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Oomycete parasites in freshwater copepods of Patagonia: effects on survival and recruitment

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ABSTRACT: Copepods are hosts to various oomycete parasite species, but the effects of pathogens on copepod populations have rarely been studied. This study aimed to characterize oomycete infection in the freshwater copepod *Parabroteas sarsi* in a temporary pond in Patagonia (Argentina). A complete hydroperiod was monitored, evaluating environmental variables as influencing factors in the oomycete infections. Laboratory experiments were performed to evaluate the susceptibility of infected copepods to consumption by predators. Although 5 species of copepods were present in the pond, only ovigerous *P. sarsi* females were parasitized by oomycetes. Two species of oomycetes were always found together: *Aphanomyces ovidestruens* and *Pythium flevoense*. Infections were detected at water temperatures >20°C, with a positive relationship between temperature and parasite prevalence. Infection occurred after a decrease in large filter-feeder densities. The pathogens were not lethal to *P. sarsi* females in the short-term, but did produce mortality of entire egg sacs, thus negatively impacting subsequent recruitment. Mean prevalence of infection in females was 53%, reaching 83% in December. Females have the capacity to release an infected egg sac and generate a new one in a few days. This infection does not affect the susceptibility of *P. sarsi* to the predator *Notonecta vereertbruggheni*. The decrease in female abundance registered towards the end of the hydroperiod could be related to a combination of factors that may have a differential effect on female survival, such as increasing temperature, the energy cost of egg sac development and carrying and oomycete infection.

KEY WORDS: Parasitism · Oomycete · Copepoda · Life cycle · Planktonic predator · Patagonia

INTRODUCTION

In natural systems, aquatic organisms can be infected by many parasite species belonging to different taxa, such as bacteria, protozoans, microsporidians, fungi, helminths and oomycetes (Rasconi et al. 2011, Stentiford et al. 2013, Adlard et al. 2015). Parasitic species cause different diseases, and they may be species-specific or able to infect a wide spectrum of species (Sarowar et al. 2013). The effect of parasites on their hosts can include behavioral alterations, increased susceptibility to predators and decreased fecundity and/or life span (Bittner et al. 2002, Decaestecker et al. 2004, Duffy & Hall 2008, Wolinska et al. 2008), thus impacting host fitness and population dynamics. Over the last 10 to 15 yr, freshwater parasitology has emerged as an important field for testing ecological and evolutionary theory (Cáceres et al. 2014). In freshwater zooplankton communities, the role of parasitism has been evaluated through studies in cladocerans, especially in the genus *Daphnia* (Little & Ebert 1999, Duffy et al. 2010, Goren & Ben-Ami 2013). The consequences of these infections include altered migration patterns (Decae-

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stecker et al. 2004), increased susceptibility to predation (Duffy & Hall 2008) and bacterial disease (Tellenbach et al. 2007), reduced fecundity (Bittner et al. 2002) and shortened life span (Wolinska et al. 2008).

The oomycetes group of fungal-like parasites belonging to the Phylum Oomycota is one of the most widespread and diverse parasitic groups in freshwater communities (Beakes & Sekimoto 2009). Commonly called 'water molds', they superficially resemble true fungi, growing by hyphal extension, obtaining nutrients via absorption and reproducing sexually through oospores (Johnson et al. 2002). These parasites infect their hosts through short-lived zoospores that actively swim, attaching to the host and invading their tissues by hyphal growth. Sporangia eventually arise from these hyphae, producing the next generation of infectious zoospores (Ebert 2005, Valois 2015). Aquatic oomycete hosts include different phytoplankton and zooplankton species, as well as vertebrates such as fishes, amphibians and reptiles (Fisher et al. 2012, Gozlan et al. 2014). Oomycetes can be very virulent, but their presence is often difficult to determine. For example, oomycete infection in cladocerans is generally characterized by inconspicuous infection hyphae (Wolinska et al. 2008), which may explain the under-representation of parasites in most zooplankton surveys. Generally, oomycete infections in freshwater copepods are concentrated in the egg sacs of females and, unlike cladocerans, the infections are conspicuous, altering egg size and appearance through the development of hyphae which can be seen with the naked eye (Burns 1980, Valois 2015). Most oomycete infections known in cladocerans and copepods belong to the genera *Aphanomyces*, *Saprolegnia* (Order Saprolegniales) or *Blastulidium* (Order Leptomitales), with few species representing the genera *Pythium* and *Lagenidium* (Order Pythiales) (Miao & Nauwerck 1999, Wolinska et al. 2008, Thomas et al. 2011, Valois 2015).

Previous studies have reported oomycete infections in 3 genera of freshwater copepods: *Boeckella* in New Zealand (Burns 1980, 1985a, 1989), *Leptodiaptomus* in North America (Redfield & Vincent 1979) and *Eudiaptomus* in Europe (Gicklhorn 1923, Miao & Nauwerck 1999, Rossetti 2005). When the oomycete infects a copepod female, it remains as an epibiont until egg development begins, at which time the oomycete penetrates the egg sac and causes its degeneration (Miao & Nauwerck 1999, Rossetti et al. 2002), with negative consequences for reproduction (Rossetti 2005, Valois 2015) and population growth (Redfield & Vincent 1979, Burns 1985b), but rarely affecting the female's life span, as in the case of *Aphanomyces* sp. parasitizing *Boeckella dilatata* (Burns 1985a). Environmental variables, such as temperature and the presence of predators, can have important influences on seasonal patterns of parasite prevalence (Valois 2015, Valois & Burns 2016).

