

Consumptive and non-consumptive effects of predation by introduced northern pike on life-history traits in threespine stickleback

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ABSTRACT

Background: Non-native, predatory northern pike (*Esox lucius*) are spreading into lakes of south-central Alaska and were illegally introduced into Scout Lake in 2001 or 2002. Pike preyed on native threespine stickleback (*Gasterosteus aculeatus*) in the lake, subjecting them to higher mortality rates.

Hypotheses: Life-history theory predicts evolutionary changes in threespine stickleback females arising from consumption by predators, including reduced body size, earlier age of reproduction, increased reproductive effort (greater clutch mass and clutch size), and, under some conditions, smaller offspring. Alternatively, energetically costly, non-consumptive predation-risk effects resembling food limitation – such as predator avoidance, reduced foraging efficiency, and chronic stress – might cause phenotypically plastic responses inconsistent with life-history theory.

Methods: We measured changes in length, body mass, clutch mass, clutch size, and egg mass during an 11-year (1999–2009) study. In analyses of clutch mass, clutch size, and egg mass, we used body mass to correct for female size.

Results: Consistent with predictions from life-history theory, the mean size and age of reproducing females declined, with one decline in size following the initial pike introduction and another after sport-fish stocking of salmonids was discontinued. The principal age at reproduction gradually shifted from two years to one year of age, with few females surviving to reproduce in a second year. Clutch mass and clutch size declined, suggesting non-consumptive predation-risk effects resembling those of nutrient deprivation. Egg mass showed an overall decline, with a moderate, temporary increase near the end of the study period.

Keywords: food limitation, invasive species, nutrient deprivation, physiological stress, predation risk, reproduction, threespine stickleback.

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INTRODUCTION

Biological invasions have dramatically altered the ecology of environments across the globe (Sakai *et al.*, 2001; Meyerson and Mooney, 2007; Leprieur *et al.*, 2008). Alien predators can exert strong selective pressure on native populations, causing rapid evolutionary change in invaded communities (Nunes *et al.*, 2014). Moreover, invasive species are a leading cause of extinction among native species of animals (Wilcove *et al.*, 1998; Pimm and Raven, 2000; Clavero and Garcia-Berthou, 2005; Garcia-Berthou *et al.*, 2005). In aquatic environments throughout the northern hemisphere, the novel introduction of predatory northern pike (*Esox lucius*) into lakes and streams is one of the most significant threats to native populations and communities (McMahon and Bennett, 1996; Byström *et al.*, 2007; Flinders and Bonar, 2008; Winfield *et al.*, 2012). In Alaska, voracious pike have altered the ecology of aquatic environments throughout the Cook Inlet Basin (Rutz, 1996, 1999; Patankar *et al.*, 2006; Haught and von Hippel, 2011) and have caused the extirpation of native fish species, including the threespine stickleback, from a number of lakes in the south-central region of Alaska (Rutz, 1996; Patankar *et al.*, 2006). Although much research has addressed the outcome of evolutionary change among prey populations (Reznick and Endler, 1982; Reznick *et al.*, 1996, 2001; Johnson and Belk, 1999, 2001; Billman *et al.*, 2011), we know little about how prey traits change during invasions and thus mediate the impact of invasive predators on naive populations (Nunes *et al.*, 2014).

Phenotypic evolution of native prey populations in response to novel predators may involve changes in behaviour, morphology, or life history (Strauss *et al.*, 2006). Predators may influence the life history of their prey in two very different ways. Consumption of prey may result in evolutionary change via selection on individuals with differing life-history traits. Alternatively, non-consumptive risk effects arising from the costs of altered prey behaviour and stress in response to predators may cause plastic effects, which may be either adaptive or non-adaptive (Creel and Christianson, 2008; Hawlena and Schmitz, 2010; McCauley *et al.*, 2011). Here we document phenotypic shifts in multiple life-history traits of the threespine stickleback (*Gasterosteus aculeatus*) in Scout Lake, Kenai Peninsula, over an 11-year period following an illegal introduction of northern pike. We examine whether the changes follow predictions for trait evolution resulting from extrinsic prey mortality by consumption in the altered predation environment, but we also consider whether the changes provide support for an alternative hypothesis – non-consumptive effects of predatory risk by pike on stickleback growth and reproduction.

Pike as predators

Pike are voracious and effective predators on small fish species (McMahon and Bennett, 1996; Haught and von Hippel, 2011; Winfield *et al.*, 2012; Sepulveda *et al.*, 2013). Pike smaller than 13 mm can eat fish larvae, but when 34–65 mm, they prey almost exclusively upon fish (Carlander, 1969; Scott and Crossman, 1973). Prey size increases with the size of the pike, which tends to consume the largest available prey first (Frost, 1954; Carlander, 1969; Scott and Crossman, 1973; Zimmerman, 2006). Soft-rayed fish, especially juvenile salmon and trout, are often the principal prey consumed by pike (Hoogland *et al.*, 1957; Eklov and Hamrin, 1989; Rutz 1996, 1999; Sepulveda *et al.*, 2013). Pike are, however, opportunistic predators, taking whatever prey are available and rapidly changing prey selection in response to changes in abundance or vulnerability of potential prey (Mann, 1985; Beaudoin *et al.*, 1999; Dominquez and Pena, 2000; Haught and von Hippel, 2011; Sepulveda *et al.*, 2013). In Alaska, the threespine stickleback becomes the target of predation as other fish species decline in abundance (Haught

and von Hippel, 2011). After the predatory pressure by pike shifts, stickleback decline in abundance as years of pike residence increase, and local stickleback populations can be driven to extinction (Patankar *et al.*, 2006).

