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Virulence of parasites in hosts under environmental stress: experiments with anoxia and starvation

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Most environments periodically impose severe stress that may cause high mortality and alter population structure, for example, by removing sick and old individuals. We examined how anoxic conditions and starvation of the host affect virulence of two closely related trematode parasites, *Rhipidocotyle campanula* and *R. fennica*. These parasites differ by prevalence of infection and by exploitation rate of individual hosts (freshwater clam, *Anodonta piscinalis*). Infection by *R. campanula* is rare ($<5\%$ prevalence of infection) and destroys on average 90% of the gonad tissue of the individual host. Infection by *R. fennica* is more common (20–60% prevalence of infection) and leads to on average 30% gonad destruction. In the end, both infections lead to host infertility. We predicted that *R. campanula* induces higher host mortality than *R. fennica* under host stress. In two laboratory experiments, we exposed naturally-infected and uninfected clams to anoxia and to starvation. Anoxia occasionally takes place during winter in eutrophic lakes, while some degree of starvation should occur seasonally. We found that mortality rate of clams was much higher under anoxia than under starvation, and that infection increased mortality rate under both types of host stress. As predicted, *R. campanula* induced higher host mortality than *R. fennica*. Host survival was population-specific, suggesting that clams of different origins carried different amount of energy reserves. Severe environmental perturbation may remove *R. campanula* infected individuals from the host population, but recolonization from the fish host is likely to prevent extinction of the parasite suprapopulation. The observed high host mortality induced by *R. campanula* may be one ecological explanation for the consistently lower prevalence of infection of *R. campanula* when compared to *R. fennica*.

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Most natural populations are subject to stochastic or periodic environmental fluctuations. Individuals weakened by age, disease or parasites are expected to be the first ones to die when population goes through a severe perturbation (Ewald 1995, McCallum and Dobson 1995, Price 1980), and parasites and environment may work together to regulate host populations (Dobson and Hudson 1986, Coltman et al. 1999). In worsened environmental conditions disease and parasites may be the proximate cause of death, while the ultimate cause is the reduced resource that is available for the host population (Kohler and Wiley 1992, Coltman et al. 1999). Here, we focus on virulence of parasites in host populations that experience a period of severe stress. We

compared two species of parasites that differ by their host exploitation rate to examine parasite-induced mortality in the host populations.

Parasites may increase their reproductive rate (fitness) by increasing the rate of host exploitation. Because an increase in the host exploitation rate may lead to increased virulence (reduction in host fitness, for example, higher parasite-induced host mortality), the gains achieved by higher exploitation rate have to be counted against the costs of a potentially shorter reproductive lifespan in the host. Low to intermediate parasite virulence may be favored when prolonged infection yields higher fitness than rapid exploitation of host resources (Bull 1994, Read 1994, Ewald 1995). Thus, optimal virulence may be context-dependent; it may vary with respect to the 'value' of each individual host, which largely depends on life-cycle characteristics, transmission probability, and host exploitation strategy of each particular parasite species (Frank 1996).

Ecological and environmental factors that are manifested through host condition may also reflect on parasite virulence (Fig. 1). For example, variation in external resources may affect host condition, leading to corresponding variation in the availability of resources for the parasite. If a prolonged infection is beneficial for the parasite, the exploitation strategy of the parasite should be flexible, so that the parasite adjusts its host exploitation rate to the availability of host resources (Fig. 1). In such a case parasite-induced host mortality

should remain low, and not respond to variation in host condition. If a prolonged infection is less beneficial than short-term production of the transmission stages, host exploitation strategy may be inflexible with respect to host condition; host is exploited at a constant rate, independent of the condition of the host (Fig. 1). In this case parasite-induced host mortality should increase when host condition decreases.

