

Body condition and reproductive capacity of three-spined stickleback infected with the cestode *Schistocephalus solidus*

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The relationship between reproductive ability and the residual index of body condition in three-spined stickleback *Gasterosteus aculeatus* from a population infected with the cestode macro-parasite *Schistocephalus solidus* in Walby Lake, Alaska was examined. In general, reproductive activities resulted in significantly lower levels of body condition in three-spined stickleback during the latter part of the breeding season, and relatively high levels of *S. solidus* infection intensified the energetic drain. Although female body condition did not differ significantly due just to the presence of *S. solidus*, increased parasite index did have a significant negative effect on female body condition. Males showed significantly lower levels of body condition in response to *S. solidus* infection alone and in association with a greater parasite index. Males had greater mean parasite indices than females. Females had significantly lower body condition than males, which may be due to discrepancies in energy expenditure between the sexes during reproduction. Females with greater body condition were significantly more likely to produce a clutch of eggs than those with lower condition, suggesting a threshold effect of body condition on reproductive capacity.

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Key words: body condition; clutch production; *Gasterosteus aculeatus*; life history; parasitism; *Schistocephalus solidus*.

INTRODUCTION

The reproductive success of an animal may be influenced by its physiological state, one correlate of which is body condition (Jakob *et al.*, 1996). For example, surplus energy which may contribute to a high level of body condition allows reproductive maturation in Arctic charr *Salvelinus alpinus* (L.) and Atlantic salmon *Salmo salar* L. when accompanied by proper environmental cues (Adams & Huntingford, 1997). Parasitism, however, may be an ecological impediment to reproductive maturation. By reducing a host's energy reserves for its own gain, a parasite may lower its host's body condition and indirectly have a negative effect on host reproduction which relies on the same energetic resources.

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Plerocercoids of the cestode *Schistocephalus solidus* Müller and the second intermediate host the three-spined stickleback, *Gasterosteus aculeatus* L., have been the focus of numerous studies considering the many deleterious effects of the macroparasite on host fish. The rapid growth rate of *S. solidus* coupled with the large size the plerocercoids may attain in relation to host size impose significant demands on the three-spined stickleback host's supply of energy and material resources (Wootton, 1984) which may result in diminished levels of body condition. Moreover, the parasite may negatively influence the foraging effort, food intake and diet composition of host fish, potentially leading to even lower levels of body condition (Wootton, 1977; Milinski, 1985; Godin & Sproul, 1988; Jakobsen *et al.*, 1988; Tierney *et al.*, 1993).

Although the presence of *S. solidus* in the perivisceral cavity of the three-spined stickleback imposes a considerable nutritional and energetic drain on its host's resources (Pennycuick, 1971a; Wootton, 1984; Tierney, 1994), only two field studies have considered the effect of the parasite on body condition in sticklebacks. Pennycuick (1971a) found that seasonal variation in the condition of three-spined stickleback from Priddy Lake was attributable to changes in food supply, breeding activity and parasite index; however, the latter influence on body condition was not examined in detail. Tierney *et al.* (1996) reported that *S. solidus* had a differential, deleterious effect on the body condition of three-spined stickleback in the autumn and spring seasons. Although Tierney *et al.* (1996) studied variation in body condition within each sex, they did not make comparisons between sexes.

Female reproductive success in the three-spined stickleback is strongly linked to both food intake and body mass (Wootton & Evans, 1976), both of which are factors that contribute to body condition. If food intake is not sufficient to cover the costs of ovum production, female three-spined stickleback often transfer resources from somatic tissues to the ovaries in order to subsidize the cost (Wootton & Evans, 1976). One laboratory experiment found that female three-spined stickleback infected by *S. solidus* had significantly lower perivisceral lipid stores and significantly larger ovaries than the controls at the end of the experiment (Barber & Svensson, 2003). Although Pennycuick (1971a) and Meakins (1974) have suggested that parasitism resulting in poor body condition delayed ovarian maturation in female three-spined stickleback, there have been no tests of the possible relationship between female reproductive capacity and body condition in a natural population.

This study analyses the effect of sex, season and parasite index on the body condition of adult male and female three-spined stickleback. In addition, the relationship of body condition of individual females and the ability to produce a clutch of eggs is tested.

MATERIALS AND METHODS

FISH COLLECTIONS

Samples of three-spined stickleback were obtained from Walby Lake (61° 62' N; 149° 21' W), which lies among many lakes and ponds in the Matanuska-Susitna Valley just north of the Cook Inlet and has been described in detail by Heins *et al.* (1999, 2002).

