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# Fecundity compensation in the three-spined stickleback *Gasterosteus aculeatus* infected by the diphyllobothriidean cestode *Schistocephalus solidus*

DAVID C. HEINS*

*Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, Louisiana, 70118, USA*

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Causal explanations for host reproductive phenotypes influenced by parasitism fit into three broad evolutionary models: (1) non-adaptive side effect; (2) adaptive parasitic manipulation; and (3) adaptive host defence. This study demonstrates fecundity compensation, an adaptive non-immunological host defence, in the three-spined stickleback fish (*Gasterosteus aculeatus*) infected by the diphyllobothriidean cestode *Schistocephalus solidus*. Both infected and uninfected female sticklebacks produced egg clutches at the same age and size. The reproductive capacity of infected females decreased rapidly with increased parasite : host body mass ratio. Body condition was lower in infected females than uninfected females and decreased with increasing parasite : host mass ratio. Females with clutches had greater body condition than those without clutches. A point biserial correlation showed that there was a body condition threshold necessary for clutch production to occur. Host females apparently had the capacity to produce egg clutches until the prolonged effects of nutrient theft by the parasite and the drain on resources from reproduction precluded clutch formation. Clutch mass, adjusted for female body mass, did not differ significantly between infected and uninfected females. Infected females apparently maintained the same level of reproductive allotment (egg mass as proportion of body mass) as uninfected females. Infected females produced larger clutches of smaller eggs than uninfected females, revealing a trade-off between egg mass and egg number, consistent with the fecundity compensation hypothesis. The rapid loss of reproductive capacity with severity of infection probably reflects the influence of the parasite combined with a trade-off between current and future reproduction in the host. Inter-annual differences in reproductive performance may have reflected ecological influences on host pathology and/or intra-annual seasonal changes.  *Biological Journal of the Linnean Society*, 2012, **106**, 807–819.

ADDITIONAL KEYWORDS: castration – manipulation – nutrient theft – parasitism – side effect – sterility.

## INTRODUCTION

Studies investigating the influence of parasites on host life history are increasingly focusing on host reproduction, spurred in part by the realization that parasites can have large effects on host reproduction with little or no effect on host survivorship (Minchella & LoVerde, 1981; Bonds, 2006; Hall, Becker & Cáceres, 2007; Heins *et al*., 2010). Hosts typically lose some measure of fitness through reduced lifetime fecundity as a cost of parasitism resulting from the exploitation of host resources (Hurd, 2001; Sorensen & Minchella, 2001; Hall *et al*., 2007). The impact of parasites on host reproduction may range from reduced reproductive output to an absolute, permanent inability to reproduce (Sorensen & Minchella, 2001; Hall *et al*., 2007; Granovitch *et al*., 2009). To avoid or mitigate the fitness loss, hosts may evolve non-immunological defences that include altered reproductive traits (Agnew, Koella & Michalakis, 2000; Hurd, 2009; Parker *et al*., 2011). Immune system-based defences and non-immunological defences interact during an infection, and over evolutionary time, to play an important role in host–parasite coevolution (Parker *et al*., 2011).

*E-mail: heins@tulane.edu

Three broad theoretical models provide a basis for understanding the evolutionary significance of host reproductive responses to parasitism and the attendant physiological mechanisms that are the proximal causes. First, reduced reproductive performance may be an incidental, non-adaptive consequence of parasitic infection resulting from the simple nutrient theft characteristic of all parasites (Polak, 1996; Heins & Baker, 2003; Heins *et al*., 2010). Second, parasite-driven manipulation of host energy away from reproduction to growth may cause host sterility (the loss of reproductive capacity), and may favour the fitness of the parasite through reproduction and transmission (Ebert *et al*., 2004; Bonds, 2006; Hall *et al*., 2007). Third, fecundity compensation may evolve as an alternative solution to the challenge of parasites, because evolving and maintaining immune systems as well as mounting an immune defence is costly (Minchella, 1985; Parker *et al*., 2011).

Fecundity compensation, which is one of a number of non-immunological defences (Parker *et al*., 2011), is a reproductive tactic that allows hosts to reproduce earlier in life or increase fecundity and realize some measure of current reproductive success before parasites reduce or stop future reproduction (Minchella & LoVerde, 1981; Minchella, 1985). Evolutionary theory predicts that where parasitism decreases individual host residual reproductive value, which is a measure of future reproductive potential, hosts should increase their current reproductive output (Williams, 1966; Minchella & LoVerde, 1981; Minchella, 1985; Agnew *et al*., 2000). Some form of fecundity compensation has been demonstrated in a number of taxa studied in the wild and in the laboratory, including most notably snails (Minchella & LoVerde, 1981; Thornhill, Jones & Kusel, 1986; Lafferty, 1993; Jokela & Lively, 1995; Krist, 2001; Granovitch *et al*., 2009), as well as insects (Polak & Starmer, 1998; Adamo, 1999; Agnew *et al*., 1999; Hurd, 2009), crustaceans (McCurdy, Forbes & Boates, 1999; Chadwick & Little, 2005), lizards (Sorci, Colbert & Michalakis, 1996), and possibly by extension of the concept, birds (Heeb *et al*., 1998; Richner, 1998; Richner & Triplet, 1999; Sanz *et al*., 2001) and mammals (Schwanz, 2008). It may occur at individual and population levels (Granovitch *et al*., 2009). At the individual level, fecundity compensation involves trade-offs in an infected organism's energy budget, whereas 'population fecundity compensation' occurs among uninfected hosts, reflecting an evolutionary trade-off in heavily infested populations (Hall *et al*., 2007; Granovitch *et al*., 2009).