Host exploitation strategy may also have important implications for the population biology of the parasite. Parasites with inflexible host-exploitation strategies may be more vulnerable to population extinction when host population experiences severe external stress and high mortality. If infected hosts are more sensitive to environmental stress, severe perturbation of the host population may effectively "purge" the host population from its parasites, and lead to extinction of the parasite population. In such cases selection is expected to favor parasite genotypes that express lower virulence, or have a more flexible host exploitation strategy (Fig. 1). Variation in the magnitude and frequency of external stress factors that the host population is likely to experience may thus be an important selective factor in the evolution of host exploitation strategies.

Few studies have estimated parasite virulence in natural environments (Herre 1993, Jaenike et al. 1995, Polak 1996, Webberley and Hurst 2002), or attempted to measure variation in parasite virulence by experimentally manipulating host condition (Lafferty

Fig. 1. A simple graphical model to illustrate how parasite virulence may respond to host stress or decreasing condition. In both panels the height of the bars indicate the amount of energy available in the host. Uninfected hosts use energy to growth/reproduction (G), and maintenance (M). Parasite uses a proportion (P) of host energy for its own growth.
(A) illustrates how realized virulence may increase as a function of host stress (indicated as decreasing amount of host energy). In this case the parasite uses a fixed amount of host energy, independent of host condition, and causes a reduction in host 'growth' that is proportionally larger that the

decrease in availability of host energy, i.e. virulence of the parasite increases as host stress increases. Obviously, this may lead to increased host mortality when parasite exploitation (P) interferes with maintenance (M). Inflexible host exploitation strategy may be beneficial when host survival is of relatively low 'value' for the parasite (e.g. transmission to the next host is easy, intrahost competition for resources likely, or expected life-span of the host is short).

In an alternative host exploitation strategy (B), the parasite adjusts its host exploitation with respect to energy available in the host. When host condition decreases, parasite uses less energy, so that virulence remains more or less constant. In this example the parasite uses a fixed proportion of host energy that was originally targeted for growth/reproduction (G), but does not interfere with the energy targeted for maintenance (M). This strategy does not jeopardize host survival. Flexible host exploitation strategies (B) are more likely to be found when each individual host has a relatively high value for the parasite (e.g. transmission to the next host is difficult, intrahost competition for resources is low, or the expected life-span of the host is long).
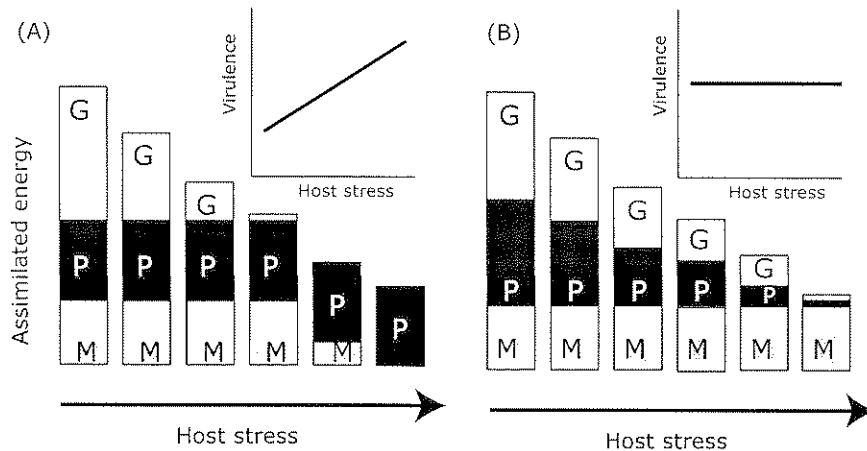
1993, Møller 1993, Oppliger et al. 1996, Brown et al. 2000). We examined how variation in host condition affects virulence of two parasite species in an aquatic mollusc−trematode system. In this system it is possible to experimentally manipulate host condition and measure the response in parasite virulence. Previous studies that have explored the survival of mollusc hosts with respect to trematode infection have given equivocal results. Trematode infections have been observed to decrease the physiological performance of the host, especially tolerance of anoxia (Becker 1980, Sousa and Gleason 1989, Jensen et al. 1996), or to increase mortality (Lafferty 1993, Jokela et al. 1999). In other studies, infection has not been found to increase mortality of the host (Goater et al. 1989, Schrag and Rollinson 1994).

