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The ecology and phylogeny of oomycete infections in *Asplanchna* rotifers

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SUMMARY

1. Recently, the potential for parasites to influence the ecology and evolution of their zooplankton hosts has been the subject of increasing study. However, most research to date has focussed on *Daphnia* hosts, and the potential for parasites to influence other zooplankton taxa remains largely unstudied.
2. During routine sampling of zooplankton in a eutrophic lake, we observed that the rotifer *Asplanchna girodi* was often infected with a parasitic oomycete. Epidemics of this parasite occurred frequently, with three separate events in a single year. Prevalence at peak infection ranged from 29 to 41% and epidemics lasted from 17 to 56 days. Our data indicate that high densities of the host population are required for epidemics to occur.
3. Our morphological and molecular analyses suggest that this parasite is in the genus *Pythium*. Most *Pythium* spp. are plant pathogens, but our study supports recent work on *Daphnia*, suggesting that *Pythium* spp. are also important parasites of zooplankton.
4. As the parasite in this study was recalcitrant to cultivation, we developed an alternative method to verify its identity. Our approach used quantitative PCR to show that the ribosomal sequences identified increased with increasing density of infected hosts and, thus, were associated with the parasite. This approach should be generally applicable to other plankton parasites that are difficult to cultivate outside their hosts.
5. Infections significantly reduced host fecundity, lifespan and population growth rate. As a result of the virulence of this parasite, it is likely to influence the population ecology and evolution of its *Asplanchna* host, and may be a useful model system for studies on host–parasite coevolutionary dynamics.

Keywords: evolution of sex, pathogens, plankton, Pythiaceae, qPCR

Introduction

In the late nineteenth and early twentieth centuries, zooplankton–parasite interactions attracted considerable attention, particularly from microbiologists (e.g. Metchnikoff, 1888; Fritsch, 1895; Budde, 1927). Nevertheless, these interactions were largely overlooked by limnologists (Wetzel, 2001), perhaps in part because infections are often difficult to detect in preserved samples. Recently, there has been a resurgence in the study of zooplankton–parasite interactions, driven largely by work on *Daphnia* and its parasites (Ebert, 2005). These studies have demonstrated that parasites can have important ecological (e.g. Decaestecker *et al.*, 2005; Duffy & Hall, 2008) and evolutionary (e.g. Decaestecker *et al.*, 2007; Wolinska & Spaak, 2009) effects on their hosts. In addition, *Daphnia* parasites have been shown to influence

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interspecific interactions (Duffy *et al*., 2005; Johnson *et al*., 2006) and even ecosystem-level properties such as water clarity (Duffy, 2007).

While it is clear that parasites are important to the ecology and evolution of *Daphnia*, the role of parasites in the ecology and evolution of other zooplanktonic taxa remains unclear. In particular, the potential for parasites to influence planktonic rotifer populations has been almost completely ignored (Wallace & Smith, 2009). However, the little evidence that exists suggests that parasites can be prevalent in rotifer populations and have dramatic effects on population densities (Miracle, 1977; Ruttner-Kolisko, 1977). Moreover, development of novel zooplankton host–parasite systems would be valuable, in part to determine whether the results of studies on *Daphnia* apply more generally.

Here, we describe an oomycete parasite of the common freshwater rotifer *Asplanchna girodi* de Guerne. Specifically, we were interested in determining the frequency of epidemics, the phylogenetic relationships of the parasite, its virulence and whether it has significant effects on host density.

Methods

Study system and field sampling

We began zooplankton sampling in Lake Clara Meer (Piedmont Park, Atlanta, GA, U.S.A.) in March 2008. Lake Clara Meer is man-made, relatively shallow (4 m maximum depth) and highly productive (Secchi depth: 0.2–1.4 m, M.A. Duffy, unpubl. data; total phosphorus: 300 μg L^{-1}, J. Lanier, unpubl. data). In April 2008, we observed *A. girodi* individuals that appeared to be infected with an oomycete. *Asplanchna girodi* is a large, common, predatory rotifer that feeds upon algae, protists, other rotifers and small crustaceans (Gilbert, 1980; Wetzel, 1983). Like most rotifers, it is cyclically parthenogenetic, and populations are generally dominated by amictic (i.e. asexual) females (Edmondson, 1959). Individuals of *A. girodi* begin reproducing after approximately 24 h and generally live *c.* 3–5 days at 25 °C (Dumont & Sarma, 1995).

