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RESEARCH ARTICLE

# The association between parental life history and offspring phenotype in Atlantic salmon

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## ABSTRACT

In many taxa there is considerable intraspecific variation in life history strategies from within a single population, reflecting alternative routes through which organisms can achieve successful reproduction. Atlantic salmon *Salmo salar* (Linnaeus) show some of the greatest within-population variability in life history strategies amongst vertebrates, with multiple discrete male and female life histories co-existing and interbreeding on many spawning grounds, although the effect of the various combinations of life histories on offspring traits remains unknown. Using crosses of wild fish we show here that the life history strategy of both parents was significantly associated with a range of offspring traits. Mothers that had spent longer at sea (2 versus 1 year) produced offspring that were heavier, longer and in better condition at the time of first feeding. However, these relationships disappeared shortly after fry had begun feeding exogenously. At this stage, the juvenile rearing environment (i.e. time spent in fresh water as juveniles) of the mother was a better predictor of offspring traits, with mothers that were faster to develop in fresh water (migrating to sea after two rather than three years of age) producing offspring that had higher maximal metabolic rates, aerobic scopes, and that grew faster. Faster developing fathers (1 year old sneaker males) tended to produce offspring that had higher maximal metabolic rates, were in better body condition and grew faster. The results suggest that both genetic effects and those related to parental early and late life history contribute to offspring traits.

**KEY WORDS: *Salmo salar*, Early development, Indirect genetic effects, Maternal effects, Parental effects, Paternal effects, Salmonid metabolism**

## INTRODUCTION

In many taxa there is considerable intraspecific variation in life history strategies within a single population (Skov et al., 2008; Chapman et al., 2011; Mehner and Kasprzak, 2011), reflecting alternative routes through which organisms can achieve successful reproduction (Roff, 2002; West-Eberhard, 2003). Intraspecific differences in life history strategies can be manifested in terms of variation in age or size at reproduction, level of reproductive investment per breeding attempt or per propagule, or strategy used to obtain a mate (e.g. fighting versus sneaking males). While some of the variation in life history strategies is genetic, some can probably be explained by differing environments experienced by the individual during its development (Monaghan, 2008). There is a growing body of literature suggesting that a considerable amount of the variation could be attributed to parental effects, whereby the conditions experienced by the parents during their own life can leave a lasting legacy on the generations to follow (Burton and Metcalfe, 2014). These non-genetic influences are sometimes termed maternal effects, but we use the term 'parental' as the father can also have an influence.

Studies to date suggest that genetic, environmental and parental effects can all contribute to intraspecific variation in life history traits (Laugen et al., 2002; Ab Ghani et al., 2012), but parental effects are arguably least well understood. Much of the work investigating parental effects has focused on understanding how the direct effect of the condition and/or current environment of the parents at the time of reproduction shapes propagule size (Mousseau and Fox, 1998), number (Einum and Fleming, 2000) and quality (Blount et al., 2002; Burton et al., 2013a,b). While it is undeniable that the current state and environment of the parent at the time of reproduction can affect the offspring in ways that have dramatic fitness consequences, their adaptive nature is often unclear (Uller et al., 2013). The early environment and life history strategy of the parents may be a better predictor for the initial performance and life history pathway of their offspring (Taborsky, 2006; Jonsson and Jonsson, 2014; Burton and Metcalfe, 2014).

Atlantic salmon *Salmo salar* (Linneaus) show extreme within-population variability in life history strategies (Fleming, 1996; Garant et al., 2002; Dodson et al., 2013). In this species, multiple discrete male and female life histories co-exist and interbreed on many spawning grounds; they are thought to arise from a mixture of genetic and non-genetic influences (Thorpe et al., 1998; Dodson et al., 2013), but the effect of the various combinations of life histories on offspring traits remains unknown. Males and females can spend between one and six years in fresh water prior to smolting (the physiological and morphological preparation for salmonids to enter sea water) and emigrating to sea, where they spend one or more winters at sea before returning to their natal freshwater stream to spawn. The range in time spent in fresh water prior to seaward migration is primarily due to variation among rivers in growth conditions, with fish migrating at a younger age in warmer rivers (Metcalfe and Thorpe, 1990), but there is also variation in age at migration within rivers. Interestingly, some males, generally those that exhibit fast early growth (Whalen and Parrish, 1999; Aubin Horth and Dodson, 2004), will become sexually mature at a small size without ever going to sea; these males, known as precocious male parr, participate in spawning as sneakers (Fleming, 1996).

Since fast-growing juveniles tend to migrate to sea earlier than their slower growing counterparts from the same population (Metcalfe, 1998), there has been interest in the intrinsic differences

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between fish that affect potential growth rate and hence generate life history variation. One trait that has received a considerable amount of attention is metabolic rate (Forseth et al., 1999), partly because it is often correlated with dominance or growth (Metcalfe et al., 1995; Álvarez and Nicieza, 2005) and partly because it constitutes the fundamental energy budget of organisms. Standard metabolic rate (SMR, usually measured in terms of oxygen consumption) is the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (so is the equivalent of the basal metabolic rate of an endotherm), and is an integrated measure of the energy expenditure involved in tissue maintenance and organism homeostasis. After controlling for temperature, body size and other sources of variation, SMR often differs by a factor of 2 or 3 between individuals of the same age, sex and species held in similar conditions (Burton et al., 2011). Aerobic scope (AS) is the difference between an animal's SMR and its maximum possible aerobic metabolic rate (MMR) under the same environmental conditions, so that AS represents the capacity of the animal to increase its rate of aerobic metabolism (Fry, 1957, 1971). Individual differences in SMR within salmon and trout populations have been linked to variation in individual growth and life history strategies (e.g. timing of smolt migration; Metcalfe et al., 1995; Forseth et al., 1999; Finstad et al., 2007). In contrast, the role of AS remains relatively understudied compared with SMR, although it has been found to be correlated with swim performance, distance of migration (Eliason et al., 2011) and survival of individuals in challenging environments (Clark et al., 2011; Killen et al., 2012).

