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Shifts in life-history traits of two introduced populations of threespine stickleback

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ABSTRACT

Background: Anadromous threespine stickleback (*Gasterosteus aculeatus*) from Rabbit Slough were introduced into Cheney and Scout Lakes, Alaska in 2009 and 2011, respectively. The introductions were intended to model colonization of freshwater habitats by oceanic stickleback following the deglaciation of the Cook Inlet region, which resulted in a widely studied system of highly variable populations.

Hypothesis: Life-history traits of females in the colonizing lake populations will change from the ancestral phenotype within the first few generations after the experimental introductions, consistent with expectations for an opportunistic life-history strategy based on models for life-history change in invasive fishes. Females in the lakes are expected to reproduce at an earlier age and smaller size than anadromous females, and to exhibit greater reproductive effort (size-adjusted clutch mass) and clutch size (size-adjusted numbers of eggs). Changes in egg mass are difficult to predict because complex factors influence this life-history trait.

Methods: We quantified length, body mass, clutch mass, clutch size, and egg mass of wild-caught females from the anadromous source population and the two introduced lake populations during the first few years after introduction. In analyses of clutch mass, clutch size, and egg mass, we used body mass to correct for female size.

Results: As expected, age at reproduction and adult body size decreased while size-adjusted clutch size and reproductive effort increased. These changes occurred abruptly in the first year after introduction. Egg mass did not change in the first year post-introduction. Changes in life-history traits in subsequent years included larger mean egg mass and a greater proportion of females reproducing at age 2 instead of age 1.

Keywords: clutch mass, clutch size, egg mass, *Gasterosteus aculeatus*, reproduction, reproductive effort, threespine stickleback.

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INTRODUCTION

The mechanisms of biological invasion have been a major area of ecological and evolutionary investigation (Williamson, 1999; Sax *et al.*, 2005, 2007; Colautti and Lau, 2015). Invading populations may be successful due to their propensity to alter phenotypes among generations originating immediately following an invasion (Reznick and Ghalambor, 2001; Fox *et al.*, 2007; Grabowska *et al.*, 2011; Westley, 2011). Many studies have investigated changes in reproductive traits among invading populations that have become separated from their ancestral populations; however, most were conducted after the focal species had occupied the invaded habitat for several generations and do not provide insight into the earliest changes (Huey *et al.*, 2005; Westley, 2011; Colautti and Lau, 2015). Phenotypic divergence often begins during the first few generations after invasion, and observation of these changes can shed light on the factors that favour establishment and subsequent proliferation of invasive species (Reznick and Ghalambor, 2001; Bohn *et al.*, 2004; Sax *et al.*, 2005; Gordon *et al.*, 2009). The changes that take place in the first few generations after invasion can account for a disproportionately large amount of the total divergence seen after many generations (Hendry *et al.*, 2008; Leaver and Reimchen, 2012).

Phenotypic shifts can occur due to phenotypic plasticity and from natural selection acting on standing genetic variation or new mutations, and they are most likely a result of a combination of these factors (Huey *et al.*, 2005; Fox *et al.*, 2007; Sax *et al.*, 2007; Hendry *et al.*, 2008). Although many examples of evolutionarily young systems suggest that phenotypic plasticity is more important for invading populations to tolerate novel conditions (Fox *et al.*, 2007; Hendry *et al.*, 2008; Grabowska *et al.*, 2011), rapid genetic change can still occur when enough heritable variation exists within a population and a new environment imposes strong selection (Huey *et al.*, 2005; Sax *et al.*, 2007).

Phenotypic variation among extant freshwater populations of threespine stickleback (*Gasterosteus aculeatus*) at high latitudes reflects divergence from ancestral marine and anadromous (oceanic) forms that established populations in freshwater habitats following the recession of northern hemisphere glaciers beginning about 22,000 years ago (e.g. Lindsey, 1962; Bell, 1976; Bell and Foster, 1994; Bell *et al.*, 2004). Freshwater populations provide an excellent example of an adaptive radiation (Schluter, 2000) and an excellent system to investigate phenotypic divergence from ancestral populations (e.g. Bell and Foster, 1994; Bell *et al.*, 2004; Furin *et al.*, 2012). Freshwater threespine stickleback populations have been investigated within a few years or decades after derivation from anadromous or marine ancestors (e.g. Bell and Aguirre, 2013; Lescak *et al.* 2015), but phenotypic divergence during the first generations after an invasion has never been studied before. In this study, we investigate changes in female life-history traits during the first few generations after experimental introduction of anadromous threespine stickleback into freshwater lakes.

Threespine stickleback from one anadromous population were introduced into two Alaskan lakes in which the native fish fauna, including threespine stickleback, had been extirpated with rotenone by the Alaska Department of Fish and Game (Bell *et al.*, 2016). These introductions were made to simulate the natural colonization process of lakes by oceanic stickleback and to observe phenotypic changes among generations immediately following establishment. The threespine stickleback is an excellent model for investigating the early generations of an invasion because the marine habitat of anadromous threespine stickleback contrasts markedly with that of lakes (Bell *et al.*, 2004; Lucek *et al.*, 2012). We use models and terminology from work on invasive species when referring to the general process of

colonization, but we refer to the populations of threespine stickleback as colonizers because they do not fit the definition of an invasive species.

Following introduction to a new environment, fish populations can exhibit phenotypic differences contrasting with the ancestral population. Some colonizing fish populations exhibit an opportunistic life-history strategy with shifts towards smaller body size, earlier sexual maturation, and greater reproductive effort compared with ancestral populations (Hutchings, 1993; Fox *et al.*, 2007; Grabowska *et al.*, 2011; Amundsen *et al.*, 2012). In contrast, other colonizing fish populations show an equilibrium strategy involving larger body size, later maturation, and lower reproductive effort (Fox *et al.*, 2007; Ribeiro and Collares-Pereira, 2010). The former response appears to be more common in the early stages of invasion, whereas the latter characteristics, if they do arise, appear more common later in the colonization process (Bohn *et al.*, 2004; Fox *et al.*, 2007; Ribeiro and Collares-Pereira, 2010).

