

Age and Heat Stress as Determinants of Telomere Length in a Long-Lived Fish, the Siberian Sturgeon

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ABSTRACT

Telomeres shorten at each cell division due to the end-replication problem but also in response to oxidative stress. Consequently, telomeres shorten with age in many endotherms, and this shortening is accelerated under stressful environmental conditions. Data in ectotherm vertebrates remain scarce so far, so our goal was to review existing data for fish and to test the influence of age and stress on telomere length in a very long-lived fish, the Siberian sturgeon (*Acipenser baerii*). Our review of the literature revealed age-related telomere shortening in approximately half of the published studies. In the Siberian sturgeon, we found a significant telomere shortening with age, both at the intraindividual level using red blood cells (−12.5% in 16 mo) and at the interindividual level using cross-sectional samples of fin over an age range of 8 yr. We also found that heat stress (30°C) significantly reduced telomere length by 15.0% after only 1 mo of exposure. Our results highlight that both age and stressful environmental conditions might be important determinants of telomere length in fish.

Keywords: fish, telomere, review, aging, stress, *Acipenser baerii*.

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Introduction

Telomeres are specialized noncoding repeated DNA sequences (TTAGGG_n) located at the end of eukaryotic chromosomes playing an important role in the protection of genome integrity (De Lange et al. 2006). Telomeres shorten at each cell division due to the end-replication problem, but they also shorten in response to oxidative stress (von Zglinicki 2002). Consequently, telomeres shorten with time, unless being elongated by a specific enzyme called telomerase (De Lange et al. 2006). In most nonproliferative somatic tissues of adult mammals and birds (with a few exceptions), telomerase activity is low or undetectable (Gomes et al. 2010), and, consequently, telomeres usually shorten with age (e.g., Haussmann et al. 2003). A critically short telomere length has been shown to induce cell death or replicative senescence, and as a result of this, it has been suggested that telomere shortening is an important cellular mechanism underpinning biological aging (Monaghan and Haussmann 2006). The causality of such a link has, however, recently been questioned (Simons 2015). Yet, telomere length or telomere shortening rates have been found to predict survival or longevity in humans (e.g., Bakaysa et al. 2007) and some bird species (see Stier et al. 2015 for a recent review). Additionally, telomere erosion rate has been associated with species longevity in mammals and birds, with long-lived species exhibiting less telomere loss with age than short-lived ones (Haussmann et al. 2003; Dantzer and Fletcher 2015).

We have little information about telomere dynamics in relation to age and environmental conditions in vertebrate ectotherms, contrary to endotherms. Interestingly, telomerase activity is maintained even at adulthood in numerous tissues of ectotherm species (Gomes et al. 2010). In reptiles, we have some cross-sectional data showing either telomere maintenance (Hatase et al. 2008; Plot et al. 2012) or shortening (Bronikowski 2008; Xu et al. 2009) with age. At present, there is no robust information about age-related variation in the length of amphibian telomeres. In fish, a few studies have looked at the influence of age on telomere length, but no clear consensus has emerged so far. Thus, our first objective was to provide an exhaustive review of the literature in fish to better understand the link between telomere dynamics, age, and species longevity. Noting some specific gaps in the literature, we were interested in obtaining data about age-related variation of telomere length in a long-lived fish species (objective 2). Indeed, we have information about only short- to medium-lived fish species to date, and patterns of telomere shortening with age might differ between short- and long-lived species, as already documented in endotherms (Haussmann et al. 2003). Our biological model, the Siberian sturgeon (*Acipenser baerii*), is a very long-lived fish with an average life span of 60 yr (Pikitch et al. 2005). Using farmed fish,

Table 1: Review of the available studies investigating the relationships between telomere length and age in fish species