The associations between parental rearing environment and offspring traits remain largely unknown, regardless of whether these are driven by genetic, environmental or parental effects. Here we use the diversity of life history strategies in Atlantic salmon and the scope they offer for controlled *in vitro* fertilizations to test (1) whether there is any association between maternal or paternal life history pathway and offspring performance (a stronger maternal influence indicating a parental effect), (2) whether the strength of any such effects weakens over time during offspring development (which would again suggest that they are parental effects), and (3) whether parents that exhibited fast juvenile growth produce offspring that themselves have higher growth and metabolic rates.

## MATERIALS AND METHODS

### Broodstock collection and crosses

In November 2012 mature sea-run Atlantic salmon undertaking their spawning migration were captured at the Loch na Croic fish trap on the River Blackwater, northern Scotland (57°60′N, 4°63′W). Males and females were held separately at the trap site until ripe in circular tanks (4 m diameter, 1.5 m deep) supplied directly with water from the River Blackwater. Precocious male parr were captured by electrofishing on the River Blackwater each day that they were required for spawning trials (see below). The life history of each fish was determined using scalimetry (whereby the pattern of circuli of a fishes scale is examined and the various stages of ontogenetic growth revealed). Results of the scalimetry analysis revealed that all females were maiden spawners and had spent either two or three years in fresh water and one (1SW) or two years (2SW) in salt water. Males had spent one, two or three years in fresh water and zero, one (1SW) or two years (2SW) in salt water prior to spawning; the males that had spent zero years in salt water were precocious male parr that would adopt a sneaking spawning strategy (Fleming, 1996). Average lengths were 578.6± 20.9 and 728.9±46.2 mm for 1SW and 2SW females, and 117.5±11.3, 578.1±47.04 and 792.9±45.6 mm (means ±s.d.) for precocious parr, 1SW and 2SW males, respectively.

In order to evaluate the associations between maternal or paternal rearing environment and offspring traits we used a factorial mating design (a diagram of the mating design and sample sizes of parents in relation to the amount of time spent in fresh water and salt water are provided in Fig. S1 and Table S1). Eggs or sperm were extruded by abdominal massage from anaesthetized ripe fish. Eggs from each female were collected, drained of ovarian fluid and weighed to the nearest 0.1 g on the day of stripping to determine clutch mass. Nine to 10 unfertilized eggs per female were then collected, frozen and later defrosted and reweighed so that the average mass of an egg from each female could be determined. A 1SW and a 2SW female (assigned to sea ages on the basis of size, later confirmed by scalimetry) were paired, and their clutches each split into three, and fertilized with sperm from a single haphazardly chosen male of each life history type (precocious parr, 1SW and 2SW). Each mating block of five fish (two female types×three male types) was then replicated nine times using different individuals (giving 54 families, derived from a total of 45 parents, nine of each parental type). All parental fish were blotted dry and measured (fork length±0.5 cm; body mass±0.1 g) after collection of their gametes. All fish spawned from 28 to 30 November 2012.

### Egg rearing, hatching and experimental procedures

Fertilized eggs were transferred from the capture site to the nearby Scottish and Southern Energy hatchery at Contin, Scotland, where they were reared as separate family groups, under ambient water temperatures (3.82±0.69°C) until the eyed stage. Dead eggs were recorded and carefully removed daily; four families had high infertility or embryo mortality during early incubation, leaving 50 families.

On 5 March 2013, 50–100 eyed eggs from each family were transferred to the aquarium facilities at the campus of the University of Glasgow, Scotland, where they were maintained as separate family groups in sections (one per family) of an experimental stream tank, which was transversely divided using 1 mm nylon mesh. Incubation temperature during this period was slowly raised from 5 to 13°C over the course of 30 days to increase developmental rate. Eggs hatched relatively uniformly across families from 25 March to 3 April 2013. On 22 April 2013, by which time they had reached the first feeding stage (the point in time when the yolk sac of the alevins is fully exhausted and individuals begin to swim up from the substrate in search of food), 10 fry per family were anaesthetized, blotted dry and measured (fork length±0.1 mm; body mass ±0.0001 g). At this stage fish continued to be held in the same experimental stream tank described above on a recirculation system, but whereas previously they had been held in darkness they were now kept under a simulated ambient photoperiod. Fish were fed *ad libitum*, several times daily, on a standard commercial salmon pellet (Biomar, Aarhus, Denmark) recommended for the particular life stage of fish.

On 30 July 2013, 10–15 fry from each of the remaining 50 families were anaesthetized, measured (fork length±0.1 mm; body mass±0.0001 g), tagged with a visible implant elastomer (Northwest Marine Technology, Inc.) and transported to the Scottish Centre for Ecology and the Natural Environment, Scotland. One individual from each family was stocked into one of ten, 15-litre (50×30×15 cm) clear plastic aquaria. The aquaria were placed inside a constant temperature room on a partial recirculation system at a temperature of 13.6±1°C (mean±s.d.), with a simulated ambient photoperiod. Fish were fed approximately 3% body mass day$^{-1}$ on a standard commercial salmon pellet (Biomar) for the remainder of the experiment. Fish were given a 2 week period to acclimate to the new rearing environment before being

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anaesthetized and re-measured on 12 August 2013. They were then anaesthetized and remeasured on 13 September 2013, so that their growth rate over the preceding 32 day period could be calculated. All fish were then subjected to metabolic measurements.