## Model system

The three-spined stickleback fish, *Gasterosteus aculeatus* Linnaeus, 1758, infected by the diphyllobothriidean cestode *Schistocephalus solidus* (Müller, 1776), provides an important model to study the impact of infections on host reproduction because the host–parasite system occurs throughout the northern hemisphere, involves a highly adaptable host, and has revealed considerable variation in pathology affecting host reproductive function (Bell & Foster, 1994; Heins & Baker, 2008; Macnab, Katsiadaki & Barber, 2009; Heins *et al*., 2010; Macnab *et al*., 2011). The complex life cycle of the trophically transmitted *S. solidus* (Smyth, 1962) includes a free-living coracidium larva, a procercoid infecting a cyclopoid copepod (first intermediate host), a plerocercoid infecting a *G. aculeatus* (second intermediate host), and an adult worm reproducing in a piscivorous bird (definitive host). Almost all of the growth of *S. solidus*, from microscopic larva to macroscopic plerocercoid, required for reproduction in the definitive host occurs in the intermediate host fish, which imposes a significant drain of energy upon the fish (Walkey & Meakins, 1970; Lester, 1971; Schultz, Topper & Heins, 2006).

Three-spined sticklebacks are usually infected when they are small and within the first year of life (Pennycuick, 1971; Heins, Singer & Baker, 1999; Christen & Milinski, 2005a; Heins, Baker & Green, 2011). The plerocercoid lives in the body cavity of the stickleback until the host fish is consumed by a bird or dies. Uninfected sticklebacks may live from 2 to 3+ years, and typically reach sexual maturity at 2+ years (Heins *et al*., 1999; Baker *et al*., 2008). Thus, *S. solidus* typically infects the stickleback before host reproduction begins, and may live within the body cavity of the host for up to 3 years (Heins *et al*., 1999, 2011; Heins & Baker, 2011). Female sticklebacks may attain reproductive maturity despite the challenge of infection, and any clutches carried by infected females are produced during the course of infection.

Prior research has shown that *S. solidus* reduces the residual reproductive value of *G. aculeatus* by taking a toll on both reproduction (Heins *et al*., 1999, 2010; Heins & Baker, 2008) and survival (Threlfall, 1968; Pennycuick, 1971; Heins *et al*., 2010). As often occurs in host–parasite interactions (Agnew *et al*., 2000), the impact of the parasitic infection increases with time after infection, as *S. solidus* grows and the parasite : host biomass ratio increases. Thus, we should expect selection for an increased current reproductive output in *G. aculeatus* because future opportunities for reproduction are expected to diminish (Agnew *et al*., 2000).

## Predictions

Nutrient theft and manipulation of energy allocation in hosts should show characteristically different

effects on host reproductive function (Hall *et al*., 2007; Heins *et al*., 2010). The mechanistic difference between the two tactics is that simple nutrient theft functionally starves the host by a general loss of energy, and may eventually shut down the host's reproduction, whereas parasitic manipulation specifically targets host energy allocation to prevent or dramatically curtail host reproduction early in an infection (Hall *et al*., 2007; Heins *et al*., 2010).

Nutrient theft should be associated with reproduction by both lightly and heavily infected hosts that have reached the size or age of reproduction. As the nutrient theft takes its toll on the energy levels of the host, measures of reproductive performance should decline. As a result, clutch mass, egg number, and ovum mass should decrease as the severity of infection, measured by the parasite : host biomass ratio, increases until reproduction ceases or the host dies before reproduction ends in very heavily infected hosts late in an infection (Javadian & MacDonald, 1974; Hurd, 2001; Hall *et al*., 2007; Heins & Baker, 2010).

Sterility arising from parasitic manipulation of host energy allocation should occur early in infections (Hall *et al*., 2007), and be evident amongst both lightly and heavily infected hosts of reproductive age or size (Hacker & Kilama, 1974; Renshaw & Hurd, 1994; Hogg & Hurd, 1995; Hurd, 2001). In *G. aculeatus*, with infections typically occurring before sexual maturity (Heins & Baker, 2011; Heins *et al*., 2011), parasitic manipulation should cause sterility among juvenile fish before reaching the size (age) at which reproduction begins. A small percentage of lightly infected fish may reproduce because sterilization may not be instantaneous (Heins & Baker, 2010).

In sum, simple nutrient theft should be associated with reproduction in adult-size hosts, although they should show decreasing metrics of reproduction as the severity of infection increases. Parasitic manipulation should result in hosts of reproductive size (age) that cannot bear clutches, excepting the small percentage of infected sticklebacks with small parasitic burdens. These phenomena should occur even if the parasite were to increase its nutrient theft at the time for transmission to the definitive host: the late activation of the innate immune system by *S. solidus* has been shown to facilitate transmission (Scharsack, Koch & Hammerschmidt, 2007).

Fecundity compensation is an inducible response to a biological enemy, allowing hosts to begin reproducing earlier in life or to elevate their reproductive output through increased fecundity. In Alaska the strong seasonal differences in weather should limit the smallest size (age) at which reproduction can occur in *G. aculeatus*. The high level of nutrient theft by *S. solidus* is likely to constrain the ability of the host fish to allocate energy to reproduction, thereby limiting any increase in reproductive effort and preventing infected females from investing in a much greater ovarian mass than uninfected females. Thus, increased fecundity should reflect a trade-off between egg number and egg mass, and eggs of infected females should be smaller.

## MATERIAL AND METHODS

### Samples and study site

Samples of *G. aculeatus* were obtained in 1997 and 1998 from Scout Lake, Alaska, using 6- and 3-mm minnow traps set near the shore under a state scientific collecting permit. Scout Lake (60°32.117′ N, 150°49.917′ W) is located in the north-west region of the Kenai Peninsula, which borders the Cook Inlet. Fish were anaesthetized until quiescent in tricaine methanesulphonate prior to fixation and storage in 10% formalin, based on an institutionally approved protocol.