Collections were taken on 22 June 1990, 28 May and 15 June 1993, and 26 May 1996. Three of the collections were obtained with 3–6 mm wire-mesh minnow traps set near the shore, whereas the May 1993 collection was obtained from fyke nets set by the Alaska Department of Fish and Game. Both the May 1993 and 1996 collections were sub-sampled to obtain larger, adult fish. All fish were anaesthetized with an overdose of MS-222 until quiescent prior to fixation in 10% formalin. The specimens were stored in 10% formalin (May 1993 and 1996) or 50% isopropyl alcohol (June 1990 and 1993) until examination.

STICKLEBACK REPRODUCTION

Three-spined stickleback were measured to the nearest 0.1 mm standard length (L_S) with digital calipers. The fish were dissected in order to determine their sex and reproductive condition and to remove any *S. solidus* plerocercoids from their perivisceral cavity. Fish were eviscerated by removing all internal organs except the kidneys and weighed to the nearest 0.01 g after being blotted dry.

To determine the incidence of reproduction in individual three-spined stickleback, females were classified into six stages of ovarian condition as described in detail by Baker *et al.* (1998) and Heins *et al.* (1999): latent (LA), early maturing (EM), late maturing (LM), mature (MA), ripening (MR) and ripe (RE). The classification system reflects the ovarian changes associated with the attainment of sexual maturity and the production of multiple clutches. Females in all stages except LA are sexually mature, and those females in MA, MR and RE stages have distinctly discernable clutches.

Testicular size and appearance were used to classify the males as latent or mature. Latent males have very small transparent or translucent testes sparsely covered with melanophores. Mature males have enlarged cloudy to opaque white or relatively cream coloured testes that are slightly to heavily covered with melanophores. Latent fish of both sexes were regarded as sexually immature juveniles, whereas specimens in all other reproductive stages were considered sexually mature adults.

BODY CONDITION

A body condition index (Jakob *et al.*, 1996) was calculated for specimens in each sample as the residual from a regression of \ln eviscerated somatic mass and $\ln L_S$. The main advantages of the residual body condition index over the commonly used ratio index are that it separates the effects of condition from 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).

PARASITE POPULATION

The *S. solidus* plerocercoids from each host were counted, blotted and weighed together to the nearest 0.01 g. When total parasite mass was <0.01 g, the parasites were weighed to the nearest mg. If the parasites weighed <1 mg, their mass was estimated as 100 μg . Parasite index (I_P), which served as an indicator of the severity of infection, was calculated by dividing the collective mass of the parasites found in each fish by the mass of the eviscerated fish carcass and expressed as a percentage.

STATISTICAL ANALYSES

JMP 3.2 and Systat 9.0 statistical software were used to conduct analyses at the $\alpha = P \leq 0.05$ accepted level of significance. An arcsine transformation of I_P was performed prior to analyses to meet assumptions of the statistical tests. The breeding season was divided into 'early' (late May samples) and 'ate' (mid-late June samples) periods because there is a well-documented seasonal effect on body condition (Pennycuik, 1971a; Tierney *et al.*, 1996) and because reproductive activities of three-spined stickleback in Alaska vary within the short (May to June) spawning season (Heins *et al.*, 1999). The

combined data from all four samples were used to compute a regression with an interactive model to analyse the effect of sex, season and arcsine I_P , as well as their two-way interactions, on host body condition. To test the influence of body condition on the presence of a clutch in females, a point biserial correlation was performed using the combined data from all four samples. A point biserial correlation measures the relationship between a dichotomous variable and a continuous variable; it is calculated by coding one of the variables in a Pearson's correlation as being either a 0 or a 1 (McNemar, 1962; Guilford & Fruchter, 1978).

A one-way ANOVA was used to analyse differences in body condition between the sexes in relation to infection status (0, not infected; 1, infected). The body condition of infected females *v.* uninfected females at all four collection dates were compared as well as the body condition of infected males *v.* uninfected males.

The non-parametric Kruskal–Wallis one-way analysis of variance was performed to test for differences in mean arcsine I_P among various groupings of the data. This analysis was conducted because arcsine transformation apparently did not result in a normal distribution of arcsine I_P among males and females combined from all four collections; there was a significant negative skew ($G1 = 1.396$, $SES = 0.077$, $P < 0.0001$) and significant positive kurtosis ($G2 = 2.512$, $SEK = 0.154$, $P < 0.0001$) observed in the distribution of arcsine I_P . Using groups of infected and uninfected specimens combined and then infected specimens only, differences in mean arcsine I_P were analysed between females *v.* males from all four samples combined and between early *v.* late season samples separately for females and males.

