

Chytridiomycosis-mediated expansion of *Bufo bufo* in a montane area of Central Spain: an indirect effect of the disease

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ABSTRACT

Emerging infectious diseases (EIDs) may indirectly influence community structure by altering biotic interactions in which affected species participate. This kind of indirect effects has received little attention to date, although recent research suggest they may not be infrequent. Chytridiomycosis is an EID that is affecting amphibian assemblages worldwide. Here we present the first (to our knowledge) field evidence of its indirect effect on assemblage structure through the disruption of a biotic interaction. We have documented an expansion of the common toad (*Bufo bufo*) after chytridiomycosis nearly extirpated the midwife toad (*Alytes obstetricans*) in Peñalara Natural Park (Central Spain). *Bufo bufo* has significantly increased its number of breeding sites. All newly occupied breeding ponds were colonized once the large numbers of large, overwintering *A. obstetricans* tadpoles they had previously held disappeared. An experiment demonstrated that breeding *B. bufo* adults strongly avoided laying eggs in water containing *A. obstetricans* tadpoles, accounting for observed patterns of pond use. A second experiment failed to show any predation by large *A. obstetricans* tadpoles on *B. bufo* eggs, but other researchers have documented reductions in condition, growth and survival of *B. bufo* larvae caused by the presence of *A. obstetricans* tadpoles. In Peñalara, chytridiomycosis attacks *B. bufo* too, but its effect on it has been quite less severe than on *A. obstetricans*. Our findings show how, besides any chytridiomycosis direct effects, interspecific differences in its impact may alter assemblage-influencing biotic interactions and, hence, assemblage structure in indirect ways.

Keywords

Batrachochytrium dendrobatidis, chytridiomycosis, emerging-diseases, expansion, indirect-effects.

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INTRODUCTION

Chytridiomycosis is an emerging infectious disease (EID; Daszak *et al.*, 2000) of amphibians that is causing mass mortality and population declines worldwide (Berger *et al.*, 1998; Bosch *et al.*, 2001; Rachowicz *et al.*, 2006). The causative agent, anon-hyphal zoosporic fungus, *Batrachochytrium dendrobatidis*, is a recently described species that presents low host specificity (Daszak *et al.*, 2003). The pattern of amphibian declines (Daszak *et al.*, 1999), and genetic studies (Daszak *et al.*, 2003; Morehouse *et al.*, 2003) support the hypothesis that the chytrid fungus has been recently introduced into native populations. However, recent evidence suggests that climate change has fostered outbreaks of the disease in some areas where the fungus could have been already present for some time (Pounds *et al.*, 2006; Bosch *et al.*, 2007). There is substantial interspecific variation in the effects of chytridiomycosis

(Lips *et al.*, 2003). In the worst cases, it may kill 90–100% of recently metamorphosed individuals of some species, causing severe reductions in their abundance up to local, or even global, extinction (Daszak *et al.*, 1999). At the other end of the spectrum, populations of some species seem to remain largely unaffected (Lips *et al.*, 2003). These differences appear related to variation among host species in traits such as immune defences, fecundity, breeding habitat etc. (Williams & Hero, 1998; Daszak *et al.*, 2003; Lips *et al.*, 2003; Woodhams *et al.*, 2006). In any case, the emergence of chytridiomycosis has severely disrupted the structure of the affected amphibian assemblages (Lips *et al.*, 2006).

So far, most information on the impact of chytridiomycosis deals with its immediate effects, documenting changes in amphibian abundances at affected sites. In contrast, information on the indirect effects of the disease is much scarcer. However, amphibian larvae play a variety of relevant functional roles in the

aquatic systems they inhabit (Petranka & Kennedy, 1999; Wissinger *et al.*, 1999; Ranvestel *et al.*, 2004; Whiles *et al.*, 2006; Altig *et al.*, 2007) and may even be keystone species in some systems (Holomuzki *et al.*, 1994; Blaustein *et al.*, 1996; Flecker *et al.*, 1999). Therefore, the collapse of the populations of many amphibian species should be expected to have a wide range of ecological consequences at the community and ecosystem levels (Ranvestel *et al.*, 2004). Thus, frog extirpation caused by chytridiomycosis has had marked effects on algae abundance, food web structure and nutrient processing in Central American streams and has also altered the flow of energy between the streams and the adjacent riparian habitat (Hunte-Brown, 2006; Whiles *et al.*, 2006).

Additionally, biotic interactions among their members can shape amphibian assemblages. Through asymmetric competition or predation, larvae of some species may reduce the growth, physical condition and abundance, up to extirpation, of hetero-specifics (Morin, 1983; Morin & Johnson, 1987; Petranka & Kennedy, 1999; Richter-Boix *et al.*, 2004). Those interactions may also influence breeding site selection by potential inferior competitors or prey species (Laurila & Aho, 1997). There is a clear potential for the emergence of chytridiomycosis to alter these community-structuring relationships and, hence, to further affect amphibian assemblages in an indirect way. Furthermore, a theoretical model has shown that the interaction between inter-specific competition or predation and disease can determine the structure of host communities (Bowers & Turner, 1997). This model has received recent empirical support as Tompkins *et al.* (2003) have shown that an EID appears to drive the replacement of the native red squirrel *Sciurus vulgaris* by the exotic grey squirrel *S. carolinensis* in the United Kingdom.

