

An unusually high frequency of Atlantic salmon x brown trout hybrids in the Loch Lomond catchment, west-central Scotland.

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ABSTRACT

A genetic study to examine population structuring of fish identified by anglers on the basis of external morphology, as Atlantic salmon, *Salmo salar*, returning as sea-migrants to the Loch Lomond catchment, recorded a rate of hybridisation with brown trout, *Salmo trutta*, of 10.4%. This is much higher than previously reported for adult fish elsewhere and considerably higher than amongst juveniles sampled from across the catchment (0.7%). The sea-migrant hybrids recorded here were F_1 hybrids derived from matings comprising one brown trout female and at least three different salmon females. Mitochondrial DNA haplotypes suggest that these are associated with spawning in different parts of the Loch Lomond catchment. The cause(s) of the high incidence among adults is uncertain. However, these findings challenge the general view, derived from existing literature, that lifetime fitness in Atlantic salmon x brown trout hybrids is low. Suggesting that, at least occasionally, hybrid survival to sexual maturity may be similar to that of individuals in the parental populations. The potential impact of high hybrid survivorship on parental species dynamics is discussed.

KEYWORDS: hybrid survival; species integrity; *Salmo salar*; *Salmo trutta*.

INTRODUCTION

Despite that most commonly applied definitions of a species require (either explicitly or implicitly) operational reproductive barriers between, species, (Mayden, 1997; Coyne & Orr, 2004), hybridisation between species of fish is relatively common (Hubbs, 1955; Chevassus, 1979; Verspoor & Hammar, 1991 and references therein).

In circumstances where reproductive isolating mechanisms in sympatric species are weak enough for hybridisation to occur, then a number of outcomes are possible. When hybridisation is frequent, and both survivorship and reproductive

competence of hybrids is high, then species can effectively merge (Taylor *et al.*, 2006) or a hybrid swarm may form (Benke, 1972). Alternatively, species may remain largely intact but introgression of genes from one species into the other may occur (Verspoor & Hammar, 1991). There have also been reported examples where a new fish taxon may have formed (see for example Sezaki *et al.*, 1994). All of these outcomes are dependent upon first generation (F_1) hybrids remaining viable (surviving and becoming reproductively competent) to reproduce, either with other hybrids, or one or both of the parent populations.

In natural systems, hybridisation is most likely to occur in closely related, recently diverged, species pairs with a common lineage (Verspoor & Hammar, 1991; Grant & Grant, 2005) presumably because the accumulation of isolating barriers is likely to be lower in such cases.

One pair of related species that share a common lineage diverging about 3.3 M years ago (Shedko *et al.* 2012) is the Atlantic salmon *Salmo salar* L. and the brown trout *Salmo trutta* L.. These species can, and do, hybridise naturally and commonly, in the wild (see Jordan *et al.*, 2007 & Makharov 2008 for reviews) and will also hybridise *in vitro* (Day, 1844; Garcia-Vazquez *et al.*, 2004) but there is evidence from the literature that in natural systems hybrid fitness may be impaired.

In the wild, hybrids of these species are frequently reported amongst the juvenile, freshwater stages of the life cycle. In 14 studies of 53 catchments where these species co-occur, hybrids have been found in 39 (Solomon & Child, 1978; Beland *et al.*, 1981; Crosier, 1984; Verspoor 1988; Garcia de Leaniz & Verspoor 1989; Hurrell and Price 1991; Jansson *et al.*, 1991, McGowan & Davidson, 1992; Jordan & Verspoor, 1993; Elo *et al.*, 1995; Hartley, 1996; Jansson & Ost, 1997; Matthews *et al.*, 2000; Garcia-Vazquez *et al.*, 2001).