Oomycete infections in *Parabroteas sarsi* (Daday) (Calanoidea: Centropagidae) have recently been observed in a fishless pond in northwestern Patagonia. This predaceous calanoid copepod inhabits both shallow temporary fishless ponds and deep fish-inhabited lakes of Patagonia and Antarctica (De los Ríos & Rivera 2008, Garcia et al. 2013). This predator can access a broad variety of prey, including rotifers and cladocerans (Dieguez & Balseiro 1998, Laspoumaderes et al. 2010), and therefore plays an important role in structuring zooplankton assemblages (Vega 1998, Reissig et al. 2004). In turn, *P. sarsi* constitutes an important food source for the upper trophic level of fishless ponds, where it is a frequent prey species for turbellarians (e.g. *Mesostoma ehrenbergii*) and aquatic insects such as notonectids, belostomatids, diving beetles and Odonata larvae (Trochine et al. 2006, Jara et al. 2012, Jara 2014). Therefore, the development of oomycete infections in *P. sarsi* and their effects in terms of survival and reproduction could have a pronounced effect on the aquatic food webs of fishless ponds.

This investigation aimed to characterize oomycete infections in the freshwater copepod *P. sarsi*, evaluating some ecological variables (e.g. water temperature, filter feeder density and the presence of predators) as factors that can have a strong influence on seasonal patterns of parasite prevalence (Burns 1985a,b, Valois 2015, Valois & Burns 2016). For this purpose, infection prevalence in the *P. sarsi* population was monitored during an entire hydroperiod in a temporary freshwater pond in northwestern Patagonia. Laboratory experiments were carried out in order to evaluate the susceptibility of infected *P. sarsi* females to predation.

MATERIALS AND METHODS

Study system

Fantasma Pond is a temporary fishless pond located in North Patagonia, Argentina (41° 5.6′ S, 71° 27′ W, 794 m a.s.l). Annual rainfall in the area is 800 to 1400 mm, which largely determines the water regime of the pond. The hydroperiod of the pond generally lasts 8 to 9 mo (May to December), with maximum water levels registered in late autumn and

winter and a maximum depth of 2 m. Water temperature ranges from 4°C in July and August (winter) to 20°C in December (Vega 1999, Garcia 2010).

The zooplankton assemblage is dominated by 3 endemic centropagid calanoid copepods: 2 genera of *Boeckella* (*B. brevicaudata* and *B. gracilis*) and *Parabroteas sarsi*, which develops exclusively from dormant eggs to adulthood (Dieguez & Balseiro 1998, Vega 1999). *B. brevicaudata* is present during the first half of the hydroperiod, coexisting briefly with adults of *P. sarsi*, whereas *B. gracilis* appears during the second half of the hydroperiod, coexisting with adults of *P. sarsi* throughout its life cycle (Garcia 2010). The cyclopoid copepods *Acanthocyclops robustus* and *Tropocyclops prasinus meridionalis* are also present in the pond but in low densities. The cladocerans are represented by 3 species: *Simocephalus serrulatus*, *Ceriodaphnia dubia* and *Daphnia commutata*. Several species of rotifers and chydorids have also been registered there (Garcia 2010).

Fantasma Pond is the only Patagonian water body where *P. sarsi* reaches a high density (up to 1 ind. l^{-1}) (Laspoumaderes et al. 2010). The population of *P. sarsi* in this pond produces 1 generation yr^{-1} (univoltine), and adults do not coexist with nauplii or first copepodite instars (Garcia 2010). Preliminary experiments in our laboratory showed that healthy *P. sarsi* females carry the same egg sac for at least 1 mo, but whether they generate more than one egg sac within each hydroperiod is not known (R. D. Garcia pers. obs.). However, under specific circumstances, such as predator attacks, females can lose their egg sacs (R. D. Garcia pers. obs.).

Field sampling and laboratory analysis

A *P. sarsi* cohort was followed throughout the entire hydroperiod, with weekly sampling carried out from May 2009 to February 2010. Dissolved oxygen, conductivity and water temperature were measured *in situ* with a YSI 85 multiprobe, and pH was measured with a Hanna HI98150 probe. The maximum depth of the pond was also recorded. We obtained whole column zooplankton samples (from the water surface to a few cm above the sediment), using a Schindler-Patalas trap (12 l) in open water. The *P. sarsi* samples were filtered through a 200 µm mesh net and preserved in a 4% formalin solution. In the laboratory, samples were quantified under an Olympus SZ30 stereomicroscope using a 5 ml Bogorov chamber. Several developmental stages of *P. sarsi* were distinguished: nauplii, copepodites (CI, CII, CIII, CIV and CV) and adults (males, non-ovigerous females and ovigerous females). The sex ratio of *P. sarsi* was calculated from field samples.

With the appearance of ovigerous females of *P. sarsi* in the pond, live copepods were collected with a hand net and immediately carried to the laboratory and placed in pre-acid-washed polypropylene bottles. The prevalence of parasitism was determined by examining random samples of 50 to 200 *P. sarsi* adults on each sampling day. Infected eggs were easily recognized by the presence of a white 'cotton-like' appearance or shiny blue color due to the growth of mycelium (Hatai & Egusa 1979, Fernández-Benéitez et al. 2008). A subset of live females with signs of infection was separated for taxonomic determination of the oomycete species (see below). Size and weight of females and egg sacs (healthy/infected) were measured to distinguish morphometric differences. The number of eggs per clutch was counted in healthy females and in females with infected egg sacs (hereinafter, infected females) throughout the hydroperiod.

