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Trajectory and rate of change in female life-history traits following colonization of a freshwater, lacustrine environment by oceanic threespine stickleback

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ABSTRACT

Question: What is the trajectory of change of female life-history traits following colonization of a freshwater lake by oceanic threespine stickleback?

Hypotheses: Based on comparative studies of many freshwater populations, we predict that (1) reproductive effort and clutch size will be reduced from the ancestral condition, and (2) the breeding pool of females will change to include greater percentages of younger breeders. The trajectory of change in egg size cannot be predicted from current knowledge.

Organism and field site: Threespine stickleback (*Gasterosteus aculeatus*). Loberg Lake, south-central Alaska, was poisoned in 1982 to remove all fish, and then restocked by the Alaska Department of Fish and Game with trout only; the lake was apparently naturally recolonized by anadromous stickleback between 1983 and 1989.

Methods: Annual small-mesh trap collections between 1992 and 2015, and trap collections of the presumed ancestral stock in a nearby stream, the latter providing the starting point for evolutionary trajectory calculations. Our study encompassed an estimated 21 generations.

Conclusions: Reproductive effort and clutch size (both standardized for female body size) declined as predicted, by 28% and 41% from the ancestral values, respectively. Although the overall downward trend in both traits was substantial and rapid, a strong cyclical pattern was also present. Female breeding age also declined as predicted, with age-1 breeders becoming more prevalent in the lake. Egg size did not change appreciably.

Keywords: clutch size, egg size, freshwater invasion, *Gasterosteus aculeatus*, rapid evolution, reproductive effort.

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INTRODUCTION

The question of how rapidly organisms can adapt to changing conditions, whether driven by environmental shifts (Bradshaw and Holzapfel, 2006) or colonization events (Sakai *et al.*, 2001), has interested biologists since the time of Darwin. Studies of rapid adaptation, or its lack, to changing climates are now common, and they are providing important insights into the adaptive process (e.g. Møller and Szép, 2005). Many of these studies have taken advantage of the fact that environmental change occurred during the course of on-going, long-term studies. Similarly, studies of colonization events are becoming more frequent, and these should also provide important, but subtly different, insights into rapid evolutionary processes (Sakai *et al.*, 2001; Lee, 2002). Nevertheless, despite the wealth of studies on biological invasions leading to successful colonization, we know surprisingly little about the trajectories and rates of evolution for populations colonizing novel environments (Williamson, 1999; Sax *et al.*, 2007; Colautti and Lau, 2015). The dearth of knowledge has resulted, in part, from studies of invasions often having focused on ecological rather than evolutionary processes (Colautti and Lau, 2015). Moreover, investigations contrasting successfully established populations to their ancestral, source populations across different time scales are lacking (Amundsen *et al.*, 2012).

Organismal traits that have been examined during invasions typically are a biased set of potentially important traits, with female life-history characteristics having been studied far less often than most other traits (Williamson, 1999). This situation is particularly unfortunate as life-history traits may be crucial to invasion success because they are more closely related to fitness than many other characteristics (Kingsolver *et al.*, 2001; Rooke and Fox, 2018). Furthermore, studies that have quantified reproductive characteristics of colonizing populations typically have been based upon data from populations that already have occupied the new habitat for many generations (Huey *et al.*, 2005; Westley, 2011; Colautti and Lau, 2015). One exception of note is the approximately 20-year study of vendace, a freshwater whitefish, colonizing a new lake in Scandinavia (Amundsen *et al.*, 2012), in which several life-history traits shifted dramatically, primarily due to natural selection in the new lake.

Colonizing populations are expected to experience strong selective pressures (Reznick and Ghalambor, 2001), and microevolutionary rates are particularly informative metrics of phenotypic evolution in such populations (comprehensively reviewed by Hendry and Kinnison, 1999; Bone and Farres, 2001; and Kinnison and Hendry, 2001). These reviews and more recent studies (Siepielski *et al.*, 2009; Baker *et al.*, 2011) demonstrate that investigations tracking populations over many generations can provide insights into the pattern and tempo of evolution that cannot be drawn from short-term studies. Long-term studies reveal important details such as the length of time during which particular rates occur and whether change occurred gradually or in short, rapid episodes. If episodes of rapid evolution are brief, long-term studies are more likely to observe them. Likewise, fluctuations and reversals (Siepielski *et al.*, 2009) are more likely to be revealed by long-term studies. Long-term studies that capture the entire trajectory of change following an invasion from initial ‘arrival’ through ‘establishment’ to ‘integration’ (Vermeij, 1996) as the colonizing population adapts to the new environment are particularly valuable but uncommon.

Many extant freshwater populations of threespine stickleback worldwide represent successful colonization of freshwater habitats following invasion by ancestral oceanic stickleback in regions that have been deglaciated in the last 12,000–22,000 years (Bell *et al.*, 1993; Bell and Foster, 1994; McKinnon and Rundle, 2002). As a result of high standing allelic variation in oceanic populations (Lescak *et al.*, 2015), stickleback possess ‘high evolvability’, and thus typically thrive and evolve rapidly due to natural selection following freshwater colonization (Carroll *et al.*, 2007; Barret and Schluter, 2008). They also possess substantial phenotypic plasticity in many traits (Foster *et al.*, 2015), including life-history traits (Baker *et al.*, 2015). Thus, this supermodel system (Gibson, 2005; Baker *et al.*, 2013a, 2013b; Foster *et al.*, 2015; Bell *et al.*, 2016) provides an excellent one in which to study phenotypic evolution following the invasion of a new habitat.

