

Prolonged life span among endemic *Gasterosteus* populations

S.J. Gambling and T.E. Reimchen

Abstract: Throughout their circumboreal coastal distribution, the threespine stickleback (*Gasterosteus aculeatus* L., 1758) typically reproduce at 1 or 2 years of age (second or third summer) and die during the year of the reproductive cycle. Extending from a previous study on the Haida Gwaii archipelago, western Canada, that identified an exceptionally long life span (8 years) in a population of large-bodied threespine stickleback, we use pelvic spine annuli to examine age of the five largest adult stickleback of 12 additional populations comprising five populations with average-sized adults (45–60 mm standard length (SL)) and seven populations with large-bodied adult stickleback (>75 mm SL). Each of the small-bodied populations had a maximum age of 1 or 2 years typical for the taxon. Among the large-bodied populations, which also reached adult size in the 2nd year, adult stickleback in the populations ranged from 3 to 6 years, indicating extended longevity. Low productivity habitats and refuge against gape-limited piscivores, each of which theoretically predicts reduced rate of senescence, are associated with the greatest longevity among these populations. These data combined with the recent full genome sequence for stickleback provide opportunities for locating genetic markers for extended longevity.

Résumé : Dans l'ensemble de leur répartition côtière circumboréale, les épinoches à trois épines (*Gasterosteus aculeatus* L., 1758) se reproduisent typiquement à l'âge de 1 ou 2 ans (deuxième ou troisième été) et meurent durant l'année de leur cycle reproductif. En prolongement d'une étude antérieure sur l'archipel Haida Gwaii, dans l'Ouest canadien, qui a découvert des durées de vie exceptionnellement longues (8 ans) chez une population de grande taille d'épinoche à trois épines, nous utilisons les annulus de l'épine pelvienne pour déterminer l'âge des cinq épinoches adultes les plus grandes de 12 populations additionnelles, dont cinq populations avec des adultes de taille moyenne (longueur standard (SL) 45–60 mm) et sept populations à adultes de grande taille (SL >75 mm). Chacune des populations de petite taille a l'âge maximal de 1 ou 2 ans typique du taxon. Parmi les populations de grande taille, qui atteignent aussi leur taille adulte la seconde année, l'âge des épinoches adultes dans la population varie de 3 à 6 ans, ce qui représente une extension de la longévité. Il y a une association entre les habitats de faible productivité et les refuges contre les prédateurs à ouverture de bouche limitée, des facteurs qui tous deux peuvent théoriquement prédire un taux réduit de sénescence, et les longévités maximales dans ces populations. Ces données combinées au séquençage complet récent de l'épinoche fournissent des occasions pour identifier les marqueurs génétiques de la longévité prolongée.

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Introduction

Across a diversity of taxonomic levels, maximum life span and body size are generally correlated (Roff 1991; Mangel and Abrahams 2001), but occasional instances emerge with exceptional longevity relative to body mass. Major research attention has focused towards the mechanisms responsible for such extended life span and for identifying useful model taxa for such research. One of the mechanisms is the “fly now, die later” hypothesis, which can emerge in life histories where morphological or behavioural attributes that result in reduced predation risk can lead to selection for reduced rate of senescence (Austad and Fischer 1991; Ridgway et al.

2011). This can account for the exceptional life span of song birds relative to mammals of equivalent mass and of spiny rodents such as porcupine relative to other rodents of similar mass (ibid). It also appears to explain some of the intraspecific variation in maximum longevity among different populations of Virginia opossum (*Didelphis virginiana* Kerr, 1792) varying in the exposure to predation on adults (Austad 1993). Although the assumption of genetic factors influencing this variation is reasonable, both theoretical and empirical evidence indicate that intraspecific variation in life-history parameters such as life span appear to have low heritability and originate from environmental plasticity (Price and Schluter 1991).

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S.J. Gambling and T.E. Reimchen. Department of Biology, University of Victoria, P.O. Box 3020, Victoria, BC V8W 3N5, Canada.

Corresponding author: T.E. Reimchen (e-mail: reimchen@uvic.ca).