Parasite prevalence was estimated in 2 ways: 'prevalence in *P. sarsi* females' (number of infected females in relation to total *P. sarsi* females, as a percentage), and 'prevalence in *P. sarsi* population' (number of infected females in relation to total *P. sarsi* population, as a percentage).

During November and December 2014, healthy and infected females of *P. sarsi* were sampled and taken to the laboratory for experiments. Adults of a well-known predator of *P. sarsi*, the backswimmer *Notonecta vereertbruggheni*, were also collected from the pond and taken immediately to the laboratory for predation experiments.

Taxonomic determination of oomycete species

Oomycete samples were obtained from infected *P. sarsi* females. Water cultures of oomycetes were obtained and placed in sterilized Petri dishes with several halves of hemp *Cannabis sativa* seeds used as bait. The hemp seeds were added to each plate, where they floated on the surface of the water and were incubated at room temperature (15 to 20°C). After 1 wk, a single hypha was separated from the water mold colony growing on the seeds and transferred to a corn meal agar medium (CMA) to obtain an axenic culture. After 3 to 4 d, a piece of agar from the edge of each colony was cut off and placed in new sterilized Petri dishes containing sterilized water. For the identification of fungal isolates, vegetative characters and asexual structures, the main

colony features, typical mode of zoosporogenesis and spore discharge, and types of zoosporangia were considered at the genus level. At the species level regarding sexual characteristics, we considered type of oogonia, oospores and antheridial branches, and also the presence of ornamentation on the outer wall of the oogonia. Measurements and observations of these features were made with an Olympus BX 40 microscope equipped with phase contrast optics. Collection and isolation of oomycetes were carried out following the monographic contributions of Scott (1961), van der Plaats-Niterink (1981), Dick (2001) and Johnson et al. (2002).

Laboratory experiments

General setup

After sampling, the ovigerous females of *P. sarsi* and their predators (*N. vereertbruggheni*) were acclimated at 15 ± 1°C for 24 h before starting the experiments. The incubation of *P. sarsi* was carried out in 5 l bottles filled with filtered pond water, with the addition of natural co-occurring prey (rotifers, copepods and cladocerans). Backswimmers were incubated in 5 l flat plastic containers with pre-filtered pond water (50 µm mesh) in order to eliminate potential prey and ensure 24 h of starvation before setting up the experiments.

Short-term incubation

In order to evaluate differences between healthy and infected *P. sarsi* females over a short time period, 5 replicates of 6 healthy/infected ovigerous females were incubated for 8 d in 700 ml flasks filled with natural pond water. During incubation the *P. sarsi* females were fed ad libitum with natural co-occurring prey (rotifers, cladocerans, copepods and phytoplankton were added by pipette). The experiments were run in a temperature–light controlled incubator at 15°C and 12 h light:12 h dark photoperiod. All containers were checked every 24 h, and short-term survivorship of females and infection status of the eggs was evaluated visually.

Susceptibility to predation

In order to assess the vulnerability of *P. sarsi* infected females to invertebrate predation, a laboratory experiment was performed where 10 healthy or infected ovigerous females were exposed to 1 *N. vereertbruggheni* in a 700 ml flask filled with pre-filtered pond water (55 µm mesh). The treatment 'healthy females' was replicated 5 times whereas the treatment 'infected females' was replicated 9 times. A cylindrical wooden rod was placed in each flask to serve as a perch for the predator. Five containers without predators served as a control for the natural mortality of the prey. The experiments lasted 24 h and were run in a temperature–light controlled incubator at 15 ± 1°C and a of 12 h light:12 h dark cycle (simulating natural conditions). The exposure time was established following preliminary trials that showed that 24 h is the time that *N. vereertbruggheni* takes to consume 50% of the prey. After 24 h, live prey were recorded in each treatment and the predator's ingestion rate (IR) of *P. sarsi* was calculated following Hampton (2004), expressed as number of prey predator^{-1} h^{-1} (see below):

$$\mathrm{IR} = \frac{P_i - P_j}{t \times D_a} \quad (1)$$

where P_i and P_j are the initial and final number of prey, t is the exposure time and D_a is predator abundance.

Another set of experiments was carried out to evaluate the selectivity of backswimmers regarding infected/healthy females. For this purpose, 5 infected females and 5 healthy females were pooled in a 700 ml flask (as in the previous experiment) and incubated for 4 h. Nine replicates were conducted. The preference index, α, was calculated following Manly (1974) and Chesson (1978) as:

$$\alpha_i = \frac{R_i/N_i}{\sum_{j=i}^{K}(R_j/N_j)} \quad (2)$$

where N_i and N_j are the initial and final number of infected females, R_i and R_j are the initial and final number of healthy females and K is the number of prey species. α_i ranges between 0 and 1 and thus $\alpha = 0.5$ indicates neutral selectivity, α values >0.5 indicate positive selection and values <0.5 denote negative selection. This index is appropriate for experiments in which prey are not replaced after consumption.

Statistical analyses

All statistical analyses were conducted using the software SPSS v.9.0. Correlation analyses (Pearson correlation) were used to study the relationship between infected copepods, population density and water temperature. We used a *t*-test or Mann-Whitney *U*-test (depending on the data) for the mor-

phometric analysis of *P. sarsi*, and these tests were also used to compare *N. vereertbruggheni* IR of *P. sarsi*. A *t*-test was used to compare the mean Chesson's α value for healthy females with the expected value of α = 0.5 for a random prey selection. Normality and homoscedasticity were tested prior to the corresponding analysis.