### Measuring standard metabolic rate

Aquaria were vacuum siphoned to remove food and debris the day before fish were placed in respirometry chambers. This ensured that fish were unfed for at least 28 h prior to oxygen uptake measurements, and had sufficient time to evacuate their guts; 28 h post-feeding has been shown to be adequate for the specific dynamic action (SDA) response to subside in salmonids (Cutts et al., 2002). SDA is an elevation in metabolic rate from the increased energy demands associated with digestion, immediately following a meal (Rosenfeld et al., 2015), and is generally not considered part of SMR.

Oxygen uptake was measured continuously over a 9–11 h period, from approximately 14:00 h onwards, using flow-through respirometry (Van Leeuwen et al., 2011, 2012). Individual fish were placed into one of 24 separate glass respirometry chambers (either 8.0 cm length and 3.4 cm diameter, or 15 cm length and 3.4 cm diameter, depending on fish size). Chambers were submersed in a water bath housed inside a second constant temperature room kept at the same temperature (13.6±0.5°C across all measurements) as the tanks in which growth was measured. An air-stone in the header tank of the respirometer apparatus kept the inflow water fully saturated with oxygen. Chambers were wrapped in dark plastic to prevent visual contact between individual fish during measurements, and all measurements were conducted in the dark to further minimize fish activity (Cutts et al., 2002). Glass respirometers and tygon tubing were used to minimize potential issues with use of plastics and oxygen-permeable materials (Stevens, 1992). Flow to each respirometer was adjusted using a nylon bodied micro valve (The West Group, Hampshire, UK) to ensure that the oxygen tension dropped by 10–20% between the inlet and outlet of the respirometer chamber. Flow was measured by collecting the outlet water for a period of 30 s and weighing to the nearest 0.0001 g.

Oxygen concentration of the outflow water was measured using one of three oxygen meters (FireSting $O_2$ oxygen meter; Pyro Science, Aachen, Germany) each fitted with four oxygen probes (calibrated daily), which were placed in small collection tubes connected to the outlet side of each respirometer chamber. Oxygen probes were rotated from the first 12 respirometers to the remaining 12 respirometers after approximately 9–11 h of continuous measurement, allowing for 24 fish to be measured daily.

The rate of oxygen consumption was determined using the following equation (Ege and Krogh, 1914):

$$\dot{M}_{O_2} = \dot{V}_w(\Delta C_{w,O_2}), \quad (1)$$

where $\dot{V}_w$ is the flow rate of water through the respirometer and $\Delta C_{w,O_2}$ is the difference in oxygen tension between water entering and leaving the respirometer. Oxygen concentration was calculated by correcting $P_{O_2}$ (partial pressure of oxygen) for barometric pressure and multiplying by $\alpha_{O_2}$ (μmol $l^{-1}$ $Torr^{-1}$), the solubility coefficient at the observed temperature (Van Leeuwen et al., 2011). Rates of background oxygen consumption were then subtracted from the observed values by measuring the oxygen concentration of the outflow water in the absence of fish at the beginning and end of each measurement trial and assuming a linear decrease in oxygen concentration over the measurement period. Measurements of oxygen uptake were plotted graphically, allowing for periods of complete rest to be readily discriminated from spontaneous activity, which appeared as distinct spikes. Standard metabolic rate was estimated using the lowest 10 min running average of oxygen consumption observed during the respirometry trial.

### Measuring maximal metabolic

After SMR had been measured, individual fish were sequentially subjected to an exhaustive chase protocol in order to determine their MMR (Reidy et al., 1995; Killen et al., 2010; Norin, 2014). A single fish was introduced into a round circular arena and hand-chased for 3 min to exhaustion. The fish was then immediately placed into one of two glass respirometry chambers as described above. Chambers were submersed in a water bath housed inside the same constant temperature room as the apparatus used for measuring SMR. An air-stone in the water bath of the respirometer apparatus kept the water fully saturated with oxygen. Oxygen uptake was measured using intermittent flow-through respirometry (Steffensen, 1989). Once a fish was placed in the respirometer chamber, the flush pump (300 universal pump; Eheim, Deizisau, Germany), which delivered oxygenated water through the respirometer, was immediately turned 'off' and this allowed for the rate of oxygen depletion (due to fish respiration) to be measured. During this 'off' phase a peristaltic pump (Masterflex L/S; Cole-Parmer, London, UK) was used to ensure adequate mixing within each of the two respirometers. Water oxygen concentration was measured every second until oxygen saturation levels reached approximately 85%. At this point the flush pump was switched 'on' until oxygen saturation levels had restored and the cycle was repeated for a second time. Oxygen concentration within the respirometer was measured using the same oxygen meter (FireSting $O_2$ oxygen meter) fitted with the same oxygen probes as above. The rate of oxygen consumption was determined using the following equation (Ege and Krogh, 1914):

$$\dot{M}_{O_2} = V_w(\Delta C_{w,O_2})/\Delta t, \quad (2)$$

where $V_w$ is the volume of water in the respirometer and associated tubing minus the volume of the fish, and $\Delta C_{w,O_2}$ is the change in oxygen tension of the water over time period $\Delta t$ (Steffensen, 1989). Oxygen concentration was calculated by correcting $P_{O_2}$ (partial pressure of oxygen) for barometric pressure and multiplying by $\alpha_{O_2}$ (μmol $l^{-1}$ $Torr^{-1}$), as before. Maximal metabolic rate was determined as the higher oxygen consumption rate of the two measurements. In most cases this value corresponded to the first measurement immediately following the exhaustive chase protocol. Following respirometry measures all fish were anaesthetized, and weighed to the nearest 0.0001 g.