The collections were made during the annual spawning season for *G. aculeatus* in Alaska, which lasts for approximately 6 weeks (Heins *et al*., 1999). The 1997 sample was obtained on 21 June and 1 July, which was near the end of the reproductive season. The 1998 collection (2 June) was made near the beginning of the spawning season. Moreover, these samples were collected during the peak of an epizootic event (Heins *et al*., 2011), and provided relatively large numbers of infected fish upon which to base this study.

### Specimen examination

After measurement with digital calipers to the nearest 0.1 mm standard length (SL), specimens of *G. aculeatus* were dissected to remove any *S. solidus* plerocercoids and to determine sex and reproductive condition. The classification of ovaries of females into stages was conducted following Baker *et al*. (1998) and Heins *et al*. (1999): latent (LA); early maturing (EM); late maturing (LM); mature (MA); ripening (MR); and ripe (RE). During the reproductive season, uninfected adult-size females produce multiple clutches as they repeatedly cycle through the LM, MA, MR, and RE stages of the 'clutch-production cycle' (Heins & Baker, 1993; Brown-Peterson & Heins, 2009). Female fish in the MA, MR, and RE stages have easily discernable clutches of developing oocytes or ripe eggs. Eggs in each clutch were counted to determine egg number (EN). A small number of fish with extreme pathologically small clutches were not used in subsequent analyses of clutch characteristics. All eggs of RE females were dried at 40 °C for 24–28 h and weighed to measure clutch mass (CM). The CM was divided by EN to calculate the mean female ovum mass (OM), which was multiplied by $10^6$ to

express the weight in micrograms (μg). Carcasses of eviscerated (all contents of body cavity removed, excepting kidneys) fish were weighed to the nearest 0.001 g after they were blotted with a paper towel to measure somatic body mass (BM).

Plerocercoids from each host were weighed together to the nearest milligram after they were blotted. I estimated the mass of each un-weighable parasite, of weight less than 1 mg, to be 0.5 mg, based on measurements of the individual masses of a number of small parasites on a more sensitive, precise balance. The combined parasite : host biomass ratio (PI, parasite index; expressed as a percentage) for each host was calculated using the formula PI = PM/BM, where PM is the total weight of all parasites and BM is the mass of the eviscerated carcass (Arme & Owen, 1967; LoBue & Bell, 1993; Tierney, Huntingford & Crompton, 1996). PI was used as a metric for severity of infection because parasite biomass should be related to nutrient theft, and the ratio should be related to pathology arising from nutrient loss (Hurd, 2001). Moreover, trophically transmitted parasites such as *S. solidus* should show intensity-independent effects on the host, with the full extent of pathology expected in single infections as well as in multiple ones (Lafferty & Kuris, 2002; Kuris, 2003; Fogelman, Kuris & Grutter, 2009).

### Study design and statistical analysis

In Alaska, three-spined sticklebacks typically hatch in spring (from mid May to late June), are infected later that year in late summer or fall (possibly also winter), and become reproductive during their second subsequent spring (Heins *et al*., 1999, 2011). Thus, female sticklebacks are usually infected within a short period of time after birth, and grow to adult size about 2 years later. *Schistocephalus solidus* is selected to allow its host to grow after infection; further growth of the host post-infection allows the parasite to reach the size at which it becomes competent to infect and reproduce in the definitive host (Christen & Milinski, 2005b).

To determine the effect of infection on the reproductive capacity of host females, I used adult-size fish of 42 mm SL or larger that should have matured sexually and already have been reproducing (Heins *et al*., 1999). These females should have been 2 years old or possibly older: females 1 year old are typically sexually immature in this region (Heins *et al*., 1999). I first compared size distributions of uninfected and infected females bearing or not bearing clutches using length–frequency plots to determine whether the parasite shuts down reproduction early in the life cycle of the *G. aculeatus*. Whether PI had an effect on clutch presence was tested using a log-likelihood ratio test in a binary logistic regression (1 = clutch, 0 = no clutch; PI, continuous variable). For the purpose of illustration, the percentage of infected, adult-size females with clutches (±95% confidence interval; Wilson, 1927; Newcombe, 1998) was calculated after grouping females into classes of parasite : host ratio of 10% intervals (uninfected, 0%; infected ≤ 9%; 10–19%; 20–29%; 30–39%, ≥ 40%).

To determine the relationship between the clutch characteristics (EN, CM, and OM) and body size, I regressed each variable on BM separately for infected and uninfected females. Comparisons of these parameters between uninfected and infected females included an adjustment for BM using analysis of covariance (ANCOVA), notwithstanding some significant interactions between BM and infection status in the analyses. I justified the use of BM as a covariate because the covariate explained a significant level of variation, the interactions involved slopes influenced by pathology, and simulation tests have established that ANCOVA is sufficiently robust to violations of this assumption under many circumstances (Wu, 1984; Reist, 1985; Sullivan & D'Agostino, 2002). The relationship between EN and OM, adjusted for BM, was plotted using residuals from regressions of EN and OM on BM for infected and uninfected females each year. Ordinary least-squares regressions and ANCOVAs were used to test relationships among the variables.

The physiological state of an animal, one correlate of which is body condition, may influence its reproductive success (Jakob, Marshall & Uetz, 1996). Moreover, body condition appears to influence the ability of a female stickleback to produce a clutch of eggs (Bagamian, Heins & Baker, 2004). A body condition index (Jakob *et al*., 1996) was calculated for specimens as the residual from a regression of ln BM and ln SL. The primary advantages of the residual body condition index over the commonly used ratio index are that the former separates the effects of condition from the effects of body size, and it provides a direct biological interpretation with positive and negative scores showing specimens are either fatter or leaner than predicted (Jakob *et al*., 1996). Analysis of variance was used to test the influence of infection, clutch presence, and year on residual body condition, and regression analysis was used to test the relationship between body condition and PI. To test the influence of body condition threshold on the presence of a clutch, a point biserial correlation was calculated for each year. A point biserial correlation measures the relationship between a continuous variable (residual condition) and a dichotomous variable (clutch; $N = 0$, $Y = 1$) in a Pearson's correlation (McNemar, 1962; Guilford & Fruchter, 1978).

