Evolutionary Ecology Research, 2013, **15**: 947–957

Validation of daily growth increments in otoliths to age threespine stickleback (*Gasterosteus aculeatus*)

Frank A. von Hippel[1], Lauren C. Smayda[1], Christian E. Zimmerman[2] and Michael A. Bell[3]

[1]*Department of Biological Sciences, University of Alaska Anchorage, Anchorage, Alaska, USA,*
[2]*US Geological Survey, Alaska Science Center, Anchorage, Alaska, USA and*
[3]*Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York, USA*

ABSTRACT

Hypothesis: Threespine stickleback deposit daily growth increments in their otoliths.

Organism: Anadromous threespine stickleback (*Gasterosteus aculeatus*) from Rabbit Slough, Alaska.

Methods: We reared stickleback from an *in vitro* cross for 95 days in a large outdoor pool and analysed the sagittae of specimens from weekly samples.

Results: Putative daily growth increments in otoliths were significantly correlated with true age in days ($r = 0.97$). Other relationships, such as those between age and otolith radius ($r = 0.72$), and between standard length and number of increments ($r = 0.63$) or otolith radius ($r = 0.51$), were also significant though not as strong.

Conclusions: These data are consistent with published results for resident freshwater threespine stickleback from Great Britain, a different clade, continent, latitude, ecological system, and fish life history. Ageing young-of-the-year threespine stickleback with daily growth increments in their otoliths thus appears to be possible for distantly related populations with different life histories and locations. Daily growth increments in the otoliths of young stickleback provide a powerful tool for investigating life-history evolution or any subject that employs precise age as a covariate, since age of wild-caught fish can be associated with a suite of fitness measures, phenotypes, or genotypes.

Keywords: otolith, sagitta, daily growth increments, age determination.

INTRODUCTION

The threespine stickleback (*Gasterosteus aculeatus*) is a model for both field and laboratory research in evolution, behaviour, developmental genetics, ecology, and ecotoxicology (e.g. Bell and Foster, 1994; Katsiadaki *et al.*, 2002; McKinnon and Rundle, 2002; Foster and Baker, 2004; Cresko *et al.*, 2007; Östlund-Nilsson *et al.*, 2007; von Hippel, 2010; Hendry *et al.*, 2013). The extraordinary natural diversity of

Correspondence: F.A. von Hippel, Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Drive, Anchorage, AK 99508, USA. e-mail: favonhippel@uaa.alaska.edu

extant ancestral marine and derived freshwater forms combined with a wide array of tools for the threespine stickleback system led Gibson (2005) to characterize it as a 'supermodel'. The addition of sequenced genomes has enhanced its value (Jones *et al.*, 2012). As such, new tools that can be employed to provide insights into fundamental questions continually increase the utility of the threespine stickleback in a wide variety of fields.

One set of anatomical structures of the stickleback that has promise as a research tool but has received relatively little attention is the otoliths (sagitta, lapillus, and asteriscus). Otoliths contain annual patterns in growth, allowing fish to be aged, and can be unique species identifiers (Panfili *et al.*, 2002). Furthermore, the chemistry of otoliths allows the reconstruction of the thermal and chemical environments of the fish, dietary and life-history parameters, geographical source, and habitat use over time (Bell, 2001; Campana and Thorrold, 2001; Panfili *et al.*, 2002; Zimmerman, 2005).

Studies in a number of fish species have documented daily growth increments in the otoliths, allowing fine-scale ageing and growth studies in young fish (reviewed by Pannella, 1971, 1980; Brothers *et al.*, 1976; Brothers, 1979, 1987; Campana and Neilson, 1985; Jones, 1986; Geffen, 1987, 1992; Radtke, 1989; Campana, 2001). Cowen *et al.* (1991) used otolith increments in wild-caught young-of-the-year marine threespine stickleback to assess age, based on the assumption that the increments were deposited on a daily basis. Wright and Huntingford (1993) verified daily increment formation in an experimental study of sagittal otoliths of resident freshwater threespine stickleback in Great Britain, with their onset corresponding to the day of hatching. They found little error in the relationship between number of increments and number of days following hatching up to at least 130 days after hatching ($r^2 = 0.998$, $P < 0.001$). Such a tight relationship allows researchers to use otolith increments in field-caught and laboratory-reared stickleback to age fish early in life, when important developmental (Swarup, 1958) and ecological changes occur (e.g. Foster *et al.*, 1988; Cowen *et al.*, 1991) and when fitness differences can impact early growth and survival (e.g. Barrett *et al.*, 2008).

