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Early breeding protects anuran eggs from *Saprolegnia* infection

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Abstract Here, we studied the ecological significance of *Saprolegnia* infections ('saprolegniasis') on the survival and development of two populations of the endemic Patagonian anuran *Pleurodema thaul* (Anura, Leiuperidae). We found that four different *Saprolegnia* species infected eggs and embryos of *P. thaul*, indicating that the infection by these 'zoosporic fungi' was different in each anuran population and among different cohorts. Late anuran cohorts generally showed a higher incidence of infection than early cohorts, but we observed no clear overall pattern between populations. In addition, in laboratory experiments, we determined that some of the *Saprolegnia* species induce early hatching, and that hatching timing was variable between populations. In summary, we found that early breeding (by underlying priority effects) could improve the survival of the earliest cohorts of *P. thaul* by allowing them to survive the stress imposed by epidemic events of *Saprolegnia*.

Key words: breeding phenology, Patagonian anuran, priority effect, *Saprolegnia* infection.

INTRODUCTION

In general, most species that clearly reproduce in defined periods of the year link their phenology to that of other organisms and to the environment. The arrival of species or individuals occurs in a sequence that reflects the interspecific differences in breeding phenology, and arrival time can vary between and within years, affecting the temporal separation between species (Harris 1980; Semlitsch 1985; Caldwell 1987) and altering both the order of arrival of species and the temporal separation between the arrivals of the same species (by underlying priority effects). It has been proposed that arriving earlier is basically better for the offspring of several animals and that early breeding may be balanced against parent capability or environmental risks (Loman 2009). Then, if arriving early underlies advantages, we can consider ecological mechanisms as priority effects. Priority effects may be described as the advantage of early-arriving species or species that hatch sooner over late arrivals, mainly in structuring population and community dynamics (Paine 1977). However, the intensity and nature of interactions in a population can also be affected by changes in the abiotic environment. Therefore, priority effects could be seen as mechanisms improving the opportunities for a given species or cohort to survive potential stressors (both biological and environmental).

These changes in the phenology patterns (e.g. early breeding) could be important to understand the immediate and evolutionary responses of animal populations to perturbations, depending on how demographic parameters interact and how they are affected by extrinsic factors (Muths *et al.* 2011). Among extrinsic factors, emerging infectious diseases, which threaten mainly wildlife species whose population, habitat or distributional range has been reduced or modified (Daszak *et al.* 1999), have been reported as critical perturbations with consequences of death in free-living wild animals. Several emerging diseases affect organisms at different levels (Dhondt *et al.* 2005; Ozgul *et al.* 2009; Vredenburg *et al.* 2010; Wibbelt *et al.* 2010). However, although there is a link between emerging diseases and population persistence, few studies have addressed the relationships among demographic parameters to clarify how a disease affects a population (Muths *et al.* 2011).

Oomycetes ('water molds') are parasites that cause high levels of mortality in many groups of aquatic organisms such as invertebrates (Martin 1981; Cerenius *et al.* 1988; Oidtmann *et al.* 1999, 2004; Barron 2004; Ramaiah 2006; Wolinska *et al.* 2008) and vertebrates (Willoughby 1978; Noga 1993; Bangyeekhun *et al.* 2003; Robinson *et al.* 2003; Lategan *et al.* 2004;

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Zaror *et al*. 2004; van West 2006). Much attention has been given to their impact on economically valuable species such as salmonids, crayfish, shrimp, amphipods and oysters (Unestam 1973; Kiziewicz & Nalepa 2008; Phillips *et al*. 2008; Gouda & Moharram 2009), and to their negative effects on the aquatic stages of several amphibian species in Europe and North America (Banks & Beebee 1988; Beattie *et al*. 1991; Blaustein *et al*. 1994a; Kiesecker *et al*. 2001; Fernández-Benéitez *et al*. 2008; Romansic *et al*. 2009; Ruthig 2009).