RESULTS

Field study

During the study, the hydroperiod of the pond lasted 10 mo (May 2009 to February 2010). The maximum water levels were recorded in spring with values around 1.4 m, whereas minimum depths were registered in summer (0.4 m) (Table 1). At the beginning of the hydroperiod in autumn (May to June), the water level varied between 0.4 and 0.9 m. From winter to spring (June to December), sustained precipitation contributed to the increase and maintenance of the high water level, which ranged from 1.1 to 1.4 m. Finally, in summer (December to February) a steady decrease in the water level was observed, from 0.85 to 0.40 m. Water temperature varied markedly during the annual cycle, ranging from 3.4 to 21.9°C. The lowest temperatures were recorded in June (winter), when the pond surface was frozen, and the highest temperatures were registered in January (summer), coinciding with the lowest depths of the pond (Table 1). The pH values fluctuated around neutrality, and oxygen levels varied from 4 to 10 mg l^{-1}, with mean values of 7 mg l^{-1}. Conductivity ranged from 73 to 106 µS cm^{-1}, with the lowest mean value of 81 µS cm^{-1} recorded in summer (Table 1).

The predatory copepod *Parabroteas sarsi* had a univoltine reproductive cycle with the occurrence of adults 1 mo after the emergence of nauplii (hatched from dormant eggs at the beginning of the hydroperiod) (Fig. 1). This species had a longer life cycle than the other copepod species in the pond and was col-

Table 1. Mean (SE) limnological features of Fantasma Pond during the hydroperiod 2009–2010. Z_{max}: maximum depth; NA: not available

Season	Z_{max} (cm)	Water temperature (°C)	pH	Dissolved oxygen (mg l^{-1})	Conductivity (µS cm^{-1})
Autumn	67 (38)	5.6 (1.8)	6.8 (0.3)	5.2 (1.2)	NA
Winter	121 (12)	6.4 (2.6)	7.3 (0.2)	7.4 (0.8)	90.6 (6.6)
Spring	130 (14)	14.9 (3.5)	7.5 (0.2)	7.4 (1.5)	96.3 (7.8)
Summer	54 (16)	16.2 (2.5)	7.6 (0.4)	6.8 (1.5)	81.2 (6.5)

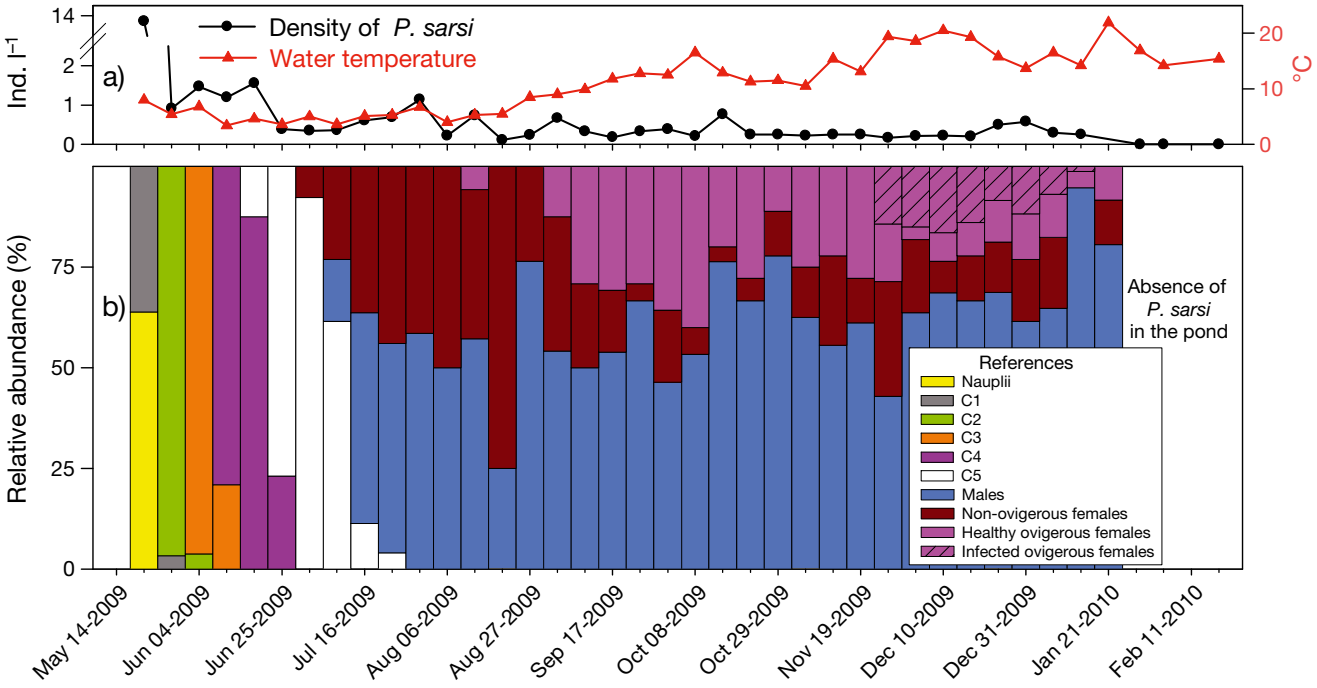

Fig. 1. (a) Water temperature and *Parabroteas sarsi* densities and (b) relative abundances of *P. sarsi* developmental stages throughout the hydroperiod in Fantasma Pond during 2009–2010. Empty dates indicate absence of this species in the pond. The prevalence of parasitism could not be measured on 21 January 2010. C: copepodite

lected during the entire flooding period, with densities between 0.1 and 13.5 ind. l^{-1} and a mean (±SD) value of 0.8 ± 2.2 ind. l^{-1}. The overlap of life history stages was low in *P. sarsi*, with only 2 different stages coexisting on any sampling date, one stage always being more abundant than the other. Males were slightly more abundant than females, especially from October onwards, when the population was composed of ~60% of males (Fig. 1). The sex ratio showed a clear predominance of males at the end of the hydroperiod (November, 5.7 males:4.3 females; December, 6.6 males:3.4 females). Remarkably, in the last 2 wk of the life cycle of *P. sarsi*, the relative abundance of females was <20%.