The purpose of the present study is to replicate the findings of Wright and Huntingford (1993) using direct validation (Wright *et al.*, 2002) in a different part of the threespine stickleback's geographical range, and using anadromous stickleback, to increase the confidence that this ageing technique can be applied in other threespine stickleback populations. Validation is a critical, yet often ignored, requirement of any ageing study and should be completed for disparate populations spanning life-history types (Beamish and McFarlane, 1983; Panfili *et al.*, 2002). This study was specifically designed to test the utility of counts of otolith growth increments in threespine stickleback from Alaska, which is rapidly becoming a centre of stickleback research. For this purpose, we tested for the existence of daily growth increments in the otoliths of stickleback from Rabbit Slough, a frequently studied anadromous population (Aguirre *et al.*, 2008) within the Cook Inlet, Alaska threespine stickleback radiation (von Hippel, 2008).

MATERIALS AND METHODS

Fish and husbandry

Anadromous threespine stickleback were trapped in Rabbit Slough, Alaska (61.534°N, 149.265°W) using 0.64 cm mesh unbaited minnow traps on 12 June 2001. These fish were used for an *in vitro* fertilization to create embryos of known age. On 14 June, 20 males were killed with an overdose of MS-222 anaesthetic. Their testes were dissected, minced in a few

drops of water immediately after removal, mixed thoroughly, and stored on ice for a few minutes. Eggs from 20 anaesthetized females were expressed into a Petri dish with several drops of water. The combined sperm preparation was spread over the combined eggs and mixed thoroughly. The eggs were allowed to sit for about 10 minutes and checked for formation of a fertilization membrane. After fertilization membranes were observed in several embryos, they were divided roughly evenly into 15 groups and placed into fifteen 5 cm diameter sections of polyvinyl chloride incubators with fine mesh bottoms. The incubators were suspended in aquaria (water temperature 20–22°C) and the developing embryos were checked daily for infection; infected embryos were removed. On 15 and 20 June [1 day and 6 days post fertilization (dpf), respectively], embryos were suspended in sulfa-treated water to minimize infection.

Embryos began to hatch on 19 June (5 dpf). On 20–21 June (6–7 dpf), fry were moved to different aquaria. Beginning on 20 June (6 dpf), fry were fed daily, alternating between a commercial *Artemia* food and brine shrimp nauplii (live and frozen), or a mix of these foods. On 27–28 June (13–14 dpf), fry were moved to a 1600 litre outdoor pool in Anchorage (61.120°N, 149.850°W), which was continuously aerated by air stones and filtered with sponge filters. Partial water changes were also performed periodically. Water temperature in the pool varied from 13°C to 20°C through the end of August, and from 9°C to 14°C in September. Beginning on 10 July (26 dpf), ground-up frozen brine shrimp was added to the feeding regime, and on 13 July (29 dpf), Golden Pearls 100–200 μm were added to the diet. Beginning on 16 July (32 dpf), Golden Pearls 200–300 μm were used instead of the 100–200 μm food, so that fish were fed daily with a mixture of Golden Pearls, ground-up frozen brine shrimp, and *Artemia* food. Beginning on 5 August (52 dpf), the frozen brine shrimp was used without first grinding it to a smaller size. Beginning on 21 August (68 dpf), fish were fed with only frozen brine shrimp. The pool also trapped or was naturally colonized by a variety of potential food species, including aquatic macroinvertebrates.

Approximately once per week (27 July; 3, 10, 19, and 27 August; 3, 10, and 17 September), 7–10 fry were netted from the pool, killed with an overdose of MS-222 anaesthetic, and frozen for otolith extraction.

Ten young-of-the-year anadromous threespine stickleback were collected from Rabbit Slough in August 2006 to compare otoliths of the 2001 laboratory-reared fish with those of wild-caught fish. The field-caught fry were killed with an overdose of MS-222 anaesthetic and frozen prior to otolith removal. Sagittae, which are the largest otoliths in threespine stickleback, were removed under a dissecting microscope and stored on glass slides in immersion oil.

Otolith preparation

Otoliths were rinsed to remove immersion oil and affixed to a glass slide with thermoplastic cement (Crystal Bond 509, Ted Pella, Inc., Redding, CA) for grinding and polishing. A small chip of Crystal Bond was placed on a microscope slide lying on a hot plate at 40–60°C. When the Crystal Bond melted, the otolith was pressed into it so that the flat surface (medial side containing the sulcus) was close and parallel to the surface of the slide. When the Crystal Bond cooled to room temperature and hardened, a few drops of distilled water were placed on the surface of the slide, and the embedded otolith was ground by sliding it over 2000 grit sandpaper. The slide was viewed occasionally during grinding using a

compound microscope to ensure that the core of the otolith was not removed. The otolith was polished with a slurry of 0.05 μm Alumina (Beuhler, Ltd., Lake Bluff, IL). Finally, to ensure that scratches were removed effectively, the polished slide was placed onto a 40–60°C hot plate. This caused the Crystal Bond to melt and smooth out the surface, which allowed for better viewing under the microscope. Both sides of the otolith were normally ground by placing the slide on a hot plate and using forceps to flip the otolith over to grind the newly exposed surface.