Since amphibians are sensitive to changes in the habitat and the environment (Stebbins & Cohen 1997), many of them have experienced severe declines (Stuart *et al*. 2004; Blaustein & Dobson 2006). Based on this evidence, during the last four decades, many researchers have studied the factors involved in their decline (Pounds 2001; de Wijer *et al*. 2002; Mendelson *et al*. 2006; Raffel *et al*. 2006; Rohr *et al*. 2008). Among these factors, three pathogens have been recognized as very harmful to amphibians: the oomycete *Saprolegnia*, the fungus *Batrachochytrium dendrobatidis* and the ranavirus (Blaustein *et al*. 1994b; Daszak *et al*. 1999; Rachowicz *et al*. 2005). However, although *Saprolegnia* infections are spread in every habitat colonized by amphibians, their effect on demographic parameters on amphibian populations has received little attention (Kiesecker & Blaustein 1997; Blaustein & Kiesecker 2002; Fernández-Benéitez *et al*. 2008).

In the present work, we studied the effect of *Saprolegnia* fungi on two populations of the endemic Patagonian frog *Pleurodema thaul* (Anura, Leiuperidae) ('four-eyed frog'). The breeding period of *P. thaul* in northern Patagonia (Argentina) begins as soon as ponds thaw and the snow melts, starting in September and continuing until December. The reproductive cycle in this anuran is almost continuous, and changes in temperature and rainfall may influence its reproduction, as in other species of temperate regions (Díaz-Páez & Ortiz 2001). During reproduction, eggs are deposited in gelatinous strings in water, sometimes forming globular masses among the aquatic plants of lagoons (Duellman & Veloso 1977; Cei 1980; Jara 2010). This species can use any body of water, but is usually observed inhabiting shallow ponds and tolerating a broad range of habitats.

During our previous field monitoring, we observed water molds growing on different organisms such as insects and zooplankton, as well as on *Pleurodema* eggs, but we could not determine whether water molds act as pathogens of the amphibian or only colonize eggs when they are dead. On the other hand, no 'zoosporic fungi' have been identified in these southern temperate lagoons so far. Thus, to determine the ecological role of water molds in the population dynamics of *P. thaul* in two wetlands of north-western Patagonia (Argentina), we combined field surveys and laboratory experiments. Our aims were: (i) to determine the water mold species infecting eggs and embryos of *P. thaul*, (ii) to analyse the proportion of infected eggs and (iii) to determine whether temporal separation among the reproductive events (underlying priority effects) through the anuran reproductive season could prevent massive anuran mortality by fungal outbreaks. In the laboratory, we studied the susceptibility of embryos to zoosporic fungal infection (mortality) and the hatching success. Finally, we tested whether anuran embryos respond to this fungal infection by hatching early. We predicted that different cohorts of *P. thaul* may have different mortality rates caused by *Saprolegnia* infections over the breeding period, depending on the temperature of the pond and the requirements of the aquatic fungus.

MATERIAL AND METHODS

Field surveys

Pleurodema thaul is a common Patagonian frog distributed around the Andes slopes in Argentina and Chile (Correa *et al*. 2007). In the most southern distribution (in Argentina), it inhabits *Nothofagus* forests and transitional areas between the Andean forest and the Patagonian steppe. *Pleurodema thaul* deposits the eggs through a gelatinous string around vegetation in the wetland shores. Clutches are individual masses of eggs, and sometimes the female deposit these egg masses close enough to find several clutches developing together. Eggs have a rapid development in the field, hatching in around 1 week.

We studied two shallow temporary lagoons (Fantasma lagoon and Mallín Cerro Otto) inhabited by *P. thaul* in the proximity of San Carlos de Bariloche city (Río Negro Province, Argentina). These ponds differ in their size and hydroperiod. Their hydroperiod is concentrated between May and January (autumn through summer in the Southern Hemisphere). The hydroperiod of the Fantasma lagoon is regulated by autumn rainfall and winter snowfall and lasts between 8 and 9 months (41°05′S, 71°27′W, 780 m.a.s.l., approximately 10 000 m^2), whereas that of the Mallín Cerro Otto is powered by a peat bog and lasts between 6 and 7 months (41°7′S, 71°22′W, approximately 200 m^2).