Here, we report on a long-term study of life-history change in a population of threespine stickleback in Loberg Lake, Alaska, that was recolonized approximately 30 years ago, following treatment to remove all fish species in 1982 (Aguirre and Bell, 2012). Our study is based upon an unplanned, natural experiment and, as a result, we did not sample the first few generations following colonization. Kurz *et al.* (2016), however, investigated the earliest stages of evolution in two experimentally introduced populations of stickleback in the same region, which have the same source population as Loberg Lake. These populations may provide general information on the earliest few years following colonization, allowing us to consider the entire invasion trajectory. The evolution of lateral plate armament and body shape of the Loberg Lake population have been studied previously (Bell *et al.*, 2004; Aguirre and Bell, 2012). Thus, our study adds an additional facet to the emerging multivariate story of adaptive change in this interesting population.

Based upon a review of life-history variation across numerous, long-established freshwater populations and multiple oceanic ancestral populations of threespine stickleback (Baker *et al.*, 2008), we were able to make several predictions regarding the long-term evolutionary trajectory in the Loberg Lake population. First, and most confidently, we predicted that the new population would evolve towards a lower reproductive effort, and concomitantly a smaller clutch size. Secondly, we expected that the proportion of age-1 breeders (uncommon in ancestral populations in Alaska) would increase, resulting in a shorter average generation time. Thirdly, due to the strong seasonality of freshwater environments in Alaska, we predicted that the mean size of reproducing females at each age would decline due to the greatly shortened growing season in contrast to oceanic fish, which appear to grow year-round. The one life-history trait whose evolution we could not predict was egg size, which appears to exhibit little congruence with any environmental feature in stickleback (Baker *et al.*, 2008). We assume that our observed phenotypes in long-established populations represent states that are well adapted to their specific environments.

METHODS

Environmental setting

Loberg Lake (61.5588 N, −149.2597 W) is a small lake in the Cook Inlet Region of Alaska (Jones *et al.*, 2003). Although listed as isolated from any surface water, the lake is only about 400 m from the nearest stream, and the lake may occasionally be connected to this stream (part of the Spring Creek drainage). The Spring Creek system flows into the Palmer Hay Flats State Game Refuge, a large tidal wetland complex through which anadromous threespine stickleback migrate each spring to spawn in Spring Creek and its tributaries. Spring Creek does not appear to have resident freshwater stickleback (J. Baker, personal observation), though sampling has been limited. The phenotypes present in Loberg Lake in 1990 suggest that the colonization event consisted almost, or entirely, of anadromous fish; no anadromous morphotypes have been observed in the lake since the early 2000s, and no new pulse of anadromous fish has been detected by our sampling. Loberg Lake has a mean depth of 5.4 m and a surface area of 4.45 ha at an elevation of 23 m. Fingerling rainbow trout (*Oncorhynchus mykiss*) and silver salmon (*Oncorhynchus kisutch*), both of which eat stickleback (Reimchen, 1994), are stocked annually for sport fishing (ADF&G records), but most of them are caught, or die, before they become large enough to eat adult stickleback (Bell *et al.*, 2004).

We used a long series of annual samples (1992–2015) to characterize female life-history shifts in the population of threespine stickleback in Loberg Lake, following colonization by anadromous, oceanic stickleback. The original Loberg Lake population, presumably founded following the most recent deglaciation of the Cook Inlet Region (Reger and Pinney, 1995), was exterminated by the Alaska Department of Fish and Game (ADF&G) using rotenone in October 1982 for the benefit of recreational fishing. Threespine stickleback were subsequently detected in the lake in 1990 during surveys associated with the annual stocking of salmonids (ADF&G Records; Bell *et al.*, 2004). The morphology of adult stickleback in Loberg Lake in 1990, which closely resembled that of the oceanic ancestor, suggested that the lake was invaded by anadromous stickleback only a few years prior to 1990, likely between 1983 and 1988 (Bell *et al.*, 2004; Aguirre and Bell, 2012). The fact that 96% of the stickleback in 1990 were still fully-plated (ancestral phenotype) suggests that colonization was closer to the latter year than the former. Thus, our annual sampling commenced 4–9 years following recolonization. In our evolutionary rate calculations, we used 1986 as the year of colonization, but calculations using 1983 or 1988 did not result in qualitatively different pictures of the trajectory.

Data collection

Dates of annual sampling varied between 20 May and 15 June (most between 27 May and 3 June), but were all well within the period of most intense breeding in south-central Alaska (Heins *et al.*, 1999). We set metal, Gee-type traps with funnels at both ends, with either 3-mm or 6-mm mesh, near the shoreline, near structures (downed trees; vegetation patches) that might attract stickleback. We spaced traps every 5–10 m in the general vicinity of the public access. We sought a minimum of about 50 gravid female stickleback per sample, though in a few years we did not achieve this goal. When very large numbers of fish were caught, we preserved a large, representative sample in addition to our focal females. We euthanized fish in MS222 and preserved and then stored them in buffered 10% formalin. We released remaining fish alive at the point of capture. Over the course of the study, we collected data on 2113 fish, with annual sample sizes ranging from 12 to 236 across the 24 annual samples, with a mean of 88 females (Table 1).

We classified female threespine stickleback into six reproductive stages following Baker *et al.* (1998) and Heins *et al.* (1999), with females having mature, ripening, and ripe ovaries being both sexually and reproductively mature. We measured standard body length (SL, to 0.1 mm) and blotted wet eviscerated somatic mass (0.001 g) on all reproductive females. Clutch size was counted directly after separating out all enlarged oocytes or eggs, which were clearly discernible in the ovaries (Heins and Baker, 1993). We measured clutch mass of ripening and ripe females (eggs fully developed) to the nearest 0.00001 g after drying for 24–28 hours at 40°C; mean egg mass of each female was then calculated to the nearest microgram by dividing dry clutch mass by clutch size. Preserved fish used in our study were stored in 10% buffered formalin for 1–3 years prior to dissection.

