Evolutionary Ecology Research, 2017, **18**: 363–382

Jacks and jills: alternative life-history phenotypes and skewed sex ratio in anadromous Threespine Stickleback (*Gasterosteus aculeatus*)

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ABSTRACT

Background: Anadromous Threespine Stickleback (*Gasterosteus aculeatus*) often have bimodal size-frequency distributions, suggesting alternative life-history phenotypes. Breeding at small size may decrease attractiveness to mates, competitiveness in territorial disputes, and fecundity, but early breeding may outweigh the fitness costs of small size if survival to reproduction is low or iteroparity is possible.

Hypotheses: (1) Two size-frequency modes exist in spawning, anadromous, male and female Threespine Stickleback, and there is sexual dimorphism in the frequencies of smaller size-class fish. (2) Smaller size-class anadromous Threespine Stickleback are younger than larger size-class fish. (3) Variation across years and populations in frequencies of smaller size-class fish and body size exist and is positively correlated with a proxy for prey abundance on the feeding grounds. (4) The sex ratio of anadromous, spawning individuals is 1:1 across years and populations.

Methods: We measured standard length (SL) and determined sex of anadromous Threespine Stickleback from Rabbit Slough in the Cook Inlet Basin, Alaska, between 1992 and 2015 and from additional sites around Cook Inlet and the Kenai Peninsula, Alaska, in 2014 and 2015. We compared sex ratio, mean SL, and size-class frequencies among years from Rabbit Slough and among four sites in 2014 and three sites in 2015. We determined ages of smaller and larger size-classes using otoliths from subsamples collected in 2010 and 2012 from Rabbit Slough.

Results: At least two size modes occurred most years in both sexes in Rabbit Slough, in three of four populations sampled in 2014, and in all populations sampled in 2015. Smaller size-class fish were younger, indicating alternative life-history phenotypes in anadromous Threespine Stickleback. The proportion of females consistently exceeded that of males in Rabbit Slough and two of three other sites, but males were more frequent than females among smaller size-class fish. Both the frequency of smaller size-class fish and mean SL varied among years in Rabbit Slough and among populations during 2014 and 2015. Prey abundance in the marine feeding grounds was not correlated with the frequency of smaller size-class fish but was

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negatively correlated with mean SL for females and for smaller size-class males across years in Rabbit Slough.

Keywords: alternative life-history strategies, body size, frequency-dependent selection, otolith, prey abundance.

INTRODUCTION

Understanding the varied life-history strategies of organisms is a fundamental endeavour in evolutionary biology. Natural selection should optimize resource allocation to reproduction, maintenance, and growth. Multiple resource allocation strategies may persist within a population if they confer equal fitness. Alternatively, multiple life histories within a population may represent plastic responses, usually to unpredictably variable environmental conditions (Blažek *et al.*, 2013). In coho salmon (*Onchorhynchus kisutch*), two alternative male life-history strategies persist – 'jacks' mature earlier (age 2) and at small body size while 'hooknose males' mature later (age 3) and at large body size. The two strategies are suggested to have a genetic basis, confer equal lifetime fitness, and be maintained in populations by both disruptive and negative frequency-dependent selection (Gross, 1985). Similar alternative life-history phenotypes exist in other fish species, including the annual killifish *Nothobranchius furzeri*, in which unpredictably variable environmental conditions favour developmentally plastic syndromes where, in response to different environmental cues, individuals adopt either relatively fast growth, early maturity (sometimes at smaller size), smaller ultimate body size, and reduced lifespan or the opposite suite of characters (Polačik *et al.*, 2014; see also Wourms, 1972; Hickman, 1975; Ellis *et al.*, 2009; Furness *et al.*, 2015).

In anadromous Threespine Stickleback (*Gasterosteus aculeatus*) in south-central Alaska, USA, both males and females returning to fresh water to breed often form two distinct size classes, where the size class of smaller-bodied fish ('smaller size-class fish') are present at low frequencies. The two size modes may represent alternative life-history strategies, where smaller-bodied, potentially fast-growing fish spawn at a younger age alongside the more frequent, large-bodied, potentially slow-growing fish that spawn when older (Aguirre *et al.*, 2008). We refer to the smaller size-class males and females as 'jacks' and 'jills', respectively. While size-frequency distributions from Alaskan populations suggest that anadromous Threespine Stickleback return to spawn at age 2 years and rarely at age 1 (e.g. reviews in Baker, 1994, Baker *et al.*, 2008), studies from Russian populations based on counts of annuli in otoliths and the operculum suggest that most anadromous spawners are ages 2 and, more frequently, 3 (reviewed in Yershov and Sukhotin, 2015). In this paper, we (1) present size-frequency data that reveal the presence of two distinct size modes in spawning, anadromous Threespine Stickleback populations in south-central Alaska, (2) determine whether distinct size modes represent distinct age classes based on otolith-derived age estimates, and (3) test for sexual dimorphism in the relative frequency of the alternative life-history phenotypes.

Investment in reproduction is assumed to occur at an age or stage (i.e. size, condition) that will optimize lifetime fitness. The benefits of earlier maturity include higher turnover due to shorter generation time and increased survival-to-maturity because organisms spend less time as juveniles. Conversely, the benefits of later maturity include higher fecundity due to a longer period of growth and attainment of a larger size prior to initial reproduction, higher adult survivorship due to attainment of larger size, and lower juvenile death rates due to greater investment in offspring (Stearns, 1992). Many factors determine the optimum age or stage at reproduction – extrinsic mortality rate [e.g. prevalence of predators and competitors (Stearns, 1977, 1992)], energetic constraints or trade-offs [e.g. migration distance, intrinsic growth rate, length of growing season, temperature (Stearns, 1977; Dutil, 1986; Snyder and Dingle, 1989; Winemiller and Rose, 1992; Lee *et al.*, 2013)], resource availability [e.g. prey, spawning grounds or territories, mates or operational sex ratio (Stearns, 1977)], and behaviour [e.g. boldness, aggressiveness, mating tactics (Gross, 1996)]. Quantification of population-level variation in optimal ages or stages at reproduction is a necessary precursor to determining the primary causal factors of the variation. Thus, we also ask whether (4) sizes at reproduction (observed mean body lengths) and (5) the relative frequencies of spawning Threespine Stickleback in the two size classes vary across years and populations. Inter-annual variation and variation across populations in mean ages/stages at reproduction would also lend support to the notion that temporal or spatial variability in the marine environment drives the persistence of alternative life-history phenotypes in Threespine Stickleback. Since operational sex ratio can influence the strength of sexual selection (Emlen and Oring, 1977) and reflect differential mortality rates between the sexes (Wootton, 1984), fluctuations can cause variation in optimal age/stage at reproduction. Therefore, we (6) look for variation in sex ratio across years and populations of spawning Threespine Stickleback.

