



Host condition as a constraint for parasite reproduction

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Environmental stress has been suggested to increase host susceptibility to infections and reduce host ability to resist parasite growth and reproduction, thus benefiting parasites. This prediction stems from expected costs of immune defence; hosts in poor condition should have less resources to be allocated to immune function. However, the alternative hypothesis for response to environmental stress is that hosts in poor condition provide less resources for parasites and/or suffer higher mortality, leading to reduced parasite growth, reproduction and survival. We contrasted these alternative hypotheses in a trematode–snail (*Diplostomum spathaceum*–*Lymnaea stagnalis*) system by asking: (1) how host condition affects parasite reproduction (amount and quality of produced transmission stages) and (2) how host condition affects the survival of infected host individuals. We experimentally manipulated host condition by starving the snails, and found that parasites produced fewer and poorer quality transmission stages in stressed hosts. Furthermore, starvation increased snail mortality. These findings indicate that in well-established trematode infections, reduced ability of immune allocation has no effect on host exploitation by parasites. Instead, deteriorating resources for the snail host can directly limit the amount of resources available for the parasite. This, together with increased host mortality, may have negative effects on parasite populations in the wild.

Parasites are often assumed to do better in individuals that are in poor condition, and it is widely accepted that deterioration of living conditions predisposes populations to disease outbreaks in the wild (reviewed by Wakelin 1989, Lloyd 1995). This presumably is a consequence of reduced host immune function as immune defence is energetically costly to maintain and use (Sheldon and Verhulst 1996, Moret and Schmid-Hempel 2000). In fact, in several parasite–host interactions, resource shortage has been shown to increase host susceptibility to infections and/or reduce host ability to resist parasite within-host growth and reproduction (Slater and Keymer 1986, Murray et al. 1998, Koski and Scott 2001, Krasnov et al. 2005). The alternative hypothesis for response to environmental stress, however, is that hosts in poor condition provide less resources for parasites, leading to reduced parasite growth and reproduction (Ebert et al. 2000, 2004, Bedhomme et al. 2004). Furthermore, the ability of hosts in poor condition to resist harmful effects of parasitism can be reduced, leading to increased host and thus also parasite mortality (Agnew and Koella 1999, Jokela et al. 1999, Brown et al. 2000, Bedhomme et al. 2004, Krist et al. 2004). Therefore, contrary to general expectations, environmental stress may have negative effects on parasite populations in such systems (Lafferty and Holt 2003, Pulkkinen and Ebert 2004), and thus further empirical studies addressing the consequences of environmental variation in parasite–host interactions are in high demand.

In addition to ecological implications that environmental stress may have in parasite–host interactions, it can also affect the evolution of parasite characteristics such as host exploitation strategy. According to the theory of virulence evolution, host exploitation rate is expected to evolve to the level where the net benefit for parasite fitness is greatest when considering also the costs due to parasite virulence for hosts (Anderson and May 1982, Bull 1994, Read 1994, Ewald 1995). Therefore, host exploitation should evolve to a lower level when prolonged host lifespan increases parasite fitness and to a higher level when rapid exploitation of host resources leads to higher lifetime reproductive success. Classical life-history theory suggests that host exploitation rate and parasite virulence should vary among parasite species according to differences in their reproductive value during the infections (Williams 1966, Stearns 1992), which is determined, for example, by parasite life cycle characteristics and transmission strategy (reviewed by Frank 1996). However, because different host phenotypes can pose different selective pressures on parasites, optimal host exploitation strategy may also vary within species on an ecological time scale depending on, for example, host physiology and ecological condition. Therefore, natural selection may, instead of favouring a single level of host exploitation rate, favour host exploitation strategy which is plastic in relation to host condition (Pfennig 2001, Thomas et al. 2002, Jokela et al. 2005).

In the present study, we experimentally investigated dependence of parasite (*Diplostomum spathaceum*; Trematoda) reproduction on host (*Lymnaea stagnalis* snail) ecological condition, and survival of infected hosts under environmental stress. Our aim was to evaluate the importance of host condition as a source of phenotypic variation in host exploitation by the parasite and in host mortality. We measured reproductive potential of the parasite (in terms of production of cercariae larvae from the snails), quality of produced transmission stages (in terms of lifespan of cercariae), and host mortality from snails manipulated for condition by maintaining them under two different food treatments: 'ad libitum food supply' and 'no food'. We used food limitation as an experimental stress factor as it is one of the most important stressors in natural snail populations because of high temporal and spatial variation in resource availability.

