Cardiac SERCA activity in sockeye salmon populations: an adaptive response to migration conditions

Katja Anttila, Anthony P. Farrell, David A. Patterson, Scott G. Hinch, Erika J. Eliason

Katja Anttila: Department of Biology, University of Turku, 20014 Turku, Finland, katja.anttila@utu.fi

Anthony P. Farrell: Department of Zoology and Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4, tony.farrell@ubc.ca

David A. Patterson: Fisheries and Oceans Canada, Science Branch, Cooperative Resource Management Institute, School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6, David.Patterson@dfo-mpo.gc.ca

Scott G. Hinch: Department of Forest and Conservation Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC, Canada, V6T 1Z4, scott.hinch@ubc.ca

Erika J. Eliason: Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, Santa Barbara, CA, USA, 93106-9620, erika.eliason@lifesci.ucsb.edu

Corresponding author: Katja Anttila, Department of Biology, University of Turku, 20014 Turku, Finland, tel: +358 29 450 4211, email: katja.anttila@utu.fi

Abstract

We show that cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) activity differs considerably among sockeye salmon populations. Variability in SERCA activity was significantly correlated with elevation gain and temperature during migration, as well as maximum cardiac stroke volume. Furthermore, because SERCA activity was not lowered during the spawning migration, this aspect of the cardiac contraction machinery is apparently spared during the senescence of these semelparous salmon, likely because it is essential for these fish to complete spawning. Only when spawning had been completed was there a significant reduction in SERCA activity, which was detectable in males at a 25°C and in females at a 15°C assay temperature. Hence, we propose that migration conditions act as a strong selective force that has resulted in local adaptation of myocardial SERCA activity among sockeye salmon populations.

Introduction

Organisms facing environmental change may respond with emigration, acclimation or adaptation. When acclimation processes are insufficient to meet the demands of the new conditions, a species' vulnerability to environmental change will fundamentally be determined by their adaptive capacity (e.g., Habary et al. 2017). In long-lived or rare species where adaptation may be challenging to directly measure, adaptive capacity may be indirectly inferred by comparing phenotypic traits across reproductively isolated populations, i.e., local adaptation and intraspecific variation (e.g., Eliason et al. 2011; Des Roches et al. 2018). The underlying mechanisms that support such phenotypic differences can also provide clues to the adaptive capacity of the species.

We assessed cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) activity in four populations of wild adult sockeye salmon (*Oncorhynchus nerka*) in relation to their upriver spawning migration. Migration conditions (e.g., distance, elevation, temperature) vary considerably for the >100 genetically and geographically isolated populations of sockeye salmon in British Columbia, Canada, depending on the location of the spawning grounds and the timing of river entry (Crossin et al. 2004). Furthermore, given that sockeye salmon are semelparous (one opportunity to spawn), the upriver migration conditions prior to spawning are predicted to exert a strong selective force. Indeed, physiology, morphology and behaviour were previously found to vary across populations and were correlated with migration difficulty (Crossin et al. 2004; Eliason et al. 2011). Since maximum cardiac performance has been determined for several populations (Eliason et al., 2011; Table S1), sockeye salmon are an excellent model organism to examine the cellular mechanisms associated with maximum cardiac capacity and assess whether

60 or not these mechanisms have been subjected to local adaptation to upstream migration
61 conditions. In doing so, this study sheds light on the adaptive capacity of cardiac function in
62 sockeye salmon.
63
64 SERCA plays a significant mechanistic role in cardiac contraction. It enables the relaxation
65 phase of cardiac contraction by re-sequestering Ca^{2+} back to sarcoplasmic reticulum after
66 contraction (Bers 2002). Given its general importance for cardiac function, SERCA activity was
67 chosen as a candidate cellular trait for local adaptation in sockeye salmon populations facing
68 significant differences during upstream migrations (distance, elevation, temperature). We also
69 related the SERCA activity to known maximum cardiac performance metrics (i.e., maximum
70 heart rate [f_H] and stroke volume [V_s]; population specific data from Eliason et al. 2011, 2013,
71 different fish than used in this manuscript; Table S1) of these populations. Specifically, we tested
72 the prediction that a challenging upstream migration (long distance, high elevation gain and high
73 temperature) is associated with greater cardiac SERCA activity. In addition, SERCA activity was
74 compared between males and females because female salmon have smaller ventricles compared
75 to males and female salmon suffer higher mortality rates than males at high temperature (e.g.
76 Martins et al. 2012). We hypothesized that males would have higher SERCA activity than
77 females. Lastly, SERCA activity was measured in fish early in the migration as well as on their
78 spawning grounds to test the hypothesis that SERCA activity decreases before spawning with the
79 onset of senescence.
80

