced – their reduced reproductive capability, survival and competitive ability are well-documented (Araki et al., 2007, Blanchet et al., 2008, Cross et al., 2007, McGinnity et al., 2009, Wachirachaikarn et al., 2011). However, the benefits of undertaking hatchery

rearing also need to be considered; for example, fish in a hatchery will have a plentiful supply of food and shelter from predation, both of which will allow more of these (potentially disadvantaged) young to survive until the point of release into the wild (Jonsson and Jonsson, 2009).

Each catchment will have its own issues, whether these are hydroelectric power and limited suitable habitat (e.g. Saltveit, 2006) or pollution and degraded habitat, such as the case in the Exe and Tamar catchments. When mitigating procedures are in progress to improve the habitat, temporary supplementation may be appropriate, but the necessity of long-term supportive-breeding in these catchments is questionable; we suggest that hatchery programmes should be monitored and decisions taken on a case-by-case basis. Assuming that stock enhancement is deemed to be an appropriate management tool for a particular catchment, it is important to maintain the genetic diversity of the hatchery strains created to reflect the overall diversity of the catchment into which they are to be released.

At both the Exe and Tamar hatcheries the number of broodstock was lower than recommended (Aho et al., 2006; Meuwissen and Woolliams, 1994; Pante et al., 2001) and the effective population size (Ne) was assessed as being even lower; similarly, Machado-Schiaffino et al. (2007) also found Ne to be lower than the number of broodstock used in their study of Spanish rivers. Koljonen et al. (2002) found the effective population size of juvenile hatchery fish to be 68% of that of their founders, while Mace and Lande (1991) assumed Ne to be < 50% of census sample size. In the current study Ne was found to be between 38.6% and 70% of the number of contributing broodstock for the LD method and 57.1% and 100% using the SA method. For the Little Exe strain, Ne was estimated at 2.8 using the LD method; this estimate is lower than that found after 12 generations of captive breeding in Finland (Aho et al., 2006). Ne is an estimation of the number of breeders, assuming random mating, HWE and Linkage equilibrium (Waples and Do, 2010). Consequently it can be affected by variation in family size, biased sex ratios and the 'population size' represented by the broodstock in the current study (Horreo et al., 2008; Koljonen et al., 2002 and references therein). Typically, a small number of broodstock or low Ne acts as a bottleneck resulting in low levels of variation; in turn, this can cause a further loss of genetic diversity (Aho et al., 2006; Pante et al., 2001) as demonstrated by the converse finding that a ten-fold increase in founding Ne could instigate a 27% increase in allelic richness (Aho et al., 2006). Aho et al. (2006) believe mating designs are crucial in preventing these bottlenecks, accentuating the importance of optimising the number

and unrelatedness of breeders. Clearly, in the hatcheries studied (Exe and Tamar), the low number of breeders is an issue. Lack of contribution, due to infertility, sperm competition or genetic incompatibility, are factors that are rarely discussed, yet could contribute to a reduction in Ne. Additionally, the differential behaviour of offspring under hatchery conditions may bias the success of particular family groups leading to lower estimates of Ne. All of these factors are relevant to the assessment of the success of hatcheries in safeguarding both numbers and genetic diversity of fish in a river, and warrant further study. Certainly, to rectify these issues, both hatcheries studied here would benefit from more closely following Environment Agency protocols, whereby sperm competition is eliminated and an increased number of cross-matings occur through partial or full factorial mating.

4.4 Hatchery records

The Environment Agency in England provides guidelines on the number of wild fish that each hatchery is allowed to catch annually for the purposes of supportive-breeding; rules for hatchery practices are not currently standardised and the Agency regulates applications for supportive-breeding programmes on a case-by-case basis. Both of the hatcheries studied work to these limits and accurate records are obviously essential. Of the twelve parents genetically inferred to have contributed to the offspring sampled from the Tamar hatchery, one individual was not expected to have contributed to the sampled raceways based on the information obtained from the hatchery records. At the Exe hatchery genetic analysis indicated that one additional (unsampled) fish was used, while another which was recorded as having been used as broodstock, apparently did not contribute at all; this latter fish may have died before it could be stripped or been infertile. The analysis of parentage at both hatcheries highlights the ability of genetic analysis to identify missing parents and to confirm or correct hatchery records. Accurate knowledge of numbers of broodstock fish used is thus essential both for maintaining genetic diversity and conforming to legal requirements.

