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Title: Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon

Authors: McLennan D. (1), Armstrong J.D. (2), Stewart D.C. (2), Mckelvey S. (3) Boner W. (1), Monaghan P. (1) & Metcalfe N.B. (1)

1) Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK.

(2) Marine Scotland – Science, Freshwater Laboratory, Faskally, Pitlochry, PH16 5LB, UK.

(3) Cromarty Firth Fishery Trust, CKD Galbraith, Reay House, 17 Old Edinburgh Road, Inverness, IV2 3HF.

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Corresponding author: Darryl Mclennan, Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK. Fax: 0141 330 5971. Email: d.mclennan.1@research.gla.ac.uk

Running title: Telomere length in the wild

ABSTRACT

A larger body size confers many benefits, such as increased reproductive success, ability to evade predators and increased competitive ability and social status. However, individuals rarely maximise their growth rates, suggesting that this carries costs. One such cost could be faster attrition of the telomeres that cap the ends of eukaryotic chromosomes and play an important role in chromosome protection. A relatively short telomere length is indicative of poor biological state, including poorer tissue and organ performance, reduced potential longevity and increased disease susceptibility. Telomere loss during growth may also be accelerated by environmental factors, but these have rarely been subjected to experimental manipulation in the natural environment. Using a wild system involving experimental manipulations of juvenile Atlantic salmon *Salmo salar* in Scottish streams, we found that telomere length in juvenile fish was influenced by parental traits and by direct environmental effects. We found that faster-growing fish had shorter telomeres and there was a greater cost (in terms of reduced telomere length) if the growth occurred in a harsher environment. We also found a positive association between offspring telomere length and the growth history of their fathers (but not mothers), represented by the number of years fathers had spent at sea. This suggests that there may be long term consequences of growth conditions and parental life history for individual longevity.

INTRODUCTION

A larger body size has many benefits, such as increased reproductive success, ability to evade predators and increased competitive ability and social status (Blanckenhorn 2000; Dmitriew 2011). Attaining a large size requires either prolonged or faster growth. However there is evidence that individuals rarely maximise their growth rates, suggesting that there are costs associated with rapid growth (Metcalfe & Monaghan 2003). It is becoming clearer that one such cost may be reduced longevity. Within species, even when comparing adults of the same size, those with faster growth rates earlier in life often exhibit faster senescence and/or shorter lifespans (Metcalfe & Monaghan 2003; Geiger *et al.* 2012).

There are likely to be multiple underlying mechanisms linking growth rate and faster senescence. Several studies have found growth rate to correlate positively with levels of oxidative damage, in both the laboratory and in the field (Alonso-Álvarez *et al.* 2007; Nussey *et al.* 2009; Carney Almroth *et al.* 2012) and oxidative damage levels are considered to be important influences on longevity and senescence (Halliwell & Gutteridge 2015). In addition, growth rate may alter trade-offs between body growth and body maintenance (i.e. antioxidant production and protein repair). McCarthy *et al.* (1994) reported such a trade-off in rainbow trout (*Oncorhynchus mykiss*), with faster growing individuals undergoing increased protein synthesis (growth) and reduced protein turnover (maintenance). The costs of a particular pace of growth may also, in turn, be partly dependent on environmental factors. For example, Stier *et al.* (2014) found that faster growth in coal tits *Periparus ater* was more costly (in terms of DNA oxidative damage) when they were reared under environmentally harsher conditions. Therefore, it may not only be the rate at which you grow, but also the environment in which you do so that has an effect on the costs and benefits of different growth trajectories.

Telomere length might be a good indicator of these costs. Telomeres cap the ends of eukaryotic chromosomes and play an important role in chromosome protection (for reviews see Blackburn 1991; Campisi *et al.* 2001). Some telomere loss inevitably occurs at each cell division as a result of the 'end replication problem' (Chan & Blackburn 2004), so telomere dynamics are affected by the pattern and rate of cell division. In addition to this, telomere loss may also be accelerated by the effects of reactive oxygen species (ROS). Telomeric DNA is vulnerable to oxidative damage for a number of reasons, particularly its high G content (Haussmann & Marchetto 2010). While the enzyme telomerase is capable of restoring telomere length, telomerase expression is down-regulated in many types of somatic cells, which is thought to be linked to tumour suppression (Collins 2006). A relatively short telomere length is indicative of poor biological state e.g. reduced potential longevity

and increased disease susceptibility (Haussmann *et al.* 2005; Ilmonen *et al.* 2008; Calado & Young 2009; Heidinger *et al.* 2012).

The determinants of variation in telomere length among and within species are still not fully understood. This is especially the case for animals living in the wild, since thus far telomere dynamics and their consequences have mostly been studied in stable laboratory conditions. An increasing number of studies have reported a heritable component to telomere length. These studies have mostly focussed on humans (for example Nordfjall *et al.* 2005; Njajou *et al.* 2007); however other taxa have also been studied, including lizards (Olsson *et al.* 2011) and many bird species (Horn *et al.* 2011; Asghar *et al.* 2015; Reichert *et al.* 2015). Results to date are inconsistent as to whether the inheritance is strongest through the father or mother (Eisenberg 2014).

Changes in telomere length are also strongly influenced by environmental conditions. As mentioned above, telomere attrition is related to the dynamics of cell proliferation and a number of studies have reported a relationship between enhanced growth rate or body size and telomere loss (Fick *et al.* 2012; Herborn *et al.* 2014; Noguera *et al.* 2015; Pauliny *et al.* 2015). These studies are mainly correlational. However using transgenic coho salmon *Oncorhynchus kisutch* with an artificially increased growth rate, Pauliny *et al.* (2015) found that these fast growing fish showed a faster telomere loss compared with maternal half sibs that grew at a rate more typical of wild fish. On the other hand, Naslund *et al.* (2015) found that wild brown trout *Salmo trutta* induced to undergo compensatory growth did not show increased telomere loss. There have also been suggestions that telomere length may help explain the previously-mentioned relationship between growth rate and longevity (Stindl 2004; Fick *et al.* 2012). Growth rate and body size are both under the influence of various environmental factors (Metcalfe & Monaghan 2001) This is especially true for ectotherms, such as fish, where fluctuations in temperature can influence myogenic processes, morphological development, growth rate and metabolism, all of which can have permanent long-term phenotypic effects (Johnston 2006; Jonsson &

Jonsson 2011). Therefore it is also possible that the environmentally conditioned plasticity of growth rate may also have some influence on telomere dynamics. Environmental stressors may also affect telomere length, most notably through their influence on the production of ROS (von Zglinicki 2002; Geiger *et al.* 2012; Kim & Velando 2015). A number of experimental studies have linked telomere dynamics to environmental stressors such as *in utero* stress (Haussmann *et al.* 2011; Marchetto *et al.* 2016), stress hormone administration and disturbance (Herborn *et al.* 2014), social position (Nettle *et al.* 2015) and social crowding (Kotrschal *et al.* 2007; Sohn *et al.* 2012). Therefore, if growth rate does have an effect on telomere dynamics, its impact may also in turn be dependent on environmental conditions.