81 Materials and methods

82

83 *Animals*

The experiments were approved by the Canadian Council on Animal Care (A11-0215 and A12-0250). Wild, upstream-migrating, adult sockeye salmon from four populations (Chilko, Early Stuart, Adams, Harrison) were collected with beach seine or gill nets early in their spawning migration (Fig. S1). The time and temperatures of capture were: Chilko (July 2015, 20.5°C), Early Stuart (July 2013, 16.6°C), Adams (September 2014, 15.1°C), Harrison (October 2013, 13.3°C). To evaluate for a change in SERCA activity during migration, the Adams population was additionally sampled in the ocean near the mouth of Fraser River (September 2014, 12.4°C) and at the Adams River spawning area (October 2014, 13.0°C) as either freshly-arrived or spawned-out individuals (Fig. S1). Water temperature in lower part of Fraser River during the population-specific migration times relative to the yearly Fraser River maximum temperature is shown in Fig. S2. Each fish was euthanized at capture before the ventricle was rapidly removed and immediately freeze clamped in liquid nitrogen. Samples were stored at -80°C prior to analysis. Each fish was also individually weighed and measured (Table S2). An adipose fin clip was used for population identification via DNA analysis (Beacham et al. 2005) to confirm that the analysis was performed only on fish from the targeted populations.

SERCA activity

SERCA activity was measured according to Aho and Vornanen (1998) with minor modifications. Briefly, ventricle samples were homogenized in 10 volumes homogenization buffer (in mM: sucrose, 200; L-histidine, 40; EDTA, 1 and NaN_3, 10, pH 7.8) with 3 volumes (by mass) of zirconium oxide beads (0.5 mm, Next Advance, Averill Park, NY, USA) by shaking twice for 2.5 min at 1700 rpm (2010 Geno Grinder, SPEX, Metuchen, NJ, USA or Tissue Lyser,

Qiagen, Austin, TX, USA). The activity of SERCA was determined as the difference in ATP hydrolysis in the presence and absence of SERCA-inhibitor thapsigargin (20 µM), i.e., nmol PO_4 liberated mg tissue^{-1} min^{-1}. The enzyme reaction was initiated by adding 180 µl of substrate solution (in mM: Hepes, 20; KCl, 200; $MgCl_2$, 15; NaN_3, 10; EGTA, 1; Na_2ATP, 5; $CaCl_2$, 1; and Triton X, 0.005%; at pH 7.5) to 20 µl of homogenate solution (with or without thapsigargin) and terminated after 10 min of incubation with 200 µl ice-cold 0.8 N perchloric acid (Walter and Seebacher 2009). After terminating the reaction, the samples were centrifuged (1000 g, 10 min at 4°C). The liberated inorganic phosphate was determined *via* the ammonium molybdate assay (Bonting et al. 1961). When comparing populations, assays of the thermal sensitivity of SERCA activity were performed at five temperatures (5, 10, 15, 20 and 25°C), but only at 5, 15 and 25°C when comparing migration state for the Adam's population. All the reagents were purchased from Sigma-Aldrich, Oakville, ON, Canada.

Statistical analyses

Statistical analyses were performed using SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA) and with SAS statistical software version 9.4 (SAS Institute Inc. Cary, NC, USA) using $\alpha \leq 0.05$ for statistical significance. Data normality and homogeneity were tested with Kolmogorov-Smirnov and Levene tests, respectively. The SERCA activity data was log-transformed in order to meet the assumptions. In order to reveal population differences in SERCA activity in the beginning of migration a 2-way ANOVA was used with population and assay temperature as factors followed with a post-hoc *Holm-Sidak* test. The influence of population specific migration difficulty (distance, elevation and capture temperature, Tables S1, S2) and cardiac capacity (mean population values for maximum f_H and V_s, Table S1) on SERCA

activity in 15 and 20°C were analysed with general linear models (GLIMMIX procedure in SAS) with lognormal distribution and identity link function. Population was used as random factor. Degrees of freedom were calculated with Kenward–Roger method and post-hoc pairwise comparisons were performed using *Tukey's* test. The influence of upstream migration was analysed merely from fish from Adam's population. 3-way ANOVA compared SERCA activity between sexes, upstream migration stage and assay temperatures. A post-hoc *Holm-Sidak* test was performed in order to detect which migration stages differed from each other. Values are presented as mean ± s.e.m. if not stated otherwise.