### Calculations and statistical analyses

Mass of females was calculated as somatic mass by subtracting egg mass from female mass to control for potential differences in reproductive investment. Offspring condition was calculated as relative condition factor, $K_{rel}$, according to Froese (2006) using the following equation:

$$K_{rel} = W/aL^b, \quad (3)$$

where $W$ is the mass of the individual offspring in grams, $L$ is the fork length in centimetres and $a$ and $b$ are the exponential form of the intercept and slope derived from the regression of mass versus length plotted on double logarithmic axes for all the offspring combined. Specific growth rates of fish (per cent per day) were calculated as $100[\log_e(\text{final mass}) - \log_e(\text{initial mass})]/\text{duration}$

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(Ricker, 1975). Aerobic scope was determined by subtracting MMR from SMR. Summaries of the mass specific metabolic rates (μmol $g^{-1}$ $h^{-1}$) are given in Table S2.

We used a linear model to test for the effects of maternal and paternal time spent in fresh water and salt water on parental traits (somatic mass, length, clutch mass and egg mass). We used linear mixed effects models (LME) to test for the effects of egg mass (i.e. mean for that family), maternal and paternal time spent in fresh water and salt water on offspring traits [length, mass, condition, growth, log(SMR), log(MMR) and log(AS)]. All LME models initially included all two way interactions between maternal and paternal traits, with maternal and paternal identities included as random factors to control for the non-independence of siblings [offspring trait=egg mass+maternal years in sea water+maternal years in fresh water+paternal years in fresh water+paternal years in sea water+(maternal years in fresh water×paternal years in fresh water)+(maternal years in sea water×paternal years in sea water)+(maternal years in sea water×paternal years in fresh water)+(paternal years in sea water×maternal years in fresh water)+random=maternal id+paternal id]. Egg mass was included because of the confounding effect of egg size on fry traits, and given the effect of size on metabolism we also used log(offspring mass) as an additional variable in the models that included either log(SMR), log(MMR) or log(AS). Variance inflation factors (VIF) for all explanatory variables were calculated prior to analysis; all VIFs were less than 3, indicating that collinearity among explanatory variables was unlikely to have affected our analyses (Zuur et al., 2009). Furthermore, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Likelihood ratio tests comparing models with and without a given term were used to sequentially compare model fit; models were progressively simplified provided that any increase in the log-likelihood ratio statistic was non-significant ($P$>0.05). For those instances where a single term could not be isolated from the model, due to a significant interaction present, we compared the single factor best-fit model with and without the single term in question. All analyses were conducted using R version 3.0.1 statistical software and the lme4 function (Bates et al., 2011). All procedures were carried out under the approval of the UK Home Office under project no. 60/4292.

## RESULTS

### Broodstock

As expected, adult males and females that spent longer at sea (2 years rather than 1 year) were heavier ($F_{2,21}$=119.92, $P$<0.001; $F_{1,13}$=292.42, $P$<0.001) and longer ($F_{2,21}$=390.16, $P$<0.001; $F_{1,13}$=87.83, $P$<0.001) at the time of spawning (full details of adult biometrics and of the final statistical models are given in Table S3). However, there was also a significant effect of time spent in fresh water on female length at the time of spawning, with females that spent longer in fresh water (3 years rather than 2 years) being longer ($F_{1,13}$=6.70, $P$=0.02); there was no such effect for male length ($F_{2,13}$=0.72, $P$=0.50) or female somatic mass ($F_{2,21}$=0.75, $P$=0.49; $F_{1,13}$=3.81, $P$=0.07). There was also a significant effect of the amount of time females spent at sea on their egg size and clutch mass: females that spent 2 years at sea produced larger eggs ($F_{1,13}$=11.95, $P$<0.004) and heavier clutches ($F_{1,13}$=31.71, $P$<0.001) compared with those that only spent 1 year at sea (see Fig. S2 for details). Lastly, females that spent longer in fresh water (3 years rather than 2 years) produced heavier clutches ($F_{1,13}$=4.85, $P$=0.05) but there was no difference in the size of their eggs ($F_{1,13}$=1.33, $P$=0.27).

### First feeding offspring

There was no effect of the amount of time that parents had spent in fresh water as juveniles on offspring length, mass or condition at the