Predictions

Based on the foregoing review of pike predation, we made two assumptions about the effect of predation by northern pike on stickleback in Scout Lake: (1) there was a high rate of predatory mortality from pike as the pike became established in the lake; (2) all size and age groups of stickleback were affected to some extent, but there was a size/age bias towards larger stickleback prey, at least among larger pike.

Predation represents one of the most important selective factors on life-history traits (Stearns, 1992; Day *et al.*, 2002). Life-history theory provides four broad, general predictions for female prey experiencing high mortality rates and/or higher adult mortality than juvenile mortality: (1) reduced size and/or age at maturity; (2) increased reproductive effort; (3) greater numbers of offspring per unit size (often a consequence of the increased reproductive effort); and (4), under some conditions, smaller offspring. A lower probability of future reproduction should select strongly for greater reproductive investment at a smaller size, hence also an earlier age at reproduction (Reznick, 1982; Reznick and Endler, 1982; Reznick *et al.*, 2001; Zimmerman, 2006). In stickleback, fecundity is positively correlated with female size (Baker *et al.*, 1998, 2008). An investment in growth, however, may never pay off if the fish is killed before reproduction occurs. In Alaska, most females reproduce at two years of age (Baker *et al.*, 2008). Because the strong seasonal climate restricts the growth season in Alaska, the evolution of a smaller size at sexual maturity in a population would be expected to lead to maturity at one year of age.

Clutch mass adjusted to some common body size is a proxy for reproductive effort (proportion of energy invested in reproduction). Selection for increased reproductive effort at a smaller size (Gadgil and Bossert, 1970; Law, 1979; Michold, 1979; Charlesworth, 1980) should thus result in a larger clutch mass at a given body size. In the threespine stickleback, clutch mass and egg number in the clutch are highly correlated; and both traits are highly correlated with female body mass (Baker *et al.*, 2008). Selection for greater fecundity (Simons, 2007, 2008), as distinct from reproductive effort, might also result in a shift in the optimum trade-off relationship between egg number and offspring size, possibly leading to smaller eggs (Reznick, 1982; Reznick and Endler, 1982; Reznick *et al.*, 2001). Nonetheless, egg size also responds to a large number of other factors, such as the early-life mortality schedule, the egg size-to-fry size function, and early-life growth rates, with more complex processes (e.g. intraspecific competition via density effects) likely complicating predictions (Jørgensen *et al.*, 2011; Baker *et al.*, 2015). Thus, shifts in egg size are difficult to predict, and egg size may not always decrease under predatory pressure.

MATERIALS AND METHODS

Study site and sampling

Collections of threespine stickleback were obtained from Scout Lake (60°32.117'N, 150°49.917'W) under scientific resource permits from the Alaska Department of Fish and Game (ADF&G). Scout Lake is located in the northwest of the Kenai Peninsula in the

south-central region of Alaska. Sampling was conducted annually in late May or early June during the spawning season for sticklebacks in this region (Heins *et al.*, 1999). We used samples taken from 1999 through 2009. Scout Lake was treated with rotenone in October 2009 to eradicate the pike (R. Massengill, personal communication), which ended our annual sampling for stickleback.

Fish were caught using 6-mm and at times 3-mm wire-mesh minnow traps set near the shore at two sites along one end of the lake. Specimens were euthanized with tricaine methanesulfonate prior to fixation and storage in 10% buffered formalin, based upon institutionally approved protocols (Animal Care and Use Committee, Tulane University; protocols 0106-1-16-047, 0219-1-16-0402, 0305-UT-C, 0305R-UT-C).

Environmental setting and predation history

Scout Lake is situated at an elevation of 75 m and is relatively small and shallow, with a surface area of 38 ha and a mean depth of 4 m. All but a small portion of the lake's basin was surrounded by hardwood forest, with little change in the landscape before and during this study. A total of about 72% of lakeside shoreline remained in a relatively natural state in 2009. Thus, we assumed there was little local anthropogenic impact on the lake environment.

With exception in a few years before the discovery of pike, Scout Lake was stocked annually for decades with fingerling rainbow trout (*Oncorhynchus mykiss*) and/or coho salmon (*Oncorhynchus kisutch*) and occasionally with chinook salmon (*Oncorhynchus tshawytscha*) as part of the recreational fishing programme of the ADF&G. Thus, we assumed that the stickleback population in the lake was adapted to salmonid predation at the time of the pike introduction.

Pike were illegally introduced into Scout Lake during the winter months of 2001 or 2002 (Alaska Wildlife Trooper, anonymous personal communication; Local Resident, anonymous personal communication). The first pike, two fish 534 and 565 mm in length respectively, were taken in September 2005, by ADF&G biologists. Sampling over the following three years documented the presence of small pike in the lake as well as a greater number of large pike. This sampling also indicated that pike had likely been reproducing in Scout Lake before the first documented catch in 2005. Sampling in 2009 just prior to the lake's rehabilitation with rotenone, showed a substantial pike population.

For lack of a known date, Haught and von Hippel (2011) used the date of discovery as their estimated date of introduction (S. Haught, personal communication). Time of residence in lakes by pike shows a significant relationship with dietary importance of different prey taxa, with shifts from soft-rayed fishes to stickleback and macro-invertebrates (Haught and von Hippel, 2011). The stomach contents of pike from Scout Lake in 2008 comprised odonates and stickleback, indicating that pike had been in the lake for some time before the discovery date (Haught and von Hippel, 2011; S. Haught, personal communication). Furthermore, ADF&G data on the sizes of the smaller pike caught in 2006 and the reduced catch per unit effort (CPUE) of sport fish in the 2005 sample also support the report from the Alaska Wildlife Trooper that pike were introduced to Scout Lake in 2001 or 2002. A pike population beginning at low initial densities may have a detectable impact on sport fisheries any time within a few years to a decade or more (R. Massengill, personal communication).