Here we study shifts in life-history traits of colonizing female threespine stickleback as they become established in populations in order to determine whether they are consistent with trait shifts observed in successful freshwater fish invasions. We also consider observed life-history patterns in native freshwater populations of threespine stickleback in Alaska (Baker *et al.*, 2008) to inform the predicted changes, under the assumption that these patterns provide clues to the selective environment that the establishing populations experience.

Most resident freshwater populations of threespine stickleback in south-central Alaska have smaller body sizes and a lower level of reproductive effort (relative clutch mass) than their anadromous counterparts; they also make smaller size-adjusted clutches than oceanic stickleback (Baker *et al.*, 2008; J.A. Baker, personal communication). Egg mass, however, is equally likely to increase or decrease, probably because of the numerous, complex environmental factors acting on this trait (Baker *et al.*, 2008; Heins *et al.*, 2016). In Scout Lake, egg size of females was greater than the ancestral ovum size [pre-pike introduction (Heins *et al.*, 2016; D.C. Heins, personal observation)].

Based upon the foregoing, we expect that female stickleback in our experimental lakes will show traits consistent with an opportunistic strategy in the first generations following introduction, including smaller body size, earlier age at reproduction, and greater reproductive effort involving larger size-adjusted clutch mass and clutch size. Although resident freshwater populations make smaller size-adjusted clutches and smaller relative clutch masses, under-exploited resources may allow greater reproductive effort. Although all of the observed life-history traits exhibit plasticity in natural and laboratory experiments, egg mass shows little plasticity in laboratory experiments and may be the least likely to change significantly in the short time span of this experiment (Baker and Foster, 2002; Baker *et al.*, 2008).

MATERIALS AND METHODS

Threespine stickleback introductions and field samples

We introduced anadromous threespine stickleback from a single source population (Rabbit Slough, 61°32′08″N, 149°15′10″W) into Cheney Lake (61°12′07″N, 149°45′36″W) and Scout Lake (60°32′05″N, 150°49′48″W); all three sites are located in south-central Alaska (Bell *et al.*, 2016). Cheney Lake has a surface area of 0.03 km^2 and a maximum depth of 4.3 m, while Scout Lake has a surface area of 0.38 km^2 and a maximum depth of 6.1 m. Both introductions followed rotenone treatments of the lakes to remove illegally introduced northern pike (*Esox lucius*) (Bell *et al.*, 2016; Heins *et al.*, 2016); the treatments also killed the resident

threespine stickleback in each lake. Trapping after both lakes were treated with rotenone failed to produce any threespine stickleback, and stickleback caught in both lakes after the introductions had morphological phenotypes of the introduced anadromous stickleback, not resident freshwater stickleback (Bell *et al.*, 2016).

Cheney Lake was treated with rotenone in the fall of 2008; we reintroduced about 2964 reproductive adult anadromous threespine stickleback between 29 May and 3 June 2009, and sampled individuals for this study in 2010, 2011, and 2012. Scout Lake was treated with rotenone in the fall of 2009; we reintroduced about 3047 reproductive adult anadromous threespine stickleback between 4 June and 4 July 2011, and sampled individuals for this study in 2012 and 2013. We also sampled threespine stickleback from Rabbit Slough in 2009 to represent the ancestral population. Threespine stickleback were captured using unbaited 0.32-cm and 0.64-cm wire mesh minnow traps set in shallow water near the shore of the lakes and across Rabbit Slough. Fish were euthanized with an overdose of MS-222 anaesthetic (tricaine methanesulfonate, Argent Chemical Laboratories, Inc., Redmond, WA) and then fixed and stored in 10% buffered formalin.

Data collection

We categorized female threespine stickleback into reproductive stages using ovarian condition following classifications of Baker *et al.* (1998) and Heins *et al.* (1992). Female threespine stickleback ovaries were classified into six stages: latent, early maturing, late maturing, mature, ripening, and ripe. Females produce multiple clutches during a breeding season, and the classification scheme incorporates the 'clutch-production cycle' as sexually mature females repeatedly cycle between the late maturing, mature, ripening, and ripe stages. Females with ovaries classified as latent are considered sexually immature. Females with ovaries that are early or late maturing are considered sexually mature because they were producing clutches in that season, but were not reproductively mature at the time of sampling, as they had not formed a discernible clutch. Mature, ripening, and ripe ovaries indicate that the female was both sexually and reproductively mature.

Specimens were dissected to determine the sex and reproductive maturity stage. We measured body length (standard length, SL) to the nearest 0.1 mm, and blotted wet eviscerated somatic mass (to 0.001 g). Clutch size is the total number of enlarged oocytes or eggs, clearly discernible in the ovaries of mature, ripening, and ripe females (Heins and Baker, 1993). We counted eggs in clutches of mature, ripening, and ripe females to determine clutch size. The clutch masses of ripening and ripe females were measured to the nearest 0.00001 g after drying for 24–28 hours at 40°C, and mean egg mass of each female was calculated to the nearest microgram by dividing clutch mass by clutch size. Length-frequency analysis was used to determine the approximate age of specimens.

Freshwater stickleback can be infected by the cestode parasite *Schistocephalus solidus*, which affects host reproduction (Heins *et al.*, 2010). Thus, infected fish were excluded from analyses. A large percentage of the threespine stickleback in Cheney Lake were infected; the parasite was not present in Rabbit Slough fish and was rare in Scout Lake fish we examined.

Data analysis

Statistical tests and computations were performed on $\log_{10}$-transformed data in SYSTAT v.10.0 (Systat Software, Inc.) and IBM SPSS Statistics v.23. The most commonly measured

indicator of fish size is SL (Baker, 1994). To compare size at reproduction, we compared SL between samples from Rabbit Slough and each of the introduced lake populations using separate one-way analyses of variance (ANOVA). To contrast the introduced populations with the ancestral population, we compared females from Cheney Lake in 2010–2012 with females from Rabbit Slough in 2009 because the population of Rabbit Slough females in 2009 was the ancestral population of females from Cheney Lake. The ancestors of females from Scout Lake in 2012–2013 would have been females from Rabbit Slough in 2011; however, we could not obtain a sample from Rabbit Slough that year. Therefore, we compared females from Scout Lake to Rabbit Slough females from 2009, under the assumption that 2009 and 2011 Rabbit Slough females would show similar trait metrics. We performed a *post-hoc* contrast to determine which yearly samples from Cheney Lake and Scout Lake were significantly different from one another.