Data analysis

We used length-frequency plots based on 1-mm-SL intervals to estimate the age at which females were reproducing, from which we derived our estimates of the number of generations that had elapsed since recolonization. The strong seasonal nature of threespine stickleback reproduction in Alaska and other high-latitude environments often creates relatively distinct size modes, allowing size-frequency plots to be an acceptable method of age estimation for the species (Baker *et al.*, 2008). Calculations using the program MIX 3.1 (Macdonald and Pitcher, 1979) were used to confirm the approximate cut-off between age-1 and age-2 females. The majority of breeding anadromous females in Alaska are two years of age (Baker *et al.*, 2008; Rollins *et al.*, 2017), and we used this as the starting generation length at colonization.

Reproductive allocation (here estimated as clutch mass) and clutch size are strongly correlated with female body size in stickleback (Baker *et al.*, 2008), so we used linear regression of log-transformed trait values and female SL to standardize all values to a common female size. Slopes of the relationship between clutch mass and SL, and clutch size and SL, were remarkably similar across our 24 annual samples, with only 1996 and 2013 differing (modestly flatter) from the others. Analysis deleting the 1996 and 2013 samples substantiated this observation, indicating no significant slope differences among the remaining 22 samples ($F_{20,874} = 1.09$, $P = 0.36$). Samples from 1996 and 2013 did not differ in slope ($F_{1,111} = 0.06$, $P = 0.80$). To standardize life-history trait values for the 22 samples with similar allometry, we used a single, common slope; we used the year-specific slopes to standardize values in 1996 and 2013. All allometric adjustments were to the overall mean female SL in Loberg Lake over the entire time series (45.5 mm SL). We note that using the common within-group slope within a simple ANCOVA using all samples gave nearly identical results. Egg size is less strongly and more variably related to body size (Baker *et al.*, 1998, 2008), thus we did not adjust egg size for body size. To estimate reproductive allocation, clutch size, and egg size of the founding, ancestral population, we used reproductive data from the presumed ancestral population in Rabbit Slough, a tributary to Spring Creek. To incorporate likely annual variation, we analysed data from a set of four annual samples ($N = 302$ females) provided by R.W. King (unpublished data).

We estimated rates of evolutionary change in phenotype in haldanes by regressing female size-adjusted annual means for clutch mass and clutch size (scaled by their pooled standard deviations) and ln-transformed raw egg size and SL against the estimated number of elapsed generations (Hendry and Kinnison, 1999). Following Hendry and Kinnison (1999), we refer to the changes we observed in this investigation as 'phenotypic evolution', although the divergence of colonizing stickleback revealed in this study likely reflect changes in the genetic variation underlying the observed traits (McGuigan *et al.*, 2011; Lescak *et al.*, 2015). All statistical analyses were performed using STATISTICA v.13 software (TIBCO Software, Inc.)

RESULTS

Age and size of reproducing females

Length-frequency plots for years with the largest sample sizes (Table 1) showed a break at around 46 mm SL (see Fig. 1B) for determining, on average, the reproductive age of stickleback in Loberg Lake, at least for the two most abundant age groups (age-1 and age-2). This estimate was confirmed by calculations using MIX 3.1 (Macdonald and Pitcher, 1979). The

Table 1. Numbers of gravid female stickleback dissected each year from Loberg Lake (total $N = 2113$)

Year	*n*	Year	*n*
1992	49	2004	78
1993	12	2005	69
1994	13	2006	141
1995	236	2007	146
1996	154	2008	148
1997	91	2009	77
1998	28	2010	81
1999	56	2011	38
2000	89	2012	50
2001	115	2013	43
2002	228	2014	50
2003	64	2015	57

length-frequency plots (Figs. 1A, B) indicated that most breeding females in 1995–1996 were age-2 (many smaller fish were collected, but none were gravid females), while in 2006–2007 a large percentage of age-1 females were also breeding. Using ages estimated by the methods above, we then estimated the weighted mean age of reproducing females as an indicator of generation time in each sample (Gaillard *et al.*, 2005). Although there is some variation across years, presumably due to differing growth rates across years, our long-term rates calculations should be relatively robust to these small fluctuations. In defining ages using this criterion, the time span of 30 years from our assumed date of recolonization (1986) encompassed an estimated 21 generations.

The size of reproductive females in our earliest samples, taken an estimated 6 years (three generations) post-recolonization, showed a dramatic decrease in average body size (Fig. 2). This initial decline probably largely represents a plastic response, as the growing season in fresh water is perhaps only half that in the ocean, where growth may occur throughout the year. Additionally, our first two Loberg Lake samples (representing 1992 and 1993) are based on only 12 and 13 adult females, respectively, so these means may not be fully representative. In our calculations of evolutionary rate of change in breeding size, we thus include only samples from 1994 onward. By the tenth generation post-colonization, mean female size had stabilized at ~45 mm SL (Fig. 2), and most of the females were age-1 breeders. Thereafter, mean female breeding size underwent a moderate increase, reaching ~48 mm SL 11 generations later, with a mix of age-1 and age-2 reproducers represented. A piece-wise regression indicated a significant trend break at 10 generations. Using this time as a cut-off, separate regressions indicated a pronounced decline in breeding size in generations 5–10 (−0.225 haldanes, $SE = 0.048$, $P = 0.003$), followed by a slow, but significant increase (0.125 haldanes, $SE = 0.019$, $P < 0.001$).