Common name	Species	Life span estimate	Tissue type	Monitoring	Method	Age-related variation	Reference
Turquoise killifish	<i>Nothobranchius furzeri</i>	1.1 ^a	Muscle, skin	C	qPCR, qFISH, and TRF	↔ (short-lived strain); ↘ (long-lived strain)	Hartmann et al. 2009
Atlantic silverside	<i>Menidia menidia</i>	2 ^a	Brain, muscle	C	qPCR	↔	Gao and Munch 2015
Eastern mosquitofish	<i>Gambusia holbrooki</i>	(3) ^a	Muscle	C	qPCR	↘	Rollings et al. 2014
Japanese medaka	<i>Oryzias latipes</i>	5 ^a	Brain, gonad, heart, intestine, kidney, liver, muscle, whole body	C	TRF	↘ (except heart and brain)	Hatakeyama et al. 2008
Japanese medaka	<i>O. latipes</i>	5 ^a	Gill, liver	C	TRF	↘	Gopalakrishnan et al. 2013
Coho salmon	<i>Oncorhynchus kisutch</i>	5 ^a	Fin	L	qPCR	↔ (wild type); ↘ (GH-transgenic)	Pauliny et al. 2015
Zebrafish	<i>Danio rerio</i>	5.5 ^a	Brain, gill, heart, intestine, liver	C	TRF	↔	Lund et al. 2009
Zebrafish	<i>D. rerio</i>	5.5 ^a	Whole body	C	TRF and qFISH	↘	Anchelin et al. 2011
Zebrafish	<i>D. rerio</i>	5.5 ^a	Fin	C	TRF	↘	Henriques et al. 2013
Brown trout	<i>Salmo trutta</i>	6 ^a	Fin	L	qPCR	↔	Näslund et al. 2015
Port Jackson shark	<i>Heterodontus portusjacksoni</i>	(12) ^a	Gonad, muscle, red blood cells	C	qPCR and TRF	↔	Izzo 2010
European sea bass	<i>Dicentrarchus labrax</i>	15 ^a	Red blood cells	C	TRF	↔	Horn et al. 2008
Blackhead seabream	<i>Acanthopagrus schlegelii</i>	15 ^b	Muscle, red blood cells	C	TRF	Muscle: ↘, RBCs: ↗ (captive); ↔ (wild)	Tsui 2005
Mangrove snapper	<i>Lutjanus argentimaculatus</i>	18 ^a	Brain, muscle, red blood cells	C	TRF	↘	Tsui 2005
Siberian sturgeon	<i>Acipenser baerii</i>	60 ^c	Fin, red blood cells	C, L	qPCR	↘	This study

Note. We reported life span estimate for the study species and the tissue type(s) being used to measure telomere length in each study. Study monitoring is divided into cross-sectional (C) and longitudinal (L) approaches; method of telomere length measurement is indicated as quantitative polymerase chain reaction (qPCR), terminal restriction fragment (TRF), or quantitative fluorescence in situ hybridization (qFISH); arrows describe telomere shortening (↘), maintenance (↔), or lengthening (↗) with advancing age.

^aAnage online database (<http://genomics.senescence.info/species/>).

^bY. Iwatsuki, personal communication, 2009.

^cPikitch et al. (2005). Life span estimates given in parentheses are those available for the closest related species.

^dWhen captive and wild fish are analyzed together, following data extraction from figure 4.18 from Tsui (2005; $R^2 = 0.20$, $P = 0.014$).

β -actin signal (S). Final calculation of telomere length (T/S ratio) was done using the telomere and control gene real efficiencies of each run (Pfaffl 2001). Both the intraplate variation based on the T/S ratio of duplicates (coefficient of variation [CV] = 5.5% \pm 0.5%) and the interplate variation based on the T/S ratio of the calibrator (CV = 5.9%) were low.

Statistics

We tested the effect of age on RBC telomere length at the intraindividual level (batch 1) using a generalized estimating equation (GEE). We used fish ID as the individual factor and age as the repeated effect. We tested the effect of age on fin telomere length at the interindividual level (batch 2) using a general linear model (GLM) with age as the fixed factor. We used Bonferroni corrected post hoc tests to investigate significant differences between the four age classes. Finally, we tested the effect of heat stress on RBC telomere length (batch 3) using a GLM with treatment as the fixed factor. Telomere data were log transformed to achieve normality assumptions, but we decided to present raw data in the figures to avoid confusion due to negative log values. Means are always presented \pm standard error, statistical tests are always two tailed, and P values ≤ 0.05 were considered significant. Statistical tests were performed using SPSS 20.0.

