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Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction

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Trophically transmitted parasites may increase their transmission efficiency by altering the behaviour of infected hosts to increase their susceptibility to predation by target hosts (the next host in the life cycle). The parasite *Diplostomum spathaceum* (Trematoda) reduces the vision of its fish intermediate hosts: its metacercariae lodge themselves in the eyes of fish and induce cataract formation, which gives them the opportunity to affect fish behaviour. We examined whether *D. spathaceum* eye flukes change the preference of fish for the surface layers of the water column or their escape behaviour, which could make the fish more vulnerable to predation by bird hosts. We also studied the influence of parasites on the susceptibility of fish to artificial aerial predators that were able to catch fish from the water surface. Infected and control fish did not differ in their preference for the surface layers but infected fish showed less escape behaviour when a black plate was drawn over the water surface. They were also more easily caught by human 'predators' dipping a net into the tank. Thus, infected fish should be easier prey for gulls and terns, implying that the ability of *D. spathaceum* eye flukes to alter fish behaviour may be a parasite strategy evolved to enhance transmission.

Individual offspring of parasites with complex life cycles have only a small probability of surviving and completing the life cycle because mortality of parasites during transmission between hosts is usually high (e.g. Dobson et al. 1992). Thus, natural selection may favour parasite genotypes that can compensate for losses by producing more offspring (e.g. Price 1974), or by being better at infecting the target hosts (the next host in the life cycle), and thus achieving higher transmission efficiency. Several complex parasite life cycles include at least one stage where the infected host has to be ingested by the target host for successful transmission. These parasites may increase their probability of transmission by changing host behaviour to make them easier prey for target hosts (Rothschild 1962; Holmes & Bethel 1972). For example, terrestrial isopods infected with *Plagiorhynchus cylindraceus* (Acanthocephala) spend more time in exposed microhabitats than unparasitized individuals do, leading to increased avian predation (Moore 1983). Differences in the behaviour of parasitized and unparasitized animals have been observed in many other parasite–host interactions (reviewed by Moore 2002), and they have often been assumed to be parasite strategies evolved to enhance transmission. In several studies, parasite-induced changes in host behaviour have been observed to increase the susceptibility of hosts to predation by target hosts (e.g. Bethel & Holmes 1973, 1977; Moore 1983; Poulin et al. 1992; Lafferty & Morris 1996; Pulkkinen et al. 2000). However, there are also cases where behavioural alterations may not enhance predation rates (e.g. Webster et al. 2000; Edelaar et al. 2003). Nevertheless, only a few studies investigating the effect of trophically transmitted parasites on the behaviour of infected hosts have evaluated the influence of observed behavioural alterations on the vulnerability of hosts to predation (Moore 2002).

We used the parasite *Diplostomum spathaceum* (Trematoda) and its fish intermediate host as a model system to study the ability of parasites to alter the behaviour of infected hosts to increase their susceptibility to predation. The parasite reproduces sexually in the intestine of fish-eating birds (definitive host). The eggs are released in water with the bird's faeces where they hatch into free-swimming miracidia (Chappell et al. 1994). The miracidia infect aquatic snails (first intermediate host) where the parasite reproduces asexually to produce free-swimming cercariae. These infect a variety of fish species (second intermediate host) by penetrating the skin or gill filaments, and migrate to the lenses of the eyes where they develop into metacercariae. For successful transmission to the definitive host, parasitized fish have to be eaten by

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a fish-eating bird. The metacercarial stages of the *Diplostomum* parasites and the opacity of the lenses caused by the parasites' metabolic wastes reduce the host's vision, which may even lead to blindness (Rushton 1937, 1938; Shariff et al. 1980). Thus, by injuring important sensory organs, *D. spathaceum* clearly has the potential to alter fish behaviour in a way that may increase vulnerability to predation by piscivorous birds. Crowden & Broom (1980) have suggested that infected fish spend more time on the surface layer of the water column, but the effect of the parasite on the susceptibility of fish to avian predation has not been studied.