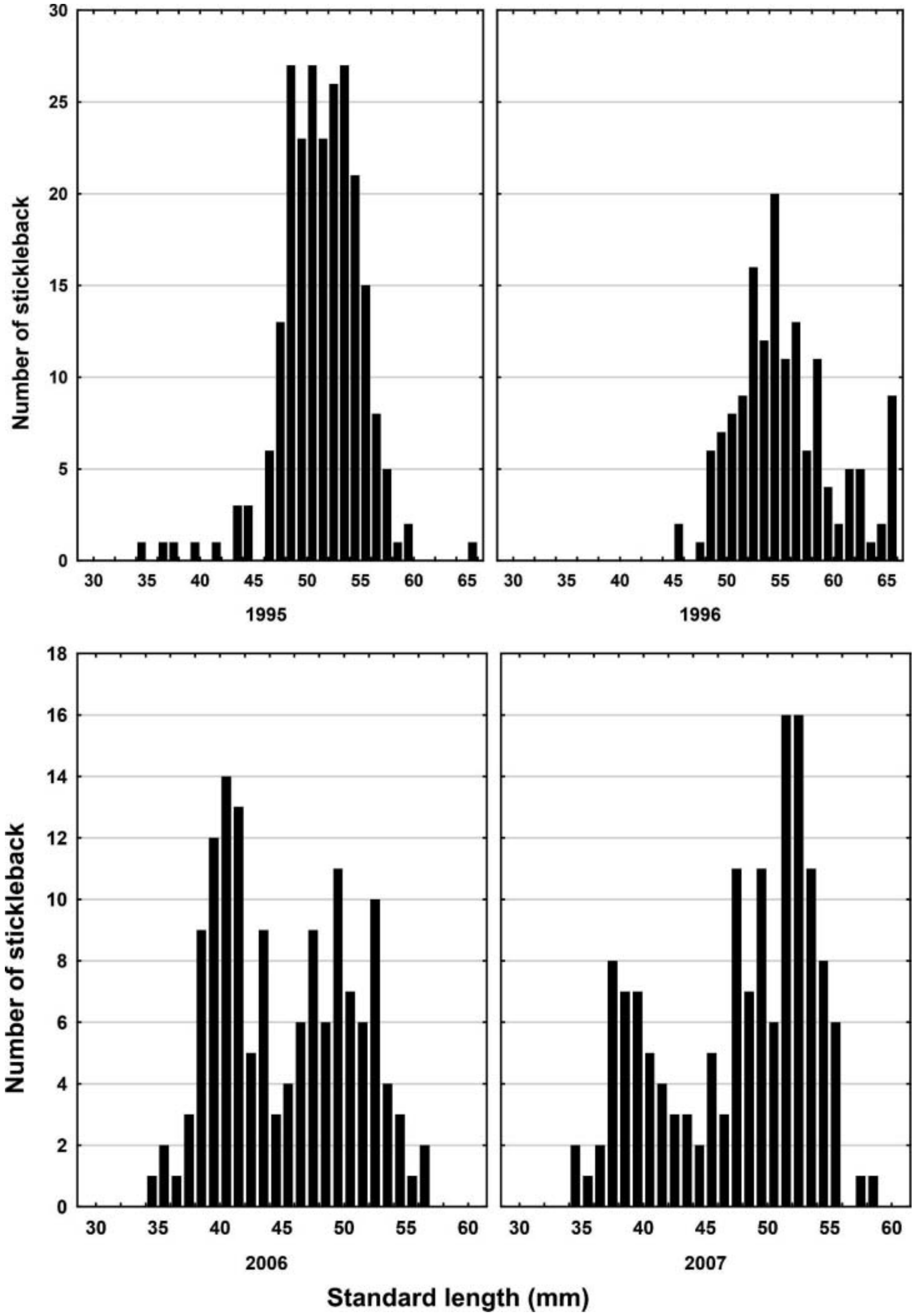

Fig. 1. Sizes of reproductive female stickleback in Loberg Lake, Alaska, in 1995 and 1996 (top panels), and in 2006 and 2007 (bottom panels). Fish less than about 46 mm SL are likely to be age-1 breeders; larger fish are age-2 (perhaps occasionally age-3) breeders. Note the considerably different estimated proportion of age-1 breeders in the two time periods.