In order to gain a better understanding of telomere length as a possible link between environmental conditions, growth rate and individual fitness, it is also important that we study these processes in animals living in the wild. Using free-living animals to tease apart parental and environmental effects can prove challenging, due to the logistics of accessing and obtaining data for parents and offspring alike. However, by utilising managed populations of wild Atlantic salmon (*Salmo salar*), it is possible to examine environmental effects on telomere dynamics, while simultaneously investigating potential parental effects. Atlantic salmon exhibit intra-population variation in their life histories (for reviews see Fleming 1996; Klemetsen *et al.* 2003). They migrate to sea and undergo a substantial increase in body mass during this time, remaining there for either one sea winter (1SW) or multiple sea winters (MSW) before returning to fresh water to reproduce. MSW fish are generally much larger than 1SW fish when they return to freshwater (Klemetsen *et al.* 2003). There is a correlation between female body size and average egg size (Fleming 1996) and therefore MSW mothers, in general, produce significantly larger eggs than 1SW mothers. It is also possible for males to become precociously mature as parr, prior to sea-migration, and thus at a much smaller body size than males that go to sea to complete their growth (Fleming 1996; Baum *et al.* 2004). Salmon will also vary in the number of years spent in fresh water prior to seaward migration, with faster growing individuals reaching the

minimum-size threshold required for sea migration earlier and thus migrating to sea at a younger age (Metcalfe & Thorpe 1990; Økland *et al.* 1993). Therefore, within a typical Atlantic salmon population there are two distinct maternal reproductive life-history variants with respect to time spent at sea (1SW and MSW) and three such variants in males (1SW, MSW and precocious parr) and within a given sea age, individuals will vary in the number of years spent in freshwater prior to migration. Since the rate of offspring growth is highly dependent on water temperature (Jonsson *et al.* 2001) spatial variation in water temperatures within a river catchment will mean that the exact location in which an egg is laid will influence the growth rate, and hence potentially the telomere dynamics, of the resulting offspring.

We used this system to examine parental and environmental effects on telomere dynamics in Atlantic salmon. We placed eggs from controlled matings of wild Atlantic salmon in contrasting streams within the same river system in order to test two hypotheses: 1) that faster growth is associated with increased telomere loss, 2) that the magnitude of telomere loss for a given growth rate is greater in harsher conditions. In addition, we were also able to simultaneously investigate the effect of parental life history and parental telomere length on offspring telomere length in the wild.

METHODS

The experiment was conducted at the River Blackwater, which is part of the larger River Conon catchment, northern Scotland (57° 60'N, 4°63'W) (Fig. S1 in supporting information). Fish movements in the River Conon have been highly impacted by the installation of hydroelectric schemes since the 1950s. In particular, this has prevented fish from reaching many of the traditional spawning areas in tributary streams. The Loch na Croic Atlantic salmon trap was constructed on the River Blackwater in the late 1950s to maintain an Atlantic salmon population in the upper regions of the River Conon (i.e. above the hydroelectric schemes) by artificial spawning. This is done by collecting returning wild adults in the trap (1790 individuals per year + s.d. 163; based on data from 1965-2012), conducting

random crosses and then planting out developing eggs (mixed at random) into tributaries throughout the upper region of the catchment. No other stocking takes place in the Conon system. Since all parent fish are collected from the same trap and their eggs are mixed thoroughly before being spread among the streams in the catchment, there is no reason to expect any genetic spatial differentiation in the catchment that would complicate this study.

This set up allowed us to access large numbers of sexually mature wild fish, which were used to generate experimental families by *in vitro* fertilisation (IVF). The experimental design (Fig. 1) was to first incubate the resulting eggs under contrasting aquarium temperatures (to examine the effect on telomeres of early embryonic conditions), then plant the eggs in contrasting tributary streams: a lower altitude stream with moderate temperature and predator density and, in contrast, a harsher high altitude stream with a relatively colder temperature and greater predator density (as a consequence of differences in stocking with salmon eggs in previous years). In both streams the mean temperature was below the optimum for growth (16 °C; Elliottand Hurley (1997)) but it was furthest from the optimum in the harsher high altitude stream. The juvenile salmon were subsequently recaptured at a later date and parental assignment was established by microsatellite analysis. This allowed the measurement of relative telomere length in parents and offspring (embryos and fry), all within a wild system.

The fish trap was first opened on 14th November 2013. The IVF matings for this experiment were all conducted between 3-5th December, using predominantly fish captured the same day (and always within one week of capture). Unripe fish that could not be used in IVF crosses on the day of capture were held at the trap site in dark circular tanks (4m diameter, 1.5m deep), supplied directly with water from the adjacent river, until they had reached spawning condition. It is unknown if fish of different life history strategies (i.e. 1SW and MSW) arrived at the loch downstream of the trap at different times. However, 1SW and MSW fish were both present by the time the trap was first opened, were treated in a similar way and were stripped over the same narrow time window. Sexually mature precocious male

parr (i.e. small sneaker males, that had matured without going to sea) were captured daily between 3-5th December, by electrofishing various tributaries of the River Blackwater. All captured parr were assessed for sexual maturity, and precocious males (indicated by the production of milt after abdominal squeezing) were retained for use that day in the IVF crosses.

In this experiment we used a split-brood IVF design. In each replication of the mating design the eggs of two female fish (one 1SW and one MSW) were fertilised with sperm from three male fish (one 1SW, one MSW and one precocious male parr) to produce six half-sib families which represented all possible parental crosses (Fig. S2). This design was repeated 14 times (using new fish each time) to produce 84 families in total. The life history of each of the anadromous parents was initially determined by size (MSW fish are generally much larger than 1SW fish (mean mass (g): MSW females = 3125 + s.e. 101; 1SW females = 1368 + s.e. 48; MSW males = 4199 + s.e. 158; 1SW males = 1606 + s.e. 123; male parr = 23 + s.e. 2). This was subsequently confirmed by analysis of the scales taken from the flank of each fish (below the adipose fin) (Shearer 1992). The scale analysis also allowed us to retrospectively determine the number of years each parent fish had spent in fresh water prior to seaward migration, and confirmed that all parent fish were virgin spawners. There was not a strong correlation between the number of years parents had spent in FW and SW (Pearson $r = 0.47$, $p < 0.001$). Prior to the stripping of gametes, all parent fish were anaesthetised, using a 5% benzocaine solution. The two female fish in each mating group (1SW & MSW) were blotted dry and manually stripped of their clutch of eggs. The eggs were drained of ovarian fluid and then weighed (to 0.01g) to obtain a clutch mass. For each female, a small sample of eggs (~15) was stored in 100% ethanol, for subsequent calculation of dry egg weight after overnight drying at 60°C.

