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Negative effects of *Rhabdias bufonis* (Nematoda) on the growth and survival of toads *(Bufo bufo)*

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Summary. The growth and survival of juvenile toads, *Bufo bufo,* infected with a common lung nematode, *Rhabdias bufonis,* were studied. Toads were raised from tadpoles in the laboratory and infected 2 months after metamorphosis. Individual toads were exposed to doses of 10, 40, 80 or 160 larvae, which enabled examination of the hypothesis that parasite-induced mortality is affected by worm numbers. Growth of infected toads began to diverge from that of uninfected controls at 6 weeks post infection (WPI) and by 12 WPI the most heavily infected toads were approximately half the mass of controls. No controls died throughout the experiment; however, mortality of infected toads was strongly affected by parasite density. A mechanism for mortality is suggested by the significant negative relationship between parasite density and dietary intake. This parasite-induced anorexia was detected at 3 WPI and persisted up to 9 WPI. Patterns of reduced host growth, survival and dietary intake provide experimental evidence of the negative consequences of parasitic infection in a natural parasite-host system which may also be present under natural conditions.

Key words: Parasitism – Growth – Survival – *Bufo* – *Rhabdias*

The effect of parasites in animal ecology has received much attention in recent years. This has been largely due to a series of theoretical models (reviewed by Anderson 1982; Hassell and May 1989) which have shown that viral, bacterial, protozoan and helminth infections can, under certain conditions, have a large effect on host population size. A critical assumption of these models is that parasites have a measurable effect, positively correlated with parasite burden, on the fitness of host individuals. The empirical support for this assumption is based primarily on laboratory host-parasite studies (e.g. Anderson 1978; Scott 1988). However, several studies have failed to detect measurable effects of parasitism on host fitness (Gill and Mock 1985; Kennedy 1987). Indeed some studies (e.g. Munger and Holmes 1988; Lincicome 1971) show that parasitic infection may sometimes benefit their hosts. It is therefore not surprising that the role played by parasites under natural conditions is controversial (Holmes 1982).

The lack of experimental data involving naturally-occurring helminth-host systems contributes to this controversy. In particular, there are very few studies which measure the effects of helminth parasites on their natural vertebrate host populations (Anderson 1980). It is helminths that are frequently studied by field parasitologists and that provide much of the empirical support for the notion that parasites evolve to be non-pathogenic to their hosts and have little effect on host population regulation. This paper contributes to this debate by experimentally measuring the effects of a nematode infection on the growth and survival of the toad, *Bufo bufo.*

Rhabdias bufonis is a common parasite of European toads and frogs (Smyth and Smyth 1980; Goater, in press). Protandrous, hermaphroditic adults live in the lungs. Eggs are coughed into the mouth, swallowed and subsequently hatch into 1st-stage larvae in the small intestine. Larvae accumulate in the large intestine and are expelled in the faeces. After a short period in the soil (3–7 days) infective 3rd-stage larvae migrate to high points in their surroundings to potentially contact toads and frogs. The larvae penetrate the skin, undergo a further molt in the musculature and then migrate to the lungs. The life-cycle and natural patterns of infection of this species have been well studied (Smyth and Smyth 1980) but experimental investigations have not been attempted. As an experimental host-parasite system, *R. bufonis* combines the advantages of a direct life-cycle, large size, hermaphroditism and short generation time (10–12 days) with its ease of manipulation in hosts which are simple to maintain.

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Materials and methods

Toads were collected as tadpoles from the Obersee (near Näfels, Kanton Glarus, Switzerland, 990 m altitude) and reared under standard conditions (Alford and Harris 1988) in the laboratory. Metamorphs were fed *ad libitum* with *Drosophila* for 8 weeks prior to infection. Prior to infection, individual toads were acclimated for 7 days in plastic aquaria (28 × 20 × 10 cm) with perforated lids. Each toad was provided with fresh water in a 30-mm Petri dish. A moist refuge was provided by placing a small clay flower pot in each container. These pots were immersed in water every 4 days. Containers were thoroughly cleaned once a week.