Increment counts and measurements of otolith radii (μm) were made from digital images captured by a QImaging Micropublisher 3.3 camera through a Nikon Eclipse E400 compound microscope under 400× magnification using Metavue 6.1r0 software. All counts were made by a single observer (L.C.S.), who did not know the age of the fish from which the otolith came, to prevent bias. Counts were recorded from the first observable increment near the centre to the last increment at the otolith edge (Fig. 1). Each radius measurement and count was made along the 2:00 o'clock radius relative to the orientation of the otolith shown in Fig. 1. In cases where the otolith was damaged along the 2:00 o'clock radius, measurements were taken as close as possible on either side of it. Increments were counted in all otoliths at least three times. Sometimes repeated counts produced different numbers of increments within a range of up to three increments, and the mean of the counts was used.

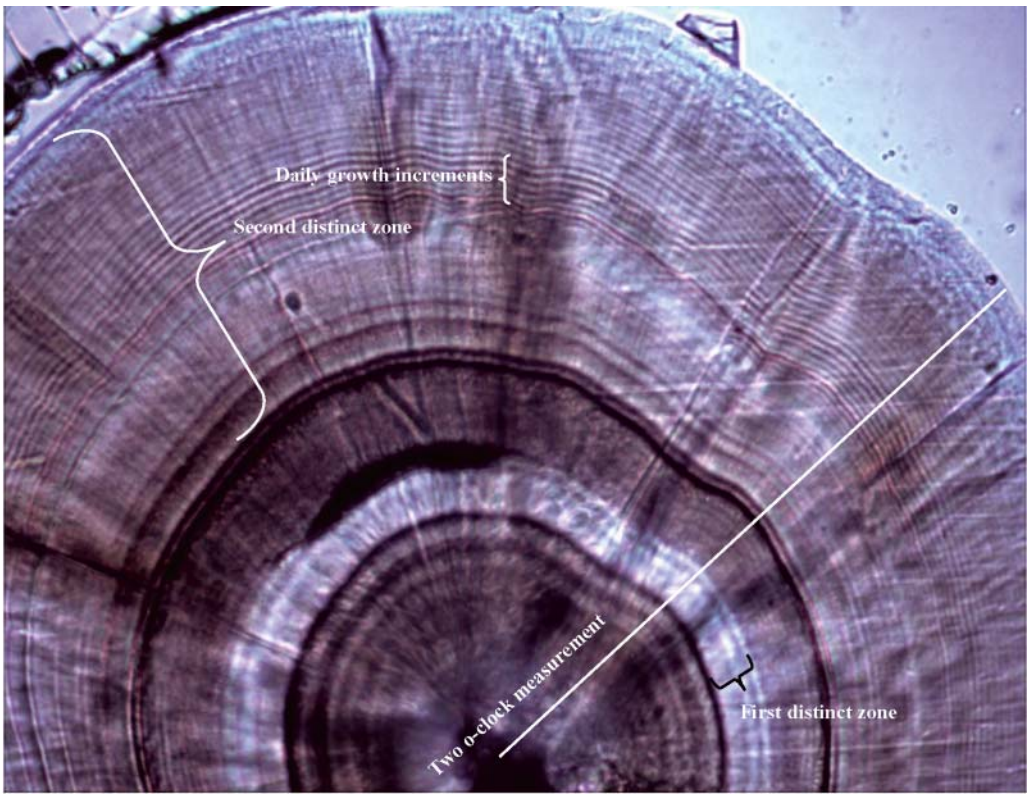

Fig. 1. Representative slide of an otolith from a laboratory-reared stickleback. Note the daily growth increments, the two distinct zones (light on this image), and the 2:00 o'clock radius of measurement along which increments were counted.

We were unable to count the increments or measure radii on 12 of the 75 otoliths extracted from the 2001 laboratory-reared fish because they were broken or the core was lost during grinding. Four broken otoliths were sufficiently intact to count the increments but not to measure the radius. All 10 otoliths from 2006 wild-caught fish were suitable for increment counts and radius measurements.