Specimen examinations and data gathering

Threespine stickleback infected by the diphyllobotriidean cestode *Schistocephalus solidus* and any females heavily infected with *Glugea* sp. or nematodes were excluded from our examinations. The standard length (SL, distance from tip of snout to base of caudal fin) of each fish was measured with digital callipers to the nearest 0.1 mm, and length-frequency analysis was used to determine age of the fish. Following measurement, each specimen was dissected to determine sex and its reproductive condition.

We followed Baker *et al.* (1998) and Heins *et al.* (1999) to determine female reproductive condition by classifying ovaries into the following stages: latent, early maturing, late maturing, mature, ripening, and ripe. During the reproductive season, adult-size females produce multiple clutches as they repeatedly cycle through the late maturing, mature, ripening, and ripe stages that comprise the 'clutch-production cycle' (Heins and Baker, 1993; Brown-Peterson and Heins, 2009). Female fish in the mature, ripening, and ripe stages have easily discernible clutches of developing oocytes or ripe eggs.

The oocytes or eggs in each clutch were counted to determine egg number ('clutch size'). All eggs of ripe females were dried at 40°C for 24–28 h and weighed together to measure clutch mass. We divided clutch mass by clutch size to calculate mean female dry egg mass (ovum mass), which was multiplied by 1,000,000 to express the weight in micrograms. The carcasses of eviscerated fish were weighed to the nearest 0.001 g after they were blotted with a paper towel to estimate somatic body mass.

Statistical analyses

All analyses were conducted using the Statistica v.12.0 software package (StatSoft® 1984–2015). Tests of annual differences in SL were conducted using analysis of variance (ANOVA). Analyses of clutch characteristics (clutch size, clutch mass, and ovum mass) were performed after the data were converted to $\log_{10}$ values to meet assumptions of the statistical analyses. To determine the pattern of change in clutch mass and clutch size, we standardized trait values to a common body mass using analysis of covariance (ANCOVA), including a term for the year $\times$ covariate interaction. If significant slope heterogeneity was detected among years, a 'separate slopes' covariate adjustment was used to calculate annual size-adjusted means and confidence limits. The results were also compared with those calculated using the more commonly employed 'common within-group slope' ANCOVA model (Reist, 1986). Owing to the large sample sizes of females in all years, and the resulting high power for declaring even relatively small differences significant, an ANOVA-specific effect size metric, partial η^2 , was computed for each model term in order to compare them based on their relative explanatory power.

Many stickleback populations exhibit a positive correlation between ovum mass and body mass, but the relationship often varies tremendously across populations and among years (Baker *et al.*, 2008). In such a situation, either the 'separate slopes' model or Reist's (1986) 'common within-group' model may be used, and these models can produce divergent patterns of change across years. Determining which represents reality may not be obvious. Furthermore, although egg size may be adjusted to a common female body size for the purposes of certain comparisons, the fitness of the egg (or the fry resulting from the egg) is a function of its actual size, not its size adjusted to some particular female size. Thus, more than one approach to evaluating changes in egg size is required.

For female SL, egg size, clutch size, and clutch mass (the latter two adjusted to a common body mass), we calculated the rate of phenotypic evolution using the linear regression approach suggested by Hendry and Kinnison (1999). We calculated generation time from the relative frequencies of age-1 and age-2 breeders within each year, estimating a total of 6.5 generations during the 11-year study period. Without knowledge of the heritability of these traits in Scout Lake, we cannot say whether the phenotypic change represented evolutionary change. Nevertheless, such rate measures are of considerable interest and value to evolutionary biologists. To quantify the overall pattern of change in life-history traits, we calculated Spearman rank order correlations for each variable across the 11-year period. For additional insight, we calculated the standardized effect size (Cohen's d statistic) between selected pairs of years, often the first and last in our series.

RESULTS

Size and age of reproductive females

Reproducing (clutch-bearing) female threespine stickleback showed a highly significant ($r_s = -0.90$, $N = 11$, $P < 0.001$) decrease in mean SL across the 11 study years of 1999–2009 (Fig. 1). There was no steady progression in the reduction in length. Instead, there were two large decreases, one from 2002 to 2003 and another between 2007 and 2008, and smaller variations among other years. The overall change in mean SL from beginning to end of the study was 9.0 mm (from 49.8 mm to 40.8 mm; 18.1%), which represented an enormous shift of more than 2.5 standard deviations (Cohen's $d = 2.65$).

As the fish became smaller, the age at which the majority of females reproduced changed from two years of age to one year of age (Fig. 2). The percentages of clutch-bearing females