We tested whether *D. spathaceum* eye flukes induce changes in the behaviour of fish that would increase their susceptibility to predation by gulls (Laridae) and terns (Sternidae), which are believed to be the most commonly used definitive hosts for *D. spathaceum* (Chappell et al. 1994). These hosts are capable of catching fish only from the water surface. Therefore, factors such as the position of a fish in the water column and its ability to respond to approaching predators by escaping to deeper water layers are essential in determining its vulnerability to predation. We studied the effect of the parasite on the preference of fish for the surface layers of the water column by introducing individual fish into elongated tanks with a light–dark gradient. We also recorded the escape responses of fish to a simulated, smoothly moving aerial predator in the same tank system, focusing on changes in the position of fish in the water column. In a second experiment, we studied the potential effect of the parasite on the susceptibility of fish to aerial predators. For this, we used groups of fish in larger tanks and rapidly moving simulated predation, which imposed even stricter demands on the visual responses of the fish.

METHODS

Study Animals and Infection Procedures

We used experimentally infected juvenile (8-month-old) rainbow trout, *Oncorhynchus mykiss*. From the variety of fish host species suitable for the parasite, we selected rainbow trout because they are relatively susceptible to infection by the parasite (Betterton 1974) and easy to maintain in laboratory conditions. Fish were obtained from a commercial fish farm where they had been reared in indoor tanks supplied with ground water, which ensured that they had no parasites. We infected the fish with *D. spathaceum* cercariae 4 and 2 months before the behaviour experiment and the predation experiment, respectively. This ensured that the metacercariae had reached the stage of being infective to birds before the experiments, and thus were ready to be transmitted (see Sweeting 1974). Infections were designed to produce fish with natural infection levels that were high enough to induce effects. In both infections, randomly chosen fish were infected under laboratory conditions with cercariae released by 10 *Lymnaea stagnalis* snails collected from natural habitats. Before the infections, the snails were allowed to produce cercariae for 4 h. We pooled all cercariae into one suspension, and estimated the cercarial density from 10 1-ml samples. For the behaviour experiment fish were infected concurrently in three 65-litre tanks each containing 150 fish. Water temperature was 17.1°C and the infection dose was 250 cercariae per fish. Age of the cercariae used varied from 30 min to 4.5 h. During the infections, water flow through the tanks was turned off, and the tanks were aerated with aquarium pumps. After 30 min, the water flow was turned on, and the amount of water in each tank was increased to 185 litres. Infected fish were maintained in these conditions until the experiment. Control fish were sham exposed and maintained in identical conditions as the infected fish. For the predation experiment fish were infected in four 65-litre tanks at 19.0°C and with a dose of 300 cercariae per fish. In other respects, the infections were the same as for the behaviour experiment. Fish were fed daily with commercial fish pellets.

Behaviour Experiment

For the behaviour experiment we used five transparent plastic tubes (205 cm high, 20 cm in diameter), where an effective light–dark gradient could be generated. Tubes were considered small enough for easy observation of the vertical position of fish in the water column, but large enough not to restrict the movement of fish. We set the height of the water column in each tube to 200 cm and the water flow through the tubes to 1.5 litres/min. Water flowed into the tubes 5 cm above the bottom and out from the surface. The bottom of each tube was covered with black gravel and each tube was illuminated from above with a 40-W lamp. Light intensity was set to produce similar light–dark gradients in the water column of all tubes (Fig. 1). Water temperature during the experiment was 6.7°C, which corresponded to natural water temperature at the time of the experiment. We conducted the experiment seven times using a different group of five fish each time. Study fish were taken evenly from all storage tanks, and we randomly placed individual fish in the experimental tubes with similar total numbers of infected and control fish in each tube over the experiment. Fish were allowed to recover from the transfer for 5 days before the experiment. Six fish died during the adjustment periods and were excluded from the data.

We divided the water column in the experimental tubes into 10 20-cm sections, and recorded the position of each fish at 1-min intervals for 2 h. We then recorded the reaction of the fish to an artificial aerial predator. We drew a black plate (21.5×10 cm) between the light and the water surface, using a thin transparent fishing line, and measured the immediate escape response of each fish to the approaching shadow (subjective scale: 0 = no reaction; 1 = restlessness; 2 = horizontal movement followed by slow movement to the deeper water layers; 3 = immediate escape by diving) and the movement of fish towards the deeper water layers after the disturbance. After the experiment, all study fish were killed with an overdose of 0.01% MS 222 (Sigma Chemical Co., St Louis, U.S.A.). We determined the intensity of parasite-induced