Methods

Study system

Lymnaea stagnalis is a holarctic freshwater snail inhabiting shallow littoral zones of stagnant and sluggish waters such as lakes, ponds and brackish waters with luxuriant vegetation. It is an important host species for several trematode parasites (Väyrynen et al. 2000). In early July 2004, we collected snails for this study from Lake Huuonjärvi in northern Finland (65°06'N, 26°08'E). We separated snails infected with *D. spathaceum* from other snails by observing the morphology and behaviour of released cercariae in vivo (Niewiadomska 1986).

The parasite *D. spathaceum* is a common trematode occurring in *L. stagnalis*. It has a three-host life cycle with a bird definitive host, and snail and fish intermediate hosts (Chappell et al. 1994). The parasite matures in the intestine of fish-eating birds, where it reproduces sexually. Eggs of the parasite are released in the water with the bird's faeces, where they hatch into free-swimming miracidia. Miracidia infect aquatic snails (mainly genus *Lymnaea*), where they penetrate into snail gonads and develop into sporocysts. Sporocysts multiply asexually and take over the gonad tissue leading to sterilization of the hosts. Thus, infected snails can be considered 'dead' in an evolutionary sense. The development of patent infection takes 4–10 weeks depending on the water temperature (Chappell et al. 1994). Cercariae larvae are formed in sporocysts through asexual reproduction, and an individual snail can produce thousands of cercariae per day for several weeks (Karvonen et al. 2004). This extensive host exploitation leads to increased mortality among infected snails (Karvonen et al. 2004). Cercariae of the parasite infect fish by penetrating the gills and skin after which they migrate to the eye lenses where they develop into metacercariae. Infectivity of cercariae produced by different snail individuals is highly variable (Seppälä et al. 2007), which suggests that parasite and/or snail characteristics affect quality of produced cercariae. For successful completion of the life cycle, infected fish has to be eaten by a piscivorous bird.

Experimental design

Before the experiment, we maintained infected snails individually in plastic containers each containing 0.7 l of lake water under natural light-dark rhythm (16L:8D) at 20°C. We fed the snails with fresh lettuce ad libitum for one week before the experiment to maximise their body condition. During that time, we transferred the snails daily into new containers with fresh water and lettuce.

We then selected 40 snails (mean \pm 1 SE shell length 44 ± 0.5 mm) that fed well on lettuce for the study. The purpose of this was to reduce the within-group variance in ecological condition of experimental snails. Developmental stage of parasite infection (i.e. time being infected) could not be controlled as we used snails infected in the field. We maintained the experimental snails as described above. During the first week of the experiment, we fed all snails daily with 3 g of lettuce, which corresponded to ad libitum feeding. Thus, snails selected for the experiment were maintained on an ad libitum food supply for a total of two weeks after collection from the field. Every day, immediately after the snails had been transferred into new containers, we determined their cercarial production from the previous 24 h by counting the number of cercariae from five 1-ml samples taken from each container. We determined cercarial densities in the containers in random order each time. Furthermore, we measured the daily amount of lettuce consumed by each snail by weighing the amount of remaining lettuce. After the first week of the experiment, 33 snails were alive and we randomly assigned them into two food treatments: 'ad libitum food supply' (16 snails) and 'no food' (17 snails). We then continued the experiment for two weeks, and maintained the snails and measured their cercarial production as described above except when cercariae were collected for the lifespan measurements (below). We followed mortality of the snails throughout the study.

