A Predator's Dilemma: prey choice and parasite susceptibility in three-spined sticklebacks

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SUMMARY

The acanthocephalan parasite *Pomphorhynchus laevis* is transmitted by a suitable intermediate host such as the amphipod *Gammarus pulex* to its definitive fish host. Parasite-induced alterations in both appearance and behaviour concur to render infected gammarids more vulnerable to predation, thus promoting parasite transmission. Experimental infection of laboratory bred full- and half-sib groups of three-spined sticklebacks (*Gasterosteus aculeatus*) provided evidence that the parasite imposes a survival cost proportional to the severity of infection on its final host. Variation among sibships in the susceptibility to infection was consistent. When given a choice, fish consumed significantly more infected than uninfected prey. Overall, more resistant fish did not prey upon infected gammarids more often than did relatively susceptible fish. Only fish with a relatively high physical condition properly adjusted prey selection to the extent of their parasite susceptibility, thus exploiting the enhanced profitability of infected prey.

Key words: *Gammarus pulex*, *Gasterosteus aculeatus*, parasite-induced trophic transmission, parasite resistance, *Pomphorhynchus laevis*, predation.

INTRODUCTION

Many parasites with complex life-cycles depend on predation on their intermediate host to reach a suitable definitive host (see Poulin, 1998 for a recent review of the evolution of parasitic life-cycles). Often, larval parasites induce phenotypic modifications in their intermediate hosts that increase the intermediate host's exposure to predation, and thus the likelihood of the parasite completing its life-cycle (see Holmes & Bethel, 1972; Moore, 1984*a*, *b*; Poulin, 1994, 1995 for reviews). While parasites trigger changes in the intermediate host's behaviour and/or appearance, selective pressure may be expected to act on potential definitive hosts towards mechanisms to avoid infected intermediate hosts. However, for any host resistance to evolve, its costs must be weighed against the costs of parasitic infection, measured in terms of life-time reproductive success. Theoretical models predict that if the parasite has a negligible effect on the host's fitness, predators may benefit from their parasites when parasite-induced modifications raise the intermediate host's profitability as a prey (Lafferty, 1992). If, however, the parasite has a severe effect on host fitness, and if its prevalence is high, the definitive host is expected to evolve selective feeding on uninfected prey (Lafferty, 1992). Hence, definitive hosts face a trade-off between parasite acquisition and easier predation, and whether or not the requisites for the evolution of selective feeding strategies are fulfilled, depends on the magnitude of the benefits of consuming infected prey as opposed to the costs of avoiding them (Holmes & Bethel, 1972).

In addition to mechanisms of recognition and avoidance of infected prey, sometimes referred to as 'behavioural resistance' (Wedekind & Milinski, 1996), adaptations that minimize the impact of infection rely on the host's genetics. Genetically controlled resistance to parasitism (mediated by aspects of the host's physiology, including the immune system) is a widespread phenomenon (Wakelin & Blackwell, 1988) with the potential of influencing the outcome of the dilemma outlined above, as resistant individuals can afford to prey upon modified infected prey regardless of the costs of infection. Yet, genetic resistance is assumed to be traded against other fitness components, such as fecundity and growth (see Gemmill & Read, 1998 and references therein). These selective antagonistic forces supposedly prevent the underlying genes from going to fixation, thus leading to a population of resistant individuals.

The infective cystacanth stage of the acanthocephalan parasite *Pomphorhynchus laevis* is distinctly visible through the cuticle of its amphipod intermediate host, *Gammarus pulex* as a conspicuous orange spot. Moreover, infected *G. pulex* specimens

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exhibit an abnormal positive phototaxis (Kennedy, Broughton & Hine, 1978; Bakker, Mazzi & Zala, 1997). Given a choice between equal amounts of infected and uninfected *G. pulex*, three-spined sticklebacks (*Gasterosteus aculeatus*) preferentially consume infected prey, whereby parasite-induced changes in both colouration and behaviour contribute to enhance the predation vulnerability of infected gammarids (Bakker *et al*. 1997). Sticklebacks benefit from the increased profitability of infected prey in terms of facilitated acquisition of energy and carotenoids, the pigments responsible for the male nuptial colouration of which gammarids are an important source (Bakker & Mundwiler, 1999). As for costs, field observations suggest that *P. laevis* lowers the fitness of its stickleback host by adversely affecting survival, physical condition and the expression of some male secondary sexual traits, such as eye colour and relative pectoral fin size (Bakker & Mundwiler, 1999).

Here, we report of a prey choice experiment designed to investigate whether laboratory bred full- and half-sib groups of three-spined sticklebacks differ in their susceptibility to an experimental infection with *P. laevis*, and whether such differences are linked to variation in prey choice behaviour.