4.5 Management implications and conclusions

The purpose of this study was to evaluate the success of the processes used in two working salmon hatcheries in South West England, specifically, to assess the ability of hatchery processes to replicate the genetic diversity of their respective adult broodstock fish in the resulting hatchery-reared juvenile fish. Finally, the study has allowed identification of key points to be considered when formulating best-practice hatchery guidelines.

The decision as to whether (or not) supplementation of any kind is required, as well as amendments as to how the practice should be conducted, must be decided on a case-by-case basis, with due consideration of local guidelines and legislation. This study was not designed to address the matter of whether or not supplementation should be carried out but to advise on best practice if supplementation is deemed necessary. The two English catchments studied here demonstrate that one-generation supportive-breeding programmes result in juveniles with a –in some cases– considerably reduced genetic diversity. Previous studies have considered the effects of sex bias (Horreo et al., 2008; Machado-Schiaffino et al., 2007), relatedness (Aho et al., 2006) and number of broodstock (Aho et al., 2006) on the diversity and representativeness of resulting hatchery-reared fish; the number of broodstock in combination with a parental bias, were identified as the main factors contributing to the reduction in genetic diversity evidenced in the current study. Aho et al. (2006) highlighted the importance of appropriate pairing designs within a hatchery and in the current study sensible polygamous breeding designs were followed; however, it seems this practice was not sufficient to maintain the genetic diversity of the resulting offspring. Thus, to ensure a more balanced contribution it may be necessary to reduce in-hatchery competition by rearing the offspring from each broodstock pair independently; however, the practicalities and costs of this for a hatchery manager may be prohibitive. A more cost effective and practical option may be to opt for full factorial mating where the eggs of all females (if maturing simultaneously) are mixed and divided between a number of raceways, each fertilised by s single male (Environment Agency, 2011), maximising the number of crosses whilst reducing bias in parental contribution by eliminating sperm competition. Sperm competition, however, is a natural process and not allowing this to occur also risks altering the natural selection process. Other factors, i.e. relatedness of individuals and Ne, can be monitored genetically and, ideally, initial pairing decisions could be made following rapid testing of candidate broodstock fish. This will be particularly important when dealing with small numbers of broodstock, but arguably less important and less practical in cases where large numbers (hundreds or thousands) of broodstock fish are to be used. If the relatedness of individuals is known, and pairings are designed it will be more crucial than ever to maintain meticulous hatchery records. Regular monitoring of Ne and sex-bias in hatchery offspring will facilitate improvements in hatchery practices until such time as measures of genetic diversity and effective population sizes are reflective of the broodstock used to produce them and of the rivers into which they are to be released.

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Table 1. Details of *S. salar* juveniles sampled at the Tamar and Exe hatcheries.

Hatchery	Strain	Raceway	NR	NS	NG	Size (cm)
Exe	Barle (Bar)	Bar1	9000	22	21	4.4 - 6.6
		Bar2	4500	11	11	4.2 - 7.5
		Bar3	6500	17	16	4.5 - 8.2
	Little Exe (LEx)	LEx1	>5000	25	24	5.0 - 7.2
		LEx2	>5000	25	25	4.7 - 6.8
Tamar	Tamar x Lyd* (TXL)	TXL	20,000	50	50	Unknown
	Lyd (Lyd)	Lyd	8,000	50	50	Unknown

NR, number of fish per raceway; **NS,** number of samples; **NG**, number successfully genotyped.

*The offspring in the Lyd raceway were produced using both Tamar and Lyd adult broodstock (juveniles were used to stock the main Tamar catchment).

Table 2. Wilcoxon signed-rank test comparing the gene diversity (He) between adult broodstock and offspring.