The program SYSTAT (Systat Software Inc.) was used for the statistical computations. I used untransformed PI values because some PIs were greater than

one, and arc sine values could not be calculated from these data. Analyses of clutch characteristics (EN, OM, and CM) were performed after the data were converted to $\log_{10}$ values to meet the assumptions of the statistical analyses.

## RESULTS

### Reproductive capacity and body condition

In Scout Lake, having reached 2 years of age, infected and uninfected females of similar sizes produced clutches of eggs (Fig. 1). Among all adult-size females used for clutch analyses, uninfected females were 42.4–66.2 mm SL (mean = 54.4 mm SL, $N = 335$); and infected females were 42.5–65.5 mm SL (mean = 54.2 mm SL, $N = 208$). Analysis with binary logistic regression shows, however, that the ability to produce a clutch of eggs declined rapidly with an increase in PI (Fig. 2) in 1997 (log likelihood = –449.630, likelihood ratio $\chi^2 = 52.060$, d.f. = 1, $P < 0.001$) and 1998 (loglikelihood = –208.706, likelihood ratio $\chi^2 = 118.687$, d.f. = 1, $P < 0.001$).

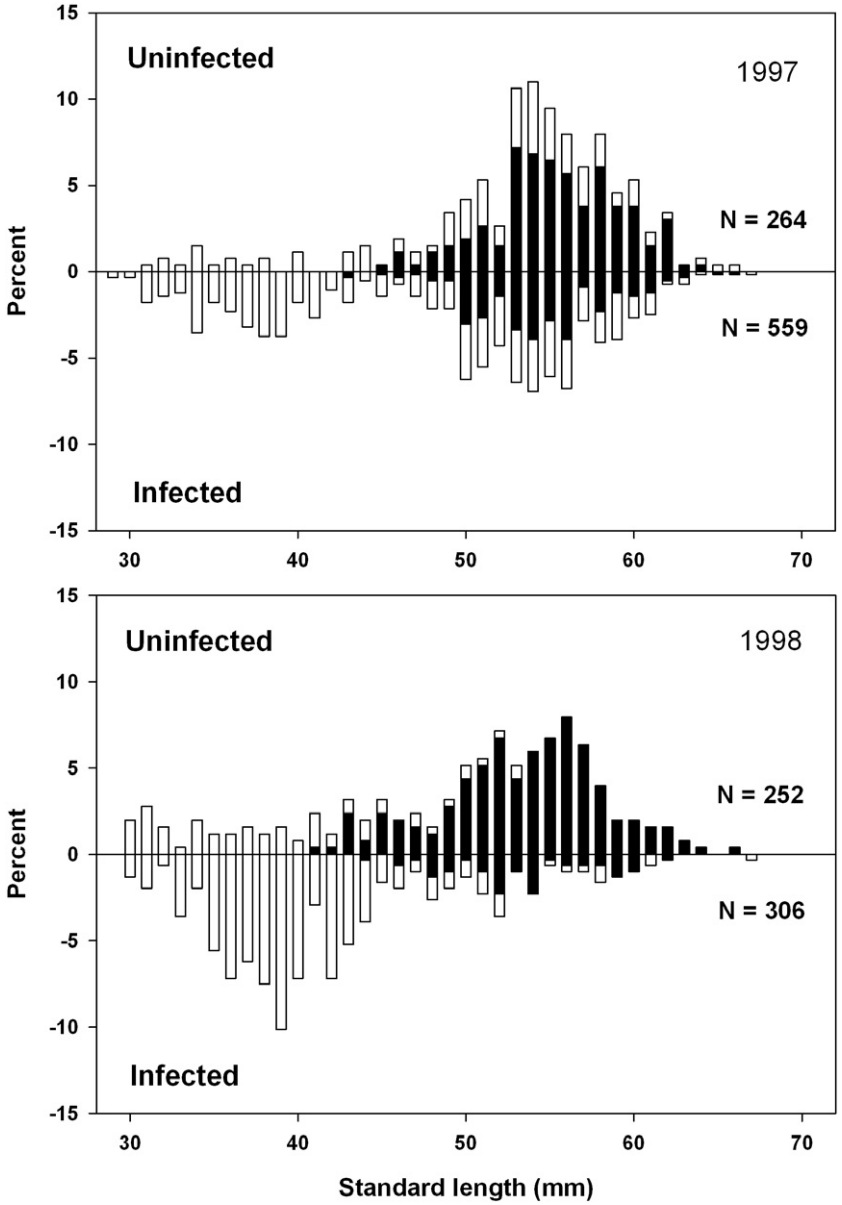


**Figure 1.** Length distributions (standard length, mm) of female *Gasterosteus culeatus* from Scout Lake, south-central Alaska, in 1997 and 1998 that were uninfected or infected by *Schistocephalus solidus*. The percentages of females with (filled bars) or without (open bars) clutches of eggs are shown using stacked bars.

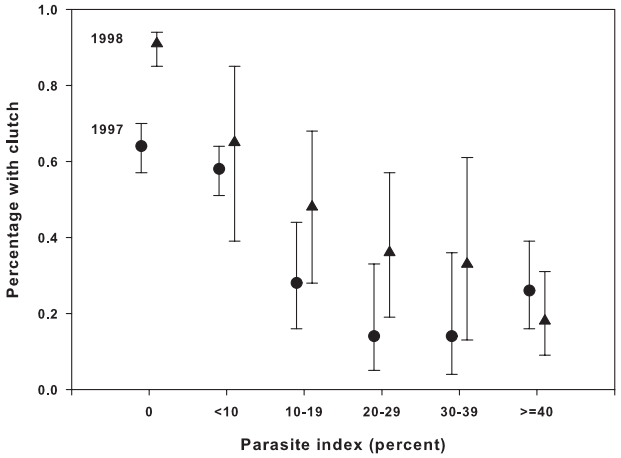


**Figure 2.** Relationship between parasite : host biomass ratio (PI) and the proportion of adult-size female *Gasterosteus aculeatus* having a clutch (±95% confidence interval) among fish uninfected (PI = 0) or infected by *Schistocephalus solidus* (0 < PI) from Scout Lake, Alaska. Sample sizes (respectively) are 247, 244, 43, 29, 22, and 61 for 1997, and 200, 17, 25, 25, 15, and 51 for 1998.