We studied trematode-induced host mortality in a long-lived (>10 years) freshwater clam, *Anodonta piscinalis*, by conducting stress experiments in the laboratory. We tested virulence of two closely related parasite species that have a similar life-cycle (mollusc−fish−predatory fish, Taskinen 1992), and that occur sympatrically in the same host populations. These two species differ by three important aspects. First, at individual level, *Rhipidocotyle campanula* is known to have higher host exploitation rate than *R. fennica*, measured as the degree of gonad destruction of the infected host (90% vs 30%, respectively, Taskinen et al. 1994). Second, at the population level, the prevalence of infection by *R. campanula* seems to be consistently lower than the prevalence of infection by *R. fennica* in natural *Anodonta* populations (less than 5% vs 20−60%, respectively, Taskinen et al. 1991). Third, the first and second intermediate host species are the same, but the definitive host species of *R. campanula* is perch (*Perca fluviatilis*) while the definitive host for *R. fennica* is pike (*Esox lucius*; Taskinen et al. 1991). In other words, we contrasted virulence of two closely related parasite species that have a very similar life-cycle and use the same long-lived intermediate host species, but that differ by the exploitation rate of the first intermediate host.

We expected *R. fennica*, which has a lower average host exploitation rate, also to have a lower virulence than *R. campanula*. To estimate context-dependent virulence, we exposed uninfected and infected clams to two different types of severe physiological stress, anoxia and starvation. Infection by *R. fennica* is known to deplete the glycogen reserves of their hosts to a much higher degree than the fat reserves of the host (Jokela et al. 1993). Glycogen is used as a primary source of energy under anoxia, while fat is a more important source of energy under starvation stress. The goal of these stress experiments was to study how host condition affects parasite virulence, and simulate the effects of severe environmental perturbation on parasite population.

Short-term anoxia may take place in eutrophic lakes and ponds during harsh winters when period of ice cover is extended preventing oxygen exchange with atmosphere. Also, the resources for filter-feeding organisms fluctuate seasonally, leading to some degree of starvation during winter. Therefore, in these experiments we manipulated the two most important stress factors facing natural populations of clams. We found that the parasite species with high host exploitation rate induced higher host mortality under both stress regimes. In both stress treatments all hosts carrying the more virulent parasite died before 50% of the uninfected hosts had died, which suggests that the parasite population in the intermediate host may go extinct in periods of high host mortality.

Methods

Study system

The host, *Anodonta piscinalis* is a common long-lived (up to 15 years) filter-feeding freshwater bivalve, inhabiting slowly running waters and littoral zones of temperate lakes in Europe (Bauer et al. 1991). *A. piscinalis* reaches maturity between 2−5 years of age and reproduces once a year thereafter (Haukioja and Hakala 1978). *A. piscinalis* is an intermediate host of two bucephalid trematode parasites, *Rhipidocotyle fennica* and *R. campanula*, which infect mainly the gonads of the host, resulting in host infertility (Taskinen and Valtonen 1995). Immature clams are not infected, but after maturity the prevalence of infection increase with host age, suggesting that a single infection lasts several years (Taskinen and Valtonen 1995, Taskinen et al. 1997). Multiple infections by both *R. fennica* and *R. campanula* are possible, but previous results suggest that they may not be frequent. Taskinen et al. (1991) observed only one concurrent infection among 384 *A. piscinalis* specimens infected by *R. fennica* and 12 individuals infected by *R. campanula* in one of the present study populations, Lake Saravesi. Clams used in this study did not carry multiple infections.

