

A small number of anadromous females drive reproduction in a brown trout (*Salmo trutta*) population in an English chalk stream

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SUMMARY

1. Brown trout, *Salmo trutta*, exhibit one of the most highly variable and polytypic life-history strategies of all salmonids. Populations may be wholly freshwater-resident or almost exclusively migratory (anadromous), or fish of a single population may exhibit varying proportions of the two life-history strategies. Both anadromous and freshwater-resident trout freely interbreed to produce fertile offspring.
2. We quantify maternal reproductive provisioning by anadromous and freshwater-resident brown trout to their offspring and assess relative parental fitness (in terms of number, size and time of emergence of offspring). Newly emerged juvenile trout (fry) were sampled ($n = 119$) over the emergence period in March–April 2007 in a lowland English chalk stream; samples of adult trout [anadromous (6F : 12M) and freshwater-resident (22F : 56M)], river-resident trout parr and macroinvertebrate prey were also collected.
3. Using a novel combination of stable isotope analysis and microsatellite genotyping we demonstrate the overwhelming contribution of anadromous parents (both female and male) to fry production, despite the obvious presence and numerical dominance of resident adults. We unambiguously identify the maternal origins of 78% of juveniles sampled and show that maternal reproductive contribution to juvenile production in the river was higher for anadromous females (76%) than freshwater-resident fish (2.5%). Offspring of anadromous females emerged earlier and at a larger body size than offspring of resident females. Similarly, while the relative contribution of resident males (37%) was higher than that of resident females, anadromous males sired considerably more offspring (63%) than resident males. This is the first study of its kind to accurately assess the reproductive contribution of anadromous male trout.
4. Overall, this study suggests that anadromous maternal traits provide offspring with an adaptive advantage and greater fitness in early ontogeny, and that a small number of anadromous females (six of 96 adults sampled) are the main drivers of reproduction in this system.

Keywords: microsatellite, parental investment, *Salmo trutta*, sea trout, stable isotope analysis

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Introduction

Brown trout, *Salmo trutta*, exhibit one of the most highly variable and polytypic life-history strategies of all the salmonids. Populations often exist where a proportion of individuals undergo smolting and migrate to the marine environment before returning to spawn in their natal stream (anadromous), whereas other individuals (freshwater-resident) complete their whole lifecycle in freshwater (Elliott, 1994). Both anadromous and freshwater-resident brown trout freely interbreed to produce fertile offspring (Frost & Brown, 1967).

Anadromous/freshwater-resident life histories will only evolve through natural selection where the gains to individual fitness (lifetime individual reproduction) exceed the costs incurred through the chosen behaviour (Gross, Coleman & McDowall, 1988); in order to maximise fitness, individual trout must adopt a strategy based on the relative costs and benefits on growth potential, reproductive output and mortality in marine/freshwater habitats. Migration to more productive environments provides anadromous brown trout with access to increased food resources and enables them to achieve a larger final body size (Jonsson, 1985; Berg & Jonsson, 1990). However, migration is energetically costly (Dieperink, Pedersen & Pedersen, 2001) and involves increased exposure to predators and disease (Bohlin, Pettersson & Degerman, 2001). The converse is true for river-resident fish.

However, the costs and benefits of migration versus residence do not affect males and females equally. Fitness is determined by the number of offspring that an individual produces and which, in turn, survive to reproductive age. In female brown trout reproductive success is typically determined by the number of eggs an individual produces, and individual fecundity has been shown to increase logarithmically with body length in both anadromous and freshwater-resident trout (Jonsson, 1985; Elliott, 1994, 1995). However, a number of studies have demonstrated a trade-off between egg size and number in trout: small individuals can increase the fitness of their offspring by producing fewer, larger eggs (Lobón-Cerviá, Utrilla & Rincón, 1997; Jonsson & Jonsson, 1999; Olofsson & Mosegaard, 1999; Acolas, Roussel & Baglinière, 2008). For males, reproductive success is determined by the number of eggs that an individual can fertilise. As sperm is relatively cheap to produce compared to eggs, even small males can produce sufficient sperm to fertilise all the eggs of the largest of females. Large size has some advantage in attracting and defending females, such that large anadromous

males are usually the principle spawners (Jones & Ball, 1954). However, smaller freshwater-resident males can adopt 'sneaking' strategies to achieve fertilisation success, thereby reducing the advantage of being large in males and the pressure to migrate. Hence, male and female trout are likely to respond differently to any changes in the costs and benefits of migration versus freshwater residence, with the potential for profound changes at the population level. The importance of understanding the influence of each parental life-history strategy and quantifying the relative reproductive contributions to future generations will play an important role in the management of this species.

An adaptive response to ensure offspring survival, and thus maximise fitness for the maternal parent, is to invest in maternal provisioning (Roff, 1992). In salmonids, the majority of studies that have investigated the influence of maternal traits have focused on the effect of egg size on offspring size at hatching (Elliott, 1984; Einum & Fleming, 1999, 2000a; Berg *et al.*, 2001), size at emergence (Elliott, 1984; Elliott & Hurley, 1998), growth rate (Wallace & Aasjord, 1984; Ojanguren, Reyes-Gavilan & Brana, 1996) and survival (Hutchings, 1991; Jonsson & Svavarsson, 2000). For *Salmo* species, however, the influence of maternal life history has typically been inferred rather than investigated directly and the fitness benefits, in terms of number, size and time of emergence of offspring between anadromous and freshwater-resident trout, remain to be explored.

Although anadromous fish undergo morphological changes that can usually distinguish them from freshwater-resident fish, independent verification of the life-history choices of individuals is generally required. A number of laboratory techniques have been used to distinguish between anadromous and freshwater-resident life histories in salmonids, including strontium concentration in scales (Eek & Bohlin, 1997) and otoliths (Rieman, Myers & Nielsen, 1994), carotenoid pigments (Youngson *et al.*, 1997) and parasites (Black, 1981). Naturally occurring stable isotopes can be used to identify marine and freshwater feeding and are now becoming a superior alternative, providing a straightforward and relatively inexpensive technique (Doucett, Hooper & Power, 1999). Stable isotopes have a further advantage over other chemical techniques: as there is a large maternal investment of organic matter into egg production, the stable isotope composition of eggs and recently emerged fry reflect the feeding environment of the maternal parent (Doucett *et al.*, 1999). Stable isotope analysis (SIA) has been used to identify eggs (McCarthy

& Waldron, 2000) and recently emerged offspring (Curry, Noakes & Morgan, 1995; Doucett *et al.*, 1999; Charles, Roussel & Cunjak, 2004; Jardine, Chernoff & Curry, 2008) as the progeny of either anadromous or freshwater-resident mothers. However, once fry begin exogenous feeding their tissues begin to reflect the freshwater feeding environment, as a consequence of new growth, replacement or repair of tissues and, for fry of anadromous mothers, the marine isotopic ratio becomes increasingly dilute (Doucett *et al.*, 1999).

Here, we present a study of the influence of maternal/paternal life history on offspring performance in a wild population of trout in a southern English chalk stream. The key aim of this study was to assess the relative contributions of the two parental life-history strategies on offspring numbers. Secondly, our findings also informed on differences in fitness between offspring from anadromous or freshwater-resident maternal parents, in terms of size and time of emergence; we also identified the relative contributions of the two paternal life-history strategies. We achieved this by applying a novel combination of field measurements, SIA and population genetics to identify recently emerged juvenile trout as the progeny of either anadromous or freshwater-resident trout parents.