Infective larvae were obtained from six adult toads collected from the Obersee. Each was naturally infected, determined by the presence of large numbers of larvae in the faeces. *Rhabdias bufonis* is the only parasite of *B. bufo* which releases larvae (as opposed to eggs) directly into the faeces (Smyth and Smyth 1980); contamination with other species is therefore avoided. Infective larvae develop directly within the faeces in 3–7 days and are then easily isolated and counted prior to infection (Goater, in press). Toads were infected over 24 h by placing them in 20-mm Petri dishes containing moist filter papers with known numbers of infective larvae.

Eighty toads were infected with 0, 10, 40, 80 or 160 infective larvae (16 toads per treatment). Each toad was assigned to one of 80 containers and randomly arranged within four spatial blocks. Containers were also randomized within blocks. Each block was placed on a shelf in a constant temperature room (20° C; 12D:12L day: light cycle). Within each infection treatment and prior to the beginning of the experiment, toads were randomly assigned to three dates for dissection. Four were to be killed (by immersion in concentrated anaesthetic) at 3 weeks post-infection (WPI), four at 6 WPI and eight at the end of the experiment. Toads dissected before the end of the experiment provided information on temporal patterns of parasite numbers and biomass. It was not possible to determine the sex of juvenile toads. More detailed descriptions of the general biological characteristics of the primary infection as well as host maintenance and infection procedures are given in Goater (in press).

Toads were also assigned to containers according to body weight. Despite the attempted uniformity in growth conditions prior to infection, there was still substantial variation in host mass ($\bar{x} = 0.45$ g $\pm$ 0.18 SD). To control for possible bias due to body size, the 40 heaviest and 40 lightest toads were separated into two containers. Large and small toads were selected alternately when assigned to containers so that each block contained equal numbers of each size class.

During the experiment, toads were maintained mainly on a diet of standard-size crickets, provided weekly. Up to 6 WPI toads received 0.10 g of 3–5 mm crickets each week (0.05 g twice per week). Beyond 6 WPI, both the mass and size (5–7 mm) of crickets was increased. Every third week, toads received an additional 0.05 g of crickets so that by the end of the experiment, toads received 0.30 g per week. These food levels were determined by previous experience with *B. bufo* juveniles in our laboratory and always provided food in excess of requirements. In addition, after 6 WPI, toads were fed 5 *Musca* per week to supplement their diet.

An estimate of the quantity of crickets eaten by each toad was obtained using the following procedure. Every third Monday, half the weekly biomass of crickets was provided to each cage and on Wednesday the second half. An estimate of the weight of individual crickets was obtained by counting the numbers of crickets provided to 10 toads prior to each feeding. On Friday the number of crickets remaining in each container was counted, converted to estimates of biomass and then subtracted from the initial biomass offered. Toads were also weighed ($\pm$0.1 g) on the Friday of every third week.

Temporal changes in host growth, survival and dietary intake were measured sequentially from the same individuals and were therefore analyzed by means of repeated measures ANOVA (StatView SE™). Mortality and diet analysis involved proportional data; these were transformed prior to analysis to arcsine (square root). The analysis of host survival was complicated by the fact that we removed toads at two specific dates during the experiment (a total of eight at each exposure dose). We took this into account when estimating mortality by only considering deaths of toads which could have died up to the end of each 3-week interval. The analysis therefore measured the cumulative survival of toads over 12 weeks. For this analysis, the four blocks of toads were used as replicates. Repeated-measures ANOVA looked for differences in the mean proportion surviving at each exposure density.

Results

All toads exposed to *R. bufonis* became infected and there was a clear positive relationship between the number of larvae administered and the subsequent number of adult worms which initially reached the lungs (Fig. 1). The infection procedure established four distinct intensities of infection which persisted until 6 WPI, after which there was a sharp decline in worm densities, especially in toads exposed to high doses of larvae (Fig. 1). Initial worm densities at 3 WPI were significantly different over the four exposure doses (one-way ANOVA with exposure dose, $F_{3,13} = 13.83$, $P = 0.0007$).