We began monitoring the dynamics of *A. girodi* (hereafter: *Asplanchna*, as we have not observed other species of *Asplanchna* in this pond) and the parasite in Lake Clara Meer in January 2009; samples were collected approximately weekly. We based our sampling regime on one that we have used to monitor populations of *Daphnia* for infections (e.g. Duffy & Hall, 2008; Duffy *et al*., 2008). On most dates, we collected three samples, two of which were used to determine the density of the *Asplanchna* population, and one of which was used to determine the prevalence of infection. The exceptions were at the beginning of the time series, where on two dates (23 January and 30 January 2009) we only collected samples for density, and on two different dates (16 January and 16 February 2009) where we only collected a sample to determine infection prevalence.

Samples were collected using a 153-μm-mesh Wisconsin bucket net. Each sample contained three separate, whole-water-column vertical tows collected from the north basin of the lake. Upon returning to the laboratory, two of the samples were preserved in 50–90% ethanol and later counted to determine *Asplanchna* density. The remaining live sample was used to determine infection prevalence in *Asplanchna*. Live samples were examined within 4 h of collection. We determined the prevalence of infection in *Asplanchna* by examining a random sub-sample of at least 500 individuals under a stereomicroscope at 15–50× magnification. *Asplanchna* is normally transparent, so infections are readily apparent through the body wall (Fig. 1). On dates with low *Asplanchna* density (<500 individuals in the sample), the entire sample was analysed. During a period of very low *Asplanchna* densities (15 May–14 September 2009), we were unable to obtain accurate estimates of infection prevalence, despite analysing all individuals in the sample. No infected *Asplanchna* were observed during this time. In general, our infection estimates are probably conservative, because early stage infections are difficult to detect, as in many other systems (e.g., Holmstad *et al*., 2003; O'Meara, Collins & Mckenzie, 2007; Duffy *et al*., 2010).

On two dates, the live sample was also used to quantify the size distributions of infected and uninfected *Asplanchna*. On 23 April 2008, a random sample of 24 infected and 145 uninfected *Asplanchna* was measured. On 21 April 2009, a random sample of 36 infected and 52 uninfected *Asplanchna* was measured. Individuals were measured using images taken at 40× magnification and Olympus DP2-BSW software (Center Valley, PA, USA). Size distributions of infected and uninfected *Asplanchna* were compared using Proc Mixed in SAS 9.1 using a two-way ANOVA.

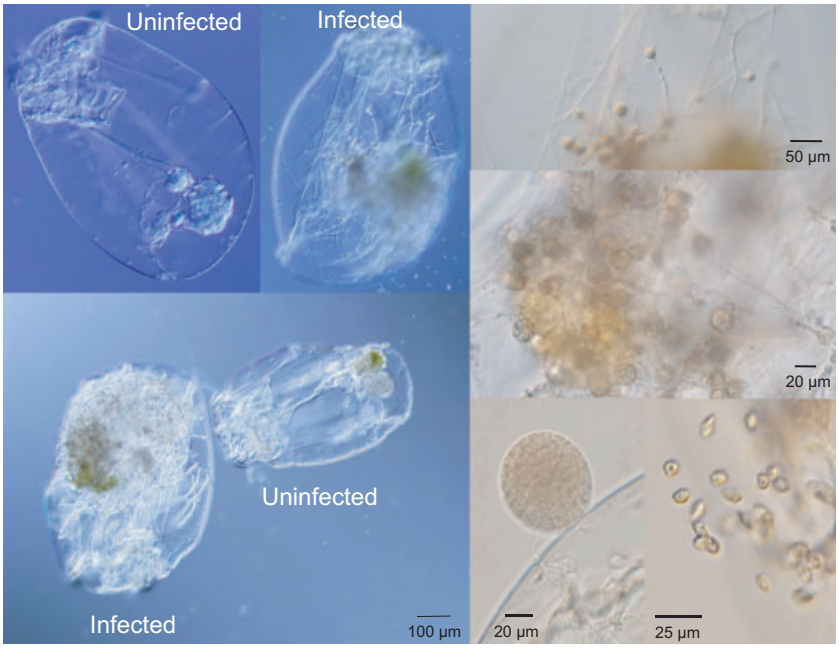

Fig. 1 Images of infected and uninfected *Asplanchna*. Infected *Asplanchna* are distinguished by the hyphal masses filling their bodies; uninfected *Asplanchna* are mostly transparent. The images on the right show the parasite morphology at greater magnification. The image in the bottom right corner shows a close-up of newly released zoospores.