Methods

Study site

The study was carried out in Tadnoll Brook, Dorset, in southwest England, a circumneutral lowland tributary of the Frome chalk catchment, fed by both groundwater and surface run-off. The confluence with the open sea, via the river Frome and Poole Harbour (tidal), is *c.* 28 km from the study area. The study reach is *c.* 6 m wide and the sediment is largely gravel and pebble, providing suitable spawning substrate for both anadromous and freshwater-resident trout; this reach was chosen as it provides some of the most suitable spawning grounds in the Tadnoll Brook and the highest proportion of nursery habitats, and is regularly used by both anadromous and river-resident trout (Ibbotson, Beaumont & Dunbar, 2006). The section upstream (*c.* 1 km length) of the study area is not suitable habitat for spawning or fry. There are no significant obstructions to fish migration between the area surveyed and the open sea.

Sample collection

The river was monitored from the bank every 3–7 days throughout the spawning period (plus one additional

date in January 2007); data were collected on spawning activity and the location and number of in-river nesting sites (redds). A positive correlation between redd size and female fork length has previously been reported (Crisp & Carling, 1989). Thus, it was possible to estimate the size of a female, and to infer their life history from the size of each redd: visual observations of both freshwater-resident and anadromous fish on redds confirmed these inferences.

Adult trout ($n = 96$; 78 river-resident and 18 anadromous fish) were caught by repeatedly electrofishing a 300 m stretch of Tadnoll Brook throughout the 2006 spawning season (November–December). Electrofishing was undertaken with an Electracatch WFC4 pulse box; the electrical waveform was 1/2 wave rectified at 50 Hz and circuit voltage *c.* 220 V and 1.25 Amps. Fish were identified visually in the field as either anadromous or freshwater-resident based on body size and colouration (Elliott, 1994). However, it was not possible to accurately identify the sex of all adults at the time of capture based on their morphology; thus, the gender of all adults was later confirmed using a molecular test. A sample of pelvic fin tissue was removed for subsequent isotopic and molecular analysis. Tissue samples were frozen and stored at -70°C before sample preparation and analysis, except for tissues collected from the first sampling date ($n = 27$) which were stored in 96% ethanol. To determine differential effects of preservation, 25 samples were divided and preserved by both techniques.

Fry ($n = 119$) were collected from the same 300 m stretch of Tadnoll Brook each week 27 February 2007 to 11 April 2007 [based on the emergence date predicted from the degree-day model of Elliott (1994)] by point sampling with electrofishing kit: no fry were caught on the first two sampling occasions (27 February 2007, 8 March 2007). Fry were mainly caught in the shallows and slow-flowing areas, typical habitats for newly emerged fry (Greenberg, Svendsen & Harby, 1996). Once captured, fry were kept alive until the whole stretch had been fished. Fry were then over-anaesthetised by an overdose of 2-phenoxyethanol (0.333 mL L^{-1}) and immediately frozen. Upon return to the laboratory, fry fork length was measured to the nearest 1 mm, the presence of yolk recorded and the digestive tract removed. A sample of caudal fin was taken for molecular analysis.

A further collection of juvenile trout and benthic macroinvertebrates was made in May 2006 to determine the isotopic composition of parr that had been feeding in the river for some time and of the prey items they were feeding on (determined by direct observation of gut contents). Three invertebrate taxa (*Baetis*, *Simuliidae*

and *Oligochaeta*) comprised the majority of prey items of newly emerged fry. These three taxa were collected by kick sampling, sorted live in the laboratory and allowed to vacate their guts prior to freezing; 16, 11 and 8 whole individuals were used for isotopic analysis respectively.

Stable isotope analysis: sample processing

All samples (whole prepared fry, fin clips and benthic invertebrates) were dried at 85 °C for 48 h, ground to a fine powder and loaded into individual 4 × 6 mm tin capsules. The samples were combusted using a Eurovector elemental analyser and analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a Micromass Isoprime IRMS. The isotopic ratio is the relative difference between the isotope ratios of the sample and that of the relevant international standard (Pee Dee Belemnite for carbon, atmospheric air for nitrogen). Results are expressed following standard notation (‰). Precision of the analysis was $\pm 0.1\%$ (derived from standards) for both elements.

It is possible that preservation may influence stable isotope values (Feuchtmayr & Grey, 2003). Hence, a correction factor was applied to account for the effect of ethanol preservation on 27 of the adult tissue samples ($\Delta^{15}\text{N} + 0.12$, $\Delta^{13}\text{C} - 0.81$), calculated from the 25 samples that were divided and stored both frozen and in ethanol. This correction compares well with previous findings of the effects of ethanol and freezing (Feuchtmayr & Grey, 2003).

Stable isotope analysis: isotope mixing model

Trout eggs are ^{13}C depleted relative to their maternal parents (Curry, 2005), a consequence of discriminative fractionation during the development of lipid-rich egg tissues. To account for this, an independently derived fractionation value (Grey, 2001) was applied to all fry samples before analysis (mother flank tissue to unreleased eggs: $\Delta^{15}\text{N} + 0.8$, $\Delta^{13}\text{C} + 1.5$).

To identify individual trout fry as having either a freshwater-resident or an anadromous mother, a single isotope, two-source mixing model was used (IsoError; Phillips & Gregg, 2001). To determine if fry had an anadromous or river-resident mother it was necessary to set the range of isotopic ratios that could be reasonably assumed to correspond to freshwater-resident or anadromous fish. The ratios that corresponded to 95% of potential observations (1.96σ , assuming a normal distribution) were calculated for freshwater-resident and anadromous adults. Any recently emerged fry that had isotopic ratios that fell within the 95% limits were assumed to be

recently emerged offspring of freshwater-resident or anadromous mothers. For $\delta^{15}\text{N}$, the 95% intervals of freshwater-resident and anadromous trout overlapped, making it impossible to discriminate between the offspring of anadromous and river resident mothers using $\delta^{15}\text{N}$. Hence, all subsequent analysis on fry size and emergence was based on $\delta^{13}\text{C}$. By the use of supporting molecular analysis, it was possible to assign many fry to family groups originating from parents of known life-history status: anadromous or river-resident (see below).

T-tests were used to determine the significance of differences in adult $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, fry size, and time of emergence. Residual plots verified the assumptions of normality and homogeneity of variance. Statistical analyses were performed using Minitab 15.

Molecular methods

DNA was extracted from pelvic fin clip tissue using a Chelex protocol (Estoup *et al.*, 1996). The total sample comprised 119 fry and 96 adults (78 river-resident, 18 anadromous). DNA from each fish was genotyped at 12 microsatellite loci (Table 1). Loci were amplified individually in a 10 μL reaction containing 1 U *Taq* polymerase (Bioline), 0.5 μM of each primer, 1× buffer, 1.5–2.0 MgCl_2 (Table 1), 0.2 M dNTPs and 1 μL of extracted DNA. The polymerase chain reaction (PCR) cycling conditions were 3 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 30 s at the optimum annealing temperature (Table 1), 72 °C for 30 s and a final elongation cycle at 72 °C for 10 min. PCR reactions were mixed and screened for size variation in two multiplexes using a Beckman Coulter CEQ8000 automatic DNA sequencer and associated fragment analysis software (Beckman Coulter, Inc., Fullerton, CA, USA); see Griffiths *et al.* (2009) for further details.