Each clutch was then divided into three approximately equal subsets. The three male fish (1SW, MSW and precocious parr) were blotted dry and manually stripped of sperm. The sperm of each male was divided in two and each half was used to fertilise one

subset of eggs from each of the two females. Each batch of eggs was left for several minutes to allow sufficient time for fertilisation to occur. Afterwards, the eggs were washed in fresh water to remove any remaining sperm. Each parent fish was measured (fork length to 0.5cm; body mass to 0.1 g). A small sample of tissue was taken from the adipose fin and stored in 100% ethanol for subsequent telomere analysis. All parent fish used in the crosses were then returned to the river, with adult salmon being returned just above the trap (so as not to be mixed up with previously un-trapped individuals) and precocious parr returned to the section of stream from which they had been electrofished (which was not fished in subsequent days to avoid recapturing the same fish).

Eggs were transferred to the nearby SSE hatchery at Contin where they were held for two months in separate family groups under ambient water temperatures (3.90 + s.d. 0.91°C) until they had reached the more stable 'eyed egg' stage of development. On February 5th 2014, 10 eggs were sampled from each family for subsequent analysis of embryo telomere length, prior to the aquarium temperature treatments (see Fig. 1). A large and a small sub-sample of eggs (n = 500 and 30 eggs respectively) was then counted from each family. The small sub-samples were held as separate families in organza pouches to allow later collection of family-specific data on telomere dynamics at the embryo stage, since the larger sub-samples were to be subsequently mixed together. On February 7th 2014 each large sub-sample of eggs was allocated to one of two temperature treatments, depending on which family it belonged to: families with odd numbers (i.e. family 1, family 3 etc.) were assigned to the 5°C group and families with even numbers were assigned to the 7°C group. This resulted in each of the 84 families being assigned randomly to only one temperature treatment, so that the aquarium temperature treatment of a fish could subsequently be known by using genotyping to determine its family. For each treatment group, the large sub-samples of eggs were mixed together to create two batches of pooled eggs (~21,000 eggs per batch). The two large batches of eggs (along with the small sub-samples of each family in the organza pouches) were then transferred to the aquarium facilities at the University of

Glasgow, where they were held in egg baskets in two separate dark tanks (100cm X 100cm X 40cm). The small sub-samples were allocated to their corresponding tank (families with odd numbers to the 5°C group and families with even numbers to the 7°C group) and remained in their pouches throughout the treatment. Both tanks were initially held at 5°C, to match the ambient temperature at the hatchery facilities. The temperature in the 7°C group was slowly raised to 7°C over a two day period. Eggs were exposed to these temperature treatments for 18 days. On February 25th the small sub-samples of eggs were removed and stored in ethanol for subsequent analysis of embryo telomere length at the end of the aquarium temperature treatment period. The mixed eggs in the baskets of the two temperature treatment tanks were combined and mixed thoroughly to create a single large batch of ~42,000 eggs. This was then transferred back to the SSE hatchery at Contin, where the eggs were kept for several days under ambient water temperature conditions until being planted in the appropriate treatment streams.

Two tributaries (Upper Meig and Allt Goibhre) of the River Blackwater were selected to represent a harsh and a benign stream respectively. While it is difficult to produce an absolute definition of 'harsh' and 'benign', we use these as relative terms to describe the conditions for growth in the two streams: the selected streams provided similar spawning substrate and water flow (Table S1), but while both showed a seasonal variation in temperature, the harsher high altitude stream (Upper Meig) was consistently colder and so further from the optimum temperature for growth (mean difference between streams = 0.72 + s.e. 0.04 °C based on recordings logged every 4h over the duration of the experiment; see Fig. S3). In addition, higher altitude tributaries often show reduced primary productivity, compared to relatively lower altitude tributaries of the same system (Elliott *et al.* 1998; Nislow *et al.* 2004). Both streams contained resident brown trout, and the Upper Meig also contained older juvenile Atlantic salmon that had been stocked as eggs in previous years. Both trout and older juvenile salmon predate Atlantic salmon fry (Henderson & Letcher 2003) and are hereafter combined and referred to as 'predators'. Subsequent electrofishing

showed that the harsher Upper Meig tributary had a ~ 3.6 x greater density of these predators than the Allt Goibhre tributary (Table S1). There was no natural salmon spawning in either stream due to migration barriers, so that the only salmon fry present were those introduced as part of the experiment.

The pooled batch of eggs was mixed again and then divided into two new batches (~21,000 eggs per batch), with each batch being assigned to one of the study streams. As a result, eggs from each of the aquarium temperature treatments were equally represented in each batch, and subsequently stream. The eggs were then planted in the benign and harsh streams on February 26th 2014 and March 1st 2014, respectively (see supporting Information for further details).

Both streams were then electrofished between 22nd and 23rd July 2014 to collect samples for telomere analysis and determine fry survival and growth rates. Beginning downstream of the treatment section, successive 10 m stretches were measured out along the stream. Each section was sampled with a single electrofishing pass. All Atlantic salmon fry (which by definition were experimental fish) were euthanized with a 10% benzocaine solution and stored in 100% ethanol for subsequent body size measurements and tissue sampling in the lab. All non-experimental fish (brown trout and older Atlantic salmon) were briefly anaesthetised, measured and then returned to the stream. For each 10 m section a number of habitat parameters were measured using the SFCC general electrofishing habitat survey (see Table S1). Consecutive sections were electrofished and analysed in this manner until ~400 experimental fry had been caught at each site. Fry density and predator density was calculated for each electrofishing site by dividing the total number of experimental fry or predatory salmonids (older brown trout and salmon) by the surface area of that site. All preserved experimental fry were weighed (0.001g), a3nd their fresh weight (mg) estimated from the following equation: M_{B1} =1.51M_{B2} +70.69, where M_{B1} and M_{B2} are the fresh and ethanol preserved values, respectively (equation from Burton *et al.* 2013).