Similar scenarios are obviously plausible for chytridiomycosis. Nonetheless, research on this area seems quite rare currently, although Blaustein & Kiesecker (2002) have stressed that adequate insight on interactions between potential determinants of amphibian populations is crucial for a useful understanding of global amphibian declines. However, we are not aware of any published evidence of chytridiomycosis effects upon biotic interactions among amphibians and, through them, upon amphibian assemblage structure in natural systems (but see Parris & Cornelius, 2004).

In this paper, we seek to illustrate that point and present just such evidence by analysing the replacement of the common midwife toad (*Alytes obstetricans*) by the common toad (*Bufo bufo*) at a number of breeding ponds in the Peñalara Natural Park (Sierra de Guadarrama, Central Spain). The near-extirpation of *A. obstetricans* from Peñalara was the first chytridiomycosis-mediated decline documented in Europe (Bosch *et al.*, 2001). Between 1997 and 1999, *A. obstetricans* almost disappeared from the area, and both pond occupancy and larval density (Bosch *et al.*, 2001; Martínez-Solano *et al.*, 2003) were dramatically reduced. More recently, chytridiomycosis has also caused mortalities of *B. bufo* and a sharp decline of the fire salamander (*Salamandra salamandra*) population in the Park (Bosch & Martínez-Solano, 2006; Walker *et al.* unpubl. data).

Shortly after the collapse *A. obstetricans* we witnessed an expansion of *B. bufo* in the area. From this pattern, we hypothesized

that the expansion of *B. bufo* could be mediated by the decline of *A. obstetricans* and that the latter species could suppress the former from potential breeding sites. Adult anurans have been repeatedly shown to avoid oviposition in sites that contain potential competitors or predators of their offspring (Petranka *et al.*, 1994; Spieler & Linsenmair, 1997; Marsh & Borrell, 2001; Murphy, 2003) and early stage, non-overwintering tadpoles of *A. obstetricans* significantly reduced mass at metamorphosis (17%), growth rate (33%) and survival (21%) of *B. bufo* tadpoles in laboratory experiments (Richter-Boix *et al.*, 2007). Additionally, *A. obstetricans* larvae in Peñalara spend more than 3 years in the water before metamorphosis and are much larger than those of *B. bufo* (J.B., unpublished data). Large tadpoles can become predators of eggs or smaller tadpoles (Crump, 1983; Tejedro, 1991; Petranka *et al.*, 1994; Spieler & Linsenmair, 1997; Petranka & Kennedy, 1999; Heusser *et al.*, 2002). In this paper, we have analysed the pattern of *B. bufo* expansion in the field and tested whether (a) *B. bufo* adults can detect the presence of *A. obstetricans* tadpoles and avoid breeding in ponds containing them and (b) *A. obstetricans* tadpoles can be predators of *B. bufo* early ontogenic stages.

METHODS

Study area

The Peñalara Natural Park is a protected alpine area (768 ha) in the Sierra de Guadarrama, Central Spain (41° N, 4° W) composed of several small glacial valleys located 1800–2430 m a.s.l. The landscape consists mainly of granitic outcrops, alpine meadows, heathlands dominated by *Cytisus oromediterraneus* and *Juniperus communis nana*, and forests of *Pinus sylvestris* below the timberline. Within the Park there are about 250 catalogued ponds (Toro & Granados, 1999) where up to 10 amphibian species breed (Martínez-Solano *et al.*, 2003).

Field surveys

Data about presence/absence and larval abundance of amphibian species in each of the 250 ponds come from ongoing, routine yearly censuses carried out by the first author supplemented occasionally with observations from Park rangers (see Bosch *et al.*, 2001; Martínez-Solano *et al.*, 2003; Bosch & Martínez-Solano, 2006; for details). From 1998 to 2006, *B. bufo* clutches and over-wintering *A. obstetricans* tadpoles were individually counted whenever possible. In larger ponds or at high tadpole densities (roughly, > 100 total), we estimated the number of tadpoles through sub-sampling (stratified when required by habitat diversity) by counting all the larvae present in predetermined areas and extrapolating to the entire pond (Jung *et al.*, 2002).

Experiment 1

To test whether breeding *B. bufo* adults are able to detect *A. obstetricans* tadpoles and respond avoiding water bodies the latter occupy, we carried out the following experiment. Eighteen

plastic containers (30 × 42 × 23 cm), filled up with 28 L of water each, were placed in an empty swimming pool at the 'El Ventorrillo' field station (MNCN, CSIC) located only a few kilometres away from Peñalara. We arranged the tanks in three columns by six rows with about 50 cm between contiguous containers. The space between tanks was filled with straw flush with the tank tops to allow *B. bufo* adults unrestricted access to the water. Half of the tanks received one large, over-wintering *A. obstetricans* tadpole from a captive breeding colony, whereas the other half remained empty in an alternate pattern.

