RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

Shorter juvenile telomere length is associated with higher survival to spawning in migratory Atlantic salmon

Darryl McLennan[1] iD | **John D. Armstrong**[2] | **David C. Stewart**[2] | **Simon Mckelvey**[3] | **Winnie Boner**[1] | **Pat Monaghan**[1] | **Neil B. Metcalfe**[1]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK

[2]Marine Scotland – Science, Freshwater Laboratory, Faskally, Pitlochry, UK

[3]Cromarty Firth Fishery Trust, CKD Galbraith, Inverness, UK

Correspondence
Darryl McLennan
Email: d.mclennan.1@research.gla.ac.uk

Abstract

1. The risk of mortality associated with a long-distance migration will depend on an animal's physiological state, as well as the prevailing ecological conditions. Here, we assess whether juvenile telomere length, which in endotherms has been shown to be a biomarker of physiological state and expected life span, predicts whether wild Atlantic salmon *Salmo salar* successfully complete their marine migration.

2. Over 1,800 juvenile fish were trapped, measured, passive integrated transponder tagged and a tissue biopsy taken when migrating as juveniles down-river towards the sea. Survivors of the marine phase of the life cycle were then re-trapped and re-sampled when returning to the river to spawn as sexually mature adults, 1.5–2.5 years later. Most individuals experienced a reduction in telomere length during the marine migratory phase of their life cycle. While the relative rate of telomere loss was greater in males than females, telomere loss was unrelated to growth at sea.

3. Contrary to expectations, salmon that had the shortest telomeres at the time of the outward migration had the greatest probability of surviving through to the return migration. This effect, independent of body size, may indicate a trade-off between investment in readiness for marine life (which favours high glucocorticoid levels, known to increase telomere attrition in other vertebrate species) and investment in telomere maintenance. Survival was also significantly influenced by the seasonal timing of outward migration, with the fish migrating downstream earliest in the spring having the highest probability of return.

4. This study reveals that telomere length is associated with survival, although in ways that contrast with patterns seen in endotherms. This illustrates that while telomeres may be universally important for chromosome protection, the potential for telomere dynamics to predict performance may vary across taxa.

KEYWORDS
fish, migration, physiological state, *Salmo salar*, sex effects, smolt

1 | INTRODUCTION

Many species are migratory between different geographical areas and/or habitats. While there can be much to gain from migration, it can be energetically demanding and a cause of significant mortality (Dingle, 2014; Gauthreaux, 1980). Performance during migration (and the associated probability of survival) may be influenced by an individual's pre-migratory body size and condition (Crossin et al., 2004; Mitchell, Guglielmo, Wheelwright, Freeman-Gallant, & Norris, 2011; Owen & Black, 1989; Schmutz, 1993; Tinbergen & Boerlijst, 1990). However, "body condition" can be hard to define and will vary with migration distance and the likelihood of feeding en route (Piersma, 1998). Moreover, faster growth in order to attain a larger size may result in subsequent mortality costs (Metcalfe & Monaghan, 2003).

We therefore examined whether telomere length could be used as an alternative marker of physiological state when assessing the likelihood of surviving a migration. Telomeres cap the ends of eukaryotic chromosomes and play an important role in chromosome protection (for reviews see Blackburn, 1991; Campisi, Kim, Lim, & Rubio, 2001; Monaghan, 2010). Telomere loss occurs at each round of cell division, partly because of the "end replication problem", but also because of environmental stress exposure (Debes, Visse, Panda, Ilmonen, & Vasemagi, 2016; Herborn et al., 2014; Simide, Angelier, Gaillard, & Stier, 2016; Watson, Bolton, & Monaghan, 2015). Telomeric DNA has a high guanine content, which is particularly susceptible to oxidative damage from reactive oxygen species (ROS), and ROS production can increase because of exposure to environmental stressors. In the absence of restoration, telomeres may eventually reduce to a "critical length", which may cause cells to enter a state of replicative senescence (Aubert & Lansdorp, 2008; Monaghan, 2014).

Partly because of the association with replicative senescence, a relatively short telomere length is indicative of poor physiological state (Monaghan, 2010). Shorter telomeres have been linked to age-related deterioration and/or increased disease susceptibility (Aubert & Lansdorp, 2008; Haussmann & Treidel, 2014; Ilmonen, Kotrschal, & Penn, 2008), and to reduced survival, both in the laboratory (Heidinger et al., 2012; Vera, Bernardes de Jesus, Foronda, Flores, & Blasco, 2012) and in the wild (Barrett, Burke, Hammers, Komdeur, & Richardson, 2013; Fairlie et al., 2016; Olsson et al., 2011; Watson et al., 2015). As such, telomere length is generally considered a good indicator of physiological state, defined as the condition of an individual's body and/ or its bodily functions. Therefore, measuring pre-migration telomere length may be an alternative way of quantifying an individual's pre-migration physiological state and therefore predicting the likelihood of a successful migration. In support of this, Angelier, Vleck, Holberton, and Marra (2013) reported that American redstarts (*Setophaga ruticilla*) with a longer telomere length pre-migration had a greater chance of returning from that migration, suggesting that telomere length may be a good biological indicator in which to study an individual's physiological fitness and the probability of successful migration. In contrast, studies on post-migration telomere length in the common tern (*Sterna hirundo*) and the dark-eyed junco (*Junco hyemalis*) found that investing in migration came at a cost to post-migration telomere length (Bauch,

Becker, & Verhulst, 2013; Bauer, Heidinger, Ketterson, & Greives, 2016). This counter-intuitive relationship may have arisen because of a trade-off between investment in migration (and associated migration success) versus investment in physiological maintenance (i.e. the energy allocated to maintaining a healthy physiology; which may involve telomere dynamics).