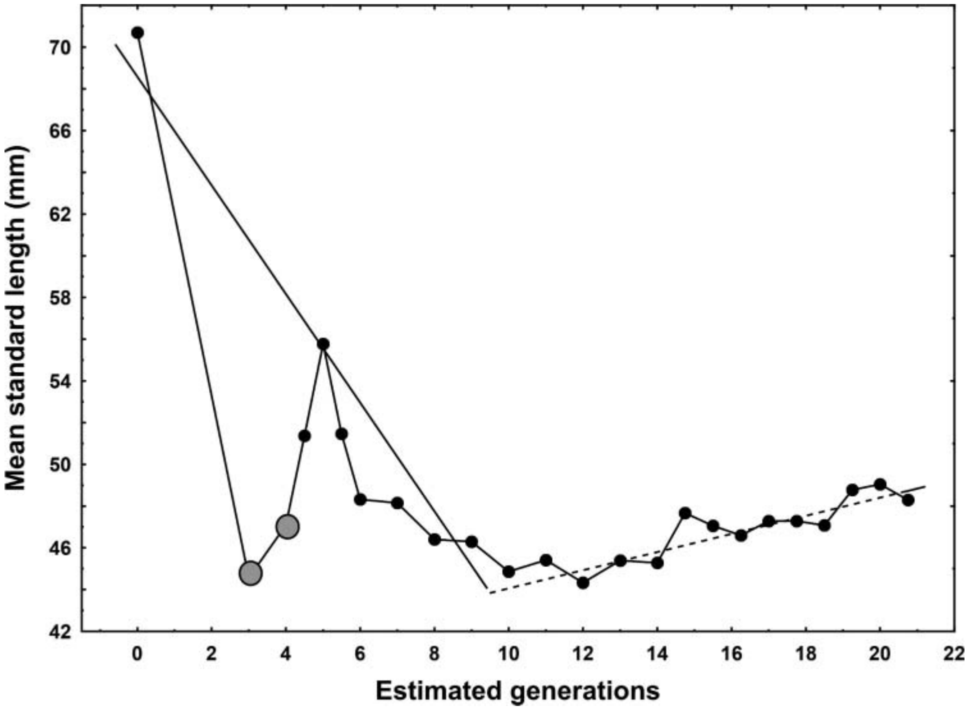

Fig. 2. Mean size of reproductive females in Loberg Lake, plotted against the estimated number of generations since recolonization of the lake by anadromous stickleback. The solid and dashed lines show the best-fit OLS regression trend for two time periods. The first data point (upper left) represents the size of females in Rabbit Slough, the presumed ancestral anadromous population. The larger, grey, symbols indicate two years of very small sample sizes; these two years were not included in the rate calculations for the first 10 generations. Because the focus is on the trend in mean values through time, error bars are not shown to reduce clutter in the figure.

Clutch mass and clutch size

Clutch mass declined significantly following recolonization (Fig. 3), with an estimated linear rate of −0.081 haldanes (SE = 0.013, $P < 0.0001$). Because haldanes express change in units of standard deviations, this overall rate represents a decline of one standard deviation per 12 generations. Clutch mass at our target SL at the end of the time series was 27.9% lower than that of the ancestral colonists, and it reached a minimum of 35.2% lower during our sampling at 16 generations post-colonization. Despite the clear general pattern, clutch mass fluctuated enormously, and possibly cyclically, over multi-generational periods. Clutch size showed an almost identical pattern as clutch mass, which was expected as the two traits are highly correlated in our data set ($r = 0.92$, $N = 24$, $P < 0.001$). The rate of divergence from the ancestral trait mean for clutch size was even more substantial than that for clutch mass (−0.094 haldanes, SE = 0.017, $P < 0.0001$), with clutch size at the end of the 21-generation period being only 40.6% of that in the ancestral population (Fig. 4). As with clutch mass, a strong cyclical variability was apparent. Back-transformed dry clutch mass of a Loberg Lake female of 45.5 mm SL (the average female length across the entire study) in 2015 was 0.050 g.

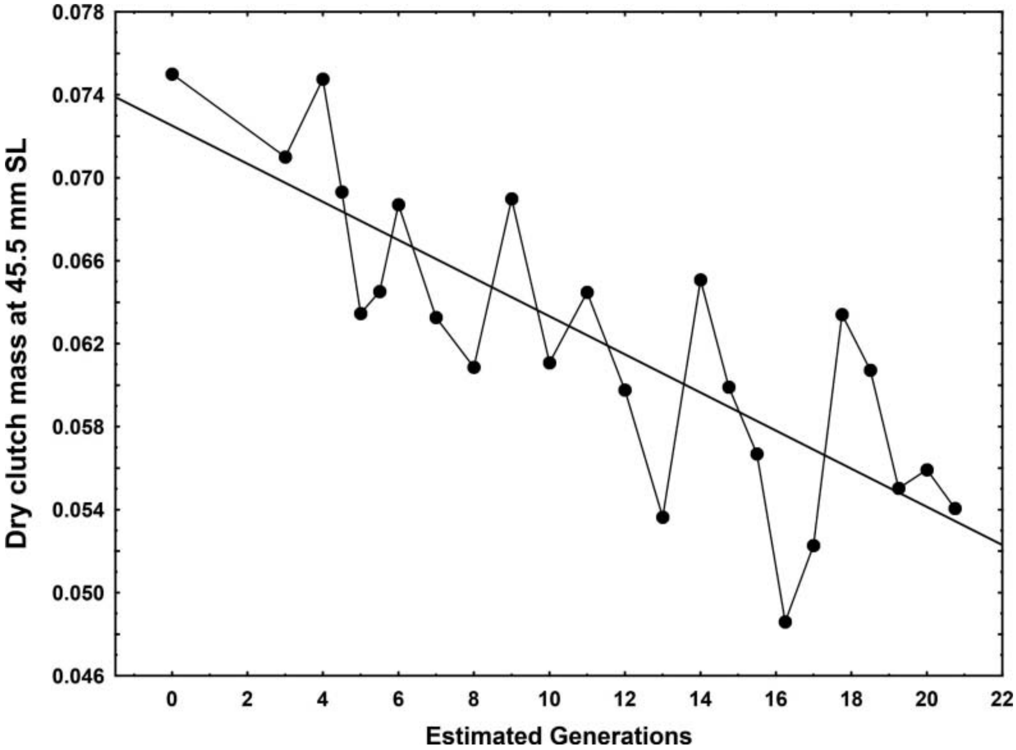

Fig. 3. Reproductive effort (as dry clutch mass, standardized to the overall mean SL of all breeding females) in Loberg Lake, plotted against the estimated number of generations since recolonization of the lake by anadromous stickleback.

Egg size

Egg size showed no consistent pattern of change across the study period (Fig. 5), and a linear regression of ln-transformed egg size on generation time produced a non-significant result ($P > 0.42$). Despite the lack of a consistent pattern, for most of the study period egg size in Loberg Lake was smaller than that in the ancestral population. During the last two generations of the study, however, egg size in Loberg Lake increased to levels approximating that of the ancestor.

