

# The impact of a vertically transmitted microsporidian, *Nosema granulosis* on the fitness of its *Gammarus duebeni* host under stressful environmental conditions

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## SUMMARY

Although purely vertically transmitted parasites are predicted to cause low pathogenicity in their hosts, the effects of such parasites on host fitness under stressful environmental conditions have not previously been assessed. Here, we investigate the effects of *Nosema granulosis*, a vertically transmitted, microsporidian parasite of the brackish water amphipod *Gammarus duebeni*, on host growth and survival under conditions of host–host competition and limited food. The parasite had no effect on host survival, but caused a reduction in juvenile growth. Stressful environmental conditions also led to a reduction in *G. duebeni* growth. However, we found no evidence to support the prediction that parasitized hosts would suffer a greater reduction in fitness than uninfected hosts under adverse environmental conditions. We interpret our results in the context of selection for successful vertical parasite transmission.

Key words: vertical transmission, microsporidia, virulence, *Nosema granulosis*, *Gammarus duebeni*.

## INTRODUCTION

Parasites generally impose some cost on their host in terms of host fitness. Parasites can adversely affect a host's growth, reproduction or survival (e.g. Möller, Allander & Dufva, 1990; Lehmann, 1993; Jaenike, Benway & Stevens, 1995). In addition, the effects of parasitism on host fitness are often more apparent under stressful conditions (Oppliger *et al.* 1998; Lafferty & Kuris, 1999). For example, the effects of parasitism on host fitness can be modulated by resource availability and intra-specific competition, with infected individuals suffering higher costs than uninfected individuals under these stressful conditions (Washburn, Mercer & Anderson, 1991).

Parasite virulence can be defined as the extent of deleterious effects caused by the parasite on host fitness (usually measured in terms of reproductive success and survival) and is dependent on the transmission strategy of the parasite (Bull, Molineux & Rice, 1991; Ebert & Herre, 1996; Dunn & Smith, 2001). Horizontally transmitted parasites are transmitted between unrelated or related hosts of the same or different generations, usually via ingestion of infective tissue. Selection for successful horizontal transmission favours a high parasite burden that is

often associated with high levels of virulence in the host (Agnew & Koella, 1997; Dunn, Terry & Smith, 2001). In contrast, vertically transmitted parasites are transmitted from parent to offspring through successive host generations. Since vertically transmitted parasites depend on host reproduction for successful transmission, they are predicted to have low virulence (Ewald, 1987; Smith & Dunn, 1991; Hurst & Majerus, 1993; Dunn *et al.* 1995) and empirical studies show that vertical transmission is often associated with low burden and virulence (Bull *et al.* 1991; Herre, 1993; Ewald, 1994; Dunn & Smith, 2001). However, most of these studies considered virulence in controlled laboratory conditions and the possibility that these effects may be modulated by other factors such as competition and resource availability is frequently ignored. Here, we investigate the effects of a vertically transmitted microsporidian parasite on host fitness under stressful environmental conditions.

The brackish water crustacean *Gammarus duebeni* is host to a number of vertically transmitted, microsporidian parasites that distort the host sex ratio in favour of the transmitting sex by converting genotypic males into functional phenotypic females (Bulnheim, 1978; Terry, Smith & Dunn, 1998; Dunn *et al.* 2001). The best studied of these is *Nosema granulosis* (Terry *et al.* 1999; Kelly, Dunn & Hatcher, 2001a; Kelly *et al.* 2001b; Kelly, Dunn & Hatcher, 2002). *N. granulosis* is present in very low burden in adult and juvenile hosts. No horizontal transmission route has been identified and the parasite is

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transmitted only via the ova of infected females to the next generation of hosts (Dunn & Smith, 2001). Previous studies have shown that infection causes little pathogenicity in *G. duebeni* (Terry, Dunn & Smith, 1997; Terry *et al.* 1998; Kelly *et al.* 2001a). However, these studies measured virulence under standard laboratory conditions with excess food and limited competition. Such favourable conditions may not be reflected in the field where *G. duebeni* are found aggregated at very high densities. For example, densities of approximately 1000 animals have been reported in an area of 0.25 m<sup>2</sup> (Kelly *et al.* 2001a). Here, we test the prediction that *N. granulosis* infection will have a greater impact on the growth and survival of juvenile hosts under conditions of host–host competition and limited food.