Results

Population differences in SERCA activity

SERCA activity, when compared at five assay temperatures and across four different populations for female fish caught early in their upstream spawning migration, revealed significant differences among populations ($F_{3,178}$=72.6, p<0.001) and among assay temperatures ($F_{4,178}$=32.7, p<0.001), with significant interactions among populations and assay temperatures ($F_{3,4,178}$=3.0, p<0.001) (Fig. 1a).

SERCA activity had a strong positive thermal dependence for all four populations (Fig. 1a). Furthermore, the precise thermal dependence of SERCA activity was markedly population-specific. While SERCA activity measured at 5°C was not significantly different among the four populations, differences progressively emerged with higher assay temperatures. For example, SERCA activity was significantly higher at 10°C compared to 5°C for the Chilko population

(p<0.001), but for no other population. However, at 20°C, SERCA activity in the Chilko population was 2.1-times greater than the next highest (Early Stuart population, p<0.001) and an impressive 4.6-times greater than that for the population with the lowest SERCA activity (Harrison; p<0.001) (Fig. 1a).

Both the migration difficulty and population specific cardiac capacities were associated with SERCA activity (Table S3, Fig. 1b-d). Migration elevation was significantly related to SERCA activity at 15°C ($F_{1,32}$=17.99, p=0.0002) and at 20°C ($F_{1,32}$=33.03, p<0.0001, Fig. 1b). The capture temperature was also positively related to SERCA activity at both temperatures (15°C: $F_{1,33}$=30.17, p<0.0001; 20°C: $F_{1,33}$=43.97, p<0.0001) (Fig. 1c). However, migration distance did not have a significant relationship with SERCA activity (Table S3). Maximum cardiac stroke volume was related to SERCA activity both at 15 and 20°C assay temperature ($F_{1,32}$=3.94, p=0.004; $F_{1,32}$=8.16, p=0.0075, respectively), while the maximum heart rate was not related to SERCA activity in either assay temperature (Fig. 1d; Table S3).

Changes in SERCA activity during migration

The influence of migration stage on SERCA activity was studied in both females and males from the Adams population that had been captured just before they entered the Fraser River, as well as at two difference stages of senescence after arrival on the spawning area. Migration stage ($F_{2,142}$=6.7, p=0.002) and assay temperature ($F_{2,142}$=61.8, p<0.001) had significant effects on SERCA activity, with significant interactions ($F_{2,2,142}$=2.6, p=0.04). There were no large differences in SERCA activity between males and females ($F_{1,142}$=3.2, p=0.078).

Migration stage affected SERCA activity depending on assay temperature and sex. At 25°C, spawned males had reduced SERCA activity when compared with early in the migration (p=0.026), unlike female fish (p=0.3). Female fish that were newly arrived on the spawning grounds had the highest SERCA activity at 15°C (p<0.035) (Fig. 2). However, there were no significant differences in SERCA activity in female fish at 5°C or male fish at 15°C.

Discussion

The present study provides clear support of the hypothesis that intraspecific variability of an important cellular trait for cardiac contraction, namely cardiac SERCA activity, is related to upstream migration difficulty among sockeye salmon populations. Specifically, the highest cardiac SERCA activity and highest maximum cardiac functional capacities were common to the population (Chilko) that was about to embark on a river migration with the highest elevation gain to reach its spawning area (Table S1; Eliason et al. 2011). This population also encounters the highest river temperatures during migration. Consequently, we provide the first intraspecific study that links environmental differences with functional cardiac differences at the cellular level (i.e., SERCA activity). The implication of this discovery is that SERCA activity could be a marker for (local) adaptation to environmental conditions across and within a broader range of fish species than studied here. This idea aligns with previous work showing that SERCA activity varies across a marine species within the same genus and may be associated with their environmental experiences (Castilho et al. 2007). Equally important is that we link enhanced cardiac SERCA activity with elevated cardiac stroke volume among sockeye salmon populations for the first time.