In order to examine the relationship between telomere length and the likelihood of surviving a migration, it is necessary to use a system in which there is likely to be variation in telomere length at the time of migration, where a significant proportion of individuals fail to complete the migration, and where it is possible to detect which animals have survived. Atlantic salmon *Salmo salar* fulfil these requirements. While the majority of the population undertakes an extensive marine migration lasting at least 18 months, there is a very strong tendency for migrants to return to their natal river to spawn (estimated 6% straying rate; Jonsson, Jonsson, & Hansen, 2003). This provides the opportunity to monitor survival rates, particularly when combined with fisheries management practices, in which all returning fish are captured in the river prior to spawning. Atlantic salmon display significant within-population variation in life-history strategy, which is linked to migratory behaviour (for reviews see Fleming, 1996; Klemetsen et al., 2003; Jonsson & Jonsson, 2011). Salmon eggs are laid in streams and rivers, where the resulting juveniles remain for up to 6 years [more typically 2–3 years in Scottish rivers (Maitland, 2007)], before transforming into the smolt stage and undertaking the spring migration to sea. The duration of the freshwater period depends largely on growth rate, as fish must reach a minimum size before smolting is triggered (Metcalfe & Thorpe, 1990; Økland, Jonsson, Jensen, & Hansen, 1993). They then spend either one or two (occasionally three or more) winters at sea (described as one sea winter (1SW) or multi sea winter (MSW) fish respectively), prior to returning to their natal river to spawn in late autumn. There are thought to be a number of determinants, both genetic and environmental, that influence how many years an individual will spend at sea (Barson et al., 2015; Fleming, 1998; Gardner, 1976).

Our study was carried out on a managed river system in northern Scotland. Approximately 6% of Scottish salmon survive the marine phase of the life cycle (from the time of outward migration until they return as sexually mature adults to the vicinity of the river mouth, prior to the impact of any coastal fisheries or estuarine predators), based on data from 2004 to 2013 (ICES, 2015). A number of studies have linked marine survival in Atlantic salmon to conditions experienced at sea (Friedland et al., 2009; Jonsson & Jonsson, 2004; Trueman, MacKenzie, & Palmer, 2012). However, conditions experienced in early life could also have long-term effects on the probability of successfully completing the return migration, due to their impact on growth rates, body size and physiological state.

In this study, we examined whether pre-migration telomere length was associated with migration survival in a wild Atlantic salmon population. Using a longitudinal mark and recapture approach, we measured body size, weight and telomere length in the same individuals, both before and after migration, in order to address two questions. Firstly, we assessed whether individuals experienced a consistent change in relative telomere length during the migratory phase of their life cycle.

Secondly, we assessed whether an individual's body size and physiological state (measured in terms of somatic weight and telomere length) at the smolt stage (prior to migration) were associated with their probability of returning to their native river to spawn (as a proxy for survival). More specifically, we predicted that a shorter juvenile telomere length would be associated with reduced survival to spawning in migratory Atlantic salmon. To our knowledge, this study is the first longitudinal analysis to link survival with telomere dynamics in a wild fish species.

2 | MATERIALS AND METHODS

2.1 | Study system

The study was conducted at the River Blackwater, which is part of the larger River Conon system, northern Scotland (57°60′N, 4°63′W). The River Conon has been highly impacted by the installation of hydroelectric schemes since the 1950s, which prevent the upstream passage of adult salmon. However, a wild population of salmon has been maintained by capturing almost all adult salmon returning to the river, conducting *in vitro* fertilizations, and then planting out eggs in artificial nests. The resulting offspring develop naturally in the river and can then pass down over the dams when undertaking their seaward smolt migration. As such, the fish can therefore complete their entire life cycle under natural conditions, until they return to the river to spawn. By utilizing this very high recapture rate of pre-spawning adults, we were able to measure the marine survival and return rate of fish in an otherwise natural population.

Atlantic salmon smolts (arising from the planted eggs) were captured during their seaward migration in April–May 2013. A temporary rotary screw trap (Riley, Ibbotson, Beaumont, Rycroft, & Cook, 2010; Thedinga, Murphy, Johnson, Lorenz, & Koski, 1994) was placed at the upstream entrance of Loch na Croic, just below the Loch na Croic dam. The dam prevents upstream migration by adults, but is no barrier to the smolts moving downstream (Figure 1). The rotary screw trap is designed to capture a proportion of the smolts moving past it. It was checked daily from 30th April until 30th May 2013 and any captured smolts removed. In total, 1806 smolts were captured for this study (*c.* 5% of the estimated

total smolt migration on the River Blackwater, S. McKelvey pers. obs.). Captured smolts were transferred to a holding tank at the nearby broodstock facilities, anaesthetized using a 5% benzocaine solution and then measured (fork length, mm; body mass, to 0.1 g). A uniquely coded passive integrated transponder (PIT) tag was inserted into the abdominal cavity, and a number of scales were sampled for subsequent scalimetry analysis, to determine the number of years spent in fresh water (Shearer, 1992). A small sample of tissue was taken from the adipose fin and stored in 100% ethanol for telomere analysis. Tagged fish were then placed in a recovery tank supplied directly with water from the adjacent river for a maximum of 4 hr until fully recovered, and then released on the same day, several metres downstream of the initial capture site.

Surviving tagged smolts were expected to return to the River Blackwater to spawn in the autumn of either 2014 or 2015. On their return they were trapped in a permanent trap incorporated into the dam at the head of Loch na Croic (Figure 1). Since the dam is impassable to large fish, the trap lures in all fish that are attempting to move upstream. There is little salmon spawning habitat downstream of Loch na Croic, and so virtually all returning adults will attempt to pass upstream through the dam and become caught. The trap is closed for the majority of the year. Therefore, Atlantic salmon returning to the River Blackwater during the summer and autumn congregate in Loch na Croic, downstream of the dam, until the trap is opened in November each year. Adult fish then move upstream into the trap and are removed daily. All returning adult fish captured in the Loch no Croic salmon trap in November–December 2014 and 2015 were scanned for the presence of a PIT tag. Tagged individuals were anaesthetized using a 5% benzocaine solution and measured (fork length to 0.5 cm; body mass to 0.1 g). A second small sample of tissue was taken from the adipose fin and stored in 100% ethanol for telomere analysis. All tagged fish were then stripped of gametes as part of the fisheries management programme and released back into the river, upstream of the trap.