The caudal fin was removed from each fry and sent to Landcatch Natural Selection Ltd (Stirling, Scotland) for microsatellite analysis of parentage (see SI). For the telomere analysis, DNA was extracted from all tissue types using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturers protocol, with a minor modification to the lysis step for each of the tissue types (see SI). Telomere length was measured in all samples using the quantitative PCR method described by Cawthon (2002), which provides a relative measure of telomere length (RTL) and is calculated as a ratio (T/S) of telomere repeat copy number (T) to a control, single copy number (S) (here the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene). Details of primers are given in the SI. Telomere PCR conditions were 15 min at 95°C followed by 27 cycles of 15 secs at 95°C, 30 secs at 58°C and 30 secs at 72°C . This was followed by the melt curve profile: temperature was slowly increased from 58°C to 95°C at a rate of 0.2°C /sec. GAPDH PCR conditions were 15 min at 95°C followed by 40 cycles of 15 secs at 95°C, 30 secs at 60°C and 30 secs at 72°C. Again this was followed by the melt curve profile (same as before). PCRs were performed on a Mx3005P qPCR system (Agilent).

The telomere (T) and single copy gene (S) assays were performed on separate 96 well plates, with each sample run in triplicate for each assay. In addition to the samples, each plate also included a six-fold serial dilution of a reference sample (1.25 - 40 ng/well), a 'golden reference' sample and a non-target control (NTC). The DNA for the serial dilution was a pool of 60 samples and included all life stages (adult, embryo and fry). The serial dilution was used to generate a standard curve and calculate assay efficiencies. The 'golden standard' was a pool of DNA from 20 samples, including all life stages, used as a reference sample. The NTC contained all reaction components apart from DNA and was included on each plate (in triplicate) to check for non-specific binding and potential contamination between sample wells. Each reaction contained 12.5µl 2x ABsolute Blue qPCR SYBR Green Mix low ROX (Fisher Scientific), forward and reverse primers and DNA (for wells containing sample, standards, gold reference) or water (for wells containing NTC) in a total volume of

25µl. Both T and S assays were performed using 10ng of DNA (equivalent to 6µl of the diluted samples). Primer concentrations were 500nM for the telomere assay (Tel1b and Tel2b) and 200nM for the GAPDH assay (salGAP8-F and salGAP8-R). The mean assay efficiencies for the telomere and GAPDH were 103.5% and 99.2% respectively and within in the acceptable range (85-115%). The average intraplate variation of the Ct values was 1.15 for the telomere assay and 0.59 for the GAPDH assay, respectively. The average interpolate variation of the Ct values was 2.59 for the telomere assay and 1.86 for the GAPDH assay, respectively. qPCR raw data was analysed using qBASE software for Windows (Hellemans *et al.* 2007). This controlled for differences in amplification efficiency between plates (assessed from the standard curve of each plate) and produces a relative quantity (RQ) for each gene of each sample. In addition, by including three inter-run calibrators (the gold reference and two points from the standard curve) we corrected for further inter-run variation. Finally, we used the software to normalise each telomere RQ by the GAPDH RQ for that sample. Therefore, for each sample, the qBASE software produced a calibrated normalized relative quantity (CNRQ). This is similar to the T/S ratio described by Cawthon (2002) but with greater control of inter-plate variation.

We measured/calculated the following four dependent variables: A) fresh weight of each fry, calculated from the ethanol preserved weight values (*fry weight*); B) the percent of fry recaptured in July, calculated separately for each family in both streams (subsequently referred to as *fry survival rate*); C) embryo relative telomere length, calculated as the mean for each family based on a pooled sample of 10 embryos (*embryo RTL*) and D) fry relative telomere length, for each individual fry (*fry RTL*). All five of the dependent variables were assessed by linear mixed models (LME) that included maternal ID and paternal ID as random effects to control for non-independence of siblings. We measured/calculated the following variables to be included as factors/covariates in analyses, where appropriate: the time point at which the embryo stage was sampled (i.e. whether before or after the aquarium temperature treatment, and subsequently referred to as *embryo time point*), the aquarium

temperature treatment each family was kept under (*aquarium temperature*), the relative telomere length of mother and father (*maternal RTL* and *paternal RTL*), the number of years each parent spent in fresh water (*maternal FW age* and *paternal FW age*) and at sea (*maternal SW age* and *paternal SW age;* note that total age = FW age + SW age), average dry egg weight for each family (*egg weight*), which experimental stream a fry was reared in (*stream*), fry density for each electrofishing section within a stream (*fry density*) and predator density for each electrofishing section within a stream (*predator density*). Tables S2-S5 describes the four full models, prior to model selection and simplification. The Akaike Information Criterion *(*AIC) was used during model fitting and variables were only removed from a model if this resulted in a relative reduction of the AIC score. We used Pearson correlation coefficient matrixes to assess potential collinearity between explanatory variables (with a cut-off coefficient of 0.8). We also used Pearson's correlation coefficient to assess the relationship between predator density and fry density. All statistical analyses were carried out using IBM SPSS 22 for Windows.

RESULTS

Fry weight and fry density

As expected, fry weight (i.e. size achieved by late July, approximately two months after first feeding) differed between the two streams, being significantly greater in the more benign (and warmer) stream (Table 1A, Fig. S4). There was also a significant correlation between fry weight and fry density, with relatively smaller fry being present in areas of increased fry density (Table 1A, Fig. S5). Both fry weight and survival were positively related to initial egg weight (Table 1A and 1B, Fig. S6 and S7). There was no significant correlation between predator density and fry density, either within streams or across streams (Pearson $r < 0.30$, $p > 0.17$)

Embryo relative telomere length

Embryo telomere length significantly increased during the 18 days of the aquarium temperature manipulation, with no difference between the two treatment groups (Table 1C, Fig. 2). Embryo telomere length was negatively correlated with paternal (but not maternal) telomere length, a pattern that was evident both before and after the temperature manipulation (Table 1C, Fig. S8).

Fry relative telomere length

Telomere length in Atlantic salmon fry was negatively correlated with body size (i.e. growth rate), but this effect was dependent on the stream from which they came (significant fry body weight x stream interaction; Table 1D). Faster growing fry thus paid a disproportionate cost, in terms of reduced telomere length, in the harsher stream (Fig. 3). In addition to this, growth was also more costly in term of reduced telomere length when fry were at higher densities (significant fry body weight x fry density interaction; Table 1D, Fig. 4). In addition, fry with the longest telomeres were found in areas with the highest predator density (Table 1D, Fig. 5). Paternal SW age also had a significant effect on fry telomere length, with males that had spent longer at sea prior to reproduction producing fry with relatively longer telomeres (Table 1D, Fig. 6). However, fry telomere length was not related to either paternal FW age or maternal FW or SW ages.