MATERIALS AND METHODS

Experimental subjects

Gammarus pulex that were uninfected or visibly infected with the acanthocephalan worm *Pomphorhynchus laevis* were sampled daily during spring 1997 from the Wohlensee, near Bern, Switzerland (46° 57′ N, 7° 28′ E) by vigorously shaking floating bundles of reed roots over a bucket of water. Gammarids were additionally collected with a long hand net at several known stickleback breeding grounds. The collected gammarids were transported to the laboratory into buckets of lake water, where they were acclimatized over a few hours to room temperature. Eventually, they were transferred in 85 l holding tanks at room temperature and a photoperiod of 16 h with lake water, a mud layer on the bottom, floating vegetation and constant aeration until required. The gammarids were always handled with insect forceps. Only amphipods of a total length between 0·5 and 0·8 cm (measured from the base of the first antennae to the base of the telson) infected with a single *P. laevis* cystacanth were used. Infected *G. pulex* are readily recognized by the orange *P. laevis* cystacanth shining through their transparent cuticle. All gammarids were used only once.

The fish used in the experiment were laboratory bred offspring of fish collected before the start of the breeding season of 1995 in the Wohlensee. Matings between the parental wild fish were arranged so that while some males were each crossed with a single female, others were given 2 females to spawn in their nest, thus yielding full-sib and half-sib groups. Following hatching, in autumn 1995, the fish were housed in 10 l aquaria under summer conditions (16 L : 8 D, temperature of 17 °C). After 1 month, 50 fry out of each group were transferred into 50 l tanks. Five months later, the surviving fish (median group size 24, range 16–32) were moved into 50 l tanks and the artificial climate was set to winter conditions (8 L : 16 D, temperature of 5 °C). At the end of January 1997, conditions were switched to late winter-early spring (10 L : 14 D, temperature of 10 °C). The storage tanks were constantly aerated and supplied with water from a well (temperature: 6–10 °C). The fish were fed daily in excess with live *Tubifex* worms or frozen chironomid larvae.

Choice experiment

The choice experiment was organized in series consisting of 30 experimental and 8 control (see below) replicates. For each series of trials, 6 fish for each of 5 randomly selected groups were used. The same 5 groups were used in 2 successive series. Between March and August 1997, eight series were performed, for a total sample size of 240 fish. Because of a few violations of the rule, dictated by fish availability, overall 19 groups with 12 fish and 2 groups with 6 fish each were used. In 5 cases, 2 groups had been fathered by the same male, thus 16 males contributed offspring to the 21 treated groups. Whenever possible, half the fish of each group were males and half were females, though the sex determination was not always unequivocal, as most fish had not yet reached sexual maturity. At the end of the experiment, all fish were dissected and sexed by visual inspection of the gonads.

The fish were individually placed in small plastic aquaria (32 × 17 × 19 cm) filled with well water to a depth of 12 cm. The water temperature was kept constant at 10 ± 1 °C. Neighbouring aquaria were separated by grey opaque partitions to prevent visual interactions. Each sextet of aquaria was illuminated for 10 h per day by a 33 W fluorescent tube, mounted 16 cm above the water surface. The aquaria were spatially interspersed in random order.

For the choice experiment, each aquarium was provided with an 'artificial' plant made up of twigs of *Elodea canadensis* (18–20 twigs of approximately 10 cm for a total dry weight of 10 ± 0·5 g) collected in the lake and placed in a conical opaque plastic cup (height 5·5 cm, diameter at the bottom 3 cm, diameter at the top 5 cm) filled with coarse gravel. Additionally, 6 circular beech (*Fagus sylvatica*) leaves with a constant surface of 10 cm^2 each, obtained by cutting leaf litter collected in the lake with an aluminium cylinder, were evenly distributed on the bottom of each aquarium. The vegetation provided gammarids with food and refuge.

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In order to standardize the feeding motivation, the fish were deprived of food on the day preceding the beginning of the experiment. Half the males and half the females of each sib group were randomly assigned to 1 of 2 treatment groups: exposed fish were given 10 infected and 10 uninfected gammarids, while non-exposed fish were given 20 uninfected gammarids. Gammarids in the 2 prey classes were matched for size and colour by eye. A Petri dish with the selected prey items in a little water was placed at the bottom of the aquarium, beside the plant. As soon as all gammarids that had not swum away upon immersion had dispersed, the Petri dish was removed. The gammarids were allowed 1 hour of acclimatization in the absence of the predator before the start of the experiment. After individual introduction into the aquaria, the fish were allowed to prey upon gammarids for 42 h, the estimated time required by an average stickleback to consume half the available prey items (personal observation). Since sticklebacks ignore dead prey, while *G. pulex* sometimes feed on dead conspecifics (personal observation), we regularly checked the aquaria for dead prey and removed them with a glass pipette, along with the occasionally excreted cystacanths that had passed through the host without establishing. The disturbance was evenly distributed over all aquaria. At the end of the experiment, the fish and the vegetation were removed and left over prey items of the 2 classes were scored.

