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Female life-history traits of a species pair of threespine stickleback in Mud Lake, Alaska

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ABSTRACT

Aim: Compare several female life-history traits in a stickleback species pair (i.e. size and age at reproduction, clutch size, egg mass, and reproductive effort) to determine whether time during the reproductive season (early or late) or breeding habitat (vegetation, open sites) significantly affect trait values.

Organisms: Sympatric ecotypes of anadromous and resident freshwater threespine stickleback (*Gasterosteus aculeatus*).

Time and place: Mud Lake, Cook Inlet region, Alaska, May–July 2003.

Results: Both resident and anadromous fish in Mud Lake bred primarily at age 2. Anadromous breeders were larger than resident breeders. Size of reproductive females declined from early to late in the breeding season for both anadromous and resident freshwater populations, but time and breeding habitat were not significant sources of variation in most life-history traits. Clutch mass showed a similar, positive correlation with female body mass in both populations, but clutch size (fecundity) rose more slowly with female size in resident fish. Both ecotypes made eggs of similar size overall, with egg size in resident females increasing modestly with female body size. Both ecotypes showed a trade-off between clutch size and egg mass after adjustment for the level of reproductive effort. The egg mass and clutch mass of both ecotypes were small compared with other Cook Inlet resident and anadromous populations.

Keywords: anadromous, benthic, breeding females, clutch size, egg mass, reproductive effort, stickleback.

INTRODUCTION

The emergence of new species, and the paths they follow during their divergence, fascinated biologists well before Darwin provided a mechanism for the process (Honeywill, 2008). Especially informative are situations in which individuals comprising the diverging or recently diverged gene pools co-exist in the same or adjacent habitats (sympatric and

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parapatric speciation). From the viewpoint of the process of speciation itself, these systems make it possible to study the interplay among details of divergence, such as the roles of selection and gene flow, the relative importance of potential isolating mechanisms, and the effect of plasticity (Orr and Smith, 1998; Knudsen *et al.*, 2006; Davis *et al.*, 2012; see also Hendry *et al.*, 2013; Baker *et al.*, 2013; Ravinet *et al.*, 2013). Even when gene flow has ceased and new species have formed, there is much we can learn by comparing the recently diverged sister species.

Often, instances of sympatric or parapatric speciation involve shifts in general lifestyle between the incipient, or newly formed, species. In aquatic systems, for example, divergence has repeatedly been observed to produce ecological axes corresponding to: bottom-oriented vs. open-water ecotypes [the benthic–limnetic axis (McPhail, 1994; Lundsgaard-Hansen *et al.*, 2012)], profundal vs. littoral forms (Knudsen *et al.*, 2006), flowing-water vs. standing-water ecotypes [the stream–lake habitat axis (Berner *et al.*, 2008; Hendry *et al.*, 2013)], pairs of parasitic and non-parasitic forms (Salewski, 2003; Espanhol *et al.*, 2007), and migratory–resident lifestyles (Northcote and Ward, 1985; Waters and Wallis, 2001; Wood *et al.*, 2008). In many instances, the newly forming (or formed) species differ in fundamental ways, yet their future evolutionary trajectories remain partially entangled due to some shared need. Often the shared need is a common resource used for only a portion of their life (e.g. a common spawning ground). These instances of ongoing or recently completed speciation are especially informative because they offer an opportunity to assess how the specific traits of the differing lifestyles evolve under variably independent conditions (e.g. Lattuca *et al.*, 2008). Traits affecting reproductive fitness, especially female reproductive investment traits, may be of particular importance. For example, early life-history traits are often of critical importance because of the high mortality rate typical at this stage (Vetter, 1988). Many fitness consequences of egg size variation result from the relative ability of the resulting fry (e.g. Phillip, 2002; Fisher and Hogan, 2007; Bashey, 2008), and large fry size is positively correlated to fry competitive ability in many species (Post and Prankevicius, 1987; Phillip, 2002; Czesak and Fox, 2003; Gagliano *et al.*, 2007; Bashey, 2008). Because of the link between female and offspring fitness (Bernardo, 1996), from the maternal perspective the best reproductive strategy is to produce offspring that are just large enough to compete well in the rearing environment, allowing for the greatest number of viable offspring to be produced (Stearns, 1992; Bernardo, 1996). In addition, early growth trajectories can carry over to affect reproductive performance later in life (Auer *et al.*, 2010; Lee *et al.*, 2012). Thus, if the diverging forms occupy the same rearing environment, selection that promotes non-competitive resource use should enhance divergence.