Results

Literature Review

We summarized the 14 available studies to date in table 1. Nine of these studies found at least partial evidence for telomere shortening with age, nine of these studies found at least partial evidence for telomere maintenance with age, and only one study showed significant telomere lengthening (table 1). Some studies found contrasting results depending on the type of tissue analyzed or the strain of fish used (see table 1 for details).

Age Effect in Siberian Sturgeon

Using the longitudinal sampling of RBCs (batch 1), we found an intraindividual shortening of telomeres with age (GEE: $\chi^2 = 31.1$, $P < 0.001$; fig. 1A). Telomeres shortened, on average, 12.5% during the 16 mo of the study, but we found important interindividual differences in the rate of telomere change (from -29.6% to +6.9%; fig. 1A).

Using the cross-sectional sampling of fins (batch 2), we found a significant effect of age on telomere length (GLM: $F = 40.2$, $P < 0.001$; fig. 1B). We found that telomeres of 3-mo-old fish were significantly longer than older age groups (all $P < 0.001$) and that fish that were older than 7 yr had significantly shorter telomeres than younger age groups (all $P < 0.001$; fig. 1B). However, fish from the intermediate age groups (i.e. 3–4 yr vs. 5–6 yr) did not differ significantly in terms of telomere length ($P = 1.0$; fig. 1B). Yet, it is important to note the pronounced interindividual differences; for example, some individuals between 3 and 8 yr old had telomere length resembling the range normally found in juvenile fish (fig. 1B).

Heat Stress Effect in Siberian Sturgeon

We found a significant effect of heat stress on telomere length (GLM: $F = 5.86$, $P = 0.021$), with fish exposed to 30°C presenting telomeres, on average, 15.0% shorter than controls after only 1 mo of exposure (fig. 2).

Discussion

The literature review that we conducted suggests that telomere shortening with age occurs in fish species in approximately half of the studies published to date. Importantly, only three studies used a longitudinal sampling to date, which limits our ability to definitively form a conclusion about age-related telomere shortening, maintenance, or lengthening in most cases.

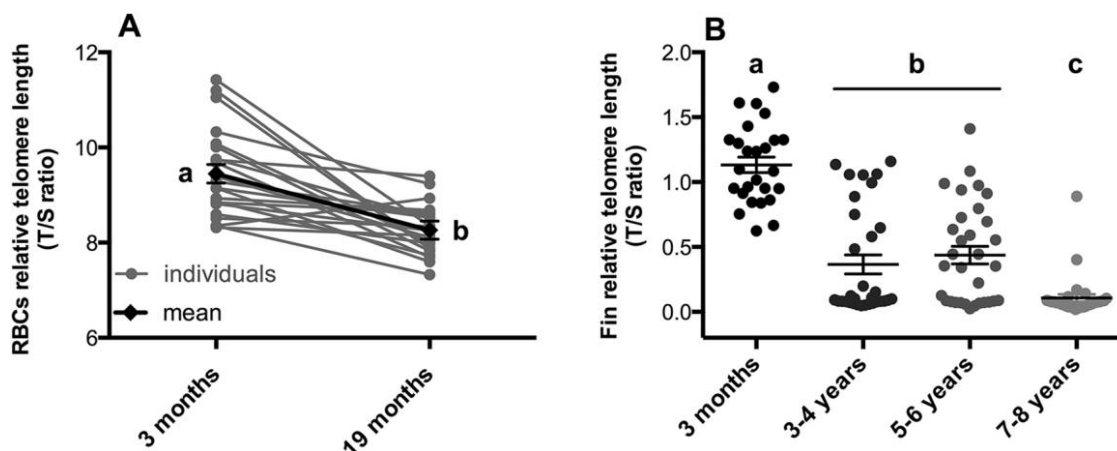


Figure 1. Relationships between relative telomere length and age at the intraindividual level using telomere length of red blood cells (RBCs; $N = 22$, $P < 0.001$; A) and at the interindividual level using telomere length measured in fin samples ($N = 121$, $P < 0.001$; B). Means are presented \pm SE, and lowercase letters indicate significant differences.