#### MATERIALS AND METHODS

*G. duebeni* were collected from the Isle of Cumbrae, Scotland and transported to the laboratory in Tupperware containers, where they were maintained under long day conditions (16 h light; 8 h dark) at 12 °C. *G. duebeni* are iteroparous breeders and produce a number of broods over the breeding season. Developing embryos are retained in the thoracic brood pouch for 3–4 weeks until the next moult at which time the juveniles are released and a subsequent brood of eggs is laid and fertilized. Paired *G. duebeni* that were carrying a brood from a previous mating were transferred to individual 150 ml pots containing brackish water (specific gravity 1005°). Rotted sycamore leaves and *Enteromorpha* (a marine green algae) were provided for food and shelter. The pairs were checked daily until mating occurred, at which time the previous brood of juveniles was released from the brood pouch and newly produced eggs could be observed. The infection status of each mother was determined by screening her newly released eggs for the presence of *N. granulosis*. The eggs were flushed from the brood pouch, permeated with 5 M HCl, rinsed in distilled water and fixed in acetone at –20 °C. The eggs were then transferred to a microscope slide and stained with DAPI (4,6-diamidino-2-phenyl-indole diluted 1:500 in 0.2 M NaH<sub>2</sub>PO<sub>4</sub>), which is fluorescent for DNA. The eggs were screened for parasites using a Zeiss Axioplan fluorescence microscope. In infected eggs, the diplokaryotic nuclei characteristic of microsporidian parasites could be seen in the cytoplasm around the host nuclei (Dunn *et al.* 1995).

At the same time as we collected eggs from the brood pouch, we collected the previous brood of juveniles released from each mother for use in our experiments. In this way we collected broods of juveniles from 40 uninfected ( $n=301$ ) and 29 parasitized mothers ( $n=215$ ). The mean ( $\pm$ S.E.) number of offspring collected from uninfected and infected mothers was  $7.7 \pm 0.6$  and  $7.4 \pm 0.6$ , respectively. In

order to avoid any potentially confounding maternal effects, offspring were pooled into 2 large groups and placed in separate Tupperware boxes (30 cm  $\times$  30 cm  $\times$  10 cm) containing brackish water. Offspring were then allocated at random to each experimental regimen (see below). Vertical transmission of *N. granulosis* is very efficient (91%, Terry *et al.* 1998) and thus we can estimate that about 90% of juveniles collected from infected mothers were themselves parasitized.

#### Experimental design

In order to investigate the effects of parasitism on growth and survival under adverse rearing conditions, *G. duebeni* offspring produced by infected and uninfected females were allocated at random to 1 of 4 experimental treatments. (1) Uncrowded/excess food; (2) uncrowded/limited food; (3) crowded/excess food; (4) crowded/limited food.

Uncrowded, 150 ml pots contained single animals, while crowded pots contained 4 animals. The excess food groups were given rotted sycamore leaves and *Enteromorpha ad libitum*. The limited food groups were given 5 mg wet weight of *Enteromorpha* and 1 cm<sup>2</sup> rotted sycamore leaf every 14 days. This approximates to the amount that 1 adult would eat in 1 week (personal observation). There were 15 replicates for each group. For the uncrowded groups, survival and the blotted wet weight (mg) of each animal was recorded every 14 days for 26 weeks. In the crowded groups, it was not possible to identify individual animals and so mean wet weights (mg) were recorded. For the growth rate analysis, only those crowded treatments in which all 4 animals survived to 26 weeks were included in the analysis.

