The effect of salinity on transovarial transmission of a microsporidian infecting *Gammarus duebeni*

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SUMMARY

This is an investigation of the impact of salinity on transovarial transmission and burden of a microsporidian sex ratio distorter in the inter-tidal crustacean *Gammarus duebeni*. Exposure of parasitized mothers to increased salinity during the gonotrophic cycle caused an increase in parasite burden in the follicle cells and a decrease in burden in the oocytes. It appears that salinity impedes parasite transmission from the follicle cells to the oocytes during host oogenesis. A lower proportion of the young were infected in broods from elevated salinity and, in infected offspring, parasite burden was lower than in control embryos. Parasite replication occurred during embryogenesis. However, the pattern of parasite growth did not differ between salinities, indicating that differences in parasite burden could be attributed to a reduction in the initial parasite burden transmitted to the gamete, rather than to a reduction in parasite replication during host embryogenesis. We discuss our findings with respect to parasite/host dynamics and the ecology of the host.

Key words: microsporidian, salinity, parasite burden, transovarial transmission, *Gammarus duebeni*.

INTRODUCTION

Parasitic sex ratio distorters may have important consequences for host evolution and ecology. They may impose selection pressures on host sex ratio (Werren, 1987; Hatcher & Dunn, 1995) and on strategies of host sex determination (Juchault & Mocquard, 1993; Dunn *et al*. 1995). In addition, parasitic sex ratio distorters may affect the size and stability of the host population and have the potential to cause population extinction (Werren, 1987).

The crustacean *Gammarus duebeni* is host to at least 2 microsporidian sex ratio distorters. These microsporidia are transovarially transmitted and feminize the host (Bulnheim, 1978; Dunn, Adams & Smith, 1993; Terry, Dunn & Smith, 1997). Previously we showed that prevalence of the microsporidian *Octosporea effeminans* (Bulnheim & Vavra, 1968) varies between natural host populations, reflecting differences in the pattern of parasite burden and transmission (Dunn & Hatcher, 1997). These patterns may be attributed to differences in host resistance to parasite transmission and growth, or to clonal differences between parasite strains in different host families and populations.

Abiotic factors may also affect parasite prevalence in the field. *G. duebeni* is a brackish water crustacean found in coastal habitats characterized by fluctuating salinity. Here we measure parasite transmission, burden and parasite growth during host development in different experimental salinities and consider the implications for parasite/host dynamics and for the ecology of the host.

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MATERIALS AND METHODS

A random collection of adult *Gammarus duebeni* was taken from a population at Budle Bay, Northumberland, UK where about 30% of females are infected with the microsporidian sex ratio distorter (Dunn *et al*. 1993). This population is found where a freshwater stream crosses the intertidal zone. Salinity is about 6·5‰, other than at high tide when the site is covered by the sea for about 2 h (approximate salinity 34‰).

Approximately 250 adults were put into each of 3 groups in 20 litre tanks of aerated water: a control group in salinity of 6·5‰; 2 treatments of increased salinity of 11·5‰ and 16·25‰. Water was made up using Instant Ocean (Aquatic Systems Inc). Growth and differentiation of the oocytes is directly synchronized with the 3–4 week moult cycle (Bulnheim & Vavra, 1968) and it appears that parasite transmission from follicle cells to gametes occurs during oocyte maturation (Terry *et al*. 1997). Animals were maintained in the experimental salinities for 6 weeks (at least 1 complete gonotrophic cycle) before transovarial parasite transmission was measured.

To measure parasite transmission to oocytes, 30 infected females from control and high (16·25‰) salinity were fixed in formalin, embedded in paraffin wax and serial transverse sections 4 μm thick stained

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Table 1. Parasite numbers in oocytes and follicle cells of infected mothers and the efficiency of transovarial parasite transmission to young

	6·5‰	11·25‰	16·25‰	Test	*P*
Oocytes					
Proportion infected	0·92 $N = 26$		0·33 $N = 24$	X^2, 1 D.F. = 18·8	< 0·001
Mean burden	19·8 $N = 26$		1·4 $N = 24$	X^2, 1 D.F. = 15·4	< 0·001
Follicle cells					
Proportion infected	0·14 $N = 278$		0·42 $N = 250$	X^2, 1 D.F. = 47	< 0·01
Mean burden	0·4 $N = 278$		1·6 $N = 250$	X^2, 1 D.F. = 25	< 0·001
Transovarial transmission	0·95 ± S.E. 0·03	0·37 ± S.E. 0·08	0·54 ± S.E. 0·09	$F_{(2,38)} = 6·78$	< 0·01

with Geimsa. Sections were examined using a light microscope and the number of parasites in follicle cells (vegetative stages and spores) and secondary and mature oocytes (vegetative stages) counted.