Anoxic stress

In December 1992 we collected several hundred clams from four populations in central Finland: (1) Siikakoski, (2) Pesiäissalmi, (3) Laukkavirta and (4) Saravesi. Detailed descriptions of the study sites are given in Taskinen et al. (1994) and Jokela and Mutikainen (1995). We randomly chose 100 reproductively mature individuals (>50 mm long) from each population knowing that some of them would be infected with trematodes. Average age of the clams in the four

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populations was 9.4, 7.7, 6.7 and 6.3 years, respectively. Age was determined from the growth rings on the shell (Haukioja and Hakala 1978).

Clams were marked by etching a population-specific code on the shell. We randomly assigned 10 clams from each population to each of ten 20-liter plastic container filled with aged tap water (total of 10 containers with 40 clams in each). Containers were stored at $+5°C$, which was only slightly warmer than the lake water ($+4°C$). After 5 days, the aeration of the containers was turned off and water temperature was allowed to raise to $+20 \pm 1°C$, rapidly leading to anoxia. No dissolved oxygen was found in any of the containers after 8 days when the mean ($\pm se$) amount of ammonium (NH_4-N) was 1.15 ± 0.24 mg l^{-1}, and mean pH 7.6 ± 0.02. We inspected each clam every morning starting at 8:00 am We removed dead clams (no valve-closing when disturbed), and immediately recorded their age, sex, and the state of infection. Sex and presence of trematodes were examined by pressing the clam tissues between two glass plates under a dissecting microscope. The experiment was terminated after 26 days when the last clam died. As we knew that in similar laboratory conditions, but in oxygenated water, both infected and uninfected clams may be maintained several months with practically no mortality (Jokela and Palokangas 1993, J. Taskinen, unpubl.), we did not include specific controls for survival in the oxygenated conditions. Our main interest was the relative survival of infected and uninfected individuals under anoxic conditions, which we knew to induce high mortality compared to the standard laboratory conditions.

Starvation experiment

In March 2001, we collected 176 and 153 mature clams from the Siikakoski and Pesiäissalmi populations, respectively. Clams were transported to the laboratory in large buckets and marked as described above.

In the laboratory, we placed groups of 27 or 28 randomly selected clams in 12 plastic baskets immersed in three oxygenated tanks initiated with aged tap water. Each basket held an equal proportion of clams from each of the two populations (13/14, or 14/14 individuals). Water in the tanks was continuously filtered using several high-volume aquaria filters filled with bioactive material, gravel, and bags of active carbon. We provided no food for the clams, and arranged the filtering to be as efficient as possible to ensure that food particles were not available in the tanks. We also held the clams at high density at relatively high temperature ($20°-25°C$) to enhance metabolic activity and competition for resources. The baskets were rotated daily when we checked the clams for mortality. Once a week the water was replaced with aged tap water, the surviving clams were

re-distributed, and returned to initial density using fewer baskets if necessary. Clams were re-distributed by emptying all baskets to one container from which clams were re-assigned randomly to baskets. All equipment were washed once a week to remove bacterial and algal cultures growing on plastic surfaces. Dead clams were immediately dissected as above.

The experiment was terminated on day 150. At that point, 61 clams (18.5%) were still alive.

Data analyses

We analyzed the differences in survival among uninfected, *R. fennica* and *R. campanula* infected hosts of different populations using Cox regression ('proportional hazards' regression, Norusis 1990). Cox regression is widely used for survival analysis in clinical trials, and allows the survival effects of several factors and their interactions to be studied simultaneously (Andersen 1991). We included population and infection status of the host (healthy *R. fennica* and *R. campanula*) as independent categorical covariates in the model. As the probability of infection increases with host age (Taskinen and Valtonen 1995), we included age as a continuous covariate. Container was treated as a block effect in the anoxia experiment.