Preference appraisal was based on a probabilistic approach proposed by Manly, Miller & Cook (1972), which allows for the depletion of prey during the course of the experiment, and thus for the change in the proportions of available prey classes as prey are eaten. Thereby preference is evaluated as the ratio of the proportionate mortalities of the 2 prey types expressed as natural logarithms. Rearrangement yields β, the probability of the next prey eaten being of type I, if the predators act in the same way with 50% presentation as they do at the experimental frequency:

$$\beta = \frac{1}{1 + \{[\ln(N_e/N)]/[\ln(N'_e/N')]\}},$$

with

N_e, N'_e = the number of prey of type I and type II eaten, and

N, N' = the number of prey of type I and type II initially available.

The index β has a scale from zero (when only uninfected prey are eaten) to 1 (when only infected prey are eaten), with a value of 0·5 for no preference.

To enable within- as well as among-treatment comparisons after the choice experiment, the fish were further fed on *G. pulex* till all exposed fish had consumed 20 infected and 30 uninfected gammarids and all non-exposed fish had consumed 50 uninfected gammarids. In order to accelerate the procedure, the second half of the treated fish had their diet reduced to a total of 20 infected and 10 uninfected gammarids (exposed fish) or 30 uninfected gammarids (non-exposed fish).

After having consumed the required amount of prey, the fish were housed individually in small plastic aquaria ($32 \times 17 \times 19$ cm, water level 14 cm) with a gravel layer and filamentous algae under summer conditions (16 L : 8 D, water temperature between 15 and 20 °C). All fish became reproductively active within a few days. The fish were fed daily with either live *Tubifex* worms or chironomid larvae.

About 2 months (median 57 days, range 37–85) after the choice experiment, the fish were killed by decapitation and dissected to search their digestive tract for parasites. Since all exposed fish were challenged with the same number of acanthocephalan larvae, the number of established parasites was taken as a measure of resistance.

A condition factor was calculated as $100 \times$ mass (g)/standard length $(\text{cm})^3$ (Bolger & Connolly, 1989). Body length and mass were recorded shortly before killing the fish. To control for differences in female ripeness, female body mass was corrected for the mass of the ovaries. Similarly, testes mass was subtracted from male body mass. No condition values were computed for fish that did not survive throughout the approximately 6-week period under summer conditions ($n = 56$), nor for those used in a parallel experiment ($n = 16$; S. Zala & T. C. M. Bakker, unpublished data).

Prey mortality

To account for differential prey mortality independent of the action of consumers causing prey availability to depart from the initial 50% presentation, 8 control replicates were randomly interspersed among experimental ones in each series of trials. Control replicates differed from experimental ones in that predation was prevented (while visual and olfactory interactions were not precluded) by enclosing the fish in a plastic beaker (height 10 cm, diameter 8·5 cm) covered with a fine-meshed net (mesh size 1 mm) fastened with 2 elastic rubber bands. Out of the 64 control replicates, we computed mean values for the natural mortality of the 2 prey classes. By subtracting these correction factors from the initial presentation densities, we obtained values for the effective availability of the 2 prey classes.

No free worms were ever sighted in control replicates, suggesting that those occasionally found in experimental aquaria had been excreted after having failed to establish themselves in the fish alimentary tract.

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Table 1. List of criteria used for comparisons within and among sibships

Within sibships
Sex
Males
Females
Infection status
Non-exposed
Exposed
Non-infected
Infected
Among sibships
Physical condition
Low physical condition
High physical condition

Statistical analyses

The distribution of the recorded variables was tested for normality using the Shapiro-Wilk test. Some non-normally distributed variables could be transformed so as to meet the assumptions of parametric statistics (see Results section), otherwise non-parametric methods were used. The latter were also preferred when sample size was small. Data organized in a hierarchical structure, with data on individual fish nested within sib-groups, were averaged per group of offspring of the same father. In such cases, given sample sizes refer to the number of fathers for which data were available for analyses. The term 'sibship' is used throughout the text for groups of offspring fathered by the same male, even though in 5 cases 2 females were involved (i.e. the 'sibship' consists of a mixture of full- and half-siblings). The criteria used for within- and among-sibships comparisons are listed in Table 1. All probabilities are two-tailed. Analyses were conducted using either Systat for the Macintosh (v. 5.2.1, Systat, 1992) or JMP IN (v. 4.0.3, SAS Institute, 2000).

RESULTS

Infection

Of the 120 exposed fish, 29 successfully resisted infection, while from all remaining exposed fish 1 or more worms were recovered at dissection. The 29 resistant fish had been fathered by 10 different males, which had median: 3, range: 1–6 resistant offspring. The resulting parasite prevalence of 76% is in fair agreement with figures derived from field studies of the Wohlensee population (Bakker & Mundwiler, 1999). The median number of worms established in infected fish was 2 (range 1–10; Fig. 1). The among-sibships difference in parasite intensity was marginally non-significant (Kruskal-Wallis one-way ANOVA, $H=24{\cdot}79$, D.F. $=15$, $n=120$, $P=0{\cdot}053$). Within sibships, susceptibility to infection did not significantly depend on sex (Wilcoxon matched-pairs signed-ranks test, $n=16$, $T=2{\cdot}0$, $P>0{\cdot}8$).