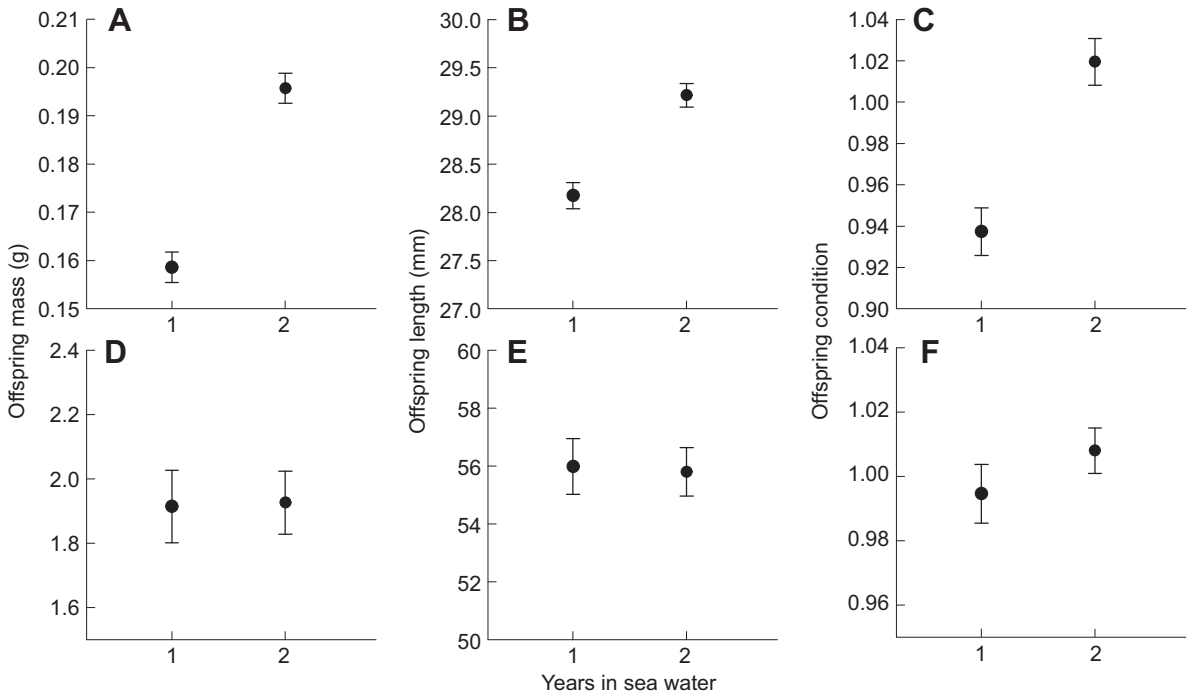


**Fig. 1. The relationship between the time Atlantic salmon mothers had spent at sea and three fundamental early fitness attributes.** Offspring length, offspring mass and offspring condition for first feeding fry (A,B,C) and 4 month old fry (D,E,F). Note the loss of statistical significance by four months of age. Error bars represent 95% confidence intervals. See text for statistical analysis.

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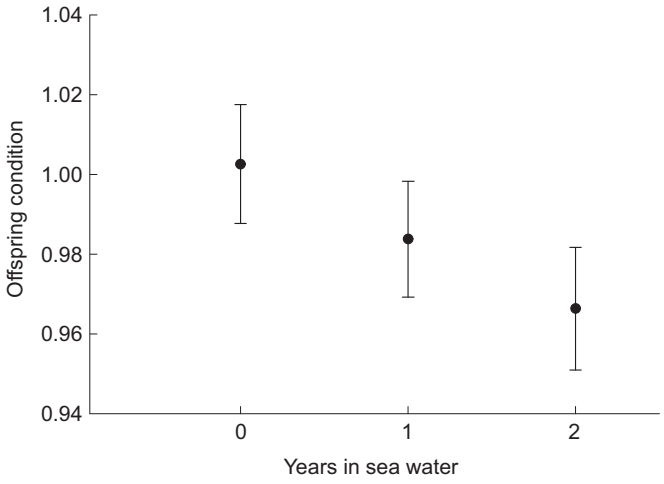


**Fig. 2. The relationship between the time fathers had spent at sea and the body condition of their offspring at first feeding.** Error bars represent 95% confidence intervals. See text for statistical analysis.

time of first feeding. Mothers that spent longer at sea prior to spawning produced longer offspring at first feeding (Fig. 1B), but this effect was due to them producing larger eggs (positive relationship between egg size and offspring length: $\chi^2$=15.04, d.f.=1, $P$<0.001; no effect of time at sea once effect of egg size was taken into account). Interestingly, while egg size influenced offspring mass at first feeding ($\chi^2$=14.36, d.f.=1, $P$<0.001), even after accounting for egg size in our analysis we found a positive relationship between the amount of time mothers had spent at sea prior to spawning and offspring mass ($\chi^2$=4.48, d.f.=1, $P$<0.034; Fig. 1A) and condition ($\chi^2$=7.90, d.f.=1, $P$=0.005; Fig. 1C). There was also a significant association between the amount of time fathers had spent at sea and the condition of their offspring ($\chi^2$=7.95, d.f.=2, $P$=0.02), with those fathered by precocious parr having a higher condition factor (Fig. 2). Lastly we also found a significant interaction between the time mothers had spent at sea and the time fathers had spent in fresh water on offspring length ($\chi^2$=9.84, d.f.=2, $P$=0.007) and condition ($\chi^2$=8.17, d.f.=2, $P$=0.017), with offspring of mothers that had spent 2 years at sea and precocious parr fathers, which had spent only 1 year in fresh water, having the greatest length and condition.

### Post-feeding offspring

The significant effects of egg size and the amount of time mothers had spent at sea on offspring mass, length and condition had disappeared by the time that the fry were 4 months old (Fig. 1D–F). Instead, it was the maternal duration in fresh water that was significant, being negatively related to offspring condition ($\chi^2$=5.86, d.f.=1, $P$=0.02; Fig. 3A), growth rate ($\chi^2$=4.28, d.f.=1, $P$=0.04; Fig. 3B), MMR ($\chi^2$=5.66, d.f.=1, $P$=0.02; Fig. 3C) and AS ($\chi^2$=4.27, d.f.=1, $P$=0.04; Fig. 3D); mothers that were faster to develop in fresh water (migrating to sea at 2 years rather than 3 years of age) produced offspring that were in better condition, had higher rates of MMR, AS and grew faster, although there was no effect on SMR (see Table S3 for details of final models). A significant interaction was found between the amount of time mothers had spent at sea and the amount of time fathers had spent in fresh water