To study the 'zoosporic fungi' infecting eggs and embryos of *P. thaul*, eggs and 'zoosporic fungi' were collected from these two wetlands, about 10 km from each other, during the reproductive season of the anuran. From September to December 2009, we weekly performed field monitoring to measure amphibian arrival at the ponds through counting the number of new clutches and fungus prevalence. We also counted the total number of clutches and, for each clutch, we took a photo to determine the total number of eggs and the total number of healthy and infected eggs (alive or killed by the fungi). After counting, the eggs were returned to the ponds. Water temperature was recorded with temperature recorders (Thermochron iButton).

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Isolation, culture and determination of *Saprolegnia*

To determine the diversity of water mold species, we collected *P. thaul* eggs with signs of saprolegniasis from the two ponds. The infected eggs were easily recognized because they have a 'cotton-like' appearance due to the growth of the *Saprolegnia* mycelium (Hatai & Egusa 1979; Fernández-Benéitez *et al*. 2008).

Samples were placed and distributed in water culture in sterilized Petri dishes containing several halves of hemp seeds (*Cannabis sativa*) and incubated at room temperature (15–20°C). After growth of the water mold on the seeds, a single hypha was isolated and transferred to corn–meal–agar medium (CMA) to obtain an axenic culture. After 3–4 days, a block of agar from the edge of each colony was cut off and placed in sterilized Petri dishes containing sterilized water. Several preparations were made for each sample and the zoosporic organisms were identified using the vegetative organs (shape and size of the hypha), the asexual organs (shape of zoosporangium and spores) and the sexual organs (structure of the oogonium and antheridium). Observations and measurements were made with an Olympus BX 40 microscope (Olympus Optical Co. Ltd, Tokyo, Japan) equipped with phase contrast optics. For collection and isolation of straminipilous organisms, we followed Seymour (1970), Seymour and Fuller (1987) and Dick (2001).

To identify fungal isolates, we considered the typical mode of zoosporogenesis and their characteristic sexual features (types of oogonia, oospores and antheridial branches).

Pre-hatching infection experiments

To evaluate susceptibility to infection (mortality) and hatching success, we performed an experiment for each population (Fantasma lagoon and Mallín Cerro Otto). Experiments consisted of a single design with fungal species as a fixed factor. We used the four isolated pure cultures of *Saprolegnia* for laboratory pathogenicity tests. All of them were isolated from infected eggs collected from the respective locations under study. *Saprolegnia ferax* and *Saprolegnia* sp. were used for the eggs from Fantasma lagoon (after isolation and determination), and *Saprolegnia diclina* type 3 (according to Diéguez-Uribeondo *et al*. 2007) and *S. ferax* for the eggs from Mallín Cerro Otto pond (as previously explained). Ten sterilized hemp seeds were used both in the control and fungus treatments, and to facilitate pathogen development, a section of the fungal culture was added in each of the treatment replicates belonging to one of the *Saprolegnia* species. Each treatment group was replicated 20 times (60 experimental units, 240 individuals per population). All the experiments were checked once a day. The numbers of healthy, dead and hatching embryos in each replicate were recorded. The time at which embryos were infected or died was also recorded. The experiment concluded when all the embryos had either died or hatched.

Different analytical procedures were applied to compare the fungal effects from the field and among experimental results. To compare the proportion of infected eggs along the reproductive season in each of the locations studied, a *t*-test was performed. Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks was applied to evaluate the effect of *Saprolegnia* species on the mortality of *P. thaul* embryos in the laboratory experiments. When significant effects of main factors were found, post-hoc multiple comparisons were performed applying Dunn's test. To compare the effect of fungal infection on hatching time across the experiment, we performed a two-way ANOVA with *Saprolegnia* species and days (after experimentation) as fixed factors. When significant effects of main factors were found, post-hoc multiple comparisons were performed applying a *t*-test (Zar 1999).