Many fishes must attain a threshold size or amount of energy reserves before environmental cues will induce sexual maturation (Hutchings and Jones, 1998). For stickleback populations in 25 lakes on Vancouver Island, minimum sizes at first reproduction for females ranged between 26 and 53 mm standard length and were heritable (McPhail, 1977). We hypothesized that in years of high prey abundance in the ocean, more small fish would reach a size threshold for spawning, and fish in general would have more energy to allocate to growth and maintenance as well as reproduction. Thus, we predict positive correlations between chlorophyll-*a* concentrations in the coastal Gulf of Alaska (Waite and Mueter, 2013), a proxy for local prey abundance on the feeding grounds of anadromous Threespine Stickleback in this study, with both (7) the relative frequency of smaller size-class fish ('jacks' and 'jills') and (8) the mean size of both small and large size-class fish.

METHODS

Samples

Anadromous Threespine Stickleback were sampled with unbaited minnow traps during May and June from the spawning grounds or an access stream around Cook Inlet, Alaska, USA. Populations included:

1. Rabbit Slough, sampled downstream of the spawning grounds between 1992 and 2015 (Matanuska-Susitna Borough; between the juncture with Spring Creek at 61.534 N, 149.266 W and a culvert under the Glenn Highway at 61.536 N, 149.253 W prior to 1999, and at the culvert site after 1999).
2. Mud Lake (Matanuska-Susitna Borough; 61.056 N, 148.149 W), sampled in 2014 and 2015.
3. Tide pools at the south end of the Seward Airport (Kenai Peninsula Borough; 60.125 N, 149.420 W), sampled in 2014 and 2015.
4. An unnamed lake at mile 85 of the Seward Highway near Girdwood (Anchorage Municipality; 60.897 N, 149.069 W), sampled in 2014 (Table 1).

Table 1. Collection sites and dates (day/month/year) for samples and numbers of each sex determined by gonadal inspection

Site	Date	# females	# males
Rabbit Slough	21/05/1992	43	52
Rabbit Slough	25/06/1997	167	92
Rabbit Slough	29/05/1998	28	6
Rabbit Slough	12/06/2000	419	159
Rabbit Slough	*/06/2001	73	51
Rabbit Slough	09/06/2003	116	38
Rabbit Slough	*2004	477	471
Rabbit Slough	*2005	610	610
Rabbit Slough	24/05/2006	88	19
Rabbit Slough	28/05/2009	99	28
Rabbit Slough	10/06/2013	509	329
Rabbit Slough	02/06/2014	409	173
Rabbit Slough	01/06/2015	302	121
Mud Lake	07/06/2014	366	197
Mud Lake	02/06/2015	179	147
Seward Highway	18/06/2014	148	87
Seward Airport	18/06/2014	379	145
Seward Airport	12/06/2015	244	49

*Multiple collection days.

Fish were euthanized with an overdose of MS-222 anaesthetic, fixed in 10% buffered formalin, and preserved in 50% isopropyl alcohol. We measured standard length (SL), the distance from the tip of the upper jaw to the end of the last vertebra (± 0.1 mm) with digital callipers and determined sex by gonadal inspection, which is reliable in sexually mature anadromous stickleback. Data from males and females were analysed separately because size in Threespine Stickleback is sexually dimorphic (e.g. Baker, 1994; Rowland, 1994; Kitano *et al.*, 2007; Aguirre *et al.*, 2008).

Hypothesis 1: Size frequency distributions for spawning, anadromous Threespine Stickleback have two distinct size classes

We used Brewer's (2003; available from http://www.bioss.ac.uk/≈markb/mixtures) method and software to determine whether the SL data from each sample [Table 1, Fig. S1 (http://www.evolutionary-ecology.com/data/2983Appendix.pdf); sexes considered separately] fit best to either a normal distribution or a mixture of two normal distributions. Briefly, a two-component mixture model of the form $f(y_i) = pf_1(y_i) + (1 - p)f_2(y_i)$ for $i = 1, 2, \ldots, n$, where f_1 and f_2 are two normal density functions with means μ_1 and μ_2 and variances $\sigma_1^2 = \sigma_2^2$, and p is the probability that an observation y_i lies in the distribution f_1, was fitted to the data using discretization of parameters and 'an efficient summation scheme' (Brewer, 2003; Hendry *et al.*, 2006). We used Akaike's Information Criterion corrected for sample size [AICc (Burnham and Anderson, 2002)] to determine whether the data fit best to a unimodal (single normal) or bimodal (two-component mixture) distribution. We computed ΔAIC as the difference between the AICc for the fitted mixture model of two normal distributions and the AICc for the fitted single normal distribution of each sample, where $\Delta AIC > 10$ represented strong and $10 \geq \Delta AIC > 4$ represented moderate support for fit to a bimodal distribution. We interpreted $4 \geq \Delta AIC \geq -4$ as equivalent support for either a unimodal or bimodal distribution, and $-4 > \Delta AIC \geq -10$ as moderate and $-10 > \Delta AIC$ as strong support for fit to a unimodal distribution (Burnham and Anderson, 2004).

Hypothesis 2: Size classes represent distinct age classes

To determine whether size classes represented fish of different ages, separate samples from Rabbit Slough were collected and frozen in 2010 and 2012 for age determination by counting annuli on sagittal otoliths at 250× magnification (Jones and Hynes, 1950; Greenbank and Nelson, 1959; Dauod *et al.*, 1985; MacColl *et al.*, 2013; otoliths prepared according to Rollins *et al.*, 2014).

Hypothesis 3: The relative frequencies of the two size classes are sexually dimorphic

The proportions (p) of fish in each mode of the bimodal samples were estimated by Bayesian inference (Brewer, 2003). These proportions represent the relative frequencies of fish in the smaller and larger size classes. We used a paired t-test to determine whether the frequency of smaller size-class fish differed significantly between males and females separately for all Rabbit Slough sample years [Table 1; BIOMstat 4.11, Exeter Software, Setauket, NY (available from http://www.exetersoftware.com/cat/biomstat/biomstat.html)]. Sample sizes for other populations sampled in 2014 and 2015 were insufficient to perform a similar analysis. Where samples fit better to a single normal distribution (unimodal), the frequency of the smaller size class was set equal to 0, as all fish in these cases were considered to be analogous to the more common larger size-class modes of bimodal samples.

Hypothesis 4: Size at reproduction varies temporally and spatially

We compared mean SL across years (1992–2015 samples) in Rabbit Slough and among populations sampled in 2014 and 2015. Sample years 2014 and 2015 for the among-population comparisons as well as sexes and size classes for both temporal (across years) and spatial (among populations) comparisons were considered separately. For bimodal samples, we used Bayesian estimates of mean sizes and variances of the two size classes from the fitted two-component mixture model (Brewer, 2003). For unimodal samples, we computed a mean and variance from the SL data and used these in larger size-class comparisons only. Since attempts to classify individuals in each of the bimodal samples into either of the two components of the mixture did not guarantee a match with the Bayesian estimates of the means and variances, the latter were used in conjunction with estimated sample sizes in each mode (taken by multiplying Bayesian estimates of the proportions in each mode with the total sample size) to compute Bonferroni-adjusted (assuming all possible pairwise comparisons) confidence intervals instead of using analysis of variance (ANOVA) to compare means. If two sample means fell outside each other's confidence intervals, then differences were considered statistically significant. We report only confidence intervals and not the results of all possible pairwise comparisons because only the overall significance of temporal and spatial variation and general trends were of interest.