One of the most common themes of divergence involves the evolution of migratory and resident forms, with the 'forms' representing a broad continuum of degrees of genetic divergence. In fishes, this form of divergence has been documented in an enormous variety of taxa, including galaxiids (Waters and Wallis, 2001), smelt [*Retropinna retropinna* (Northcote and Ward, 1985)], lampreys [*Lampetra* (Espanhol *et al.*, 2007)], charr [*Salvelinus alpinus* (Klemetsen *et al.*, 2003)], whitefish [*Coregonus* (Lundsgaard-Hansen *et al.*, 2012)], rainbow trout [*Oncorhynchus mykiss* (Hecht *et al.*, 2012)], Atlantic salmon [*Salmo salar* (Páez *et al.*, 2011)], threespine stickleback [*Gasterosteus aculeatus* (Karve *et al.*, 2008)], and even coastal marine fishes such as *Apogon notatus* (Fukumori *et al.*, 2008). Some of the best-studied examples of the migratory–resident dichotomy are of salmonids, particularly sockeye salmon (*Oncorhynchus nerka*). Within the Pacific basin, sockeye have repeatedly given rise to genetically differentiated land-locked forms known collectively as kokanee (Wood *et al.*, 2008). The sea/river form of sockeye salmon is anadromous, acquiring most of their nutrition during the oceanic phase of their life cycle, but spending the early part of their life in their natal stream. A lake form is also anadromous, but spends more time in a rearing lake prior to migrating to sea. Kokanee populations are founded by sockeye that fail to migrate to the ocean, and they spend their entire adult life in a lake. Thus, the forms are subject to markedly different selective regimes in their primary growth habitats. In contrast to their growth habitats, the forms of *O. nerka* often use the same streams as spawning and rearing habitats (Wood and Foote, 1996), and their juveniles may compete in the natal stream and the rearing lake habitat (Hutchings, 1991). The literature comparing the life histories of these two lifestyles has contributed much to our understanding of how they diverge.

The threespine stickleback species complex exhibits an array of life-history forms, including marine, anadromous, and resident freshwater populations of a variety of types (Wootton, 1984; Bell and Foster, 1994). The threespine stickleback adaptive radiation provides several examples of this life cycle overlap between migratory and resident forms, yet compared to salmonids their respective life histories have received little attention. In this paper, we report on one of these migratory–resident pairs of threespine stickleback – the pair that breeds in Mud Lake, Alaska. Although there is good evidence (below) that the freshwater resident and anadromous fish represent distinct gene pools, and thus are good biological species, in this paper we refer to the two as 'ecotypes'.

Anadromous–lake pairs are rare (Karve *et al.*, 2008) and thus the Mud Lake system provides an excellent opportunity to compare life-history traits of lake-resident fish with those of their probable, recent ancestor. In this paper, we compare several life-history traits in Mud Lake resident and anadromous females, including size and age at reproduction, clutch size, egg mass, and reproductive effort. In addition, we examine the potential trade-off between egg mass and clutch size, and we investigate the allometric relationships between life-history traits and female body size.

MATERIALS AND METHODS

Study system

Mud Lake (61.70°N, 149.09°W) is one of several low-lying lakes on the floodplain between the Matanuska and Knik Rivers in south-central Alaska, a few kilometres southeast of Palmer, Alaska. It has an area of 1.73 km^2 and a mean depth of only 3 m (A.D. Karve, unpublished data). Lakes in this region are usually covered with ice from October to April (Woods, 1985). Mud Lake resident freshwater stickleback are benthic in their trophic ecology with deep bodies, small eyes, short snouts, and few gill rakers (Karve *et al.*, 2008). Based on skull morphology, Mud Lake resident freshwater stickleback represent an extreme benthic ecotype (Willacker *et al.*, 2010). In the late spring, anadromous oceanic stickleback migrate from their wintering habitat within Cook Inlet or the neighbouring north Pacific Ocean into the Knik River, then up Jim Creek into Mud Lake. Mud Lake anadromous fish make a long migration, estimated to be at least 45 km (Google Maps, 2013). The two ecotypes are easily distinguished based on body size (resident fish are much smaller than anadromous fish) and plate morphology [resident fish are all the low-plate morph while anadromous fish are the complete morph (Karve *et al.*, 2008)]. Although the two ecotypes occur at the same time and at the same sites in Mud Lake during the breeding season, gene flow between them is absent or very low, indicating that they represent biologically separate species (Bell *et al.*, 2010).

Data acquisition

Stickleback were collected from Mud Lake every week from 11 May until 29 July 2003. Ten sampling sites were chosen around the lake. Sites were reached by canoe, and GPS coordinates were recorded for each site to ensure that sampling occurred at the same place each time. Fish were trapped using 0.32 and 0.64 cm mesh unbaited minnow traps. All fish collected were killed with an overdose of MS-222 anaesthetic and fixed in 10% formalin until analysis.