RESULTS

Water mold species

Following observation of the typical mode of zoosporogenesis, the water mold isolates were identified as *S. ferax* and *Saprolegnia* sp. for Fantasma lagoon and as *S. diclina* type 3 (according to Diéguez-Uribeondo *et al*. 2007) and *S. ferax* for Mallín Cerro Otto.

Saprolegnia infection in the field

The proportion of infected eggs showed significant differences between populations (localities) and among sampling months over the season (population: $F = 29.79$, $P < 0.001$; month: $F = 34.92$, $P < 0.001$), although the interaction was not significant ($F = 0.18$, $P = 0.91$). There were also significant differences between the locations in each of the months (September, $t = 5.76$, $P < 0.001$; October, $t = 3.61$, $P < 0.001$; November, $t = 3.03$, $P = 0.003$; December, $t = 2.1$, $P = 0.04$). In general, clutches were more infected during late spring and early summer (October to December, late cohorts), in coincidence with high mean temperatures (Fig. 1).

In Fantasma lagoon, *Saprolegnia* species were present in approximately 90% of the clutches and, when present, killed 54% of the eggs, on average. These results varied over the reproductive season. In this pond, the proportion of infected eggs was significantly different across months (Kruskal–Wallis, $H = 45.29$, $P < 0.001$), with the lowest proportion of infected eggs in September (Fig. 1). Surveys in Mallín Cerro Otto showed the same pattern as that observed in Fantasma lagoon with *Saprolegnia* species present in 93% of the clutches and killing approximately 34% of the eggs, on average. Also, September was the month with the lowest proportion of infected eggs and marginal differences across months ($P = 0.05$; Fig. 1).

Saprolegnia infection experiments

Mortality across the experiments of infection by *Saprolegnia* species showed differences according to the

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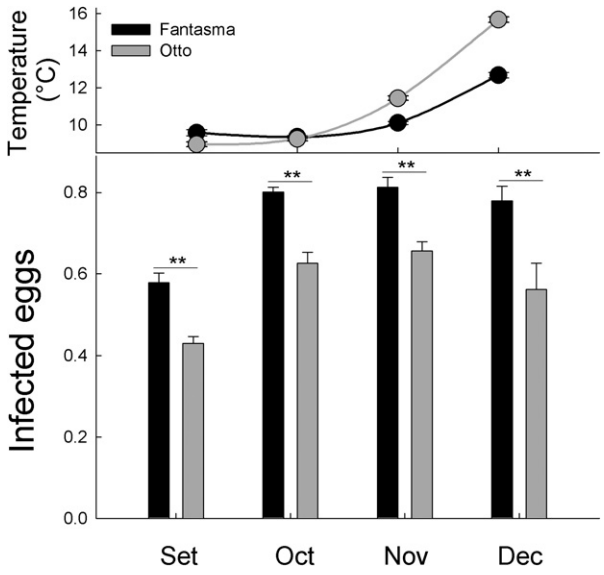

Fig. 1. Proportion of infected eggs (mean ± SE) of *Pleurodema thaul* from two populations, Fantasma lagoon and Mallín Cerro Otto, during the reproductive season (September and December 2009). The upper plate shows mean water temperatures in the wetlands. The asterisks denote statistical significance.