Hypothesis 5: Relative frequencies of the two size classes vary temporally and spatially

We compared the relative frequencies of fish in the two size classes across years in Rabbit Slough and among populations sampled in both 2014 and 2015. As above, we considered sexes and the two sample years for among-population comparisons separately. We used sample sizes and Bayesian estimated proportions (p) to compute the estimated number of fish in the two size modes for each sample. We used these raw frequencies in G-tests to determine overall significance of temporal and spatial variation in relative frequencies of the two size classes (BIOMstat 4.11).

Hypothesis 6: Sex ratio varies temporally and spatially

We performed separate G-tests for differences in sex ratios among years in Rabbit Slough, among populations in 2014, and among populations in 2015 (Table 1; BIOMstat 4.11). Samples from 2004 and 2005 were excluded from the analysis for differences among years in Rabbit Slough because they were not random with respect to sex.

Hypotheses 7 and 8: Local prey abundance on the feeding grounds is associated with (a) the frequency of jacks and jills and (b) fish size

We hypothesized that in years of high prey abundance in the marine feeding grounds of anadromous Threespine Stickleback, higher frequencies of smaller size-class fish would reach the minimum size required to induce spawning, and fish in general would be larger. We used chlorophyll-*a* concentrations in the Gulf of Alaska as a proxy for prey abundance in the feeding grounds. Chlorophyll-*a* concentrations for the Gulf of Alaska were extracted from SeaWiFS remotely sensed data available from the Goddard Space Flight Center OceanColor website (http://oceancolor.gsfc.nasa.gov) according to Waite and Mueter (2013). We used the mean of the monthly composite chlorophyll-*a* concentrations, from June of each fish collection year $i - 1$ to May of collection year i, for the years between 2000 and 2009 for which we had both fish and chlorophyll-*a* data. Mean annual chlorophyll-*a* concentrations excluded November through January for all years due to lack of data for those months. We tested the significance of product–moment correlation coefficients between mean annual chlorophyll-*a* concentrations ($\mathrm{mg \cdot m^{-3}}$) and (a) the frequency of smaller size-class fish, and (b) the estimates of mean SL of larger and smaller size-class fish separately, across sample years in Rabbit Slough (BIOMstat 4.11).

RESULTS

Hypothesis 1: Size frequency distributions for spawning, anadromous Threespine Stickleback have two distinct size classes

All Rabbit Slough samples fit best to a mixture of two normal distributions with strong support except 1992 males, 1998 males and females, 2001 females, and 2006 males, many of which had small sample sizes (Tables 1 and 2). Females in 2001 fit best to a bimodal distribution but with moderate support, while the other exceptions fit best to a unimodal distribution. Among populations sampled in 2014 and 2015, only Mud Lake samples from 2014, Seward Airport tide pool males from 2015, and males from the unnamed lake at mile 85 of the Seward Highway were not bimodal (Table 2). Thus, across

Table 2. Modality of size-frequency distributions for each sample (population/year) and sex

Sample	ΔAICc	Modality	Support	$\overline{SL}_{\mathrm{small}}$[1]	$\overline{SL}_{\mathrm{large}}$[1]	σ_{SL}[2]	p_{small}[3]	σ_{P}[4]
Females								
RS1992	19.77	bimodal	strong	59.98	73.77	2.78	0.112	0.047
RS1997	40.89	bimodal	strong	52.13	69.28	2.90	0.021	0.011
RS1998	−5.68	unimodal	moderate	n/a	69.68	5.71	0	n/a
RS2000	116.84	bimodal	strong	50.50	67.58	2.97	0.020	0.007
RS2001	4.08	bimodal	moderate	64.01	72.16	2.40	0.079	0.126
RS2003	59.88	bimodal	strong	54.55	72.26	2.27	0.025	0.014
RS2004	73.09	bimodal	strong	62.34	71.55	2.61	0.060	0.014
RS2005	125.03	bimodal	strong	62.28	70.93	2.59	0.184	0.019
RS2006	41.80	bimodal	strong	61.81	72.75	2.42	0.336	0.050
RS2009	141.93	bimodal	strong	53.07	69.15	2.82	0.070	0.025
RS2013	340.37	bimodal	strong	56.43	70.54	2.42	0.030	0.008
RS2014	87.27	bimodal	strong	60.01	70.76	2.39	0.015	0.007
RS2015	180.72	bimodal	strong	57.18	71.79	2.77	0.016	0.007
MD2014	−21.87	unimodal	strong	n/a	70.76	2.39	0	n/a
MD2015	23.94	bimodal	strong	60.27	71.50	2.46	0.025	0.013
SA2014	63.81	bimodal	strong	56.79	67.57	3.36	0.395	0.030
SA2015	66.14	bimodal	strong	40.88	67.34	4.66	0.025	0.016
SH2014	−3.70	equivalent	equivalent	n/a	68.72	10.67	0	n/a
Males								
RS1992	−4.71	unimodal	moderate	n/a	68.83	5.56	0	n/a
RS1997	18.44	bimodal	strong	52.13	65.48	2.67	0.040	0.023
RS1998	−40.97	unimodal	strong	n/a	64.70	6.10	0	n/a
RS2000	49.33	bimodal	strong	50.04	64.35	3.11	0.056	0.019
RS2001	21.97	bimodal	strong	54.42	67.23	2.22	0.057	0.032
RS2003	14.93	bimodal	strong	53.99	67.33	2.66	0.089	0.048
RS2004	78.49	bimodal	strong	58.42	66.61	2.49	0.109	0.018
RS2005	138.08	bimodal	strong	58.19	66.20	2.32	0.262	0.020
RS2006	−5.81	unimodal	moderate	n/a	64.44	29.99	0	n/a
RS2009	15.62	bimodal	strong	53.50	64.84	2.28	0.302	0.084
RS2013	191.99	bimodal	strong	53.97	65.92	2.22	0.078	0.015
RS2014	17.53	bimodal	strong	56.00	66.06	2.10	0.019	0.016
RS2015	20.21	bimodal	strong	57.94	67.09	2.32	0.057	0.023
MD2014	−5.04	unimodal	moderate	n/a	66.14	5.24	0	n/a
MD2015	8.08	bimodal	moderate	59.00	67.28	2.19	0.037	0.076
SA2014	16.16	bimodal	strong	49.47	62.24	4.39	0.136	0.061
SA2015	3.16	equivalent	equivalent	n/a	59.36	57.78	0	n/a
SH2014	9.90	bimodal	moderate	54.57	64.83	2.86	0.066	0.058

Note: The standard length data fit best to a bimodal distribution when ΔAICc was positive and to a unimodal distribution when ΔAICc was negative. The strength of support for either modality was determined using the magnitude of the ΔAICc as explained in the text. RS = Rabbit Slough, MD = Mud Lake, SA = Seward Airport tide pools, SH = lake off mile 85 of the Seward Highway. n/a = not applicable.