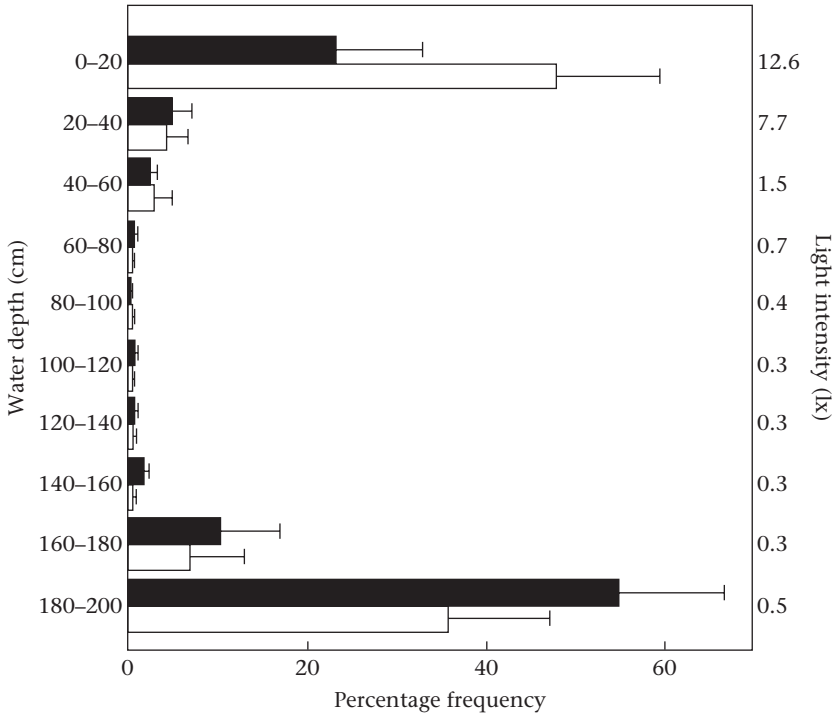

Figure 1. Average proportional distributions of fish in the water column + SE for infected (■; $N = 14$) and control fish (□; $N = 15$), and light intensities in different depth layers.

cataracts with a Kowa Portable Slit Lamp SL-14 microscope (Wall & Bjerkås 1999), and counted the *D. spathaceum* metacercariae by dissecting the lenses. We measured the length (± 1 mm) and mass (± 0.1 g) of each fish. The average body length and mass $\pm$ SE of infected fish were 99 ± 1.3 mm and 8.3 ± 0.4 g. Corresponding values for control fish were 96 ± 1.4 mm and 8.0 ± 0.4 g. Body length and mass did not differ between infected and control fish (independent samples t test: length: $t_{27} = -1.19$, $P = 0.244$; mass: $t_{27} = -0.43$, $P = 0.671$).

Data from positions of individual fish in the water column were pooled up to produce average distributions for infected and control fish. To analyse the data we primarily used two-tailed parametric tests, but nonparametric tests were also used when the assumptions of parametric tests could not be met. We analysed the effect of parasites on the preference of fish for the surface layers of the water column by comparing the number of observations made between 0 and 60 cm of depth, using an independent-samples t test. Other depth layers were ignored, since observations in mid-water layers were scarce, and the fish in the bottom layers were unavailable for aerial predators. Similarly, the effect of parasites on fish escape behaviour was analysed using only the fish that were between 0 and 60 cm of depth before the disturbance. The escape response of fish to the disturbance was analysed with an independent-samples Mann–Whitney U test and the movement of fish to the deeper water layers with an independent-samples t test with Bonferroni corrections. Thus, observed P values were compared to the significance level of 0.025.

Predation Experiment

The predation experiment was conducted in five round tanks, each with a diameter of 140 cm and a water depth of 90 cm. Water flow through the tanks was set to 15 litres/min, and water was directed to a depth of 30 cm to keep the water surface calm. The surroundings of the experimental tanks were darkened. Each tank was illuminated from above with a 40-W lamp placed 70 cm above the water surface. Light intensity was set to 250 lx, measured 40 cm underneath each lamp. The bottom of each tank was covered with black gravel to produce a light–dark gradient in the water column. We randomly selected fish from all the storage tanks and placed them at random into the experimental tanks so that each tank received 10 infected and 10 control fish. The fish were allowed to recover from the transfer for 5 days before the experiment. We conducted the experiment twice 1 week apart, using new fish each time. The respective water temperatures were 15.5 and 14.5°C, corresponding to water temperatures in nature at the time of the experiment. One fish died during the adjustment periods and was excluded from the data.