The occurrence of Atlantic salmon x brown trout hybrids in the freshwater life stages in some catchments can be high. Verspoor (1988) for example, recorded hybridisation rates of up to 11.1% in parr (juvenile fish in their freshwater phase in their second year or older) in catchments in Newfoundland. In Sweden, 22.8% of parr in the River Gronan (Jansson *et al.*, 1991) and 66.7% in the River Dalalven, (Jansson & Ost, 1997) were hybrids. However, the reported incidence of hybrids at later life stages is significantly lower. Verspoor (1988) found 0.3% F_1 hybrids amongst Atlantic salmon smolts (the seaward migration phase of the life cycle) in 331 individuals, from 4 catchments in Newfoundland (the maximum occurrence was 1.75% in the River Trepassey). In England, only two F_1 hybrid smolts were identified from a large sample of salmon and trout smolts (the sea migration phase) from the River Piddle (Solomon & Child, 1978)

Amongst sea-migrant adult fish, Youngson *et al.*, (1992) recorded a single returning Atlantic salmon x brown trout hybrid, from the River Don, Scotland in a sample from the recreational fishery. Payne *et al.* (1972) examined a sample of 4431 adult apparent Atlantic salmon in commercial inshore fisheries around the UK and Ireland and found 0.4% of these fish were hybrids. In a sample of 198 returning sea-migrant adults from 3 rivers in Newfoundland rivers, Verspoor (1988) found no hybrids, despite that hybridisation had been recorded amongst parr sampled from these rivers.

Thus the pattern emerging from previous studies is that of a much lower frequency of Atlantic salmon x brown trout hybrids at later life stages, compared with earlier life stages, strongly suggestive of low hybrid survivorship and impaired long-term hybrid fitness.

During a study to examine population structuring of Atlantic salmon we had the opportunity to examine hybridisation frequencies between Atlantic salmon x brown trout amongst returning marine migrants and freshwater stages in a single Scottish catchment, Loch Lomond. These data we present here.

MATERIALS & METHODS

The study site

The Loch Lomond catchment, in west-central Scotland (56°07'N 004°38'W), comprises a large lake (Loch Lomond) with one major afferent tributary navigable by migratory salmonids (the Endrick Water), a number of minor afferent streams (the largest being the Rivers Fruin, Luss and Blane) and a single efferent river (the River Leven). Both Atlantic salmon and freshwater-resident (brown) and migratory (sea) forms of trout co-exist naturally, occupying a similar range within the catchment.

Fry collection

Fry (age 0+), identified superficially as salmon, were collected by electrofishing in summer 2005, from 6 parts of the Lomond catchment known to support high densities of fish. A total of 281 fry were collected comprising: 70 from the lower Endrick Water (3 sites), 62 from the upper Endrick Water (3 sites), 37 from the Blane Water (2 sites), 41 from the River Luss, 33 from the River Fruin and 38 from the efferent River Leven (Fig.1).

Sea migrant collection

A sample of sea returning migrants showing the external characteristics of Atlantic salmon were collected from the rod fishery in 2006 from the Loch Lomond catchment. Fifteen individuals were collected from the fishery in the main loch, 31 from the fishery in efferent River Leven and 1 each from the rod fishery in the rivers Fruin and Endrick. The adipose fin was removed from captured fish and stored in 100% ethanol.