DISCUSSION

The ability of the threespine stickleback to colonize freshwater habitats, and the adaptive changes that it undergoes in a variety of phenotypic traits, are well established (Bell and Foster, 1994; Foster and Baker, 2004). For example, in Alaska alone, multiple colonist populations have exhibited evolutionary adaptation in pelvic structures, body shape, and numerous aspects of life history as these traits diverged from the ancestral phenotypes (Bell and Ortí, 1994; Walker, 1997; Baker *et al.*, 2008). Almost all of these comparisons, however, are between the ancestral type and current, presumably well-adapted derived lake populations, which may

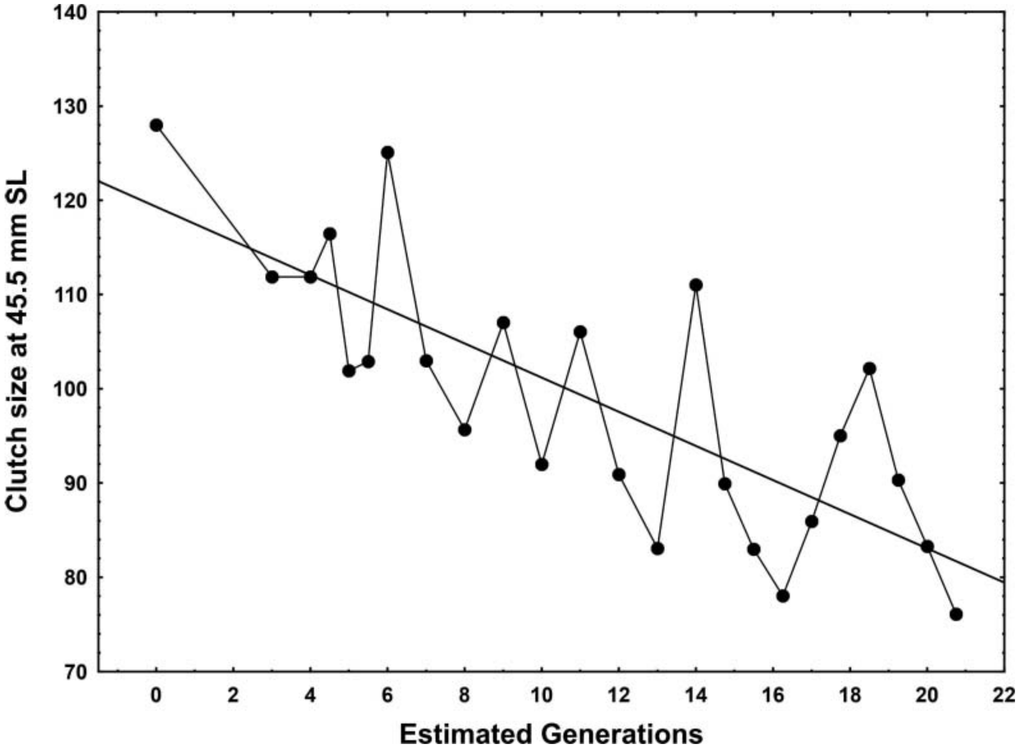

Fig. 4. Clutch size (number of eggs in at one spawning, standardized to the overall mean SL of all breeding females) in Loberg Lake, plotted against the estimated number of generations since recolonization of the lake by anadromous stickleback.

bracket a span of several thousand generations. What is rarely known is the trajectory of adaptation to fresh water, which requires both knowledge of the original state and consistent assessment throughout the colonization process. For such studies, the Loberg Lake system previously has been used to explore the pattern and rate of adaptation in both lateral plate phenotype (Bell *et al.*, 2004) and body shape (Aguirre and Bell, 2012). For both traits, substantial adaptive changes occurred within just two decades, concomitant with the changes we have observed here for life history. Although we have no direct evidence that the traits we assessed are genetically based in our study population, life-history traits are known to generally have positive additive heritability (Mousseau and Roff, 1987; Snyder, 1991).

The complete trajectory of life-history change in Loberg Lake from initial colonization to circa 20 generations thereafter can be visualized by combining our long-term findings with those of Kurz *et al.* (2016), who studied the first few years post-colonization in two other lakes in the same area. Doing so suggests that the evolutionary trajectory, at least in the initial stages, may be more complex than anticipated. For example, our long-term study, in which we could accurately estimate the ancestral condition, but had no data for the lake fish for the first few years, indicated an initial, large decrease in body size and age at reproduction, followed by more modest longer-term shifts in body size. Age of reproduction showed a similar pattern, with a mixture of fish breeding at age-1 and age-2 by about 10 years