To determine the age or ages at which females were reproducing, we created length-frequency plots in SigmaPlot (Systat Software, Inc.) showing the percentage of all fish that fell into each 1-mm SL interval. The strong seasonal nature of threespine stickleback reproduction in Alaska and other high-latitude environments usually creates distinct size classes between ages, allowing size-frequency plots to be the most common method of age estimation for the species (Baker *et al.*, 2008).

Body mass is correlated with these life-history traits in female threespine stickleback and must be accounted for as a covariate (Baker *et al.*, 2008). We tested for significant relationships between three reproductive traits (clutch size, egg mass, and clutch mass) and female body mass (eviscerated blotted wet mass). Both $\log_{10}$-transformed clutch size and $\log_{10}$-transformed clutch mass showed significant correlations ($P < 0.05$) with $\log_{10}$-transformed body mass in all of the samples from Cheney Lake, Scout Lake, and Rabbit Slough. There was a significant correlation between $\log_{10}$-transformed egg mass and $\log_{10}$-transformed body mass in all of the samples from Cheney Lake, but not in Rabbit Slough or Scout Lake.

We used analysis of covariance (ANCOVA) to compare clutch size, egg mass, and clutch mass among years within each lake, with body mass as a covariate. Regression slope equality is an assumption of ANCOVA. We used a general linear model to test for homogeneity of slopes, which revealed that the slopes of regressions for clutch size vs. body mass, egg mass vs. body mass, and clutch mass vs. body mass were homogeneous among Cheney Lake and Rabbit Slough samples ($P > 0.05$). In Scout Lake, the slopes of regression for clutch size vs. body mass and clutch mass vs. body mass were heterogeneous ($P < 0.05$), but were homogeneous for egg mass vs. body mass ($P > 0.05$). We used ANCOVA for the comparisons of Scout clutch size and clutch mass despite heterogeneity because body mass accounts for much of the variation in these traits in the majority of threespine stickleback populations, and simulations have shown that ANCOVA is robust to this type of violation under most circumstances (Wu, 1984; Reist, 1985). Subsequent to the ANCOVA, Least Significant Difference (LSD) pairwise comparisons were used to determine which Cheney Lake and Scout Lake samples differed significantly from Rabbit Slough and from other years within the same lake, with LSD *P*-values adjusted based on false discovery rate (FDR) (Benjamini and Hochberg, 1995). Finally, we computed Cohen's *d* for each sample comparison to represent effect size.

RESULTS

Body size

Reproducing females from the ancestral population in Rabbit Slough were larger than those sampled from Cheney Lake in each of the first 3 years post-introduction (ANOVA: $F_{1,163} = 2794.33$, $P < 0.05$–0.0001). In the first year, reproducing females from Cheney Lake averaged 43 mm SL and were 40% smaller than females from the parental population, which averaged 71 mm SL (Fig. 1). Mean SL of reproducing females varied among years within Cheney Lake, increasing from 43 mm in 2010 to 45 mm in 2011 ($P < 0.0001$, FDR-adjusted; Table 1), and then decreasing to 42 mm in 2012 ($P < 0.0001$, FDR-adjusted). The means for 2010 and 2012 did not differ significantly ($P = 1.000$, FDR-adjusted).

Reproducing females from Rabbit Slough were also larger than reproducing females from Scout Lake in 2012 and 2013 (ANOVA: $F_{2,258} = 870.00$, $P < 0.0001$; Table 1). In 2012, reproducing females in Scout Lake averaged 52 mm SL and were 27% smaller than the mean size (71 mm SL) of adult females from Rabbit Slough (Fig. 1). Reproducing females from Scout Lake were significantly larger in 2013 (mean = 59 mm SL) than in 2012 (mean = 52 mm SL; $P < 0.0001$, FDR-adjusted).

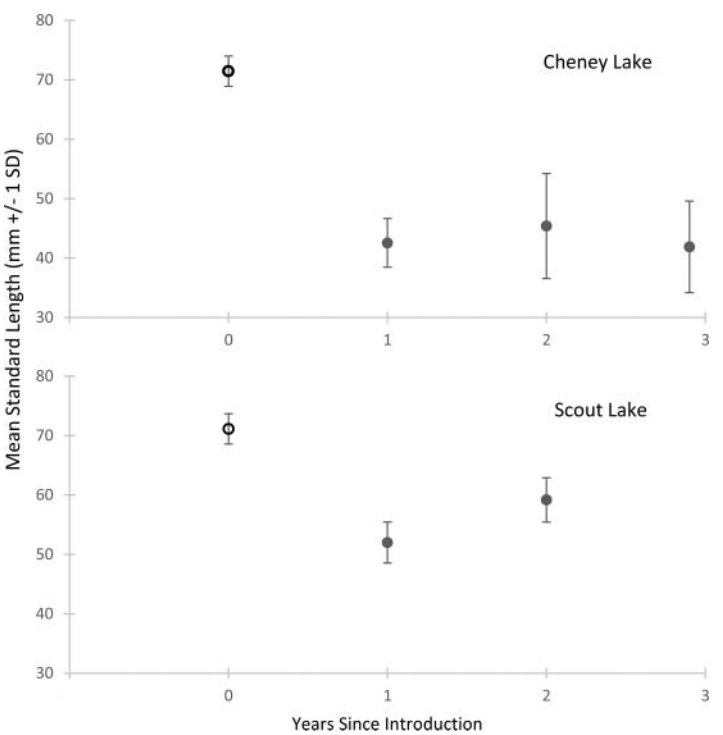

Fig. 1. Mean standard length (anti-logs, mm) of female threespine stickleback from Rabbit Slough in 2009 (open circles, $n = 69$); Cheney Lake (upper panel, solid circles) in 2010 ($n = 105$), 2011 ($n = 112$), and 2012 ($n = 54$); and Scout Lake (lower panel, solid circles) in 2012 ($n = 123$) and 2013 ($n = 69$). Bars represent one standard deviation from the mean, and years since introduction correspond to calendar years for samples from each lake.