To measure parasite transmission to young, early-stage embryos (2–128 cell) were flushed from the marsupium of infected mothers from the different salinities. Clutch size and blotted wet weight of the mother were recorded. Embryos were freeze fractured, fixed and stained with DAPI (4,6-diamidino-2-phenyl-indole), a fluorescent dye for DNA, and examined using a Zeiss Axiovert 10 fluorescent microscope. This enabled us to see host nuclei and the nuclei of vegetative parasite stages lying in the cytoplasm of the embryo cells (Dunn & Hatcher, 1997).

Each embryo was scored for the presence or absence of the parasite to provide an estimate of parasite transmission efficiency (the proportion of embryos of an infected mother which had inherited the infection). Parasite burden was measured by counting total parasite load in infected embryos. The infection status of each mother was determined from the status of the offspring and subsequent examination of the mother by light microscopy (Dunn *et al*. 1993).

Data were analysed using the generalized linear modelling package GLIM (GLIM 3.77, Numerical Algorithms Group, Oxford 1985). Significance was assessed by examining the reduction in deviance caused by deletion of a term from the maximal model. Data for parasite growth in embryos were log transformed. Data for clutch size and for parasite burden are count data, therefore we specified a Poisson error distribution. We corrected for overdispersion with a heterogeneity factor (Hf = Pearson's X^2/D.F.; Crawley (1993)). Changes in deviance caused by removing a factor from the model were compared with X^2 tables. Data for parasite transmission efficiency were proportion data and were analysed specifying a binomial error structure, taking the total number of eggs as the binomial denominator. A heterogeneity factor (Hf) was used to correct for overdispersion and changes in deviance caused by removing a factor from the model were assessed using an F test.

RESULTS

Parasite transmission efficiency

The proportion of oocytes infected with the microsporidian was significantly lower in females from high salinity treatment (16·25‰) than in females from the control group. Salinity also caused a significant reduction in parasite burden in the oocytes. Conversely, the proportion of follicle cells containing microsporidia and the parasite burden per follicle cell were significantly *higher* in females from high salinity than in control females (Table 1, Fig. 1).

Salinity also significantly affected the efficiency of parasite transmission to embryos. At 6·5‰, 95% of the young of infected females inherited the microsporidian; at 11·5‰, 37% inherited the microsporidian; at 16·25‰, 54% of young inherited the infection (Table 1). Simplification of the model to a factor with 2 levels: control (6·5‰) and elevated (11·5‰ and 16·25‰) salinity did not significantly alter the fit of the model ($F_{(1,39)} = 0·01$, $P > 0·05$). The efficiency of parasite transmission to young was lower in elevated than in control salinity and followed closely the frequency of infection in oocytes in the different treatments (Table 1).

Parasite burden in embryos

Parasite burden increased with host developmental stage ($F_{(6,231)} = 25·65$, $P < 0·01$) indicating that the microsporidian undergoes replication during early host development (Fig. 2). Parasite burden increased 2 to 3-fold during 5 host cell divisions, in accord with previous studies (Dunn *et al*. 1995).