Daphnia commutata was present in the pond from June to early December, but its abundance was low (<0.5 ind. l^{-1}) until October, when it reached a level of 1 ind. l^{-1}. Its disappearance from the aquatic community coincided with the beginning of the oomycete infection (Fig. 2). *Ceriodaphnia dubia* was present from September to November with an abundance between 0.1 and 1.0 ind. l^{-1}, whereas *Simocephalus serrulatus* was present in low numbers (<0.5 ind. l^{-1}) from November to January, but increased in abundance at the end of the hydroperiod (January and February), once *P. sarsi* was absent (Fig. 2). Adults of *Notonecta vereertbruggheni* were present in the pond from spring (September) until the end of the hydroperiod, co-occurring with *P. sarsi* and presenting densities of <0.01 ind. l^{-1}.

The first infected females of *P. sarsi* were recorded in late November, when the water temperature of the pond reached 20°C (Figs. 1 & 2). Although 3 other species of copepods coexisted with *P. sarsi* at the time of infection (the calanoid *Boeckella gracilis* and the cyclopoids *Acanthocyclops robustus* and *Tropocyclops prasinus meridionalis*), only ovigerous females of *P. sarsi* were affected by the pathogen. The mode of zoosporogenesis, obtained through the culture of oomycetes from the isolated water mold, was identified as typical of *Aphanomyces ovidestruens* (=*A. scaber*) (Order Saprolegniales) and *Pythium flevoense* (Order Pythiales) (Table 2). Both species, which belong to Phylum Oomycota, were always found together in this study, so for this reason their prevalence and effects were evaluated together. Mean oomycete prevalence over time in the *P. sarsi* population was 19.5 ± 9.3% (assessed on the total number of adults), with a maximum prevalence of 31.9%

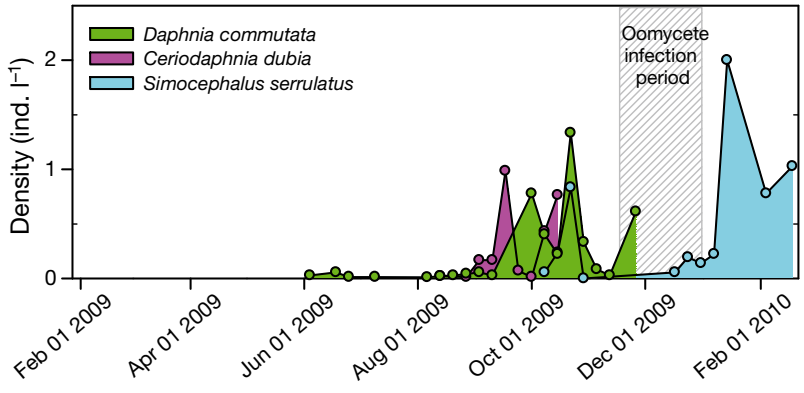

Fig. 2. Cladoceran densities throughout the hydroperiod in Fantasma Pond during 2009–2010. Oomycete infection period is shown in dashed pattern

Table 2. Main diagnostic features of the 2 parasitic oomycetes recorded in *Parabroteas sarsi*

	Aphanomyces ovidestruens	*Pythium flevoense*
Discharge mode	Achlyoid (apical clusters of primary zoospores)	Zoospores differentiated outside the sporangium. Sporangium contents discharged into a vesicle, in which the zoospores are differentiated
Sporangia	Filamentous, unbranched. Spores produced in a single row	Filamentous (non-inflated sporangia); with vesicle just before the discharge of zoospores
Flagellated zoospores	Dimorphic (primary lack flagella)	Monomorphic (secondary)
Oogonia type/size	Smooth and ornamentated ogonial wall (roughened to papillulate, crenulate, tuberculate, slightly spiny); 23–26 µm diam.	Smooth oogonial wall; 17–23 µm diam.
Oospore type/size	Single, eccentric (with a big central oil globule) brownish; 17–24 µm diam.	Single, aplerotic or sometimes nearly plerotic; 15–20 µm diam.
Antheridial branches	Diclinous or monoclinous	Diclinous
Colony type	Homothallic species	Heterothallic species

during December (Fig. 3). However, prevalence in *P. sarsi* females alone was much higher; the mean value was 53.0 ± 18.4 %, with high values (>50 %) from November to December (coinciding with the highest water temperatures), and reaching maximum prevalence (82.6 %) in December (Fig. 3). Therefore, both prevalence in *P. sarsi* females and prevalence in *P. sarsi* population showed a positive relationship with water temperature (Pearson correlation, prev. females: r = 0.78, p < 0.05, n = 10; prev. population: r = 0.81, p < 0.05, n = 10), but there was no significant correlation between prevalence of infected females and *P. sarsi* female density (Pearson correlation, r = 0.28, p = 0.43, n = 10), or between infection prevalence in *P. sarsi* population and host density (Pearson correlation, r = 0.19, p = 0.58, n = 10).