There was a significant increase in the size of toads throughout the experiment but growth was not directly influenced by parasite exposure dose (Fig. 2, Table 1). However, there was a significant interaction between time and exposure dose showing that toads with different levels of infection had different temporal patterns of growth. For example, masses of toads infected with 160 larvae began to diverge from lightly infected toads at 6 WPI (Fig. 2). Divergence in mass in all infection classes was especially marked at 9 WPI and 12 WPI.

The survival of infected toads declined over time, but survival was not significantly influenced by the dose of larvae administered (Fig. 3, Table 1). A significant interaction between time and exposure dose demonstrates that infection produced a gradual, rather than an im-

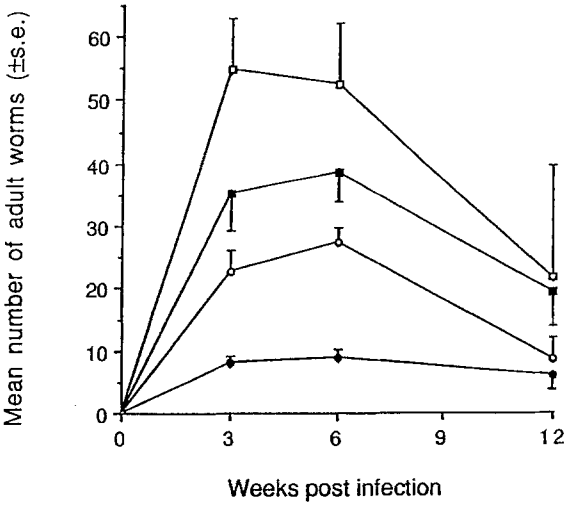

Fig. 1. Temporal changes in the density of *Rhabdias bufonis* in the lungs of *Bufo bufo* exposed to 4 doses of infective larvae. Sample sizes from infection doses 10, 40, 80, 160 respectively, are at 3 weeks post-infection (WPI) – 3, 4, 3, 4; at 6 WPI – 4, 4, 3, 2; at 12 WPI – 5, 4, 5, 3. *Symbols:* —●— 10; —○— 40; —■— 80; —□— 160

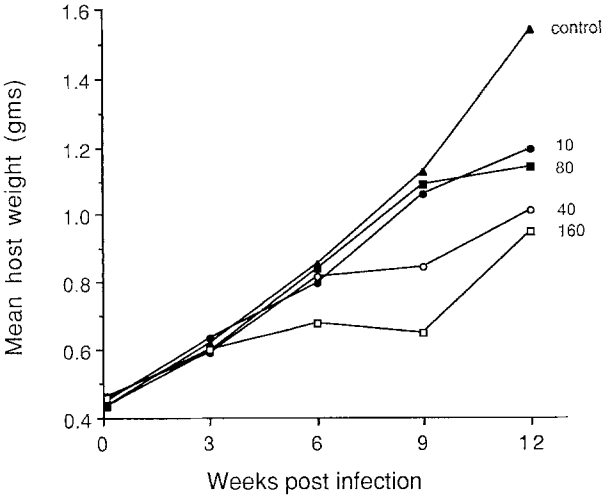

Fig. 2. The growth of *B. bufo* infected with 4 doses of infective larvae of *R. bufonis*. *Points* represent mean growth at each exposure dose. Sample sizes from infection doses 0, 10, 40, 80, 160 respectively, are at 0 WPI – 16, 16, 16, 16, 16; at 3 WPI – 16, 15, 15, 15, 15; at 6 WPI – 12, 10, 11, 9, 11; at 9 WPI – 8, 6, 7, 6, 5; at 12 WPI – 8, 5, 5, 5, 3