2.2 | Telomere analysis

For the telomere analysis, DNA was extracted from the fin tissue (of both smolts and adults) using the DNeasy Blood and Tissue Kit
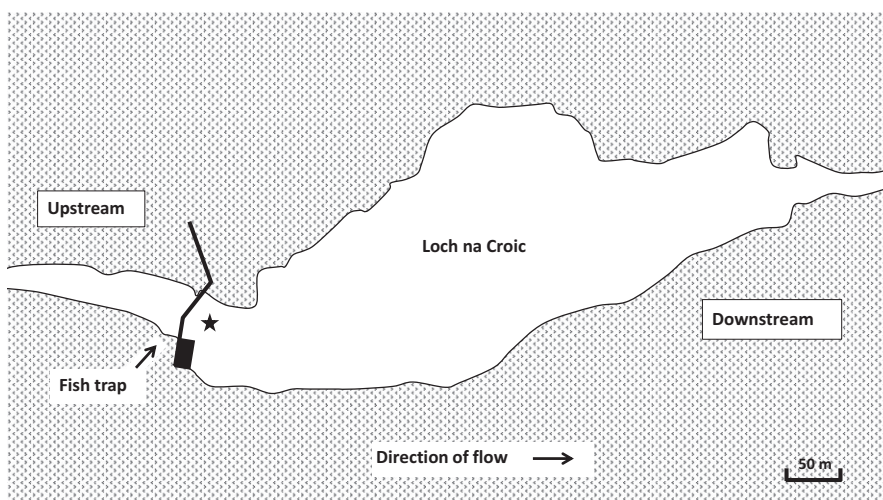

FIGURE 1 Diagrammatic outline of Loch na Croic (situated on the River Blackwater) and its associated dam (with incorporated fish trap). The star outlines approximate location of the temporary rotary screw trap, used for the purposes of this experiment

(Qiagen), following the manufacturer's protocol. Each set of DNA extractions also included a negative control, which contained all of the reagents, but without tissue. This was used to check for contamination during the lysis and extraction steps. DNA concentration and purity was measured spectrophotometrically using a Nanodrop 8000.

Telomere length was measured in all samples using quantitative PCR (Cawthon, 2002), and data were analysed using qBASE software for windows (Hellemans, Mortier, De Paepe, Speleman, & Vandesompele, 2007), as described in McLennan et al. (2016). In brief, this qPCR method 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 gene number (S). For each sample, the qBASE software produces the RTL values as calibrated normalized relative quantities (CNRQs). This is similar to the more commonly calculated T/S ratio (described by Cawthon, 2002), but with greater control of inter-plate variation. The Atlantic salmon Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was chosen as the single copy gene (Genbank accession number: NM_001123561). Please refer to McLennan et al. (2016) for primer list and the PCR conditions used. The samples (23 adults and 69 smolts) were randomly distributed across four sets of PCR plates. In addition to the samples, each plate also included a six-fold serial dilution of a reference sample (1.25–40 ng/well) and a non-target control (NTC). The DNA for the reference sample was a pool of 24 samples that included both life stages (smolt and adult). The serial dilution was used to generate a standard curve and calculate assay efficiencies. Three points from the standard curve (5, 10 and 20 ng/well) were also used as inter-run calibrators during qBASE analysis, to help correct for inter-run variation. 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. The mean assay efficiencies for the telomere and GAPDH were 103.5% and 99.2%, respectively, and well within the acceptable range (85%–115%). The average quantification cycle (C_q) for the telomere assay was 16.62 ± SD 0.10 (intra-assay coefficient of variability in telomere C_qs = 1.15). The average C_q for the GAPDH assay was 24.91 ± SD 0.11 (intra-assay coefficient of variability in GADPH C_qs = 0.59). The six points of the standard curve were used to calculate the inter-assay coefficient of variability in the CNRQs (which was 7.87).

2.3 | Data analysis

The following variables were included in statistical models as dependent/independent variables as appropriate: the downstream migration date of the smolts, i.e. the Julian date on which an individual was captured and PIT tagged (subsequently referred to as *timing of smolt migration*, covariate), whether or not each individual was recaptured as an adult post-migration (*migration survival*, binary), the fresh body weight of smolts at capture on their outward migration (*smolt weight*, covariate), fresh body weight of returning adults at recapture (*adult weight*, covariate), the total age of each individual, as determined by scalimetry (*total age*, covariate), the number of years that each individual had spent in fresh water prior to migration

(*FW age*, factor), the sex of each returning individual (*sex*, factor), the smolt and adult relative telomere lengths (*smolt RTL* and *adult RTL*, respectively, covariates), the life stage at which relative telomere length was measured (i.e. whether at the smolt or adult stage; subsequently referred to as *stage*, factor) and the relative change in telomere length, calculated for each returning individual as the difference between smolt RTL and adult RTL (*RTL change*, covariate). Within-individual RTL change could only be measured for surviving individuals, thus precluding any analysis of RTL change in relation to survival probability.

All statistical analyses were carried out using IBM SPSS 22 for Windows. Body weight and body length were highly collinear at both smolt and adult stages (Pearson $r > .96$, $p < .001$), and so only body weight was used in statistical models. Inclusion of smolt RTL in the model explaining variation in RTL change ran the risk of the statistical artefact known as regression to the mean (Barnett, van der Pols, & Dobson, 2005; Verhulst, Aviv, Benetos, Berenson, & Kark, 2013). Thus, while raw values for RTL change (adult RTL– smolt RTL) were significantly negatively correlated with those for smolt RTL (Pearson $r = -.76$, $p < .001$), this relationship became non-significant after correcting RTL change values for regression to the mean, using the procedure of Verhulst et al. (2013). Therefore, as the apparent effect of smolt RTL on RTL change was a statistical artefact, smolt RTL was not included as an explanatory variable in the analyses.

Raw data were analysed for outliers, following the "outlier-labelling rule" proposed by Hoaglin, Iglewicz, and Tukey (1986). Telomere values
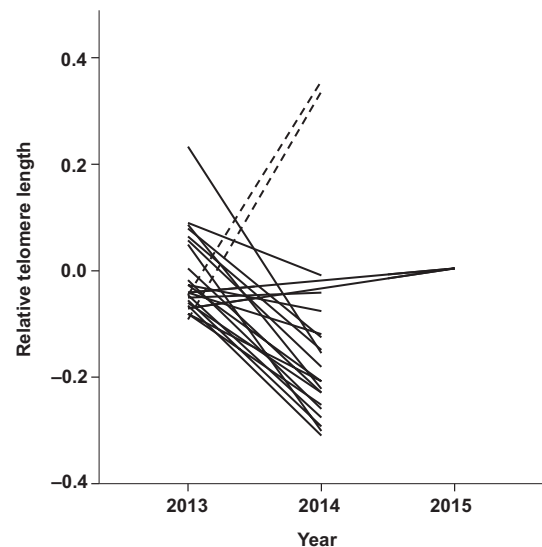

FIGURE 2 The relative change in telomere length during the marine phase of the life cycle in salmon. The lines join the values for individual fish first sampled as juveniles during their seaward migration in 2013, and then recaptured as sexually mature adults on their return to the river in either 2014 or 2015. Dashed lines represent the two individuals that were identified as outliers. They are included here for presentation purposes; however, they were excluded from all analyses that included adult RTL or RTL change. RTL, relative measure of telomere length

at the adult stage were extremely large for two fish, and were subsequently identified as outliers by the boxplot outlier-labelling rule applied to the raw data. As a result, the two fish were excluded from all analyses that included adult RTL or RTL change. Although we decided to remove these two fish from particular analysis, we cannot discount the possibility that these extremely large telomere values are real. As such, we have included these two individuals in Figure 2.