The model included date (random effect), infection status (fixed effect) and their interaction. We tested for the significance of random effects by using differences in the −2 restricted log likelihood, which are χ^2 distributed with 1 degree of freedom, between models with and without the particular random effect included (Littell *et al.*, 2006).

Parasite morphology

Live field- and laboratory-infected individuals were used for morphological analyses. Infected *Asplanchna* were mounted on slides and examined at 100–1000× using an Olympus BX51 microscope with differential interference contrast (DIC). Photomicrographs were captured using Olympus DP2-BSW software.

Molecular methods

Morphological identification of oomycetes can be difficult, and molecular techniques have increasingly been used to identify oomycetes (Hulvey, Padgett & Bailey, 2007; Ruthig, 2009). DNA was extracted from 25 live, infected, field-collected *Asplanchna* individuals with a Power Soil DNA Extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA, U.S.A.). This kit uses bead beating for cell lysis and, thus, is expected to increase DNA yield from oomycetes (Fredricks, Smith & Meier, 2005). The whole body of infected organisms was used because parasite material was difficult to isolate from the gelatinous host body.

We used 18S internal transcribed spacer (ITS) and large subunit (LSU) rRNA gene sequences (White *et al.*, 1990; Moon-Van Der Staay *et al.*, 2000) to identity the parasite to genus. Primer sequences are given in Table 1. PCR amplification started with an initial denaturation at 94 °C for 5 min followed by 30 cycles of the following conditions: denaturation at 94 °C for 1 min, primer annealing at 55 °C for 2 min and extension at 72 °C for 3 min, with a final extension at 72 °C for 10 min (Moon-Van Der Staay, De Wachter & Vaulot, 2001).

18S rRNA gene. Each amplification reaction took place in a total volume of 20 µL containing 1× colourless Go Taq Flexi reaction buffer (TOPO, Madison, WI, U.S.A.), 500 nM of each primer, 250 µM deoxynucleotide triphosphate mixture, 1.5 mM $MgCl_2$, 2.5 U Taq DNA polymerase, 7.8 µL nuclease free water and 2 µL isolated DNA (template).

LSU and ITS. Each amplification reaction took place in a total volume of 20 µL containing 1× colourless Go Taq reaction buffer (Promega), 1000 nM of each primer, 500 µM deoxynucleoside triphosphate mix-

Table 1 PCR primer sequences used in this study and their targets

Target group	Primer name	Sequence	References
Total eukaryotes (18S rRNA gene)	18SFwdMV	ACCTGGTTGATCCTGCCAG	Moon-Van Der Staay *et al.* (2000)
	18SRevMV	TGATCCTTCYGCAGGTTCAC	
Total eukaryotes (internal transcribed spacer) region of rRNA units)	ITS1	TCCGTAGGTGAACCTGCGG	White *et al.* (1990)
	ITS4	TCCTCCGCTTATTGATATGC	
Total eukaryotes (large subunit rRNA gene)	C′1	ACCCGCTGATTTAAGCAT	Leclerc *et al.* (2000)
	D2	TCCGTGTTTCAAGACGG	
Pythium sp.	Pyth712Fwd	GAGGATGTTCTTCTGCCATTCAG	This study
	Pyth1758Rev	GTTCCCATAAGTCCACTCGC	
	Pyth664Fwd	GCCCTTTCGGGTGTGTTACTAG	
	Pyth712Rev	CTGAATGGCAGAAGAACATCCTC	

ture, 2 U Taq DNA polymerase, 4.6 μL nuclease free water and 2 μL isolated DNA (template).

Products of all PCR reactions (5 and 1 μL of loading dye; Promega) were separated and visualised via gel electrophoresis (90 V, 60 min) on an ethidium bromide-stained 1.5% agarose gel. PCR products were purified with a Gel Extraction kit from Promega. Newly purified PCR products were cloned and sequenced using the Invitrogen TOPO TA cloning kit (Life Technologies, Carlsbad, CA, U.S.A.). PCR products generated from M13 amplification of transformed cell colonies were sent to Nevada Genomics Center for sequencing. 18S rRNA, ITS, and LSU gene sequences were submitted to GenBank under accession numbers GU270938, GU270939 and GU270940, respectively.