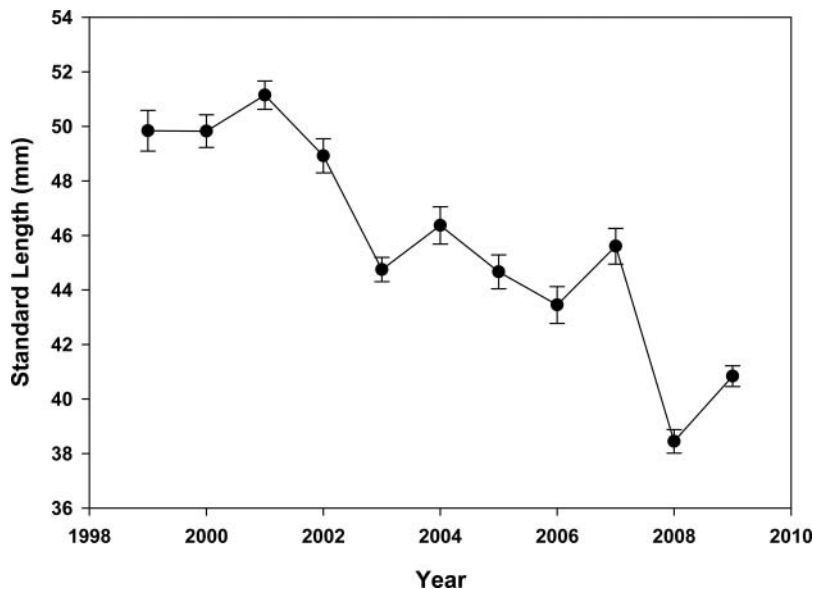


Fig. 1. Mean standard length (mm, $\pm 95\%$ CI) of clutch-bearing female threespine stickleback from Scout Lake, Alaska, 1999–2009.

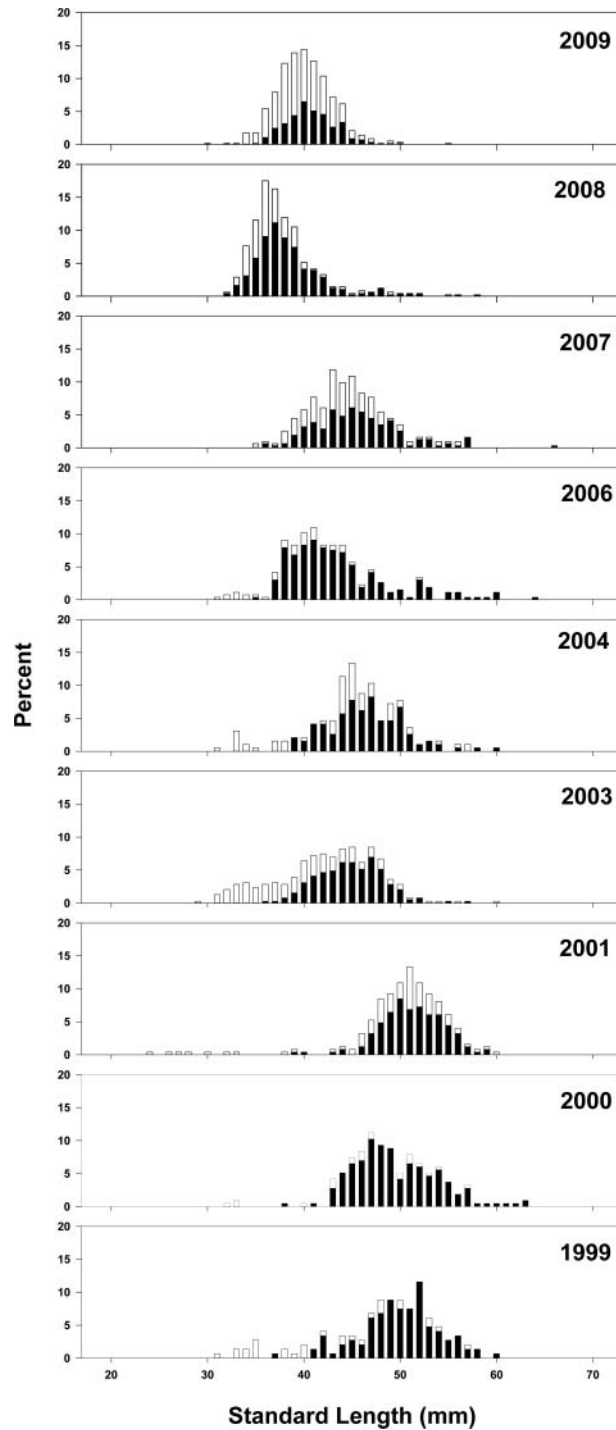


Fig. 2. Length-frequency distributions of female threespine stickleback from Scout Lake, Alaska. Solid bars = clutch-bearing females, open bars = non-clutch-bearing females.

less than 42 mm SL (approximate size differentiating one- and two-year-old fish) in 1999 and 2000 were only 3% and 1%, respectively. The percentages had increased to 85% and 64% in 2008 and 2009, respectively.

Clutch size

The slope comparison test within the ANCOVA indicated significant heterogeneity of the 11 annual regressions relating clutch size to body size ($F_{10,2009} = 3.2$, $P = 0.0005$). Nevertheless, the effect of the heterogeneity was small compared with the effects of female somatic mass and year (partial $\eta^2 = 0.015$, 0.451, and 0.084, respectively). Year-to-year differences were highly significant ($F_{10,2009} = 18.5$, $P < 0.0001$). The pattern of adjusted annual mean clutch sizes (Fig. 3) was nearly identical for the ‘separate slopes’ ANCOVA as for the more widely used ‘common within-group slope’ model. Clutch size, adjusted to the overall mean female somatic mass of 0.82 g, remained consistent from 1999 to 2003. After this time, clutch size declined, albeit erratically, through the end of the study in 2009 (Fig. 3). By 2009, clutch size had declined from 63 to 43 eggs at the average female size, a decline of 31.7% overall, and one representing a shift of one standard deviation (Cohen’s $d = 1.00$). The overall trend is one of declining clutch sizes, as evidenced by a strong correlation between adjusted clutch size and year ($r_s = -0.86$, $N = 11$, $P < 0.001$).