Using the entire sample of tagged smolts ($n = 1,806$), we analysed migration survival in relation to smolt weight and timing of smolt migration using binary logistic regression. The logistic regression model was fitted by maximum likelihood, with a logit link function, and the significance of terms in the model was evaluated with the Wald statistic (distributed as chi-squared). We started with a full model (including both smolt weight and timing of smolt migration) and performed backwards model selection; removing the least significant variable until all remaining variables are statistically significant. Please see Table S1 for an outline of the initial full model.

Using only the 1SW fish ($n = 19$), variation in adult RTL and RTL change was assessed in relation to sex, total age, adult weight and timing of smolt migration by general linear models (GLM), fitted by least squares. Due to the small sample size, each variable was initially tested singly in order to select variables for inclusion in the final model, with only variables that had a significant association ($p < .05$) being included in the final model. Please see Table S2 for an outline of the individual GLMs. Again using only the 1SW fish, we analysed RTL in relation to life stage by a paired t test, to test whether or not telomere length changed over time within an individual.

Lastly, to test the hypothesis that smolt RTL is associated with migration survival, we also compared relative telomere lengths at the smolt stage in the fish that did return, to a comparable sample of smolts that did not return. For logistical reasons, it was not possible to analyse the telomere lengths of all smolts and so we used a subsample, controlling for variation in the timing of smolt migration by selecting non-returners that had been tagged on the same days as returning individuals. For each returner we therefore selected two smolts that failed to return (the two individuals that had been tagged immediately prior to and immediately after the returned individual). If the returned fish was the first/last individual to be tagged that day, the subsequent/prior two fish were used instead. Because of the association between relative body size and telomere dynamics (e.g. McLennan et al., 2016; Näslund, Pauliny, Blomqvist, & Johnsson, 2015) we controlled for possible smolt weight effects by conducting a GLM with weight as the dependent variable and return status as a fixed factor (returned vs. non-returned). There was no significant difference in smolt weight between the fish that returned and the matched sample of fish that undertook the smolt migration over the same time period but failed to return ($F_{1,67} = 0.221$, $p = .640$), indicating that our matched samples were equivalent in body size at the time of outward migration. We then analysed migration survival in relation to smolt RTL (log-transformed) and FW age using binary logistic regression. The logistic regression model was fitted by maximum likelihood, with a logit link function, and the significance of terms assessed by the Wald statistic as before. We started with a full model (including both smolt RTL and FW age) and performed backwards model selection. Please see Table S3 for an outline of the initial full model.

3 | RESULTS

3.1 | Summary of characteristics of returning fish

Of the smolts that were tagged in the spring of 2013, 21 were recaptured as 1SW adults at the Loch na Croic salmon trap in early winter 2014 (16 males and five females), and a further two as MSW adult fish in the next year's spawning migration (one male and one female). In total, this represents 1.3% of the individuals tagged as outgoing smolts. Male and female returning fish did not differ in body length or weight (at the smolt or returning adult stage) nor in age or the timing of smolt migration (Table S4).

3.2 | The effect of migration on telomere dynamics

Telomere length significantly decreased between the smolt and adult stage ($T_{18} = 7.33$, $p < .001$), with 85.7% of the returned individuals, all 1SW fish, experiencing a relative telomere loss (Figure 2). Only three fish experienced a relative increase in telomere length, and two of these were the fish that had spent multiple years at sea, but this small sample size precluded us from including time at sea as a factor in the analysis.

Variation in adult RTL and RTL change was separately assessed in relation to sex, total age, adult weight and the timing of smolt migration. The number of years in SW significantly affected physiological weight ($F_{1,21} = 118.8$, $p < .001$) with MSW fish being much larger than 1SW fish. Therefore, to be able to test the effect of weight on adult telomere dynamics independently of this effect of time at sea, only adult fish that had spent 1 year at sea were included in the adult RTL and RTL change models, thus removing the two individuals that had spent multiple years at sea. Total age (3–5 years), adult weight and timing of migration were not significantly associated with telomere dynamics (either adult RTL or RTL change). However, adult telomere length and the rate of telomere change (between smolt stage and adult stage) both significantly differed between the sexes (Adult RTL $F_{1,17} = 11.78$, $p = .003$; RTL change $F_{1,17} = 4.85$, $p = .04$). Males had similar telomere lengths to females at the smolt stage (Table S1) but experienced a relatively greater rate of telomere loss while at sea, and as a result had significantly shorter telomeres at the returning adult stage (Figure 3).

3.3 | Migration survival

When using the entire sample of smolts ($n = 1,806$), migration survival (i.e. the probability of an outgoing smolt returning as a spawning adult and being caught in the trap) was not significantly affected by the weight of the smolts on their outward migration. However, it was significantly influenced by the timing of smolt migration (Logistic regression, Wald statistic = 6.99, $df = 1$, $p = .008$). Migrating to sea later in the smolt run was associated with reduced migration survival (Figure 4).