Molecular sexing of adult fish was undertaken using a duplex PCR approach using primers for Exon 2 of the male-specific *sdY* gene, and primers for the nuclear 18S ribosomal RNA gene (amplification positive control) (Yano *et al.*, 2013; Eisbrenner *et al.*, 2014). The 18S primers amplify a product of 371 base pairs (bp) from both male and female fish, whereas the *sdY* primers amplify a product of 118 bp in male fish only. Amplifications were performed in a 10 μL volume, containing 5 μL of HotStar Taq Master Mix Kit (Qiagen), 0.1 μM each 18S primer (18S S and 18S AS, Yano *et al.*, 2013), 0.4 μM each *sdY* primer (Exon 2F, Eisbrenner *et al.*, 2014 and E2AS4, Yano *et al.*, 2013) and 1 μL of extracted DNA. PCR cycling conditions were 95 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s

Table 1 Details of microsatellite loci used for molecular parentage analysis.

| Locus | Annealing temp (°C) | MgCl ₂ (mM) | Size range (bp) | Multiplex | Dye | Volume (µL) | Reference |
|-------------------|---------------------|------------------------|-----------------|-----------|-----|-------------|---|
| Str85 | 53 | 1.5 | 154–182 | 1 | D3 | 0.8 | Presa & Guyomard (1996) |
| SsHaeIII 14.20 | 57 | 2.0 | 286–322 | 1 | D4 | 1.5 | Genbank U10050 |
| Str60 | 60 | 2.0 | 98–102 | 1 | D3 | 0.7 | Estoup <i>et al.</i> (1993) |
| SS11 | 60 | 1.5 | 331–337 | 1 | D3 | 1.3 | Martinez, Moran & Garcia-Vazquez (1999) |
| Strutta58 | 55 | 1.5 | 102–166 | 1 | D4 | 0.7 | Poteaux (1995) |
| Str15 | 55 | 1.5 | 222–230 | 1 | D4 | 0.5 | Estoup <i>et al.</i> (1993) |
| Ssa197 | 60 | 1.5 | 130–166 | 1 | D4 | 0.8 | O'Reilly <i>et al.</i> (1996) |
| SsosL417 | 56 | 1.5 | 174–194 | 2 | D4 | 0.6 | Slettan, Olsaker & Lie (1995) |
| SsosL25 | 59 | 1.5 | 114–134 | 2 | D3 | 2.0 | Slettan <i>et al.</i> (1995) |
| SsosL85 | 55 | 2.0 | 163–177 | 2 | D3 | 2.0 | Slettan <i>et al.</i> (1995) |
| SsosL438 | 57 | 1.5 | 100–110 | 2 | D4 | 0.7 | Slettan <i>et al.</i> (1995) |
| Str73 | 57 | 1.5 | 144–152 | 2 | D4 | 0.7 | Estoup <i>et al.</i> (1993) |

and a final extension at 72 °C for 10 min. Products were visualised on ethidium bromide stained, 1.8% agarose gels. A negative control (water only template) and two positive controls (DNA from one male and one female trout) were included in each batch of amplifications.

Molecular data analysis

COLONY v2.0 (Jones & Wang, 2010) was used for parentage assignment and sibship inference. This programme implements a maximum likelihood method to simultaneously assign sibship and parentage among individuals using their multilocus genotypes. The programme was run three times, under different random number seeds, run lengths and likelihood precision to check for consistency of results. Run 1 – high precision, medium length run, 0.5 probability that an actual father or mother is included in the candidate parental fish; Run 2 – high precision, medium length run, 0.25 probability that a parental fish is included in the data set; Run 3 – very high precision, long run, 0.25 probability that a parental fish is included in the data set. We assumed both male and female polygamy without inbreeding and a 1% error rate for both microsatellite scoring and allelic dropout.

Limitations of the combined stable isotope–genetic approach

A $\delta^{13}\text{C}$ mixing model was used to attribute fry to anadromous or freshwater-resident mothers, i.e. if the $\delta^{13}\text{C}$ of fry was within the range covered by 95% of observations for adults. Confidence in attribution of maternal life history to fry was strengthened for individuals that still had yolk present and by use of parallel

genetic analysis to identify sibling groups. Previous studies have demonstrated that progeny feeding endogenously retain the isotopic ratio of their maternal parent (McCarthy & Waldron, 2000; Charles *et al.*, 2004). In this study, some individuals had yolk but fell outside the range of observations for adults, suggesting that either the range of accepted observations was too restrictive or that some fry with yolk had started feeding exogenously; it is not uncommon for salmonid fry to begin feeding before the yolk is fully exhausted (Hutchings, 1991; Vignes & Heland, 1995). Although it is possible to determine maternal parentage once offspring have begun feeding (Charles *et al.*, 2004; Jardine *et al.*, 2008), there is limited time before organic matter is taken up from the surrounding biome, gradually replacing the maternal isotopic ratio with one characteristic of feeding in the freshwater environment (Doucett *et al.*, 1999). Therefore, initial identification of fry of freshwater-resident maternal origin was based on a combination of body size and isotopic ratio. Fry with signs of endogenous feeding outside the 95% interval may have been erroneously placed outside the limits if the value used for fractionation between mother and offspring (Grey, 2001) was incorrect; certainly, on the basis of the sibling groups defined by microsatellite genotyping (see below), many of the SIA 'near misses' were in fact the offspring of an anadromous mother.

Results

Redd production

Freshwater-resident females were smaller than anadromous females: fork length 189–248 mm, cf. 460–748 mm (*t*-test: d.f. = 14, *t* = 9.23, *P* < 0.001). Out of the 12 redds

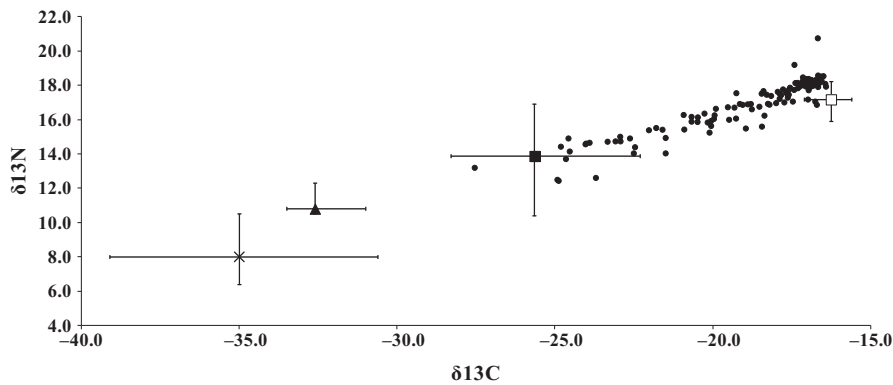


Fig. 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of recently emerging trout fry (●) collected in the Tadnoll Brook during the period of emergence in March–April 2007. Isotopic ranges of adult anadromous (□) and freshwater-resident (■) brown trout, river feeding 0+ trout parr (▲) and macroinvertebrate prey (×) are also shown; vertical and horizontal lines indicate the range of isotopic values (mean, minimum, maximum).

recorded, nine were cut by large females (redd tail length ≥ 1.6 m) and three by smaller females (redd tail length ≤ 1.5 m). The first redds produced were large; small redds were first observed 3 days later (Figure S1). However, there was no distinction in the time of production of small and large redds, both being produced throughout the spawning period.