Clutch-bearing (gravid) resident and anadromous females were chosen from seven sampling dates and from two of the 10 sites (61°45′N, 149°10′W; 61°12′N, 149°44′W) where breeding fish were most abundant. Resident and anadromous stickleback were identified based on morphology, including body size, shape, foraging traits, and armour. A complete description of morphological features that distinguish the two ecotypes can be found in Karve *et al.* (2008). To evaluate potential temporal and spatial variation in life-history traits, data were separated into two time periods (early and late) and two habitat types (vegetated and unvegetated). Although the breeding seasons of the two ecotypes overlapped broadly, the time periods varied for resident and anadromous fish due to differences in length of their breeding cycles, which was longer in resident fish. For resident fish, the ‘early’ period comprised samples collected on 26 May and 3, 10, and 17 June, while the ‘late’ period comprised samples collected on 1, 15, and 21 July. For anadromous fish, the ‘early’ period comprised samples collected on 26 May and 3 June, and the ‘late’ period comprised samples collected on 10 and 17 June and 1 July. Numbers of fish analysed from each sampling date are provided in Table 1.

Size and age of breeding females

Standard length (SL; nearest 0.1 mm) was used to determine age of breeding resident females. Size–frequency plots were constructed using standard length for a sample of 600 resident fish collected on 12 May, 17 June, and 15 July (200 fish from each date). Standard length of breeding resident females was compared to this background size distribution to estimate age. In strongly seasonal environments such as Alaska, size–frequency plots generally provide a reasonable method to age fish because year classes are produced over a short period during the summer (J.A. Baker, unpublished data). In addition, size-related overwinter mortality (Schultz *et al.*, 1998) may accentuate this effect by eliminating the smallest members of each year class. Since all anadromous fish in Mud Lake are in breeding condition, breeding age could not be determined using size–frequency histograms. Standard length and somatic dry mass of breeding anadromous females in Mud Lake were compared with data for other anadromous stickleback populations in the Cook Inlet Basin of Alaska (provided by R.W. King) to estimate their age. For both the resident and anadromous populations, an initial two-way

Table 1. Numbers of fish analysed on each sampling date from Mud Lake in 2003

Ecotype	26 May	3 June	10 June	17 June	1 July	15 July	21 July
Resident	11*	17*	2*	11*	25#	2#	2#
Anadromous	9*	22*	11#	7#	1#	–	–

Note: Asterisks (*) indicate samples from the ‘early period’ and hashes (#) those from the ‘late period’.

analysis of variance (ANOVA) was used to determine whether female size differed between the two time periods and between the two habitat types.

Female reproductive traits

All clutch-bearing (gravid) females were dissected to determine reproductive stage of maturity. Following Heins *et al.* (1992), Heins and Baker (1993), and Baker *et al.* (1998), females were assigned to one of three categories based on the development of oocytes – namely, mature stage (MA), ripening stage (MR), and ripe stage (RE). Eviscerated blotted wet mass was determined by weighing blotted wet specimens after removal of ovaries and digestive tract and used as a measure of female somatic mass. Clutch size was determined by making complete counts of the developing oocytes or eggs in each female sampled (from all three reproductive stages of females). Egg dry mass and clutch dry mass were calculated for ripe stage and ripening stage females only, because these are the only stages for which growth of the eggs is certain to have been completed (Heins *et al.*, 1992). Eggs were dried in an incubator for 24 h at 40°C and weighed. Mean egg mass was calculated by dividing the clutch mass by clutch size. Reproductive effort, which is the proportion of the total energy budget of an organism devoted to reproduction (Hirshfield and Tinkle, 1975), was estimated from regressions of clutch mass on somatic mass. We use somatic mass rather than standard length as our measure of female 'size' because we believe that mass better estimates the energetic state of a female stickleback relative to her ability to produce eggs (Wootton, 1979).

All data were $\log_{10}$-transformed prior to analyses. For clutch size, egg mass, and clutch mass, we initially performed a two-way analysis of covariance (ANCOVA), with female somatic mass as the covariate, to determine whether time during the season or habitat type significantly affected trait values. If no effect was detected, data were pooled to generate an overall description of the life history. For the analysis of the relationship between egg mass and clutch size, we adjusted both clutch size and egg size to a common female body mass separately for each ecotype. This was done because the number of eggs produced, and sometimes the size of eggs, is a function of female size (Baker, 1994; Baker *et al.*, 1998). Following this, the relationship between residual clutch size and egg mass was examined using a partial correlation analysis, in which the effect of variation in reproductive effort was considered. ANCOVAs and typical regressions use the ordinary least squares (OLS) procedure to estimate the slope and intercept describing the relationship between traits and the co-variate. However, we also calculated reduced major axis regression (RMA) slopes of the relationships of clutch size, clutch mass, and egg mass with female body mass. This model assumes that the error variance on both axes is proportional to their true variances, and it has been shown to be a more accurate descriptor of allometric slopes between variables such as those comprising life-history traits (Harvey and Pagel, 1991; McArdle, 1988; Baker *et al.*, 1998).