FIGURE 3 The relationship between sex (F = female, M = male) and (a) relative telomere length as sexually mature adults, and (b) relative change in telomere length between the seaward migrating and returning adult stage (negative values indicate reductions in telomere length). Data plotted as individual data points with means indicated by horizontal bars
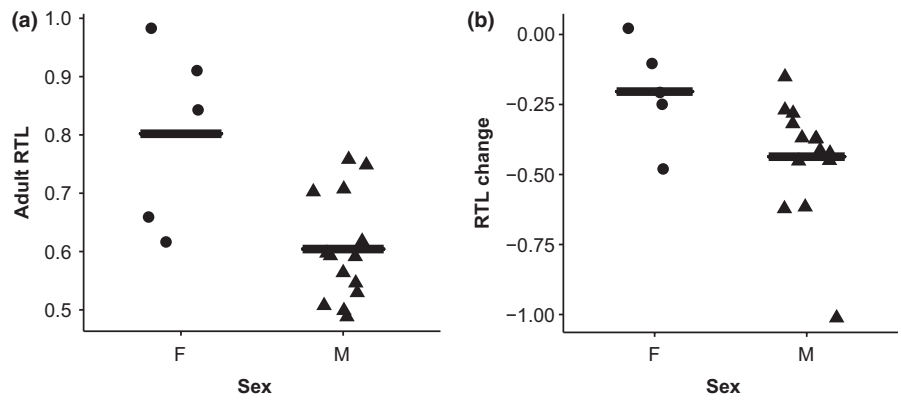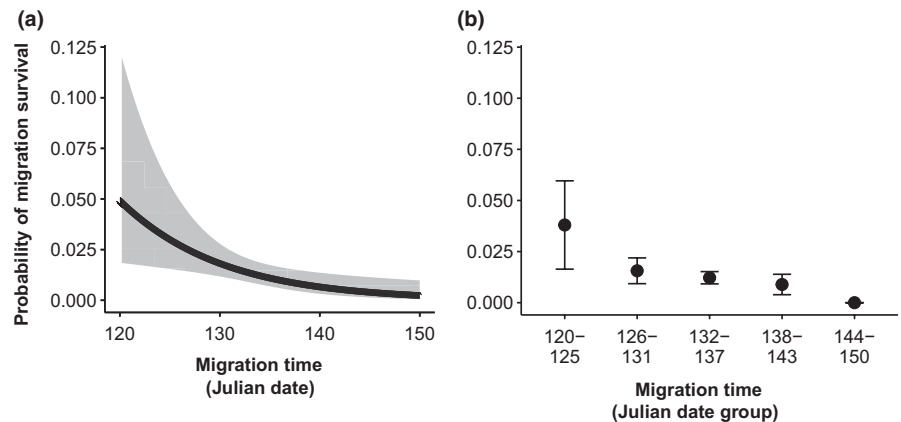

FIGURE 4 The relationship between the timing of a fish's seaward migration as a juvenile (Julian date on which it was trapped and released) and the probability of it surviving the marine phase and migrating back to the river as a sexually mature adult. The logistic regression curve (with 95% confidence bands) is shown in (a), while in (b) the data are plotted for presentational purposes as mean probabilities ± 1*SE* within 6 day time bins

When comparing tagged individuals that had successfully returned to a subsample of individuals that did not return (while controlling for smolt weight and timing of migration—see Materials and methods), FW age did not significantly affect the probability of surviving the migration. However, smolt RTL did have a significant effect on migration survival (Logistic regression, Wald statistic = 4.08, *df* = 1, *p* = .043), but the effect was the opposite to that expected, as individuals with shorter telomeres at the time of migrating to sea were more likely to return (Figure 5).

4 | DISCUSSION

Based on data collected from a wild population of salmon, we found that most individuals experienced a reduction in telomere length during the migratory phase of their life cycle. The relative rate of telomere loss was dependent on sex, with males experiencing a relatively greater loss. This was not related to age, body mass or to the seasonal timing of the initial seaward migration. In contrast to our hypothesis, we found that juvenile salmon that had the shortest telomeres at the time of outward migration had the greatest probability of surviving through to the return migration. Migration survival was also significantly influenced by the timing of outward smolt migration, with the smolts migrating earliest in the spring having the highest probability of return.

In general, telomeres have been found to shorten with age, at least in many mammal and bird studies to date (Haussmann et al., 2003;

Henriques & Ferreira, 2012). Many mammal and bird species appear to down-regulate telomerase expression in post-embryonic physiological tissue and instead use replicative ageing as a tumour suppression mechanism (Gomes, Shay, & Wright, 2010). In contrast, telomerase expression has been detected in post-embryonic physiological tissue in reptiles, amphibians and fish (Gomes et al., 2010) suggesting that telomere attrition with age is not as common in ectotherms. Simide et al. (2016) reported that around half of the fish telomere studies to date have reported telomere shortening with age, while the rest reported no effect, or even telomere elongation. We found that most individuals migrating to sea for 1 year experienced a telomere loss during their time at sea (18 of 19 individuals). The two returning individuals that had remained at sea for multiple winters both experienced an increase in telomere length.

Telomere length in the returning adults differed significantly between the sexes, with males undergoing a greater rate of telomere loss at sea than females, and having significantly shorter telomeres in adulthood. In birds, mammals and XY-sex determined reptiles, adult life span tends to be lower in the heterogametic sex (Barrett & Richardson, 2011; Liker & Székely, 2005), as does telomere length (Barrett & Richardson, 2011). In salmon, it is males that are the heterogametic sex (Davidson, Huang, Fujiki, von Schalburg, & Koop, 2009), so this pattern also holds.

The most surprising aspect of our study is that, contrary to expectations, juvenile salmon with the shortest telomeres at the start of their migration had the greatest probability of returning from it.
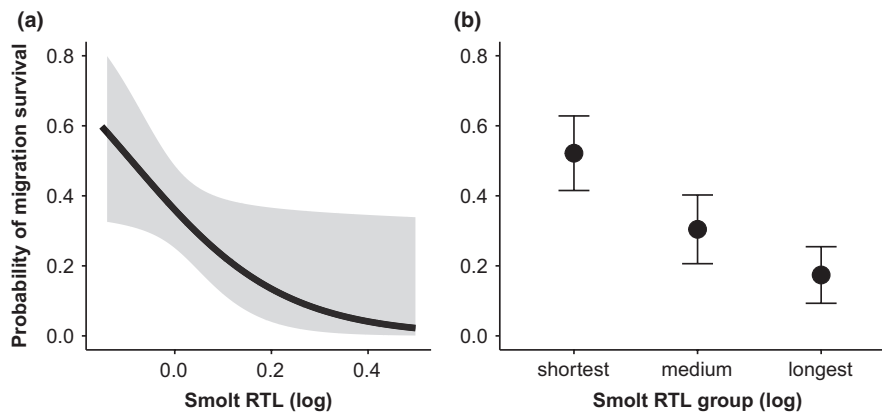
FIGURE 5 The relationship between relative telomere length of salmon smolts at the time of their seaward migration and the probability of surviving the marine phase and migrating back to the river as a sexually mature adult. The logistic regression curve (with 95% confidence bands) is shown in (a), while in (b) the data are shown for presentation purposes as mean probabilities ± *SE* in three telomere length categories of equal sample size (*n* = 23 for each group). Note that these probabilities are relative (and are much higher than the true survival probabilities) as the subsample of fish on which this analysis was based only included two non-returning fish for each returner in order to make the sample size for telomere analysis manageable—see text for selection criteria and analysis