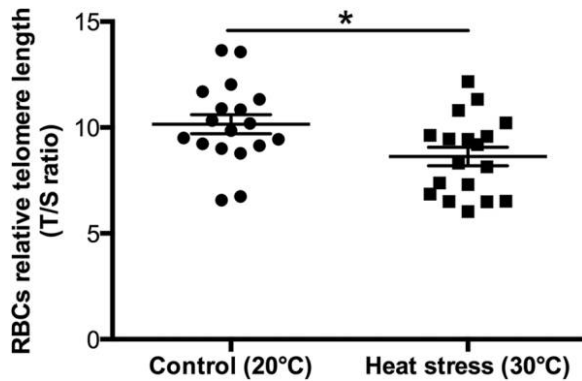


Figure 2. Effect of 1-mo exposure to heat stress (30°C) on red blood cell (RBC) telomere length of 6-mo-old Siberian sturgeons ($N = 36$, $P = 0.021$). Means are presented \pm SE, and asterisk indicates significant difference between experimental groups.

Interestingly, the longevity of the species under investigation does not seem to influence the likelihood of observing telomere shortening with age, which contrasts what is known in endotherms (Haussmann et al. 2003). As a remarkable example, the longest-lived species (i.e., Siberian sturgeon) in our review exhibits an age-related decline in telomere length, while the short-lived strain of the shortest-lived fish species does not (table 1). However, gathering precise information on telomere shortening rate (i.e., base pair loss per unit of time) between species will be useful in the future to ascertain such ideas. According to our review of the literature, it seems that the discrepancies between studies are more likely linked to species-specific patterns rather than methodological considerations (i.e., type of tissue, longitudinal studies vs. cross-sectional studies, method of telomere length measurement).

We found evidence of telomere shortening with age in a long-lived fish species, using both longitudinal sampling of RBCs and cross-sectional sampling of fin samples. Considering the exceptional longevity of this species, it might seem counterintuitive at first glance that we found evidence of telomere shortening with age, especially considering the limited time window of our study compared to the longevity of the species in the wild. Still, our results agree with those of many other studies in fish, as shown in table 1.

It is worth noting that we found marked individual differences in the rate of telomere shortening (fig. 1A) but also in telomere length per se among age-matched individuals (fig. 1B). Such heterogeneity might have multiple causes, such as individual differences in physiological stress levels (e.g., glucocorticoids, oxidative stress), in telomerase expression levels, or even in infectious status (see Asghar et al. 2015 for an example in birds). However we have no information so far about the biological relevance of such heterogeneity in this species, for instance, in predicting survival, as shown in human and birds (Bakaysa et al. 2007; Stier et al. 2015), or even fecundity, as shown recently in a short-lived fish species (Gao and Munch 2015). Investigating potential relationships between telomere

length and fecundity will be of particular interest in the case of farmed sturgeon, considering the economic value of caviar.

Environmental stress is likely to be an important determinant of telomere shortening in fish, since we found significantly shorter telomeres in sturgeons exposed to chronic heat stress. Despite the relatively brief exposure to heat stress (i.e., 1 mo), telomeres were 15.0% shorter in fish exposed to this treatment than in control fish. Rollings et al. (2014) found no significant differences in telomere length between mosquitofish being raised at 20° or 30°C. However, while mosquitofish were well within their normal thermal range (i.e., 0°–45°C; Pyke 2008), our Siberian sturgeons were slightly above their normal thermal range (i.e., 1°–27°C; Williot 2002), possibly explaining this discrepancy. This suggests that sturgeons in our study were exposed to a substantial heat stress (as confirmed by an increased expression of heat shock protein 90; Simide et al. 2016), while mosquitofish in Rollings et al. (2014) were exposed only to a warmer temperature. According to our results, telomere length has the potential to be used as an indicator of chronic stress in sturgeons by fish farmers. However, gathering information about the impact of other stressors such as crowding or infection will be of tremendous importance in validating telomere length as an integrative marker of stress and welfare in fish. Finally, our results highlight the need to work with fish of known age and history for future studies looking at the impact of environmental conditions on telomere length.

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