Otolith analysis

We used simple linear regression to compare the number of increments counted and the otolith radius to the true age in days of laboratory-reared specimens following the methods of Wright *et al.* (2002). We also counted increments and measured radii out to distinct zones that differed in light transmission, and we measured the width of the zones themselves (Fig. 1). Data for wild-caught fish are presented only for the centre of the otolith to the inner edge of the first zone because the second zone in wild-caught young-of-the-year either did not exist or its start was not as well defined as in laboratory-reared specimens.

RESULTS

The slope of the linear regression of increment count on true age for laboratory-reared young-of-the-year stickleback was not significantly different from 1 (slope = 0.92, $t = 2.703$, d.f. = 2; Fig. 2), and the number of increments was highly correlated with true age in dpf through the tested age of 95 days ($r = 0.97$, $n = 63$, $P < 0.001$). The relationship between

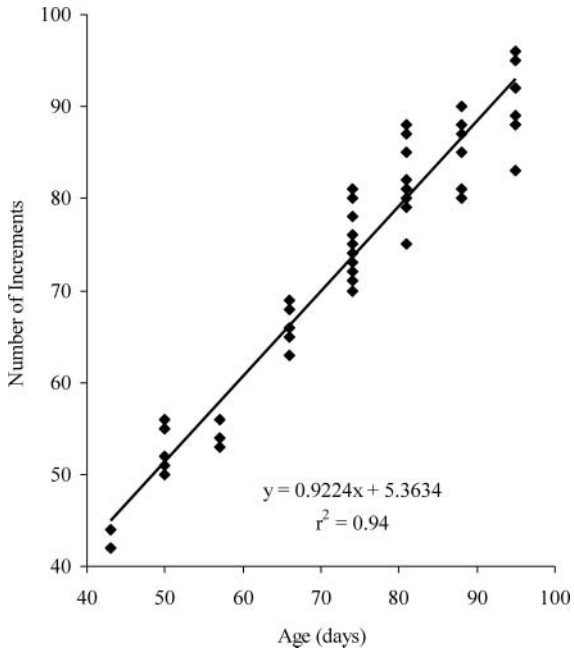

Fig. 2. Relationship between the number of otolith increments and true age in days post fertilization for laboratory-reared stickleback.

otolith radius and age ($r = 0.72$, $n = 59$, $P < 0.001$) was not as strong as the association between number of increments and age.

All otoliths from laboratory-reared fish had two distinct zones (dark when viewed with transmitted light, light when viewed on a dark background with reflected light; Fig. 1). The first zone began between increments 12 and 14, and the second zone between increments 30 and 32 (Fig. 3). In contrast, the otoliths of wild-caught young-of-the-year stickleback had between one and three distinct zones, but we compare only the first zone with that of laboratory-reared fish because of greater variability in number and distinctness of subsequent zones (Table 1).

The standard length of laboratory-reared fish accounted for 39% of the variance in the number of increments deposited in the otolith ($r = 0.63$, $n = 61$, $P < 0.001$) and 27% of the variance in the radius of the otolith ($r = 0.51$, $n = 60$, $P < 0.001$) (Fig. 4). Within age classes, as defined by weekly sampling date, standard length and otolith radius did not show a clear relationship (Fig. 4). Although increment counts tended to overestimate age early in life, as fish aged, ageing error increased and increment count underestimated age (Fig. 5).

DISCUSSION

The number of putative daily increments in the sagittal otoliths of laboratory-reared young-of-the-year threespine stickleback was strongly correlated with true age in days post fertilization. In this study, fish age accounted for 94% of the variance in number of

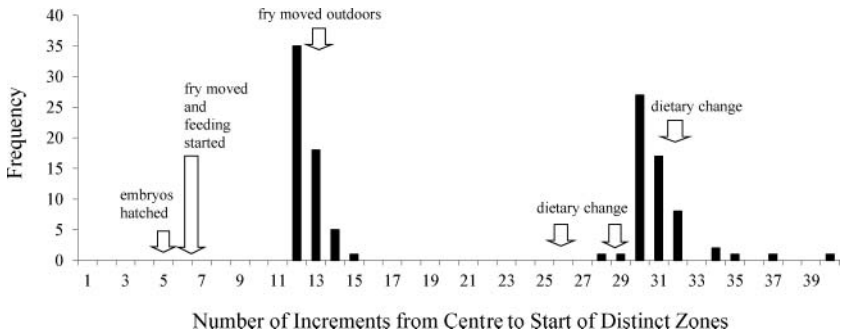

Fig. 3. Frequency of distinct zones found to begin at increments between 12 and 13 days and between 30 and 32 days post fertilization in laboratory-reared stickleback.