Parasite burden differed between embryos at the different salinities (Fig. 2). The difference in burden could be attributed to a difference between control salinity and the two higher salinities (aggregation of the salinity factor into these two new levels did not cause a significant increase in deviance $F_{(1,230)} = 0·09$, $P > 0·05$). Parasite burden was significantly greater at control salinity than at high salinity. Regressions of parasite burden against host stage had significantly different intercepts for the different salinities ($F_{(2,229)} = 33·4$, $P < 0·01$), but there was no

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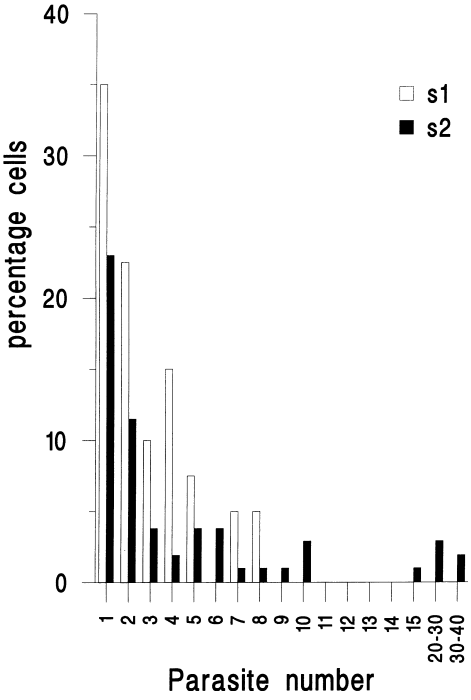

Fig. 1. Parasite distibution in follicle cells of infected *Gammarus duebeni* females in control (s1) and elevated (s2) salinity.

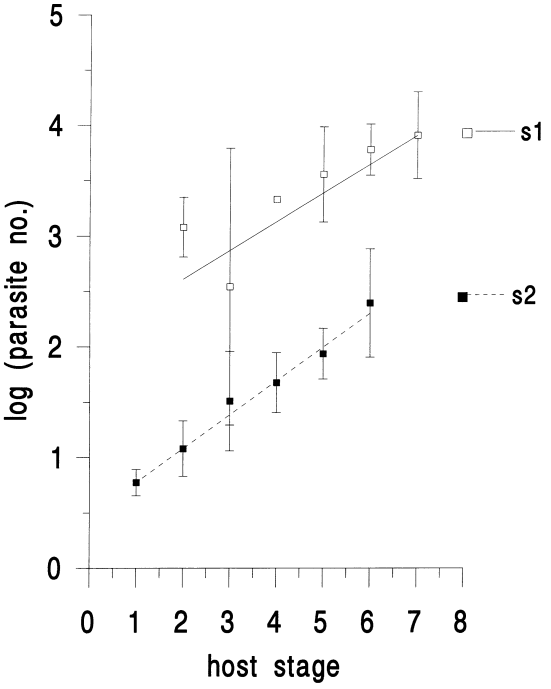

Fig. 2. Parasite burden in early stages of *Gammarus duebeni* embryogenesis. Stage indicates the number of host cell divisions (i.e. 1 = 2 cell embryo, 7 = 128 cell embryo). Mothers and embryos kept in control (s1, 6·5‰) or elevated salinity (s2, 11·5 and 16·25‰).

significant difference between the slopes ($F_{(4,230)}$ = 0·42, $P > 0·05$) suggesting that the difference in burden can be attributed to differences in initial dose of the parasite at different salinities, rather than to an effect of salinity on parasite growth rate.

Effects of parasitism and salinity on host fitness

We also looked for possible effects of parasitism and salinity on host fitness. There was no difference in the weights of infected and uninfected adult females collected from the field (Table 2). Clutch size was significantly related to the weight of the mother (X^2, 1 D.F. = 9·15, $P < 0·01$) and this is in accord with previous studies (e.g. Dunn & McCabe, 1995). We found no evidence for a reduction in the clutch size of infected mothers (Table 2).

However, female fecundity was affected by salinity (X^2, 2 D.F. = 12·81, $P < 0·01$, female weight taken as a covariate). Figure 3 illustrates that clutch size was greatest at the intermediate salinity of 11·5‰. These differences in fecundity could not be attributed to differences in female weight at the different salinities: females used in the experiment were allocated at random to the 3 salinity treatments and a subsequent analysis showed no difference in female weight between the 3 treatments ($F_{(2,84)}$ = 2·64, $P > 0·05$). We found no evidence for an effect on clutch size of the interaction between parasitism and salinity (X^2, 2 D.F. = 3·33, $P > 0·05$).