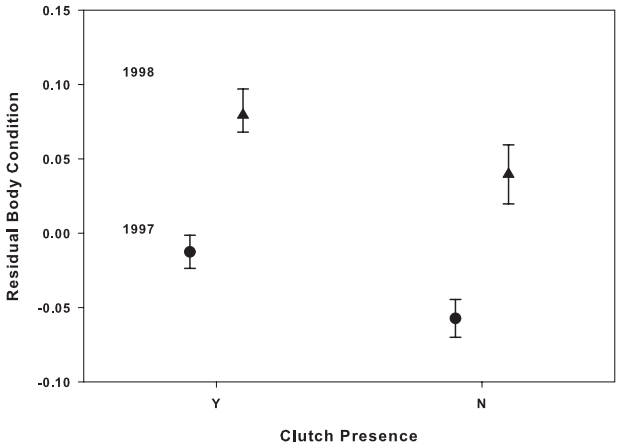


**Figure 3.** Residual body condition (±95% confidence interval) for *G. aculeatus* females with and without clutches in 1997 (filled circles) and 1998 (filled triangles).

Residual body condition (Fig. 3) was lower among infected females than uninfected females ($F = 7.234$; d.f. = 1975; $P < 0.01$), was greater among females with clutches than among females without clutches ($F = 11.653$; d.f. = 1975; $P < 0.001$), and was lower in 1997 than in 1998 ($F = 118.861$; d.f. = 1975; $P < 0.001$); there were no significant interactions among these factors ($P > 0.05$). Regression analysis showed that among all adult-size females body condition decreased with increasing PI: the decline was significant in both 1997 ($F = 18.520$; d.f. = 1647; $P < 0.001$) and 1998 ($F = 21.021$; d.f. = 1332; $P < 0.001$). A point biserial correlation showed that there was a significant relationship between residual body condition and the presence of a clutch in both 1997 ($r = 0.202$, $\chi^2 = 26.859$, d.f. = 1, $P < 0.001$) and 1998 ($r = 0.197$, $\chi^2 = 13.165$, d.f. = 1, $P < 0.001$). Thus, the correlation between residual body condition and reproductive capacity in female *G. aculeatus* from Scout Lake shows they must exceed a threshold level of body condition to become capable of producing a clutch of eggs.

### Infection parameters

The total parasite mass per clutch-bearing host female ranged from 0.001 to 2.265 g (mean = 0.160 g). The parasite : host mass ratio varied from 0.0003 to 1.252 (mean = 0.102). The mean parasite mass per host was 0.001–0.359 g (mean = 0.035 g). The intensity of infection ranged from 1 to 48 (mean = 7.24) *S. solidus* per host fish.

### Clutch mass

The CM increased significantly with BM among both infected and uninfected female fish each year (Table 1). Overall, the CM increased significantly from 1997 to 1998 ($F = 12.400$; d.f. = 1187; $P = 0.001$). The CM was not significantly different between uninfected females and infected females ($F = 0.082$; d.f. = 1187; $P = 0.775$). The adjusted least squares mean CM (anti-log) for uninfected females in 1997 was 0.063 g, and in 1998 it was 0.072 g. Among infected females the adjusted least squares mean CM was 0.063 g in 1997 and 0.074 g in 1998. None of the interactions between independent variables were significant ($P > 0.05$).

### Egg number and ovum mass

The EN of both infected and uninfected female fish increased significantly with BM each year (Fig. 4; Table 1). The overall EN adjusted to the mean BM was significantly greater in 1998 than in 1997 ($F = 68.975$; d.f. = 1536; $P < 0.001$). Infected females had clutches with greater numbers of eggs than uninfected females ($F = 42.422$; d.f. = 1536; $P < 0.001$). The adjusted least squares mean EN (anti-log) for uninfected females in 1997 was 87, and in 1998 it was 103. Among infected females the adjusted least squares mean EN was 107 in 1997 and 140 in 1998. The percentage increase in egg number in response to infection was 23% in 1997 and 36% in 1998. There were significant interactions between year and infection status ($P = 0.044$), and between year and BM ($P < 0.001$). The interaction between infection status and BM was not significant ($P > 0.05$).

**Table 1.** Statistics for ordinary least squares regressions of $\log_{10}$ clutch number, egg weight, and clutch mass on $\log_{10}$ somatic mass for uninfected and infected *Gasterosteus aculeatus* females from Scout Lake, Alaska, in 1997 and 1998