DISCUSSION

Our study demonstrates the complexity of factors that can influence telomere dynamics in early life. We found that faster growth resulted in shorter telomeres but this effect depended on the relative harshness of the environment. We also found significant paternal effects, with males with longer telomeres producing embryos with shorter telomeres, and males that had spent longer at sea producing fry with relatively longer telomeres.

At the time of field site selection, we based our identification of the harsh and benign river systems on differences in altitude, with the rationale that a higher altitude stream would provide a harsher environment as a consequence of having a lower primary productivity (so lower invertebrate food supply for the fish) and being colder (so further from the optimal temperature for growth). The higher altitude Upper Meig tributary was colder, and also had a greater density of predators, than the Allt Goibhre tributary. Together, these factors contribute to making the Upper Meig a harsher environment for growth for young salmon than the Allt Goibhre. As predicted, fry growth rate was faster in the more benign stream. It is possible that the difference in growth rate between the streams was also partially due to the difference in predator density, since an increased predator density may result in reduced foraging (Ward *et al.* 2011). Fry density had a negative effect on fry growth rate in both streams, as would be expected since density-dependent growth is well established in fish (for salmonid example see Jenkins *et al.* 1999).

Interestingly, embryo telomere length increased during the 18 day aquarium temperature manipulation, between the age of ~65 days and ~83 days post fertilization (Fig. 5). Telomerase is often upregulated during various stages of embryogenesis (e.g. Mantell & Greider 1994; Schaetzlein *et al.* 2004), most likely due to the high cell proliferation rate at this stage (for review see Gomes *et al.* 2010). This may also help explain why we did not see a difference in telomere length between the two temperature treatments, since a higher telomerase expression may help buffer any possible environmental effects. It may also be the case that the 18 day manipulation was too short a treatment period to produce any effect, especially if there is little embryonic variance in growth rate.

Telomere length in Atlantic salmon fry was negatively correlated with mass gain, but the effect was dependent on the stream they inhabited, with a shorter telomere length for a given mass gain in the harsher stream. This suggests that faster growing fry pay a higher cost in terms of telomere length in harsher environments. There are several mechanisms that might individually or collectively underlie this effect. Firstly, environmental temperature

may affect trade-offs between cell division (hyperplasia) and cell growth (hypertrophy) in fishes (reviewed by Arendt 2007). Only hyperplasia has direct consequences for telomeres, since telomere loss occurs at each cell division. In Atlantic salmon, several studies have found warm-incubated hatchlings to have fewer but larger muscle fibres, indicating that hyperplasia is having a relatively smaller contribution towards growth when salmon develop in warmer conditions (Stickland *et al.* 1988; Usher *et al.* 1994). A possible cause of this effect is that dissolved oxygen is lower at warmer water temperature, which will limit an individual's capacity for cell proliferation (Matschak *et al.* 1997). Therefore, fish developing in warmer water may achieve somatic body growth most efficiently by increasing the size of their existing cells (hypertrophy).

Temperature may also affect the trade-off between growth and self-maintenance: individuals that allocate greater resources to growth will have a reduced allocation of resources to self-maintenance, e.g. tissue repair and antioxidant production (Zera & Harshman 2001). Temperature has been shown to affect components of the energy budget in the closely-related brown trout (*Salmo trutta*), with the amount of energy allocated to body materials (i.e. body growth and body maintenance) decreasing either side of the temperature at which growth is maximised (Elliott 1976). The optimal temperature for growth in juvenile Atlantic salmon is thought to be around 16°C (Elliott & Hurley 1997) which was only just reached by the warmer (benign) stream by mid-summer (the end of the experiment). Therefore while neither stream provided ideal conditions for growth, the thermal conditions in the harsh stream were further from the optimum. For a given temperature (and food intake) body growth rate can only vary through an alteration of the relative amount of energy allocated to body growth vs body maintenance (McCarthy *et al.* 1994). Therefore achieving growth at a relatively colder temperature may come at a cost to body maintenance. A reduction in body maintenance (e.g. protein and DNA repair) could in turn affect telomere dynamics.

Ambient temperature may play an additional role in the telomere dynamics of ectotherms since it can affect the exposure of telomeres to oxidative damage through its effect on mitochondrial functioning. Telomeric DNA is vulnerable to oxidative damage from Reactive Oxygen Species (ROS) and most ROS are produced in the mitochondria, which are affected by temperature in several ways. For example, it is often the case in endotherms that mitochondrial density increases as a phenotypic response to lowered temperature (for reviews see Guderley 2004; O'Brien 2011). In ectotherms mitochondrial respiration rate (and hence ATP production) decreases at lower temperatures (Schulte 2015), and it may be that an individual compensates by increasing mitochondrial density, thus helping to maintain ATP production (O'Brien 2011). An increase in mitochondrial density may also result in increased ROS production (Crockett 2008). Therefore fish growing in colder environments might be exposed to more ROS and hence have more telomere attrition.

In addition to identifying a growth-environment effect on telomere length between the two streams, we also found growth-environment effects within streams. Electrofishing was conducted within 10m long sections and for each section we were able to calculate fry density. Although overall fry density did not differ significantly between the two streams, there was sufficient variation in density between the different sections within each stream to reveal a significant growth-fry density effect on telomere length. In the benign stream, fry density had no significant effect on the relationship between fry weight and fry telomere length. However in the harsh stream, it was beneficial in terms of telomere length for small, but not large fry to be at higher densities. The contrast between streams may reflect resource availability, with the harsher high altitude stream likely being more oligotrophic with reduced food availability. There were also more predators in the harsher stream. Therefore, while high densities may confer benefits to smaller fish from increased predator protection, one would expect increased intraspecific competition for resources (e.g. food) as individuals grow larger. Previous studies in other taxa have linked density to physiological stress (Montero *et al.* 1999; Trenzado *et al.* 2006) and telomere attrition (Kotrschal *et al.* 2007;

Sohn *et al.* 2012). However, these studies have mostly involved experimental manipulations involving extreme differences in density (crowded vs non–crowded), whereas here the effect was found among naturally-occurring variation in density over relatively small spatial scales. Overall, the results are consistent with the hypothesis that growth in more challenging environments comes at a greater cost to telomere length.