	BarHeA – BarHeJ	LExHeA – LExHeJ	TXLHeA – TXLHeJ	LydHeA – LydHeJ
Z	-3.6365	-3.5933	-3.5933	-3.5062
p (Asymp. Sig., 2-tailed)	**0.0003**	**0.0003**	**0.0003**	**0.0005**

BarHeA – BarHeJ represents the comparison of gene diversity between adults and offspring of the Barle strain; LExHeA – LExHeJ represents the comparison of gene diversity between adults and offspring of the Little Exe strain; TXLHeA – TXLHeJ represents the comparison of gene diversity between adults and offspring of the TXL Tamar/Lyd crossed strain; and LydHeA – LydHeJ represents the comparison of gene diversity between adults and offspring of the Lyd strain. All within-strain comparisons of gene diversity between broodstock and offspring were found to be significant. *p*-values are shown in bold.

Table 3. Twelve paired *t*-tests of adult–juvenile genetic diversity.

Adult–juvenile pairs	Paired Differences				*t*	*df*	*p* (2-tailed)
	Mean	Standard error	95% CI of the difference				
			Lower	Upper			
BarNaA – BarNaJ	0.7222	0.8948	0.2772	1.1672	3.4244	17	**0.0032**
BarArA – BarArJ	2.1682	1.6262	1.3600	2.9769	5.6566	17	**0.0000**
BarFisA – BarfisJ	0.0388	0.1683	-0.0448	0.1225	0.9792	17	0.3412
LExNaA – LExNaJ	0.7778	0.7321	0.4137	1.1418	4.5074	17	**0.0003**
LExArA – LExArJ	1.7624	1.0376	1.2464	2.2784	7.2062	17	**0.0000**
LExFisA - LExFisJ	0.1589	0.2364	0.0374	0.2805	2.7718	16	0.0136
TXLNaA - TXLNaJ	0.7778	0.9428	0.3089	1.2466	3.5000	17	**0.0027**
TXLArA - TXLArJ	2.4639	1.1031	1.9153	3.0125	9.4762	17	**0.0000**
TXLFisA - TXLFisJ	0.0583	0.1261	-0.0044	0.1210	1.9606	17	0.0665
LydNaA - LydNaJ	0.3333	0.6860	-0.0078	0.6745	2.0616	17	0.0548
LydArA - LydArJ	2.6467	1.5654	1.8683	3.4252	7.1733	17	**0.0000**
LydFisA - LydFisJ	0.1588	0.2523	0.0333	0.2842	2.6701	17	0.0162

Adults (A) and juveniles (J) were compared within each strain with respect to the number of alleles (Na), allelic richness (Ar), and inbreeding coefficient (F_{IS}). Significant *p*-values are shown in bold, *t* is the test statistic, *df* indicates degrees of freedom within the test and *p* (2-tailed) gives the *p* value for the 2-tailed paired *t*-test.

Table 4. Genetic differentiation between samples within hatcheries.

	BB	BJ	LEB	LEJ
BB	0	-0.0124	-0.0060	***0.0805***
BJ	0.9750	0	***0.0255***	***0.0925***
LEB	0.8833	***0.0083***	0	-0.0213
LEJ	***0.0083***	***0.0083***	0.3167	0
	TLB	TLJ	LB	LJ
TLB	0	-0.0104	-0.0246	***0.0488***
TLJ	0.4333	0	***0.0529***	***0.1275***
LB	0.925	***0.0083***	0	0.0084
LJ	***0.0083***	***0.0083***	0.1167	0

The upper triangle shows the F_{ST} values while the lower triangle show the *p* values. For both hatcheries, *p* values were obtained after 120 permutations and at both hatcheries the indicative adjusted nominal level (5%) for multiple comparisons was: 0.0083. Significant values are shown in bold. Key: BB = Barle broodstock; BJ = Barle juveniles; LEB = Little Exe broodstock; LEJ = Little Exe juveniles; TLB = broodstock used in the Tamar/Lyd cross; TLJ = juveniles resulting from the Tamar/Lyd cross; LB = Lyd broodstock; LJ = Lyd juveniles.

Table 5. Estimates of effective poplation size (Ne) and number of breeders (Nb).