RESULTS

Size and age of breeding females

Resident fish captured to assess the general size/age distribution ranged from 21 mm to nearly 60 mm SL (Fig. 1), with modes indicating that the majority were in their second and third summers (ages 1 and 2). Size frequencies of resident stickleback in May and June were similar, but by July (near the end of the breeding season) most of the presumed age 2 fish

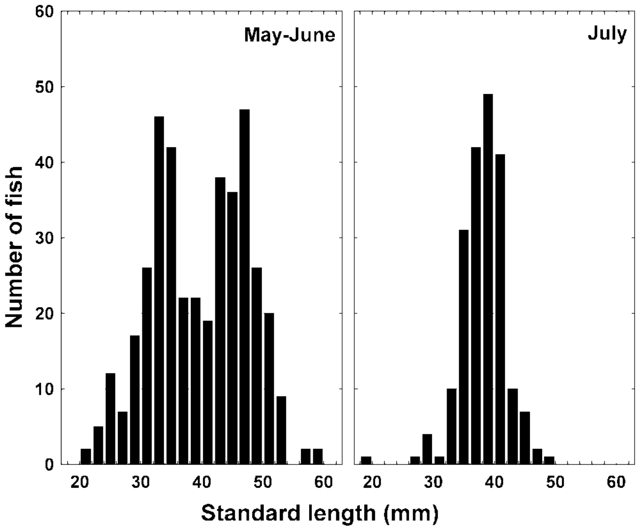

Fig. 1. Size–frequency plots of Mud Lake resident fish during the 2003 breeding season.

had died, leaving a single size mode of age 1 fish. Breeding resident females ranged from 37.8 to 63.9 mm SL (0.62 to 3.38 g), and thus the primary breeding stock consisted of age 2 fish. The comparison of breeding female size with the background size distribution indicates that only small numbers of resident females breed at age 1 or survive to age 3. Mean reproductive size of breeding resident females was considerably and significantly larger earlier in the breeding season (1.62 g vs. 1.17 g; $F_{1,72} = 9.67$, $P < 0.002$), but only a weak difference was detected between the vegetated and unvegetated habitats (vegetated 1.34 g vs. unvegetated 1.46 g; $F_{1,72} = 3.88$, $P = 0.053$).

The only anadromous fish occurring in Mud Lake are breeding adults. Anadromous females ranged from 61.5 to 77.8 mm SL (2.81 to 5.09 g), and are thus likely to represent mostly age 2 fish (Fig. 2). Mean size of breeding anadromous females was significantly larger earlier than later in the breeding season (3.72 g vs. 3.34 g; $F_{1,45} = 5.16$, $P = 0.028$), but no difference was detected between the two habitats (vegetated 3.55 g vs. unvegetated 3.51 g; $F_{1,45} = 0.18$, $P = 0.67$).

Female reproductive traits

Clutch mass

Mean dry clutch mass of anadromous females was 0.11 g, and ranged from 0.05 g to 0.19 g; the mean for resident females was 0.05 g, with values for individual females ranging from 0.01 to 0.21 g. Clutch mass was strongly related to female size in both resident and anadromous stickleback (Fig. 3). The ANCOVA indicated that the two ecotypes had similar relationships (interaction term: $F_{1,86} = 0.944$, $P = 0.334$). Resident and anadromous females did not differ significantly in clutch mass at a common female size of 2 g ($F_{1,86} = 0.584$, $P = 0.447$). Both ecotypes showed an increase in adjusted clutch mass later in the breeding season ($F_{1,86} = 8.380$, $P = 0.005$), with the pooled values increasing by 24.1% in the late-season samples (early 0.054 g vs. late 0.067 g).

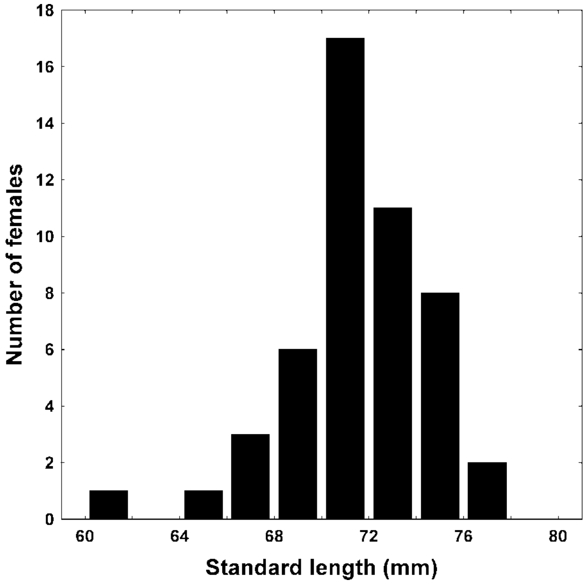

Fig. 2. Size–frequency plots of breeding anadromous stickleback in Mud Lake during the 2003 breeding season.