Data were analysed using the generalized linear modelling package GLIM 3.77 (Crawley, 1993). Survival was analysed as proportion data specifying a binomial error structure with the total number of offspring in each treatment used as the binomial denominator. The effects of parasitism on weight under adverse rearing conditions were analysed using factorial ANOVA in GLIM for unequal sample sizes. Treatments were (1) parasitized or non-parasitized, (2) excess food or limited food and (3) crowded or uncrowded conditions. Significance was determined by comparison of the change in deviance, on removing a factor with *F* tables or  $\chi^2$  tables as appropriate.

#### RESULTS

Survival at 26 weeks was high in all groups, ranging from 73 to 100% (Fig. 1). There was no significant difference in survival between uninfected or infected treatments ( $\chi^2_1=0.23$ ,  $P>0.05$ ) or between uncrowded and crowded treatments ( $\chi^2_1=0.98$ ,  $P>0.05$ ). Surprisingly, survival was higher in the limited food treatments (mean survival = 92%) than

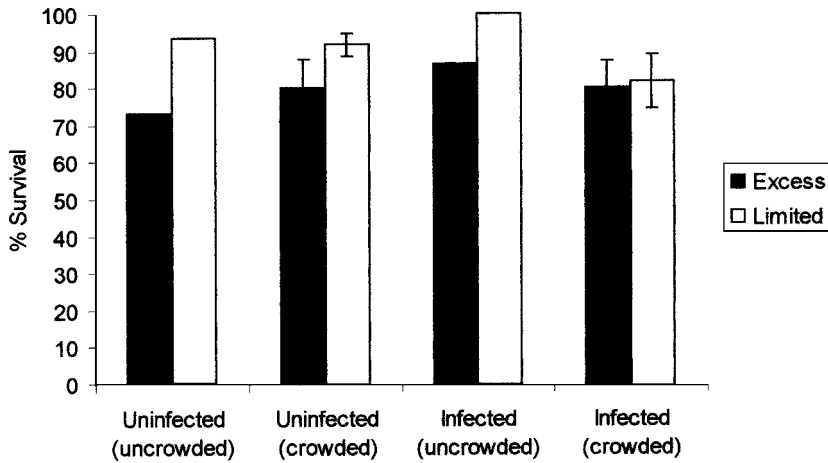


Fig. 1. The mean survival (data back transformed) of uninfected and infected offspring in uncrowded ( $\times 1$ ) and crowded ( $\times 4$ ) conditions under excess and limited food regimes. Error bars represent 1 s.e. of the mean.