Choice experiment

The vast majority of the fish whose preference could be assessed (105 out of 117, i.e. *ca* 90%) consumed more infected than uninfected gammarids (Fig. 2). The median preference index of 0·77 (range 0·25–1) significantly departed from the null expectation of no discrimination (Wilcoxon one-sample test, $n=16$, $T=13{\cdot}35$, $P<0{\cdot}001$). The preference index did not significantly differ among sibships (Kruskal-Wallis one-way ANOVA, $H=14{\cdot}04$, D.F. $=15$, $n=120$, $P>0{\cdot}5$). Within sibships, the 2 sexes did not significantly differ with respect to prey selection (Wilcoxon matched-pairs signed-ranks test, $n=16$, $T=30{\cdot}0$, $P>0{\cdot}1$), nor did fish that resisted infection and their infected siblings (Wilcoxon matched-pairs signed-ranks test, $n=10$, $T=10{\cdot}5$, $P>0{\cdot}3$).

Prey consumption significantly differed among sibships, and within sibships, fish having the choice between infected and uninfected *G. pulex* ate significantly more than their siblings administered only uninfected prey (two-way ANOVA on log(intake + 2), $F_{15,223}=3{\cdot}39$, $P<0{\cdot}001$ for the effect of father, and $F_{1,223}=6{\cdot}32$, $P=0{\cdot}01$ for the effect of treatment; Fig. 3).

Prey choice, parasite susceptibility and physical condition

Sibships significantly differed in their physical condition, and males were in significantly better condition than females (two-way ANOVA, $F_{15,133}=1{\cdot}93$, $P=0{\cdot}026$, and $F_{1,133}=91{\cdot}46$, $P<0{\cdot}0001$ for the effect of father and sex, respectively). The condition factor (corrected for differences between the sexes) did not significantly differ between exposed and non-exposed fish (Wilcoxon matched-pairs signed-ranks test, $n=16$, $T=2{\cdot}0$, $P>0{\cdot}9$), nor between exposed infected and exposed uninfected fish ($n=7$, $T=8{\cdot}0$, $P>0{\cdot}2$). When sibships with a higher than average mean physical condition were considered, a significantly negative relationship was found between mean preference index and mean parasite intensity ($r_s=-0{\cdot}750$, $n=7$, $P=0{\cdot}050$; Fig. 4). No such pattern applied to sibships with lower than average mean physical condition ($r_s=0{\cdot}228$, $n=8$, $P>0{\cdot}5$). Groups with higher and lower than average mean physical condition did not significantly differ in parasite intensity (Mann-Whitney U test, $n_1=7$, $n_2=8$, $U=21{\cdot}5$, $P>0{\cdot}4$), nor in prey choice ($U=28{\cdot}0$, $P>0{\cdot}9$), nor in intake rate ($U=32{\cdot}0$, $P>0{\cdot}6$).

Parasite-induced host mortality

The mortality rate, expressed as the ratio of the number of fish that died throughout the period spent under summer conditions to the number of fish initially present, varied greatly among sibships, ranging from 0 to 70% (median 25%). Within sib-groups, exposed fish suffered significantly higher mortality

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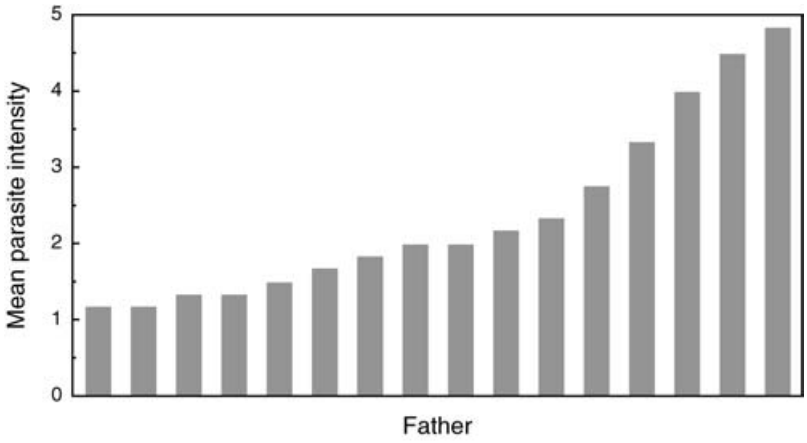

Fig. 1. Variation in parasite susceptibility: mean parasite intensity per sibship, plotted in ascending order.