In other taxa, a relatively short telomere length has been linked to reduced survival, both in the laboratory (Heidinger et al., 2012; Vera et al., 2012) and in the wild (Barrett et al., 2013; Fairlie et al., 2016; Olsson et al., 2011; Watson et al., 2015). Angelier et al. (2013) reported a correlation between longer pre-migration telomeres and a greater chance of migration survival in American redstarts (*S. ruticilla*). However, Bauch et al. (2013) and Bauer et al. (2016) both found that individuals investing in migration also had shorter telomeres at the end of migration, perhaps as a trade-off between investment in migration (and associated migration success) and physiological maintenance (and associated telomere dynamics). It may also be the case in this study that we are witnessing a trade-off, with a greater investment in physiological preparedness for life at sea coming at an increased cost in terms of pre-migratory telomere attrition. The process of smolting requires many metabolic and biochemical changes in preparation for life at sea (Jonsson & Jonsson, 2011). Smolt "physiological preparedness" can be quantified by measuring the abundance of Na$^+$-K$^+$2Cl$^-$ cotransporter (a protein associated with ion regulation) and/or the activity of the associated Na$^+$-K$^+$2Cl$^-$ATPase enzyme, both of which are up-regulated in Atlantic salmon smolts prior to sea entry (Pelis & McCormick, 2001; Stich, Zydlewski, & Zydlewski, 2016). Moreover, it has been found that individuals investing more in physiological preparedness immediately prior to migration (in terms of higher K$^+$2Cl$^-$ATPase activity) subsequently experience reduced mortality (Stich, Zydlewski, Kocik, & Zydlewski, 2015). However, these physiological changes are also associated with changes in circulating hormone levels during smolting. For example, cortisol is known to increase gill Na$^+$-K$^+$2Cl$^-$ cotransporter abundance (Pelis & McCormick, 2001) and therefore has positive effects on smolt development (Hoar, 1988). However, there is also mounting evidence of an association between glucocorticoids and telomere attrition (for review see Haussmann & Marchetto, 2010), and so a greater investment in the physiological process of smolting may be beneficial in terms of survival over the period of entry to sea water, but come at a cost to

telomere length. It is also possible that smolts with the shortest telomere length at the time of migration were those that had experienced more exposure to stress. This is in line with other studies that show that stress exposure, including growing in a relatively harsh environment, is associated with reduced telomere length (e.g. Herborn et al., 2014; McLennan et al., 2016; Olsson, Pauliny, Wapstra, & Blomqvist, 2010). It is possible that this stress exposure increased the resilience of the fish, thus improving their survival at sea.

There may also be a behavioural basis to the relationship between juvenile salmon telomere length and subsequent migration survival. Reduced telomere length has been found to be associated with altered behaviours, such as elevated impulsive foraging (Bateson, Brilot, Gillespie, Monaghan, & Nettle, 2015) and enhanced foraging and aggression (Adriaenssens, Pauliny, Blomqvist, & Johnsson, 2016). It is arguable that these behaviours require a greater activity level and a higher metabolic rate, which in turn may lead to greater exposure to oxidative stress and telomere attrition (Chan & Blackburn, 2004). Nonetheless, behaviours such as boldness and aggression are generally found to positively correlate with food intake and growth (for review see Biro & Stamps, 2008) and there is a strong link between growth and survival in Atlantic salmon (Friedland, Hansen, Dunkley, & Maclean, 2000). Therefore, it is plausible that we are again witnessing a trade-off between individual fitness and telomere dynamics: juvenile salmon that display bolder and more aggressive behaviour may then go on to have a better chance of survival at sea, but do so at the expense of telomere length.

We found that the likelihood of completing the return migration was also significantly influenced by the timing of outward smolt migration: smolts migrating earliest in the spring had the highest probability of return. Mortality is thought to be high in salmon smolts, both during movement to the mouth of the river, and when they first reach the sea (Thorstad et al., 2012). Many populations of smolts must pass through lakes during their downstream migration, and a previous study on the River Conon found that the chances of migrating successfully across

a lake depended on the timing of entry to the upstream end of the lake, with the earliest migrating individuals having the best chance of completing their migration through the lake (E. Rush & S. Mckelvey, unpubl. data). Predation is one of the biggest natural causes of smolt mortality during downstream migration (Thorstad et al., 2012), and it may be that predators (such as pike *Esox lucius*) take time to cue in on the arrival of smolts, thus giving early migrants an advantage. There may also be a difference in the physiological fitness between early and late migrants. The transformation into the migratory smolt stage appears to be triggered only when the fish reach a minimum size (Metcalfe & Thorpe, 1990; Økland et al., 1993). It is possible that more successful individuals, having achieved a faster growth rate in the preceding months, are able to start migrating early in the spring, while smaller, less successful individuals remain on their feeding territories in fresh water for as long as possible, prior to starting migration. A further factor linking timing of smolt migration to survival is the timing of entry to the sea (McCormick, Hansen, Quinn, & Saunders, 1998). Reports suggest that ocean temperature at the time of sea entry is one of the key determinants of survival (Friedland, 1998; Friedland et al., 2000). Therefore, it may be that the earlier migrating smolts from this study were met with more favourable sea entry conditions. Of course, predicting sea conditions will be difficult for smolts, and annual fluctuations in sea temperature may favour either early or late migrants, depending on the conditions in a particular year.

Although our sample size of surviving fish was relatively small, we have identified a clear association between early-life telomere length and subsequent survival over the migratory part of the Atlantic salmon life cycle. The mechanisms underlying this relationship are currently unclear. However, we have shown that indicators of physiological state, other than size or body condition, can be taken into consideration when assessing the relationship between an individual's pre-migratory physiological state and the likelihood of successful migration.

AUTHORS' CONTRIBUTIONS

D.M., J.D.A., S.M., P.M. and N.B.M. contributed to experimental design. D.M. and S.M. carried out the field study (PIT tagging of juvenile fish and recapturing of adults). D.C.S. performed scalimetry analysis. The telomere assays were performed by D.M., after having been optimized by D.M. and W.B. D.M. performed the data analysis, with additional comments from P.M. and N.B.M. The paper was written by D.M., with additional comments from W.B., J.D.A., P.M. and N.B.M.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.rk030 (McLennan et al., 2017).

REFERENCES

Adriaenssens, B., Pauliny, A., Blomqvist, D., & Johnsson, J. I. (2016). Telomere length covaries with personality in wild brown trout. *Physiology & Behavior, 165*, 217–222.