We estimated quality of produced cercariae by measuring their lifespan. This gave a sufficient estimate of parasite quality because infectivity of *D. spathaceum* cercariae, which is also tightly connected to parasite fitness, is known to correspond to their lifespan (Karvonen et al. 2003). We measured lifespan of cercariae three times during the experiment: after the first week (all snails fed ad libitum), after the second week (after one week of manipulation) and at the end of the experiment. To collect fresh cercariae for the measurements, we placed the snails individually into glass jars containing 0.2 l of water for one h. We placed 20 cercariae from each jar on 96-well plates, one cercariae in each well containing 200 μ l of water. After this, we transferred the snails back into their containers into 0.5 l of water and added the water from the glass jars to the containers to ensure that the estimates of cercarial production from that day would not be distorted. We then followed survival of cercariae in 4-hour intervals until the death of the cercariae. We used age at last observation when the cercariae were alive as a measure of their lifespan. We observed cercariae from different snails in random order each time.

Statistical analyses

We analysed the effect of food supply (treatments: ad libitum, no food) on cercarial production using repeated measures ANOVA with daily cercarial production from each snail as a unit of observation. We tested the first week of the experiment separately to ensure that snails in different treatments did not differ before manipulation started. We used repeated measures ANOVA also to analyse the effect of manipulation on lifespan of the cercariae (measurements done after weeks two and three) with the average lifespan of cercariae produced from each snail as a unit of observation. We analysed the first measurement of cercarial lifespan (before manipulation started) using independent-samples t-test. We performed the analyses using the snails that survived until the end of each period. We analysed the effect of feeding on snail survival (during weeks two and three) using Cox regression with the average amount of lettuce consumed per day as a covariate.

Results

During the first week of the experiment, when all snails were fed ad libitum, the number of cercariae released from snails or lifespan of cercariae did not differ between the treatment groups (number of cercariae: repeated measures ANOVA, $F_{1,30} = 0.288$, $p = 0.595$, Fig. 1; lifespan of cercariae: independent samples t-test, $t_{30} = -1.382$, $p = 0.177$, Fig. 2). After the manipulation started (weeks two and three), the numbers of cercariae released from food limited snails decreased (repeated measures ANOVA: $F_{1,16} = 6.046$, $p = 0.026$, Fig. 1). Also, the lifespan of cercariae was affected by the food treatment (Fig. 2). Lifespan of cercariae released from starved snails was shorter compared to those released from individuals fed ad libitum (repeated measures ANOVA: $F_{1,15} = 8.579$, $p = 0.010$, Fig. 2) as the lifespan of cercariae from snails fed ad libitum increased during the second week of the experiment but remained constant in starved individuals (Fig. 2). During the food manipulation (weeks two and three), mortality of

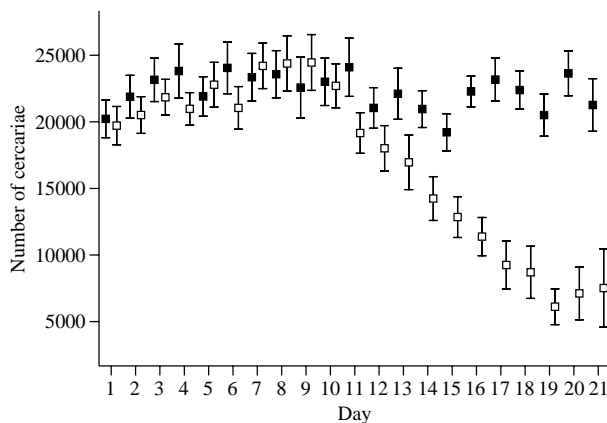


Fig. 1. Daily production of *D. spathaceum* cercariae (mean \pm 1 SE) by snails in 'ad libitum food supply' (■) and 'no food' (□) treatments. Food manipulation started after day seven of the experiment. Before that, all snails were fed ad libitum. Data from all snails that were alive each day is presented.