Identification of maternal life history

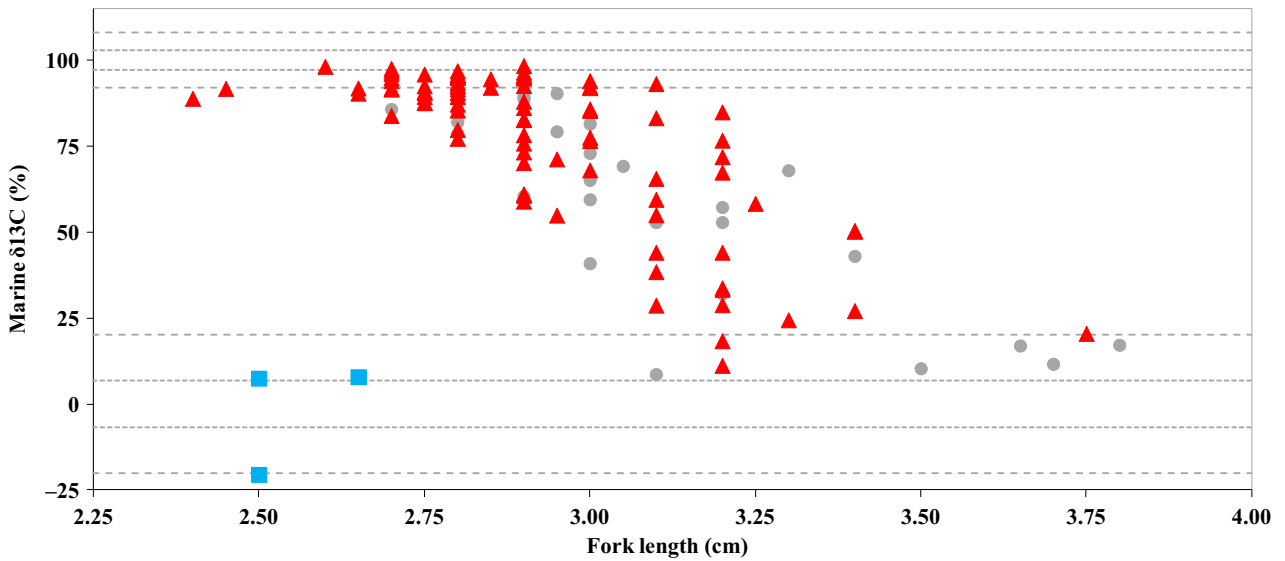
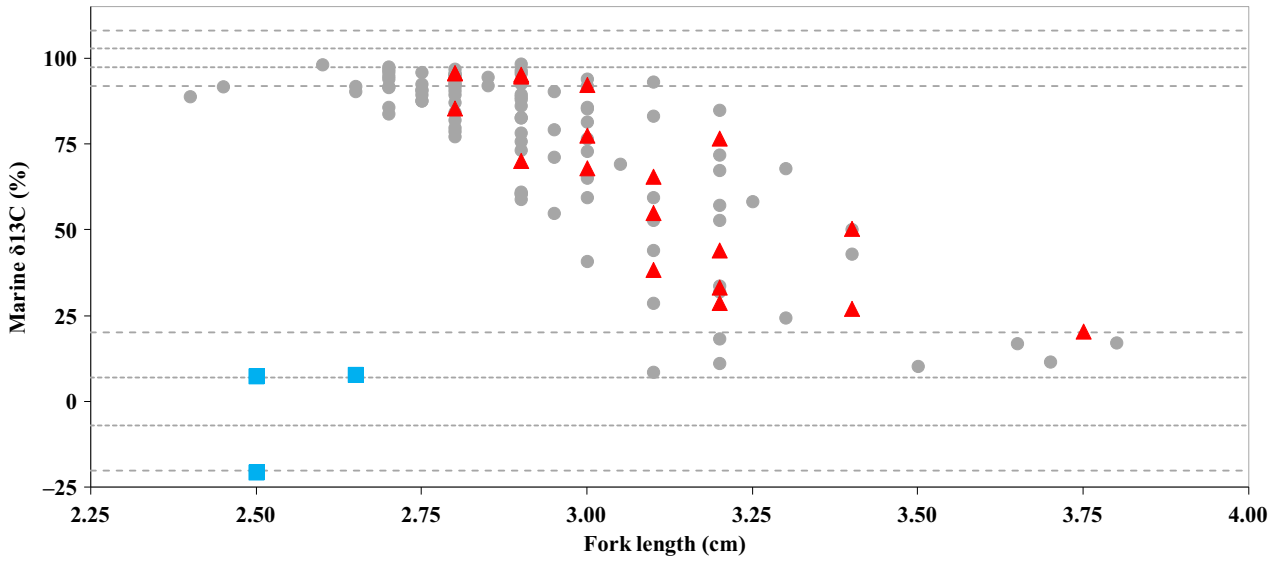
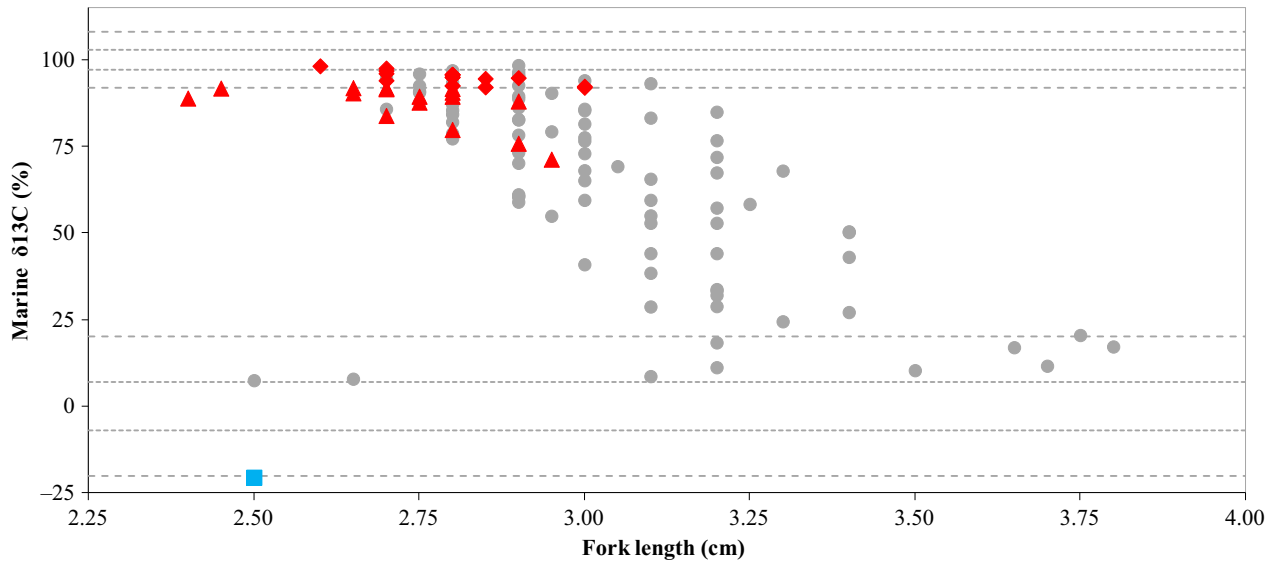
Adult samples were split into two distinct groups by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 1). Freshwater-resident trout were depleted in both heavy isotopes compared with anadromous trout (*t*-test, $\delta^{13}\text{C}$: d.f. = 73, $t = 37.02$, $P < 0.001$; $\delta^{15}\text{N}$: d.f. = 73, $t = 8.30$, $P < 0.001$). Compared with river-feeding juvenile trout (parr), freshwater-resident and anadromous adult trout were enriched, respectively, by *c.* 4.5‰ and *c.* 7.8‰ for $\delta^{15}\text{N}$, and by *c.* 8.2‰ and *c.* 17.6‰ for $\delta^{13}\text{C}$. It was not possible to determine the maternal life history of fry using $\delta^{15}\text{N}$; due to high variability in $\delta^{15}\text{N}$ of freshwater-resident adults, the 95% ranges for anadromous and freshwater-resident mothers overlapped. Since $\delta^{15}\text{N}$ is influenced by trophic position (*c.* 3–5‰ per trophic level; Fry, 2008), the large variance of $\delta^{15}\text{N}$ of freshwater-resident trout is likely to be a consequence of a varied diet. Nevertheless, it was possible to separate fry of anadromous and freshwater-resident maternal origin using $\delta^{13}\text{C}$.