Strain	N	Nb (M:F)	Ne(LD)	Ne(SA)	Ne(SA) hatch
Barle (BarJ)	9	10 (6:4)	6 (5.2-6.7)	8 (4-24)	10 (5-28)
Little Exe (LExJ)	4	4 (2:2)	2.8 (2.6-3.1)	4 (2-12)	
TXL (TXLJ)	7	7 (4:3)	3.3 (3.1-3.6)	5 (2-20)	8 (4-24)
Lyd (LydJ)	6	7 (4:3)	2.7 (2.5-3)	4 (2-12)	

The table includes the number of broodstock used per strain (N) as recorded by hatchery staff, the number of breeders per strain as infered using the program Colony v2.0.1.4, including the male: female ratio (Nb(M:F)), the effective population size (and jackknife confidence intervals) of each strain as estimated by the linkage disequilibrium method in the program LDNE (Ne(LD)), the effective population size (and 95% confidence intervals) of each strain as estimated by the sibship assignment method in the program Colony (Ne(SA)) and the effective population size (and 95% confidence intervals) of each hatchery as estimated by the sibship assignment method in the program Colony (Ne(SA)hatch).

Table 6. Correlation of estimates of effective population size (Ne) and the number of breeders (Nb).

	LD	SA	N	Nb
LD	1			
SA	0.9961	1		
N	0.8544	0.8882	1	
Nb	0.8398	0.8627	0.9806	1

Measures of effective population size, the number of breeders and the numbers of fish recorded for each strain have been abbreviated in this table to: LD, Ne(LD); SA, Ne(SA); N, number of broodstock recorded as used by hatchery staff; Nb, number of broodstock inferred using Colony v2.0.1.4.

Figure legends

Figure 1. Map showing the locations of the Exe and Tamar hatcheries. Catchment names are marked at respective river mouths.

Figure 2. Exe parentage per raceway based on the results of genetic analysis. Circles represent females and squares represent males. Raceways Bar1, Bar2 ,Bar4 from the Barle and LEx1 and LEx2 from the Little Exe are presented seperately, although some broodstock are common to more than one raceway (e.g. BC1). Male broodstock are represented by squares and females by circles. Barle broodstock are represented by the codes BH1, BH2A, BH2B, BH3, BH4 (females) and BC1-BC4 (males). Little Exe broodstock are represented by the codes LEH1-2 (females) and LEC1-2 (males). Parental crosses are coded a-p.

Figure 3. Parent-pair contribution to each raceway and strain at the Exe hatchery. Columns indicate the proportion of each parent-pair to raceways Bar1, Bar2, Bar3, LEx1, LEx2, and the Barle (Bar1-3 combined) and Little Exe (LEx1-2 combined) strains. Barle broodstock are represented by the codes BH1, BH2A, BH2B, BH3, BH4 (females) and BC1-BC4 (males). Little Exe broodstock are represented by the codes LEH1-2 (females) and LEC1-2 (males). Tamar broodstock are represented by codes TH2-4 (females) and TC1-2 (males). Lyd broodstock are represented by codes LC1-4 (males) and LH1-3 (females).

Figure 4. Parentage inferred at the Tamar hatchery. Circles represent females and squares represent males. Raceways TXL and Lyd are presented seperately, although some broodstock are common to both. Tamar broodstock are represented by codes TH2-4 (females) and TC1-2 (males). Lyd broodstock are represented by codes LC1-4 (males) and LH1-3 (females). Parental crosses are coded a-k.

Figure 5. Parent-pair contribution to each raceway sampled (TXL and Lyd) at the Tamar hatchery. Columns indicate the porportion of each parent-pair to raceways TXL and Lyd respectively. Tamar broodstock are represented by codes TH2-4 (females) and TC1-2 (males). Lyd broodstock are represented by codes LC1-4 (males) and LH1-3 (females).