[1] $\overline{SL}_{\mathrm{small}}$ and $\overline{SL}_{\mathrm{large}}$ = when bimodal, Bayesian estimates for the means of smaller and larger size-class fish, respectively; when unimodal, p_{small} was assumed 0, and $\overline{SL}_{\mathrm{large}}$ was the mean standard length of the sample.

[2] σ_{SL} = when bimodal, Bayesian estimates for the common standard deviation of the two modes; when unimodal, the standard deviation for the sample.

[3] p_{small} = when bimodal, the Bayesian estimate of the proportion of the sample in the first, smaller size-class mode; when unimodal, set to 0.

[4] σ_{P} = the Bayesian estimate of the standard deviation of p_{small} where applicable (for bimodal samples).

years in Rabbit Slough (22 of 26 samples) and in samples among other populations (6 of 10), spawning, anadromous Threespine Stickleback indeed exhibit two distinct size classes (2983Appendix.pdf, Fig. S1; Table 2).

Hypothesis 2: Size classes represent distinct age classes

Smaller size-class fish of both sexes were either 1+ or 2+ years old, while those in the larger size class were 2+, 3+, or 4+ (Fig. 1). The majority of spawning fish were age 3+, and most 3+ individuals were members of the larger size class, with some small-bodied outliers within the smaller size class. Age 2+ fish were either small or large and appeared to be bimodally distributed; the frequency of age 2+ fish within the smaller size class was roughly equivalent to that within the larger size class for both sexes. Age 1+ and 4+ fish were relatively rare and were always within the small and large size modes, respectively. Thus, on average, the smaller size-class fish were younger (age 1+ and half the 2+ fish) than the larger size-class fish (age 3+ and half the 2+ fish). However, the size classes do not represent distinct age classes, since age 2+ fish were present in both modes in approximately equal frequencies.

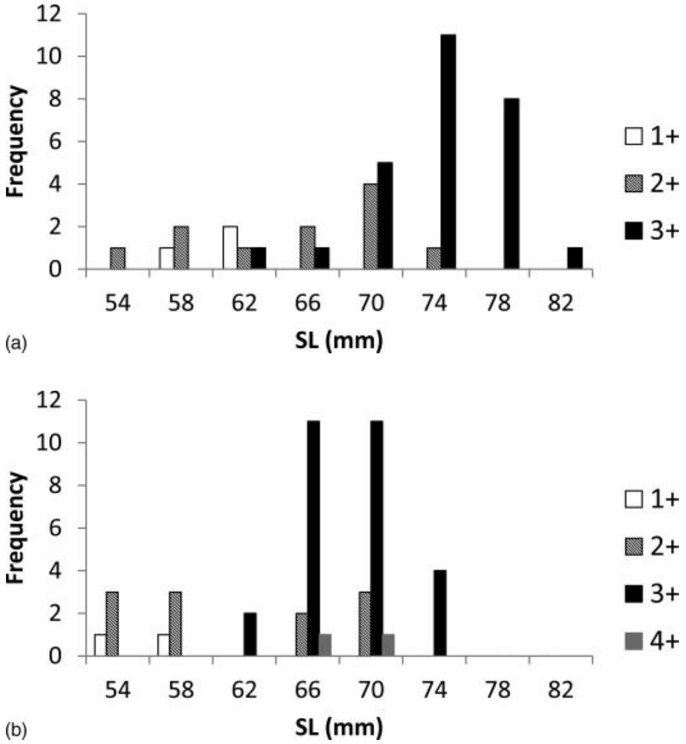

Fig. 1. Size-frequency distributions of aged (a) female and (b) male Threespine Stickleback from Rabbit Slough. Ages are indicated by bar shading.

Hypothesis 3: The relative frequencies of the two size classes are sexually dimorphic

With all samples from Rabbit Slough included in the paired *t*-test, there was no significant difference between female and male frequencies of smaller size-class fish ($t_{\text{female-male}} = -0.218$, $df = 12$, $P_{\text{two-tailed}} = 0.831$). However, smaller sample sizes among males relative to females in some years were a cause for concern; in some cases with small sample size, the AIC analyses indicated support for a unimodal distribution, suggesting that perhaps not enough male fish were collected in those samples to obtain any of the rarer smaller size class. To account for this bias due to low sample size, we ran another paired *t*-test excluding sample years (i.e. 1992, 1998, 2003, 2006, and 2009) where the sample size for one or both sexes was less than 50. In this analysis, males had significantly larger relative frequencies of smaller size-class fish than females ($t_{\text{female-male}} = -2.886$, $df = 7$, $P_{\text{two-tailed}} = 0.023$), suggesting sexual dimorphism in the frequency of smaller size-class spawners (Fig. 2a).

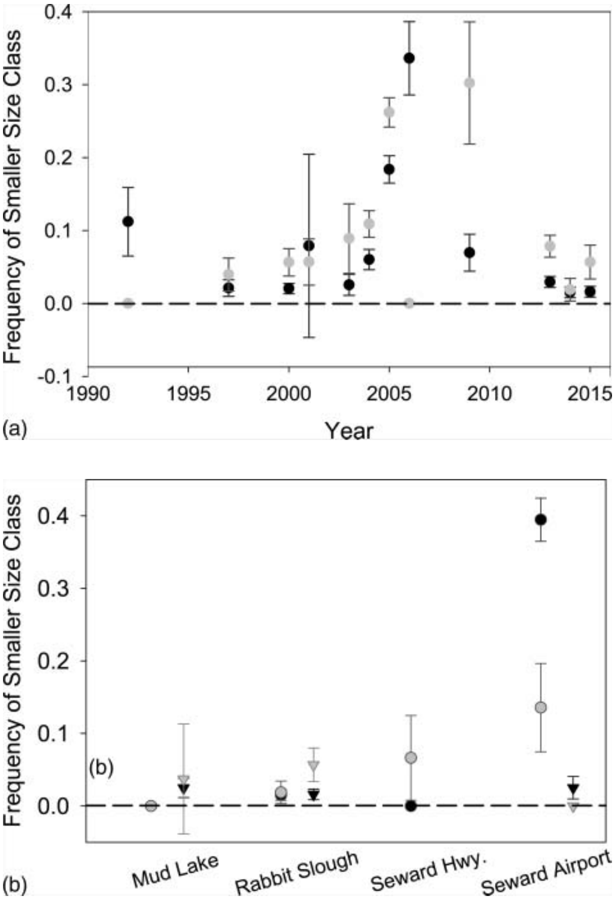

Fig. 2. Proportion of smaller size-class females (black) and males (grey) (a) among years of anadromous Threespine Stickleback in Rabbit Slough and (b) among populations sampled in 2014 (circles) and 2015 (triangles). Error bars are ± standard deviation.