Storage of specimens in isopropyl alcohol causes the specimens to lose mass, and in many cases would necessitate the use of adjustment factors when data based on fish held in different fluids were used (Baker *et al.*, 1998). In the present study, however, this was not necessary as body condition was calculated using residuals from the length–mass regression for fish within each sample. Analysis of mass loss among 20 populations of three-spined stickleback held in isopropyl alcohol as contrasted with formalin storage revealed that all fish in any one population lost mass proportionally, the elevations of the length–mass regression lines being reduced while the slopes of the regression lines and the variation around the lines were unchanged (J. Baker, pers. obs.)

RESULTS

BODY CONDITION

Overall there were significant differences in body condition of three-spined stickleback with both sex and season (Table I). Females generally had a lower body condition than males, and condition was higher early in the breeding season than it was late in the season (Table I). The interaction of sex \times season was non-significant (Table I). Body condition was similar in both uninfected and infected females (*t*-test, d.f. = 679, $P = 0.124$), however, infected males had significantly (*t*-test, d.f. = 326, $P < 0.03$) lower mean body condition (0.0146) than uninfected males (0.0549).

PARASITE INDEX

The I_P had a significant and consistent negative relationship with body condition throughout the breeding season (Table I). Although the negative effect of I_P did not vary with season, Kruskal–Wallis ANOVA showed that average I_P in the late season were significantly lower than those of the early season (Table II) for both males and females (Mann–Whitney *U*-tests, $P < 0.001$). The average I_P of the females was significantly lower than the

TABLE I. Results of interactive regression of sex, season and parasite index, and their two-way interactions on body condition of all adults, including equation constant, slope coefficients, *t*-test, standard error, and probability level of each main effect variable and their two-way interactions ($R^2 = 0.211$)

Effect	Coefficient	<i>t</i>	S.E.	<i>P</i>
Constant	0.145	9.19	0.016	<0.001
Sex (0 = male, 1 = female)	-0.111	-6.44	0.017	<0.001
Season (0 = early, 1 = late)	-0.139	-6.87	0.020	<0.001
I_P	-0.101	-2.55	0.039	0.011
Sex \times season	0.0284	1.29	0.022	0.197
Sex \times I_P	0.0204	0.43	0.473	0.667
Season \times I_P	-0.130	-1.92	0.068	0.056

average I_P of the males (Mann-Whitney *U*-test, $P < 0.001$; Table II). This relationship was also observed when only infected fish were considered; infected females had a significantly lower mean I_P than their infected male counterparts (Mann-Whitney *U*-test, $P < 0.000$; Table II).

BODY CONDITION AND FEMALE REPRODUCTION

Among adult females from the four combined samples, the point biserial correlation showed a weak but significant positive relationship between body condition and the presence of a clutch ($r = 0.127$, d.f. = 679, $P < 0.01$). Thus, females in better condition were more likely to be capable of producing a clutch of eggs.

DISCUSSION

BODY CONDITION DIFFERENCES BY SEASON AND SEX

The lower body condition observed in uninfected three-spined stickleback late in the breeding season most likely reflects the investment of both sexes in reproduction. Female fish are capable of spawning multiple clutches within a breeding season, and males may sequentially raise multiple broods (Wootton, 1984). These activities are energetically draining of both storage components and somatic tissue (Wootton, 1984).

Both the uninfected and infected females of Walby Lake had a significantly lower body condition than males presumably because egg production is more

TABLE II. Mean \pm S.D. per cent parasite index for all males and all females grouped by season and as all adults or all infected adults. Sample size (*n*) is given in parentheses

Season	Early	Late	All adults	Infected adults
Males	29.1 \pm 26.9 (125)	17.6 \pm 14.0 (202)	22.0 \pm 0.2 (327)	27.0 \pm 19.8 (267)
Females	17.4 \pm 20.1 (478)	2.1 \pm 4.6 (202)	12.9 \pm 18.4 (680)	20.7 \pm 19.5 (422)