Clutch mass

As with clutch size, the slope comparison test within the ANCOVA indicated significant heterogeneity of the 11 annual regressions relating clutch size to body size ($F_{10,896} = 3.0$, $P < 0.0001$), and a highly significant year effect ($F_{10,896} = 13.41$, $P < 0.0001$). Again, the

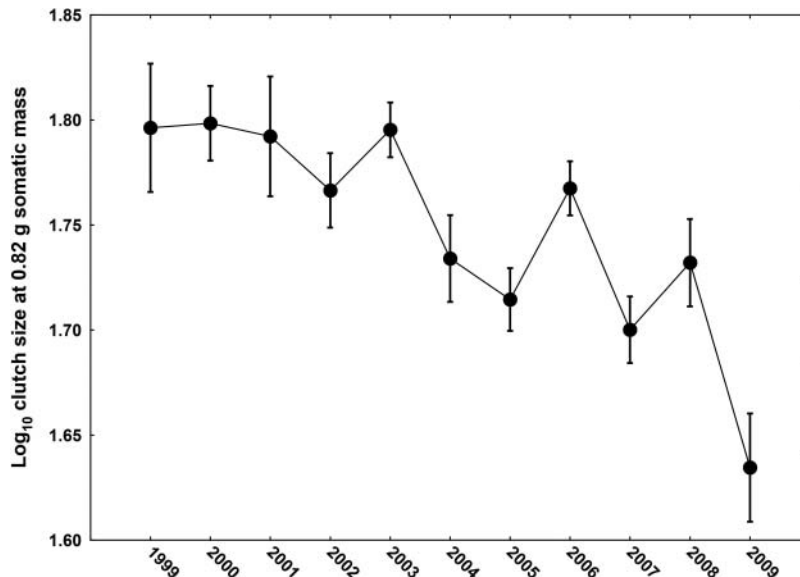


Fig. 3. Clutch size of breeding female threespine stickleback in Scout Lake, Alaska, 1999–2009, adjusted to a common female somatic mass of 0.82 g. Error bars represent 95% confidence intervals.

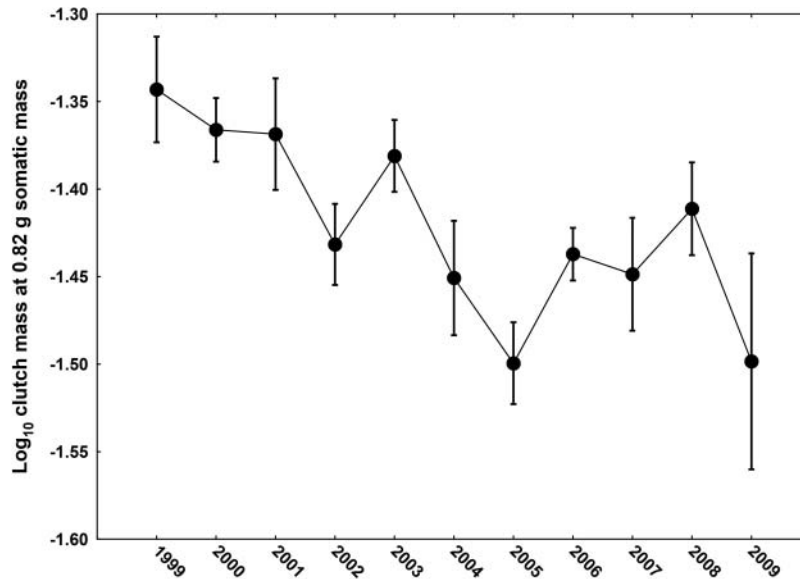


Fig. 4. Clutch mass, an index of the level of reproductive effort, of breeding female threespine stickleback in Scout Lake, Alaska, 1999–2009, adjusted to a common female somatic mass of 0.82 g. Error bars represent 95% confidence intervals.

effect of the heterogeneity was small (partial $\eta^2 = 0.032$) compared with the effects of female somatic mass and year (partial $\eta^2 = 0.488$ and 0.130 , respectively). Also similarly, the pattern of adjusted annual mean clutch masses (Fig. 4) was nearly identical for the ‘separate slopes’ ANCOVA as for the more widely used ‘common within-group slope’ model. Except for a moderate decline in 2002, clutch mass, adjusted to a female somatic mass of 0.82 g, remained relatively consistent at about 0.043–0.045 g dry mass from 1999 to 2003 (Fig. 4), mirroring the pattern shown by clutch size. Over the following two years, clutch mass declined substantially (by 22.0%, from 0.041 g to 0.032 g dry mass), rebounded moderately over the next three years, and finally declined to 0.032 g in 2009. The overall decline in clutch mass at a common body size from 1999 to 2009 was 28.9% (from 0.045 to 0.032 g), giving an effect size of more than one standard deviation (Cohen’s $d = 1.13$). The overall trend is clearly one of declining reproductive effort, as evidenced by a strong correlation between adjusted clutch mass and year ($r_s = -0.74$, $N = 11$ years, $P = 0.03$).