Table 1. Cohen's *d* pairwise comparisons for female standard length and size-adjusted clutch size, egg mass, and clutch mass

Sample comparison	Standard length (mm)	Clutch size	Egg mass (μg)	Clutch mass (g)
Rabbit Slough vs. Cheney, 2010	10.487	−0.374	0.684	−0.369
	(0.982)	(−0.184)	(0.324)	(−0.181)
Rabbit Slough vs. Cheney, 2011	4.002	−0.445	−0.309	−0.726
	(0.895)	(−0.219)	(−0.152)	(−0.341)
Rabbit Slough vs. Cheney, 2012	5.067	−0.213	−0.413	−0.833
	(0.930)	(−0.106)	(−0.202)	(−0.384)
Cheney, 2010 vs. Cheney, 2011	−0.304	−0.207	−1.017	−0.592
	(−0.150)	(−0.103)	(−0.453)	(−0.284)
Cheney, 2010 vs. Cheney, 2012	0.172	0.074	−1.266	−0.739
	(0.086)	(0.037)	(−0.535)	(−0.347)
Cheney, 2011 vs. Cheney, 2012	0.362	−0.216	−0.061	0.000
	(0.179)	(−0.108)	(−0.031)	(0.000)
Rabbit Slough vs. Scout, 2011	7.089	−3.786	0.690	−4.568
	(0.962)	(−0.884)	(0.326)	(−0.916)
Rabbit Slough vs. Scout, 2012	4.211	−1.405	−0.524	−2.212
	(0.903)	(−0.575)	(−0.254)	(−0.742)
Scout, 2011 vs. Scout, 2012	−2.316	2.375	−1.378	2.696
	(−0.757)	(0.764)	(−0.565)	(0.803)

Note: The size-effect correlation, *r*, is indicated in parentheses.

Size distribution, age, and reproductive condition

Females from Cheney Lake in the first year following introduction (2010, F_1) were smaller than females from the anadromous, parental population used for the introduction in 2009 (Fig. 2). Apparently none of the introduced parents survived the winter, as we did not catch any in our traps. The smallest female we examined from Rabbit Slough was 64 mm SL, whereas the largest female examined from Cheney Lake in 2010 was 56 mm SL (Fig. 2) (also see Bell *et al.*, 2016). Among the Cheney Lake females, 50% were 43 mm SL or smaller. Of those females ≤ 43 mm SL, only 26% were reproductively mature; but 95% of females > 43 mm SL were reproductively mature (Fig. 2).

The size distribution in our sample of Cheney Lake females from the second year following introduction (2011) indicates that the population was likely comprised of first- and second-generation descendants of the introduced parents (Fig. 2). We assume that individuals falling into the group with larger body size were the two-year-old, F_1 offspring of the anadromous parents while individuals in the smaller group were the one-year-old, F_2 offspring of the F_1 generation. Both F_1 and F_2 fish appeared to be reproducing, although the frequency of reproducing females was higher for two-year-old, F_1 females. Assuming females smaller than 45 mm SL (Fig. 2) were one-year-old, F_2 females, 22% of the one-year-old females ($n = 93$) were reproductively mature. Among the two-year-old, F_1 females ≥ 45 mm SL ($n = 108$), 99% were reproductively mature.

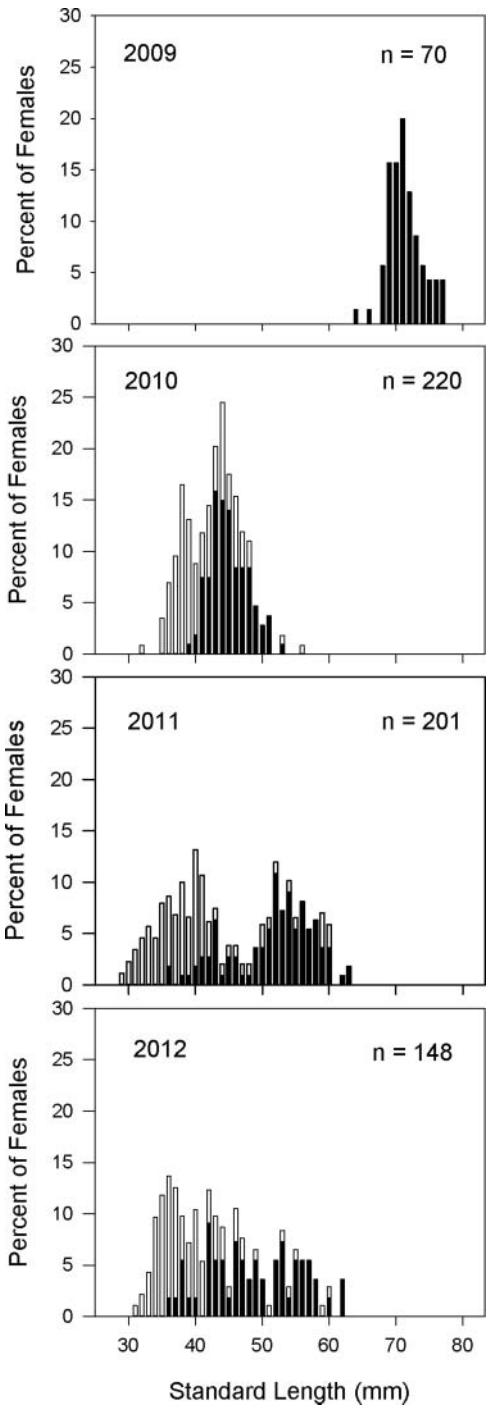

Fig. 2. Length-frequency distributions (stacked bars) of female threespine stickleback from Rabbit Slough in 2009 and from Cheney Lake in 2010, 2011, and 2012. Solid bars represent females with clutches, while open bars represent females without clutches.