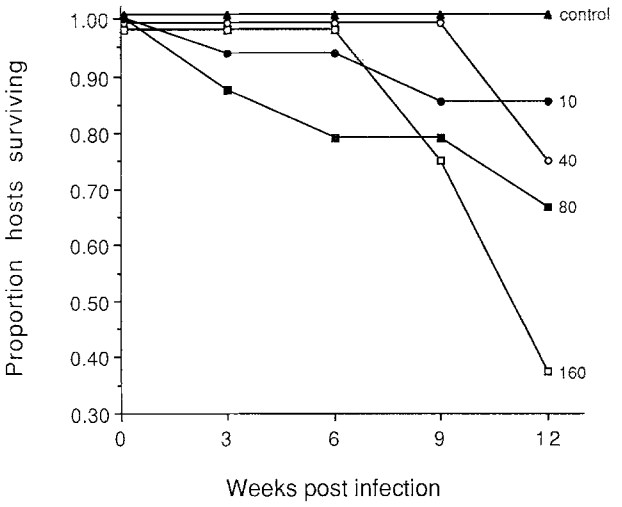

Fig. 3. The survival of *B. bufo* infected once with 4 doses of the infective larvae of *R. bufonis*. *Points* represent the cumulative proportion of hosts surviving at the end of each 3-week interval within each exposure dose

Table 1. Summary of repeated-measures ANOVA for analysis of the effects of time and parasite exposure dose on the weight, survival and dietary intake of toads infected with *R. bufonis*

Source	*df*	MS	*F*	*P*
Host weight				
Exposure dose	4	0.172	0.848	0.512
Error	20	0.203		
Time	4	2.535	100.152	0.0001
Exposure dose × time	16	0.053	2.086	0.0169
Error	80	0.025		
Host survival				
Exposure dose	4	0.315	1.659	0.2117
Error	15	0.190		
Time	4	0.582	11.054	0.0001
Exposure dose × time	16	0.128	2.086	0.0067
Error	60	0.053		
Host dietary intake				
Exposure dose	4	0.494	5.233	0.0036
Error	24	0.094		
Time	3	0.978	16.740	0.0001
Exposure dose × time	12	0.119	2.031	0.0333
Error	72	0.058		

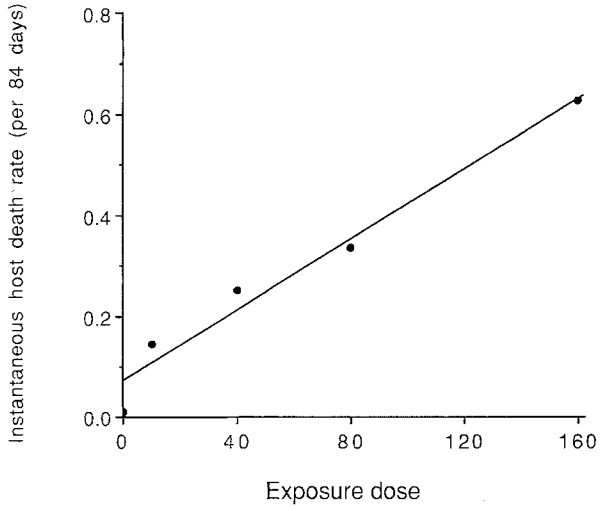

Fig. 4. The relationship between the estimated instantaneous rate of mortality of *B. bufo* infected with 4 doses of the infective larvae of *R. bufonis* and exposure dose. The *line* is the best-fit linear model and the *points* represent average host mortality within blocks of infected toads

mediate decline in host survival. Importantly, none of the 16 controls died during the experiment. After 6 WPI there was a decline in host survival, especially in the most heavily infected treatment. There was also a significant linear relationship between parasite exposure dose and the calculated estimate of the instantaneous host death rate (Fig. 4, $y = 0.0701 + 0.0035x$, $r^2 = 0.966$, $df = 4$, $t = 9.271$, $P = 0.0027$). The slope of the line relating host death rate to parasite burden has been used by Anderson (1978, 1980) to estimate the death rate of hosts induced by parasitic infection.