Sequence alignment and phylogenetic analysis. Phylogenetic analyses were conducted in MEGA4 using default parameters (Tamura *et al.*, 2007). Alignments were performed using CLUSTALW. Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values were calculated based on 500 replicates (Felsenstein, 1985). *Saprolegnia ferax* (Gruith.) Nees was used as an outgroup for 18S rRNA gene phylogeny, *Pythium aphanidermatum* (Edson) Fitzp. was used as an outgroup for ITS phylogeny. *Lagenidium thermophilum* Nakamura *et al.*, *Lagenidium callinectes* Couch, *Lagenidium chthamalophilum* Johnson and *Phytophthora undulata* (Petersen) Dick were used as outgroups for LSU phylogeny.

Pythium-specific primer design. Specific PCR primers targeting conserved regions of the 18S rRNA gene of our *Pythium* sp. were designed based on the nearly complete 18S rRNA gene sequences obtained from clones, as described earlier, and related oomycetes. The 18S rRNA gene sequences were aligned using the MEGALIGN programme of the LASERGENE software package (DNA Star Inc., Madison, WI, U.S.A.). Primers were selected based on a region of the 18S rRNA gene specific to our *Pythium* parasite and BLAST analysis against the NCBI non-redundant database suggested primer specificity (Table 1). The expected size of Pyth712F/Pyth1758R amplicons is 1046 bp.

Quantitative PCR. The Pyth664F/Pyth712R primer pair (Table 1) was designed to be used with SYBR Green detection chemistry (Invitrogen, Carlsbad, CA, U.S.A.). The expected size of the amplicon is 48 bp, a small size which promotes more efficient amplification (ABI, 2005). Amplification was performed using the ABI 7500 Fast Real-Time PCR System (Life Technologies Corporation, Carlsbad, CA, USA). Default parameters for SYBR Green amplification with melting curve analysis were used according to Software version 2.0.1. Each reaction included SYBR Green PCR Master Mix (1×), 500 nM of each primer, and 2 μL of template per 20 μL reaction. Eight-point calibration curves containing known copy numbers of the *Pythium* sp. 18S rRNA gene were prepared using pCR2.1 plasmid constructs generated using a TOPO TA cloning kit (Invitrogen). Samples with detectable fluorescence that fell below the lowest point on the calibration curve were considered below the quantitation limit and conservatively estimated at one half of the lowest standard in the linear calibration curve.

Infection correlation experiment. To support data suggesting that *Pythium* was the parasite of interest in the *Asplanchna* population, an artificial population of infected and uninfected animals was established using laboratory-infected and uninfected individuals

from laboratory cultures. Twenty animals were included for each of five data points including 0, 25, 50, 75 and 100% infected animals in the groups; for example, the 50% group contained 10 infected and 10 uninfected *Asplanchna*, and the 100% infected group contained 20 infected *Asplanchna*. DNA was extracted from each of these groups of 20 animals using the MoBio Powersoil extraction kit, and qPCR was utilised to assess the numbers of *Pythium* gene copies in each experimental group.

Culturing efforts

Attempts were made to culture the oomycete parasite based on published culturing techniques (Wolinska *et al*., 2008). Hemp seed baits with Sabaraud agar, potato dextrose agar, corn meal agar and V8 agar were used with and without choramphenicol. Crushed hemp seeds were autoclaved in clean water as well as in EPA freshwater medium (EPA, 2002). Infected animals (both alive and macerated) and mid-epidemic pond sediment and water samples were incubated in separate treatments in autoclaved hemp seed suspensions at 20 °C. Hemp seeds that developed signs of infection (halos) were transferred, via sterile forceps, to fresh plates (media listed earlier) and incubated at 20 °C. Growth on plates was evaluated via microscopy and species-specific PCR to screen for the *Pythium* identified from infected *Asplanchna*. In addition, peptone yeast-extract glucose seawater agar (PYGSA) and glucose yeast-extract seawater agar (GYSA) were modified for freshwater organisms and culturing was attempted, according to Hatai & Lawhavinit (1988). As a positive control for our infection protocols, we used the same techniques to grow an oomycete parasite isolated from infected amphipods (Kestrup *et al*., in press).