Unlike previous reports (Doucett *et al.*, 1999; McCarthy & Waldron, 2000; Charles *et al.*, 2004), fry did

not form two distinct groups based on their isotopic ratios but were scattered along a cline between the ratios obtained for anadromous and freshwater-resident adults (Fig. 1).

Recently emerged fry could be identified by the presence of visible yolk reserves. Once yolk is exhausted fry can only sustain growth by exogenous feeding. Most fry with yolk had an isotopic ratio that corresponded to a marine source (Fig. 2a). However, one individual was found with yolk that had an isotopic ratio corresponding with freshwater-resident fish (Fig. 2a, blue squares). Fry of larger size and without obvious yolk tended to have progressively less of a marine ratio with increasing size. However, two fry that did not have obvious yolk were identified as being within the freshwater-resident isotope ratio interval but not part of the cluster of other, larger fry (Fig. 2a), indicating a putative freshwater-resident maternal origin for these fish. Genetic analysis confirmed that these two fish, together with the individual with yolk and a freshwater-resident isotopic ratio, were the product of freshwater-resident mothers [Supporting information, Spreadsheet 1, Fish A104, A116 (Family 42), Fish A78 (Family F34)]. Fry within the isotopic ratio limits set for anadromous origin that still had obvious yolk reserves were certainly of anadromous origin. However, 16 fry with yolk were identified as just outside or close to the isotopic limit defined for anadromous origin (Fig. 2a, red triangles). These fry were designated as being of putative anadromous origin as their isotopic ratio was not likely to have been derived from

Fig. 2 Relationship between the proportion of marine derived carbon in individual offspring (from mixing model) and fork length for trout fry in the Tadnoll Brook (0% marine carbon corresponds to mean freshwater-resident adult trout, 100% marine carbon corresponds to mean anadromous adult trout). The ranges corresponding to 95% (large dashed line; 2 SD) and 50% (small dashed line; 1 SD) of adult observations were used to define intervals that corresponded to the respective life-history strategies. (a) Results based on SIA data alone: fry with yolk sac falling within anadromous range (◆), fry with yolk sac falling outside anadromous range (▲), fry with yolk sacs falling outside resident range (■), all other samples (●); (b) results based on genetic analysis alone: fry with known anadromous mother (▲), fry with known resident mother (■), all other samples (●); (c) combined SIA and genetic results: fry of anadromous maternal origin (▲), fry of resident maternal origin (■), all other samples (●).



freshwater-resident adults (Doucett *et al.*, 1999) and either they had not started feeding exogenously or had not been doing so for very long; genetic analysis using the program COLONY confirmed that nine of these 16 juveniles were derived from an anadromous mother (Supporting Information, Spreadsheets 1 and 7). Twelve fry within the isotopic limit set for anadromous origin were identified (gut content analysis) as having begun exogenous feeding.

With the exception of the three fry identified as being of putative freshwater-resident maternal origin, the relationship between fry length and % marine isotopic ratio appeared to follow a clear growth curve. Smaller fry feeding endogenously had a typical marine ratio (anadromous maternal origin) that became progressively dilute with increasing size, as the fry built body tissues using organic matter derived from freshwater prey. Thus, the classification of larger fry could be equivocal: fry within the 95% freshwater-resident interval may be of freshwater maternal origin or they may be anadromous origin fry with a $\delta^{13}\text{C}$ reflecting freshwater feeding (Fig. 2a). Fry that could not be identified unequivocally as being of anadromous or freshwater-resident maternal origin from their isotopic composition were defined as being of undetermined source, though many of these were subsequently confirmed by molecular-based sibship analysis using the program COLONY as being the offspring of an anadromous mother (Supporting Information, Spreadsheets 1 and 7).

Size at emergence

Only those fry of putative anadromous maternal origin with obvious yolk were used to determine size at emergence (Fig. 2a). Two groups of fry of anadromous maternal origin were used, those fry with yolk that were within the 95% interval ('within') and all fry with yolk within and outside the 95% interval ('pooled'). Both groups of fry of anadromous maternal origin were larger (within: FL = 2.80 cm \pm 0.11 SD, pooled: FL = 2.77 cm \pm 0.13 SD) than fry of putative freshwater-

resident maternal origin (FL = 2.55 cm \pm 0.09 SD, *t*-test: Within, d.f. = 18, *t* = 3.89, *P* = 0.001, pooled, d.f. = 34, *t* = 2.80, *P* = 0.008).