Contrary to our hypothesis, mass-specific SERCA activity displayed only minor differences between sexes. Thus, differences in SERCA activity are unlikely to contribute to the higher mortality observed in female salmon at high temperature (e.g. Martins et al. 2012). Remarkably, SERCA activity did not decrease until an advanced state of senescence during spawning and did not decline with the known decline in physiological condition during river migration (Hruska et al. 2010). Actually in female fish measured in 15°C assay temperature the SERCA activity even increased during migration. We interpret this result as a sparing of the cellular cardiac contraction machinery during migration presumably because cardiac function is essential for completing the once-in-the-life-time spawning migration and spawning behaviours.

From an ecological perspective, the conditions encountered during the adult upriver migration likely act as a strong selective force for enhanced whole animal function and cardiac capacity, which have to be supported at the cellular level. Understandably, a salmon facing a river reach with high river velocities will require an elevated maximum swimming capacity (Hinch and Bratty 2000) that is supported by an elevated cardiac performance (Eliason et al. 2011). Elevation gain is a primary determinant of the water velocity against which the salmon swim. This study found that SERCA activity correlated with the river temperature when the fish were sampled as well as with elevation gain during migration, i.e., enabling maximum cardiac performance at high temperatures in fast flowing river reaches. Importantly, a training effect associated with performing either a difficult or an easy river migration can be eliminate as a potential explanation for these intraspecific differences for two reasons: Fish were sampled at the start of their river migration and SERCA activity was largely unchanged during the river migration to the spawning area. Nevertheless, we cannot exclude the possibility that behavioural

differences among populations during their ocean migration prior to the spawning migration could have influenced SERCA activity.

The thermal sensitivity of SERCA activity also differed appreciably among populations, which is an important novel discovery. SERCA activity significantly increased between 5°C and 10°C only for the Chilko population. This result may be related to the need for this population to sustain an elevated cardiac performance during the challenging, cold river sections (7°C during September prior to spawning) (Patterson et al. 2007). All the populations studied encounter temperatures ranging between 12 and 20°C during their migration in the mainstream Fraser River (Patterson et al. 2007; Fig. S2) and population differences in SERCA activity were clearly evident at these assay temperatures. However, intraspecific differences were most pronounced at the highest assay temperatures, which is perhaps not surprising given that Chilko and Early Stuart can routinely encounter temperatures near 20°C whereas Adams and Harrison encounter cooler temperatures (Fig. S2). Although the highest assay temperature (25°C) exceeds current peak temperatures (>22°C) in the Fraser River (Fig. S2b), sockeye salmon populations migrating in the Snake River, Idaho have encountered 24°C (Keefer et al. 2008) and peak summer Fraser River temperature has increased by ~2°C over the last 60 years (Patterson et al. 2007; Fig. S2). It remains to be seen whether these populations can adjust to the increasing temperatures in the future via changes in behaviour (e.g. Hague et al. 2011), or adaptations to physiological tolerance (Eliason et al. 2011).

Population variability in SERCA activity was found to positively correlate with maximum stroke volume, but not maximum heart rate. The intuitive explanation of this result is that SERCA is

more important for stroke work (stroke volume × mean arterial pressure) than heart rate because the force of cardiac muscle contraction depends partly upon how much Ca^{2+} is cycled between contractions (e.g. Westerblad and Allen 1996). Ca^{2+} cycling and SERCA activity are intimately related, and they may be enhanced by β-adrenergic stimulation through phosphorylation of phospholamban, which activates SERCA (MacLennan and Kranias 2003). Such modulation could prove to be an important mechanism to enhance cardiac function and aerobic scope in Chilko sockeye salmon because they have an especially high density of cardiac β-adrenoceptors (Eliason et al. 2011). All the same, there are several other calcium handling proteins involved in cardiac contraction (as well as their regulatory proteins) and further study could reveal similar associations between these proteins, migration difficulty and cardiac contraction capacity. It also needs to be stated that the cardio-physiological measurements were done from different fish than the SERCA activity measurements i.e. connection was made merely at population level. Since the river temperature varies between years (Fig. S2) and as we showed the environmental temperature is connected to SERCA activity this could have influence on our results. In future studies analyses of both SERCA activity and cardiac capacities needs to be made at individual level when estimating the connection between SERCA activity and cardiac capacities of the fish.