Laboratory studies

We quantified the virulent effects of the oomycete on survival, fecundity and population growth rate of *Asplanchna* using field-collected individuals. We measured effects on population growth rate by placing single infected ($n = 18$) or uninfected ($n = 30$) *Asplanchna* in wells of two 24-well tissue culture plates. We intentionally included more 'uninfected' individuals at the start, expecting some of these actually to be early-stage infections. Indeed, seven of the 'uninfected' individuals later developed signs of infection. These individuals were treated as infected in the final analysis, yielding sample sizes of 25 infected and 23 uninfected individuals. Infected and uninfected individuals of similar sizes were chosen and were distributed haphazardly among the wells of the two plates. Food (1 mL concentrated *Paramecium bursaria* Ehrhart) was added to each well initially; on subsequent days, each well was fed 0.5 mL concentrated *P. bursaria*. The plate was incubated at 23 °C and 12 : 12 light : dark. The number of *Asplanchna* per well was counted daily for 8 days.

Population growth rate integrates effects on survival and fecundity. To determine the separate effects of infections on survival and fecundity, we conducted a second experiment. The set-up was identical to the first experiment, except in numbers of replicates (infected: $n = 8$; uninfected: $n = 15$). Survival and the number of offspring were monitored daily until all individuals had died; offspring were removed from wells daily to avoid confusing them with their mothers. Offspring from infected mothers were placed in separate wells and monitored for 6 days to determine whether they too became infected.

Results

Field infection patterns

Infected *Asplanchna* are distinguished by hyphae growing throughout the body cavity (Fig. 1) and occasionally protruding through the body wall. Sporangia and zoospores of the parasite were observed (Fig. 1) and are morphologically similar to those produced by species by the genus *Pythium* (Webster & Weber, 2007).

There were three epidemics of the oomycete over the course of 1 year (Fig. 2a). Peak infection prevalence ranged from 29 to 41% and epidemics lasted *c.* 17–56 days. Epidemics occurred at high *Asplanchna* densities (Figs 2 & 3, $r = 0.59$, $P < 0.0001$), though the relationship appears to be non-linear. Host density declined during the first and second epidemics, but remained high throughout the third epidemic (Fig. 2b).

Infected *Asplanchna* were significantly larger than uninfected animals ($F_{1,254} = 6.31$, $P = 0.013$); the mean size of infected *Asplanchna* was 701 μm [95% confidence interval (CI): 666, 735 μm] compared with

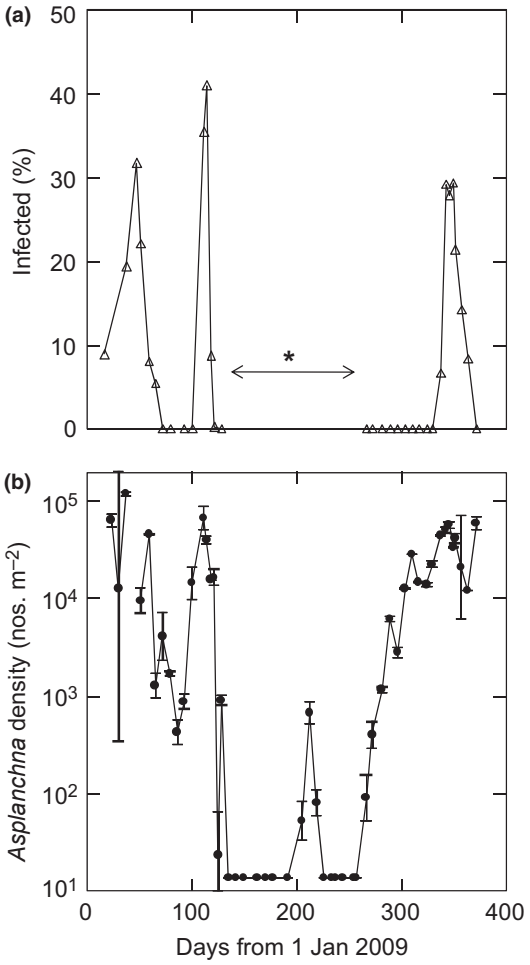

Fig. 2 Dynamics of oomycete infections and density of *Asplanchna*. (a) % Infected *Asplanchna* (infection prevalence could not be accurately measured between days 135 and 257, indicated by double-headed arrow and asterisk, because of very low *Asplanchna* density). (b) Density of *Asplanchna* (mean ± SE). One-half the lowest observed density (13.55) was added to all estimates to allow for a log-scale.