Time of emergence

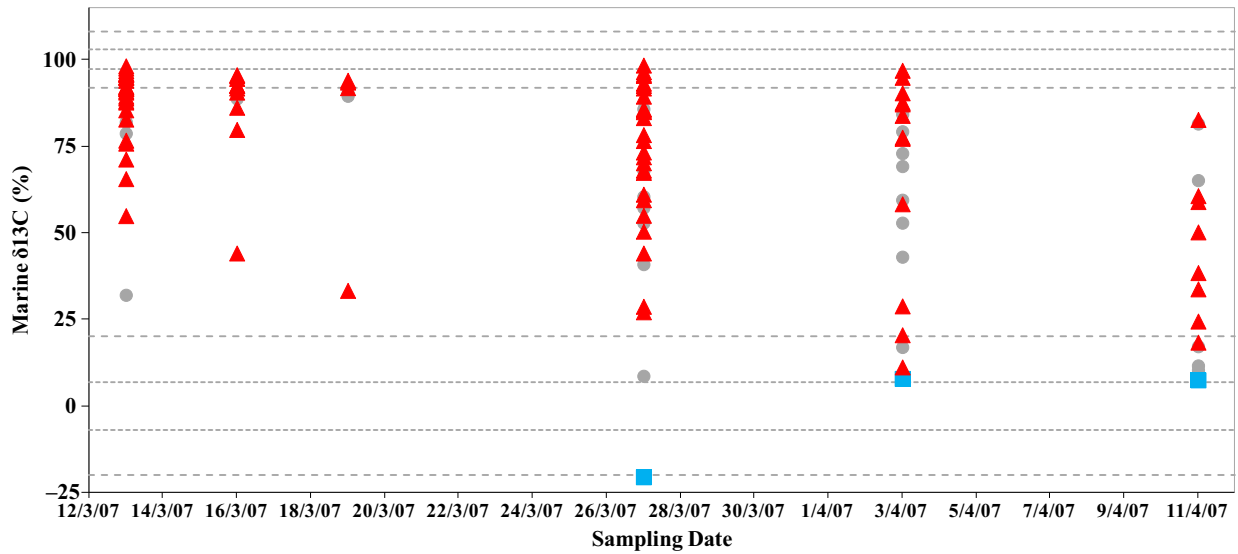
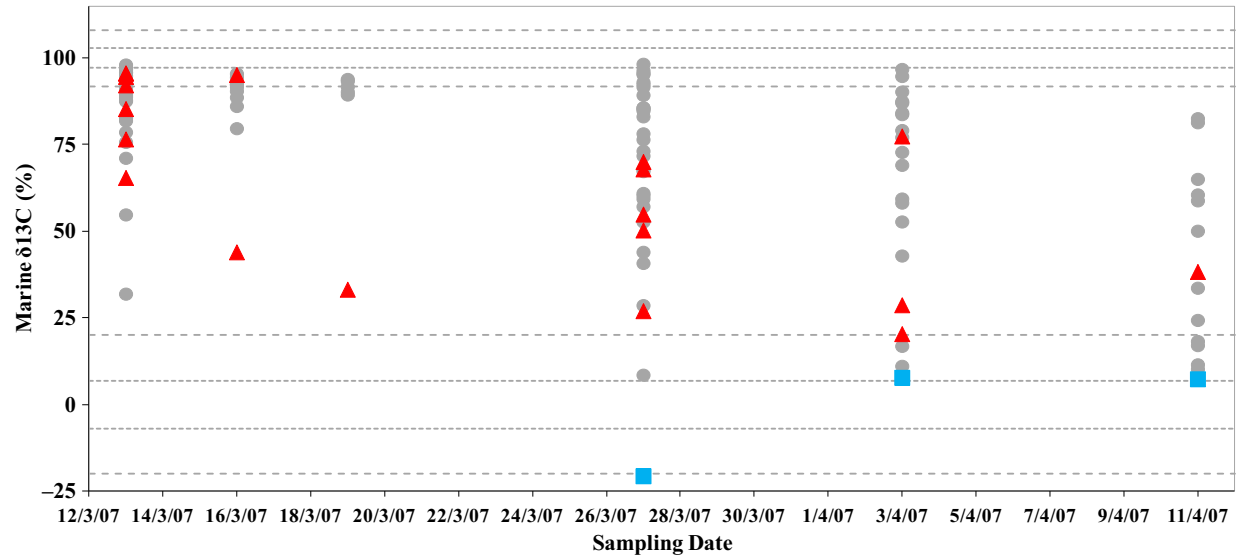
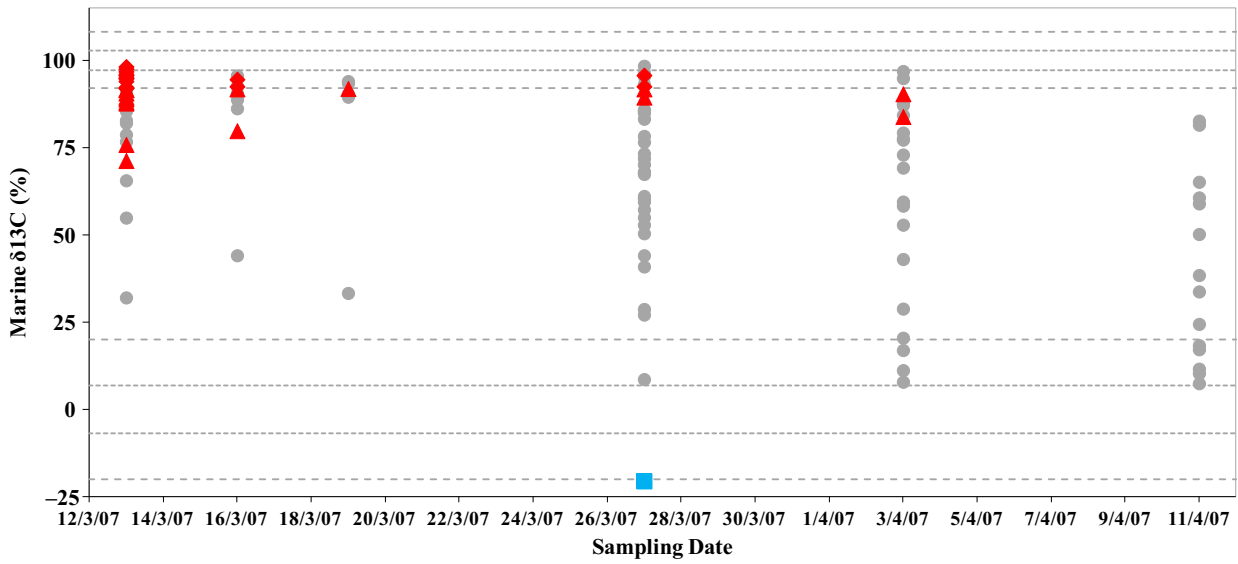
Fry derived from anadromous mothers were first caught on 13 March 2007 and were collected throughout the period of emergence (Fig. 3). Fry feeding endogenously, indicating recent emergence, were caught on all but the final occasion (Fig. 3). Endogenous feeding fry of putative freshwater-resident maternal origin were first collected on 27 March 2007 (Fig. 3); the remaining fry of putative freshwater-resident maternal origin were collected later. On the first three sampling visits a small number of fry were collected that had isotopic ratios between those of anadromous and freshwater-resident fish, suggesting some degree of exogenous feeding and an earlier date of emergence.

COLONY results

The duplex PCR method (Yano *et al.*, 2013; Eisbrenner *et al.*, 2014) indicated that, of the 78 freshwater-resident adult trout sampled, 22 were female and 56 were male. Of the 18 anadromous adult trout sampled, six were female and 12 were male.