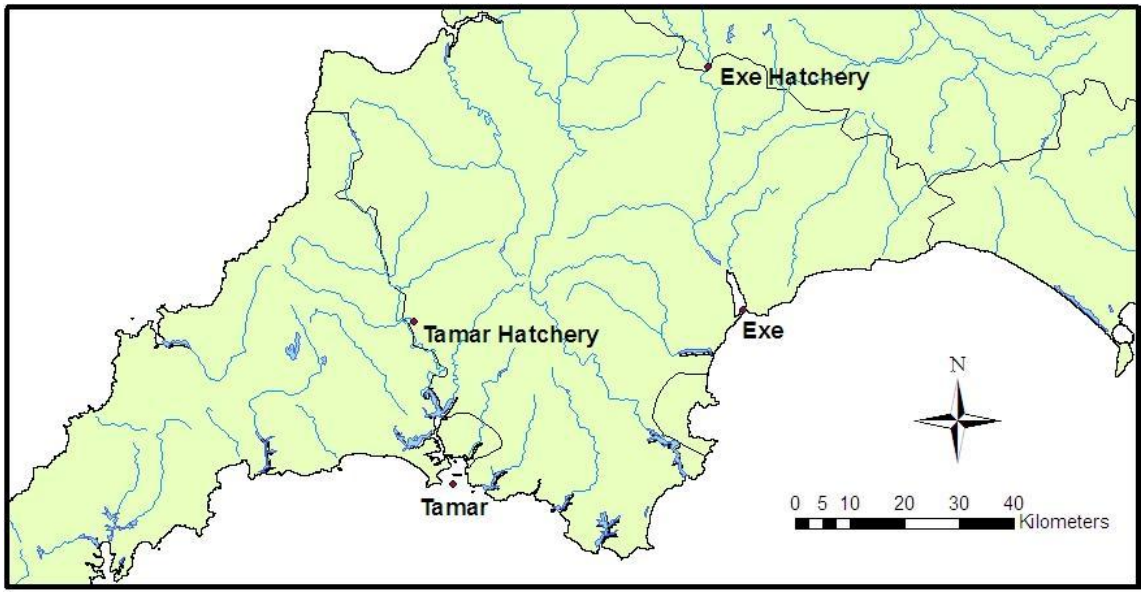
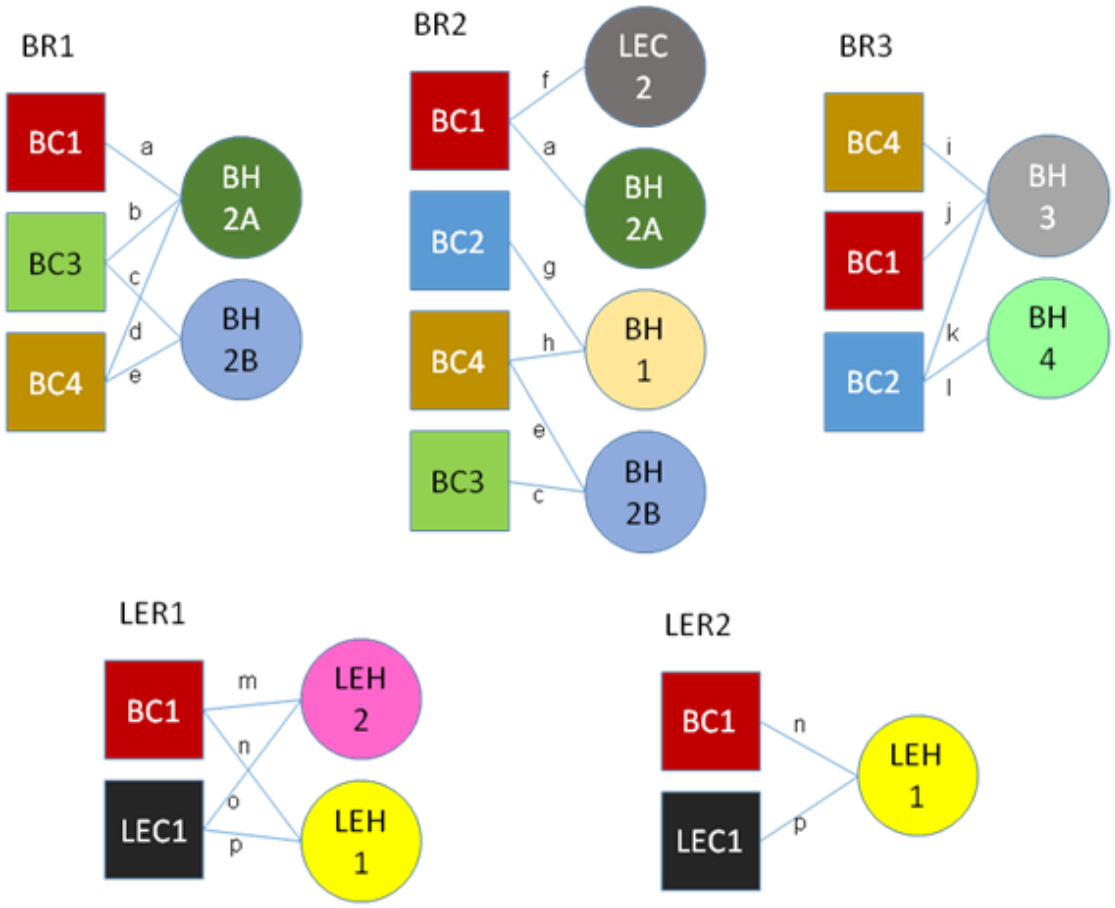