Five people who were unaware of the purpose of the experiment were allowed to catch fish from the surface layer (0–20 cm) of the water column using round dip-nets (diameter 20 cm). Fish were caught from each shoal until either 10 fish were caught, or a total of 30 attempts were made. We allowed the fish to recover for 1 h between attempts except for attempts 15 and 16, when there was a 9-h break. At each time, the persons tried to catch one

fish from the shoal with one rapid sweep. The attempt had to be made during a 5-min period, and if the time was up without an attempt a disturbance similar to the attempt was made at the water surface without trying to catch any fish. The five persons were assigned randomly to the tanks at the beginning of each replicate of the experiment and moved to the next tank after each attempt. After the experiment, all study fish were killed as described previously, and we recorded the intensity of parasite-induced cataracts, the number of *D. spathaceum* individuals, and fish length and mass as in the behaviour experiment. The average body length and mass ± SE of infected fish were 103±0.9 mm and 9.7±0.3 g, and correspondingly for control fish 105±0.7 mm and 10.4±0.2 g. Body length and mass did not differ between infected and control fish (independent samples t test: length: $t_{197} = 1.70$, $P = 0.090$; mass: $t_{197} = 1.88$, $P = 0.062$). Furthermore, because the water temperature during the predation experiment was relatively high and thus favourable for ectoparasite infections, we determined the infections from one pectoral fin and a mucus sample (see methodology in Rintamäki-Kinnunen & Valtonen 1997) taken from each fish. Low-level *Gyrodactylus salaris* and *Ichthyopthirius multifiliis* infections were observed in infected fish (*G. salaris*: $\overline{X}\pm\mathrm{SE} = 0.7\pm0.2$ per fish; *I. multifiliis*: 2.9±0.4) and in control fish (*G. salaris*: 0.2±0.1; *I. multifiliis*: 2.4±0.3), but these are unlikely to have affected the results, because the levels of infections were very low (see Rintamäki-Kinnunen & Valtonen 1997).

We calculated values of Manly's α (Manly 1974) for each tank to assess the relative predation susceptibility of infected and control fish:

$$\alpha = \frac{\ln((n_i - r_i)/n_i)}{\ln((n_i - r_i)/n_i) + \ln((n_c - r_c)/n_c)}$$

where n_i is the number of infected fish at the beginning of the experiment, and r_i is the number of infected fish caught in the experiment. Correspondingly, n_c is the number of control fish at the beginning of the experiment, and r_c the number of control fish caught. The values of α range between 0 and 1, and values higher than 0.5 indicate that infected fish were caught more often than control fish. Observed values of α were compared to a situation of equal susceptibility ($\alpha = 0.5$) using a two-tailed nonparametric sign test, since the assumptions of parametric tests could not be met.

Ethical Note

We used experimentally infected fish instead of naturally infected ones, because fish in nature are commonly infected with other parasite species as well as with *D. spathaceum*. Our earlier studies in this system provided information on the infection levels necessary to produce fish with natural infection levels that were high enough to induce effects. The average infection levels in the experiments were high (54.6, range 35–80, and 107.9, range 20–214, in the behaviour and predation experiments, respectively) but corresponded to parasite burdens observed in wild fish populations. The average parasite burden in fish populations can vary from 0 to over 100 metacercariae per fish and parasite load in individual fish can be even higher (e.g. Valtonen & Gibson 1997; Marcogliese et al. 2001). For example, Marcogliese et al. (2001) recorded a maximum mean infection level of 151 eye flukes per fish from a white sucker, *Catostomus commersoni*, population from Canada. Furthermore, Chappell (1969) recorded over 200 eye flukes from an individual stickleback, *Gasterosteus aculeatus*, and Wootten (1974) even 550 metacercariae from an individual rainbow trout. In our study, no mortality of fish occurred during the infections, but low mortality was observed afterwards. However, this mortality corresponded to the mortality of uninfected juvenile rainbow trout kept in optimal laboratory conditions, where individual fish die every now and then without any particular reason. All infected fish appeared healthy to the human eye. A total of 117 infected and 118 control fish were used in the experiments described, but more fish than this were infected for use in other experiments. All study fish were killed at the end of the experiments by introducing them into water containing 0.01% of MS 222, a commonly used fish anaesthetic. Experiments were conducted with permission of the Lab-Animal Care and Use Committee of the University of Jyväskylä.