Results were very similar across the three runs of COLONY: we present the results for Run 2. The 119 fry were assigned to 44 full-sib families (Supporting information: Spreadsheet 1 and Figure S2). The number of fry per family ranged from 1 to 13 (mean 2.7). Paternity was assigned to 52 fry with 19 having freshwater-resident fathers and 33 anadromous fathers. In total, 15 fish (nine freshwater-resident and six anadromous) acted as a male parent. Maternity was assigned to 22 fry with three having a freshwater-resident mother and 19 an anadromous mother (Figure S3). Five fish (two freshwater-resident and two anadromous) acted as a female parent. For 16 fry both the paternal and maternal parents were determined. Unfortunately, of the three largest

Fig. 3 Relationship between proportion of marine derived carbon in individual trout fry (from mixing model) and date of capture in the Tadnoll Brook (0% marine carbon corresponds to mean freshwater-resident adult trout, 100% marine carbon corresponds to mean anadromous adult trout). The ranges corresponding to 95% (large dashed line; 2 SD) and 50% (small dashed line; 1 SD) of adult observations and were used to define intervals that corresponded to the respective life-history strategies. Any offspring that fell between the bounds were accepted as having a mother with that particular life history. (a) Results based on SIA data alone: fry with yolk sac falling within anadromous range (◆), fry with yolk sac falling outside anadromous range (▲), fry with yolk sacs falling outside resident range (■), all other samples (●); (b) results based on genetic analysis alone: fry with known anadromous mother (▲), fry with known resident mother (■), all other samples (●); (c) combined SIA and genetic results: fry of anadromous maternal origin (▲), fry of resident maternal origin (■), all other samples (●).



family groups identified (Families 3, 25, 35) only one parent fish (a male) was sampled (Family 25) (additional date-specific information on these family groups is provided separately in Supporting information: Figures S4–S6). Nonetheless, the presence of some of the earliest caught fry from family groups 3 and 35 within the stable isotope ratio limits characteristic of anadromy unambiguously identified these two families as being the offspring of an anadromous mother.

The decay profile of the marine isotopic ratio in family groups 3, 25 and 35 can be tracked (Fig. 4). Critically, these genetic data demonstrate that fry caught later in the sampling period (e.g. Family 3: 11 April 2007) with a largely freshwater isotopic profile are related to juvenile fish sampled c. 1 month earlier (e.g. Family 3: 13 March 2007) which displayed a strongly marine isotopic ratio; thus, these fish appear to derive from the same, apparently anadromous, mother.

Maternal/paternal reproductive contribution

In combination, SIA and molecular analysis could confidently ascribe 90 fry (76%) to anadromous mothers and 3 fry (3%) to freshwater-resident mothers, i.e. a total of 93/119 fry were assigned (78%; Fig. 2c). The maternal origin of the remaining 26 fry (22%) was undetermined as (i) their isotopic ratios fell outside of the limits set for either putative anadromous or putative freshwater-resident maternal origin, and (ii) no member of their family group – as determined by sibship analysis of their microsatellite profiles – fell within the 95% limits set for putative anadromous or putative freshwater-resident maternal origin.

Stable isotope-based identification of maternal origin was confirmed by genetic analysis for 5/30 fry of

anadromous origin and 3/3 fry of freshwater-resident origin (Supporting Information: Spreadsheet 1). Of the remaining 86 fry (72%) that were unassigned using SIA alone, 42 were assigned to anadromous mothers by microsatellite analysis. In addition, the assignment of fry to genetically defined family groups that included at least one individual whose isotope ratio identified it/them as anadromous, indicated that a further 17 juveniles were derived from anadromous mothers, despite these juveniles having isotopic ratios outside the 95% limits defined *a priori*. Finally, a further eight fry were identified as the offspring of an anadromous mother by genetic analysis; these fish were part of small sib-groups (of 1–3 fish) which had no members of their sib-group within the anadromous isotope ratio limits, but which through microsatellite genotyping and parentage assignment were shown to have an anadromous mother.

Of the remaining 26 fry (22%) whose maternal origin could not be confidently determined, the cline in observed isotopic ratio values (Fig. 2c) suggests that these fish were from anadromous mothers, but had been feeding exogenously, as they followed a similar size/isotope trajectory to the other 90 fry (76%) identified as being derived from an anadromous mother. However, the assignment of five larger, late-caught fry (4%) with freshwater isotopic ratios remained ambiguous (Fig. 2c). Overall, including fry with intermediate isotopic ratios, we suggest – albeit with less confidence – that 97.5% of the fry caught were derived from anadromous mothers. This finding is somewhat at variance with the proportion of fry identified by genetic analysis as having an anadromous father.

The number of fry for which parentage could be genetically assigned was correlated with the size of each individual adult fish. Fork length was used as a proxy

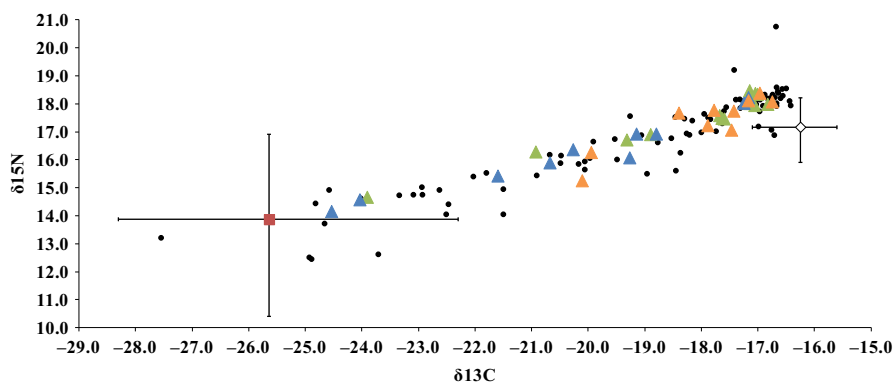


Fig. 4 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of recently emerging trout fry for the three largest sib-groups detected using the program COLONY [families: 3 (\blacktriangle), 25 (\blacktriangle) and 35 (\blacktriangle)]. Data for all other fry (\bullet) are also included. Isotopic ranges of adult anadromous (\diamond) and freshwater-resident (\blacksquare) brown trout are also shown; vertical and horizontal lines indicate the range of isotopic values (mean, minimum, maximum). Additional date-specific information on the members of each of the family groups sampled is provided in Supporting information.

for fish size and was measured for all adult fish. Spearman's rank correlations were non-significant for both resident (Spearman's $\rho = -0.276$, $P = 0.472$; Figure S7a) and anadromous males (Spearman's $\rho = 0.464$, $P = 0.354$; Figure S7b). There was a highly significant positive correlation between size and number of offspring for anadromous females (Spearman's $\rho = 1.0$, $P < 0.001$; Figure S7c). Only three juveniles were genetically assigned to two resident females and no correlation was calculated.

In summary, while comprising only 21% of females sampled (6/28 females), anadromous females contributed a disproportionately high component (76–97.5%) of the juvenile population. For male adult trout, the ratio of anadromous to freshwater-resident males (12 : 56) was approximately equal to the ratio observed for adult females (6 : 22). However, in the case of adult males, the balance of juveniles assignable to one or other life-history morph, was less skewed towards the anadromous male parent (33 : 19) than was apparent for adult females (90 : 3). Thus, while we were unable to evaluate the direct effects of having an anadromous father on offspring fitness, numerically, adult sea trout males also appear to be making a disproportionately large contribution to the number of juvenile trout in the next generation at this study site. It is also clear that females were mating with multiple males (Figure S2).