energetically expensive than sperm production (Wootton, 1984, 1994) and because females mobilize more glycogen and lipid than males prior to the breeding season (Huntingford *et al.*, 2001). An alternative explanation of lower body condition in females is the sexual difference in the mobilization of energy reserves for reproduction. Chellappa *et al.* (1989) discovered that males first experience a massive depletion of liver glycogen, then successive depletions of gonad and liver lipid, followed by carcass glycogen, and finally, to a lesser extent, carcass lipid. In contrast, females experience a direct depletion of lipids and glycogen from both the liver and the carcass when the energetic costs of egg production are not met by ingested food (Wootton *et al.*, 1978; Wootton, 1994). The residual index of body condition calculated in this study included eviscerated carcass mass comprising muscle and fat tissue but excluded the mass of organs such as the liver and gonads which are the sites of the lipid and glycogen reserves that males preferentially deplete during breeding. This procedure may have resulted in higher values of body condition for males than females. Two other possible explanations for the sexual difference in body condition (cf. Wootton, 1984) may be that males in the samples were not actively guarding nests or they were engaged in sneaking behaviour rather than conventional nest-holding reproduction. Spawning male three-spined stickleback are known to stay close to the nest they are guarding (Wootton, 1984), therefore, they would be less likely to be caught in the traps used for collection than those males either sneaking or not engaged in reproductive activities.

Parasite indices would be expected to increase through the breeding season as seasonal temperatures increase and parasite growth accelerates. The mean parasite index was, however, significantly lower late in the breeding season (11.5% lower for males and 15.3% lower for females). The plerocercoids found in Walby Lake fish early in the season were too large to have been from infection from earlier in the year (Heins *et al.*, 1999). Thus, these fish were infected well before breeding and were able to support the infections until spawning. The decrease in the mean I_P during the late season may, therefore, reflect the deaths of more heavily infected fish who could not survive the combined energetic costs of a heavy parasite burden and reproductive investment. This agrees with the findings of Pennycuick (1971a), who observed that fish that died in Priddy Lake had a significantly lower body condition and a significantly higher I_P than live specimens, leading to her conclusion that the deaths of three-spined stickleback were caused by poor condition due to the combined effects of parasitism and breeding (Pennycuick, 1971a).

BODY CONDITION DIFFERENCES BY SEX IN RELATION TO PARASITISM

In agreement with the results of Tierney *et al.* (1996), there was no significant difference in body condition between uninfected and infected adult females in Walby Lake. Infected adult males in Walby Lake had a significantly lower body condition than their uninfected counterparts whereas Tierney *et al.* (1996) found that adult males that were infected had greater body condition than uninfected ones. Although the presence of the *S. solidus* was not enough to decrease body condition in infected females relative to that of uninfected females, high parasite

index did have a significant negative effect on female body condition. In males, the presence of *S. solidus* was enough to cause a highly significant difference in body condition between infected and uninfected males. This relationship may be the result of sex-biased parasitism, as the males in this study had a significantly higher mean I_P than the females. The immunocompetence handicap model (Folstad & Karter, 1992) suggests that while enhancing secondary sexual characters, testosterone simultaneously depresses the immune system, thereby increasing parasite susceptibility and severity of infection in reproductive males. Increased parasitism and elevated mortality in males relative to females is not uncommon (Moore & Wilson, 2002). Although male three-spined stickleback may have a higher prevalence of parasitism (Reimchen & Nosil, 2001), female fish are the ones that usually suffer heavier and more consequential *S. solidus* infection (Pennycuik, 1971b; Tierney *et al.*, 1996; Reimchen & Nosil, 2001). In their 15 year evaluation of parasitism levels in Boulton Lake, Reimchen & Nosil (2001) found that the differential rates of infection between the sexes results from ecological differences in habitat use and diet rather than from sexual selection. Female three-spined stickleback consumed significantly more copepods (primary host of *S. solidus*) than males (Reimchen & Nosil, 2001). Thus, female fish were significantly more likely to be infected by *S. solidus* (Reimchen & Nosil, 2001), and to undergo behavioural modification and subsequent predation (Jakobsen *et al.*, 1988; Godin & Sproul, 1988; Tierney *et al.*, 1993; Reimchen & Nosil, 2001). Although the males of Walby Lake seemed to have more severe infections than the females, the possibility can not be ruled out that more heavily infected reproducing females were not present in Walby Lake because either they were easy targets for predators or they had such poor body condition that much further loss of condition could not occur without death.

BODY CONDITION AND FEMALE REPRODUCTION

A positive relationship between body condition and the presence of a clutch was observed in the present study among female three-spined stickleback from Walby Lake, a phenomenon that has been observed in other animals. For example, Chastel *et al.* (1995) found that in blue petrels *Halobaena caerulea* (Gmelin) the body condition of females producing eggs was higher than in non-breeding females. For the Australian python *Liasis fuscus* Peters, females showing greater body condition in excess of a threshold value were more likely to reproduce (Madsen & Shine, 1996, 1999). Thus, the correlation between body condition and reproductive capacity in female three-spined stickleback from Walby Lake suggests that they too may have to exceed a threshold level of body condition in order to produce a clutch of eggs.

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