Hypothesis 4: Size at reproduction varies temporally and spatially

Based on Bonferonni-adjusted 95% confidence intervals, there were significant differences in the mean SL of smaller size-class and larger size-class fish across years (within sexes) in Rabbit Slough (Fig. 3). The mean size of smaller size-class fish is significantly and marginally positively correlated with that of larger size-class fish across years for females and males, respectively ($r_{\text{females}} = 0.712$, $df = 10$, $P < 0.01$; $r_{\text{males}} = 0.620$, $df = 8$, $P = 0.056$). Mean SL differed significantly among populations for (a) smaller size-class females in both 2014 and 2015 (Fig. 4a) and (b) smaller size-class males in 2014 but not 2015 (Fig. 4b). Mean SL also differed significantly among populations for larger size-class females and males in both 2014 and 2015 (Fig. 5). (The 2014 Mud Lake and 2015 Seward Airport tide pool samples were unimodal, so no fish were in the smaller size class, and these could not be compared with other populations. In addition, the unnamed lake at mile 85 off the Seward Highway was only sampled in 2014.)

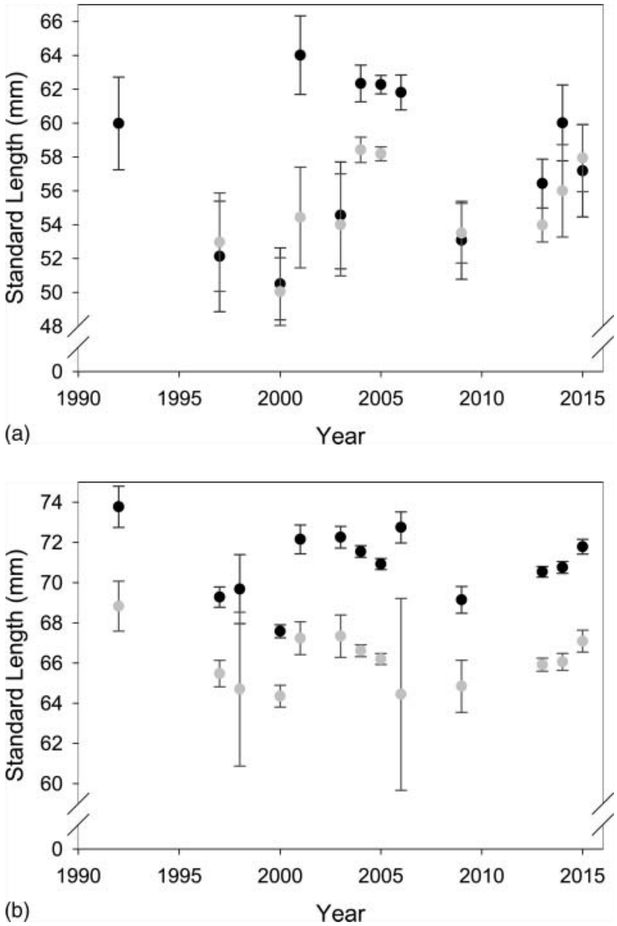

Fig. 3. Mean standard length (± 95% Bonferroni-adjusted confidence interval) among years for female (●) and male (●) smaller size-class (a) and larger size-class (b) anadromous Threespine Stickleback from Rabbit Slough.

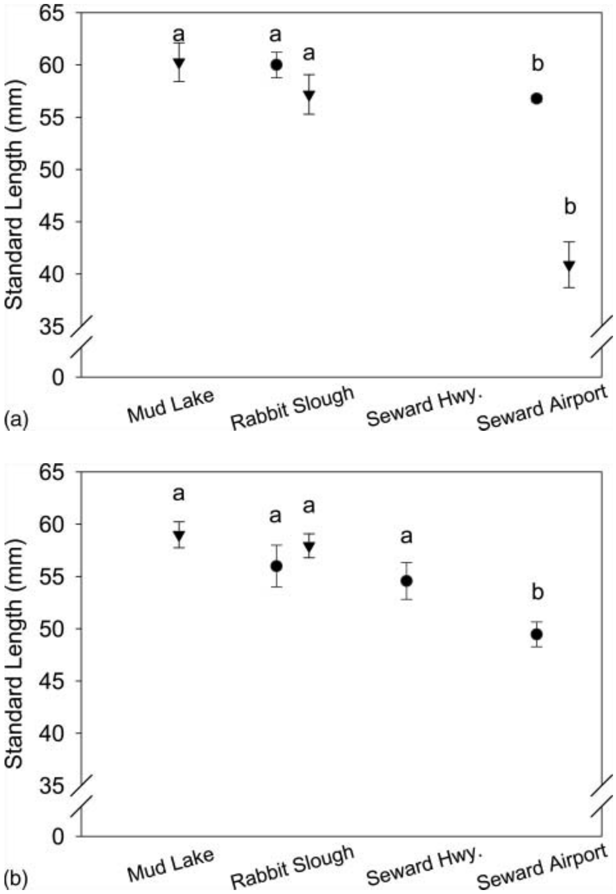

Fig. 4. Mean standard length (± 95% Bonferroni-adjusted confidence interval) of smaller size-class female (a) and male (b) anadromous Threespine Stickleback among populations sampled in 2014 (●) and 2015 (▼). Different letters above points indicate significant differences among populations within years at $P < 0.05$.

Hypothesis 5: Relative frequencies of the two size classes vary temporally and spatially

Relative frequencies of smaller and larger size classes were significantly different among years in Rabbit Slough for both females and males ($G_{\mathrm{females}} = 234$, $df = 10$, $P < 0.001$; $G_{\mathrm{males}} = 155$, $df = 8$, $P < 0.001$; Fig. 2a). The analyses excluded sample years with low samples sizes where expected frequencies for the *G*-test were less than five (1992 and 1998 for females; 1998, 2003, 2006, and 2009 for males). Relative frequencies of smaller and larger size classes were significantly different among populations sampled in 2014 for both females and males ($G_{\mathrm{females}} = 279$, $df = 3$, $P < 0.001$; $G_{\mathrm{males}} = 200$, $df = 3$, $P < 0.001$), but not in 2015 ($G_{\mathrm{females}} = 0.419$, $df = 1$, $P = 0.518$; $G_{\mathrm{males}} = 0.868$, $df = 1$, $P = 0.351$; Fig. 2b). Mud Lake females and Seward Airport tide pool males in 2015 were excluded from analyses due to low sample sizes.