| Trait | Year | Infection status | *a* | *b* | *r* | *F* | *P* |
|---|---|---|---|---|---|---|---|
| Clutch number | 1997 | Uninfected | 1.779 | 0.891 | $r = 0.687$ | $F_{1,154} = 137.602$ | $P < 0.001$ |
| | | Infected | 1.883 | 0.808 | $r = 0.581$ | $F_{1,162} = 82.439$ | $P < 0.001$ |
| | 1998 | Uninfected | 1.914 | 0.553 | $r = 0.636$ | $F_{1,177} = 119.997$ | $P < 0.001$ |
| | | Infected | 2.053 | 0.517 | $r = 0.543$ | $F_{1,42} = 17.593$ | $P < 0.001$ |
| Egg weight | 1997 | Uninfected | 2.814 | 0.168 | $r = 0.316$ | $F_{1,43} = 4.754$ | $P = 0.035$ |
| | | Infected | 2.737 | 0.064 | $r = 0.081$ | $F_{1,41} = 0.273$ | $P = 0.604$ |
| | 1998 | Uninfected | 2.810 | 0.213 | $r = 0.470$ | $F_{1,83} = 23.542$ | $P < 0.001$ |
| | | Infected | 2.611 | 0.500 | $r = 0.472$ | $F_{1,19} = 5.432$ | $P = 0.031$ |
| Clutch mass | 1997 | Uninfected | –1.413 | 1.122 | $r = 0.758$ | $F_{1,43} = 58.093$ | $P < 0.001$ |
| | | Infected | –1.384 | 0.976 | $r = 0.771$ | $F_{1,41} = 60.126$ | $P < 0.001$ |
| | 1998 | Uninfected | –1.289 | 0.798 | $r = 0.750$ | $F_{1,83} = 106.543$ | $P < 0.001$ |
| | | Infected | –1.315 | 0.965 | $r = 0.684$ | $F_{1,19} = 16.664$ | $P = 0.001$ |

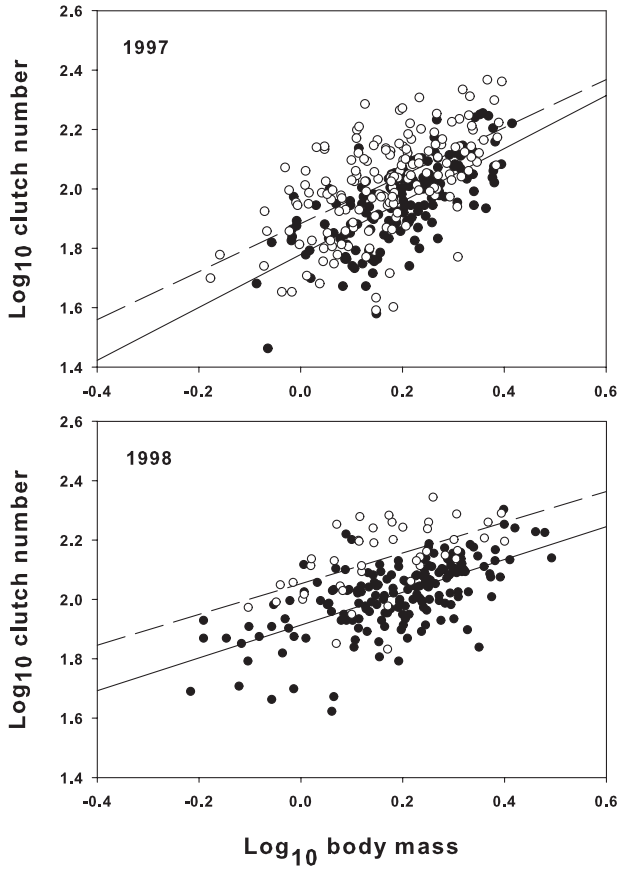


**Figure 4.** Relationship between $\log_{10}$ body mass and $\log_{10}$ egg number of uninfected (filled circles, solid lines) female *Gasterosteus aculeatus* and females infected by *Schistocephalus solidus* (open circles, dashed lines) from Scout Lake of south-central Alaska in 1997 and 1998.

The OM showed positive correlations with BM among three of four groups divided by year and infection status (Table 1). The overall OM adjusted for the covariate effect was significantly greater in 1997 than in 1998 ($F = 4.760$; d.f. = 1187; $P = 0.030$). The OM showed a highly significant difference between uninfected females and infected females ($F = 30.096$; d.f. = 1187; $P < 0.001$), with infected females having a lower overall OM. The adjusted least squares mean OM (anti-log) for uninfected females was 700 μg in 1997 and 708 μg in 1998. Among infected females the adjusted least squares mean OM was 564 μg in 1997 and 513 μg in 1998. Thus, the percentage decrease in egg mass associated with infection was 19% in 1997 and 28% in 1998. None of the interactions between independent variables was significant ($P > 0.05$).

Residuals from regressions of OM and EN on BM for uninfected and infected females in each year show the relationship between EN and OM (Fig. 5). Infected fish showed a decrease in OM with an increase in EN, when compared with uninfected females. Ordinary least squares regression showed that in 1997 the regression slope for uninfected females was negative and non-significant ($b = –0.1369$; $F = 3.2965$; d.f. = 1, 43; $P > 0.05$), whereas the slope for infected females was negative and significant ($b = –0.4892$; $F = 29.4217$; d.f. = 1, 41; $P < 0.001$). The regression slopes for females in 1998 were negative and non-significant for both uninfected ($b = –0.1207$; $F = 3.5126$; d.f. = 1, 83; $P > 0.05$) and infected ($b = –0.3319$; $F = 1.1206$; d.f. = 1, 19; $P > 0.05$) females. An ANCOVA showed the slopes of the lines were significantly different in 1997 ($F = 8.7106$; d.f. = 1, 84; $P < 0.01$), but not in 1998 ($F = 0.9565$; d.f. = 1, 102; $P > 0.05$). The effects of both infection