There was also a significant predator density effect: areas of higher predator density within each stream were associated with increased fry telomere length. This is somewhat surprising since predation risk has previously been linked to a number of physiological stress parameters (Hawlena & Schmitz 2010; Clinchy *et al.* 2013) including oxidative stress (Slos & Stoks 2008; Janssens & Stoks 2013) and telomere loss (Olsson *et al.* 2010). However, predatory salmonid parr will exhibit greater movement within a stream compared to the limited movement of salmon fry. Therefore our assessment of predatory parr density may only be a snapshot of parr distribution at the time of electrofishing. With this in mind, a more controlled laboratory manipulation is needed to elucidate potential predator effects on physiological stress pathways and telomere dynamics. The predator densities recorded here may actually have been an index of environmental quality. Predator distribution is partially dependent on prey abundance, and fry abundance would be expected to be greater in good quality habitat (e.g. plentiful shelter, food availability etc.). Therefore better quality habitats within each stream may be simultaneously producing healthier offspring, with an associated reduction in telomere attrition, while at the same time attracting more predators due to the numbers of fry. Thus a greater local predator density could actually provide a more benign environment for surviving fry by reducing fry densities and hence intra-cohort competition, although the situation is complicated by the evidence that fry and older fish may at times compete for the same resources (Kaspersson *et al.* 2012). Notwithstanding this possibility, we did not detect a significant correlation between predator density and fry density.

Offspring telomere length was not affected by any of the maternal traits included in the models. In contrast however, it was significantly influenced by paternal telomere length and paternal life history. There was a negative relationship between paternal telomere length and offspring (embryo) telomere length. This effect was evident both before and after the temperature manipulation, but the reasons for it are unclear. Since there were no maternal effects, and we used an in vitro fertilisation design, assortative mating is not involved in generating this pattern, and it might possibly relate to some differences in telomerase activity during the embryo stage, but this would require further investigation. There was a positive relationship between the number of years fathers (but not mothers) had spent at sea (ranging between 0 and 2) and offspring (fry) telomere length. A number of studies report significant paternal inheritance of telomere length, mostly in humans (i.e. De Meyer *et al.* 2007; Njajou *et al.* 2007) but also in lizards (Olsson *et al.* 2011). This effect is often attributed to paternal age, since telomere length has been shown to increase with age in human sperm cells (Allsopp *et al.* 1992). This may help explain our paternal effect in Atlantic salmon since, on average, MSW males will be oldest and precocious parr will be youngest. However, no effect was found of time spent by fathers in fresh water, and these paternal life history variants differ in many other ways than just age. Perhaps the most striking difference is in body size: MSW males used in this experiment were >2.5 times heavier than 1SW males and almost 200 times heavier than the precocious parr. If the larger fathers produce offspring that grow faster, their offspring might have been expected to have shorter telomere lengths; this pattern of reduced telomere length in larger individuals has been reported, for example in birds (Ringsby *et al.* 2015). However, our results suggest the opposite pattern, but we cannot separate time spent at sea from age and body size in this study. This variation in size and associated growth rate may be associated with differing levels of telomerase expression. A number of fish studies have detected telomerase activity in various adult tissues (Klapper *et al.* 1998; Hatakeyama *et al.* 2008; Gomes *et al.* 2010). It has also been reported that telomerase expression in fish is positively correlated with cell proliferation (Yap *et al.* 2005; Peterson *et al.* 2015). In fish, muscle fibre recruitment continues beyond

embryogenesis (Weatherley *et al.* 1988) and active cell proliferation is still detectable in Atlantic salmon at least 6 months after transfer to sea water (Johnston *et al.* 2003). Therefore, it is possible that the higher growth rate of MSW fish results in a greater expression of telomerase, which in turn may affect germline telomere dynamics, and subsequently offspring telomere dynamics.

This study demonstrates the complexity of parental and environmental factors that can influence telomere dynamics in early life, but highlights the fact that paternal effects may be stronger than maternal, and that growth in harsher conditions has greater costs in terms of telomere length. It may be that individuals growing in harsher early environments are required to invest more in telomere maintenance at a later stage, if they are to avoid an acceleration of senescence. However, we now need to examine which features of a harsher environment have the greatest impact on telomere dynamics. Doing so will allow more accurate predictions of the effect of environmental conditions on patterns of senescence.

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DATA ACCESIBILITY

Data deposited in the Dryad repository: doi:10.5061/dryad.2r6r4

AUTHOR CONTRIBUTION

DM, JDA, SM, PM and NBM contributed to experimental design. DM, DCS and SM carried out the field experiment and DM performed the laboratory analysis. Telomere data was analysed by DM and WB. The paper was written by DM, with additional comments from WB, PM and NBM.

Table 1. Summary of the four final linear mixed-effect models explaining variation in: A) fry weight (g), B) fry survival, C) embryo telomere length and D) fry telomere length. See SI for a full list of the main effects and interactions initially included in each model.

Model	Explanatory variable	Numerator df	Denominator df	F	p
A	Stream	1	763.12	1199.89	<0.001
	Fry density	1	765.17	11.34	0.001
	Egg weight	1	50.08	20.44	<0.001
B	Egg weight	1	18.53	11.66	0.003
C	Embryo time point	1	100.84	22.37	<0.001
	Paternal RTL	1	41.58	4.38	0.042
D	Stream	1	733.96	2.52	0.113
	Fry weight	1	732.79	1.22	0.269
	Fry density	1	733.14	14.53	<0.001
	Predator density	1	736.60	14.98	<0.001
	Paternal SW age	3	106.11	3.859	0.012
	Fry weight x stream	1	728.81	13.02	<0.001
	Fry weight x fry density	1	731.55	8.51	0.004

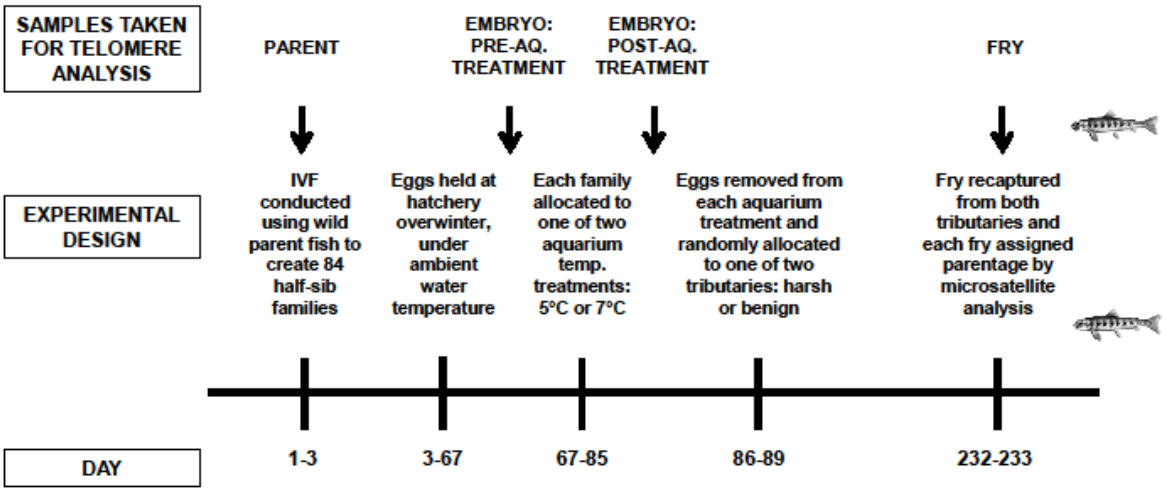

Fig. 1. Outline of experimental design. Day 1 = 3rd December 2013.