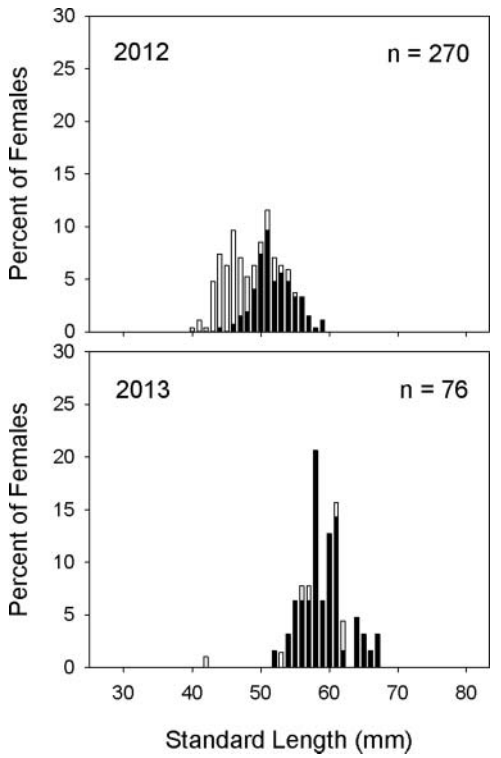

Fig. 3. Length-frequency distributions (stacked bars) of female threespine stickleback from Scout Lake in 2012 and 2013. Distributions of females examined from Rabbit Slough are illustrated in Fig. 2 for comparison. Solid bars represent females with clutches, while open bars represent females without clutches.

In the third year following introduction in Cheney Lake (2012), the population likely was largely comprised of fish born in the lake in the prior 2 years (i.e. one- and two-year-old, F_3 and F_2 individuals) because few threespine stickleback in freshwater habitats within south-central Alaska live beyond 2 years (Baker *et al.*, 2008). In 2012, 28% of females smaller than 45 mm ($n = 100$, one-year-old, F_3) were reproductively mature, whereas 98% of two-year-old, F_2 females ≥ 45 mm ($n = 48$) were reproductively mature.

The first annual sample from Scout Lake post-introduction in 2012 also showed a single size group of one-year-old, F_1 fish, indicating that the parents did not survive the winter (Fig. 3). Among females in the sample ($n = 270$), 58% were reproductively mature. All females except one caught in the second year post-introduction in 2013 ($n = 76$) were ≥45 mm SL and were likely two-year-old, F_1 fish. Of those larger females, 100% were reproductively mature.

Clutch size

Reproductive females in Rabbit Slough (64.2–78.3 mm SL) produced 145–531 eggs per clutch, whereas reproductive females caught in Cheney Lake in 2010–2012 (36.1–62.9 mm

SL) produced 24–377 eggs per clutch. A difference in size-adjusted least squares mean clutch size was detected among samples of Rabbit Slough females from the parental population and females caught in Cheney Lake (ANCOVA: $F_{3,335} = 649.348$, $P < 0.0001$; Table 1). The size-adjusted mean clutch sizes (anti-logs) were greater in Cheney Lake each year (2010 = 166, 2011 = 177, 2012 = 162) compared with Rabbit Slough ($n = 148$) ($P < 0.0001$, FDR-adjusted). The mean size-adjusted clutch sizes for Cheney Lake females did not differ significantly among years ($P > 0.05$, FDR-adjusted; Fig. 4).

Females caught in Scout Lake in 2012 and 2013 (38.4–67.4 mm SL) produced 103–474 eggs per clutch. There was a significant difference in size-adjusted clutch sizes among samples of females from Rabbit Slough and females from Scout Lake (ANCOVA: $F_{2,255} = 157.940$, $P < 0.05$; Table 1). The size-adjusted mean clutch sizes (anti-logs) were larger in Scout Lake each year (2012 = 361, 2013 = 231) compared with Rabbit Slough ($n = 149$) ($P < 0.05$, FDR-adjusted). The size-adjusted mean for Scout Lake in year 2 (2013) was significantly greater than the size-adjusted mean in 2012 ($P < 0.05$, FDR-adjusted).

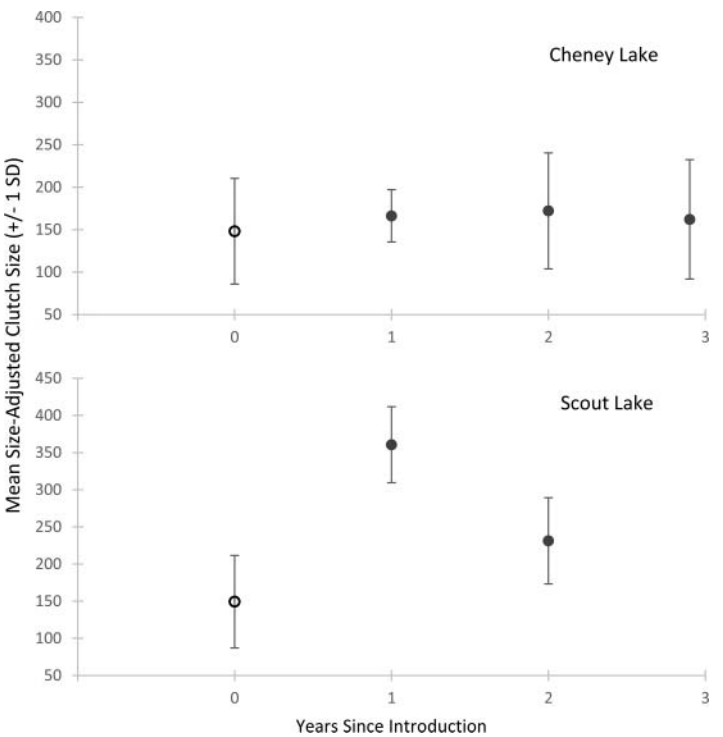

Fig. 4. Mean size-adjusted clutch size (anti-logs) of female threespine stickleback from Rabbit Slough in 2009 (open circles, $n = 69$); Cheney Lake (upper panel, solid circles) in 2010 ($n = 105$), 2011 ($n = 112$), and 2012 ($n = 54$); and Scout Lake (lower panel, solid circles) in 2012 ($n = 123$) and 2013 ($n = 69$). Bars represent one standard deviation from the mean, and years since introduction correspond to calendar years for samples from each lake.