zoosporic fungus treatment. *Pleurodema thaul* eggs from Fantasma lagoon inoculated with *Saprolegnia* sp. and *S. ferax* showed that mortality was significantly different between the fungal treatments (Kruskal–Wallis, $H = 38.44$, $P < 0.001$). Post-hoc analysis showed differences both between *Saprolegnia* sp. and *S. ferax*, and between the fungal treatments and the controls (Dunn's test, *S. ferax vs. Saprolegnia* sp., $Q = 3.80$, $P < 0.05$; *Saprolegnia* sp. *vs.* control, $Q = 8.31$, $P < 0.05$; *S. ferax vs.* control, $Q = 5.10$, $P < 0.05$) (Fig. 2a). Approximately 50% of the individuals died in the *Saprolegnia* sp. treatment, whereas approximately 20% of the eggs died in the *S. ferax* treatment. In particular, *Saprolegnia* sp. caused the highest mortality levels on days 3 and 4 of the experiment, with significant differences with respect to day 1 ($P < 0.001$). The highest levels of mortality observed in the *S. ferax* treatment were observed on days 2, 3 and 4 ($P < 0.001$; Fig. 2a). Furthermore, *P. thaul* eggs from Mallín Cerro Otto exposed to *Saprolegnia* fungi showed significant differences between the fungal treatments (Kruskal–Wallis, $H = 21.11$, $P < 0.001$). Post-hoc analysis showed differences between the fungal and control treatments (Dunn's test, *S. ferax vs. S. diclina*, $Q = 2.64$, $P < 0.05$; *S. ferax vs.* control, $Q = 0.71$, $P < 0.05$; *S. diclina vs.* control, $Q = 3.31$, $P = 1$) (Fig. 2b). Only *S. diclina* type 3 caused almost approximately 40% of the mortality observed on days 2 and 3 of the experiment, whereas no mortality was observed for the eggs exposed to *S. ferax* ($P < 0.001$; Fig. 2b).

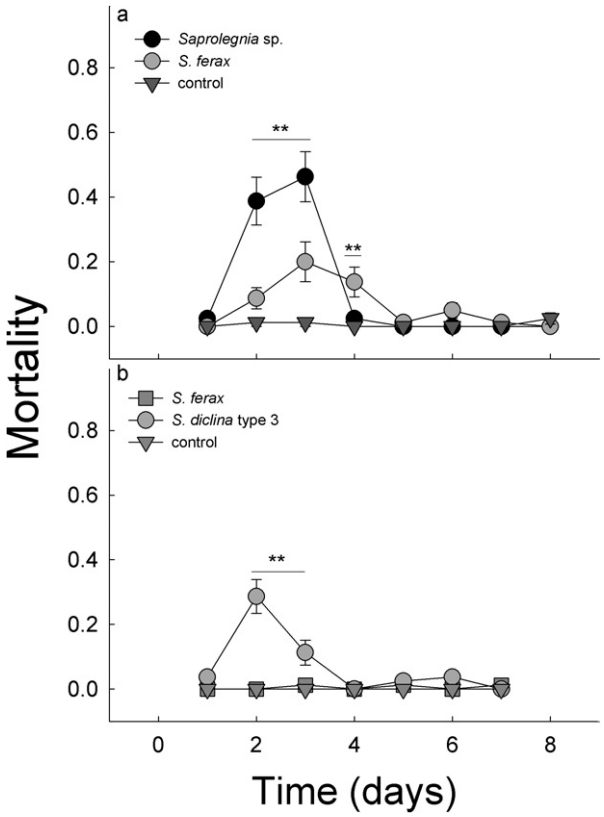

Fig. 2. Mortality during the course of the experiment (proportion of dead individuals) from individuals exposed to *Saprolegnia* water molds: (a) Fantasma lagoon and (b) Mallín Cerro Otto (mean ± SE), n = 240 individuals per population. The asterisks denote statistical significance.