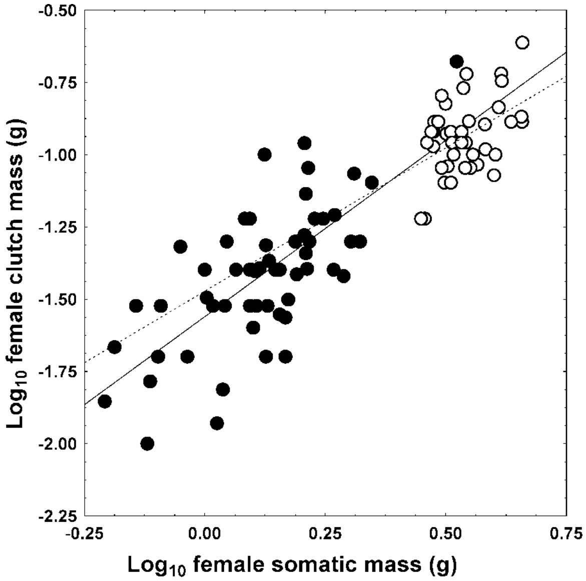

Fig. 3. The relationship between female size and clutch mass for resident and anadromous female threespine stickleback breeding in Mud Lake in 2003. Solid circles and the solid trend line represent resident females, while open circles and the dashed trend line represent anadromous females. The pooled ordinary least squares (OLS) and reduced major axis (RMA) regression slopes (the allometries are nearly identical) are: OLS = 1.143 (S.E. = 0.063); RMA = 1.288 (S.E. = 0.063); pooled $r = 0.887$.

Clutch size

As with clutch mass, both resident and anadromous populations showed a strong, positive relationship between clutch size and body size. In contrast to clutch mass, the allometric relationships differed, with anadromous females having a significantly steeper slope (interaction term in ANCOVA: $F_{1,110} = 8.899$, $P = 0.004$) (Fig. 4).

In the resident females, clutch size averaged 87 eggs and ranged from 27 to 214 eggs. In the anadromous females, clutch size averaged 235 eggs and ranged from 128 to 433 eggs. However, clutch sizes adjusted to a common female somatic mass of 2.0 g (a log somatic mass of 0.30) did not differ significantly (pooled slope ANCOVA: $F_{1,110} = 0.182$, $P = 0.670$); nor did clutch sizes differ between the two time periods ($F_{1,110} = 0.995$, $P = 0.321$).

Egg mass

Mean dry egg mass of resident females was 498 µg, and ranged from 313 µg to 758 µg. Egg mass showed a modest, but non-significant increase over time ($F_{1,87} = 2.876$, $P = 0.093$), observed more so in anadromous fish than in resident fish. Eggs in anadromous females were slightly (mean 536 µg, range 438–829 µg), though not significantly heavier (ANOVA: $F_{1,87} = 2.484$, $P = 0.119$). Eggs produced by resident females were, on average, about 7.1% lighter than those produced by anadromous females. This comparison did not adjust for a moderate trend for egg mass to increase with female size in resident fish ($r = 0.357$,

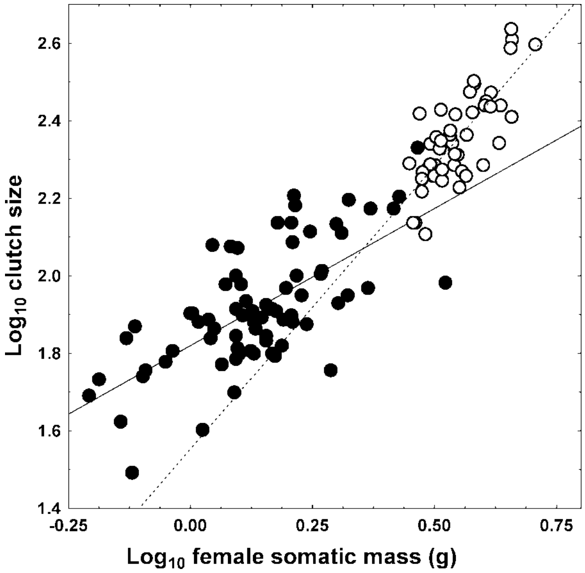

Fig. 4. The relationship between female size and clutch size for resident ($r = 0.615$) and anadromous ($r = 0.774$) female threespine stickleback breeding in Mud Lake in 2003. Solid circles and the solid trend line represent resident females, while open circles and the dashed trend line represent anadromous females. Clutch size was determined by making complete counts of the developing oocytes or eggs in each female sampled (from all three reproductive stages of females). Anadromous: OLS = 1.461 (S.E. = 0.179), RMA = 1.829 (S.E. = 0.179). Resident: OLS = 0.708 (S.E. = 0.102), RMA = 1.111 (S.E. = 0.102). Note that because not all clutch-bearing females had fully developed eggs (ripening or ripe stage), the number of females available for these clutch size regressions exceeds those for clutch mass in Fig. 3.