Discussion

Using a combination of stable isotopes and population genetics we have demonstrated the effect of maternal provisioning on offspring numbers and fitness in brown trout, and the differential reproductive success of anadromous and river-resident trout. Uniquely, we were able to demonstrate the distinction between maternal and paternal contributions, showing that the relative contribution of river-resident males to offspring production was higher than that of resident females. However, despite sampling many more male than female adult trout at our study site (for both river-resident and anadromous fish), our combined analyses demonstrate how, ultimately, a relatively small number of anadromous females (only six of the 96 adults sampled) can drive reproduction in a chalk stream trout population.

The skewed sex ratio we observed among adult trout seems not atypical for salmonids; Consuegra & García de Leániz (2007) report a range of skews (both male and female biased) in Atlantic salmon populations in rivers in northern Spain. However, our findings are at odds

with the work of Bekkevold, Hansen & Mensberg (2004) who identified a *c.* 3 : 1 female bias in anadromous trout from six Danish rivers, though their overall conclusions regarding the importance of females in mediating gene flow were not dissimilar to ours.

Offspring of anadromous trout emerged on average earlier and at a larger body size than offspring of freshwater-resident fish (although the latter were fewer in number). These results do not correspond with previous findings. Thériault & Dodson (2003) observed resident brook charr spawning *c.* 15 days before anadromous charr, with fry of resident mothers emerging earlier. Similarly, Jardine *et al.* (2008) reported that the progeny of resident brook trout emerged earlier than progeny of anadromous individuals. However, in both cases, emerging progeny of anadromous parents were larger than the progeny of freshwater-residents.

Maternal traits are known to affect egg size in salmonids and, further, egg size is positively related to fry size (Ojanguren *et al.*, 1996; Lobón-Cerviá, 2000), with fitness benefits to juveniles of being larger. The volume of yolk resources increases with egg size (Elliott & Hurley, 1998) and larger egg size has been reported as affording hatchery-reared brown trout fry with greater energy reserves (Ojanguren *et al.*, 1996), whereas larger fry have greater survival and can feed on a wider range of prey (Hutchings, 1991). However, there are disadvantages of large egg size. During incubation, eggs may be subjected to low concentrations of dissolved oxygen (Sear *et al.*, 2014); as egg size increases, the efficiency of oxygen transfer declines (Berg *et al.*, 2001). Therefore, larger eggs may experience higher mortality. With such antagonistic pressures acting on egg size and emergence, the relative advantage of a maternal anadromous or freshwater-resident life history is likely to vary among sites dependent upon local conditions. Here, a very small proportion of offspring (<3%) were attributed to freshwater-resident mothers. Such a small proportion may be a true reflection of the contribution of freshwater-resident females or could be due to smaller fry with smaller energy reserves facing higher rates of mortality (Einum & Fleming, 1999); either way, they represent a low contribution of freshwater-resident mothers to the next generation of juveniles, *i.e.* low reproductive fitness.

Differences in juvenile fitness in salmonids vary greatly within the same species (Elliott, 1984; Acolas *et al.*, 2008) and the occurrence of both anadromous and freshwater-resident life histories in a single brown trout population suggests no overall advantage of either life-history strategy (Pettersson, Hansen & Bohlin, 2001). However, it is apparent that a small number of anadro-

mous females contributed a disproportionately large number of offspring to the next generation of the trout population at the study site; moreover, these anadromous females also produced the largest emerging fry. For successfully returning females, the benefit (and risk) of migrating to a more productive environment (the sea) appears to outweigh that of remaining in the river and the larger fry produced by anadromous females suggests they pass these benefits on to their progeny. Accordingly, we hypothesise that in this population anadromous mothers invest more energy into reproduction (in proportion to their size) and, assuming offspring size is linearly related to maternal size (L'Abèe-Lund & Hindar, 1990), offspring of anadromous adults should be larger. On the face of it, such findings are at odds with the trade-off relationship between fecundity and offspring size previously described for salmonids (Fleming & Gross, 1990; Einum & Fleming, 2000b). We did not collect data on egg numbers for the two life-history morphs and it may be that selection against smaller eggs had already occurred by the time we measured numbers of emerging fry.

In addition, a larger proportion of anadromous males than resident males contributed to the juvenile population of the river. However, the imbalance between life histories was not as marked as that exhibited by female trout and it appears that the advantages of (and selection for) migrating to sea for males are not so great as for females. Given that the benefits of anadromy to male trout are not derived through enhanced sperm competition (Vladić, 2006), males do not directly pass on the benefits of anadromy to their offspring in the same manner as females. Thus, the selective advantages of anadromy to male trout (and our data indicate considerable advantages) must manifest through other mechanisms, e.g. the ability to compete for females during mating and to hold breeding territories (Pettersson & Järvi, 1997; Berg *et al.*, 2001; Vladić & Järvi, 2001). Other strategies, e.g. sneaker mating by precocious parr, are known to offer distinct advantages to non-migrating male Atlantic salmon, though to what extent this strategy is utilised by brown trout is unclear (Garcia-Vazquez *et al.*, 2001; Vladić, 2006). It is clear from our data, that anadromous females mated with multiple males, including freshwater-residents.

Varying degrees of genetic divergence have been reported between and within brown trout populations (Ferguson, 1989; Pettersson *et al.*, 2001); however, in rivers where life-history strategies overlap, within-population genetic variation does not appear to be directly related to life-history strategy (Charles *et al.*, 2006). Nonetheless, if life history is genetic and maternal provi-

sioning does influence offspring fitness (at least in the early growth stages) then, clearly, some aspects of this fascinating system remain to be resolved. Whether life history is predetermined by genotype or environment, or to some extent by both, the fitness benefits to offspring have – as shown in this study – undoubtedly been shaped by the experiences of the preceding generation.

Acknowledgments

We thank William Beaumont (Game and Wildlife Conservation Trust) for help with sampling and fieldwork, and Dr Rasmus Lauridsen (Game and Wildlife Conservation Trust) for advice on stable isotope analysis. This work was supported by a Natural Environment Research Council (UK) PhD studentship (NER/S/A/2005/13773); additional funding was provided by the Game and Wildlife Conservation Trust and the University of Exeter. J. I. Jones was supported by NERC grant NE/C511905/1. All tissue collection was carried out under UK Home Office licence PPL 80/1913. We thank three anonymous referees for constructive comments on an earlier draft of this manuscript. The authors declare no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Number of new anadromous (shaded) and freshwater-resident (unshaded) trout redds created in the 300 m study section of the Tadnoll Brook during the spawning period 2006–2007.

Figure S2. Fry-parental schema, derived from microsatellite genotyping and analysis of allele frequencies using the COLONY software.

Figure S3. Maternal assignment analysis of juvenile trout arranged according to their $^{13}\text{C} : ^{15}\text{N}$ isotope ratios.

Figure S4. Family 3: date-specific isotope ratios for individual family 3 juvenile trout

Figure S5. Family 25: date-specific isotope ratios for individual family 25 juvenile trout

Figure S6. Family 35: date-specific isotope ratios for individual family 35 juvenile trout

Figure S7. Correlation between adult size (as measured by fork length) and number of offspring for: (a) male-resident trout (Spearman's $\rho = -0.276$, $P = 0.472$); (b) male anadromous trout (Spearman's $\rho = 0.464$, $P = 0.354$); and (c) female anadromous trout (Spearman's $\rho = 1.0$, $P < 0.001$).

Spreadsheets 1-7. Additional supporting data files.

(Manuscript accepted 9 March 2016)