The hatching time of eggs from the Fantasma lagoon population was significantly different between the fungal treatments and days (treatments, $F_{2,179} = 22.85$, $P < 0.001$; days, $F_{2,179} = 42.72$, $P < 0.001$), and the interaction was also significant ($F_{4,179} = 12.13$, $P < 0.001$). Individual comparisons showed differences in hatching time between the *S. ferax* treatment and the control ($t = 2.39$, $P = 0.018$), with embryos exposed to *S. ferax* infection hatching 24 h before those of the control treatment. Also, we found differences in hatching time between the fungal treatments (*S. ferax vs. Saprolegnia* sp., $t = 3.79$, $P < 0.001$). The highest proportion of hatching embryos was observed in the control treatment (approximately 80%), while the hatching success in the fungal treatments varied (*S. ferax* approximately 50%, *Saprolegnia* sp. approximately 10%). *Saprolegnia ferax* accelerated hatching approximately 12% compared with the control treatment (Fig. 3a). Differences in hatching time were mainly after days 6 and 7 of development. On day 6, the first hatchings were observed in the *S. ferax* treatment (control *vs. S. ferax*, $t = 2.39$, $P = 0.018$; *S. ferax vs. Saprolegnia* sp., $t = 3.79$, $P < 0.001$), while on day 7 hatching success was high in all the

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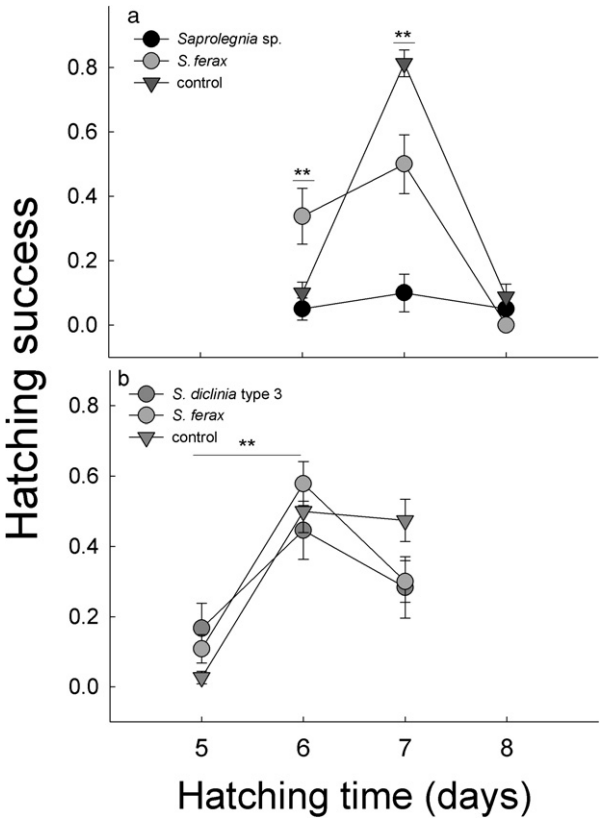

Fig. 3. Hatching success during the course of the experiment (proportion of hatched individuals) from individuals exposed to *Saprolegnia* water molds: (a) Fantasma lagoon and (b) Mallín Cerro Otto (mean ± SE), n = 240 individuals per population. The asterisks denote statistical significance.

treatments, but with significant differences among them (control *vs. Saprolegnia* sp., $t = 8.69$, $P < 0.001$; control *vs. S. ferax*, $t = 3.49$, $P < 0.001$; *S. ferax vs. Saprolegnia* sp., $t = 5.19$, $P < 0.001$) (Fig. 3a).

Eggs from the Mallín Cerro Otto population exposed to fungal infection showed significant differences in hatching time among days but not between fungal treatments (days, $F_{2,176} = 42.87$, $P < 0.001$; fungal treatments, $F_{2,176} = 2.28$, $P < 0.1$). The interaction was also significant ($F_{4,176} = 3.60$, $P < 0.008$). Individual comparisons showed no significant differences between the fungal treatments and the controls (*S. diclina vs.* control, $t = 1.67$, $P < 0.096$; *S. ferax vs.* control, $t = 1.35$, $P < 0.18$; *S. diclina vs. S. ferax*, $t = 0.32$, $P < 0.75$). Almost all the embryos hatched on days 6 and 7 after oviposition, although a high proportion of embryos hatched on day 6 in all the treatments (Fig. 3b). On day 6, the highest proportion of hatching was observed in the *S. ferax* treatment (approximately 60%), while in the *S. diclina* type 3 and control treatments, hatching success was slightly lower (approximately 50%). Both fungal treatments had the highest proportion of hatching on day 6 ($P < 0.001$), while in the control treatment the hatching success on days 6 and 7 was similar ($P < 0.85$) (Fig. 3b).