RESULTS

Behaviour Experiment

The preference of infected fish for the surface layers of the water column did not differ from that of control fish (t test for the number of observations between 0 and 60 cm of depth: $t_{27} = 1.52$, $P = 0.141$; Fig. 1). However, the escape response of infected fish after the disturbance was less than that of control fish (Mann–Whitney U test: $U = 1$, $N_1 = 4$, $N_2 = 8$, $P = 0.004$; Fig. 2). The change in the position of infected fish towards deeper water layers after the disturbance was also smaller than that of control fish (t test: $t_{10} = 3.36$, $P = 0.009$; Fig. 3). All infected fish were parasitized and the average *D. spathaceum* burden ± SE in infected fish was 54.6±3.3, causing parasite-induced cataract formation observed as grey opacity of the lenses. Control fish were free of parasites.

Predation Experiment

A total of 91 fish were caught in 258 attempts. Infected fish were more likely to be caught than control fish (sign test for index α: $M = 5$, $N = 10$, $P = 0.002$; Table 1). All infected fish were parasitized and the average *D. spathaceum* burden ± SE in infected fish was 107.9±5.7, causing intense parasite-induced cataract formation observed as white opacity of the lenses. In addition, 45% of the control fish had a low-level *D. spathaceum* infection ($\overline{X}\pm\mathrm{SE} = 1.5\pm0.1$), but no cataract formation was observed.

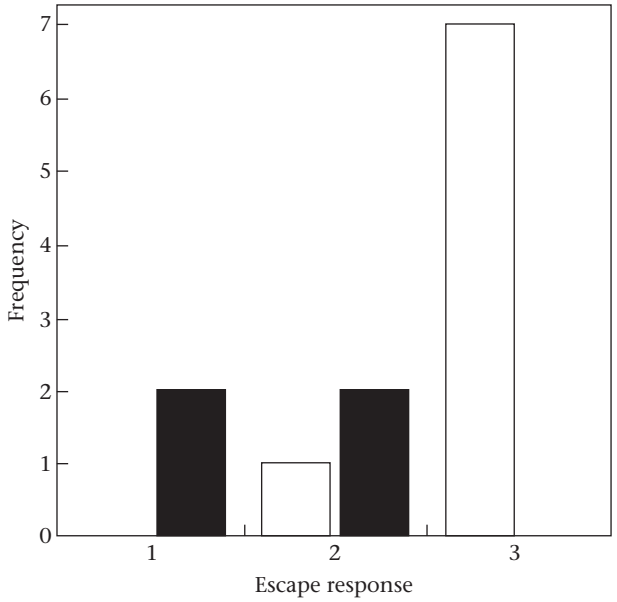

Figure 2. Frequencies of escape responses of infected (■) and control fish (□) to a disturbance by an artificial predator. Responses are: 1 = restlessness; 2 = horizontal movement followed by slow movement to the deeper water layers; 3 = immediate escape by diving. Only the data from fish that were between 0 and 60 cm of depth before the disturbance are shown.

DISCUSSION

Trophically transmitted parasites may increase their transmission efficiency by altering the behaviour of infected hosts so that their susceptibility to predation by target hosts is increased. We suggest that this phenomenon also occurs in the *D. spathaceum*–fish interaction, since in this study, fish infected with *D. spathaceum* eye flukes had decreased escape responses and increased susceptibility to artificial aerial predators. We did not observe parasite-induced preference of fish for the surface layers of the water column as suggested by Crowden & Broom (1980). These results indicate that, because of reduced escape behaviour, infected fish should be easier prey for gulls and terns, which may enhance parasite transmission. The behaviour experiment was conducted in elongated tubes where we could easily observe vertical changes in the position of the fish as a response to disturbance. This may have reduced possible horizontal movements of fish which may also be important in predator avoidance. However, we assumed that vertical responses would be most effective against surface-feeding avian predators.