Table 1. Number of increments and measurements of otoliths for wild-caught ($n = 10$) versus laboratory-reared ($n = 63$ for increments and 59 for measurements) stickleback

	Laboratory-reared		Wild-caught	
Trait	mean	S.E.	mean	S.E.
No. of increments from otolith centre to inner edge of first distinct zone	12.5	0.8	21.0	8.5
Width from otolith centre to inner edge of first distinct zone (μm)	22.9	5.8	21.5	1.9
Width of first distinct zone (μm)	3.0	4.7	3.3	1.6

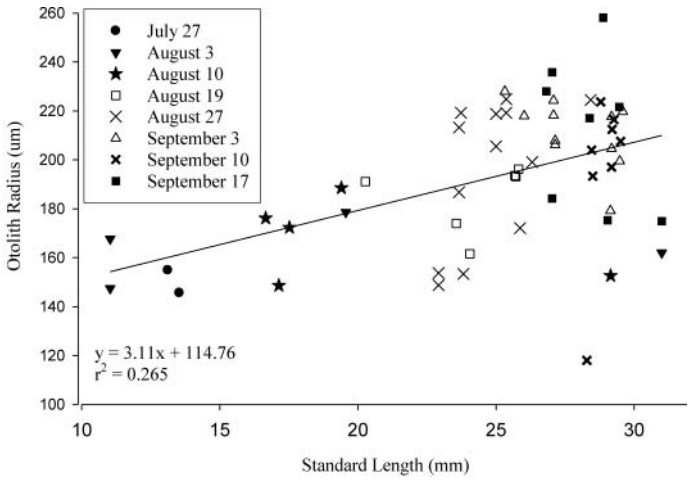

Fig. 4. Relationship between otolith radius and standard length within and among age classes (as indicated by sampling date) of lab-reared stickleback.

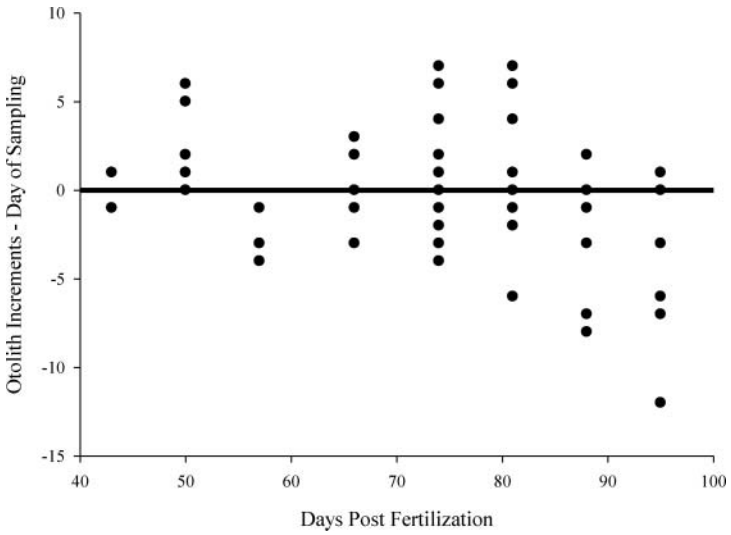

Fig. 5. Ageing error and age in days post fertilization.

increments (Fig. 2). This is comparable to the 99.8% of the variance in number of increments accounted for by age reported by Wright and Huntingford (1993), although the strength of our relationship is weaker. The difference in accuracy could be due to different laboratory methods (interpretation error), or different stickleback populations might show more or less variation in increment number (e.g. resident freshwater vs. anadromous, Great Britain vs. Alaska, etc.) due to an incomplete growth record along the measurement radius [process error (Campana, 2001)]. Regardless, both studies show that daily increment count is an accurate tool for ageing young-of-the-year threespine stickleback up to at least 95–130 days. We arrived at the same conclusion as Wright and Huntingford (1993) that because our fish

were raised in a large outdoor pool under ambient light and temperature conditions, and with a diversity of foods, these results could apply to wild threespine stickleback (see also Campana, 2001). Such an accurate method to age young stickleback should have broad applications in ecological and evolutionary studies that concern early life-history events or natural selection on juvenile phenotypes.