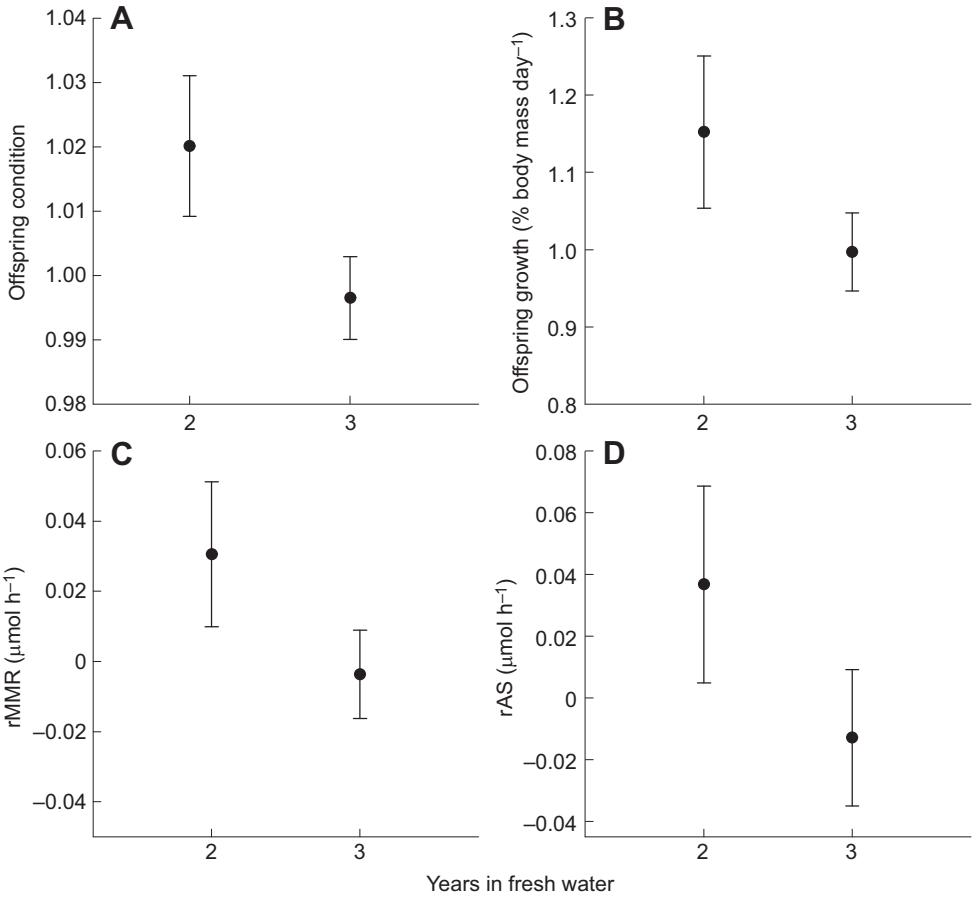


**Fig. 3. The relationship between the time mothers had spent in fresh water and attributes of their offspring.** (A) Body condition of offspring at 4 months of age; (B) growth; (C) maximal metabolic rate (MMR); (D) and aerobic scope (AS). For clarity of presentation measures of metabolic rate are expressed as residuals, after correction for body mass (rMMR; rAS); residuals were calculated from the regression equation of absolute oxygen consumption (MMR or AS, μmol $h^{-1}$) on body mass (g) for the full sample size of fish, plotted on double logarithmic axes. Error bars represent 95% confidence intervals. See text for statistical analysis.

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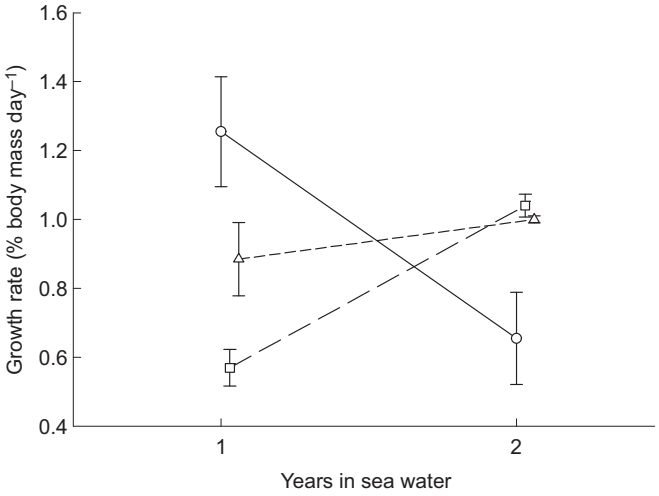


**Fig. 4. The relationship between the time mothers had spent at sea, fathers had spent in fresh water and the growth rate of their fry at 4 months of age.** Mothers: 1 or 2 years at sea. Fathers: open circles and continuous line denote 1 year in fresh water; open squares and interrupted line denote 2 years in fresh water; open triangles and interrupted line denote 3 years in fresh water. Error bars represent 95% confidence intervals. See text for statistical analysis.