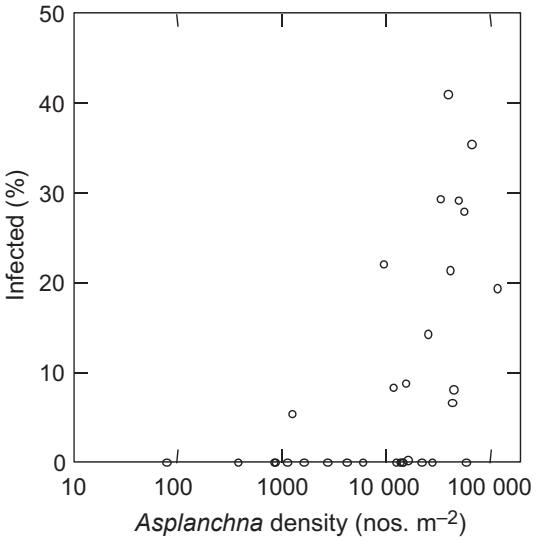

Fig. 3 Relationship between *Asplanchna* density and prevalence of oomycete infections in the *Asplanchna* population. It was not possible to accurately determine infection prevalences when *Asplanchna* densities were very low, so these data are not plotted.

607 μm (95% CI: 589, 625 μm) for uninfected hosts. The rotifers were larger in 2009 than 2008 ($\chi^2 = 3.8$, $P = 0.051$); the mean length was 572 μm (95% CI: 555, 590 μm) in 2008 and 738 μm (95% CI: 718, 758 μm) in 2009. There was no significant interaction between year and infection ($\chi^2 = 0$, $P = 1.0$).

Molecular identification

Of the 37 18S rRNA gene clones analysed from infected *Asplanchna*, 32 were identified as belonging to the same oomycete species, according to BLAST analysis. The remaining five sequences were rotifer sequences. Forty-three clones were sequenced from the LSU clone library, of which eight were oomycete sequences belonging to the same species and the remaining 35 were rotifer sequences. Finally, owing to low transformation efficiency, only two clones were obtained from the ITS cloning, and both of these were oomycete sequences belonging to the same species. As all of the DNA in the *Asplanchna*–parasite consortium belonged to either *Asplanchna* or an oomycete, these data confirm preliminary morphological observations, suggesting that the parasite was an oomycete. Phylogenetic analysis of the 18S rRNA gene, the LSU gene and ITS sequences indicated that the parasite should be classified as a *Pythium* sp (Figure S1). BLAST analysis demonstrated that the *Asplanchna* parasite is closely related to clone sequences of *Daphnia* parasites (the *Pythiaceae* sp. PHY2 strains, with 98 and 96% 18S rRNA gene and ITS sequence identity, respectively; Wolinska, Giessler & Koerner, 2009). Additionally, the sequences are closely related to those of *Pythium capillosum* Paul and *Lagenidium myophilum* Hatai & Lawhavinit.

The sequence alignment of the 18S rRNA gene was used to design specific primers for traditional and quantitative PCR (qPCR) to verify future identification of parasites (Table 1). According to BLAST analysis, the most characteristic region of the 18S

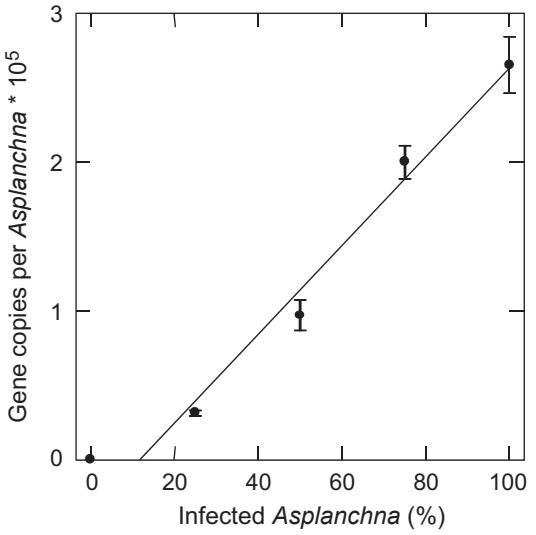

Fig. 4 Correlation between the infection prevalence in artificially constructed *Asplanchna* assemblages (20 individuals per assemblage) and the abundance of 18S rRNA gene copies of *Pythium* sp.