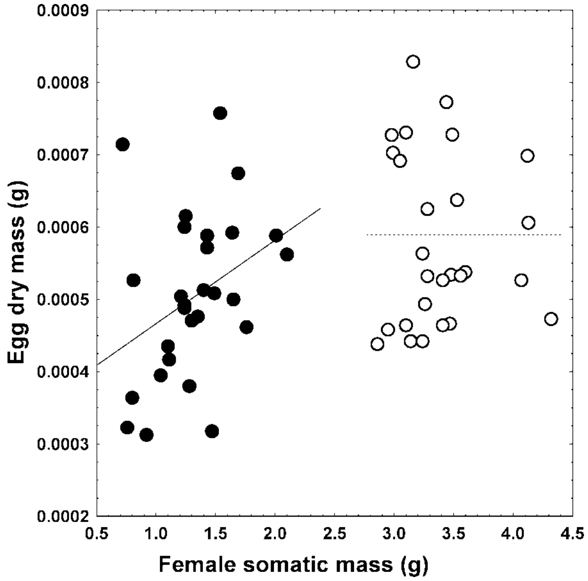

Fig. 5. The relationship between egg mass and female size in resident and anadromous threespine stickleback in Mud Lake in 2003. Solid circles and the solid trend line represent resident females, while open circles and the dashed trend line represent anadromous females. The plot uses females that had ripening and ripe eggs only.

$P = 0.006$; Fig. 5), a trend that was not observed in anadromous fish. Although the relationship in resident females was weak, the average difference in egg mass between relatively small and large breeding females (0.75 g vs. 1.5 g) represented approximately a 35% increase in egg mass across the two sizes (400 μg vs. 540 μg).

After taking into account variation in reproductive effort, there was a strong trade-off between clutch size and egg mass (both adjusted for female somatic mass) in both anadromous and resident females (anadromous: $r = -0.909$, $n = 31$, $P < 0.001$; resident: $r = -0.911$, $n = 44$, $P < 0.001$). Both this trade-off and the overarching importance of level of reproductive effort are apparent in Fig. 6.

DISCUSSION

One of the strengths of the threespine stickleback adaptive radiation is that the extant ancestral form permits determination of both the direction and extent of evolutionary change in freshwater derivatives (Bell and Foster, 1994). When most stickleback populations in lakes are founded, the opportunity for gene flow with the ancestral population is likely to be reduced or eliminated rapidly as drainage to the ocean becomes intermittent or stops. However, in some instances ancestral and derived stickleback continue to share a common habitat during the reproductive season, as in Mud Lake (Karve *et al.*, 2008). Such 'species pairs' are of particular interest to the study of behavioural reproductive isolation (Hagen, 1967; Zyuganov and Bugayev, 1988; Karve *et al.*, 2008), but they are also valuable for the study of life-history evolution. Despite this, ancestor-derived pairs have rarely had their life-history traits compared (Hagen, 1967; Zyuganov and Bugayev, 1988; Snyder, 1991).

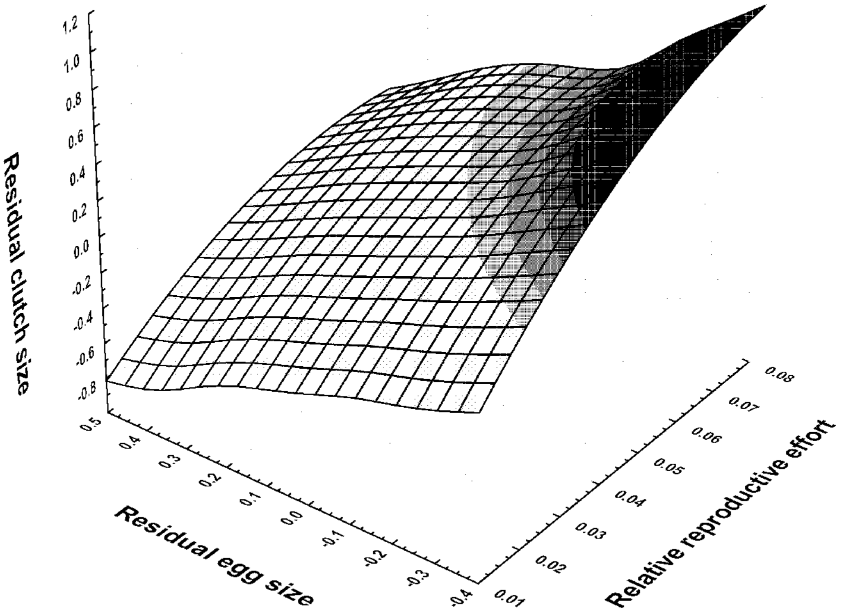

Fig. 6. The relationship among three components of female reproductive investment.