Egg mass

Egg mass of females from Rabbit Slough ($n = 70$) was 435–789 μg, whereas females from Cheney Lake ($n = 281$) produced egg masses between 313 and 699 μg. Females from Rabbit Slough and Cheney Lake showed differences in size-adjusted mean egg mass among the samples (ANCOVA: $F_{1,131} = 17.648$, $P < 0.05$; Table 1). None of the size-adjusted mean egg masses in the annual samples from Cheney Lake differed significantly (2010 = 492 μg, 2011 = 563 μg, 2012 = 568 μg, anti-logs) from the mean size-adjusted egg mass for Rabbit Slough (520 μg, anti-log; $P > 0.05$, FDR-adjusted; Fig. 5). Within Cheney Lake, size-adjusted egg mass was larger in years 2 and 3 (2011 and 2012) than in year 1 (2010; $P < 0.05$, FDR-adjusted).

Scout Lake females ($n = 200$) produced egg masses between 378 and 673 μg. There were significant differences in size-adjusted mean egg mass among the samples from Scout Lake and Rabbit Slough (ANCOVA: $F_{2,103} = 3.989$, $P < 0.05$; Table 1). Neither of the Scout Lake samples differed significantly from Rabbit Slough (540 μg, anti-log; $P > 0.05$, FDR-adjusted; Fig. 5). In the first year after introduction (2012), Scout Lake females had a lower mean egg mass (476 μg) than in the second year, 2013 (555 μg; $P < 0.05$, FDR-adjusted).

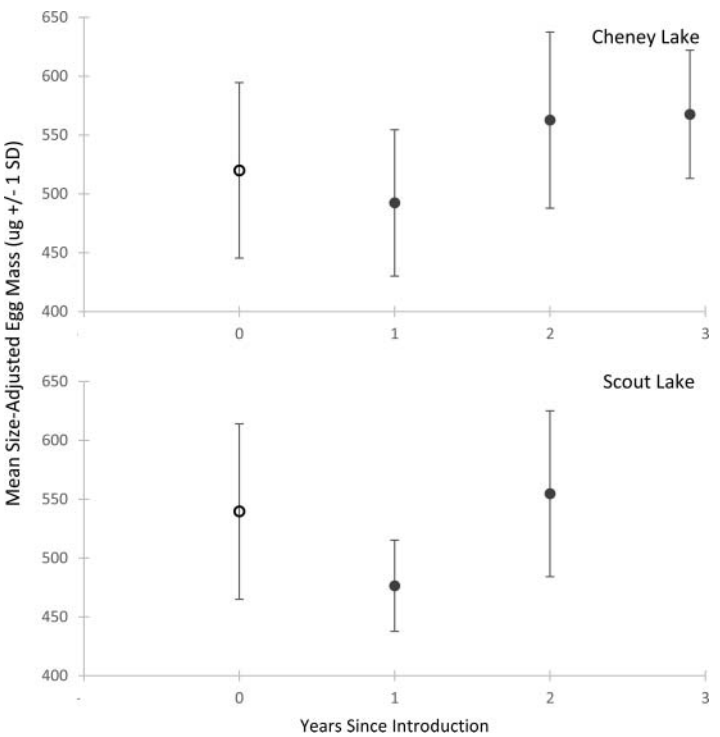

Fig. 5. Mean size-adjusted egg mass (μg) of female threespine stickleback from Rabbit Slough in 2009 (open circles; $n = 46$); Cheney Lake (upper panel, solid circles) in 2010 ($n = 33$), 2011 ($n = 34$), and 2012 ($n = 22$); and Scout Lake (lower panel, solid circles) in 2012 ($n = 37$) and 2013 ($n = 26$). Bars represent one standard deviation from the mean, and years since introduction correspond to calendar years for samples from each lake.

Clutch mass

Rabbit Slough females ($n = 70$) had unadjusted clutch masses ranging from 0.092 to 0.252 g, whereas Cheney Lake females ($n = 281$) produced clutch masses between 0.023 and 0.217 g. After adjusting for body size, adult females showed significant differences in mean clutch mass among samples from Rabbit Slough and Cheney Lake (ANCOVA: $F_{1,131} = 391.209$, $P < 0.0001$; Table 1). Size-adjusted mean clutch mass in Cheney Lake was larger in all years (2010 = 0.094 g, 2011 = 0.115 g, 2012 = 0.110 g, anti-logs) compared with the ancestral Rabbit Slough population (0.085 g; $P < 0.05$, FDR-adjusted; Fig. 6). Mean clutch mass rose significantly in Cheney Lake females from the first (2010) to the second year (2011; $P < 0.05$, FDR-adjusted), but years 2 and 3 did not differ significantly from one another ($P > 0.05$, FDR-adjusted).

Females from Scout Lake ($n = 200$) had clutch masses between 0.056 and 0.193 g. A significant overall difference in mean size-adjusted clutch mass was detected for all samples from Scout Lake and Rabbit Slough (ANCOVA: $F_{3,103} = 96.003$, $P < 0.0001$; Table 1). The size-adjusted mean clutch mass of Scout Lake females in 2012 (0.21 g, anti-log) and 2013 (0.14 g) was greater than that of females from the ancestral Rabbit Slough population (0.09 g; $P < 0.05$, FDR-adjusted). Females from Scout Lake had a larger mean clutch mass in year 1 than in year 2 ($P < 0.05$, FDR-adjusted).