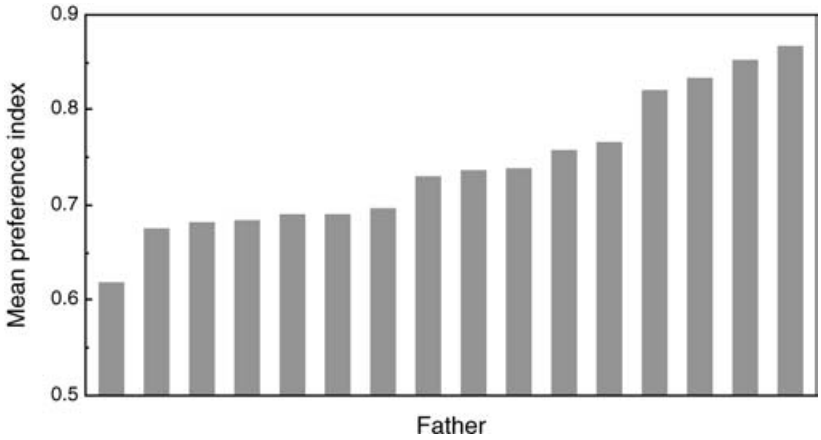

Fig. 2. Variation in prey choice: mean preference index per sibship, plotted in ascending order. Values above 0·5 reflect a preference for infected prey.

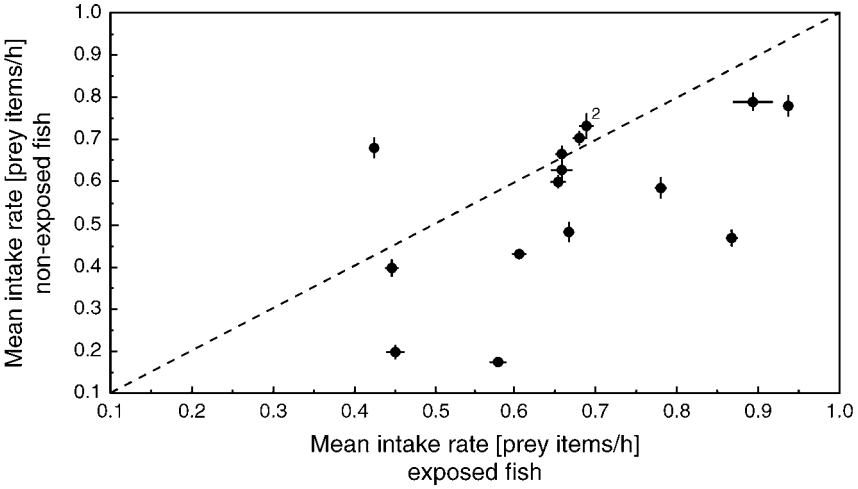

Fig. 3. Mean (±S.E.) intake rate of exposed and non-exposed offspring of different fathers. The dashed line represents the line of equality. The number indicates overlapping data points.

than non-exposed ones (Wilcoxon matched-pairs signed-ranks test, $n=16$, $T=25{\cdot}0$, $P=0{\cdot}050$; Fig. 5). Furthermore, the mortality rate increased with the intensity of infection ($r_s=0{\cdot}53$, $n=16$, $P=0{\cdot}034$; Fig. 6). Mortality significantly negatively correlated with physical condition for exposed fish ($r_s=-0{\cdot}57$, $n=16$, $P=0{\cdot}019$), and tended to do so for non-exposed fish ($r_s=-0{\cdot}43$, $n=16$, $P=0{\cdot}098$). Mortality was not significantly sex-biased (Wilcoxon matched-pairs signed-ranks test, $n=16$, $T=3{\cdot}5$, $P>0{\cdot}7$, and $n=16$, $T=7{\cdot}0$, $P>0{\cdot}4$, for exposed and non-exposed fish, respectively).

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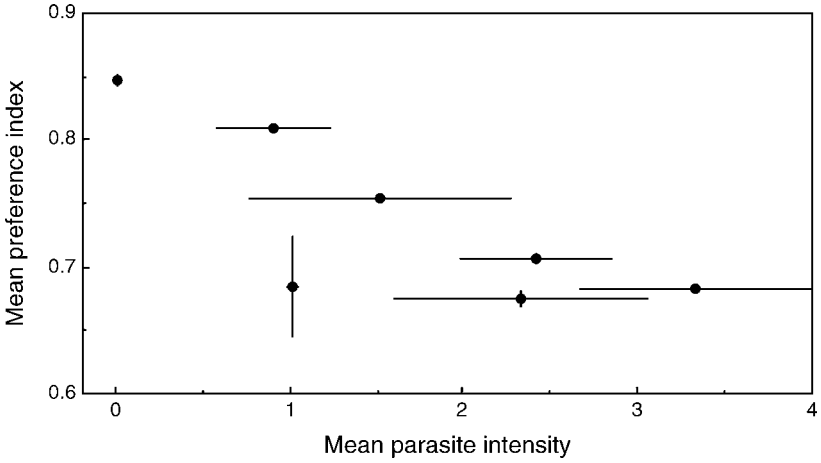

Fig. 4. Relationship between mean (±S.E.) preference index and mean (±S.E.) parasite intensity for sibships with higher than average mean physical condition.