Results

Survival under anoxia

As we expected based on earlier studies, prevalence of infection by *R. fennica* was higher than that of *R. campanula* in all populations (Table 1). The survival analysis indicated that *R. campanula* was clearly more virulent than *R. fennica* (Fig. 2, Table 2), and that the uninfected clams had the highest survival under anoxic conditions. The low number of *R. campanula* infected clams did not allow estimation of host survival by parasite species in each population of clams, but the sample size was large enough to estimate the overall difference in parasite-induced host mortality between the parasite species. The last clam infected by *R. campanula* died on day 9, when 56% of the uninfected clams were still alive. Therefore, only a short period of anoxia may lead to complete extinction of *R. campanula* in natural populations of clams. The last clam infected with *R. fennica* died on day 15, when only 4% of the uninfected clams were still alive. Hence, it is unlikely that a short period of anoxic conditions will eradicate *R. fennica* from the host population. Significant interaction between age and population (Table 2) indicates that the age-specific survival differed by population (data not shown). Overall, the survival rate decreased with age. Note that including age as a covariate in the analysis

Table 1. Median (min. −max.) age, prevalence of infection (%), and 95% confidence interval for the prevalence estimates by parasite species of *Anodonta piscinalis* clams in the anoxia experiment. Note that the confidence intervals for the prevalence esimates of the parasite species do not overlap.

Population	n	age	R. fennica	95% CI	R. campanula	95% CI
Siikakoski	100	9 (4−15)	31.0	22.1−41.0	3.0	0.6−8.5
Pesiäissalmi	100	8 (4−12)	26.0	17.7−35.7	2.0	0.2−7.0
Laukkavirta	100	7 (3−13)	58.0	47.7−67.8	1.0	0.03−5.5
Saravesi	100	6 (4−16)	70.0	60.0−78.8	2.0	0.2−7.0
Total	400	7 (3−16)	46.3	41.2−51.2	2.0	0.9−3.9

effectively corrects for possible effects of age when clams of different infection status are compared.

Survival under starvation

As in the anoxia experiment, prevalence of infection by *R. fennica* was much higher than the prevalence by *R. campanula* in the material collected for the experiment (Table 3). It is noteworthy that although the clams for the starvation experiment were collected nine years later than for the anoxia experiment, the prevalence of infection by both parasite species in Siikakoski and Pesiäissalmi was very similar to year 1992 (Table 1, 3). While the clams were more resistant to starvation stress than to anoxia, the relative differences in survival between hosts infected by the two trematodes species were qualitatively similar to those found in the experiment under anoxic conditions (Fig. 2). *R. campanula* infected hosts died earlier than hosts infected by *R. fennica*, and uninfected hosts had the highest survival (Table 4, Fig. 2). The last clam infected with
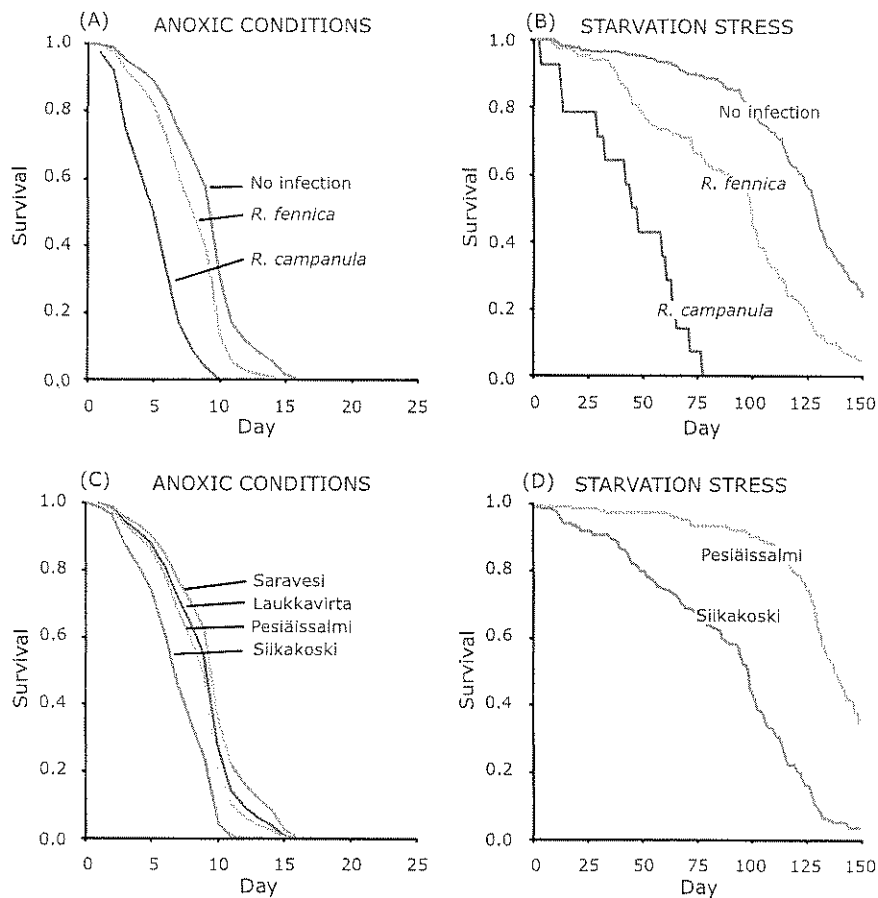