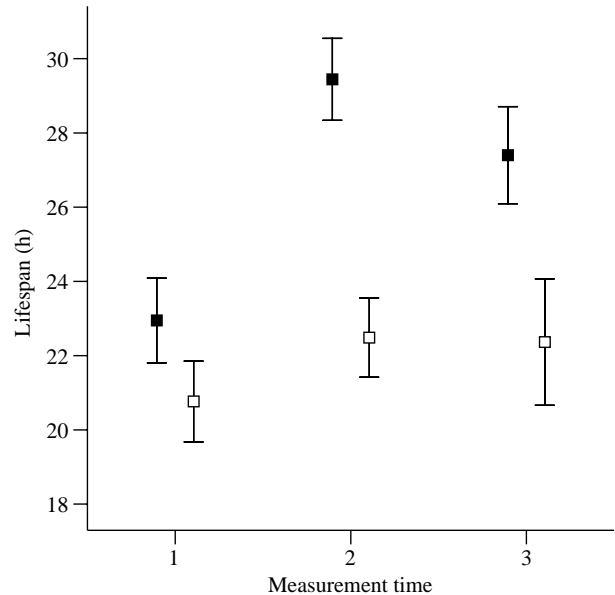


Fig. 2. Lifespan of *D. spathaceum* cercariae (mean \pm 1 SE) produced by snails in 'ad libitum food supply' (■) and 'no food' (□) treatments. First measurement was conducted after first week of the experiment, when all snails were fed ad libitum. Second and third measurements were conducted after weeks two and three, when food manipulation was in progress. Data from all snails that were alive during each measurement time is presented.

starved snails was higher than that of snails fed ad libitum (Cox regression: Wald = 3.945, DF = 1, $p = 0.047$, Fig. 3).

Discussion

Parasites are generally assumed to do better in hosts in poor condition, presumably as a consequence of reduced host

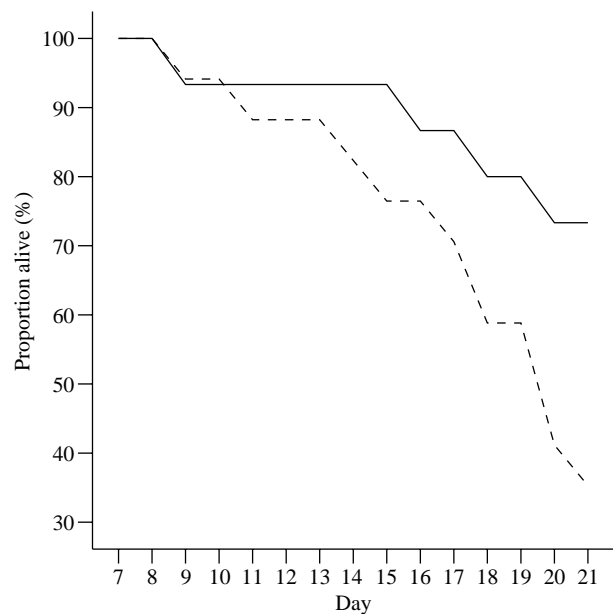


Fig. 3. Survival of snails in 'ad libitum food supply' (solid line) and 'no food' (dashed line) treatments after food manipulation had started (weeks two and three of the experiment).

immune allocation, or due to interaction between physiology of host stress responses and the immune system. Stress, in the broad sense, can increase host susceptibility to infections and/or reduce host ability to resist parasite within-host growth and reproduction (Slater and Keymer 1986, Murray et al. 1998, Agnew and Koella 1999, Koski and Scott 2001, Krasnov et al. 2005). Our results somewhat contradict with this view as starvation of *D. spathaceum*-infected snails reduced both production and quality (lifespan) of parasite cercariae larvae as compared to those in well-fed snails. Cercarial production decreased gradually from the beginning of the food limitation while it remained constant in snails fed ad libitum. Lifespan of the cercariae released from the starved snails, however, remained constant but increased in individuals with ad libitum food supply. The latter result may be, for example, due to increase in the quality of cercariae when infection proceeded, or shortness of the acclimation period so that snails had not yet reached maximal body condition before the manipulation started. For example, the snails may have been predisposed to food stress before the study in the field, or the parasites may have infected mainly individuals with poor genetic or ecological background. In other words, host quality may have inhibited the lifespan of cercariae before the study, and the ad libitum conditions of the experiment lead to increased lifespan of the cercaria in the food treatment. Our findings suggest that variation in the amount of external resources may not affect hosts' ability to resist exploitation by a parasite which has successfully infected the host, but instead it can directly reduce the amount of resources available for the parasite reproduction. In the terminology of life-history theory, such parasites can be called 'income breeders' (Calow 1983).