The threespine stickleback (*Gasterosteus aculeatus* L., 1758) is a short-lived species and well known for variability in body size. Throughout most of their circumboreal distribution, the stickleback reach reproductive age in their second or third summer, at approximately 12 or 24 months and at body sizes ranging from 30 to 80 mm standard length (SL) (reviews in Wootton 1976). Under optimal growth conditions, the larger adult body sizes can be reached within 12 months (Mori and Takamura 2004). Adults typically die shortly after one or two breeding cycles with maximum life spans of 2 or 3 years but occasionally reaching 4 years (Pennycuick 1971; Moodie 1984; Baker 1994). During studies of an endemic population of threespine stickleback at Drizzle Lake, on the Haida Gwaii archipelago, western Canada, Reimchen (1992) observed that reproductive age was reached in their third summer (age = 2 years) at a body size from 75 to 90 mm SL. However, rather than senescent mortality at this stage, these fish had a life span up to 8 years. Despite numerous life-history studies of stickleback over the last 7 decades throughout Europe, Asia, and North America (Wootton 1984 for review), such great longevity had not been previously reported. Part of this may be methodological, as otoliths or pelvic spine annuli are rarely used in aging stickleback and consequently other instances of extended longevity may be present but not reported. Conversely, there may be unique biophysical attributes in Drizzle Lake that facilitate the remarkable longevity in this population. Drizzle Lake is exceptionally tannin stained, has low productivity with cool summer temperatures, and has average area (100 ha) and depth (20 m) (Reimchen 1989). Perhaps of more importance, the extended life span is consistent with the predator-refuge hypothesis of Austad, as these stickleback have a very robust defense morphology and are largely outside the swallowing ability of the multiple gape-limited piscivores observed in the lake (Reimchen 1988, 1994).

In this paper, we test for extended longevity among other large-bodied endemic lake stickleback populations from the Haida Gwaii archipelago. Emerging from geographical surveys and collections of stickleback from 140 allopatric populations in the archipelago, mean adult body size of stickleback was 58 mm SL with a range from 30 to 90 mm SL (Moodie and Reimchen 1976a; Reimchen 1994; Spoljaric and Reimchen 2011). Based on adult size and length frequencies, most of these probably have a 2-year life span, which is representative across the broad geographical distribution of the taxon (Baker 1994). Using 75 mm SL as an arbitrary break between “typical” and “large-bodied” stickleback, as this exceeds the maximum size observed in the majority of lake populations in Europe, Asia, and North America (Bell 1984; Baker 1994), there are eight lakes on Haida Gwaii, each in a different watershed, that exhibit gigantism in stickleback. One of these, Mayer Lake, has the largest stickleback currently known in the circumboreal distribution of the species, reaching 100 mm SL and these sizes appear to be achieved within their third summer (Moodie 1984; Moodie and Reimchen 1976a). Mayer Lake is deeply stained and dystrophic, with major abundance of predatory trout that have influenced the evolution of major morphological defenses in the stickleback (Moodie 1972). These attributes are very similar to those observed at Drizzle Lake where the initial instance of extended longevity was reported (Reimchen