Fig. 2. Cumulative survival of infected and uninfected *Anodonta piscinalis* under anoxia and under starvation stress in laboratory. Top panels show survival by parasite species (clams of different populations pooled). Lower panels show survival by population (clams of different infection status pooled). Examination of the infection status was not possible by population due to low prevalence of infection by *R. campanula*.

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Table 2. Survival analysis of the infected and uninfected *Anodonta piscinalis* clams of the four study populations (POP) in anoxic conditions. Clams were exposed to anoxia in ten containers (a categorical block-effect, Block). Infection status (INF) contrasts survival of healthy clams to that of clams infected with either *R. campanula* or *R. fennica*. Age of the clam was included as a continuous covariate. The best model was searched using forward stepwise method utilizing likelihood ratio significance tests for evaluation of each effect. Procedure is available in COXREG procedure of SPSS statistical package. Model fitting was initiated with a model that included all main effects and interactions POP × AGE and INF × AGE. Odds ratio indicates the ratio of survival probabilities of individuals of specific classes, e.g. value of 6.28 for "*R. campanula*" indicates that mortality of *R. campanula* infected clams was more than six-fold when compared to that of the uninfected clams. Cox regression is specifically designed for survival analysis (Andersen 1991).

The best model Effect	β (se)	Wald	df	p	Odds ratio
Infection (INF)		38.77	2	<0.001	
R. fennica	0.502 (0.108)	21.40	1	<0.001	1.65
R. campanula	1.838 (0.373)	24.23	1	<0.001	6.28
Population (POP)		44.88	3	<0.001	
AGE	0.081 (0.027)	9.13	1	0.003	1.09
POP × AGE		8.50	1	0.004	

Variables not in the best model		Score	df	p	
POP		1.02	3	0.797	
BLOCK		7.22	9	0.614	
INF × AGE		3.57	2	0.168	

R. campanula died on day 77, when 89% of the uninfected clams were still alive. Hence, *R. campanula* infected clams seem to tolerate starvation stress poorly. On day 150, when the experiment was terminated, only 4.8% of *R. fennica* infected clams were alive, whereas 24% of uninfected clams were still alive. As under anoxic conditions, tolerance of starvation stress decreased with age, and populations of origin differed by their average mortality rate (Table 4, Fig. 2). Also, the degree of damage (exploitation rate) was higher in clams infected by *R. campanula* than in clams infected by *R. fennica*. At the point of dissection all *R. campanula* infections occupied 100% of the gonad and carried transmission ready cercaria, while for *R. fennica* the corresponding figure was 45% at Siikakoski population and 56% at Pesiäissalmi population. Exploitation rate did not differ by population (*R. campanula*, Mann–Whitney U-test, P = 1.000; *R. fennica*, M–W U-test, P = 0.236), but the exploitation rate of *R. campanula* was higher than that of *R. fennica* (M–W U-test, P = 0.001).