Exe Hatchery
Tamar Hatchery
Exe
Tamar
N
0 5 10 20 30 40
Kilometers

Figure 1.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BC4
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BH 2A
BH 1
BH 2B
f
a
g
h
e
c
BR3
BC4
BC1
BC2
BH 3
BH 4
i
j
k
l
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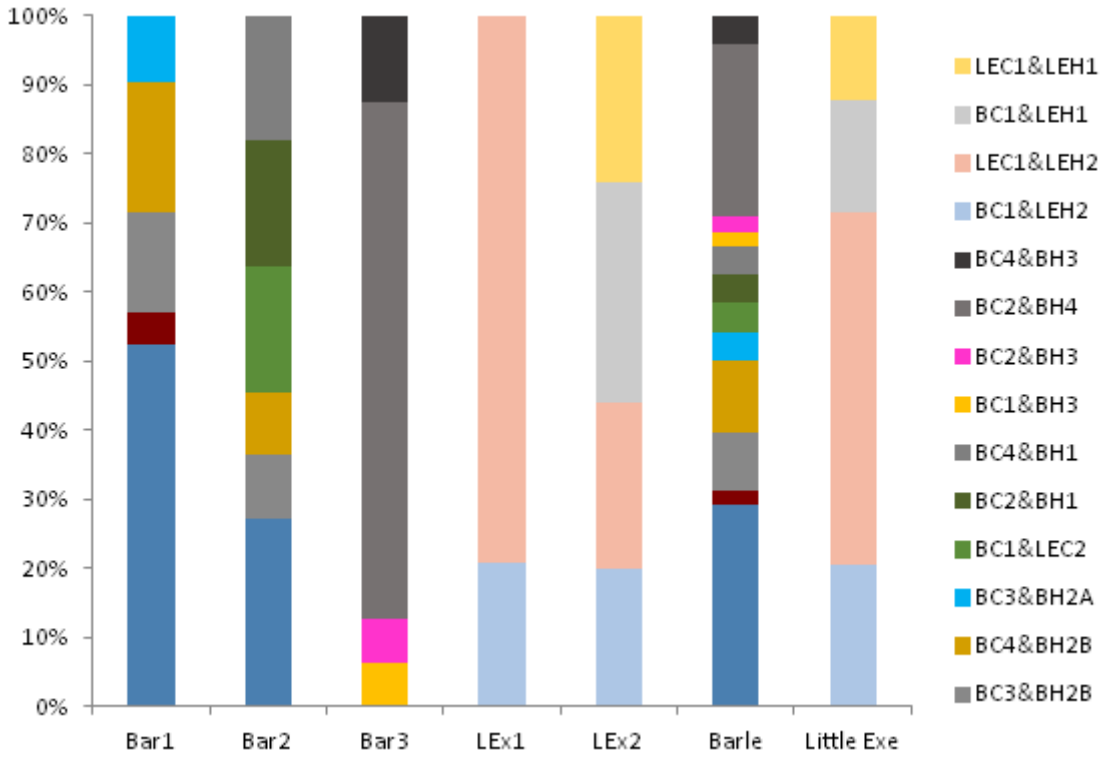
Figure 2.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Bar1
Bar2
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LEx2
Barle
Little Exe
LEC1&LEH1
BC1&LEH1
LEC1&LEH2
BC1&LEH2
BC4&BH3
BC2&BH4
BC2&BH3
BC1&BH3
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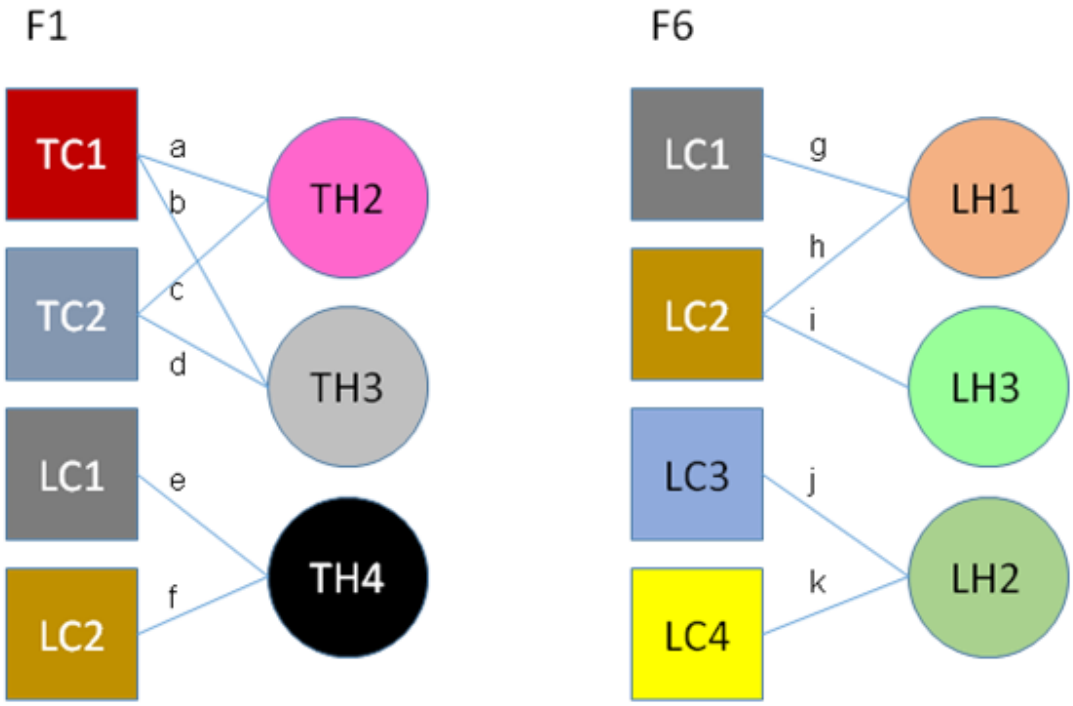
BC3&BH2B