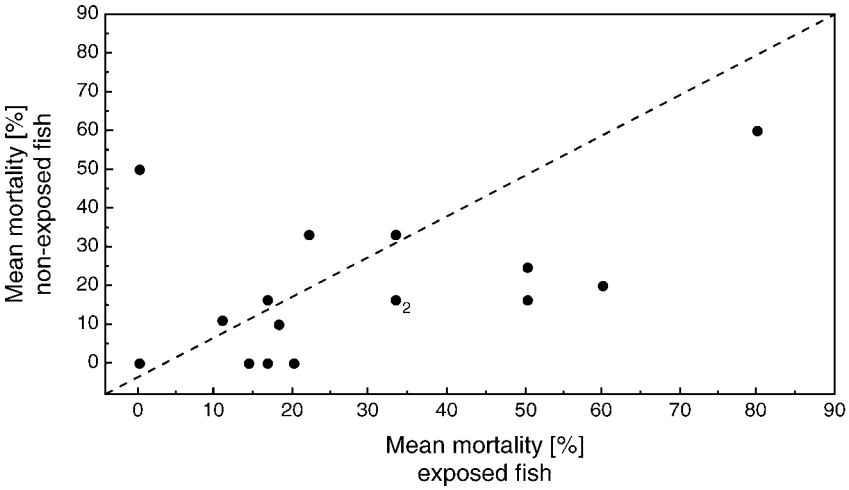

Fig. 5. Mean mortality of exposed and non-exposed offspring of different fathers. The dashed line represents the line of equality. The number indicates overlapping data points.

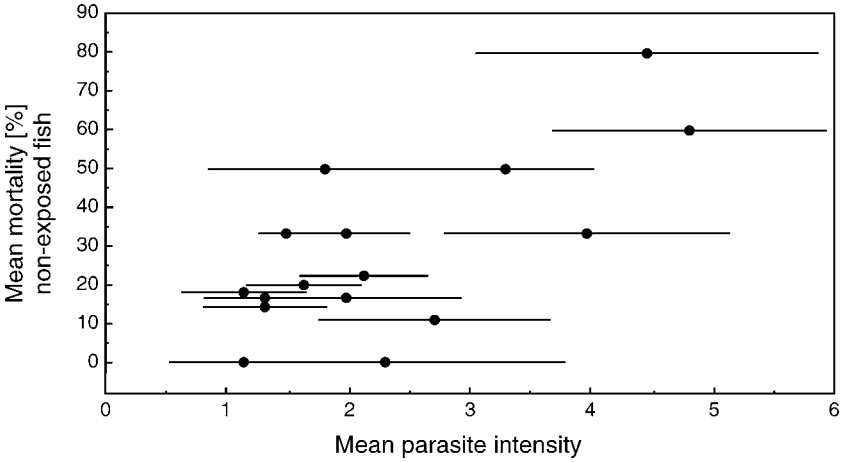

Fig. 6. Relationship between mean mortality and mean (±S.E.) parasite intensity per sibship.

Prey mortality

Infected *G. pulex* experienced significantly higher mortality than uninfected ones (Wilcoxon matched-pairs signed-ranks test, $n=64$, $T=90{\cdot}5$, $P=0{\cdot}002$). On average, 0·625 infected and 0·203 uninfected *G. pulex* per control replicate died during a 42 h interval. Accordingly, corrected prey availabilities entering the formula for the calculation of the preference indices were 9·375 and 9·797 for infected and uninfected gammarids, respectively.

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DISCUSSION

Experimental infection of laboratory bred full- and half-sib groups of three-spined sticklebacks revealed that the acanthocephalan parasite *P. laevis* adversely affects the survival of its final host. We ignore what mechanisms cause the higher mortality observed in infected fish. The damages imposed by acanthocephalan parasites upon their fish hosts are mainly of a mechanical nature, along with secondary bacterial infection, while significant withdrawal of nutrients probably only occurs in the case of heavy infestation (see Schäperclaus, 1990 for details). The immune response mounted by fish hosts is unspecific and essentially limited to the formation of a haemocyte capsule around the worm's proboscis. The positive correlation between the mortality of exposed fish and parasite burden suggests that the inflicted damages and the encapsulation reaction involve costs, for instance in terms of energy that is no longer available for other purposes. Possibly the entailed costs lead to an overall weakening, and eventually the death of infected fish. However, other causes of mortality, such as an increased susceptibility to other diseases, cannot be ruled out.

If survival is at stake, why do sticklebacks keep eating infected prey, rather than systematically avoiding them? One obvious reason is that the parasite-driven modifications in appearance and behaviour render infected gammarids more rewarding as prey, i.e. searching for and capturing infected gammarids entails lower costs than handling uninfected gammarids, despite similar energy contents. A comparison of the intake rates of exposed and unexposed fish shows that, on average, significantly more prey are eaten when infected prey are available.

The evolution of genetically based resistance to *P. laevis* would enable sticklebacks to benefit from the parasite-induced enhancement of prey profitability without paying the costs of infection. The great among-sibships variation in susceptibility to infection is suggestive of such a counter-adaptation. This variation may be at least partially genetic in origin. However, common environmental influences due to sib-groups being raised together cannot be ruled out, despite efforts to standardize breeding conditions.