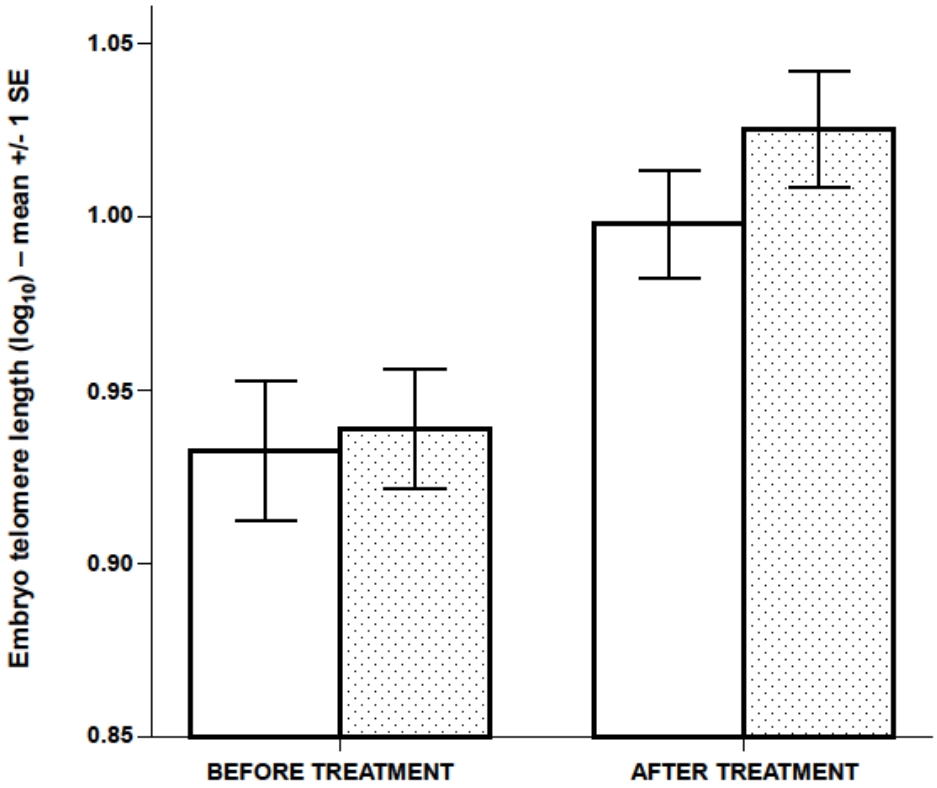

Fig. 2. The mean relative embryo telomere length before and after the aquarium temperature treatment. Blank columns represent the 5°C group and the patterned columns represent the 7°C group.

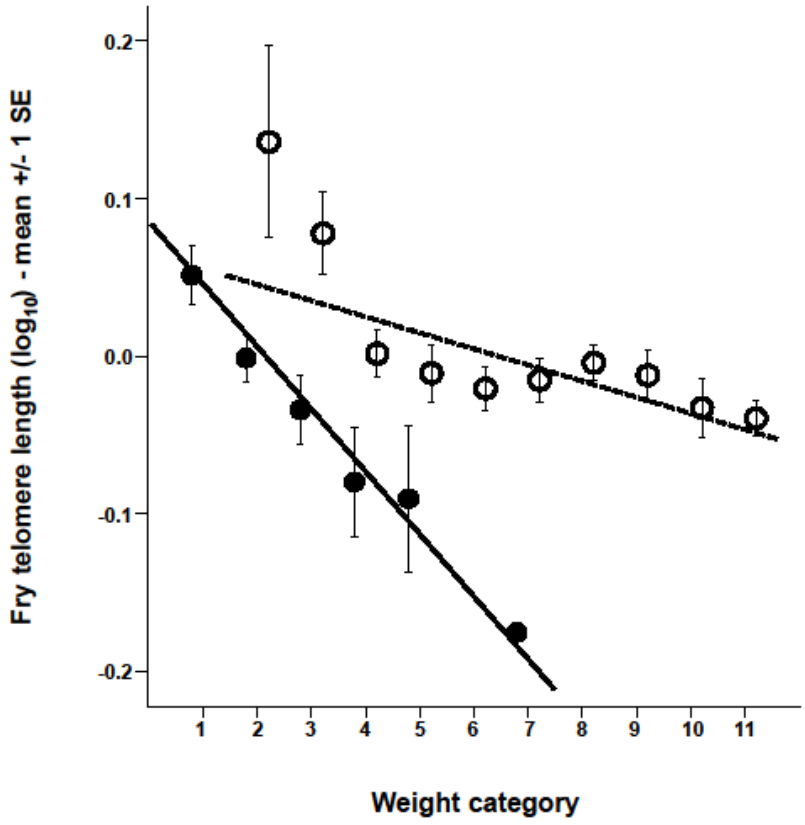

Fig. 3. The relationship between fry weight (g) and fry telomere length. Closed circles = harsh stream and open circles = benign stream. Weight data grouped for ease of presentation (analysis based on original data); definition of weight groups (g): group 1 = <0.40, group 2 = 0.41-.0.50, group 3 = 0.51-0.60, group 4 = 0.61-0.70, group 5 = 0.71-0.80, group 6 = 0.81-0.90, group 7 = 0.91-1.00, group 8 = 1.01-1.10, group 9 = 1.11-1.20, group 10 = 1.21-1.30, group 11 = >1.31.