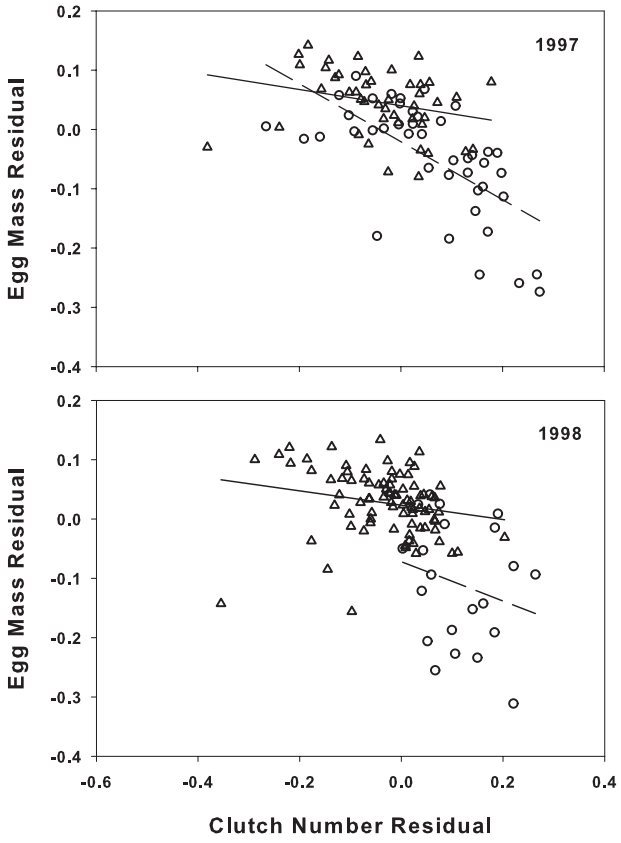


**Figure 5.** Relationship between residuals from regressions of $\log_{10}$ egg number and $\log_{10}$ ovum mass on $\log_{10}$ body mass of uninfected (open triangles, solid lines) female *Gasterosteus aculeatus* and females infected by *Schistocephalus solidus* (open circles, dashed lines) from Scout Lake of south-central Alaska in 1997 and 1998. The lines illustrate the regressions for each set of data.

status and EN on OM were significant in each year ($P < 0.001$–$0.05$). Notwithstanding the non-significant slope for infected females and the non-significant difference in slopes for infected and uninfected females in 1998, the pattern of variation was the same in 1997 and 1998. The results for 1998 may have been influenced by range restriction in the data for infected females (Fig. 5).

## DISCUSSION

### Body condition and reproduction

The data from Scout Lake show that the reproductive capacity of infected females declined as parasite : host mass ratios increased. Body condition decreased with increasing PI among all female sticklebacks and was lower among infected females than uninfected females. Bagamian *et al*. (2004) did not find a significant difference in body condition between infected and uninfected females in the population of *G. aculeatus* in Walby Lake, Alaska, although they did find that the body condition of infected females declined with the severity of infection, which was quantified using PI. The point biserial correlation between clutch presence and body condition among Scout Lake females indicates that there is a threshold in body condition necessary for females to produce an egg clutch, just as has been observed for sticklebacks in Walby Lake (Bagamian *et al*., 2004), and for other animals (Madsen & Shine, 1996, 1999).

Infected stickleback females in Scout Lake lost reproductive capacity rapidly with increasing PI, especially compared with females in Walby Lake (Heins *et al*., 2010: fig. 2). The rapid loss of reproductive capacity among females in Scout Lake may have been associated with parasitized females investing reproductive effort at the same level as their uninfected counterparts, and it apparently reflects a trade-off between current and future reproduction (Stearns, 1976; Minchella & LoVerde, 1981; Richner & Triplet, 1999). Infected females appear to have allocated energy to reproduction away from growth and maintenance. The difference in body condition of infected females vis-à-vis uninfected females in Scout Lake, as compared with those in Walby Lake, appears to support the conclusion that Scout Lake females were allocating relatively greater energy to reproduction, revealing greater reproductive effort. The combined, cumulative effect of reproduction and parasitism may have taken a heavy toll on body condition, and hence on the reproductive capacity of host females.

Body condition was greater for both uninfected and infected females in 1998 than in 1997. The difference in body condition appears to be associated with between-year differences in measures of reproductive performance. The 1997 sample was collected near the end of the reproductive season when body condition should be low because of the loss of energy during the reproductive season, and the 1998 sample was made near the beginning of the season when condition should be high. A decrease in female body condition during the reproductive season has been observed in other populations of *G. aculeatus* (Poizat, Rosecchi & Crivelli, 1999; Bagamian *et al*., 2004), and energy expended in reproduction may result in reduced performance with time during the breeding season (Baker, 1994; Brown-Peterson & Heins, 2009). Thus, the differences observed for body condition, reproductive competency, and clutch characteristics between 1997 and 1998 are likely to have been influenced by the within-season timing of sampling. Nonetheless, inter-annual differences in resource availability may have influenced the results.

### Fecundity compensation in *G. aculeatus*

Fecundity compensation should be more common among host–parasite systems where parasites cause a complete reduction in reproductive capacity (Minchella & LoVerde, 1981; Krist, 2001). Fecundity compensation should also occur in cases where parasites increase mortality or reduce fitness late in life (Polak & Starmer, 1998), as appears to be the case in the stickleback populations studied in Alaska.

This investigation has shown that the diphyllobothriidean cestode parasite *S. solidus* had a deleterious effect on the ability of adult females of *G. aculeatus* in Scout Lake to produce a clutch of eggs. The impact of the parasitism increases with time after infection. The effect of *S. solidus* on the capacity of female sticklebacks to produce egg clutches appears to result from the prolonged effect of nutrient theft by the parasite, which is compounded with the energy drain of host reproduction. Until the cost of infection prohibited clutch production as the parasite : host mass ratio increased, infected female sticklebacks appear to have actively produced clutches during the spawning season (Brown-Peterson & Heins, 2009; Heins & Brown-Peterson, 2010). These results, demonstrating a deleterious effect of parasitism on the future reproductive potential of stickleback females, appear to be consistent with the results of studies on the population of *G. aculeatus* from Walby Lake (Heins *et al*., 1999, 2010; Bagamian *et al*., 2004; Schultz *et al*., 2006; Heins & Baker, 2008; Heins & Brown-Peterson, 2010).