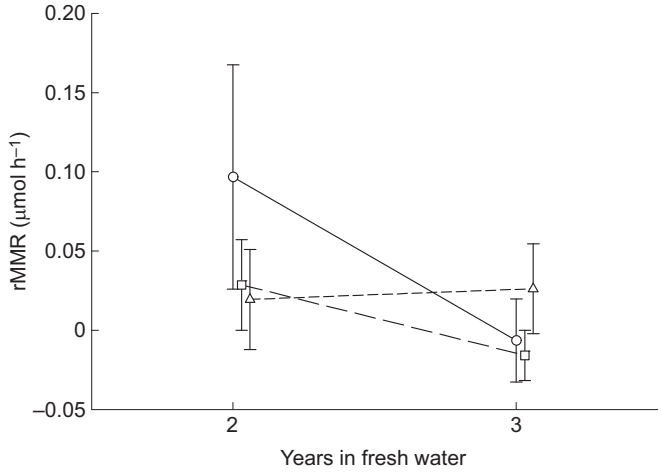


**Fig. 6. The relationship between the time spent in fresh water by mothers and fathers and the maximum metabolic rate of their offspring at 4 months of age.** Mothers: 2 or 3 years in fresh water. Fathers: open circles and continuous line denote 1 year in fresh water; open squares and interrupted line denote 2 years in fresh water; open triangles and interrupted line denote 3 years in fresh water. For clarity of presentation measures of metabolic rate are expressed as residuals, after correction for body mass (rMMR), as in Fig. 3. Error bars represent 95% confidence intervals. See text for statistical analysis.

on offspring growth ($\chi^2$=6.78, d.f.=2, $P$=0.034), with offspring of mothers that had spent 1 year at sea and precocious parr fathers that had spent only 1 year in fresh water having the fastest growth (Fig. 4; note that the only males that spent a single year in fresh water prior to maturation were precocious parr). Furthermore, in addition to the significant main effect of maternal time in fresh water there was a significant interaction between the amount of time the two parents had spent at sea on offspring SMR ($\chi^2$=8.93, d.f.=2, $P$=0.01), with offspring from the slowest developing parents at sea (2 years) having the highest SMR (Fig. 5). Lastly, in addition to the significant main effect of maternal time in fresh water on offspring MMR there was a significant interaction with the time spent by the father ($\chi^2$=7.30, d.f.=2, $P$=0.03), with offspring from the fastest developing parents in fresh water having the highest MMR (Fig. 6).

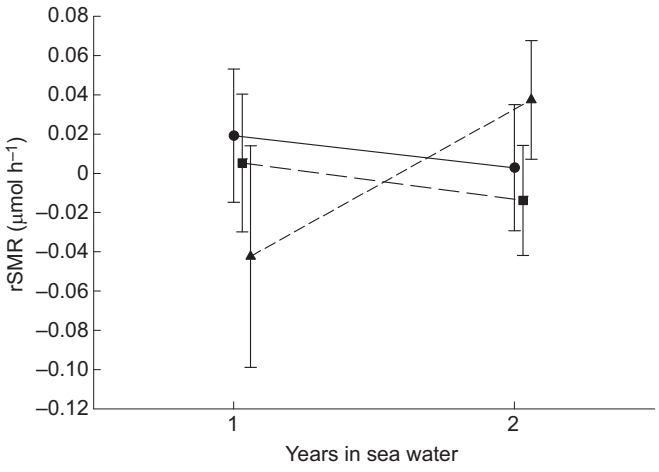


**Fig. 5. The relationship between the time spent at sea by mothers and fathers and the standard metabolic rate of their offspring at 4 months of age.** Mothers: 1 or 2 years at sea. Fathers: filled circles and continuous line denote 1 year at sea; filled squares and interrupted line denote 2 years at sea; filled triangles and interrupted line denote 3 years at sea. For clarity of presentation measures of metabolic rate are expressed as residuals, after correction for body mass (rSMR), as in Fig. 3. Error bars represent 95% confidence intervals. See text for statistical analysis.

## DISCUSSION

Our study demonstrated that the life history strategy of the mother, and to a lesser extent of the father, was significantly associated with a range of offspring traits. The mother's life history was found to be significant throughout early offspring development, with the duration of time spent by the mother in both fresh water and salt water being associated with offspring traits related to early growth and metabolism. Mothers that had spent longer at sea (2 years versus 1 year) were larger, produced larger eggs and offspring that were heavier and in better condition at the time of first feeding, even after accounting for the effect of egg mass. These effects are likely to be mediated through the provisioning of the egg, since up until this point in development the embryo has been dependent on egg reserves. Because mothers that spend longer at sea are generally larger [probably due to the higher productivity in marine environments compared with adjacent freshwater environments (Gross et al., 1988)], the egg size effect was not surprising given that larger female Atlantic salmon tend to produce larger offspring (Burton et al., 2013b). Similar effects of maternal state on egg investment are found in other taxa, for example the migratory duration and condition of female snow geese *Chen caerulescens atlantica* (Linneaus) can influence the clutch size of eggs (Bêty et al., 2003). The relationships in the present study between egg mass, maternal marine life history and offspring traits had disappeared by the time the fry were 4 months old, a finding consistent with the idea that they were mediated through egg provisioning, and which may suggest that they were largely maternal effects [note that Donelson et al. (2009) found that maternal effects on juvenile size in the spiny chromis damsel fish *Acanthochromis polyacanthus* (Bleeker) were evident up to 29 days post-hatching, but had disappeared by 50 days].

Much of the work investigating parental effects has focused on understanding how a mother's state around the time of reproduction can affect the phenotype of her offspring (Bernardo, 1996). The

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most obvious non-genetic way that mothers providing no parental care can affect offspring traits is through egg or embryo provisioning, but this is not simply in terms of the overall amount of nutrients. It is noteworthy that even after accounting for the effect of egg size in our study, there was still a positive relationship between the amount of time mothers had spent at sea prior to spawning and the mass and condition of their offspring. This indicates that these effects on early development were driven by more than just variation in the size of egg produced: females with different life histories were also potentially altering the composition of their eggs. Although not measured in this study, there can be variation in specific modifiers such as maternal hormones that are deposited in the eggs, so influencing factors such as offspring metabolism. Rossignol et al. (2010) and Régnier et al. (2012) found that the routine metabolic rate of Atlantic salmon fry and the SMR of brown trout *Salmo trutta* (Linnaeus) embryos respectively decreased with increasing egg size (an indicator of general nutrient investment), while Sloman (2010) showed that SMR in brown trout fry could be affected by maternal cortisol deposited in the egg. Even the position of the egg within the ovary can influence metabolic and growth traits in the resulting offspring (Burton et al., 2013a).