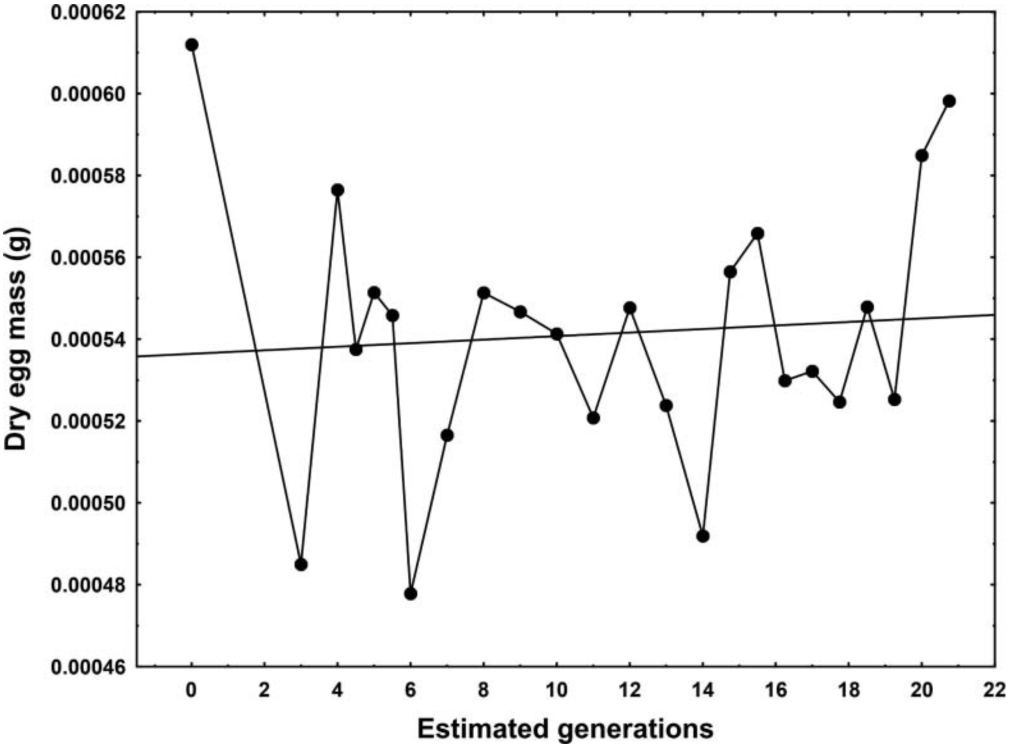

Fig. 5. Mean egg size (dry mass) in Loberg Lake, plotted against the estimated number of generations since recolonization of the lake by anadromous stickleback.

post-colonization. We expected age at reproduction upon colonization, and for several years thereafter, to average about two years, which is the age at which females in oceanic populations most often breed (Baker *et al.*, 2008). Kurz *et al.* (2016), however, found that large percentages of one-year-old fish in their two artificial colonization experiments bred in the first year after introduction, whereupon most fish then reproduced at two years of age during the subsequent few years. These combined results are consistent with a strong initial plastic response, possibly due to a low initial density of stickleback. Indeed, age at reproduction appears to be plastic in many species (Grift *et al.*, 2003), including stickleback (Baker *et al.*, 2015). In a previous study of another Alaskan Lake – Solid Rock Lake (Baker *et al.*, 2011) – we observed the same rapid decline in age and size at first reproduction.

As suggested above, the distinct 'broken stick' pattern of breeding female size is best explained by a combination of initial plasticity, followed by slower adaptive change to 'fine tune' the population to the new environment. We have evidence (J. Baker, personal observation) from controlled rearing of numerous freshwater and oceanic populations that anadromous fish grow more rapidly in their first year than do freshwater fish, but the advantage is relatively minor. Thus, it appears that the larger size-at-age in wild oceanic stickleback compared with freshwater populations is simply due to a longer, possibly year-round, growing season. This unavoidable, environmentally driven limit on first-year growth should, then, produce a strong phenotypic response upon colonization of fresh water (much smaller size-at-age), which then should produce strong selection on life-history traits (Roff, 1984), affecting both subsequent plastic (Stearns and Koella, 1986) and adaptive responses (McGuigan *et al.*, 2011).

Similarly to size and age of first reproduction, our interpretation of the trajectory of change in reproductive effort must also take into account the findings of Kurz *et al.* (2016), which indicated no decline, or even small increases, in reproductive effort in the first few generations following colonization. Nevertheless, our overall results are consistent with data presented in the reviews by Baker (1994) and Baker *et al.* (2008). The latter review, on long-established populations of threespine stickleback in south-central Alaska, indicated that more than 92% of freshwater populations exhibited lower reproductive effort than the oceanic ecotype from which they were derived. These findings formed the basis of our prediction of a decline in reproductive effort in Loberg Lake over our long study period. Baker *et al.* (2008) also observed a very wide range in reproductive effort in 83 surveyed freshwater (descendant) populations, with individual populations exhibiting as much as a 61% decline from the ancestral state, and with freshwater populations averaging 25% lower. Thus, although a decline was predictable, the magnitude of the decline seems to be determined by environmental conditions in a lake-specific manner. Preliminary calculations based on our unpublished data show a significant ($P < 0.0001$), negative relationship between a composite index of lake productivity and female reproductive effort ($b = -1.342$, $SE = 0.0086$; $n = 63$ lakes; $r^2 = 0.58$), and based on this relationship females within Loberg Lake should continue to decline in reproductive effort, as this lake exhibits relatively low productivity (J. Baker, unpublished data). Although this relationship seems, on the face of it, counterintuitive, the entire life history is under selection, and thus sometimes counter-intuitive changes in individual traits are 'mandated' by the cumulative fitness of the constellation of traits involved.

A potential explanation for the cyclic 'zig-zag' changes in clutch mass and clutch size that we observed is that the cycles reflect density-dependent selection, with cycle minima and maxima reflecting conditions of high and low density, respectively, likely with a short lag phase (e.g. Wootton, 2007). Predictable changes in life-history traits in response to population cycles are not uncommon in nature in a wide variety of taxa (e.g. Stefan and Krebs, 2001; Karell *et al.*, 2009; Klyashtorin *et al.*, 2009). Baker and colleagues have shown that clutch mass and clutch size are plastic traits in threespine stickleback (Baker *et al.*, 2013a, 2015). Thus, they could respond rapidly to density-related environmental changes (e.g. less food per individual in years of high density). Population density should be low soon after arrival of the breeding adult anadromous stickleback in the lake, and densities might remain below carrying capacity for several generations. If so, females making a high reproductive effort, or those producing a larger number of smaller offspring, would be favoured early in the colonization process, as they would on average leave more offspring (Rejmanek, 1999). As the population density approaches carrying capacity, females having lower reproductive allocation, or those producing fewer, larger offspring, could be favoured (Smith and Fretwell, 1974; Bernardo, 1996; Svensson and Sinervo, 2000; Sinervo *et al.*, 2000). Fluctuations in population density due to differing offspring survival rates during the first summer might then select for or against females with these differing traits on multiple-year time scales, leading to the cycles we observed. Baker *et al.* (2013b) found that most Alaskan stickleback populations they studied ($n = 23$) showed substantial apparent fluctuations in population size across a 20-year time span, based on catch per unit effort in small-mesh trap catches.