Wright and Huntingford (1993) reported that increment deposition began at hatching (5 dpf). Swarup (1958) reported the presence of otoliths in unhatched embryos at about 3.5 dpf (i.e. 88 hours), and otoliths were consistently visible in cleared and stained (alcian and alizarin) Rabbit Slough fry produced by our methods within 6 dpf (i.e. 149 hours), 2 days (48 hours) after hatching began (M.A. Bell *et al.*, unpublished data). The intercept of our relationship, however, was equal to 5 (Fig. 2), indicating that 5 increments were present at day 0 even though the otoliths had yet to form. This may have been caused by formation of sub-daily increments. It has been suggested that otolith increment formation can be influenced by photoperiod (Eckmann, 2000) with sub-daily increments forming in fish that do not experience a strongly distinct light and dark period each day (Huuskonen, 1999). In the early stages of our experiment, day length was over 19 hours, and no distinct dark period is experienced near the summer solstice at this latitude. At older ages, however, ageing error in our study increased and increment counts tended to underestimate age (Fig. 5). Such a relationship suggests that increment formation at older ages is less than one per day in some cases. More work is needed to assess the role of photoperiod in determining increment formation rates.

The relationship between the radius of an otolith and true age in days ($r^2 = 0.52$) was not as strong as the association between number of increments and age ($r^2 = 0.94$; Fig. 2). This is expected because the thickness of individual increments and thus the radius of the otolith on any day depend on growth rate or metabolism (Pannella, 1971; Mosegaard *et al.*, 1988; Brothers, 1990). Clearly, the number of increments should be used preferentially over otolith radius to determine a stickleback's age.

Otolith zones varied between laboratory-reared and wild-caught fish (Table 1). This may have been caused by environmental variation, such as food availability and temperature, or by developmental milestones (Fig. 3), and such variables often differ between the lab and field (Campana, 2001). Greater knowledge of the causes of these zones, and the checks between them, would help to pinpoint life-history events or environmental changes.

The relationships between standard length and the number of otolith increments ($r^2 = 0.39$) or otolith radius ($r^2 = 0.27$; Fig. 4) were not as strong as were the relationships between otolith radius or number of increments and true age. This may be expected because of the potentially confounding factor of negative allometry on increment width; fast-growing fish typically have wider increments, but perhaps not as wide as would be expected for the amount of growth that day if there were a 1:1 relationship between fish growth and otolith growth (Mosegaard *et al.*, 1988). Such an allometric relationship is not apparent, however, when age classes of fish are considered separately (Fig. 4). [See also Radtke (1989), who found that patterns of daily growth in Atlantic cod (*Gadus morhua*) could be estimated from daily increment widths.]

Potential allometry of increment width deserves further study because a definable relationship would allow researchers to use the width of daily growth increments to reconstruct the growth history of individual stickleback with different phenotypes, such as different lateral plate or pelvic phenotypes from populations where those traits are highly variable (e.g. Lescak *et al.*, 2013). For example, fish with more bone (more lateral plates, more robust pelvic

girdle) might be expected to grow more slowly when the bones start to develop, which is a potential cost of armour development (Giles, 1983; Bell *et al.*, 1993; Bourgeois *et al.*, 1994; Marchinko and Schluter, 2007; Barrett *et al.*, 2008).

Furthermore, the ability to quantify the number of daily growth increments creates new opportunities to study ecological and evolutionary processes, including offshore dispersal (Cowen *et al.*, 1991) and selection against juvenile hybrids between members of sympatric species pairs (Jones *et al.*, 2006; Gow *et al.*, 2007). Similarly, Cargnelli and Gross (1996) studied the pronounced effect of birth date on overwinter survival in bluegill sunfish (*Lepomis macrochirus*), an analysis that could also be conducted for stickleback populations. These kinds of problems illustrate the utility of employing otolith data to address questions in stickleback evolution and ecology.

The relationship between number of daily growth increments and true age observed in our study of anadromous threespine stickleback is consistent with the results of Wright and Huntingford (1993) for resident freshwater threespine stickleback from Great Britain, a different clade (Ortí *et al.*, 1994), continent, latitude, ecological system, and fish life history. Ageing young-of-the-year threespine stickleback with daily growth increments in the otoliths thus appears to be possible for distantly related populations with different life histories and locations. Further validation is needed for populations at the extremes of the geographic range (e.g. northern Mexico and Mediterranean populations) and habitats (e.g. thermal pools in Iceland, ponds on the North Slope of Alaska) where threespine stickleback occur. Additionally, validation is needed for other life-history types (e.g. limnetic threespine stickleback, the 'white' threespine stickleback of Nova Scotia) and for the Sea of Japan threespine stickleback, the sister group of most other populations in the species complex (reviewed by Mattern, 2007). Future studies should also investigate the accuracy of using daily growth increments to age stickleback through their entire lifespan.