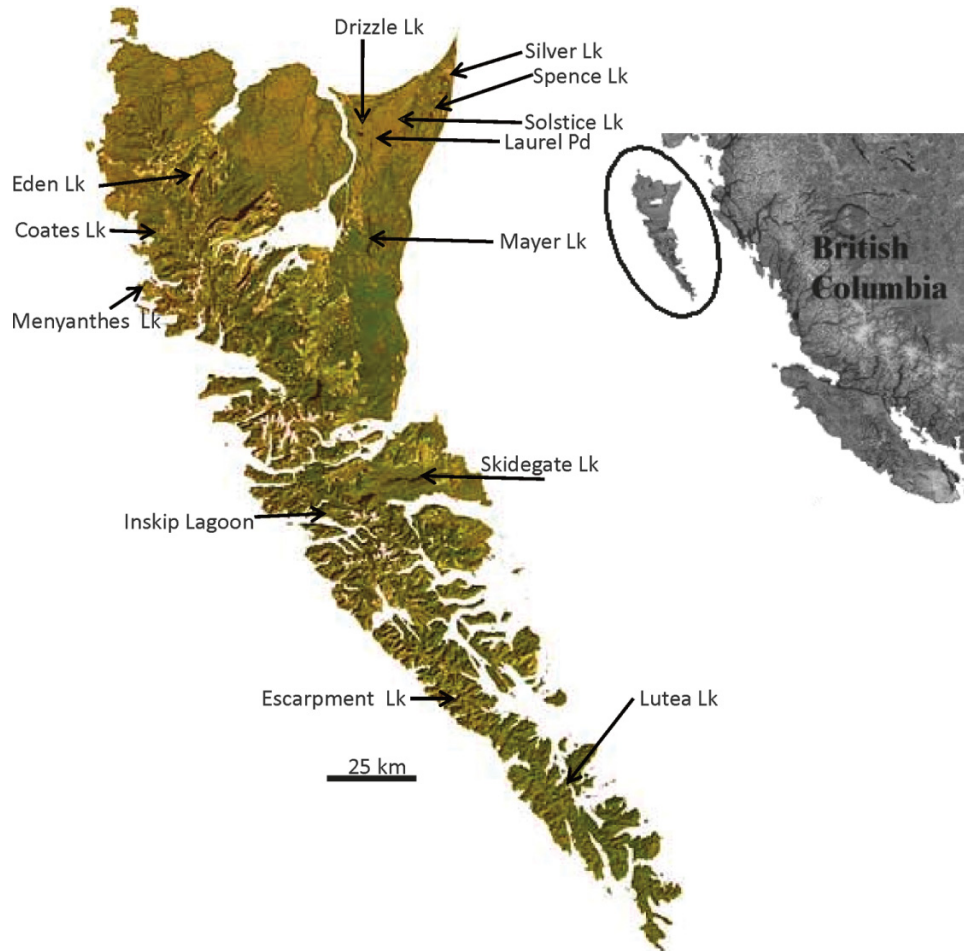
1992). The six remaining giant populations occur in a diversity of lake habitats including large, clear oligotrophic mountain lakes through to a shallow dystrophic pond. We also include representatives from five populations with “typical” adult sizes (45–60 mm SL) from geographically diverse habitats in the archipelago including a marine population. Vertebrates including diving birds that prey on stickleback occur in all of the localities on Haida Gwaii, but it is primarily the giant adult stickleback that are outside the swallowing ability of most the piscivores (Reimchen 1994). We use pelvic spine annuli for quantifying age of the largest bodied individuals from each locality. Because the large sizes in the giant populations are reached in their third summer (2 years of age), we refer to any fish ≥ 3 years of age as having extended longevity. Extending from the “fly now due later” hypothesis, we predict that extended longevity should occur in each of the giant populations and will be greatest in Mayer Lake, which has the largest adults.

Materials and methods

Stickleback were initially collected between May and August 1966 to 2003, fixed in a 10% formaldehyde solution followed by a 95% ethanol solution for long-term preservation. To increase the likelihood of detecting the oldest fish in the samples, we chose the five largest adults, most of which were females, from each sample for the 13 localities (Fig. 1). In no cases were the largest fish outliers but rather were part of a continuous distribution of adult sizes. Nine of the 13 populations were sampled on two separate years, while two of the giant populations (Drizzle, Mayer) were sampled on multiple years (>10) between 1967 and 2010, and in all cases, the mean adult sizes of the samples were conserved and varied $<5\%$. Maximum SL (mm) and approximate total sample size of adult fish from which the largest fish were extracted are respectively: Inskip Lagoon (SL = 49, total = 150), Solstice (SL = 56, total = 200), Silver (SL = 57, total = 200), Menyanthes (SL = 58, total = 200), Lutea (SL = 62, total = 400), Spence (SL = 77, total = 50), Coates (SL = 81, total = 200), Escarpment (SL = 87, total = 150), Eden (SL = 90, total = 30), Laurel (SL = 90, total = 180), Skidegate (SL = 94, total = 150), Drizzle (SL = 96, total = 100), Mayer (SL = 106, total = 300). Inskip is a marine full-plated population, whereas all remaining samples are freshwater low-plated populations.

Stickleback ages were determined through counts of pelvic spine annuli (methodology in Reimchen 1992). In summary, the left pelvic spine was removed and decalcified for 3–4 h (RDO rapid bone decalcifier), dehydrated with ethanol, and infused with 2 mL of 1:1 ratio of 100% ethanol and plastic polymer for 2 h. This was replaced with more concentrated plastic polymer, left for 12 h, and then exchanged with additional plastic monomer and hardener II and left to polymerize. The embedded spines were cut immediately above the basal lateral flanges of the spines $\sim 15\%$ along the length of the spine. From this initial cut, about five successive 4–5 μm sections were made towards the distal part of the spine. The sections were floated onto a glass slide, dried, and then infused with Richardson’s stain. Annuli were counted on all stickleback. We used Pearson’s correlation to examine the relationship between adult body size and longevity.