There was a preliminary indication that the size of toads at infection affected their survival. Of the first 7 toads that died up to 9 WPI, 6 were from the small size class; at 12 WPI an equal number of large and small toads died. Repeated-measures ANOVA of toad survival from 0 WPI to 9 WPI gave a marginally significant interaction between time, exposure dose and size at infection ($F_{12,90} = 1.904$, $P = 0.044$) indicating that smaller toads are more susceptible (at least initially) to mortality and that the effect of one of these factors may be dependent on the presence of the other two. More detailed consideration of host size in future experiments is required to examine further its precise influence on parasite-induced mortality.

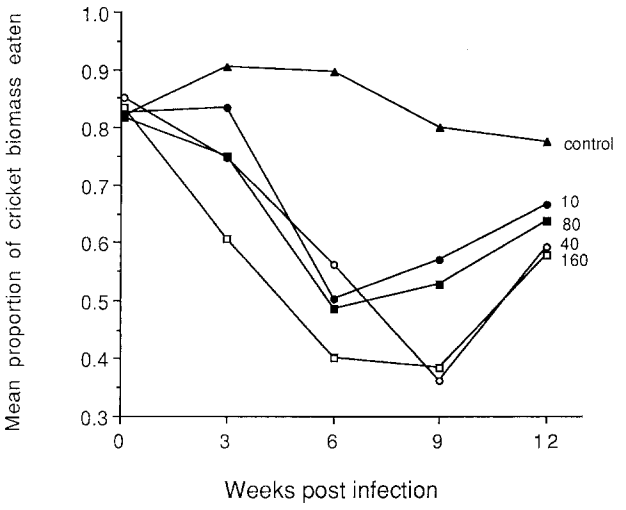

Fig. 5. The proportion of crickets eaten by *B. bufo* infected with 4 doses of infective larvae of *R. bufonis*. *Points* represent the average proportion of cricket biomass eaten per toad, estimated from the numbers of crickets remaining after each feeding trial. Sample sizes at each infection dose are listed in Fig. 2. For further details see text

Infected toads ate a progressively smaller proportion of the crickets offered to them over time (Fig. 5) but this was weakly associated with parasite exposure dose over the 12 weeks of the experiment (repeated-measures ANOVA with time: $F_{4,76}=10.43$, $P=0.0001$; and exposure dose: $F_{4,19}=2.41$, $P=0.0853$). However, at 12 WPI there was a convergence in the parasite burdens of the groups (Fig. 1). The results were therefore analysed for data collected up to 9 WPI (Table 1), when the groups still had markedly different burdens. These results provided stronger evidence that decreased dietary intake was dependent on time and exposure dose. The interaction of time and exposure dose was also significant (Table 1). This shows that a parasite-induced effect on the intake of crickets by toads is more pronounced during the initial stages of infection. Figure 5 also shows a marked divergence in dietary intake between control and infected toads as early as 3 WPI. At 12 WPI there is a tendency towards convergence in dietary intake (Fig. 4), which may indicate that the loss of parasites is associated with a compensatory increase in dietary intake.

Discussion

Under experimental conditions, *Rhabdias bufonis* significantly affects the growth and survival of juvenile *Bufo bufo*. Infected toads grow more slowly (Fig. 2) and have lower survival (Figs. 3, 4) than uninfected controls. These results provide support for the negative role of helminth parasites in natural host populations as envisioned by Crofton (1971) and Anderson (1978, 1982) and also support their contention that mortality is correlated with worm density. In addition, these results suggest a simple mechanism for the pathogenic effects of infection; infected toads eat up to 50% less biomass than controls during the initial stages of infection (Fig. 5).