ACKNOWLEDGEMENTS

We thank Jenifer Rollins, Andrew Hendry, and anonymous reviewers for helpful comments on the manuscript. Stickleback were collected under Alaska Department of Fish and Game permits SF-2001-062 and SF-2006-017. All work was approved by the UAA IACUC. This work was supported by National Science Foundation grants DEB 0320076, 0422687, 0522059, and 0618551 to F.A.vH., DEB 0211391 to M.A.B., and DEB 0322818 to M.A.B. and F.J. Rohlf. Use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the US Government. This is contribution 1229 from Ecology and Evolution at Stony Brook University.

REFERENCES

Aguirre, W.E., Ellis, K.E., Kusenda, M. and Bell, M.A. 2008. Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: implications for postglacial adaptive radiation. *Biol. J. Linn. Soc.*, **95**: 465–478.

Barrett, R.D.H., Rogers, S.M. and Schluter, D. 2008. Natural selection on a major armor gene in threespine stickleback. *Science*, **322**: 255–257.

Beamish, R.J. and McFarlane, G.A. 1983. The forgotten requirement for age validation in fisheries biology. *Trans. Am. Fish. Soc.*, **112**: 735–743.

Bell, M.A. 2001. Fish do not lie about their age . . . but they might lose count. *Trends Ecol. Evol.*, **16**: 599–600.

Bell, M.A. and Foster, S.A. 1994. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press.

Bell, M.A., Ortí, G., Walker, J.A. and Koenings, J.P. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. *Evolution*, **47**: 906–914.

Bourgeois, J.F., Blouw, D.M., Koenings, J.P. and Bell, M.A. 1994. Multivariate analysis of geographic covariance between phenotypes and environments in the threespine stickleback, *Gasterosteus aculeatus*, from the Cook Inlet area, Alaska. *Can. J. Zool.*, **72**: 1497–1509.

Brothers, E.B. 1979. Age and growth studies on tropical fishes. In *Stock Assessment for Tropical Small-scale Fisheries* (S.B. Saila and P.M. Roedel, eds.), pp. 119–136. Kingston, RI: University of Rhode Island Press.

Brothers, E.B. 1987. Methodological approaches to the examination of otoliths in aging studies. In *Age and Growth of Fish* (R.C. Summerfeld and G.E. Hall, eds.), pp. 319–330. Ames, IA: Iowa State University Press.

Brothers, E.B. 1990. Otolith marking. *Am. Fish. Soc. Symp.*, **7**: 183–202.

Brothers, E.B., Mathews, C.P. and Lasker, R. 1976. Daily growth increments in otoliths from larval and adult fishes. *Fish. Bull.*, **74**: 1–8.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.*, **59**: 197–242.

Campana, S.E. and Neilson, J.D. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.*, **42**: 1014–1032.

Campana, S.E. and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.*, **58**: 30–38.

Cargnelli, L.M. and Gross, M.R. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). *Can. J. Fish. Aquat. Sci.*, **53**: 360–367.

Cowen, R.K., Chiarella, L.A., Gomez, C.J. and Bell, M.A. 1991. Offshore distribution, size, age, and lateral plate variation of late larval/early juvenile sticklebacks (*Gasterosteus*) off the Atlantic coast of New Jersey and New York. *Can. J. Fish. Aquat. Sci.*, **48**: 1679–1684.

Cresko, W.A., McGuigan, K.L., Phillips, P.C. and Postlethwait, J.H. 2007. Studies of threespine stickleback developmental evolution: progress and promise. *Genetica*, **129**: 105–126.

Eckmann, R. 2000. The influence of photoperiod and feeding frequency on the distinctness of daily otolith increments in larval whitefish (*Coregonus lavaretus* L.). *Limnologica*, **30**: 102–105.

Foster, S.A. and Baker, J.A. 2004. Evolution in parallel: new insights from a classic system. *Trends Ecol. Evol.*, **19**: 456–459.

Foster, S.A., Garcia, V.B. and Town, M.Y. 1988. Cannibalism as the cause of an ontogenetic shift in habitat use by fry of the threespine stickleback. *Oecologia*, **74**: 577–595.

Geffen, A.J. 1987. Methods of validating daily increment deposition in otoliths of larval fish. In *Age and Growth of Fish* (R.C. Summerfelt and G.E. Hall, eds.), pp. 123–240. Ames, IA: Iowa State University Press.

Geffen, A.J. 1992. Validation of otolith increment deposition rate. In *Otolith microstructure examination and analysis* (D.K. Stevenson and S.E. Campana, eds.). *Can. Spec. Publ. Fish. Aquat. Sci.*, **117**: 101–113.

Gibson, G. 2005. The synthesis and evolution of a supermodel. *Science*, **307**: 1890–1891.

Giles, N. 1983. The possible role of environmental calcium levels during the evolution of phenotypic diversity in Outer Hebridean populations of the three-spined stickleback. *J. Zool.*, **199**: 535–544.