rRNA gene is complimentary to primer sequence Pyth664Fwd (Table 1). To ensure that *Pythium* is the parasite (and not simply associated with *Asplanchna*), we created artificial consortia with known numbers of infected and uninfected *Asplanchna*. The proportion of infected individuals in the sample was strongly correlated with the number of gene copies of the target *Pythium* sp. 18S rRNA gene sequence ($R^2 = 0.96$, $P < 0.0001$; Fig. 4) indicating that *Pythium* is the parasite. The qPCR analysis also indicated that an infected animal contains, on average, about $2.65 \times 10^5 \pm 1.5 \times 10^4$ parasite gene copies.

Cultivation attempts

While many fungal and oomycete species were cultivated in association with field-collected infected *Asplanchna*, PCR analyses with specific primers indicated that none of them matched the parasite. Attempts at cultivating a different oomycete parasite (isolated from infected amphipods) were successful, indicating that the *Asplanchna*-associated species is recalcitrant to cultivation methods that have worked for other zooplankton (Wolinska *et al*., 2008) and crustacean (Kestrup *et al*., in press) hosts. Cultures can be maintained in the laboratory indefinitely (at 10–20 °C) by adding uninfected rotifers to cultures containing infected individuals (M.A. Duffy unpubl. data).

Laboratory studies of virulence

Pythium infection decreased the growth of *Asplanchna* populations in the laboratory (Fig. 5a). Population sizes at the end of the experiment were significantly different (uninfected treatment: 95% CI: 10.5, 18.0; infected treatment: 95% CI: 2.0, 7.7). Additionally, only 9% (2/23) of the uninfected populations went extinct, while 52% (13/25) of the infected populations went extinct. *Pythium* infection reduced host survival and fecundity (Fig. 5b,c). Reproduction was significantly lower in the infected treatment (95% CI: −0.14, 0.64) when compared with the uninfected treatment (95% CI: 4.1, 6.5). Offspring were removed daily and maintained separately; none became infected, suggesting that *Pythium* is horizontally, rather than vertically, transmitted.

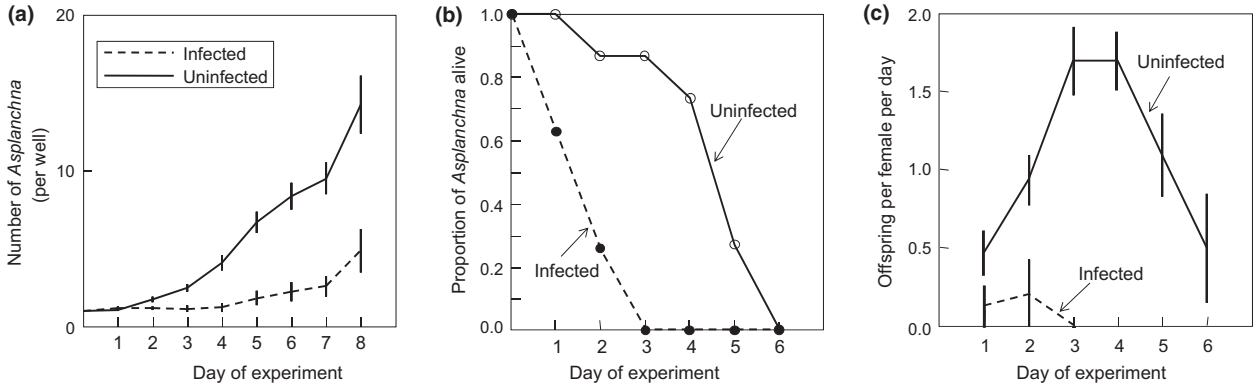

Fig. 5 Effects of *Pythium* sp. on (a) population size (±SE), (b) survival and (c) fecundity (mean offspring per female ± SE). Infected (dashed line) and uninfected (solid line) animals were collected from Lake Clara Meer.