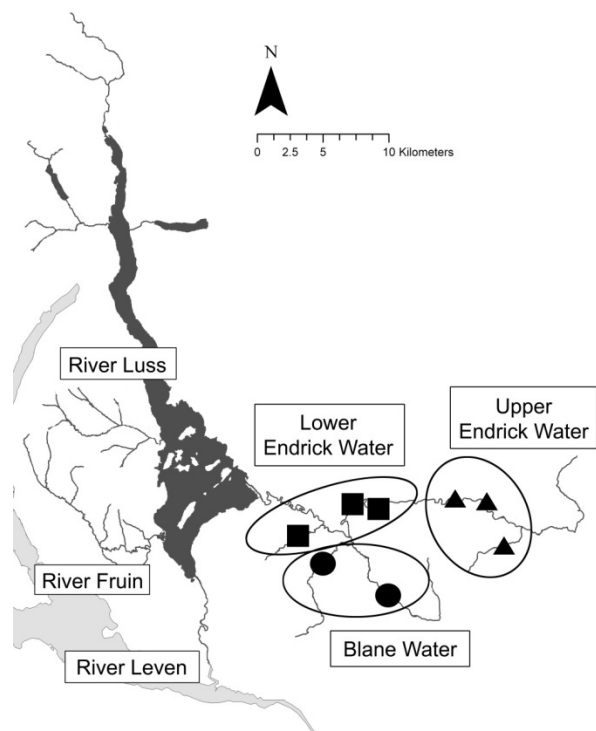


Fig. 1. The Loch Lomond catchment showing principal sampling areas; the lower Endrick Water (3 discrete sampling sites), the upper Endrick Water (3 discrete sampling sites), the Blane Water (2 discrete sampling sites), the River Luss, the River Fruin, the River Leven and Loch Lomond.

Genetic analysis

DNA was extracted from fin tissue from all individuals following the method of Knox *et al.* (2002). All samples were amplified at the diagnostic 5S rDNA locus to confirm the identity of their species of origin using the procedure of Pendas *et al.*, 1995. In addition, the ND1 region of the mtDNA was amplified by PCR (Youngson *et al.* 1992) and restricted with Hae III restriction enzyme which gives diagnostic fragment patterns for Atlantic

salmon and brown trout (unpublished; Fig. 2). In addition mtDNA was amplified at the mitochondrial ND1 region and screened with five restriction enzymes for DNA sequence polymorphisms that are known to be common in Atlantic salmon from Europe (*AvalI*, *DraI*, *HaeIII*, *HinfI*, *RsaI*) (Knox *et al.*, 2002).

RESULTS

Of the 281 juvenile, freshwater stage salmon collected in six spawning streams from across the Lomond catchment 0.7% (2 individuals) were found to be hybrids.

Table 1. The capture site, mass and sex of five Atlantic salmon x brown trout hybrids from the Loch Lomond catchment.

Sex	Capture site	Capture date	Mass (kg)
Female	Loch Lomond	08 July 2006	2.27
Female	River Leven	15 Aug 2006	2.49
Male	River Leven	30 June 2006	4.54
Male	River Leven	29 May 2006	6.12
Female	River Leven	27 May 2006	5.44

In contrast, 5 of the 48 (10.4%) sea migrant fish with externally salmon-like morphological characteristics

were found to be Atlantic salmon x brown trout hybrids. Three of the sea-migrant hybrids were females and two were male fish. They comprised a broad size range (2.3 to 6.1 kg) strongly suggesting that they spanned multiple age classes. Four of the five were caught in the efferent River Leven and 1 in Loch Lomond itself.

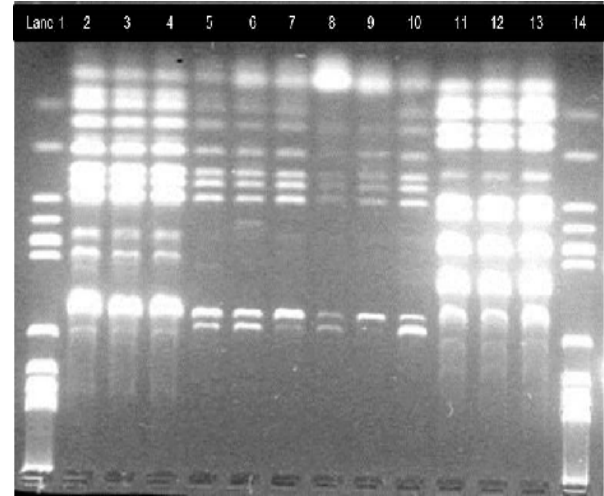


Fig. 2. The restriction fragment patterns obtained following digestion of the amplified ND1 gene with Hae III. Lanes 2 -10 - Atlantic salmon; brown trout lanes 11-13. Molecular size marker shown in the lanes 1 and 2 is phiX174 Hae III digest.