Figure 3.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g
h
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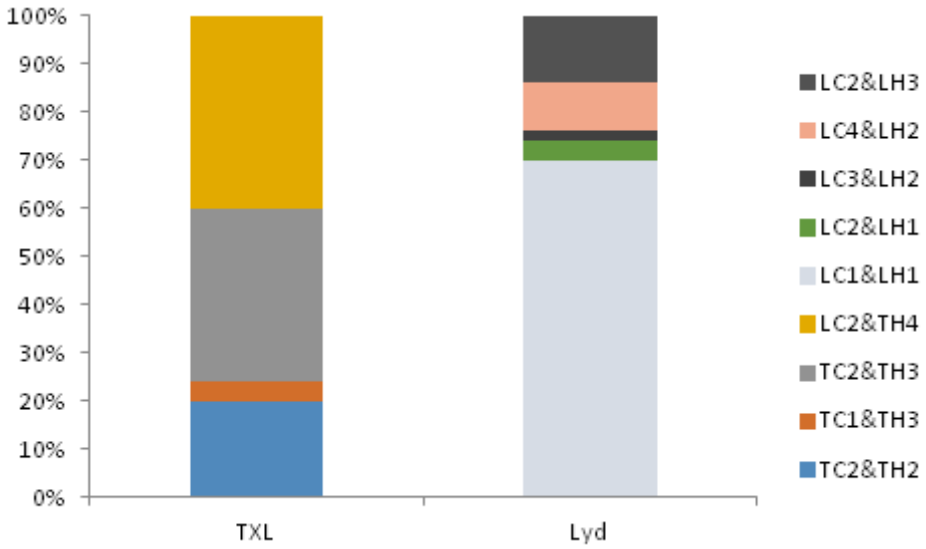
LH1
LH3
LH2

Figure 4.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LC4&LH2
LC3&LH2
LC2&LH1
LC1&LH1
LC2&TH4
TC2&TH3
TC1&TH3
TC2&TH2

Figure 5.

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