Morphometric analysis showed that the egg sacs of infected *P. sarsi* females were significantly larger than healthy ones (infected: 1.11 ± 0.13 mm, n = 8; healthy: 0.97 ± 0.08 mm, n = 19; Mann-Whitney *U* = 57.500, p = 0.004), but the healthy egg sacs were heavier than the infected ones (infected: 82 ± 34 µg, n = 23; healthy: 154 ± 23 µg, n = 15; *t* = –4.190, p = 0.002). The mean number of eggs in healthy females was 35 ± 7 eggs clutch^{-1} (n = 85) throughout the hydroperiod. However, it was not possible to count the eggs of infected females since the oomycetes cause individual eggs to lose their integrity and become indiscernible. No size difference was found between healthy and infected females (healthy: 5.39 ± 0.12 mm, n = 106; infected: 5.38 ± 0.14 mm, n = 31; *t* = 0.39, p = 0.695).

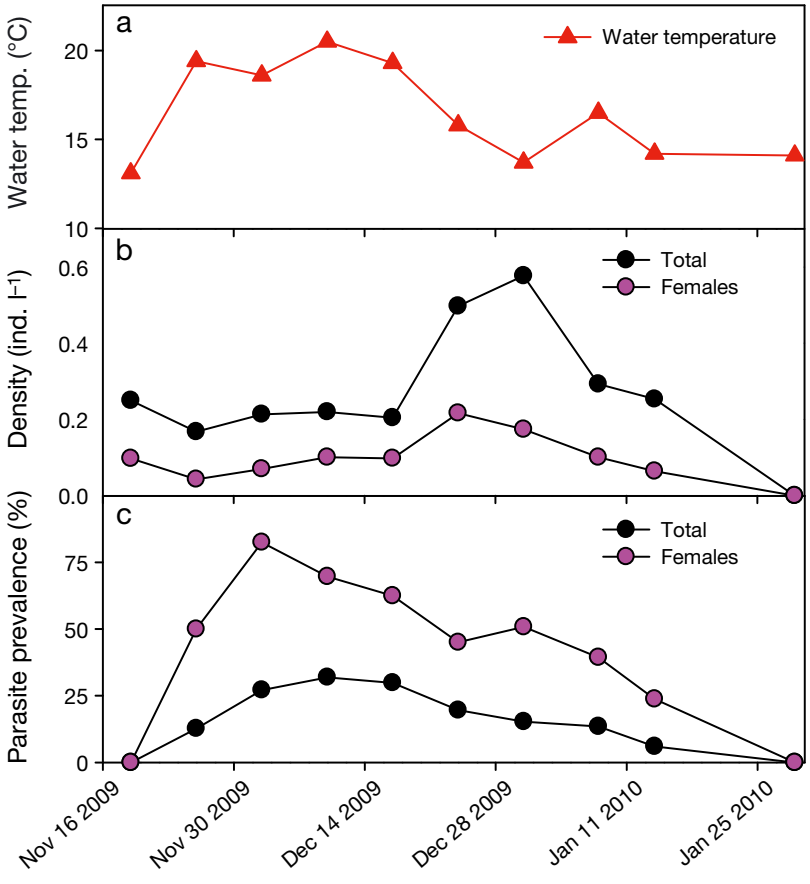

Fig. 3. (a) Fantasma Pond water temperature during oomycete infection period; (b) total and female densities of *Parabroteas sarsi* during oomycete infection period; and (c) parasite prevalence in *P. sarsi* females and in the *P. sarsi* population

Laboratory experiments

Short-term incubation

Female survival after 8 d incubation was 100 % in both healthy and infected individuals. This experiment revealed that infected *P. sarsi* females have the ability to release the infected egg sacs and generate a new one, without visual signals of infection, on the same day. In fact, in only 5 d of incubation, all the infected females released their infected egg sacs and then either generated a new one or remained as non-ovigerous females (Fig. 4). On the 6th day of incubation, one originally infected female released a recently regenerated egg sac which appeared visually healthy. No egg sacs were released in the controls consisting of healthy ovigerous females.

Predation experiments

In the predation experiments, *P. sarsi* survival was 100 % in the controls, indicating that natural mortality was negligible. The *N. vereertbruggheni* consumption rate of *P. sarsi* was similar for infected and healthy females (*t* = 0.86, p = 0.40), with a mean IR of 0.2 ± 0.1 prey pred.$^{-1}$ h^{-1} (Fig. 5a). The backswimmer *N. vereertbruggheni* showed no preference for infected or healthy females when both prey were offered simultaneously (1-sample *t*-test, *t* = 0.59, p = 0.56; Fig. 5b).