Bell *et al.* (2004, figure 3) found a similar 'zig-zag' pattern of lateral plate counts in Loberg Lake for the low-plate morph between 1992 and 2001, which they suggested could be due to the presence of odd- and even-year cohorts. This is unlikely to explain our fluctuations. The 'zig-zags' found by Bell *et al.* (2004) were for alternate years, whereas many of our fluctuations spanned several years. Thus, our pattern more closely resembled that for population size and body size determined by Wootton (2007), which was attributed to density dependence. Secondly, the shifts in clutch mass and clutch size do not match with differences in average breeding female size, which we would expect them to do if we had alternating age-1 and age-2 breeders cycling in alternate years.

We made no prediction about the changes in egg mass because freshwater populations exhibit an extremely wide range of mean egg masses, with some populations making larger eggs than the ancestral populations and some making smaller eggs (Baker *et al.*, 2008). There is no apparent relationship between habitat and egg mass among populations (Baker *et al.*, 2008). Kurz *et al.* (2016) did not find a difference between mean egg mass in the two populations artificially introduced into Alaska lakes within the first few years after introduction. In the present investigation, we observed a cyclical pattern of change with no apparent directional trend.

Rates of evolution

The evolutionary rates we calculated for threespine stickleback inhabiting Loberg Lake are well within those calculated for microevolutionary divergence of a variety of biological characteristics from prior multi-generation studies [overall range = 0.001–0.742 haldanes (Hendry and Kinnison, 1999)]. Compared with published rates, the population in Loberg Lake showed relatively rapid changes in size at reproduction during the approximately 30 years post-colonization. For age or size of breeding fish, mosquitofish (*Gambusia affinis*) in Hawaii showed rates of 0.001–0.007 haldanes overall for female age and length (Stearns, 1983), and mosquitofish in Nevada showed microevolutionary rates of 0.001–0.009 haldanes for length at maturity (Stockwell and Weeks, 1999). Rates of evolution for female age and size in Trinidadian guppies (*Poecilia reticulata*) were 0.014–0.043 haldanes (Reznick *et al.*, 1997). Female body length of sockeye salmon in Washington showed evolutionary rates between 0.005 and 0.074 haldanes (Hendry and Quinn, 1997). Overall, these rates range from 0.001 to 0.074 haldanes, whereas we observed a rate of −0.225 haldanes for breeding size over the first 5–10 generations in Loberg Lake. Even the rate of increase in mean length at reproduction in the last 11 generations (0.125, generations 11–21) was relatively high.

Rates of change in clutch mass (−0.081 haldanes) and clutch size (−0.094 haldanes) are also high compared with the calculated evolutionary rates for gonadosomatic index of chinook salmon in New Zealand [0.021–0.026 haldanes (Kinnison *et al.*, 1998)]. Life-history shifts in another lake in Alaska – Solid Rock Lake (Baker *et al.*, 2011) – were faster for clutch size (0.207 haldanes) but slower for clutch mass (0.042 haldanes) compared with those for the same traits in Loberg Lake. In sum, both Loberg and Solid Rock lakes have shown rapid rates of evolutionary change compared with similar life-history characteristics for other fish populations. Rates of microevolution in life-history traits of stickleback from Loberg Lake are similar to the rates Bell and colleagues (2004) calculated for change in lateral plate phenotypes for the same population. The calculated values in that study (−0.104 to −0.237 haldanes) were generally comparable to one another given the large confidence limits (Bell *et al.*, 2004).

Initial and perhaps recurring plasticity, coupled with subsequent strong selection modified by plasticity with respect to environmental features such as population density, likely underpin the trajectory of shifts in life-history traits we observed. That genetic drift, either via small founding population size or large population size fluctuations, may also have played a role, at least early in the colonization history, also seems likely. In Loberg Lake itself, intensive sampling indicated a decline in population size during 1990 and 1991 (Aguirre and Bell, 2012), although the actual population size is not known. Additionally, the reintroduced populations of stickleback in Cheney and Scout lakes experienced large decreases in population size just a few years post-establishment – in the fourth and fifth year after reintroduction in Cheney and in the third year after introduction in Scout Lake (Bell *et al.*, 2016). We know the number of stickleback reintroduced into the artificially colonized lakes (~3000), but we do not know how many oceanic threespine stickleback recolonized Loberg Lake. The rapidity of the shifts is not unexpected, given the substantially different environments inhabited by the ancestral (oceanic) stickleback and newly founded lake populations. In particular in stickleback, successful colonization of freshwater habitats may be enhanced by the accumulation of alleles that are adaptive to freshwater environments [cryptic genetic variation (Lee, 2002; Schlichting, 2008; McGuigan *et al.*, 2011)] within oceanic ancestral stickleback populations (Bell and Aguirre, 2013; Lescak *et al.*, 2015).

Summary

Anadromous, oceanic stickleback colonized Loberg Lake, Alaska, and showed an overall decrease in body length and size at sexual maturity over about 21 generations, as predicted based upon prior research on populations from Alaska. We observed a rapid and substantial decline across the first 10 generations, followed by a modest but significant increase over the last 11 generations. As expected, size-adjusted clutch mass (a proxy for reproductive effort) and clutch size also decreased over 21 generations, with cyclic fluctuations suggesting density dependence. Previous research suggests, however, that the decreases in clutch mass and clutch size followed initial, early stability, or even slight increases during the first few generations, which we were unable to observe in our study. The rates of evolution we calculated for life-history traits quantified herein are consistent with rates calculated for morphological traits in Loberg stickleback. Compared with published values for life-history traits in other species, our rates were relatively rapid.

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