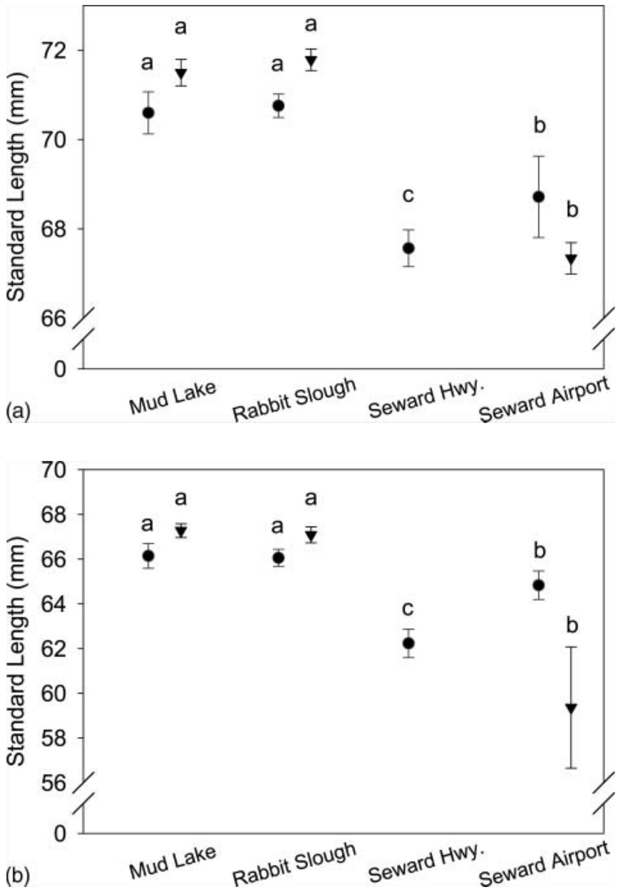

Fig. 5. Mean standard length (± 95% Bonferroni-adjusted confidence interval) of larger size-class female (a) and male (b) anadromous Threespine Stickleback among populations sampled in 2014 (●) and 2015 (▼). Different letters above points indicate significant differences among populations within years at $P < 0.05$.

Hypothesis 6: Sex ratio varies temporally and spatially

Sex ratios differed significantly among years in Rabbit Slough ($G = 80.0$, $df = 10$, $P < 0.001$). The percentage of females was higher relative to males every year except 1992 and 2001 (Fig. 6). Sex ratios differed significantly among populations in 2014 ($G = 11.9$, $df = 3$, $P < 0.01$); Seward Airport tide pools (72.9 ± 5.0%; Bonferroni-adjusted 95% Wald confidence interval) had a significantly higher female-to-male ratio than Mud Lake (65.0 ± 5.2%), and the percentage of females in all four populations ranged from 63.0 to 72.9% (data not shown). Sex ratios also differed significantly among populations in 2015 ($G = 60.4$, $df = 2$, $P < 0.001$); Seward Airport tide pools (83.3 ± 5.1%) had a significantly higher female-to-male ratio than Rabbit Slough (71.2 ± 5.1%), which had a significantly higher ratio than Mud Lake (54.9 ± 6.4%).

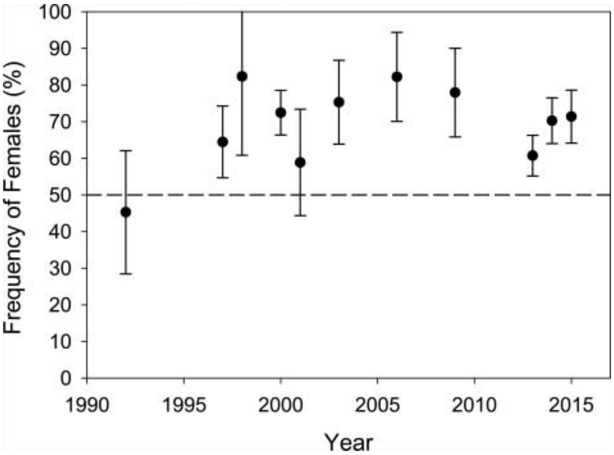

Fig. 6. Sex ratio variation among years for anadromous Threespine Stickleback from Rabbit Slough. Error bars are ±95% Bonferroni-adjusted Wald confidence intervals. Dashed line indicates a 1 : 1 sex ratio.

Hypotheses 7 and 8: Local prey abundance on the feeding grounds is associated with (a) the frequency of jacks and jills and (b) fish size

The correlation between chlorophyll-*a* concentration and the frequencies of smaller size-class fish in Rabbit Slough across years was not significant for either sex ($r_{\text{females}} = -0.551$, $df_{\text{females}} = 5$, $P_{\text{females}} = 0.200$; $r_{\text{males}} = 0.230$, $df_{\text{males}} = 5$, $P_{\text{males}} = 0.621$; Fig. 7a). Contrary to predictions, mean SL of both smaller and larger size-class females were significantly negatively correlated with chlorophyll-*a* concentration ($r_{\text{smaller}} = -0.906$, $df_{\text{females}} = 5$, $P_{\text{smaller}} < 0.01$; $r_{\text{larger}} = -0.911$, $df_{\text{females}} = 5$, $P_{\text{larger}} < 0.01$; Fig. 7b,c). Although relationships for males were similar to those for females, fish size was only significantly correlated with chlorophyll-*a* concentration in smaller size-class males ($r_{\text{smaller}} = -0.874$, $df_{\text{males}} = 4$, $P_{\text{smaller}} = 0.023$; $r_{\text{larger}} = -0.926$, $df_{\text{males}} = 5$, $P_{\text{larger}} = 0.181$; Fig. 7b,c).

An additional measure of nutrient availability, the National Oceanic and Atmospheric Administration's (NOAA) Pacific Fisheries Environmental Lab upwelling index [monthly estimates from the 60°N, 149°W location within the Gulf of Alaska used to compute annual means from June in year $i - 1$ to May in fish collection year i (available from https://www.pfeg.noaa.gov/products/PFELData/upwell/monthly/upindex.mon)], was not correlated with the frequency of smaller size-class fish or the size of either size class (data not shown). NOAA's Oceanic Niño Index [global monthly estimates used to compute annual means from June in fish collection year $i - 1$ to May in year i (available from https://www.climate.gov/news-features/understanding-climate/climate-variability-oceanic-ni%C3%B1o-index)] was only correlated with the mean SL of smaller size-class males ($r = 0.684$, $df = 8$, $P = 0.029$).