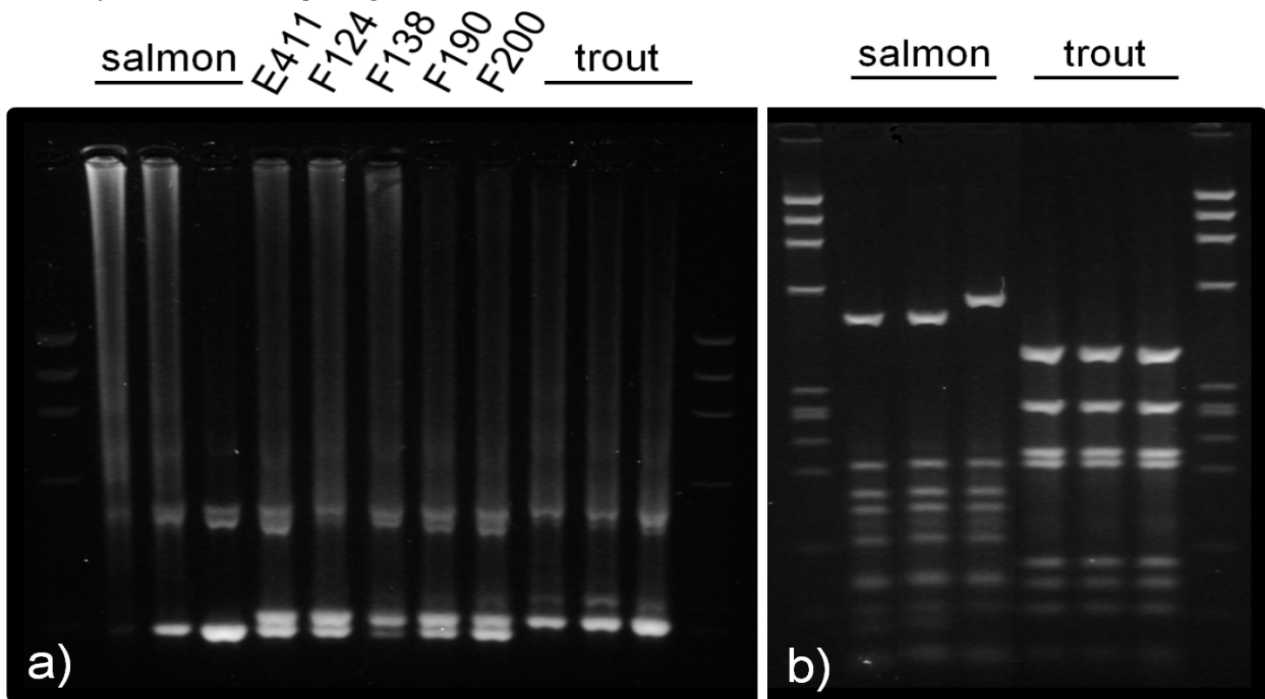


Fig. 3. Agar gel electropherograms showing patterns used to identify a) hybrids and pure salmon and trout based on 5sRNA assay and b) HaeIII ND1 assay; see text for details.

The direction of the hybridisation of the sea-migrants varied across fish in this study. Of the 5 hybrids, one (E411 in Fig. 3) had a trout female parent and the remaining 4 had salmon female parents. Of the 4 hybrids with salmon female parents, 3 distinct composite haplotypes were identified from restriction fragment length polymorphisms at the 5 mtDNA restriction sites in the ND1 region. Thus at least 3 different female salmon parents gave rise to the 4 hybrids with female salmon parents.

DISCUSSION

The incidence of sea-migrant hybrids found among adult "salmon" angled in the Leven system is significantly higher than has been reported for any other catchment where Atlantic salmon and brown trout co-exist (Payne *et al.*, 1972; Verspoor, 1988; Youngson *et al.*, 1992). It is also significantly higher than the frequency of hybrids observed for freshwater stage juveniles from the six salmon nursery areas in the catchment sampled, which represent the main known areas of salmon production.