Discussion

We found that two closely related parasite species differed fundamentally in their virulence independent of the type of host stress. *R. fennica* that exploits host resources to a lower degree than *R. campanula* expressed lower virulence both under anoxia and under starvation stress when compared to *R. campanula*. This result suggests that the degree of parasite-induced host mortality may be directly related to parasite-inflicted damage on host tissues. In this sense, the parasites seem to have different host exploitation strategies. In a variable environment, where some degree of host stress occasionally takes place, these strategies are likely to lead to different average residence time in the host. Probability of survival for *R. campanula* infected hosts is likely to be much lower than for *R. fennica* infected hosts.

Based on these results, it appears that the population biology of these parasites species may be affected by periods of severe host stress. It is even possible that *R. campanula* population in clams is completely eradicated when the host experiences environmental perturbation, while *R. fennica* population is only affected by the very worst conditions, which also threaten the existence of the host population. Therefore, given the low prevalence of infection, it seems that the virulence strategy of *R. campanula* involves a higher risk of extinction than that of *R. fennica*. Before committing on this view, one should note that both species harbor high prevalence of infection in the second intermediate host, the roach (*Rutilus rutilus*; Taskinen et al. 1991, Gibson et al. 1992, Taskinen 1998), where infections may

Table 3. Median (min. −max.) age, prevalence of infection (%), and 95% confidence interval for the prevalence estimate by parasite species of *Anodonta piscinalis* clams in the starvation stress experiment. Note that the confidence intervals for the prevalence esimates of the parasite species do not overlap.

Population	n	Age	*R. fennica*	95% CI	*R. campanula*	95% CI
Siikakoski	176	11 (4–16)	29.0	22.4–36.3	5.7	2.8–10.2
Pesiäissalmi	153	7 (5–16)	21.6	15.3–28.9	2.0	0.4–5.6
Total	329	10 (4–16)	25.5	20.9–30.6	4.0	2.1–6.7

Table 4. Survival analysis of the infected and uninfected *Anodonta piscinalis* clams of the two study populations (POP) under starvation. Infection status (INF) contrasts survival of healthy clams to that of clams infected with either *R. campanula* or *R. fennica*. Age of the clam was included as a continuous covariate. The best model was searched using forward stepwise method utilizing likelihood ratio significance tests for evaluation of each effect. Procedure is available in COXREG procedure of SPSS statistical package. Model fitting was initiated with a model that included all main effects and interactions POP ×AGE and INF ×AGE. Odds ratio indicates the ratio of survival probabilities of individuals of specific classes, e.g. value of 13.68 for "*R. campanula*" indicates that mortality of *R. campanula* infected clams was more than 13-fold when compared to that of the uninfected clams. Cox regression is specifically designed for survival analysis (Andersen 1991).

The best model Effect	β (se)	Wald	df	p	Odds ratio
Infection (INF)		95.95	2	<0.001	
R. fennica	1.040 (0.142)	53.58	1	<0.001	2.83
R. campanula	2.616 (0.328)	63.69	1	<0.001	13.68
Population (POP)		83.06	1	<0.001	
AGE	0.146 (0.025)	34.36	1	<0.001	1.157
Variables not in the best model		Score	df	p	
POP × AGE		0.07	1	0.782	
INF × AGE		1.891	2	0.389	