The notion that a finite pool of resources prevents individuals from optimizing all behaviours simultaneously has been the focus of much research (see Sih, 1987 and Lima & Dill, 1990 for reviews). Intrinsic (e.g. age) and extrinsic factors (e.g. population density) are known to affect behavioural decisions, and to constrain the set of available decisions. Physical condition is related to mate choice decisions in sticklebacks (Bakker, Künzler & Mazzi, 1999), and is a likely candidate for a mediator of diet choice. When given a mixture of infected and uninfected prey, fish from sibships with rather high mean physical condition (or a correlate thereof) aptly adjusted feeding to their extent of parasite susceptibility: relatively resistant sibships profited from the more obvious, infected prey, whereas relatively susceptible sibships prudently relied upon the more evasive, uninfected prey. In contrast, the diet selected by fish from sibships with lower mean physical condition was not significantly related to the susceptibility to parasitic infection, likely reflecting a lack of ability or willingness to exert a choice.

Fish that did not survive the 6-week period under summer conditions were omitted from all analyses involving physical condition. No correlation was found between preference index and parasite intensity for fish that died during that time, neither in groups with higher nor in groups with lower than average condition. Thus, fish that behaved according to our expectations had better survival chances, implying that selection should favour selective feeding mechanisms tailored to the extent of parasite susceptibility.

The use of mortality and prey consumption as currencies for measuring the costs of infection and the benefits of easy predation cannot claim to be exhaustive, as costs other than reduced survival and payoffs other than increased energy intake will contribute to determining optimal foraging strategies. Gammarids are a valuable source of carotenoids (Simpson, Katayama & Chichester, 1981; Bakker & Mundwiler, 1999), which they obtain by feeding on carotenoid-rich vegetation. Carotenoids make up the orange-red breeding colouration of male sticklebacks (Czeczuga, 1980). Like any other animal, sticklebacks are not able to synthesize carotenoids themselves, and are therefore dependent on the supply in their diet (Simpson *et al.* 1981). The expression of the nuptial colouration may consequently reflect the male's foraging ability, or else its capability of efficiently turning carotenoids into visual signals. Females are expected to use such condition-dependent traits indicating male competence or quality as criteria in their mate choice decisions. Indeed, carotenoid-derived colour cues are established determinants of female choice in fish, with females often preferring brighter males (e.g. Endler, 1983; Kodric-Brown, 1985; Milinski & Bakker, 1990).

Hamilton & Zuk (1982) predicted a negative relationship between the expression of secondary sexual traits and the intensity of parasitic infection. However, tests of this hypothesis have produced mixed results (reviewed by Møller, 1990). Milinski & Bakker (1990) showed that the intensity of the red breeding colouration of male three-spined sticklebacks is weakened by infection with the ciliate parasite *Ichthyophthirius multifiliis*, and that females preferentially select healthy males because of their brightness. In contrast, in the Bakker & Mundwiler (1999) study, the intensity of the breeding colouration and the severity of infection with *P. laevis* were not significantly correlated. It is questionable

whether the nuptial colouration actually retains its reliability as a signal when parasites are transmitted through carotenoid-rich food. The consumption of gammarids increases a male's carotenoid stores and thus its showiness, but at the same time it increases the risk of infection. If the brightest males are not consistently immunologically superior, the intensity of the breeding colouration may become positively associated with parasite burden. Females choosing on the basis of this sexual display are then deceived into mating with heavily infested males, thus securing neither the immediate advantage of a healthy and vigorous mate, nor the indirect advantage of heritable resistance for their offspring. Hence, varying levels of parasite exposure and parasite susceptibility might interact to give rise to either positive or negative relationships between parasite load and the intensity of the breeding colouration. Folstad *et al*. (1994) suggested that in systems involving intermediate hosts containing carotenoids, such relationships depend on the parasite's pathogenicity, with the more pathogenic parasites most adversely affecting trait expression. Accordingly, they observed that the intensity of the red breeding colouration of male threespined sticklebacks was positively correlated with the intensity of infection with relatively innocuous *Diphyllobothrium* spp. parasites, but negatively associated with the more harmful cestode *Schistocephalus solidus*. They argue that the red nuptial colouration may simultaneously signal both the degree of exposure to prevalent parasites and genetic resistance towards such parasites.

There exists some evidence pointing at a possible beneficial function of carotenoids for females as well (Tacon, 1981 and references therein). Egg pigmentation and egg mortality are purportedly negatively correlated, eggs with many pigments being more tolerant against hypoxia, increased water temperature and ammonium concentration. The suggested respiratory function of carotenoids in eggs may be important for stickleback females, as the survival of their broods is often limited by insufficient dissolved oxygen supply (T. C. M. Bakker, D. Mazzi & S. B. M. Kraak, unpublished data), and suggests that females too face a trade-off between carotenoid acquisition and infection risk.