Angelier, F., Vleck, C. M., Holberton, R. L., & Marra, P. P. (2013). Telomere length, non-breeding habitat and return rate in male American redstarts. *Functional Ecology, 27*, 342–350.

Aubert, G., & Lansdorp, P. M. (2008). Telomeres and aging. *Physiological Reviews, 88*, 557–579.

Barnett, A. G., van der Pols, J. C., & Dobson, A. J. (2005). Regression to the mean: What it is and how to deal with it. *International Journal of Epidemiology, 34*, 215–220.

Barrett, E. L., Burke, T. A., Hammers, M., Komdeur, J., & Richardson, D. S. (2013). Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology, 22*, 249–259.

Barrett, E. L. B., & Richardson, D. S. (2011). Sex differences in telomeres and lifespan. *Aging Cell, 10*, 913–921.

Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., ... Karlsson, S. (2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature, 528*, 405–408.

Bateson, M., Brilot, B. O., Gillespie, R., Monaghan, P., & Nettle, D. (2015). Developmental telomere attrition predicts impulsive decision-making in adult starlings. *Proceedings of the Royal Society of London B: Biological Sciences, 282*, 20142140.

Bauch, C., Becker, P. H., & Verhulst, S. (2013). Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society of London B: Biological Sciences, 280*, 20122540.

Bauer, C. M., Heidinger, B. J., Ketterson, E. D., & Greives, T. J. (2016). A migratory lifestyle is associated with shorter telomeres in a songbird (*Junco hyemalis*). *The Auk, 133*, 649–653.

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution, 23*, 361–368.

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature, 350*, 569–573.

Campisi, J., Kim, S.-H., Lim, C.-S., & Rubio, M. (2001). Cellular senescence, cancer and aging: The telomere connection. *Experimental Gerontology, 36*, 1619–1637.

Cawthon, R.M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research, 30*, e47.

Chan, S. R. W. L., & Blackburn, E. H. (2004). Telomeres and telomerase. *Philosophical Transactions of the Royal Society B: Biological Sciences, 359*, 109–121.

Crossin, G. T., Hinch, S. G., Farrell, A. P., Higgs, D. A., Lotto, A. G., Oakes, J. D., & Healey, M. C. (2004). Energetics and morphology of sockeye salmon: Effects of upriver migratory distance and elevation. *Journal of Fish Biology, 65*, 788–810.

Davidson, W. S., Huang, T. K., Fujiki, K., von Schalburg, K. R., & Koop, B. F. (2009). The sex determining loci and sex chromosomes in the family salmonidae. *Sexual Development, 3*, 78–87.

Debes, P. V., Visse, M., Panda, B., Ilmonen, P., & Vasemagi, A. (2016). Is telomere length a molecular marker of past thermal stress in wild fish? *Molecular Ecology*, 25, 5412–5424.

Dingle, H. (2014). *Migration: The biology of life on the move*. Oxford, UK: Oxford University Press.

Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, 15, 140–148.

Fleming, I. A. (1996). Reproductive strategies of Atlantic salmon: Ecology and evolution. *Reviews in Fish Biology and Fisheries*, 6, 379–416.

Fleming, I. A. (1998). Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences*, 55(Suppl 1), 59–76.

Friedland, K. (1998). Ocean climate influences on critical Atlantic salmon (*Salmo salar*) life history events. *Canadian Journal of Fisheries and Aquatic Sciences*, 55(Suppl 1), 119–130.

Friedland, K. D., Hansen, L. P., Dunkley, D. A., & Maclean, J. C. (2000). Linkage between ocean climate, post-smolt growth, and survival of Atlantic salmon (*Salmo salar* L.) in the North Sea area. *ICES Journal of Marine Science*, 57, 419–429.

Friedland, K. D., MacLean, J. C., Hansen, L. P., Peyronnet, A. J., Karlsson, L., Reddin, D. G., … McCarthy, J. L. (2009). The recruitment of Atlantic salmon in Europe. *ICES Journal of Marine Science*, 66, 289–304.

Gardner, M. L. G. (1976). A review of factors which may influence the sea-age and maturation of Atlantic salmon *Salmo salar* L. *Journal of Fish Biology*, 9, 289–327.

Gauthreaux, S. A. (1980). *Animal migration, orientation, and navigation*. London, UK: Academic Press.

Gomes, N. M. V., Shay, J. W., & Wright, W. E. (2010). Telomere biology in Metazoa. *FEBS Letters*, 584, 3741–3751.

Haussmann, M. F., & Marchetto, N. M. (2010). Telomeres: Linking stress and survival, ecology and evolution. *Current Zoology*, 56, 714–727.

Haussmann, M.F., & Treidel, L.A. (2014). Senescence: Integrating biology from cradle to the grave. *Integrative organismal biology* (pp. 257–275). Hoboken, NJ: John Wiley & Sons.

Haussmann, M.F., Winkler, D.W., O'Reilly, K.M., Huntington, C.E., Nisbet, I.C.T., & Vleck, C.M. (2003). Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 270, 1387–1392.

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 1743–1748.

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, 8, R19.

Henriques, C. M., & Ferreira, M. G. (2012). Consequences of telomere shortening during lifespan. *Current Opinion in Cell Biology*, 24, 804–808.

Herborn, K. A., Heidinger, B. J., Boner, W., Noguera, J. C., Adam, A., Daunt, F., & Monaghan, P. (2014). Stress exposure in early post-natal life reduces telomere length: An experimental demonstration in a long-lived seabird. *Proceedings of the Royal Society of London B: Biological Sciences*, 281, 20133151.

Hoaglin, D. C., Iglewicz, B., & Tukey, J. W. (1986). Performance of some resistant rules for outlier labeling. *Journal of the American Statistical Association*, 81, 991–999.

Hoar, W. (1988). 4. The physiology of smolting salmonids. *Fish Physiology*, 11, 275–343.

ICES. (2015). *Report of the working group on North Atlantic salmon (WGNAS)*. Moncton, Canada: ICES.

Ilmonen, P., Kotrschal, A., & Penn, D. J. (2008). Telomere attrition due to infection. *PLoS ONE*, 3, e2143.

Jonsson, B., & Jonsson, N. (2004). Factors affecting marine production of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 2369–2383.

Jonsson, B., & Jonsson, N. (2011). *Ecology of Atlantic salmon and Brown trout: Habitat as a template for life histories* (1st edn). Dordrecht, The Netherlands: Springer.