Fig. 1. Collection sites of threespine stickleback (*Gasterosteus aculeatus*) on Haida Gwaii, British Columbia, Canada.



Results

Of the 65 largest fish from 13 localities (range 47–106 mm SL), the mean age was from 1 to 5 years (Fig. 2). Maximum age is 1–2 years for each of the small-bodied populations (SL < 60 mm) including the marine population (Inskip Lagoon). Three or 4-year-old stickleback occurred in each of the giant populations and three of these (Coates, Laurel, and Drizzle) also have stickleback that are 5 or 6 years.

Among the largest five fish from each of the 13 localities, there was a positive correlation between body size and life span ($r_{[65]} = 0.75$, $P < 0.001$), but among the giant populations, there was only marginal association between size and age ($r_{[40]} = 0.29$, $P = 0.07$). Coates Lake, with stickleback at the lower size range (75–80 mm SL) have some of the oldest fish (6 years), whereas Mayer Lake, with the largest stickleback (100–106 mm SL), have no fish above 4 years of age.

There are large differences in age-specific body size among populations but only marginal differences within populations (Fig. 3). Both 1-year and 2-year adults can range from 45 mm SL (Inskip Lagoon) to 85 mm SL (Escarpment). Three-year-old stickleback from Spence Lake averaged 75 mm SL, whereas those from Mayer Lake were about 100 mm SL. Among the populations with multiple adult year classes, yearly increments of body growth were small (<5 mm/year) beyond 2 years.

Discussion

Life-history characteristics including age of first reproduction exhibit considerable variation among populations of threespine stickleback, even within small geographical areas, and typically indicate a life span ranging from 1 to 2 years (Wootton 1976; Baker 1994). Based on a previous result for a greatly extended maximum life span of large-bodied stickleback at Drizzle Lake, in coastal British Columbia (Reimchen 1992), we confirm the presence of extended life histories in seven additional giant populations of stickleback from the region. Four-year-old fish occurred in six of the seven localities, with 6-year-old fish in three localities. As these fish were generally drawn from small samples (~100 adults), the maximum potential life span in the population will probably be older. For the Drizzle Lake stickleback, we used a random subsample of 100 adults to obtain the largest adults from which a maximum age of 6 years was identified, whereas earlier analyses of 492 adults from this locality indicate ages up to 8 years (Reimchen 1992). We also compared the giant populations to five populations from the same geographical region that had mean adult sizes (45–60 mm SL) predicting that extended longevity would only occur in the large-bodied populations. This was the case as all of the small-bodied stickleback populations were either 1 or 2 years of age, which is typical for stickleback populations elsewhere in the distribution of the taxon including the adjacent Alas-

Fig. 2. Mean age of the largest adult threespine stickleback (*Gasterosteus aculeatus*) from localities in Haida Gwaii, British Columbia, Canada, with giant stickleback (>75 mm SL) and typical body sizes (45–60 mm SL). Populations are ranked from smallest (Inskip) to largest (Mayer) mean adult body size. All populations are from lakes except Inskip, which is a marine lagoon.