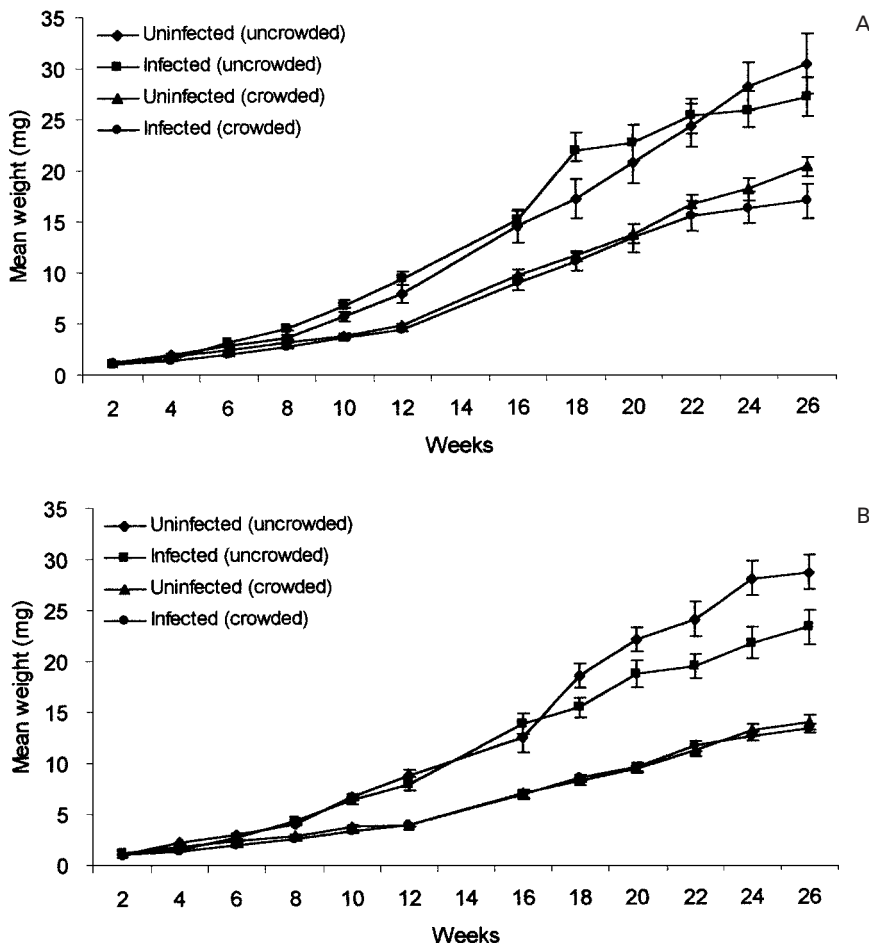


Fig. 2. (A) The mean weight (mg) over 26 weeks for offspring from uninfected and infected broods in uncrowded ( $\times 1$ ) and crowded ( $\times 4$ ) conditions with excess food. Error bars represent 1 s.e. of the mean. (B) The mean weights (mg) over 26 weeks of offspring from uninfected and infected broods in uncrowded ( $\times 1$ ) and crowded ( $\times 4$ ) conditions with limited food. Error bars represent 1 s.e. of the mean.

in treatments where food was provided in excess (mean survival = 80%,  $\chi^2_1 = 4.32$ ,  $P < 0.05$ ). We found no evidence that parasitized animals suffered reduced survival under stressful conditions. The interactions between infection status and food availability ( $\chi^2_1 = 1.04$ ,  $P > 0.05$ ) and between infection status and rearing density ( $\chi^2_1 = 2.47$ ,  $P > 0.05$ ) were

both non-significant. Similarly, there was no significant 3-way interaction between infection, food availability and rearing density ( $\chi^2_1 = 1.18$ ,  $P > 0.05$ ).

We measured the weights of the developing juveniles in each treatment over 26 weeks (Fig. 2A, B). Between 2 and 8 weeks there appeared to be very little difference in weight between animals kept in the

Table 1. The mean weight (and standard error) at 26 weeks for offspring from uninfected broods (U) and infected broods (I) for uncrowded ( $\times 1$ ) and crowded ( $\times 4$ ) treatments under excess and limited food

(For crowded pots,  $N$  refers to the number of replicates in which all 4 individuals survived to 26 weeks.)

	Excess food			Limited food		
	$\bar{X}$	s.e.	$N$	$\bar{X}$	s.e.	$N$
U ( $\times 1$ )	30.2	2.9	11	28.4	1.6	14
I ( $\times 1$ )	26.8	1.9	13	23.1	1.7	15
U ( $\times 4$ )	20.1	0.9	9	13.7	0.7	10
I ( $\times 4$ )	16.7	1.7	9	13.1	0.5	9

Table 2. ANOVA table for the effect of parasitism (infection), rearing density (density) and food availability (food) on host weight at 26 weeks

Source	ss	D.F.	ms	$F$ -ratio	$P$
Infection	334.5	1	334.5	10.31	<0.01
Density	2961	1	2961	91.25	<0.001
Food	392.5	1	392.5	12.1	<0.01
Infection.Density	60.36	1	60.36	1.86	>0.05
Infection.Food	4.22	1	4.22	0.13	>0.05
Infection.Density.Food	11.68	1	11.68	0.36	>0.05
Error	2475	82	$s^2 = 32.45$		
Total	6239	88			

different treatment regimes, suggesting that rearing conditions had not yet become limiting. However, from 8 weeks onwards, differences could be seen between the mean weights of animals kept in each treatment with animals in crowded conditions showing slower growth than animals kept singly and infected animals showing lower growth than uninfected animals.