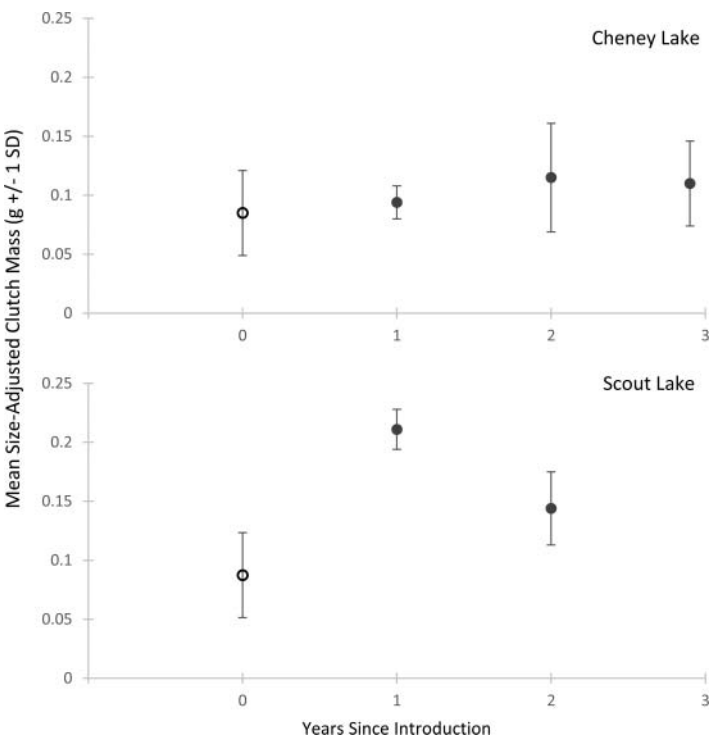

Fig. 6. Mean size-adjusted clutch mass (g) of female threespine stickleback from Rabbit Slough in 2009 (open circles; $n = 46$); Cheney Lake (upper panel, solid circles) in 2010 ($n = 33$), 2011 ($n = 34$), and 2012 ($n = 22$); and Scout Lake (lower panel, solid circles) in 2012 ($n = 37$) and 2013 ($n = 26$). Bars represent one standard deviation from the mean, and years since introduction correspond to calendar years for samples from each lake.

DISCUSSION

The postglacial colonization of freshwater habitats by oceanic threespine stickleback is a useful model for adaptive radiation (Bell and Foster, 1994; Bell *et al.*, 2004). Our goal was to investigate early phenotypic shifts in female life-history traits following the reintroduction of anadromous threespine stickleback into two lakes with the aim of simulating the postglacial colonization that preceded the variability seen within and among populations in south-central Alaska today. This study provides insights into the immediate changes in reproductive traits that occur in the first generations subsequent to colonization. Although the study populations do not fit the definition of invading populations, we use terminology from invasion studies to describe these colonizers.

Consistent with expectations for an opportunistic strategy, female threespine stickleback in both Cheney Lake and Scout Lake showed significantly smaller body sizes, larger size-adjusted clutch sizes, and greater size-adjusted clutch masses than the ancestral Rabbit Slough population (Figs. 1, 4, 6). Cohen's *d* calculations indicate large effect sizes for standard length and clutch mass (Table 1). These changes were apparent in the first generation in both lakes and occurred over subsequent generations. The shift in body size is consistent with the expectation that invading populations would shift to a smaller body size at sexual maturity, as well as the smaller sizes of resident freshwater threespine stickleback in south-central Alaska (Davis, 2005; Baker *et al.*, 2008; Burton *et al.*, 2010; Grabowska *et al.*, 2011; Amundsen *et al.*, 2012). Size-adjusted egg mass (Fig. 5) displayed a distinct pattern compared with clutch size and clutch mass. Ovum mass did not differ between the ancestral population and the colonizing populations during any years post-introduction. Within each colonizing population, however, egg mass increased significantly beginning with the second year.

These observed shifts towards an opportunistic strategy occurred suddenly within the first generation after introduction in both lake populations. Phenotypic shifts towards smaller body size at sexual maturity, larger clutch sizes, and greater reproductive effort have been observed during the establishment of a number of invading or colonizing species (Bohn *et al.*, 2004; Davis, 2005; Fox *et al.*, 2007; Amundsen *et al.*, 2012). We conclude that in the first few generations following colonization, threespine stickleback are capable of shifting these traits in a manner similar to invasive species.

Most oceanic threespine stickleback, including anadromous threespine stickleback in south-central Alaska, breed after reaching age 2 (Baker *et al.*, 2008), but a majority of one-year-old females from both lakes were reproductively mature in the first year after introduction (Figs. 2, 3). Although the colonizing lake females were breeding at a significantly smaller body size relative to ancestral Rabbit Slough females, our results are not entirely consistent with our hypothesis that the colonizing threespine stickleback populations would shift towards reproducing at an earlier age. Among reproducing females in Cheney Lake, there were more females breeding at age 2 than age 1 one year post-introduction (Fig. 2). We cannot draw definitive conclusions about average breeding age in Scout Lake. All but one female caught in Scout Lake in the second year was age 2, suggesting that few offspring produced by the one-year-old females in the first year survived the winter (Fig. 3).

A larger relative clutch mass indicates a higher allocation of energy towards reproductive effort in female threespine stickleback (Baker, 1994; Baker *et al.*, 2008) and greater reproductive effort appears to be common among invading fish species (Bohn *et al.*, 2004; Fox *et al.*, 2007; Burton *et al.*, 2010; Amundsen *et al.*, 2012). The relative clutch masses of most resident freshwater threespine stickleback populations in south-central Alaska are smaller than the relative clutch

masses of ancestral, oceanic populations (Baker *et al.*, 2008). In Scout Lake, size-adjusted mean clutch mass before the pike introduction was 0.043 g (Heins *et al.*, 2016), representing a lower reproductive effort than both Rabbit Slough (0.09 g) and the new Scout Lake population (2012 = 0.21 g, 2013 = 0.14 g). We do not have data on Cheney Lake females before the pike introductions. The observation that the colonizing Scout Lake population exhibits higher reproductive effort than both the native Scout Lake population and the Rabbit Slough population suggests that, in the short term, reproductive success is maximized via an opportunistic life-history strategy that differs from the fitness pay-offs achieved well after population establishment. The combined changes of smaller body size at maturation and greater reproductive effort fit the assumption that there is a trade-off between body size and reproductive effort (Stearns, 1980; Baker, 1994; Baker *et al.*, 2008).