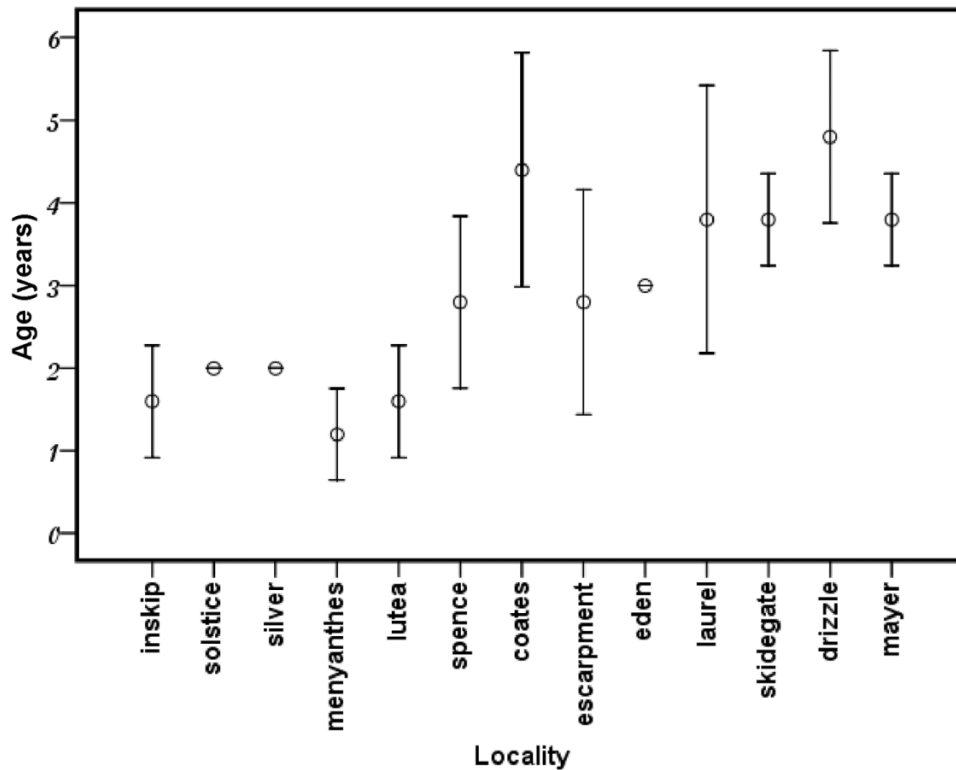
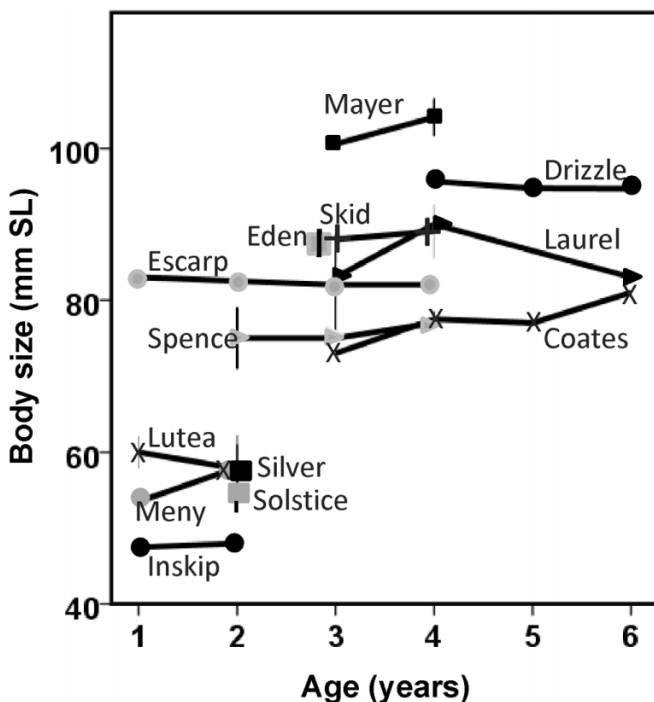


Fig. 3. Relationship between age and body size of the five largest adults observed in each of eight lake populations of giant threespine stickleback (*Gasterosteus aculeatus*) and five populations of typical adult body sizes. Inskip is a marine lagoon.



kan populations (Baker 1994; Baker et al. 2008). Each of the large-bodied populations is thought to have a separate evolutionary origin from the smaller bodied marine (Moodie and Reimchen 1976b; Deagle et al. 2011) and indicates parallel development of extended longevity.

We predicted that the maximum life spans of the giant stickleback would be greater in populations with the largest size, but this had limited support with our sample sizes. The largest fish (100–106 mm SL) from Mayer Lake did not exceed 4 years of age, whereas much smaller adults (~80 mm SL) from Laurel and Coates lakes were 6 years. Size differences among the populations were present even in 3-year-old fish indicating large differences in growth rate, possibly reflecting trophic competition in the habitat, as has been recently identified for large-bodied stickleback populations in Japan (Mori and Takamura 2004).

What factors might contribute to the occurrence of the exceptional lifespan? There is clear evidence that these fish with extended longevity are reproductive. We cannot ascertain that individuals are breeding each year, but our samples include gravid females that are 5 and 6 years of age, whereas those investigated previously from Drizzle Lake can be gravid at 8 years of age (Reimchen 1992; T.E. Reimchen, unpublished observations). Delayed reproduction or iteroparity can be favoured by “habitat uncertainty” during reproduction (Mann and Mills 1979). The majority (7 out of 8) of the giant populations in our study are found in large lakes that are subject to major winds and turbulence (T.E. Reimchen, unpublished data) and this would impact littoral nesting success of the stickleback. Drizzle Lake and Coates Lake, which contain stickleback with the greatest longevity, have the most