Gow, J.L., Peichel, C.L. and Taylor, E.B. 2007. Ecological selection against hybrids in natural populations of sympatric threespine sticklebacks. *J. Evol. Biol.*, **20:** 2173–2180.

Hendry, A.P., Peichel, C.L., Matthews, B., Boughman, J.W. and Nosil, P. 2013. Stickleback research: the now and the next. *Evol. Ecol. Res.*, **15**: 111–141.

Huuskonen, H. 1999. Is otolith microstructure affected by latitude? *Mar. Ecol. Prog. Ser.*, **177**: 309–310.

Jones, C. 1986. Determining age of larval fish with the otolith increment technique. *Fish. Bull.*, **84**: 91–104.

Jones, F.C., Brown, C., Pemberton, J.M. and Braithwaite, V.A. 2006. Reproductive isolation in a threespine stickleback hybrid zone. *J. Evol. Biol.*, **19**: 1531–1544.

Jones, F.C., Grabherr, M.G., Chan, Y.F., Russell, P., Mauceli, E., Johnson, J. *et al.* 2012. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, **484**: 55–61.

Katsiadaki, I., Scott, A.P. and Mayer, I. 2002. The potential of the three-spined stickleback (*Gasterosteus aculeatus* L.) as a combined biomarker for oestrogens and androgens in European waters. *Mar. Environ. Res.*, **54**: 725–728.

Lescak, E.A., von Hippel, F.A., Bernhardt, R.R. and Bell, M.A. 2013. Pelvic girdle reduction and asymmetry in threespine stickleback from Wallace Lake, Alaska. *Evol. Ecol. Res.*, **15**: 155–170.

Marchinko, K.B. and Schluter, D. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. *Evolution*, **61**: 1084–1090.

Mattern, M.Y. 2007. Phylogeny, systematics, and taxonomy of sticklebacks. In *Biology of the Three-spined Stickleback* (S. Östlund-Nilsson, I. Mayer and F.A. Huntingford, eds.), pp. 1–40. Boca Raton, FL: CRC Press.

McKinnon, J.S. and Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.*, **17**: 480–488.

Mosegaard, H., Svedäng, H. and Taberman, K. 1988. Uncoupling of somatic and otolith growth rates in Arctic charr (*Salvelinus alpinus*) as an effect of differences in temperature response. *Can. J. Fish. Aquat. Sci.*, **45**: 1514–1524.

Ortí, G., Bell, M.A., Reimchen, T.E. and Meyer, A. 1994. Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. *Evolution*, **48**: 608–622.

Östlund-Nilsson, S., Mayer, I. and Huntingford, F.A., eds. 2007. *Biology of the Three-spined Stickleback*. Boca Raton, FL: CRC Press.

Panfili, J., de Pontual, H., Troadec, H. and Wright, P.J., eds. 2002. *Manual of Fish Sclerochronology*. Brest, France: Ifremer-IRD.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. *Science*, **173**: 1124–1127.

Pannella, G. 1980. Growth patterns of fish sagittae. In *Skeletal Growth of Aquatic Organisms* (D.C. Rhoads and R.A. Lutz, eds.), pp. 519–560. New York: Plenum Press.

Radtke, R.L. 1989. Laval fish age, growth, and body shrinkage: information available from otoliths. *Can. J. Fish. Aquat. Sci.*, **46**: 1884–1894.

Swarup, H. 1958. Stages in the development of the stickleback *Gasterosteus aculeatus* (L.). *J. Embryol. Exp. Morph.*, **6**: 373–383.

von Hippel, F.A. 2008. Conservation of threespine and ninespine stickleback radiations in the Cook Inlet Basin, Alaska. *Behaviour*, **145**: 693–724.

von Hippel, F.A. 2010. *Tinbergen's Legacy in Behaviour: Sixty Years of Landmark Stickleback Papers*. Leiden, The Netherlands: Brill Academic Publishers.

Wright, P.J. and Huntingford, F.A. 1993. Daily growth increments in the otoliths of the three-spined stickleback, *Gasterosteus aculeatus* L. *J. Fish Biol.*, **42**: 65–77.

Wright, P.J., Panfili, J., Folkvord, H., Mosegaard, H. and Meunier, F.J. 2002. Direct validation. In *Manual of Fish Sclerochronology* (J. Panfili, H. de Pontual, H. Troadec and P.J. Wright, eds.), pp. 114–127. Brest, France: Ifremer-IRD.

Zimmerman, C.E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. *Can. J. Fish. Aquat. Sci.*, **62**: 88–97.