Egg mass

A ‘separate slopes’ ANCOVA revealed an overall significant relationship between egg size and female size ($F_{1,896} = 35.4$, $P < 0.0001$), significant slope heterogeneity ($F_{10,896} = 2.4$, $P = 0.009$), and a significant year effect ($F_{10,896} = 9.8$, $P < 0.0001$). Partial η^2 indicated year to have the strongest effect on egg size (partial $\eta^2 = 0.098$, 0.018 , and 0.026 for year, female size, and the year $\times$ female size interaction effect, respectively). The pattern generated by the separate slopes analysis indicated a steady decline in adjusted egg size from 1999 to 2005, followed by a steady return to the starting egg size by the end of the study in 2009 (Fig. 5A). From largest to smallest, adjusted egg size in individual years ranged from 693 to 608 μg , a

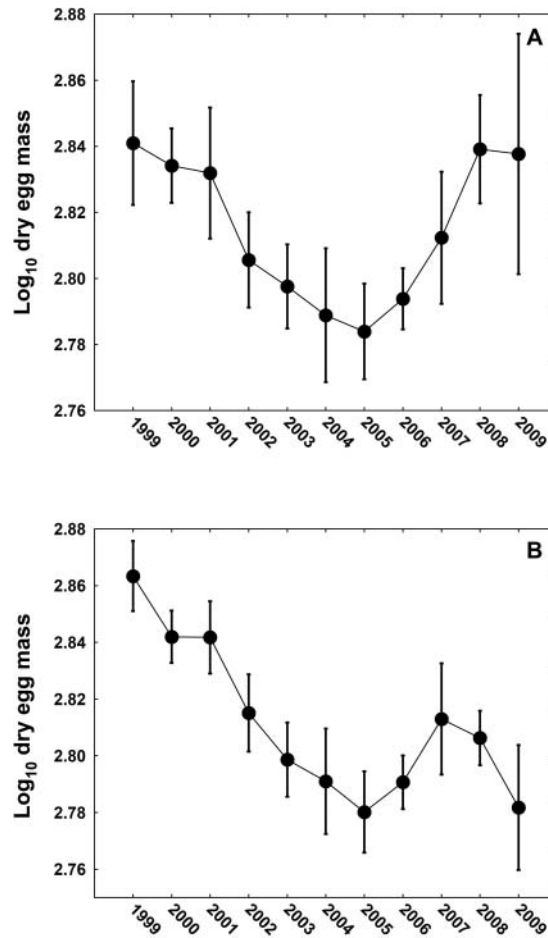


Fig. 5. (A) Egg dry mass of breeding female threespine stickleback in Scout Lake, Alaska, 1999–2009, adjusted to a common female somatic mass of 0.82 g. Error bars represent 95% confidence intervals. The adjustment procedure within ANCOVA employed a separate slope calculation for each of the 11 years in the data set. (B) Egg dry mass comparisons across years without adjustment for female size.

difference of 12.3%. The pattern of change in actual egg size (not adjusted for female size) was somewhat different (Fig. 5B). Egg size declined almost linearly between 1999 and 2005, increased modestly over the following two years, then declined again in the last two years, giving a correlation of $r_s = -0.74$ ($N = 11$ years, $P = 0.03$). From 1999 to 2009, unadjusted egg size declined from 734 to 613 μg , a reduction of 16.5%, producing a Cohen's $d = 1.39$. The pattern of egg size change estimated using the 'common within-group slope' model (not shown) was intermediate between the former two, showing a steady decline to 2005, a strong rebound, and then an abrupt decline in 2009. In the present situation, we believe that unadjusted egg size best reflects the true pattern of change.

Rates of phenotypic change

Rates of phenotypic evolution for all life-history traits were rapid, statistically significant, and similar across traits over the estimated 6.5-generation interval covered by our study. Rate estimates, 95% confidence limits, and P -values for individual traits were: SL ($h_p = -0.134$, 95%CI = -0.090 to -0.178 , $P < 0.0005$); egg dry mass ($h_p = -0.162$, 95%CI = -0.054 to -0.270 , $P = 0.015$); clutch mass ($h_p = -0.148$, 95%CI = -0.052 to -0.244 , $P = 0.013$); and egg number ($h_p = -0.151$, 95%CI = -0.091 to -0.211 , $P < 0.0008$).

DISCUSSION

Our results demonstrate significant, directional phenotypic changes in life-history traits of threespine stickleback over time following the introduction of northern pike into Scout Lake. All life-history traits showed substantial rates of phenotypic evolution, from -0.134 to -0.162 haldanes. Haldanes measure evolutionary rates in standard deviations per generation; thus, over the approximately 6.5 generations covered by our study, each trait shifted by almost one full standard deviation. Over such an interval, these rates and shifts would be considered relatively large (Hendry and Kinnison, 1999; Hendry *et al.*, 2008). The rapidity of phenotypic evolution should not be surprising, given that the change in the Scout Lake ecosystem was a man-made one, and human alterations of natural communities have been shown to produce more rapid shifts than natural alterations (Palumbi, 2001; Stockwell *et al.*, 2003; Hendry *et al.*, 2008). Baker *et al.* (2011) documented similarly rapid phenotypic evolution in life-history traits in an Alaskan stickleback population, due apparently to human-induced changes in aquatic productivity.

Local physico-chemical factors and global climate change are unlikely to have driven the changes observed in this study. There was little change in an otherwise natural landscape surrounding the lake during the study period. Moreover, mean annual air temperatures varied $<1.5^\circ\text{C}$ from the overall mean for 2000–2009, except for 2006 when a 2.6°C decrease was observed (data from www.wunderground.com).

Predation on threespine stickleback by salmonid fishes, aquatic birds, and aquatic insects preceded the introduction of pike and presumably continued to occur after the introduction of pike. There was no reason to assume there was continuously increasing predatory pressure from these sources driving the observed changes in stickleback life history. The introduction of pike, however, brought a very different predator to the lake, one that is voracious and preys upon stickleback as other sources of food diminish in availability. Our data, therefore, demonstrate the apparent strong effect of introduced pike through increasing predatory pressure on the stickleback population over time, driving substantial shifts in stickleback life history. The life-history shifts appear to stem from both consumptive and non-consumptive effects of predatory pressure. In addition, the decrease in salmonid populations following the cessation of stocking in 2005 (R. Massengill, personal communication) may have led to a subsequent acceleration of the effects on the stickleback population.