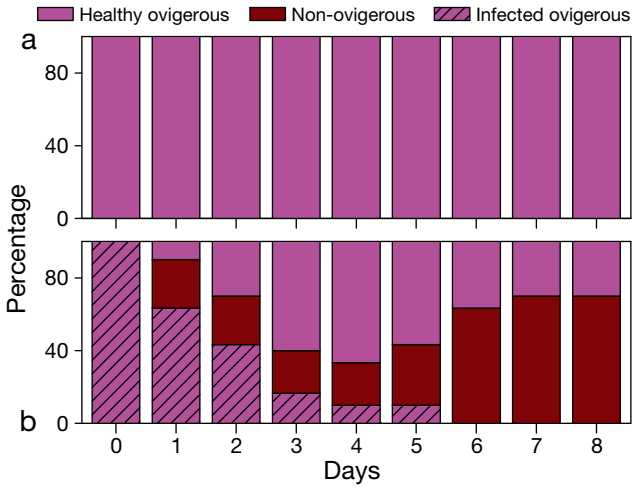

Fig. 4. Condition of *Parabroteas sarsi* females during short-term incubation. (a) Control; (b) females initially infected

DISCUSSION

Oomycete infection in Fantasma Pond occurred only in ovigerous females of *Parabroteas sarsi* from November until January, the end of the hydroperiod. Oomycetes infect the brood pouches of females, hence the infections occur only in ovigerous females (Valois 2015). In our study, oomycete infection was not detected in 4 other copepods that coexist with *P. sarsi* in Fantasma Pond. Due to its low tolerance to high temperatures (Garcia & Diéguez 2014), *Boeckella brevicaudata* was not present in the pond when the oomycetes infected *P. sarsi*. However, *B. gracilis*, *Acanthocyclops robustus* and *Tropocyclops prasinus meridionalis* did coexist with infected *P. sarsi* females, but were not infected by the pathogen. Infectious zoospores are postulated to be attracted by chemical signals from the live eggs (Lawrence 2000, Songe 2015) and/or by species-specific pheromones produced by female copepods for mate finding (Rossetti 2005). Therefore, it is possible that infectious zoospores are specifically attracted by *P. sarsi*-specific substances, which could explain the lack of infections found in other copepod species and the immature stages and adult males of *P. sarsi*. However, little is known regarding this aspect and further investigation is required on the subject.

Two oomycete species were found together parasitizing *P. sarsi* egg sacs. They were identified as *Aphanomyces ovidestruens* (=*A. scaber*), which is the first record of this parasite in American copepods, and *Pythium flevoense* (Table 2). Until now there have been no records of *Pythium* spp. in freshwater copepods, whereas the genus *Aphanomyces*, one of the earliest identified animal parasites in the Oomycota, was first documented in copepods in Germany in 1923 (Gicklhorn 1923). A summary of current reports of *Aphanomyces* spp. parasitizing freshwater copepods revealed a patchy distribution, with most records centering on the alpine regions of Italy, Austria and Germany; there are a number of records from Norway and New Zealand, but none in North or South American systems (Valois 2015). Oomycete infection was previously reported in Fantasma Pond in the endemic Patagonian anuran *Pleurodema thaul* (Anura, Leiuperidae) during late spring and early

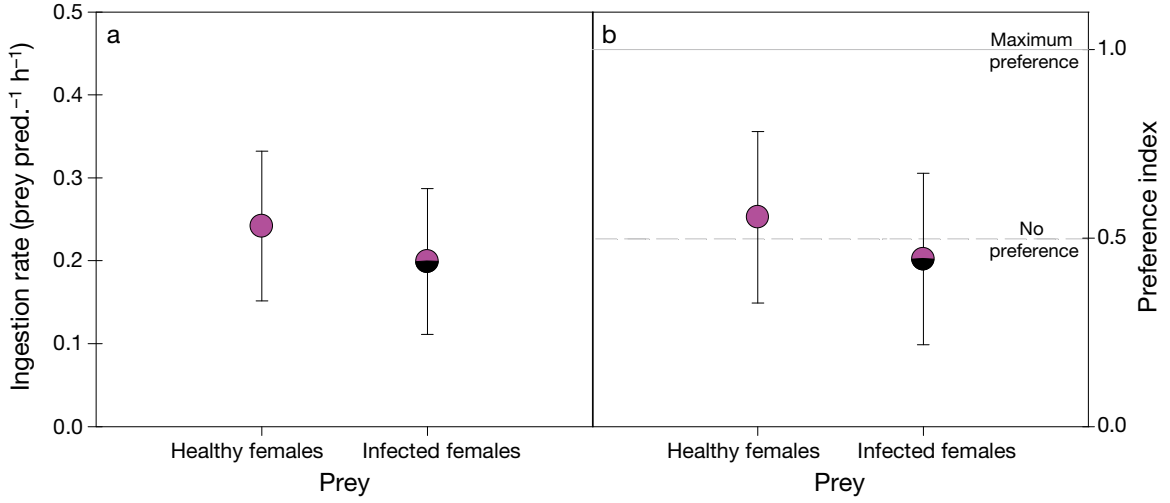

Fig. 5. (a) Ingestion rate of the backswimmer *Notonecta vereertbruggheni* preying on *Parabroteas sarsi* (healthy and infected females); (b) preference index (α) of *N. vereertbruggheni* for healthy (α) and infected (i–α) *P. sarsi* females, when exposed to both prey species together. Data are mean ± SD

summer (Perotti et al. 2013). However, we ruled out any potential interspecific infection in the pond since these anurans were infected by the species *Saprolegnia ferax*.

In our study, we found a mean oomycete prevalence of 53% in *P. sarsi* females, representing 19% of the total *P. sarsi* population (Fig. 3). This prevalence in the total *P. sarsi* population is similar to that found for *Eudiaptomus intermedius* attacked by *Aphanomyces* sp. in different water bodies (approx. 15%) (Rossetti 2005). Oomycete prevalence in zooplankton is highly variable (0.8 to 89%), with marked variation across space and time (Valois 2015). This wide variation has been related to differences in parasite virulence, host susceptibility and environmental variables, temperature in particular (Valois 2015).