In general the frequency of Atlantic salmon x brown trout hybrids reported in the literature is very low at later life stages compared with that of fry and parr (Solomon & Child, 1978; Beland *et al.*, 1981; Crosier, 1984; Verspoor, 1988; Garcia de Leaniz & Verspoor, 1989; Hurrell & Price, 1991; Jansson *et al.*, 1991; McGowan & Davidson, 1992; Jordan & Verspoor, 1993; Elo *et al.*, 1995; Hartley, 1996; Jansson & Ost, 1997; Matthews *et al.*, 2000; Garcia-Vazquez *et al.*, 2001). In most cases, where frequencies amongst juveniles and adults from the same river or region are compared, frequencies in the former are significantly lower. For example, in the study by Verspoor, (1988) in Newfoundland, parr hybrid frequency was significantly higher than the mature adult hybrid frequency ($\chi^2 = 6.2$, $P < 0.05$), both overall and when analysed on a river-specific basis. Indeed, no adult hybrids were found among the 196 adults screened. This strongly suggests that the fitness of hybrids in the wild is impaired. A study of survival of artificial crosses in semi-natural conditions also suggests that salmon x trout hybrids have reduced survivorship (Garcia-Vasquez *et al.*, 2002), with crosses having brown trout mothers showing particularly low survivorship. However, this situation does not appear to be the case under hatchery conditions, where survival is equal to that of parental types and no difference is seen for trout and salmon mothers (Chevassus 1979).

The returning adult sea-migrant Atlantic salmon x brown trout hybrids observed here are not the result of a single successful hybridisation event but the product of at least 4 separate pairings. There is some evidence of a bias in the direction of hybridisation with four of the five pairings resulting from salmon female and trout male parentage. The

hybrids also appear to derive from different locations in the catchment. Juvenile salmon in Loch Lomond show significant genetic structuring among sub-catchments, based on both mtDNA haplotype and microsatellite locus allele frequencies (Thompson & Verspoor, 2007). Of the four hybrids with salmon female parents, the observed mtDNA haplotypes suggest that the female parent of one originated from the afferent River Endrick, two from the efferent River Leven and one could have been from either the River Leven or the afferent Luss Water. Thus the observed hybrids do not appear to be the product of a single hybrid event and, on first impressions, to derive from a single part of the Leven catchment.

There are several possible explanations for the high incidence of salmon x trout hybrids observed among the adult "salmon" here. If the low survival rate of hybrids in Lomond are broadly similar to the survivorship of hybrids elsewhere (see e.g. Verspoor, 1988), then this suggests a very high frequency of hybrids amongst juveniles in the catchment. This was not found. However, the 0+ fry examined in this study (in 2005) did not comprise the same age cohort as the return sea-migrants in 2006. Thus for the observed pattern of sea-migrant hybrid frequency to occur, a much higher juvenile hybridisation rate must have occurred in the years before 2005. Alternatively, the observed frequencies may be an accurate reflection of earlier hybridisation rates in the parts of the catchments they came from but the hybrids derive from parts of the catchment not sampled in this study. These explanations are not mutually exclusive. It is very unlikely that there is a major part of the catchment that consistently supports a very large juvenile population of hybrids that was not sampled during this study. The evidence of studies from other catchments suggest that it would require a juvenile hybrid frequency (whether this was spatial or temporal) exceeding that reported before to achieve the hybridisation rate of 10% in adult fish observed here (see Payne *et al.*, 1972; Verspoor, 1988; Youngson *et al.*, 1992). Given that the hybrids represented 10% of the catch, it suggests that a part of the catchment producing much more than 10% of the production of adult fish in the system has been missed.