exposed shorelines and minimal protection from prevailing winds compared with other lakes, consistent with an “uncertainty” factor. This could not account for the extended longevity at Laurel Pond, which is small and is highly protected from the winds. An additional factor in longevity could be sources of mortality. Herczeg et al. (2009) suggest that the occasional presence of gigantism and extended longevity in ninespine stickleback (*Pungitius pungitius* (L., 1758)) is favoured where predation and interspecific competition are reduced. The majority of the large-bodied adult sticklebacks in our study have very robust defense morphology and are generally outside the swallowing ability of the multiple gape-limited piscivores observed in the majority of these lakes (Moodie 1972; Moodie and Reimchen 1976a; Reimchen 1988, 1991, 1994). Such a predator refuge could produce a selection regime favouring development of intracellular antioxidative mechanisms that reduce rate of senescence and facilitate extended longevity (Austad and Fischer 1991; Ridgway et al. 2011; Ungvari et al. 2011).

Although “habitat uncertainty” and “predator-refuge” hypotheses implicate heritable components to delayed senescence, both empirical and theoretical evidence suggest that population differences in longevity generally have low heritability (Price and Schluter 1991). Consequently, environmentally induced plasticity may contribute to variability in maximum life span of these stickleback populations. From yeast to mammals, experimental and prolonged calorie restriction is one of the major correlates of prolonged life span and appears to result from multiple physiological and neuroendocrine mechanisms that delay aging (Smith et al. 2004; Innes and Metcalfe 2008; Terzibasi et al. 2009). Specifically, in fishes, low feeding rates and lower water temperatures can lead to slower growth and greater longevity (Craig 1985; Jonsson et al. 1991; Valenzano et al. 2006). All of the lakes in the current study with giant stickleback are either oligotrophic or dystrophic and would be expected to have slow growth rate yet the same conditions also occur in the localities with small-bodied stickleback. Longevity in aquatic taxa can be accentuated by dissolved organic compounds such as humic substances that are prevalent in dystrophic waters (Steinberg et al. 2006, 2010). Some of the benefits of humic substances in fishes are the strengthening of the mucosa barrier including ion regulation and defence against pathogens and this appears to facilitate extension of life span (Meinelt et al. 2004; Steinberg et al. 2006, 2007). In this context, the most extended longevity, which occurred in stickleback from Drizzle Lake, was correlated with relatively slow growth rate, low summer temperatures (<15 °C) (Reimchen 1990), and high levels of dissolved organic compounds (Reimchen 1989), suggestive of a nonheritable influence to longevity in this lake. Although reasonable, the same conditions are also present in small-bodied populations such as Silver Lake, which have the typical 1- or 2-year life span.

Carotenoids, derived from primary producers, have important antioxidative and immunosuppressant roles in animals and can influence longevity (Olson and Owens 1998). In stickleback, female preference for enhanced carotenoid expression among courting males has been proposed as both a correlate and causal mechanism for extended life span in this species (Pike et al. 2007). If applicable to the stickleback from Haida Gwaii, such a proposal predicts accentuated carotenoid displays

in these populations with extended longevity. Relative nuptial color expression is known for 60 populations from the Haida Gwaii area and is greatest in clear oligotrophic mountain lakes and least in stained dystrophic lakes (Reimchen 1989). Among the 13 populations in the current study, nuptial colour is available from eight lakes and suggests limited association with longevity. Accentuated carotenoid displays were observed in one of the giant populations (Escarpment Lake) and two of the small-bodied populations (Inskip, Lutea), but the remainder had either average (Coates), weak (Mayer), or an absence of carotenoid expression (Drizzle Lake), of which the latter has the greatest longevity.

Small-bodied fish offer a useful vertebrate model for research on rates of aging (Herrera and Jagadeeswaran 2004). Our study indicates that endemic populations of threespine stickleback from Haida Gwaii, coastal British Columbia, exhibit exceptional variability in relative longevity among closely situated lakes, including several instances of greatly extended life span relative to body size. Recent investigations of Alaskan populations show rapid decadal shifts in stickleback life histories, including age of reproduction, some of which comprise heritable effects (Baker et al. 2011). The recent advances in genetic mapping in the threespine stickleback (Kingsley et al. 2004; Hohenlohe et al. 2010), as well as the current sequencing of the Drizzle Lake stickleback, may allow identification of genetic markers that influence rate of senescence and longevity.

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