In conclusion, our study demonstrated that cardiac cellular function (SERCA activity) and its thermal sensitivity differ substantially across sockeye salmon populations and these differences can be related to differences in their ecology (migration difficulty and thermal environment) and physiology at population level (maximum stroke volume). We suggest that the migration difficulty has acted as strong selection force and has induced local adaptation that is reflected in intraspecific cellular and functional cardiac performance seen here. We, therefore, propose that enhanced SERCA activity is an important component of the cellular mechanisms conferring

increased cardiac performance and may be a common target for adaptation across taxa. Understanding the mechanistic basis of these intraspecific differences and their association with migration difficulty will be useful in the management of wild sockeye salmon populations.

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References

Aho, E., and Vornanen, M. 1998. Ca^{2+}-ATPase activity and Ca^{2+} uptake by sarcoplasmic reticulum in fish heart: effects of thermal acclimation. J. Exp. Biol. **201**: 525–532.

Beacham, T.D., Candy, J.R., McIntosh, B., MacConnachie, C., Tabata, A., Kaukinen, K., Deng, L., Miller, K.M., Withler, R.E., and Varnavskaya, N. 2005. Estimation of stock composition and individual identification of sockeye salmon on a Pacific Rim basis using microsatellite and major histocompatibility complex variation. Transact. Am. Fish. Soc. **134**: 1124–1146.

Bers, D.M. 2002. Cardiac excitation–contraction coupling. Nature **415**: 198-205.

Bonting, S.L., Hawkins, N.M., and Simon, K.A. 1961. Studies on sodium-potassium-activated adenosine triphosphatase 1. Quantitative distribution in several tissues of cat. Arch. Biochem. Biophys. **95**: 416-423.

Castilho, P.C., Landeira-Fernandez, A.M., Morrissette, J., and Block, B.A. 2007. Elevated Ca^{2+} ATPase (SERCA2) activity in tuna hearts: comparative aspects of temperature dependence. Comp. Biochem. Physiol. A **148**: 124-132.

Crossin, G.T., Hinch, S.G., Farrell, A.P., Higgs, D.A., Lotto, A.G., Oakes, J.D., and Healey, M.C. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. J. Fish Biol. **65**: 788-810.

312 Des Roches, S., Post, D.M., Turley, N.E., Bailey, J.K., Hendry, A.P., Kinnison, M.T.,
313 Schweitzer, J.A., and Palkovacs, E.P. 2018. The ecological importance of intraspecific variation.
314 Nat.Ecol. Evol. **2**: 57-64.
315
316 Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale,
317 M.K., Patterson, D.A., Hinch, S.G., and Farrell, A.P. 2011. Differences in thermal tolerance
318 among sockeye salmon populations. Science **332**: 109-112.
319
320 Habary, A., Johansen, J.L., Nay, T.J., Steffensen, J.F., and Rummer, J.L. 2017. Adapt, move or
321 die – how will tropical coral reef fishes cope with ocean warming? Glob. Change Biol. **23**: 566–
322 577.
323
324 Hague, M.J., Ferrari, M.R., Miller, J.R., Patterson, D.A., Russell, G.L., Farrell, A.P., and Hinch,
325 S.G. 2011. Modelling the future hydroclimatology of the lower Fraser River and its impacts on
326 the spawning migration survival of sockeye salmon. Glob. Change Biol. **17**: 87-98.
327
328 Hinch, S.G., and Bratty, J. 2000. Effects of swim speed and activity pattern on success of adult
329 sockeye salmon migration through an area of difficult passage. Transact. Am. Fish. Soc. **129**:
330 598-606.
331
332 Hruska, K.A., Hinch, S.G., Healey, M.C., Patterson, D.A., Larsson, S., and Farrell, A.P. 2010.
333 Influences of sex and activity level on physiological changes in individual adult sockeye salmon
334 during rapid senescence. Physiol. Biochem. Zool. **83**: 663–676.