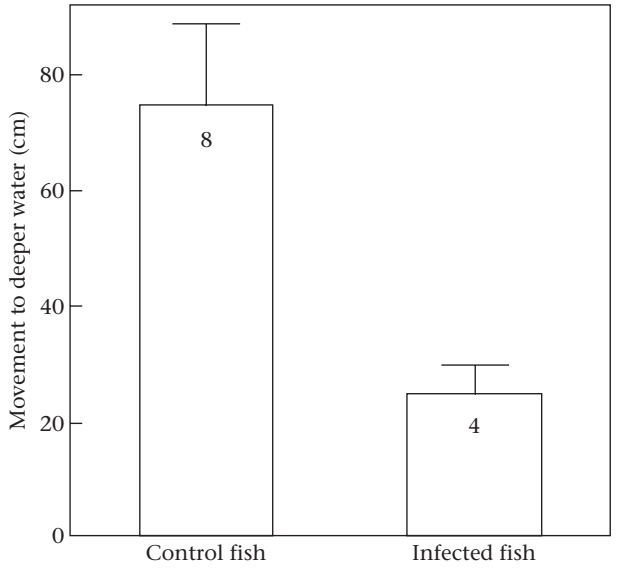

Figure 3. Average movement of fish to the deeper water layers after a disturbance by an artificial predator + SE for infected and control fish measured from fish between 0 and 60 cm of depth before the disturbance. Numbers refer to sample sizes.

Table 1. Numbers of infected and control fish caught from each shoal and values of Manly's α

Shoal	Infected fish	Control fish	Manly's α*
A	10	0	1.000
B	8	2	0.878
C	9	0	1.000
D	10	0	1.000
E	9	1	0.956
F	8	0	1.000
G	6	1	0.897
H	9	0	1.000
I	8	0	1.000
J	9	1	0.956

Number of available fish in both fish groups was 10, except for shoal I where there were nine infected fish.
*Manly's α indicates relatively susceptibility to being caught for infected and control fish.

The ability of *D. spathaceum* parasites to make infected fish more vulnerable to predation may be an evolutionary adaptation of the parasite to enhance its transmission to bird hosts. However, infected fish may also be more vulnerable to dead-end nonhost predators such as piscivorous fish as a consequence of altered behaviour. Therefore, the effect of behavioural alterations on parasite fitness depends on proportional changes in susceptibility to predation by different predator species. *Microphallus* (Trematoda) parasites, for example, cause infected aquatic snails to stay on the upper sides of rocks in the early morning hours, when they are exposed to intensive predation by waterfowl, and hide for the rest of the day, when predation by nonhost fish is increased (Levri & Lively 1996; Levri 1998). To be fully adaptive, behavioural changes should take place only when parasites are infective to target hosts, as observed in the *Microphallus*–snail interaction (Levri & Lively 1996). These aspects indicate that transmission efficiency of the parasite is increased as a consequence of altered snail behaviour. In our study, we found the effect of fully developed *D. spathaceum* metacercariae on the vulnerability of fish to aerial predators. Brassard et al. (1982) have observed that penetration of cercariae increases the vulnerability of fish to predation by nonhost piscivorous fish, but they did not consider the effect of metacercarial stages. Therefore studies that explore the susceptibility of infected fish to nonhost predators and the effect of developing

metacercariae on fish are needed to evaluate the adaptive nature of parasite-induced behavioural alterations in this system. We also need studies on the effect of varying parasite load, since even low-level infections may affect the fish (Owen et al. 1993).

Decreased escape behaviour as well as other parasite-induced changes in fish behaviour and colouration may increase their vulnerability to predators. For instance, Milinski (1990) suggested that *D. spathaceum* parasites cause fish to become darker and hence more conspicuous, and other trematode parasites impair shoaling behaviour of infected fish (Radabaugh 1980; Ward et al. 2002). Furthermore, the effect of the parasite on host phenotype may be host specific (see e.g. Poulin 1993), which could explain the surface-seeking behaviour observed in dace, *Leuciscus leuciscus*, parasitized with *D. spathaceum* eye flukes (Crowden & Broom 1980).

We suggest that the definitive mechanism whereby *D. spathaceum* parasites alter fish behaviour and increase susceptibility to predators is the reduction in vision. Thus, it is possible that parasites that live in the eyes have been favoured by natural selection if they have had higher transmission efficiency (see Poulin 1993; Barber & Crompton 1997). Alternatively, the selective pressure may have acted more strongly on parasite survival in fish, and may have favoured parasites escaping the host's immune defence by living in the eyes (see Szidat 1969). The ability of *D. spathaceum* eye flukes to induce cataract formation on the lenses may be a key process leading to increased susceptibility of fish to predators. However, since cataract formation may be a consequence of metabolic wastes excreted by the parasites, it is tightly connected to other traits that depend on the rate of parasite metabolism. Thus, strong cataract formation may be an adaptation to increase fish susceptibility to predators or a side-effect of selection favouring, for example, parasites growing faster and reaching infectivity to bird hosts sooner. Nevertheless, natural selection may still favour features of metacercariae that enhance parasite transmission, even if these features were originally selected for other purposes.

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