The mean sizes of offspring at the end of the experiment (26 weeks) are given in Table 1. We used a factorial ANOVA to compare the weights of the animals under the different treatments at 26 weeks. Parasite status, food availability and competition all affected the size of the young. Offspring from infected broods were significantly smaller than offspring from uninfected broods ( $F_{1,82} = 10.3$ ,  $P < 0.01$ ). Animals provided with limited food were significantly smaller than those provided with excess food ( $F_{1,82} = 12.1$ ,  $P < 0.01$ ). Finally, animals kept under crowded conditions were significantly smaller than those in uncrowded treatments ( $F_{1,82} = 91.25$ ,  $P < 0.001$ ). Rearing density had the greatest impact on size at 26 weeks, explaining 47% of the variance whereas infection status and food availability explained 5% and 6% of the variance respectively. However, we found no evidence that parasitized animals suffered more than unparasitized animals when food was limiting or when conditions were crowded. There were no significant interactions between infection status, rearing density and food availability (Table 2).

## DISCUSSION

Infection with the vertically transmitted parasite *N. granulosis* caused a reduction in the growth of *G. duebeni* hosts during development from hatching to the adult stage (26 weeks), but had no effect on host survival over this period. The weight of infected *G. duebeni* was reduced by up to 20% in comparison with uninfected hosts, a finding that is in keeping with previous studies of the parasite–host relationship which were carried out under optimum laboratory conditions (Terry *et al.* 1998). Fecundity is dependent on female size (Dunn & McCabe, 1995). Therefore the parasite-induced reduction in growth rate is likely to lead to a decrease in host fecundity and a concomitant decrease in opportunities for vertical transmission of the parasite to hosts of the next generation.

Stressful rearing conditions also caused a reduction in the growth of both parasitized and unparasitized juveniles and led to a lower weight in young adults. In contrast, survival was not reduced under stressful rearing conditions. Indeed, survival was lower when food was provided in excess than when food was limited. We suggest that the presence of decaying food may have led to higher mortality when food was provided in excess.

We found no evidence to support our prediction that parasitism should cause a greater reduction in host fitness under stressful environmental conditions.

Limited food caused an increase in survival of both uninfected and infected *G. duebeni*, whilst competition had no effect on the survival of either parasitized or unparasitized animals. Although parasitized animals showed lower growth than unparasitized animals, there was no evidence that the effects of parasitism were more severe when resources were limiting or when competition was high. The impact of *N. granulosis* on host fitness was not exacerbated under stressful conditions.

*N. granulosis* is a feminizing parasite which displays an unusual life-cycle. The parasite is present in low burden throughout host development. In the adult hosts, the parasite is localized in the ovary and forms spores characteristic of 'early' or autoinfection spore (Iwano & Ishihara, 1991; Iwano & Kurtti, 1995). Germination of these spores leads to infection of the gametes during vitellogenesis (transovarial transmission, Terry *et al.* 1999). However, in contrast with other microsporidia of the genus *Nosema*, there is no evidence in the life-cycle for production of a second spore type associated with horizontal transmission, suggesting that this parasite has lost its capacity for active horizontal transmission (Terry *et al.* 1999; Dunn *et al.* 2001).

A parasite which depends solely on vertical transmission will be under strong selection to limit the metabolic burden and pathogenicity imposed on the host, as the parasite relies upon successful host development and reproduction for transmission to new hosts (Dunn *et al.* 1995, 2001). Previous studies demonstrated small, but significant, deleterious effects on host fitness under controlled laboratory conditions (Terry *et al.* 1998; Kelly *et al.* 2001*a*). However, these conditions might not reflect field conditions where food limitation and intraspecific competition are likely to be frequent events. The current study shows that deleterious effects on host fitness appear to be minimized even under conditions more likely to be experienced, on average, in natural populations.

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