However, while it is likely that maternal state in the time leading up to egg production influenced early offspring traits, it is not possible to rule out the influence of genetic effects. Indeed, the fact that offspring fathered by precocious parr had a higher body condition at the time of first feeding indicates that genetic effects can also play a role in egg resource utilization, since fathers play no role in egg provisioning. There is evidence from other studies that life history variation in salmon is partially under genetic control (Páez et al., 2010, 2011a,b), but it is also known that the early performance of the fish is influenced by non-genetic (parental and environmental) factors (Dodson et al., 2013), so further research is needed to partition these contributions to early offspring traits.

There is a growing body of literature that suggests that non-genetic influences on offspring phenotype may also arise as a consequence of the conditions that parents of both sexes experienced in their own early life (Taborsky, 2006; Burton et al., 2013b; Burton and Metcalfe, 2014). In this study we found that offspring traits were related to the early life, as well as the pre-reproductive period of the parents. Indeed, the relative importance of the parents' early and later life in predicting offspring traits shifted as the offspring became older. The time spent by the mother at sea prior to reproduction was more important in predicting features of offspring early development (e.g. egg mass, mass and condition at first feeding), whereas by the time that the fry had been feeding on exogenous food for 4 months, the early life history of the mother in fresh water was a better predictor of offspring traits. Thus mothers that were faster to develop in fresh water (migrating to sea at 2 years rather than 3 years of age) produced offspring that had higher maximal metabolic rates and aerobic scopes and that grew faster; some fry traits were also affected (but to a lesser extent) by an interaction between maternal life history and the early life history of the father (i.e. time spent in fresh water).

Given that the age of seaward migration in salmonids is principally determined by growth rate, whereby fast-growing juveniles tend to migrate to sea at a younger age than slower-growing counterparts (Metcalfe and Thorpe, 1990), and given the role of metabolism in shaping growth potential, our data suggest that offspring of mothers that spent the minimal period in fresh water may themselves be more likely to migrate at a young age, provided that they experience an environment favourable for growth. While we cannot exclude the possibility that these are additive genetic effects, the fact that the measured offspring traits were more related to maternal than paternal life histories suggests that parental effects were more important. There is experimental evidence of a significant non-genetic component to migration age in salmon (Metcalfe, 1998) and so it is possible that mothers may be producing offspring that have traits that suit the environment the mothers themselves experienced when young, so that they are tailoring the life history trajectory of their offspring (Jonsson and Jonsson, 2014).

There were some significant associations with the father's reproductive strategy. Males of many species display alternative mating tactics (Brockmann, 2001; Dodson et al., 2013). Two of the most common tactics include the territorial tactic (which would naturally be adopted by 1SW and 2SW male Atlantic salmon), whereby a male defends and monopolizes a single female against other males, and the non-territorial/sneaker tactic (adopted by precocious parr) whereby a male (generally considerably smaller in size) steals fertilizations away from the territorial male by darting in and out of the nest area at the time of egg release (Garant et al., 2002; Neff, 2004). While both male reproductive tactics can be successful there remains a paucity of studies examining the relationship between a male's reproductive tactic and the viability and performance of his offspring. However, there is some evidence that females may derive considerable benefits from mating with non-territorial males, despite their apparently lower social status. For example, studies done on Atlantic salmon (Garant et al., 2002) and Bluegill sunfish *Lepomis macrochirus* Rafinesue (Neff, 2004) found significantly higher growth rates and swim performance in offspring fathered by sneaker males, consistent with the higher condition, growth and MMR of offspring fathered by precocious parr males in our study (although the higher growth and MMR found in our study was also related to the life history of the female). However, whether mating with a non-territorial or sneaker male results in higher fitness overall for the mother is still unclear, since there are many components that will determine the viability and reproductive success of her offspring [similar uncertainty over their adaptive value surrounds the analogous situation of extra-pair offspring in species that form pair bonds, despite the intensity of research on that topic (Forstmeier et al., 2014; Hsu et al., 2014 and references therein)]. Moreover, the mechanism through which a male's reproductive tactic is associated with offspring traits and performance is unclear, since it could be through additive genetic or epigenetic means.

In conclusion, this study demonstrates that the duration of key stages in the early and later life of the mother (and to a lesser extent the father) are significant predictors of a range of performance-related metabolic and growth-related traits of their offspring. The durations of these life history stages are partly determined by environmental conditions, with each life stage taking longer in a poorer quality of environment. While the mechanisms underlying these parent–offspring relationships are not yet known, it is possible that parents may optimize offspring traits to suit the quality of environment they themselves experienced as a juvenile, so that they potentially have the capacity to influence the life history trajectory of their offspring.

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**Competing interests**
The authors declare no competing or financial interests.

**Author contributions**
T.E.V.L., N.B.M. and C.E.A. conceived the study; D.M., S.M. and D.C.S. collected the broodstock and carried out the crosses; T.E.V.L. undertook the experiments, analyzed the data and drafted the manuscript; N.B.M., C.E.A. and D.M. contributed to manuscript revisions.

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**Supplementary information**
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