DISCUSSION

Both our field studies in two ponds (Fantasma lagoon and Mallín Cerro Otto) of north-western Patagonia (Argentina) and our experimental studies showed that *Saprolegnia* infections have strong effects on different populations of the endemic anuran *P. thaul*. We determined that the mortality rates were variable depending on the anuran population and fungal infection. We identified three different species of *Saprolegnia*: *Saprolegnia* sp., *S. diclina* type 3 and *S. ferax*. Water molds infected clutches of *P. thaul* in the field at levels approximately 40% on average, a value close to the mortality values observed in the experimental trials (46% and approximately 30%).

The virulence of *S. diclina* towards amphibians has been demonstrated only in two anuran species in the Northern Hemisphere (*Pelobates cultripes* and *Bufo calamita*) (Fernández-Benéitez *et al.* 2008, 2011), while *S. ferax* is a widespread pathogen affecting many amphibian species (e.g. Kiesecker & Blaustein 1999).

We found that fungi induced early hatching in *P. thaul* embryos. Similar results have been described in several North American and European anuran species (Gomez-Mestre & Buchholz 2006; Touchon *et al.* 2006; Uller 2008; Uller *et al.* 2009). Also, we observed variation in both the pathogenicity of the fungi and the embryonic hatching response to different pathogen strains. However, this variability does not modify our conclusion that infection with pathogenic *Saprolegnia* can induce early hatching in both populations tested.

Uller *et al.* (2009) found that tadpoles hatching from clutches exposed to *Saprolegnia* have 20% decreased mass at metamorphosis, even after no further exposure subsequent to hatching. It has also been found that surviving embryos of *P. cultripes* and *B. calamita* that had been removed from their jelly coat and exposed to the water mold hatched earlier and were slightly less developed than uninfected ones, although the presence of the water mold did not alter the hatching time of eggs in normal jelly masses (Fernández-Benéitez *et al.* 2011). These results allow the conclusion that the jelly coat could be a protective barrier against fungi. Although we did not test the protective effect of jelly coats on the potential infection by aquatic fungi, we found that individuals hatched early even when presenting their protective jelly.

Our results show that both *P. thaul* populations studied (Fantasma lagoon and Mallín Cerro Otto) present different levels of infection over the breeding period, with the highest levels in late spring and summer, but that this temporal variation of the pathogenic impact may not be too strong.