One possible explanation is that the incidence of hybrids in the system was elevated due to the increased incidence of escaped farm "salmon" in the system, as was reported in 2006 (Anon 2006). Most simply, the majority of the hybrids observed could be escapes from a farm. Though hybrids are not normally used, they have been inadvertently produced on some farms due to misidentification of sea trout as salmon when broodstock are collected (EV, unpublished data). Alternatively, it is known that the incidence of hybrids increases where farm escapes are present (Youngson *et al.* 1993; Hindar and Balstad 1994; Matthews *et al.* 2000). 2006 is not

the only year that farm escapes have been reported in the system. They were reported in 1998 as well (Anon 2006) and conceivably may also have been present in the years 2000-2003, when the hybrid adults would have been spawned. If so, then it may be that there have also been other spikes in the frequency of hybrids in the system prior to and after this date (from spawning of farm salmon in 2006) that have not been recorded. Alternatively, these hybrids may comprise fish released as a part of a stock enhancement programme where trout and salmon were accidentally or deliberately crossed. Large sea-run sea-trout and salmon can occasionally be confused with each other and there was a stocking programme in operation at this time. Thus this possibility cannot be ruled out.

Another potential explanation is that the survivorship of hybrids from the Lomond catchment is unusually high, at least in the years prior to 2006 and significantly greater than reported elsewhere. However, there have been no studies that have attempted to identify sources of mortality in salmon x trout hybrids and the fundamental mechanisms underlying hybrid fitness remain unknown. However, if increased hybrid survival is important it is likely that some element of environmental change or reduced abundance of salmon and trout in the system seem the most likely candidate.

Increased hybridisation does not mean that the species integrity of Atlantic salmon and brown trout is at risk. Although sexually mature viable hybrids have been produced *in vitro*, (Jones, 1947) and hybrid backcrosses with Atlantic salmon have been recorded under experimental conditions (Garcia-Vazquez *et al.*, 2003). The two species differ in their chromosome number (Philips & Rab 2001). In Europe, the Atlantic salmon normally has 29 pairs of chromosomes (2N=58) with 74 chromosome arms (NF) while brown trout in western Europe appear to most typically have 42 chromosome pairs and 102 arms (e.g. Garcia-Vasquez *et al.*, 1995). The maintenance of chromosomal function in hybrids despite these differences suggests that pairing of brown trout chromosomes with corresponding chromosomal regions on salmon chromosomes during mitosis is possible, allowing a stable and full gene complement to be inherited by most somatic cells. However, this stability appears to breakdown in subsequent generations, due to mitotic and meiotic disturbances associated with imbalanced chromosome sets, and mixing of different co-adapted gene complexes (e.g. Cauwelier *et al.* 2012) and probably underlies the failure of most backcross and F_2 individuals to survive. However, natural triploid female hybrids have been observed and can produce diploid eggs which can be successfully fertilised by males of the parental species (Makharov 2008). A recent study of single nucleotide polymorphism variation in the River Tweed, S.E. Scotland has found some evidence for natural

backcrosses (EV, unpublished) and there is also circumstantial evidence suggesting that introgression may have occurred in some circumstances in the past. However, a conclusive case for hybridisation commonly leading to introgression between these species is lacking.

Overall, the available evidence would suggest that a direct genetic impact of increased hybridisation on either the trout or salmon population from the Lomond catchment is unlikely. However, an indirect ecological impact through competitive interactions cannot be ruled out, based on empirical and modelling evidence of interactions between stocked or farmed fish and native fish in the wild (McGinnity *et al.*, 2003, 2009). The presence of Atlantic salmon x brown trout hybrids may well have similar negative impacts on the wild parental populations at some stages of the lifecycle, beyond the loss of production associated with the hybridisation itself, if hybrids are particularly abundant and survivorship is high. Thus factors which increase hybridisation rates, such as increased levels of farm escapes or depressed abundance of one or both parental populations, may add to an already large list of factors threatening the abundance of salmon and trout in Scotland.

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