Keefer, M.L., Peery, C.A., and Heinrich, M.J. 2008. Temperature-mediated *en route* migration mortality and travel rates of endangered Snake River sockeye salmon. Ecol. Freshwat. Fish **17**: 136-145.

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G., and Healey, M.C. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. J. Exp. Biol. **206**: 3239-3251.

MacLennan, D.H., and Kranias, E.G. 2003. Phospholamban: a crucial regulator of cardiac contractility. Nat. Rev. Mol. Cell Biol. **4**: 566–577.

Martins, E.G., Hinch, S.G., Patterson, D.A., Hague, M.J., Cooke, S.J., Miller, K.M., Robichaud, D., English, K.K., and Farrell, A.P. 2012. High river temperature reduces survival of sockeye salmon (*Oncorhynchus nerka*) approaching spawning grounds and exacerbates female mortality. Can. J. Fish. Aquat. Sci. **69**: 330-342.

Patterson, D.A., Macdonald, J.S., Skibo, K.M., Barnes, D., Guthrie, I., and Hills, J. 2007. Reconstructing the summer thermal history for the lower Fraser River, 1941 to 2006, and implications for adult sockeye salmon (*Oncorhynchus nerka*) spawning migration. Can. Techn. Rep. Fish. Aquat. Sci. 2724. 43 pp.

Walter, I., and Seebacher, F. 2009. Endothermy in birds: underlying molecular mechanisms. J. Exp. Biol. **212**: 2328-2336.

Westerblad, H., and Allen, D.G. 1996. Mechanisms underlying changes of tetanic $[Ca^{2+}]_i$ and force in skeletal muscle. Acta Physiol. Scand. **156**: 407–416.