Although we have no direct information on trait heritability in Scout Lake stickleback, the shifts probably represent a combination of both genetic change and phenotypic plasticity, a common finding in studies of rapid phenotypic change (Hendry *et al.*, 2008). In the present case, the life-history shifts that were most likely due to plasticity seem to have been maladaptive (e.g. the decrease in reproductive effort as opposed to the predicted increase).

Nonetheless, we do not know whether changes in any particular trait reflected phenotypic plasticity, genetic change, or both. Evaluating the changes with respect to established life-history predictions, however, permits us to propose several reasonable inferences. Below, we consider whether the phenotypic changes are consistent with life-history theory for adaptive change in response to predation, and we infer underlying genetic change from literature addressing the genetic basis of variation in the life-history traits. We also consider the physiology of reproduction in threespine stickleback better to understand possible environmental influences on phenotypic plasticity in the traits. Predator–prey relationships provide some of the strongest demonstrations of adaptive life-history evolution in response to environmental pressures (Day *et al.*, 2002). Thus, we should expect that some of the observed changes reflect adaptive shifts in response to the introduced predator. Alternatively, risk effects arising from the non-consumptive costs of altered prey behaviour and stress in response to predators can have large effects on life-history traits which are non-adaptive (Creel and Christianson, 2008; McCauley *et al.*, 2011).

Body size, age, and maturation

Consistent with life-history theory, the size of reproducing threespine stickleback females declined following pike introduction; and the majority of females shifted from reproducing at two years of age in 1999–2001 to reproducing at one year of age in 2008–2009. The first of two decreases in body size occurred within a few years of the introduction of pike and likely was driven by a large and rapid increase in pike abundance due to reproduction of the individuals introduced into the lake. It also probably reflected a time lag (Crooks, 2005) following pike introduction. The number of introduced pike was probably small (<50 individual fish), and the earliest effects on the stickleback should not have been evident until a few years after introduction. The second decrease in length likely reflected continued reproduction of the pike within the lake coupled with the cessation of sport fish stocking.

Although the shift in average length of breeding females seems dramatic and large over such a short interval, we have previously documented such a change in an Alaskan stickleback population (Baker *et al.*, 2011). In addition, Conover and Munch (2002) have shown that shifts of even larger magnitude can be produced by size-selective mortality in even fewer generations.

The dramatic shift in the age of breeding females in Scout Lake likely resulted from a combination of two selective forces – increased predation on the largest fish as pike increased in both size and number, and increased fitness of females that matured at one year of age. The decreases in length due directly to predation on larger individuals may reflect simple phenotypic change; however, the shift in frequency of females breeding at age 1 probably represents genetic – hence evolutionary – change in the population. Following McGuigan *et al.* (2011), who revealed expression of cryptic genetic variation in a novel environment, perhaps the predation and effects of predation risk created by pike exposed genetic variation for growth rate or size at maturity, allowing the rapid shift we observed. An interpretation of adaptive plasticity is also plausible.

As the mean length of females decreased, the size threshold for maturation (Berner and Blanckenhorn, 2007) in stickleback females also appears to have decreased. The threshold mass (estimated as 2.5 standard deviations below mean mass) for females captured from 1999 to 2002 was about 0.63 g. From 2003 to 2007, it had decreased to approximately 0.45 g. And in 2008–2009, the threshold averaged 0.32 g, with a low in 2008 of 0.25 g.

Reproductive traits

Reduction in adult size results in lower egg number per spawning episode (Baker *et al.*, 2008) and likely lowers lifetime fecundity if the egg number–body size relationship and number of spawns per reproductive season remain unchanged. Life-history theory predicts that reproductive effort (size-adjusted clutch mass) should increase in the face of predation (Reznick, 1982; Reznick and Endler, 1982; Reznick *et al.*, 2001). As a correlated consequence, egg number should also increase, as a means of compensation for reduced survivorship of larger (older) fish (Simons, 2007). Under some conditions, egg number could increase with a decrease in egg/offspring size arising from changes in the trade-off function relating egg number and offspring size (Reznick, 1982; Reznick and Endler, 1982; Reznick *et al.*, 2001). Both mechanisms serve to buffer individual reproductive value (production of offspring), the first by increasing the energetic commitment to reproduction, and the second by decreasing the commitment to individual offspring. Contrary to these theoretical predictions, clutch mass and egg number, adjusted for body size, declined overall throughout the study. Given these counter-intuitive changes, the decline in egg size may be an adaptive shift, maintaining fecundity in the face of stress-induced, non-adaptive changes in reproductive effort. In a previous study of rapid evolution in stickleback by Baker *et al.* (2011), changes in fecundity were in the predicted direction, giving additional support to the hypothesis that stress-induced changes due to predatory pressure were acting in a different manner in Scout Lake.