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In both localities, the highest density of *P. thaul* clutches was observed in early spring, in contrast with the highest infections and mortalities observed in late spring and early summer coincident with warmer temperatures (Fig. 1). In Fantasma lagoon, this pattern was clear, showing that the amphibian was not strongly affected by the 'zoosporic fungus' in September, when the highest levels of egg density were recorded (around 50–70% more than in the rest of the season). The number of eggs in September was around 1200 eggs (per clutch), while that between October and December was around 500 eggs. This suggests that epidemic outbreaks could not be a threat for these anuran populations. This mismatch between early massive oviposition and the apparent suboptimal environmental requirements for water molds could be advantageous for the anuran. Given that breeding phenology shapes many of the ecological interactions in amphibian communities through priority effects (Alford & Wilbur 1985; Dayton & Fitzgerald 2005), it would be important to take it into account when other factors interact, as the case of anuran infectious diseases. For example, *Rana sylvatica* emerges from hibernation very early in the season and breeds soon after the ponds thaw, while temperatures are still very low (Gomez-Mestre *et al.* 2006). This early parental breeding behaviour, combined with a short embryonic period, ensures that *R. sylvatica* embryos develop when water temperatures inhibit rapid mold growth, and this can be the case for *P. thaul*. Several studies have documented sources of variation in host viability at pathogen exposure within and among amphibian populations depending on their biology (genetic origin) and environmental factors (e.g. temperature) (Sagvik *et al.* 2008). It has been suggested that low water temperature during breeding is associated with a higher incidence of *Saprolegnia*-infected clutches, which could lead to seasonal and geographic variation in the degree of infection (Banks & Beebee 1988; Beattie *et al.* 1991). In this regard, it has been suggested that low temperatures can lead this trend of variable degree of infection due to changes in the relative growth rates of embryos and fungi or reduced defence of the developing embryos. However, in contrast to this pattern, we found that what is called 'low temperature' in the northern hemisphere represents high temperatures in our study sites at high latitudes (north-western Patagonia). It is possible that the environmental characteristics of Patagonian wetlands lead to the mismatch between the biology (phenology) of *P. thaul* and the phenology of the cosmopolitan *Saprolegnia* which has different thermal requirements to develop. There is some evidence that eggs are less susceptible to infection once they reach the tail bud stage (*Rana temporaria*; Robinson *et al.* 2003). Then, early cohorts of *P. thaul* may have the advantage that embryos reach stages and sizes that are less susceptible to infection before wetlands reach optimal thermal requirements for the spread of fungi (late spring). Also, other characteristics may improve survival. Considering that in early spring in our study sites there is an overlap of environmental and biological conditions such as low temperature, low predation risk (Jara 2010) and low competition, we believe that early cohorts may behave as active feeders, leading to increase in growth and development rates and improving the survival of juveniles, as observed in other studies (Altwegg 2002; Altwegg & Reyer 2003; Orizaola *et al.* 2012).

We concluded that the phenology of the populations of *P. thaul* studied could be an important strategy to reduce the risks imposed by pathogens such as *Saprolegnia*. The water mold infection of egg clutches observed is substantially less frequent at early spring than at late spring and summer. Then, if levels of infection have been consistently high during the late spring, it might be expected that mechanisms as priority effects can allow successful recruitment for early cohorts. However, there may also be temporal variation both within and between years depending on the climatic fluctuations. Accordingly, priority effects may be an important mechanism to understand the population dynamics of *P. thaul* initiated by the individuals' arrival, the environment and pathogens. Also, our data showed earlier hatching when embryos are at high risk of infection. We interpret this behaviour as a compensatory strategy allowing embryos to escape from the pathogen when the risk increases (late cohorts). Warkentin (2011) has proposed that, in some cases, the responses of embryos are congruent with a variation in the mortality rate. This could be the case for *Pleurodema* recruitment. *Pleurodema thaul* embryos showed oomycete-induced acceleration of hatching when they were at high risk of infection, which, in nature, is coincident with an increased growth rate for oomycetes with temperature. Similar results have been observed in two amphibians with different temperature requirements and exposed to water molds (Gomez-Mestre *et al.* 2006).

These results allow us to predict that *Saprolegnia* infections may have strong implications for anuran population dynamics in Patagonia along with environmental and biological changes. The increase in temperature is projected to affect wetlands at high altitudes in southern South America and high latitudes as some portions of Patagonia (piedmont and steppe wetlands) (Brinson & Malvarez 2002). Given the pressing need to explain and predict the impact of these climatic changes on the Patagonian biota, additional studies are necessary to better understand the relationship between the phenology of amphibians and potential stressors such as pathogenic fungi. The knowledge of the strategies used by anuran populations exposed to pathogenic fungi is essential to determine the limits of the organisms' responses to a

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hypothetical warm climate scenario and how this scenario can affect these interrelations.

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