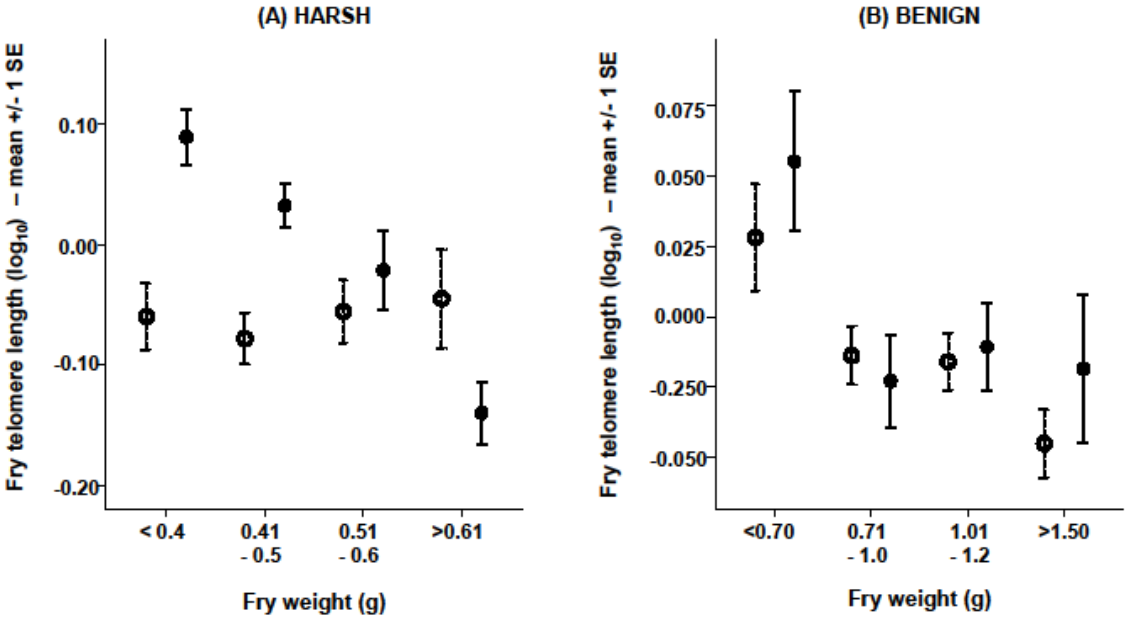

Fig. 4. The relationship between fry weight (g) and fry telomere length in (A) the harsh stream and (B) the benign stream. For presentation purposes, open circles represent fry that were captured in areas below the mean fry density (fry/m^2) and closed circles represent fry that were captured in areas above the mean fry density, although analysis was based on original continuous data. Mean fry density was calculated separately for each stream.

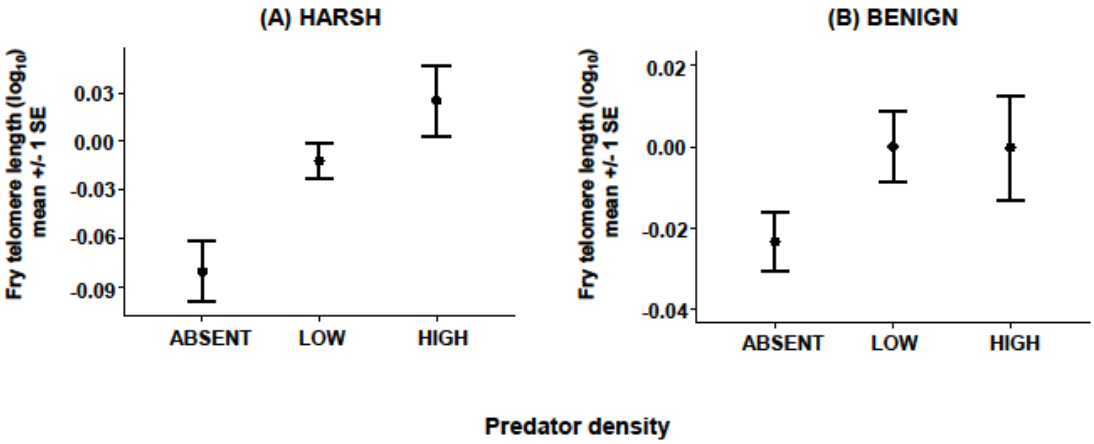

Fig. 5. The relationship between predator density and mean fry telomere length in (A) the harsh stream and (B) the benign stream. Predator density categorised for clarity of presentation, but treated as a continuous variable in the analysis. Definitions of predator density: ABSENT = 0, LOW = below the mean salmonid parr density (parr/m^2), HIGH = above the mean parr density (calculated separately for each stream).

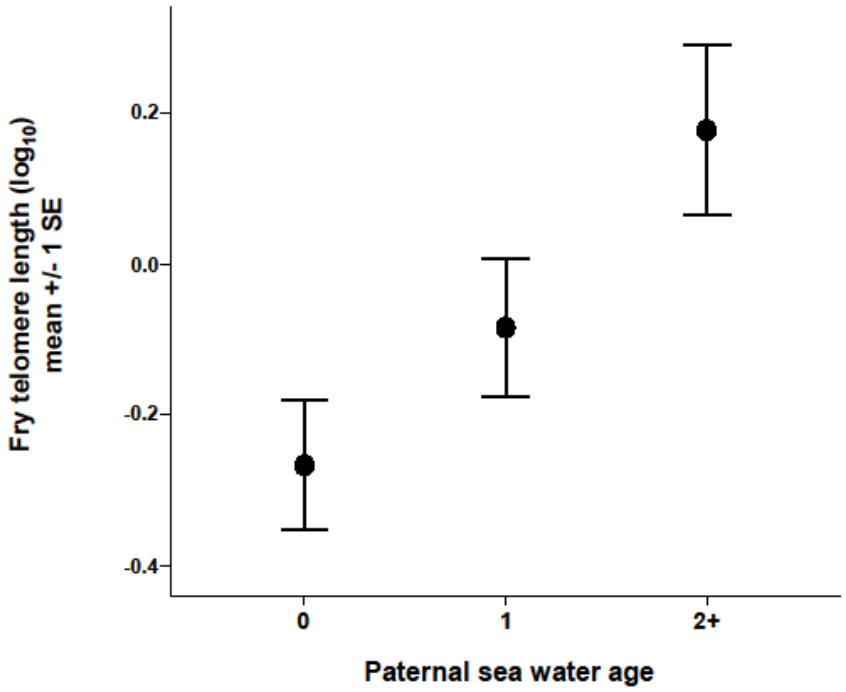

Fig. 6. The relationship between paternal sea water age and average fry telomere length. Paternal sea water age definitions: 0 = precocious parr fathers (which have not migrated to sea), 1 = 1SW fathers (which have spent 1 winter at sea), 2+ = MSW fathers (which have spent 2+ years at sea).