We tested for additional correlations between chlorophyll-*a* concentration, upwelling index, and Oceanic Niño index averaged across two years prior to fish collections (from June in year $i - 2$ to May in fish collection year i) with the mean SL of larger size-class fish. We found no significant correlations except for upwelling and mean SL of larger size-class males ($r = 0.652$, $df = 11$, $P = 0.016$). We found no significant pairwise correlations between

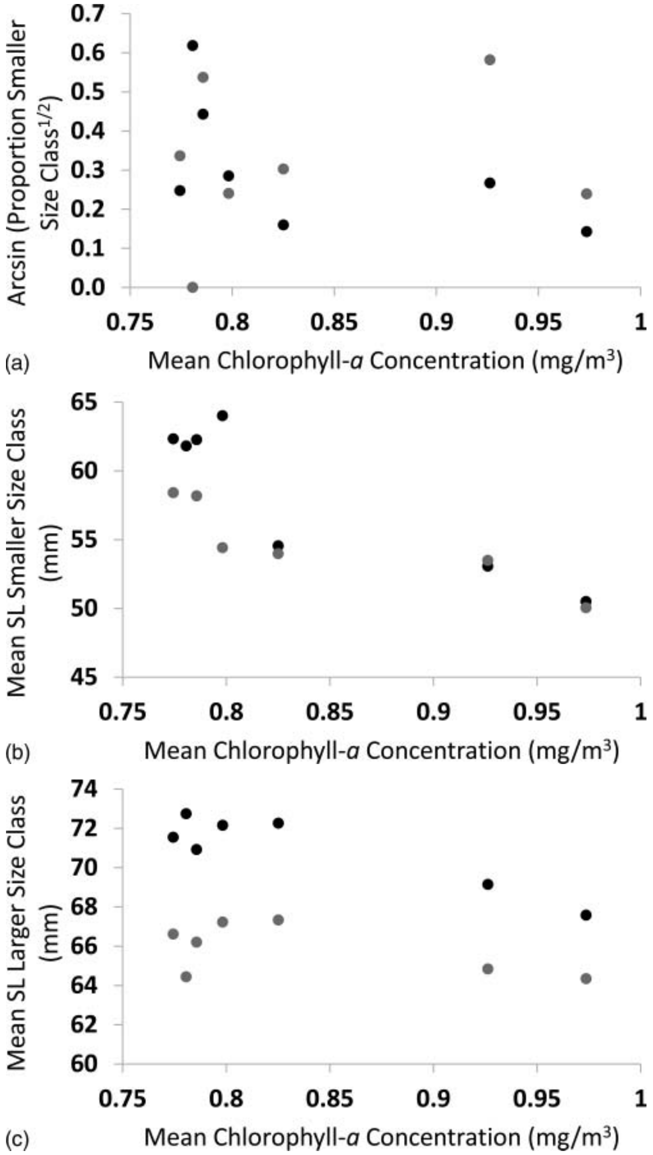

Fig. 7. Association between mean annual chlorophyll-*a* concentration and (a) the proportion of smaller size-class fish, (b) mean standard length (SL) of smaller size-class fish, and (c) mean SL of larger size-class fish. See text for significance of correlation coefficients. ●, females; ●, males.

chlorophyll-*a* concentration, upwelling index, and Oceanic Niño index values used in any of our analyses (data not shown).

DISCUSSION

We found evidence for alternative life-history phenotypes for both male and female anadromous Threespine Stickleback; while most anadromous stickleback returning to spawn were 2+ to 3+ years old and >65 mm SL, some members of both sexes returned to spawn earlier (1+ and 2+; 2983Appendix.pdf, Fig. S1; Table 2, Fig. 2) and at smaller sizes (<65 mm SL) across years and populations (Figs. 3, 4, and 5). Although it is not known whether anadromous Threespine Stickleback are iteroparous, it is unlikely that iteroparity can entirely explain the co-occurrence of smaller and larger size-class spawners because older and larger size-class spawners are present in higher frequencies than younger and smaller size-class spawners, suggesting that most fish begin reproduction when older and larger. Even if some of the older and larger size fish are returning to spawn a second time, this certainly cannot be the case for all of them or we would expect much larger frequencies of younger and smaller-bodied fish.

Although the lower-frequency, smaller-bodied mode of spawners consisted of fish that were on average younger than their larger-bodied counterparts, distinct size classes did not appear to represent distinct age classes. It has often been assumed that the smaller-bodied mode is comprised of fish that mature to spawn at age 1+ while the larger-bodied mode represents the more common strategy of spawning at age 2+ in Alaskan Threespine Stickleback populations (e.g. reviews in Baker, 1994; Baker *et al.*, 2008). However, we found that the smaller size class contained both 1+ and 2+ fish, while the larger size class contained mostly 3+ fish, a few rare 4+ fish, and about half the 2+ fish in the sample. These findings are more similar to those from Russian stickleback populations (reviewed in Yershov and Sukhotin, 2015). Collectively, our findings suggest that (a) it is important to validate estimates of age distributions that are based on size-frequency distributions by using a method of age determination such as annuli in calcified structures, and (b) many or most Pacific anadromous Threespine Stickleback mature, migrate, and spawn at age 2+ and 3+. It is interesting that age 2+ fish appear to have bimodal size-frequency distributions for both sexes. Additional samples across multiple years should be examined to determine whether this was a chance occurrence, an artefact of combining across two sample years (2010 and 2012), or a common occurrence with some underlying biological cause.

Variation in the relative frequencies and sizes at reproduction of the alternative life-history phenotypes across years and among populations (Figs. 2, 3, 4, and 5) may reflect variation in environmental conditions in the ocean or on the breeding grounds, which may contribute to the maintenance of alternative life histories. However, it is thought that the ocean is a more stable environment without the large seasonal temperature changes and associated fluctuations in prey abundance found in temperate and boreal freshwater habitats (Gross *et al.*, 1988; Ydenberg, 1989). The marine microhabitat and home range of anadromous Threespine Stickleback are unknown, so it is difficult to assess whether they experience large variation in conditions with correlated variation in resource availability that would favour alternative life histories. Anadromy may be favoured when rates of extrinsic mortality in the ocean are high or highly variable – fresh water may be relatively safe nursery grounds away from the risk of heavy predation in the ocean, especially for a small-bodied species with correspondingly low fecundity like Threespine Stickleback (Winemiller and Rose, 1992). High or highly variable juvenile mortality, in particular, also favours iteroparity (Stearns, 1992) as well as a syndrome of delayed maturation, smaller reproductive effort, and longer lifespan (Stearns, 1977). It is unknown whether anadromous or resident freshwater stickleback with a lifespan longer than one year are iteroparous. However, anadromous stickleback have delayed maturation and longer lifespans relative to freshwater resident sticklebacks (J.L. Rollins, unpublished data). Thus, it is probable that high or highly variable juvenile mortality in the oceanic environment has favoured anadromy, delayed maturation, and longer lifespan in oceanic Threespine Stickleback. It is also possible that the same variability has favoured plasticity in age at reproduction such that some fish may grow large enough in their first year of life to spawn early and hedge their bets by reproducing in multiple years (iteroparity). Conversely, high variation in extrinsic mortality among adults may contribute to the existence of alternative life histories. Adult oceanic Threespine Stickleback are relatively small-bodied fishes and have many potential predators (Reimchen, 1994); fluctuations in predator abundance across time or space may cause large variation in adult mortality. High variability in adult mortality favours earlier maturation, and enhancing the probability of survival to reproduction may outweigh the benefits of greater fecundity due to attainment of larger size at reproduction associated with maturing later in some years or in some populations (Stearns, 1977). This selection pressure may oppose those that favour later maturation, or it may act alone to favour the evolution of plastic life-history responses to environmental conditions.