lay resident over the period of perturbation in the clam population (Taskinen et al. 1991). Hence, while the parasite population may go extinct from the clam host, the suprapopulation of the parasite (consisting of parasite individuals resident in all hosts of the life cycle at any given time) may not be severely affected. *Rhipidocotyle* spp. metacercariae may live in roach (*Rutilus rutilus*) for several months, or even years, which is sufficient to overcome the environmental perturbation facing clam population. For example, Taskinen et al. (1991) found alive metacercariae of *R. fennica* one year after an experimental infection in roach. It is therefore possible that in this case a life cycle with multiple hosts stabilizes the (supra) population dynamics of the parasite, a point that is rarely considered in host–parasite literature. A new generation of parasites may infect clams from the stages that are carried by the second intermediate and definitive hosts. Therefore, the clam population might have to go through a long period of stress before the parasite suprapopulation crashes detrimentally. Such a stabilizing effect ("extinction refugia") may be one important factor selecting for multiple host life cycles, which are common in many parasite groups.

Extinction refugia in other hosts of the life cycle may allow evolution of higher average host exploitation rate in a particular host species than would be optimal in a case of direct life cycle. For example, consider two parasite lineages that differ by host exploitation rate as *R. fennica* and *R. campanula* differ. Fitness of these lineages can be estimated as the number of parasite propagules produced per year. If the life cycle was direct, the parasite lineages should adjust the exploitation rate to a level where production of transmission stages is unlikely to fail. In such a case fitness of the lineage would be best estimated as the geometric mean fitness calculated over years (e.g. following the theory considering fitness under temporally variable environments, Thoday 1953, Cohen 1966), where years of failure would

lead to zero fitness, and extinction of the lineage. The evolution of host exploitation rate would then be driven by the maximization of the geometric mean fitness of the parasite. However, if utilization of multiple hosts allows refugia for the parasite for stochastic failure in one host, fitness of the lineage may be best estimated as the arithmetic mean fitness over years (e.g. following the theory considering fitness in spatially variable environment, Stearns 1976, Houston and McNamara 1992, Kawecki 1993). Optimal host exploitation rate would then be higher, as the optimal rate would follow maximization of arithmetic mean fitness.

The availability of refugia, i.e. variation in average residence time in the other hosts during the parasite's life-cycle, may be an important factor that has led *R. campanula* and *R. fennica* to have such different host exploitation strategies. Note that the definitive host species are different; perch, *Perca fluviatilis*, for *R. campanula*, and pike, *Esox lucius*, for *R. fennica* (Taskinen et al. 1991, Gibson et al. 1992). This idea remains open for future studies. We suggest that the availability of extinction refugia in multiple hosts may be one factor selecting for higher virulence in those hosts that are primarily used for the production of transmission stages.

Clams respond to anoxia and starvation stress using different physiological pathways; this was our motivation for using two different stress factors in the experiments. Anoxic stress is best survived using glycogen reserves, while fat reserves are important under resource shortage. Infection by *R. fennica* is known to deplete the glycogen reserves to a much higher degree than the fat reserves of the host (Jokela et al. 1993). Therefore, one might expect that the type of physiological stress would modulate the virulence of the parasite to a much higher degree than was observed in our study. In fact, we expected that starvation stress would not lead to as high increase in parasite-induced host mortality by *R. fennica*

as by *R. campanula*. Contrary to our expectation, the results suggest that the fat reserves known to be carried by infected hosts were not allocated to host survival; instead, the parasites must have continued exploiting the host by shedding cercaria. Indeed, all *R. campanula* infected clams and almost half of *R. fennica* infected clams carried transmission ready cercaria in the end of the experiment. We have no knowledge of the fat content of *R. campanula* infected host, but both parasites appear to have rather inflexible host exploitation strategies in the sense that they induce host mortality similarly under different stress factors.

Even if it appears that *R. campanula* may be eradicated from the host population by high mortality, it seems that the clam hosts are expendable. Both types of stress produced similar, parasite-species specific response in the host, and parasite species appeared to be rather inflexible in their host exploitation strategies. The refugia offered by the multiple-host life cycle may function to stabilize parasite suprapopulations against perturbations in particular hosts, and may allow evolution of higher host exploitation rates.

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