In summary, our findings validate earlier studies reporting that the increased conspicuousness and reversed phototaxis induced by the acanthocephalan parasite *P. laevis* make infected *G. pulex* more vulnerable to fish predation (e.g. Bakker *et al*. 1997). We further show that the parasite imposes costs in terms of impaired survival on its definitive stickleback host, and that these survival costs grow with the severity of infection. Finally, we document (possibly genetic) variation in the ability of sticklebacks to withstand infection, and argue that the link between parasite susceptibility and prey selection is mediated by the fish physical condition.

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REFERENCES

BAKKER, T. C. M., KÜNZLER, R. & MAZZI, D. (1999). Condition-related mate choice in sticklebacks. *Nature, London* **401**, 234.

BAKKER, T. C. M., MAZZI, D. & ZALA, S. (1997). Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology* **78**, 1098–1104.

BAKKER, T. C. M. & MUNDWILER, B. (1999). Pectoral fin size in a fish species with paternal care: a condition-dependent sexual trait revealing infection status. *Freshwater Biology* **41**, 543–551.

BOLGER, T. & CONNOLLY, P. L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology* **34**, 171–182.

CZECZUGA, B. (1980). Carotenoids in fish. *Pungitius pungitius* (L.) and *Gasterosteus aculeatus* L. (Gasterosteidae). *Hydrobiologia* **74**, 7–10.

ENDLER, J. A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* **9**, 173–190.

FOLSTAD, I., HOPE, A. M., KARTER, A. & SKORPING, A. (1994). Sexually selected color in male sticklebacks: a signal of both parasite exposure and parasite resistance? *Oikos* **69**, 511–515.

GEMMILL, A. W. & READ, A. F. (1998). Counting the cost of disease resistance. *Trends in Ecology and Evolution* **13**, 8–9.

HAMILTON, W. D. & ZUK, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384–387.

HOLMES, J. C. & BETHEL, W. M. (1972). Modification of intermediate host behaviour by parasites. In *Behavioural Aspects of Parasite Transmission* (ed. Canning, E. U. & Wright, C. A.), pp. 123–149. Academic Press, New York.

JMP (2000). *Version 4*. SAS Institute Inc., Cary, NC, USA.

KENNEDY, C. R., BROUGHTON, P. F. & HINE, P. M. (1978). The status of brown and rainbow trout, *Salmo trutta* and *S. gairdneri* as hosts of the acanthocephalan, *Pomphorhynchus laevis*. *Journal of Fish Biology* **13**, 265–275.

KODRIC-BROWN, A. (1985). Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology* **17**, 199–205.

LAFFERTY, K. D. (1992). Foraging on prey that are modified by parasites. *American Naturalist* **140**, 854–867.

LIMA, S. L. & DILL, L. M. (1990). Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**, 619–640.

MANLY, B. F. J., MILLER, P. & COOK, L. M. (1972). Analysis of a selective predation experiment. *American Naturalist* **106**, 719–736.

MILINSKI, M. & BAKKER, T. C. M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature, London* **344**, 330–333.

MØLLER, A. P. (1990). Parasites and sexual selection: current status of the Hamilton and Zuk hypothesis. *Journal of Evolutionary Biology* **3**, 319–328.

MOORE, J. (1984*a*). Altered behavioral responses in intermediate hosts – an acanthocephalan parasite strategy. *American Naturalist* **123**, 572–577.

MOORE, J. (1984*b*). Parasites that change the behavior of their host. *Scientific American* **250**, 82–89.

POULIN, R. (1994). Meta-analysis of parasite-induced behavioural changes. *Animal Behaviour* **48**, 137–146.

POULIN, R. (1995). 'Adaptive' changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology* **25**, 1371–1383.

POULIN, R. (1998). *Evolutionary Ecology of Parasites: From Individuals to Communities*. Chapman and Hall, London.

SCHÄPERCLAUS, W. (1990). *Fischkrankheiten*, 5th Edn. Akademie-Verlag, Berlin.

SIH, A. (1987). Predators and prey lifestyles: an evolutionary and ecological overview. In *Predation. Direct and Indirect Impacts on Aquatic Communities* (ed. Kerfoot, W. C. & Sih, A.), pp. 203–224. University Press of New England, Hanover, NH, USA.

SIMPSON, K. L., KATAYAMA, T. & CHICHESTER, C. O. (1981). Carotenoids in fish feeds. In *Carotenoids as Colorants and Vitamin A Precursors* (ed. Bauernfeind, J. C.), pp. 463–538. Academic Press, New York.

SYSTAT (1992). *Statistics, version 5.2*. Systat, Evanston, IL, USA.

TACON, A. G. J. (1981). Speculative review of possible carotenoid function in fish. *Progressive Fish-Culturist* **43**, 205–208.

WAKELIN, D. & BLACKWELL, J. M. (1988). *Genetics of Resistance to Bacterial and Parasitic Infection*. Taylor & Francis, London.

WEDEKIND, C. & MILINSKI, M. (1996). Do three-spined sticklebacks avoid consuming copepods, the first intermediate host of *Schistocephalus solidus*? – an experimental analysis of behavioural resistance. *Parasitology* **112**, 371–383.