DISCUSSION

The microsporidian infection is localized within ovarian tissue of the adult host (Bulnheim & Vavra, 1968; Dunn *et al*. 1993) and may be transmitted to oocytes during host oogenesis (Bulnheim & Vavra, 1968). Investigation of a second microsporidian sex ratio distorter in *G. duebeni* showed transmission of the parasite from follicle cells to the oocytes during oogenesis (Terry *et al*. 1997) and this mechanism of gamete infection has also been reported for *Nosema* infecting locusts (Raina *et al*. 1995). In elevated salinity, we found that infection frequency and parasite burden were *higher* in follicle cells but *lower* in oocytes. Hence, it appears that elevated salinity impedes transmission of the microsporidian from follicle cells to the developing oocytes. Inhibition of cell to cell transmission of *Nosema* by culture medium of high osmolarity has also been demonstrated *in vitro* (Kurtti, Tsang & Brooks, 1983).

Decreased transmission to oocytes resulted in decreased vertical transmission to the young (in broad agreement with Bulnheim (1978)) and in lower parasite burden in infected embryos. The reduction in parasite burden at high salinity may decrease the efficiency of transmission to the gonadal tissue in the developing host (parasites in other tissues are not transmitted, Dunn *et al*. (1995); Hatcher Tofts & Dunn (1997)) as well as lowering the efficiency of feminization of the host. Therefore, salinity may be an important determinant of parasite prevalence at the population level and may contribute to the mismatch between predicted and

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Table 2. Mean weight (±S.E.) and clutch size for uninfected *Gammarus duebeni* females and for females infected with the microsporidian sex ratio distorter

	Uninfected females	Infected females	Test	*P*
Weight (mg)	22·50±0·63	20·50±0·68	$F_{(1,83)} = 2·99$	> 0·05
Clutch size	16·90±0·81	17·24±1·47	X^2, 1 D.F. = 0·953	> 0·05

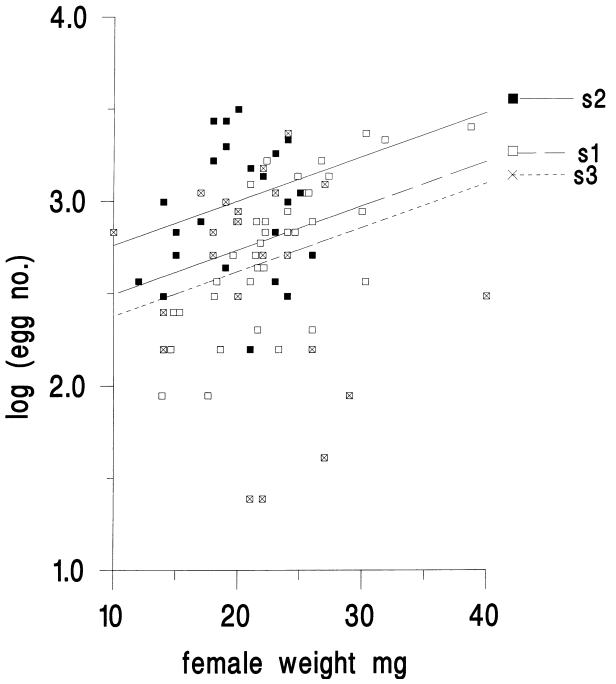

Fig. 3. Fecundity (number of eggs per clutch) of *Gammarus duebeni* females at control and elevated salinity. s1 = control salinity, 6·5‰; s2 = 11·5‰; s3 = 16·25‰. Lines indicate regression of best fit with equations:

salinity 1: ln egg no. = (0·024±S.E. 0·008)weight+(2·256±S.E. 0·196),

salinity 2: ln egg no. = (0·024±S.E. 0·008)weight+(2·521±S.E. 0·098),

salinity 3: ln egg no. = (0·024±S.E. 0·008)weight+(2·139±S.E. 0·103).

Although both female weight and salinity significantly affect clutch size they explain only 9·4% and 12·8% of the deviance, respectively, and there is considerable variation in clutch size which is not explained by the model.

observed prevalence of the sex ratio distorter (Hatcher & Dunn, 1995).