Jonsson, B., Jonsson, N., & Hansen, L. (2003). Atlantic salmon straying from the River Imsa. *Journal of Fish Biology*, 62, 641–657.

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F., & Mortensen, E. (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): A review of aspects of their life histories. *Ecology of Freshwater Fish*, 12, 1–59.

Liker, A., & Székely, T. (2005). Mortality costs of sexual selection and parental care in natural populations of birds. *Evolution*, 59, 890–897.

Maitland, P. S. (2007). *Scotland's freshwater fish: Ecology, conservation & folklore*. Oxford, UK: Trafford Publishing (UK) Limited.

McCormick, S. D., Hansen, L. P., Quinn, T. P., & Saunders, R. L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, 55(Suppl 1), 77–92.

McLennan, D., Armstrong, J. D., Stewart, D. C., McKelvey, S., Boner, W., Monaghan, P., & Metcalfe, N. B. (2016). Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. *Molecular Ecology*, 25, 5425–5438.

McLennan, D., Armstrong, J. D., Stewart, D. C., Mckelvey, S., Boner, W., Monaghan, P., & Metcalfe, N. B. (2017). Data from: Shorter juvenile telomere length is associated with higher survival to spawning in migratory Atlantic salmon. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.rk030

Metcalfe, N. B., & Monaghan, P. (2003). Growth versus lifespan: Perspectives from evolutionary ecology. *Experimental Gerontology*, 38, 935–940.

Metcalfe, N. B., & Thorpe, J. E. (1990). Determinants of geographical variation in the age of seaward-migrating salmon, *Salmo salar*. *The Journal of Animal Ecology*, 59, 135–145.

Mitchell, G. W., Guglielmo, C. G., Wheelwright, N. T., Freeman-Gallant, C. R., & Norris, D. R. (2011). Early life events carry over to influence pre-migratory condition in a free-living songbird. *PLoS ONE*, 6, e28838.

Monaghan, P. (2010). Telomeres and life histories. the long and the short of it. *Annals of the New York Academy of Sciences*, 1206, 130–142.

Monaghan, P. (2014). Organismal stress, telomeres and life histories. *Journal of Experimental Biology*, 217, 57–66.

Näslund, J., Pauliny, A., Blomqvist, D., & Johnsson, J. I. (2015). Telomere dynamics in wild brown trout: Effects of compensatory growth and early growth investment. *Oecologia*, 177, 1221–1230.

Økland, F., Jonsson, B., Jensen, A. J., & Hansen, L. P. (1993). Is there a threshold size regulating seaward migration of brown trout and Atlantic salmon. *Journal of Fish Biology*, 42, 541–550.

Olsson, M., Pauliny, A., Wapstra, E., & Blomqvist, D. (2010). Proximate determinants of telomere length in sand lizards (*Lacerta agilis*). *Biology Letters*, 6, 651–653.

Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., Miller, E., & Blomqvist, D. (2011). Sexual differences in telomere selection in the wild. *Molecular Ecology*, 20, 2085–2099.

Owen, M., & Black, J. M. (1989). Factors affecting the survival of barnacle geese on migration from the breeding grounds. *Journal of Animal Ecology*, 58, 603–617.

Pelis, R. M., & McCormick, S. D. (2001). Effects of growth hormone and cortisol on Na^+-K^+-$2Cl^-$ cotransporter localization and abundance in the gills of Atlantic salmon. *General and Comparative Endocrinology*, 124, 134–143.

Piersma, T. (1998). Phenotypic flexibility during migration: Optimization of organ size contingent on the risks and rewards of fueling and flight? *Journal of Avian Biology*, 29, 511–520.

Riley, W. D., Ibbotson, A. T., Beaumont, W. R. C., Rycroft, P., & Cook, A. C. (2010). A portable, cost effective, pass-through system to detect downstream migrating salmonids marked with 12 mm passive integrated transponder tags. *Fisheries Research*, 101, 203–206.

Schmutz, J. A. (1993). Survival and pre-fledging body mass in juvenile emperor geese. *The Condor*, 95, 222–225.

Shearer, W. M. (1992). *Atlantic salmon scale reading guidelines*. Copenhagen, Denmark: International Council for the Exploration of the Sea Copenhagen.

Simide, R., Angelier, F., Gaillard, S., & Stier, A. (2016). Age and heat stress as determinants of telomere length in a long-lived fish, the Siberian sturgeon. *Physiological and Biochemical Zoology*, *89*, 441–447.

Stich, D. S., Zydlewski, G. B., Kocik, J. F., & Zydlewski, J. D. (2015). Linking behavior, physiology, and survival of Atlantic salmon smolts during estuary migration. *Marine and Coastal Fisheries*, *7*, 68–86.

Stich, D. S., Zydlewski, G. B., & Zydlewski, J. D. (2016). Physiological preparedness and performance of Atlantic salmon *Salmo salar* smolts in relation to behavioural salinity preferences and thresholds. *Journal of Fish Biology*, *88*, 595–617.

Thedinga, J. F., Murphy, M. L., Johnson, S. W., Lorenz, J. M., & Koski, K. V. (1994). Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. *North American Journal of Fisheries Management*, *14*, 837–851.

Thorstad, E. B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A. H., & Finstad, B. (2012). A critical life stage of the Atlantic salmon *Salmo salar*: Behaviour and survival during the smolt and initial post-smolt migration. *Journal of Fish Biology*, *81*, 500–542.

Tinbergen, J. M., & Boerlijst, M. C. (1990). Nestling weight and survival in individual great tits (*Parus major*). *Journal of Animal Ecology*, *59*, 1113–1127.

Trueman, C. N., MacKenzie, K. M., & Palmer, M. R. (2012). Stable isotopes reveal linkages between ocean climate, plankton community dynamics, and survival of two populations of Atlantic salmon (*Salmo salar*). *ICES Journal of Marine Science*, *69*, 784–794.

Vera, E., Bernardes de Jesus, B., Foronda, M., Flores, J. M., & Blasco, M. A. (2012). The rate of increase of short telomeres predicts longevity in mammals. *Cell Reports*, *2*, 732–737.

Verhulst, S., Aviv, A., Benetos, A., Berenson, G. S., & Kark, J. D. (2013). Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for 'regression to the mean'. *European Journal of Epidemiology*, *28*, 859–866.

Watson, H., Bolton, M., & Monaghan, P. (2015). Variation in early-life telomere dynamics in a long-lived bird: Links to environmental conditions and survival. *Journal of Experimental Biology*, *218*, 668–674.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.