According to life-history theory, shifts in size and age of reproduction may be expected during periods of population growth due to the advantage of shorter generation time (Stearns, 1980; Roff, 1992). This may explain why expanding invasive fish populations, such as pumpkinseed (*Lepomis gibbosus*), eastern mosquitofish (*Gambusia holbrooki*), and vendace (*Coregonus albula*) sometimes exhibit these plastic changes (Bohn *et al.*, 2004; Fox *et al.*, 2007; Benjamini *et al.*, 2008; Amundsen *et al.*, 2012). Although invasive fish populations have been shown to exhibit abrupt shifts towards opportunistic life-history strategies which they maintain through multiple generations, other populations shift towards equilibrium strategies over time (Bohn *et al.*, 2004; Fox *et al.*, 2007; Ribeiro and Collares-Pereira, 2010; Grabowska *et al.*, 2011; Amundsen *et al.*, 2012). Shifts to either strategy may occur among different populations of the same species (Fox *et al.*, 2007; Ribiero and Collares-Pereira, 2010), suggesting that life-history variability in response to different environmental pressures is a common characteristic among successful invaders (Burton *et al.*, 2010). The success of invading or colonizing populations may be most dependent on rapid phenotypic shifts in life-history traits because they are intimately linked with fitness (Bohn *et al.*, 2004; Fox *et al.*, 2007).

Populations in the two lakes were not compared statistically here because a more informative comparison can be made when data have been collected for several years post-introduction and an assessment as to whether any initial differences persist can be made. We expected life-history traits to shift in a manner consistent with an opportunistic strategy; but Cheney and Scout Lake are different in several ways, so we did not necessarily expect identical patterns in both lakes. For example, Scout Lake is more than ten times larger than Cheney Lake. Scout Lake females appeared to be larger than Cheney Lake females in the first two years post-introduction. Scout Lake females produced clutches that were 2.4 times larger than the ancestral size, whereas Cheney Lake females produced clutches that were 1.1 times larger. The pattern that stands out as consistent between the lakes is egg mass (Fig. 5). Within the two introduced lake populations, eggs were heavier after the first year. In the first year, all reproducing females were one-year-olds that produced smaller eggs, whereas in later years a significant proportion was two years old. In a situation where females must reproduce at a small size and young age, reducing offspring size and increasing offspring number may be favourable when it is possible to reproduce again at a later time and larger size (Hutchings, 1993). In salmon, the optimal strategy for females travelling farther during migration is to prioritize larger clutches over larger eggs (Kinnison *et al.*, 2001). Perhaps this trade-off occurs among females when the environment is entirely novel.

The capacity for abrupt phenotypic change in anadromous threespine stickleback has probably resulted from their accumulation of alleles that are adaptive for fresh water for at least 10 million years (Bell *et al.*, 2009). Anadromous and freshwater-resident threespine stickle-

back often breed in sympatry, and behavioural isolating mechanisms may fail and permit freshwater alleles to flow into populations of the anadromous ancestor (Colosimo *et al.*, 2005; Schluter and Conte, 2009). There is tentative evidence that these alleles are recessive to those for life in the ocean. Thus, they can be carried at low frequencies in oceanic populations, rarely be expressed, and experience purifying selection only in rare homozygotes (Bell and Aguirre, 2013). Accordingly, Jones *et al.* (2012) identified hundreds of loci in freshwater threespine stickleback populations that have alleles that must have been carried in oceanic populations to be used again and again to adapt to freshwater conditions after colonization. Since these alleles are adaptive for fresh water and exist as multiple copies, not as single mutations, selection can rapidly increase their frequencies in fast-growing freshwater isolates founded by oceanic stickleback (Barrett and Schluter, 2007). The changes that occur in the early years after threespine stickleback colonize lakes could be due to natural selection acting on plasticity, new genetic variation, standing genetic variation, or a combination of these (Colosimo *et al.*, 2005; Fox *et al.*, 2007; Hendry *et al.*, 2008; Grabowska *et al.*, 2011; Lucek *et al.*, 2012, Ghalambor *et al.*, 2015). To our knowledge, this type of genetic analysis has not been done for rapid shifts in life-history traits in this species. Such analyses could potentially distinguish between plasticity, new genetic variation, and standing genetic variation as causal factors underlying rapid life-history change in the early years following colonization.

Phenotypic plasticity is a probable component of the early changes observed in the life-history traits of threespine stickleback in Cheney Lake and Scout Lake, given the short time period this study encompassed. Of the life-history traits we observed, female body size, clutch size, and clutch mass have all been shown to have significant adaptive plasticity in threespine stickleback populations, both in the wild and in laboratory experiments (Baker, 1994; Baker *et al.*, 2008, 2015). Egg mass does not show appreciable short-term plasticity compared with other female threespine stickleback life-history traits when diets and other conditions are manipulated in the lab (Baker *et al.*, 2015). Our results suggest that plasticity played a role in the differences in egg mass observed within both lakes between the first and second years of breeding in the new environment. This is especially apparent in Scout Lake, where the females breeding in the first and second year are likely members of the same generation but produced significantly different egg masses in the two years. If egg mass is solely based on heritable variation, we would not expect to see an appreciable difference between those two years. Our results are also consistent with data for stickleback in established populations in which two-year-old stickleback produce larger eggs than one-year-old stickleback (Baker *et al.*, 2008). The capacity of the threespine stickleback for phenotypic plasticity in many traits may be a major advantage this species possesses during the colonization of a new environment.

The changes in life-history traits studied here may continue in the coming years as the colonizing threespine stickleback become fully integrated into the Cheney Lake and Scout Lake ecosystems. The population crashed after the last year of this study in each lake. In 2013, only 13 fish were caught in Cheney Lake (Bell *et al.*, 2016). Fish were almost as difficult to catch in Scout Lake in 2014. We do not know what caused the populations to crash, but the new environments may have imposed hard selection. This may be especially evident in the data from Scout Lake. In 2013, there were no one-year-old reproductive females. Reproduction apparently was poor in 2012 and produced very few one-year-old reproductive females in 2013. This scenario apparently happened again in 2013, so there were few recruits in 2014. In the summer of 2015, the threespine stickleback populations in both Cheney Lake and Scout Lake appeared to be larger and to have produced numerous fry, based on visual observations and trapping success (Bell *et al.*, 2016), which indicates

adaptation and recovery of the populations. Additional study in the coming years should provide a chance to observe further shifts in life-history traits, perhaps more towards what is expected for established freshwater populations.

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