We found some evidence for sexual dimorphism in the frequency of smaller size-class fish, with higher frequencies of 'jacks' than 'jills' (Fig. 2). Smaller-bodied males are inferior in territorial defence and less attractive to females (Rowland, 1989). However, smaller males of other fish species, called 'jacks' in salmonids, use sneak fertilization (Gross, 1984). Indeed, the sneaker strategy is common for stickleback males in some populations (Wootton, 1984; Goldschmidt *et al.*, 1992; Rico *et al.*, 1992; Largiadèr *et al.*, 2001), although sneaking in stickleback is not a tactic restricted to smaller-bodied fish (Goldschmidt *et al.*, 1992; Mori, 1995). A higher frequency of jacks than jills may be due to the option for small males to sneak fertilizations, which may favour males returning at smaller sizes to spawn. Alternatively, a higher frequency of smaller size-class males may reflect greater mortality rates for males than for females as they age or grow larger (Wootton, 1984). These alternative hypotheses are difficult to disentangle because greater proportions of males expending energy towards reproduction early in life or at smaller sizes may cause the higher age- or size-specific mortality among males, or the higher mortality may select for earlier maturation in males (Stearns, 1992). It is also possible that males returning to spawn at smaller sizes experience high reproductive success later in the breeding season once larger, more competitive nesters stop courting females (Wootton, 1984; cf. Perrin, 1995). An over-abundance of spawning females relative to males (Fig. 6) also may contribute to the success of smaller males (Owens and Thompson, 1994).

Frequency of smaller size-class fish and size differences among four populations sampled in 2014 and three populations sampled in 2015 (Figs. 2b and 4) may reflect genetic differentiation, which would require either that anadromous Threespine Stickleback populations are philopatric (i.e. home or return to their natal breeding grounds) and genetically divergent or that size influences how far Threespine Stickleback migrate to spawn and, therefore, their ultimate breeding location. Without citing a primary source, McPhail (2007) reported that a mark-recapture study in British Columbia indicated that anadromous Threespine Stickleback home. Furthermore, the *Ectodysplasin* locus shows allelic divergence between Mud Lake and Rabbit Slough anadromous populations, which are in the same drainage and only a few kilometres apart (Bell *et al.*, 2010). Thus, variation in the size of larger size-class fish of both sexes among populations and of the frequency of smaller size-class individuals may reflect genetic divergence for size among populations.

Sex ratios varied significantly across years and among populations, and were significantly female-biased in all years except 1992 and 2001 (Fig. 6), for all populations in 2014 and for all populations in 2015 except Mud Lake. Female-biased ratios were not an artefact of trapping on male territories for many of the years in the across-year analysis, since we trapped and caught all fish swimming upstream through a culvert in Rabbit Slough from 1999 to 2015. Before 1999, fish in Rabbit Slough were also caught during the upstream migration and potentially prior to establishment of territories. Females may outnumber males in the ocean due to higher male-specific mortality rates (suggested by Wootton, 1984), but we lack sufficient data to support this. Female-biased sex ratios are not unique to spawning anadromous Threespine Stickleback from the Gulf of Alaska; Aneer (1973) and Yershov and Sukhotin (2015) also observed female-biased sex ratios for Baltic and White Sea anadromous Threespine Stickleback on the spawning grounds.

We hypothesized that years of high prey abundance on the feeding grounds would allow a higher frequency of smaller-bodied or younger anadromous Threespine Stickleback to reach a size or energy reservoir threshold for spawning. However, we did not find a significant correlation between chlorophyll-*a* concentrations in the coastal Gulf of Alaska and the frequency of smaller size-class fish (Fig. 7a). We also predicted that the mean size of both smaller and larger size-class fish would be positively correlated with mean annual chlorophyll-*a* concentrations because years with higher prey abundance should allow for greater somatic growth. However, we found a significant negative correlation between mean SL and chlorophyll-*a* concentrations for females and smaller size-class males, with similar but non-significant negative trends for larger size-class males (Fig. 7b,c). Because oceanic stickleback eat zooplankton (Hart and Gill, 1994) and zooplankton abundances usually track phytoplankton abundances (e.g. Polovina *et al.*, 1995), it is difficult to explain this negative correlation. Indeed, the body size of many fish species is positively correlated with primary productivity (e.g. Basilone *et al.*, 2004; Hernandez-Miranda and Ojeda, 2006; Huston and Wolverton, 2011). Future research might examine possibilities such as size-selective mortality in years of high prey abundance, changes in reproductive migration behaviour associated with depauperate growing conditions, and migration energetics associated with body size.

In conclusion, we found evidence for alternative life-history phenotypes for both male and female anadromous Threespine Stickleback; in both sexes, a relatively low number of younger, smaller-bodied fish migrate to fresh water to spawn alongside older, larger-bodied stickleback. This occurred across multiple years and populations of anadromous Threespine Stickleback in the Gulf of Alaska. Fitness advantages of early reproduction in anadromous Threespine Stickleback are still unclear, but may include advantages due to sneaking in males and optimizing lifetime fitness across years by ensuring reproduction before death or via iteroparity for both sexes. We add to previous evidence that spawning, anadromous Threespine Stickleback populations have female-biased sex ratios. It will be difficult to determine whether this is due to differential mortality between the sexes and at which ontogenetic stage(s) this difference manifests in the ocean. Finally, we found a relationship between body size of spawners and prey abundance on the feeding grounds across years, though not in the predicted direction. This still suggests the potential for spawning Threespine Stickleback populations to respond to environmental variation, which could contribute to the maintenance of alternative life-history phenotypes.

ACKNOWLEDGEMENTS

We thank R. Baldi, C. Sanges, H. Tsang, P. Ubba, and J. Babinski for measurement and sex determination of fish, and J. Walker and M. Cashin for otolith preparation. Many people helped collect anadromous stickleback, including P.J. Park in particular. Specimens were sampled and euthanized under several annual permits from the Alaska Department of Fish and Game and with annual approvals from the Institutional Animal Care and Use Committee to M.A.B. Sampling was supported some years by NSF grants BSR-8905758, DEB-0320076, DEB-0211391, and DEB-0322812, an NSF GRFP to J.L.R. (ID#: 2010100659), and Stony Brook University URECA fellowships for undergraduate field assistants.

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