The relationship between salinity, fecundity and parasitism has interesting implications for host fitness and consequently for the distribution of the host population (Minchella & Scott, 1991). *G. duebeni* tolerates a wide range of salinities but growth, fecundity and offspring survival are highest at intermediate salinity (Fenchel & Kolding, 1979). However, parasitic sex ratio distortion may cause host population instability (Werren, 1987). Hence, population persistence may be greater in regions of high salinity where the risk of parasitism is reduced. Further field investigation is necessary to investigate possible adaptations of the parasite to salinity in different host populations and the implications for host fitness.

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REFERENCES

BULNHEIM, H. P (1978). Interaction between genetic, external and parasitic factors in sex determination of the crustacean amphipod *Gammarus duebeni*. *Helgoländer wissenschaftliche Meeresuntersuchungen* **31**, 1–33.

BULNHEIM, H. P. & VAVRA, J. (1968). Infection by the microsporidia *Octosporea effeminans* sp. n., and its sex determining influence in the amphipod *Gammarus duebeni*. *Journal of Parasitology* **54**, 241–248.

CRAWLEY, M. J. (1993). *GLIM for Ecologists*. Blackwell Scientific Publications, Oxford.

DUNN, A. M., ADAMS, J. & SMITH, J. E. (1993). Transovarial transmission and sex ratio distortion by a microsporidian parasite in a shrimp. *Journal of Invertebrate Pathology* **61**, 248–252.

DUNN, A. M., HATCHER, M. J., TERRY, R. S. & TOFTS, C. (1995). Evolutionary ecology of vertically transmitted parasites: strategies of transovarial transmission of a microsporidian sex ratio distorter in *Gammarus duebeni*. *Parasitology* **111**, S91–S110.

DUNN, A. M. & HATCHER, M. J. (1997). Prevalence, transmission and intensity of infection by a microsporidian sex ratio distorter in natural *Gammarus duebeni* populations. *Parasitology* **114**, 231–236.

DUNN, A. M. & MCCABE J. R. (1995). Resource allocation to young: seasonal patterns within and between *Gammarus duebeni* populations. *Oikos* **73,** 199–202.

FENCHEL, T. M. & KOLDING, S. (1979). Habitat selection and distribution patterns of five species of the amphipod genus *Gammarus*. *Oikos* **33**, 316–322.

HATCHER, M. J. & DUNN, A. M. (1995). Evolutionary consequences of sex ratio distortion by cytoplasmically inherited feminizing factors. *Philosophical Transactions of the Royal Society*, *London*, *B* **348**, 445–456.

HATCHER, M. J., DUNN, A. M. & TOFTS, C. (1997). The effect of the embryonic bottleneck on vertical

microparasite transmission In *Computation in Cellular and Molecular Biological Systems* (ed. Cuthbertson, R., Holcombe, M. & Paton, R.), pp. 339–351. World Scientific, Singapore.

JUCHAULT, P. & MOCQUARD, J. P. (1993). Transfer of a parasitic sex factor to the nuclear geneome of the host: a hypothesis on the evolution of sex-determining mechanisms in the terrestrial Isopod *Armadillidium vulgare* Latr. *Journal of Evolutionary Biology* **6**, 511–528.

KURTTI, T. J., TSANG, R. & BROOKS, M. A. (1983). The spread of infection by the microsporidian *Nosema disstriae* in insect cell lines. *Journal of Protozoology* **30**, 652–657.

MINCHELLA, D. J. & SCOTT, M. E. (1991). Parasitism: a cryptic determinant of animal community structure. *Trends in Ecology and Evolution* **6**, 250–253.

RAINA, S. K., DAS, S., RAI, M. M. & KHURAD, A. M. (1995). Transovarial transmission of *Nosema locusta* (Microsporida: Nosematidae) in the migratory locust *Locusta migratoria migratorioides*. *Parasitology Research* **81**, 38–44.

TERRY, R. S., DUNN, A. M. & SMITH, J. E. (1997). Cellular distribution of a feminizing microsporidian infecting *Gammarus duebeni*. *Parasitology* (in the Press).

WERREN, J. H. (1987). The coevolution of autosomal and cytoplasmic sex ratio factors. *Journal of Theoretical Biology* **124**, 313–334.