Marked variation was observed in water temperature in Fantasma Pond during our study on the annual cycle, with values of ~3°C in winter and ~22°C in summer. Although the first ovigerous *P. sarsi* females appeared at temperatures of 9°C, no signs of infection were found until water temperature reached 20°C (Fig. 1). This, together with the positive correlation between prevalence of infected females and water temperature, indicates that temperature has a positive impact on parasite development. Temperature can alter host susceptibility, parasite virulence and the growth rates of both host and parasite (Mitchell et al. 2005, Hall et al. 2006, Perotti et al. 2013). Temperature may also play a key role in the dynamics of parasite species (Marano et al. 2008, Ruthig 2009) and had a significant effect on *Aphanomyces* sp. development, with increasing temperature favoring parasite growth and rapid zoospore production (Valois 2015). However, we cannot rule out other factors that may have led to the late appearance of oomycetes in Fantasma Pond. For example, the possibility of an immunity effect in younger females cannot be disregarded (Burns 1985a), or biological control of the infection due to infectious spores being grazed on by cladoceran filter-feeders (e.g. *Daphnia* sp.) (Valois & Burns 2016). In fact, the highest abundances of cladocerans *Ceriodaphnia dubia* and *Daphnia commutata* were recorded during October and November; they then disappeared from the pond in early December, coinciding with the appearance of infections in *P. sarsi* (Fig. 2). Therefore, since large cladoceran grazers like *Daphnia* can consume zoospores, leading to a decrease in infection rates (Valois & Burns 2016), we propose that *Daphnia* may act to control oomycete infections in the months before December. However, further investigations are necessary for a deeper understanding of this point. We reject the existence of a density-dependent mechanism as a trigger for infection (Decaestecker et al. 2002, Cáceres et al. 2014), since *P. sarsi* female density was similar before and after the infection occurred (around 0.1 ind. l^{-1}) (Fig. 3), and no correlation was found between density of *P. sarsi* females and prevalence of infected females.

The development of oomycete infections in copepods is easily recognized by changes in the external appearance of eggs due to growth of the mycelium. Oomycete development is characterized by hyphal growth and the development of sporangia, followed by the release of mobile zoospores (Rossetti et al. 2002, Rossetti 2005). We detected hyphae attached only to the egg sacs of *P. sarsi*, consistent with observations by Burns (1985a), Miao & Nauwerck (1999) and Rossetti et al. (2002). The pathogens were not lethal to *P. sarsi* females, at least in the short-term, but oomycete colonization produced mortality of entire egg sacs. The infected egg sacs were lower in weight (approx. 53% of the mean weight) but larger in size (approx. 14% of the mean diameter) due to hyphal growth, and in appearance clearly showed non-viable eggs. Therefore, the infection negatively impacts the next recruitment of *P. sarsi* by reducing the number of resistant eggs. Healthy *P. sarsi* females carry the same egg sac for at least 1 mo (Garcia 2010); however, our short-term incubation showed that infected females released infected egg sacs after a few days of incubation, ovipositing new sacs (without visual signals of infection) immediately thereafter (Fig. 4). In natural conditions, females without eggs were observed in the pond throughout the year. The reason for this could include late maturation of some females, or females that induced early release of eggs due to predator attacks (e.g. turbellarians and aquatic insects) or oomycete infection. Although further studies should be carried out to assess the viability of the new clutches of eggs, as well as the likelihood of reinfection under natural conditions, the reproductive potential of females seems not to be entirely jeopardized by infection, due to the behavior of releasing infected egg sacs.

The proportion of *P. sarsi* females in Fantasma Pond decreased at the beginning of spring (Fig. 1), coinciding with the appearance of the predatory insect *N. vereertbruggheni* in the pond (Jara et al. 2012). This parasitic infection can reduce swimming performance and increase visibility of parasitized females, which may be of importance when they are exposed to visual predators (Burns 1985b, Brown & Pascoe 1989). However, our predation experiments showed that infection did not affect the susceptibility

of infected *P. sarsi* females to *N. vereertbruggheni* predation (Fig. 5). Furthermore, no differences in the consumption of males and healthy females of *P. sarsi* by backswimmers were found, despite their difference in size (R. D. Garcia & F. G. Jara unpubl. data). For copepods, the proportion of males and females are expected to be almost equal. However, skewed sex ratios have been recorded in many populations related to selective predation on females, due to their larger size compared to males (Maly 1970, Hairston et al. 1983). Selective predation pressure by *N. vereertbruggheni* did not explain the decrease of *P. sarsi* females at the end of the hydroperiod; however, the effects of other factors could explain this. For example, temperatures >15°C can affect copepod survival (Hall & Burns 2001, Garcia & Diéguez 2014). In conjunction with temperature increases in spring, females have an extra energy cost due to the generation and retention of dormant eggs (Dahms 1995). Furthermore, the oomycete infection not only generates an additional cost due to re-development and oviposition of new egg sac, but it could also reduce the life expectancy of females in the long-term, as has been seen in other studies (Burns 1985a). In fact, it is possible that a combination of these factors imposes severe restraints on *P. sarsi* females from spring onwards, differentially affecting their survival in Fantasma Pond.

Our work represents the first report of oomycetes infection of South American copepods, representing an increase in the known geographical range of these parasites. Accurate identification of parasites is a critical starting point for wildlife management (Tompkins et al. 2015), but information on transmission, host specificity, pathogenicity and environmental tolerance is also necessary for the development of mechanisms to control potentially harmful parasites (Adlard et al. 2015). Further studies should try to follow the infectious process and its causal mechanisms, in combination with its effects on the survival of infected females, to make more accurate predictions about the impact and spread of these oomycete parasites.

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