In Mud Lake in 2003, 2-year-old fish comprised most of the breeding resident females and all of the breeding anadromous females. Age of breeding resident females in Mud Lake is typical of that of other stickleback populations in the Cook Inlet region of Alaska, where freshwater stickleback most commonly breed at age 2, with fewer breeding at ages 1 and 3 (Baker *et al.*, 1998, 2008; Heins *et al.*, 1999). Oceanic stickleback in Alaska also mainly breed at age 2 (Baker *et al.*, 2008), with small numbers of age 1 females participating in some years (R.W. King and J.A. Baker, unpublished data), which is consistent with European (Munzing, 1963; Aneer, 1973) and St. Lawrence River estuary (Craig and FitzGerald, 1982) populations. Both Mud Lake ecotypes also show a decline in mean breeding female size through the spawning season, suggesting that the larger females breed early in the season, a phenomenon commonly observed in fish (e.g. Templeman, 1948; Shuter and Post, 1990; Danylchuk and Fox, 1994). Thus, in terms of their typical reproductive age, resident Mud Lake stickleback have diverged little if at all from the ancestral condition. Resident females breed at much smaller sizes than do anadromous females, but this phenomenon is likely due to the much-reduced annual growth period in fresh water [~6 months per year vs. year-round in oceanic stickleback (Gross *et al.*, 1988)]. If stickleback males choose females at least partly based on size (Nagel and Schluter, 1998; Conte and Schluter, 2013), then early in the divergence of the Mud Lake resident ecotype from the anadromous ancestor, behavioural isolation may have been important in the isolation of the gene pools.

Clutch mass is an indicator of reproductive effort, which is the proportion of the total energy budget of an organism devoted to reproduction (Hirshfield and Tinkle, 1975; Reznick and Yang, 1993; Baker *et al.*, 1998). Although the clutch mass of resident females was slightly larger than that of anadromous females at a common size, we could not distinguish them statistically. Thus, as with breeding age, our conclusion is that the Mud Lake resident females are largely scaled-down anadromous females, as they make a size-appropriate reproductive effort that has not evolved appreciably from the ancestral state. Both ecotypes breeding in Mud Lake make low reproductive efforts compared with other stickleback of their type in the region (resident, 0.030; anadromous, 0.036; Fig. 3) (Baker *et al.*, 2008; R.W. King and J.A. Baker, unpublished data). For example, reproductive efforts across 83 populations of Alaskan threespine stickleback range from 0.020 to 0.075, with freshwater and oceanic means of 0.048 and 0.064, respectively. The low level of reproductive effort in the anadromous population is surprising because with one possible exception (Mori, 1987), all anadromous populations studied to date (Baker *et al.*, 1998, 2008; R.W. King and J.A. Baker, unpublished data) show substantially higher values. It is likely that the relative rigour of the migration they make from the oceanic feeding grounds to Mud Lake may be involved. Mud Lake anadromous females are about 9% lighter, for any given standard length, than females from other Cook Inlet anadromous populations (R.W. King, personal communication), which is expected if they undergo a relatively difficult migration. A similar effect of migratory rigour on reproductive effort has been documented in Pacific salmon (Kinnison *et al.*, 2001).

Mud Lake resident and anadromous fish have diverged in their trajectories relating clutch size to body size. The number of offspring produced per reproductive bout is a strong correlate of overall lifetime fitness, and therefore clutch size is a particularly important life-history trait (Roff, 2002). In stickleback, clutch size increases as a strong power function of body size (Baker *et al.*, 2008), and across nearly 100 Alaskan threespine stickleback populations body size alone accounts for approximately 75% of the overall variation in clutch size (J.A. Baker, unpublished data). The shallower slope in the Mud Lake resident fish appears to stem from their positive allometric relationship between egg mass and body size. Given their comparable clutch mass vs. body size relationships, this positive allometry necessarily reduces the clutch size for larger resident females, thus lowering the slope of the relationship between clutch size and body size.

Baker *et al.* (2008) found a positive relationship between egg mass and body size in about one-half of 83 populations. Our conclusion of a lack of a positive egg mass–body size relationship for the Mud Lake anadromous ecotype should be considered tentative because several other Cook Inlet anadromous stickleback populations show a positive relationship between egg mass and female size (Baker *et al.*, 1998; R.W. King and J.A. Baker, unpublished data). Lack of correlation between egg mass and body size in Mud Lake anadromous stickleback may be due to the presence of only one age class of breeding females (age 2); only the largest (and oldest) females may be capable of making the long migration required. In populations in which both age 1 and 2 anadromous fish breed, a strong, positive relationship between egg mass and body size is found, driven by the considerably smaller eggs produced by the younger females (R.W. King, personal communication).