Figure Captions

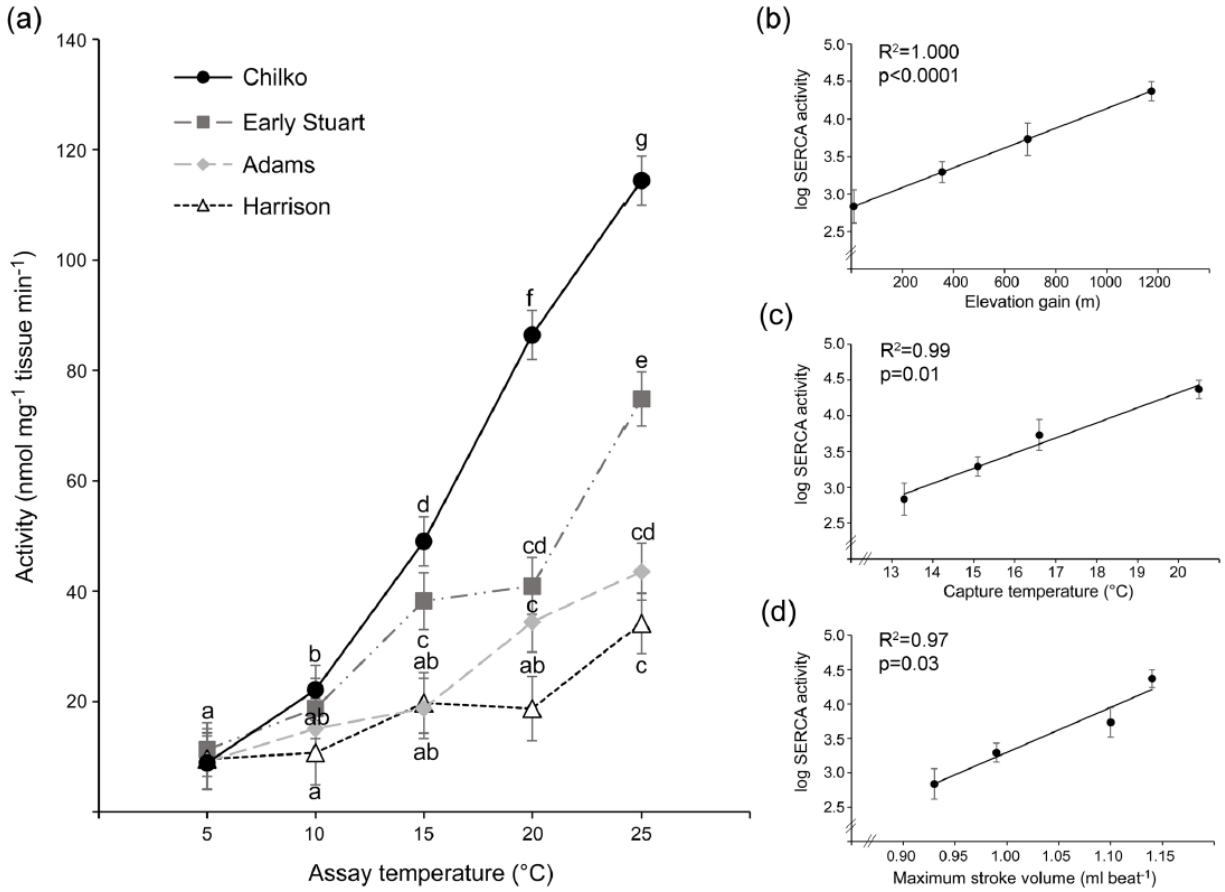
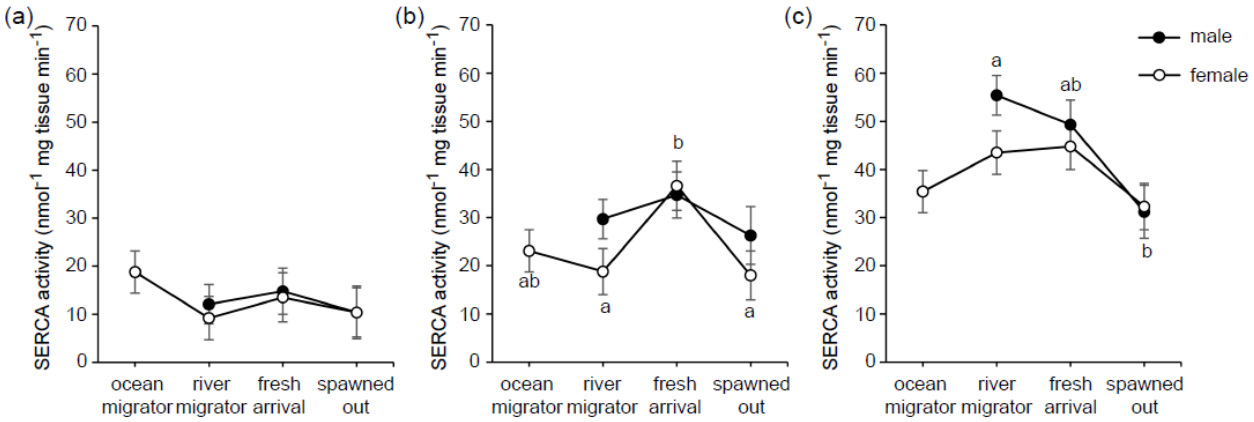


Fig. 1. (a) The activity of SERCA in ventricles of female fish from four different populations caught in freshwater during the early part of their upstream migration. The population differences of SERCA activities were measured at 5 different assay temperatures. Different letters indicate significant differences between groups (p<0.05). Relationship between SERCA activity in 20°C assay temperature and population specific migration elevation (b), capture temperature (c) and maximum stroke volume (d). The relationships were calculated with general linear models (GLIMMIX procedure in SAS). Values are means ± s.e.m., n=10 for Early Stuart, n=12 for Chilko, n=10 for Adams and n=8 for Harrison population.



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375 **Fig 2.** The activity of SERCA (nmol mg^{-1} tissue min^{-1}) using three different assay temperatures
376 in ventricles of female and male sockeye salmon from the Adams population sampled at different
377 times during the upstream migration. (a) 5°C, (b) 15°C and (c) 25°C assay temperature. The
378 assay temperature had significant effects on SERCA activity ($F=61.8$, $p<0.001$). Differences in
379 SERCA activity between males and females did not reach statistical significance ($F=3.2$,
380 $p=0.078$). Different letters indicate significant differences between migration status in female
381 fish in 15°C assay temperature and in male fish in 25°C assay temperature ($p<0.05$). No other
382 significant differences were found. Values are means ± s.e.m. n = 10 for ocean migrators, n = 9
383 and 11 for female and male river migrators, respectively, n = 8 for Fresh arrivals and n = 8 and 6
384 for spawned out females and males, respectively.