Discussion

Here, we report multiple epidemics of an oomycete parasite in a lake population of the rotifer *A. girodi* in a single year. We used molecular approaches to demonstrate that this parasite is in the genus *Pythium*. We show that this *Pythium* species is highly virulent, reducing fecundity and population growth rate, and increasing mortality rates of infected individuals. Based on the frequency of epidemics and the virulence of the parasite, we propose that this parasite has the potential strongly to influence the ecology and evolution of *Asplanchna* populations.

Pythium infections have previously been reported in *Asplanchna priodonta* Gosse collected from the Volga Delta in Russia (Gorbunov & Kosova, 2001). This suggests that *Pythium* infections of *Asplanchna* may be widespread, yet overlooked. Our molecular analyses suggest that this parasite may be a novel species of *Pythium*, but further evaluation by oomycete taxonomists is warranted before such an assignment can be made. While supported by morphological and molecular evidence, the phylogenetic placement of our parasite is somewhat surprising, because most animal-pathogenic oomycetes are in the subclass Saprolegniomycetidae (Phillips *et al*., 2008). The subclass Peronosporomycetidae (which contains the genus *Pythium*) contains mostly plant parasites (Phillips *et al*., 2008), although recent studies report *Pythium* sp. infections of *Daphnia* in the United States and Europe (Wolinska *et al*., 2008, 2009). Further studies of *Pythium* pathogenic to animals could lead to interesting insights into how parasites pass from plant to animal hosts.

This *Pythium* parasite appears to be recalcitrant to cultivation, even when using techniques that succeed with closely related parasites (Wolinska *et al*., 2008). Therefore, and as is commonly the case (e.g. Jacomo, Kelly & Raoult, 2002; Rodrigues *et al*., 2008), we were unable to fulfil Koch's postulates (used to establish a particular organism as the cause of a disease; Black, 2008). Rather, we developed an alternative method to verify the identity of the parasite. We created artificial consortia of *Asplanchna* that varied in the prevalence of infected hosts. We then used quantitative PCR to verify that the identified ribosomal sequences increased with increasing prevalence of infected hosts, indicating that those sequences are associated with the parasite. This approach should be generally applicable to other plankton parasites that are difficult to cultivate outside their hosts.

The frequency of epidemics observed in this study was greater than that normally observed in *Daphnia* hosts (Wolinska *et al*., 2006; Duncan & Little, 2007; Duffy & Hall, 2008). The difference may be driven, in part, by the shorter generation time of *Asplanchna* and/or because this lake is warm (compared to the northern lakes in which *Daphnia* parasites have generally been studied). It appears that a high density of *Asplanchna* is required for epidemics to occur; infections have also been found to be most common at high host density in a different rotifer-parasite system (Miracle, 1977). However, the effects of epidemics on host density are unclear. Two of the three epidemics were associated with a large decline in host density, whereas it remained high in the third epidemic. Thus, experimental manipulations are required to determine whether *Pythium* drives host density changes in lake populations.

Even if the parasite does not always have effects on host density, it may still have large effects on host genetics and evolution (Yoshida *et al*., 2007). Such 'cryptic population dynamics' have been shown in *Daphnia* populations affected by a virulent yeast parasite (Duffy & Hall, 2008). Given the high virulence of this parasite, and the frequency and magnitude of epidemics, we suspect that it will affect the evolution of *Asplanchna*. *Asplanchna* has a fast generation time and a cyclically parthenogenetic life history, so this could make it an interesting system for studies of host–parasite coevolutionary dynamics and of the effects of parasitism on investment in sexual reproduction (such as those in Dybdahl & Lively, 1998; Duncan, Mitchell & Little, 2006; Decaestecker *et al*., 2007; Wolinska & Spaak, 2009).

For years, most limnologists have overlooked parasites and their potential impacts on zooplankton populations (Wetzel, 2001). This is unfortunate, because existing evidence suggests that parasites have the potential strongly to influence the density, evolution and interspecific interactions of their zooplankton hosts. Here, we report that epidemics of an oomycete parasite are common in a North American population of *Asplanchna*, and present evidence suggesting that this parasite is likely to be important to the ecology and evolution of *Asplanchna* populations. Given the importance of rotifers in freshwater systems (Wallace

& Smith, 2009), these effects could have consequences for the wider food web.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogenetic trees based on 18S, ITS and LSU gene sequences.

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