The precise mechanisms linking parasite density with mortality are unknown in this and other systems (see review by Thompson 1990). Two likely possibilities are that mortality is related to nutritional demands imposed by parasites or to the release of toxic by-products, both of which may increase with density. In this system, an additional effect of infection may be associated with decreased lung function and interference by the parasites with oxygen utilization. However, the simplest mechanism relating reduced survival to increasing worm density may be the decrease in dietary intake associated with infection (Fig. 5). Crompton (1984) reviewed the interaction between host food intake and parasitic infection and indicated that the degree to which parasite-induced anorexia occurs is dependent on parasite numbers. Here, parasite-induced anorexia may result from interference with specific activity patterns (especially foraging behaviour) or may be associated with a more general lethargy imposed by the drain of nutrients. A further possibility is that anorexia is an adaptive response by the host to infection (Holmes and Zohar 1990). Conceivably, reductions in dietary intake coupled with inactivity may allow the host to wait out the pathological effects of infection, especially if the parasites are short-lived and if reduced activity is correlated with decreased chances of further infection. Alternatively, decreased activity may also be associated with increased risk of becoming heavily infected by larvae from the host's own faeces. In the present system, 3 of 8 toads infected with 160 larvae, outlived their period of infection (Fig. 3) and there is an indication that these were resuming normal growth (Fig. 2) and feeding patterns (Fig. 5). A longer-term study is required to clarify the precise mechanism of parasite-induced anorexia and to determine whether toads that survive heavy infections can then resume normal growth. It is noteworthy that reduced feeding can occur at low infection densities (Fig. 5). This suggests that reduced diet may be important under natural conditions, where 60–100% of toads are infected with 6–14 worms per host (range 1–183; summarized from three studies involving a total of 435 adult toads: Cox 1971; Kozak 1973; Frandsen 1974), especially when they are under conditions of resource limitation.

The significant interactive effects between parasite density and time on host growth and survival are not surprising; they indicate that the pathological effects of *R. bufonis* are not immediate but increase as the infection proceeds. The further importance of interactive effects is indicated by preliminary evidence suggesting that initial size at infection is also important in determining survival. The complex interaction between size, time and exposure dose suggests that smaller toads are at increased risk of dying from parasitic infection, especially earlier in the course of infection. This result may again be associated with host diet. Not surprisingly, toads which were small at the beginning of the experiment ate relatively less than large ones. However, Goater (in press) has shown that host size has no effect on the numbers of larvae which ultimately reach maturity in the lungs. Thus, small toads have the same number of worms as large ones, but eat less. This may provide a simple explanation for reduced

survival of small toads. This experiment was not specifically designed to examine the effects of size on subsequent mortality. However, small size at metamorphosis is known to be correlated with decreased survival and reproductive success (Berven and Gill 1983; Smith 1987) and our preliminary results suggest that an additional cost may be increased risk of death due to parasitism.

We interpret the pathological effects of *R. bufonis* in toads in the following manner. Juveniles, which are extremely susceptible to this nematode, are infected by about 70% of the larvae they are exposed to, regardless of larval dose (Goater, in press). Worms rapidly mature in the lungs with per worm and per host worm fecundity reaching peak levels very early in the infection (Goater, in press). If worm reproduction is correlated with either the drain of nutrients from hosts, the release of toxins or impaired oxygen consumption, then it is probable that the pathological effects of infection begin shortly after exposure. Thus, as early as 3 WPI, there is a significant dose-related decline in the biomass of food eaten, manifested in reduced host growth approximately 3 weeks later. After 6 WPI there is a decline in host survival, particularly among heavily infected hosts. We do not know the mechanism responsible for the relationship between parasite density and survival although it is probably associated with a density-related reduction in dietary intake.

Previous studies on helminth parasites have concentrated on those which are of veterinary or medical importance or on the characteristics of specific parasite-host combinations, such as seasonality and host specificity. The former have little relevance to natural parasite-host systems and the latter seldom address the issue of parasite-induced effects on host fitness. Although the results presented here cannot directly address the issue of the potential impact of *R. bufonis* on natural toad populations, the clear difference in survival between infected and uninfected hosts (Fig. 3) indicates that mortality of juveniles induced by *R. bufonis* can act in addition to other forms of mortality.

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