One of our more interesting comparative findings is not a difference between the ecotypes, but rather a commonality – the production of eggs of a similar size. This is the opposite of what is found for anadromous sockeye salmon and sympatric-rearing kokanee (Wood and Foote, 1996). Stickleback fry almost certainly feed on planktonic food items, at least for the first few weeks of life. In Mud Lake, whether fry feed in ecotype-specific groups or mixed groups, they would compete for food unless there is habitat partitioning of which we are unaware. The anadromous females spawning in Mud Lake produce eggs more than 14% lighter than most other anadromous populations studied to date (Baker *et al.*, 2008). Given the long migration made by the Mud Lake anadromous fish, which appears to reduce reproductive effort, producing a smaller egg would permit greater fecundity, and this 'cost of migration' may explain the similarity in egg size of the two ecotypes. However, the benefit of the increased fecundity in this instance likely would be offset to some extent by increased competition with resident fry. If our speculation about 'forced' competition is correct, it has two potential outcomes that could be tested in this system. The first is that the competition has led to fry from one ecotype shifting to rear in a different (the vegetated) habitat. This is plausible, as benthic fry shift to small benthos quite early in life (J.A. Baker, personal observation), and benthos is likely more abundant in the vegetation. Benthic fish are also possibly better suited morphologically for foraging in a complex environment (Walker, 1997), though this has been indicated only for adults thus far. The second possibility is that the anadromous fry avoid prolonged competition by initiating seaward migration sooner than they might in the absence of competition. This 'fry competition' scenario is similar to a situation described for the benthic–limnetic ecotype pair in Benka Lake, Alaska (Baker *et al.*, 2005). That study found that the benthic ecotype appeared to be required to produce larger eggs than the limnetic ecotype because it breeds in warmer parts of the lake. Less efficient development at warmer temperatures leads to smaller fry for any given egg size, producing a less competitive offspring when fry of both ecotypes begin feeding in a single rearing habitat.

Both ecotypes showed a strong, negative trade-off between egg size and clutch size after accounting for the effect of level of reproductive effort. The trade-off can be visualized as a series of contours running parallel to the residual egg size axis (Fig. 6). As reproductive effort increases, these lines become steeper, which indicates that females making larger efforts have more 'options'. By making a large effort, for example, they are able to make more competitive fry (the result of larger eggs) while keeping fecundity high.

Comparisons of the life histories of migratory and resident ecotypes of fish that use a common environment at some point in the life cycle suggest that there will be some commonalities, but also many variations. A comprehensive review is beyond the scope of this paper, but we provide one example of the complexity involved. One of the most directly comparable studies to ours is that by Wood and Foote (1996), who compared anadromous sockeye salmon and resident kokanee in a single system in British Columbia. Commonalities included maturation at the same age but with the anadromous ecotype reaching much larger sizes and overall fecundities, slightly higher reproductive effort in the anadromous ecotype, and a broadly overlapping spawning period and habitat. However, Wood and Foote (1996) found that the anadromous fish had much larger eggs than the resident form. In contrast, Olofsson and Mosegaard (1999) noted that resident brown trout (*Salmo trutta*) produced much larger eggs than the migratory form. It is probable that selection via ecological factors such as habitat preference and/or competitive ability of the juveniles (Morinville and Rasmussen, 2006) may drive these differences in egg size (Perry *et al.*, 2005). These attributes, in turn, determine the fitness of females exhibiting either the migratory or resident lifestyle.

The life history of the Mud Lake resident population can be summarized as follows. The estimated productivity of Mud Lake is well above the average for Cook Inlet lakes based on standard measures of chlorophyll, phosphorus, and nitrogen in open water (Jones *et al.*, 2003). Due to the short annual growing season, resident fish breed at much smaller sizes than their oceanic ancestor. The fact that most females delay reproduction to age 2 (beyond which survival is low), combined with their relatively low reproductive effort, suggests that the lake is a poor environment for large juvenile and adult stickleback, despite its high productivity. This in turn suggests that density-dependent processes may be important in reducing the overall production of eggs via effects on population dynamics (e.g. Wootton, 2008). The relatively small egg mass [compared with other Cook Inlet freshwater populations (Baker *et al.*, 2008)] indicates that either the environment is better for fry than for juveniles and adults, or that producing large eggs would reduce clutch size to an unacceptable level [selection directly on fecundity (Simons, 2007)]. Because reproductive effort increases allometrically with size with an exponent exceeding unity, larger females can produce heavier eggs (and therefore larger, more competitive fry) while still maintaining fecundity.

Our results represent data collected during a single breeding season, and several years of data may be necessary to better represent reproductive traits of the two populations (Seigel and Fitch, 1985). The size of the anadromous stickleback run appears to alternate yearly in Mud Lake with large runs during odd-numbered years (this study) and small runs during even-numbered years (A.D. Karve, unpublished data). Hence, the results of this study of small clutches with small eggs may not be the trend for even-numbered years when fewer anadromous fish are competing on the breeding grounds. Annual comparisons of life-history traits in Mud Lake resident and anadromous fish populations would provide a better understanding of their overall breeding activity, and possibly illuminate regular cyclical trends based on the size of the anadromous fish run.

Anadromous (migratory) and resident forms, no matter what their evolutionary status, provide unusually useful systems for understanding the traits and selective factors important in divergence. Their value is maximized when the forms share a common resource during their life cycle. This common resource is often a shared spawning/rearing habitat critical to juvenile fitness, and the precise manner in which selection acts on juvenile fitness can alter selection on numerous other aspects of the life history, including gonadal investment, fecundity, and the fecundity–egg size trade-off.

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