Our study is one of the first to demonstrate decreased parasite reproduction in hosts in poor condition as similar results have been reported only in a limited number of study systems (Ebert et al. 2000, 2004, Bedhomme et al. 2004). Interestingly, also those earlier studies have been conducted in invertebrate-parasite systems, whereas increased parasite reproduction in poor conditioned hosts is typically described from vertebrate hosts (Koski and Scott 2001, Krasnov et al. 2005). This suggests that parasites of vertebrates and invertebrates may differ in their general response to host condition, which could be explained by the nature of these interactions. In invertebrates, parasites generally consume a higher proportion of host resources than in vertebrates, in other words, size difference between the host and the parasite is smaller (Pulkkinen and Ebert 2004). Following this, parasites of invertebrates may be more strongly dependent on their hosts and environmental stress can have a strong negative effect on their within-host growth and reproduction.

Furthermore, environmental stress can cause high host mortality (Agnew and Koella 1999, Jokela et al. 1999, Brown et al. 2000, Bedhomme et al. 2004, Krist et al. 2004), and also in the present study, host mortality was higher among the starved snails. It is important to note, however, that further examination of the effect of host condition on survival of uninfected snails is needed to separate the role of starvation, parasitism and their interaction in snail mortality. Both decreased parasite reproduction and increased host mortality under stress have consequences that can escalate

even to parasite population dynamics, as the size of a parasite population is determined by a balance between parasite within-host growth and reproduction, host and parasite mortality, and parasite transmission rate (Anderson 1978, Anderson and May 1978). If environmental stress causes reduced parasite reproduction and increased mortality rather than increased host susceptibility to infection, the net effect of stress may reduce parasite population size (Lafferty and Holt 2003, Pulkkinen and Ebert 2004). In our study system, however, effect of stress on snail susceptibility to parasite infection is not known, and results from other snail-trematode interactions are conflicting (Abrous et al. 2001, Krist et al. 2004). Thus, some uncertainty still remains whether the overall effect of reduced snail nutritional state has a positive or negative effect on parasite fitness. Furthermore, in parasites with complex life cycles, the effect of environmental stress on parasite population dynamics may depend on its joint effects on all stages of the life cycle.

The observed host condition dependence of parasite reproduction can either be completely due to environmental constraint (reduced resource availability) or a combination of resource availability and plastic host exploitation strategy by the parasite. In general, plastic host exploitation could enhance host and thus parasite survival by reducing parasite within-host growth and reproduction under stressful environmental conditions (Pfennig 2001, Jokela et al. 2005). Separation between these two hypotheses depends on whether reduction in host exploitation is adaptive for the parasite. In other words, does it promote parasite future reproduction? In the present study, parasite reproduction decreased gradually during the two weeks period of starvation. Furthermore, as starvation also led to increased mortality of the hosts, our results do not appear to support the view that reduction in parasite reproduction would have been an adaptive strategy. It is likely that a more effective way for the parasite to enhance host survival under stress would have been to reduce reproduction more rapidly. In several free living animals, for example, reproduction has been shown to cease immediately after the beginning of food depletion (Kaitala 1991, Ernande et al. 2004). Therefore, we conclude that the observed reduction in parasite reproduction was more likely to be due to reduced energy budget of the snail hosts and reflects the 'income breeding' strategy of the parasite (Calow 1983). The increased mortality of the starved snails also suggests that parasites may exploit their hosts to a degree that they risk losing them. In fact, it is possible that parasites increase their relative host exploitation rate under food shortage even if their absolute reproductive output is constrained. This is because increased host exploitation may lead to highest parasite fitness in poor conditioned hosts as it enables effective exploitation of host resources before death (terminal investment, Williams 1966, Clutton-Brock 1984).

To conclude, we found that reproduction by the parasite *Diplostomum spathaceum* was dependent on host condition as the number and quality of produced cercariae was lower in food limited snails. Furthermore, starvation led to increased host mortality. These results suggest that environmental stress may have important ecological and evolutionary implications for parasite-host interactions. First, stress has the potential to affect parasite population dynamics by reducing parasite population size (see also

Lafferty and Holt 2003, Pulkkinen and Ebert 2004). Second, stress may exert selective pressure on parasites favouring plastic host exploitation strategy if optimal host exploitation rate varies according to host condition (see also Pfennig 2001, Jokela et al. 2005).

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