Unlike the observations for Walby Lake, however, infected females in Scout Lake had the same CM adjusted for body mass, hence reproductive allotment (egg mass as proportion of body mass), as uninfected females in each year of study. Infected females in Walby Lake showed a lower CM than uninfected females, in each of the 4 years of study (17–32% reduction; Heins *et al*., 2010), and CM declined with PI (Heins *et al*., 2010). Infected females in Scout Lake, therefore, appeared to be showing greater reproductive effort (proportion of energy invested in reproduction) than their counterparts in Walby Lake. Among all infected clutch-bearing females, the relative clutch mass (RCM), a measure of reproductive effort, was 29% greater among females from Scout Lake than among those from Walby Lake (RCM = 0.044 versus 0.034, respectively; D.C. Heins, pers. observ.) The decline in CM among infected females from Walby Lake was accompanied by declines in EN and OM (Heins *et al*., 2010), whereas infected females in Scout Lake produced more but smaller eggs in each of 2 years. Thus, the trade-off between EN and OM resulting in larger clutches associated with fecundity compensation in Scout Lake sticklebacks appears to reflect a fundamentally different response to infection than in Walby Lake sticklebacks.

Data on the survival probability of the eggs produced by infected females are not available, nor am I aware of data that would allow direct comparisons. Data for OM of 65 freshwater populations of *G. aculeatus* in Alaska (Baker *et al*., 2011) show that the eggs produced by infected females in Scout Lake were within the size range for populations in the region. Optimal offspring size from the mother's perspective may be different from the size that is best for each offspring (Jørgensen, Auer & Reznick, 2011). The maximization of maternal fitness by sacrificing offspring size and survival may explain the trade-off between the size and number of offspring in highly fecund organisms (Einum & Fleming, 2000). Parasite-induced selection may have shifted the optimum OM without parasitism to an optimum with parasitism (Richner, 1998). Tests of the optimal OM should include these considerations, as well as the importance of ovum quality and the influences of interacting environmental factors, such as temperature and food, occurring in the wild.

That there are differences in the response to infection among populations of *G. aculeatus* is a plausible hypothesis. Population-level responses to infection and local adaptation of hosts to their parasites have been shown in other host–parasite systems (Krist, 2001; Parker *et al*., 2011). *Gasterosteus aculeatus* is a highly adaptable species complex that shows a wide range of adaptations to local environments, including morphological, behavioural, and life-history differences among populations (Bell & Foster, 1994). Growing evidence suggests that there are also differences among *G. aculeatus* populations in response to parasitism (McPhail & Peacock, 1983; Tierney *et al*., 1996; Heins & Baker, 2008; Macnab *et al*., 2009, 2011; Heins *et al*., 2010), and there may be cryptic species of *S. solidus* in different regions (Nishimura *et al*., 2011), which may help to explain some of the variation observed in host pathology. Fecundity compensation may also show variation among populations. Because fecundity compensation is not a generalized life-history response to parasitism (Moore, 2002), we may expect to find populations, such as the one in Walby Lake, that do not evolve fecundity compensation.

### Alternative hypothesis

Fecundity compensation may not represent a different response to infection among populations of stickleback. An alternative hypothesis is that females in Walby Lake did not show a trade-off because annual (temporal) differences in ecological conditions or possibly environmental (habitat) differences between the

lakes constrained the reproductive physiology of infected females and kept them from showing the trait. A key aspect of the difference in the response to infection between the two lakes appears to be the difference in clutch mass adjusted for female body mass. Infected females in Scout Lake showed the same clutch mass as uninfected females, whereas infected females in Walby Lake showed a lower clutch mass than their uninfected counterparts. This appears to have been an important factor in determining whether females would or would not show fecundity compensation.

Two lines of evidence suggest that the differences are robust, despite temporal and habitat differences. First, the available data show that the pattern of response involving clutch mass in each of the two lakes was the same, despite any temporal differences in ecological conditions among 4 years in Walby Lake and 2 years in Scout Lake. Second, Scout Lake is a seepage lake, whereas Walby Lake is a drainage lake. Among the lakes on the Kenai Peninsula and in the Mat-Su Valley, seepage lakes generally had lower pH, salinity, and alkalinity than drainage lakes, and lakes of both types on the Kenai Peninsula had lower values than the lakes in the Mat-Su Valley (Jones *et al*., 2003). Although spatial heterogeneity could affect the relationships between these values and lake productivity (Jones *et al*., 2003), these results suggest that Walby Lake should be more productive than Scout Lake. Thus, infected females from Walby Lake would have been expected to be able to maintain clutch mass, vis-à-vis uninfected females, and to show fecundity compensation had it evolved as an inducible defence in the population.

Another alternative hypothesis is that the observed differences between populations may be explained by parasite : host mass ratios. In Scout Lake the mean parasite : host mass ratios of infected females (7%) were lower on average than in Walby Lake (15%; D.C. Heins, unpubl. data). Thus, infections in Walby Lake may have had a large effect on current reproduction, in addition to the effect on future reproduction, which constrained the response to infection. Had infections comprised a greater percentage of the host mass in Scout Lake, clutch mass may have declined in infected fish. A decline in clutch mass might have been associated with declines in both egg number and ovum mass, and a trade-off may not have occurred. If this is the case, fecundity compensation should be observable only when infections show low to moderate parasite : host mass ratios.

### Conservation of stickleback

The population of *G. aculeatus* in Scout Lake is one of many populations representing a valuable, unique adaptive radiation in the Cook Inlet region of Alaska. In the fall of 2009, Scout Lake was treated by the Alaska Department of Fish and Game to remove illegally introduced northern pike from the lake. This treatment also resulted in the extirpation of the population of *G. aculeatus*.

Concerns over the conservation of this evolutionary radiation have been raised in previous reports (Foster, Baker & Bell, 2003; von Hippel, 2008). The assemblage of populations in the Matanuska-Susitna Valley and on the Kenai Peninsula presents many different morphological, behavioural, and life-history phenotypes that have resulted from parallel evolution among the many populations. The discovery of fecundity compensation adds to the breadth of diverse phenotypes having evolved in the region and serves as a basis for a renewed plea for conservation of the Cook Inlet biological radiation.

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