The proximate mechanisms for shifts in reproductive traits are unknown, but the source of energy used to provision reproductive bouts may have played a role. Life-history theory distinguishes between income breeders and capital breeders, the difference being defined by the source of energy used to provision reproductive attempts. Income breeders produce offspring using energy gained from foraging concurrent with reproductive episodes. Capital breeders provision offspring from energy accumulated at an earlier time. Energetic studies of captive, well-fed stickleback (Wootton, 1994; Fletcher and Wootton, 1995; Ali and Wootton, 1999) indicate that stickleback are largely an income breeder, although assimilated, somatic energy may be used to supplement energy from foraging (Wootton, 1994; Fletcher and Wootton, 1995; Ali and Wootton, 1999). Stickleback females appear to produce the first clutch each reproductive season from capital energy, whereas the energy for subsequent clutches comes from food obtained during the breeding season (R.J. Wootton, personal communication). Poizat *et al.* (1999) and Bagamian *et al.* (2004) have shown that size-adjusted carcass mass (body condition) in wild populations decreased during the breeding season, suggesting that natural populations rely upon capital energy more so than well-fed laboratory fish. Apparently, then, females in the wild rely on concurrent energy income to provision gametes during the spawning season, especially as capital sources dwindle. Moreover, many animals exhibit energetic phenotypes between the capital vs. income extremes, which may represent the influence of foraging strategies on energy allocation in reproduction rather than evolved strategies (Stephens *et al.*, 2009; Van Dyke *et al.*, 2012). Reproductive performance may, therefore, depend heavily upon foraging success fuelling immediate energy expenditure. If this is the case, it may explain the seemingly maladaptive changes we observed in reproductive traits of female stickleback in the presence of pike predators.

The coincidental decline in clutch mass and clutch size suggests that reduced reproductive investment caused these changes. A plausible explanation for the reduction in reproductive effort is that predatory stress and energy expended to avoid predation decreased energy acquisition rate. This proximal cause may have been mediated by time spent avoiding

pike and limitation on the use of food-rich habitats by the presence of the predator. Altered prey behaviours manifested in response to predators, including perceived predation risk, may have large costs (Creel and Christianson, 2008; Zanette *et al.*, 2011). Prey organisms experience considerable physiological stress (Pfeiffer, 1962; Hawlena and Schmitz, 2010). Behavioural avoidance of risk may involve changes in habitat use, vigilance, and foraging (Creel and Christianson, 2008). Behaviourally mediated risk effects may result in reduced reproduction, which may be mistaken for effects of food limitation (Fraser and Gilliam, 1992; Creel and Christianson, 2008). Moreover, increased mortality due to predation may not lead to compensatory increases in reproduction (Fraser and Gilliam, 1992).

Our hypotheses explaining phenotypic shifts in reproductive traits postulate risk effects arising from extreme environmental conditions resulting from altered prey behaviour and physiological stress. The changes in reproductive traits appear similar to the changes one might expect under conditions of food limitation. Moreover, there may have been interactions between environmental conditions and rapid evolutionary changes in life-history traits.

The phenotypic changes in clutch mass, egg number and egg mass may, however, reflect some undetermined level of underlying genetic change. Egg number and egg mass should be influenced by the balance between selection for large offspring (larger egg mass) favouring greater survivorship and high maternal fecundity resulting in smaller offspring (Smith and Fretwell, 1974; Einum and Fleming, 2000). These polygenic traits should have genetic and environmental influences. Although heritability estimates are unavailable for these traits in Scout Lake stickleback, estimates for life-history traits including offspring size and egg number among animal populations typically range from 0.1 to 0.4 with an average of 0.25 (Mousseau and Roff, 1987). Heritability of egg mass and egg number in a California population of the stickleback was consistent with this range (Snyder, 1991). Unpublished data on egg size from Alaska stickleback populations differing in mean egg mass provide additional evidence for heritability of offspring size in threespine stickleback (Baker *et al.*, 2011). Moreover, egg mass does not show large environmental phenotypic effects as compared with genetic effects (Fletcher and Wootton, 1995; Baker *et al.*, 2011). We do not think genetic drift played a significant role in the life-history changes because our observations indicate the population of stickleback in Scout Lake was large (tens of thousands or more) prior to the pike introduction. Large numbers of stickleback remained in the lake with the pike, although the catches of stickleback were reduced.

Extirpation mechanisms

Haught and von Hippel (2011) highlighted abiotic and biotic factors influencing the co-existence of stickleback with invasive pike. Our data suggest that non-consumptive influences on reproductive performance of individual females may play a major, if not final, role in the local extinction of stickleback populations.

The minimum age at reproduction for stickleback in Alaska appears to be one year because strong seasonal periodicity in temperature and a short spawning season prohibit females from growing large enough to reproduce within the spring/summer after hatching. In Scout Lake, the average mass of the reproducing one-year-old stickleback females in 2008 and 2009 was 0.5 and 0.6 g, respectively. These averages are comparable to the smallest average mass for populations of threespine stickleback in Alaska (Baker *et al.*, 2008). Thus, the

population in Scout Lake appears to have been at or near the lower physiological extreme for reproducing females in Alaska.

In Scout Lake, the age of reproduction changed from two years to one year within a 10-year period, whereas naturally occurring populations characterized by Baker *et al.* (2008) presumably had been reproducing at one year of age for many years. Physiological processes affecting reproductive performance in Scout Lake may not have adjusted at the same rate to allow optimal performance. Moreover, females in Scout Lake experienced risk effects stemming from predator avoidance, stress, and limited foraging success. The combination of risk effects and rapid genetic change might have led to large percentages of females without clutches in 2009 [64% vs. 8–42% in other years; see Fig. 2 (D.C. Heins, personal observation)]. The interaction of these phenomena may be the eventual cause of extirpation of naive stickleback populations exposed to sudden, intense predation.

Risk effects are not widely appreciated in conservation, although they are increasingly well recognized in basic ecology (Creel and Christianson, 2008). Conservation of the adaptive radiation among threespine stickleback populations in Alaska is a concern (Foster *et al.*, 2003; von Hippel, 2008; Heins, 2012). The extirpation of stickleback populations due to consumptive and non-consumptive effects of predation presents a challenge in conservation of this biological diversity.

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