We collected 14 *B. bufo* pairs in amplexus at the Laguna Grande de Peñalara on different nights during the breeding season and immediately transported them to the experimental arena. Every morning, we checked the containers for clutches. For each clutch we found we recorded whether it was in a tank with or without *A. obstetricans* tadpole, then removed it and deposited it in the Laguna Grande. Only clutches with at least 90% of the egg mass in a single tank were considered for analyses. We also looked for and collected toads no longer in amplexus and took them back to the Laguna Grande. Pairs in amplexus that did not lay eggs after 5 days in the experimental arena were also returned to the collection site.

We used the binomial test to assess if egg masses were randomly distributed among tanks. We used a one-tailed exact binomial probability test because there was an a priori expectation on the direction of the response (tadpole avoidance).

Experiment 2

To test whether *A. obstetricans* over-wintering tadpoles can predate on *B. bufo* eggs, we placed five newly released *B. bufo* eggs from three different clutches into each of ten 250 mL plastic containers and then added a single, large *A. obstetricans* tadpole from the same captive stock as in the previous experiment to each tank. Tadpoles were not fed during the experiment and no alternative food source was available to them in the containers. We inspected the tanks daily and, after a week, we counted the remaining eggs and tadpoles and returned them to their places of origin.

RESULTS

Field surveys

In the period 1998–2004, *B. bufo* in Peñalara passed from only being known to lay eggs at the Laguna Grande de Peñalara (surface area 5452 m², maximum depth 4.7 m) to breeding in five ponds (Fig. 1). At all four new breeding sites, marked increases in the number of *B. bufo* clutches occurred after the *A. obstetricans* over-wintering tadpoles that previously lived in those ponds disappeared (Fig. 1). In two instances, one and two *B. bufo* clutches appeared whereas a few *A. obstetricans* larvae were still present, but really substantial increases in the number of *B. bufo* clutches only occurred once no *A. obstetricans* tadpoles remained in all four cases (Fig. 1). Colonization events were not synchronous and occurred over a 5-year period (1999–2004). New breeding ponds were colonized in the order (1) Laguna Chica (1999,

surface area 739 m², maximum depth 0.7 m) (2) Laguna de los Pájaros (1999 for first occurrence, but 2001 for a substantial increase, particularly considering the large size of the water body, surface area 4866 m², maximum depth 1.3 m); (3) Charca de la Mariposa (2002, surface area 483 m², maximum depth 0.65 m) and (4) Charca de las Piedras (2004, surface area 71 m², maximum depth 0.6 m). Numbers of *B. bufo* clutches in the Laguna Chica (closest to the original breeding nucleus in the Laguna Grande and in the same hydrographic basin) were quite high from the beginning and have shown little change along time, whereas we have observed gradual increases in the other three new sites (Fig. 1). In 2005 and 2006, *B. bufo* has continued breeding at the five sites (Fig. 1), but has not occupied any new ponds.

From 1998 to 2006, the total number of *Alytes* tadpoles has dramatically decreased in the area (Kendall rank correlation, $\tau = -0.743$, $P < 0.003$), whereas the number of ponds occupied by *B. bufo* has significantly increased (Kendall $\tau = 0.897$, $P < 0.00001$). The total number of *Bufo* clutches showed an overall increase (60 in 1999, 95 in 2006) that was only marginally significant (Kendall $\tau = 0.423$, $P < 0.090$). A significant inverse relationship between the number of *B. bufo* clutches and of *Alytes* tadpoles existed for each of the breeding sites (Kendall $\tau = -0.502$ to -0.873 , $P < 0.0381$), save the Charca de las Piedras (Kendall $\tau = -0.345$, $P > 0.1281$). When analysing all sites together, this negative correlation was also apparent (Kendall $\tau = -0.340$, $P < 0.0011$).

Experiment 1

Breeding *B. bufo* adults avoided laying eggs in containers holding over-wintering *Alytes* tadpoles. Ten *B. bufo* females laid eggs in the tanks. Nine clutches were collected from empty tanks and one from a tank with tadpoles (binomial test: $P = 0.0107$). Two clutches had some eggs in both tanks holding tadpoles and tanks lacking them, but in both cases more than 90% of the eggs were in the tanks with no tadpoles. One egg mass was found on land and therefore was not considered in the analysis. Finally, clutches laid in tanks lacking tadpoles were randomly distributed across them ($\chi^2 = 14$, d.f. = 8, $P < 0.0818$).

Experiment 2

Over-wintering *Alytes* tadpoles did not feed on *B. bufo* eggs. After a week, all tanks still contained the initial five eggs. Some of them had lost their gelatine outer layer, presumably as a consequence of tadpole movements. However, the eggs appeared undamaged otherwise and continued their normal development after the experiment ended.

DISCUSSION

To our knowledge, our findings constitute the first field evidence of indirect effects of chytridiomycosis through disruption of biotic interactions among amphibian species. As such, they agree with recent theoretical and empirical research showing that such kind of indirect effects of EIDs may be more

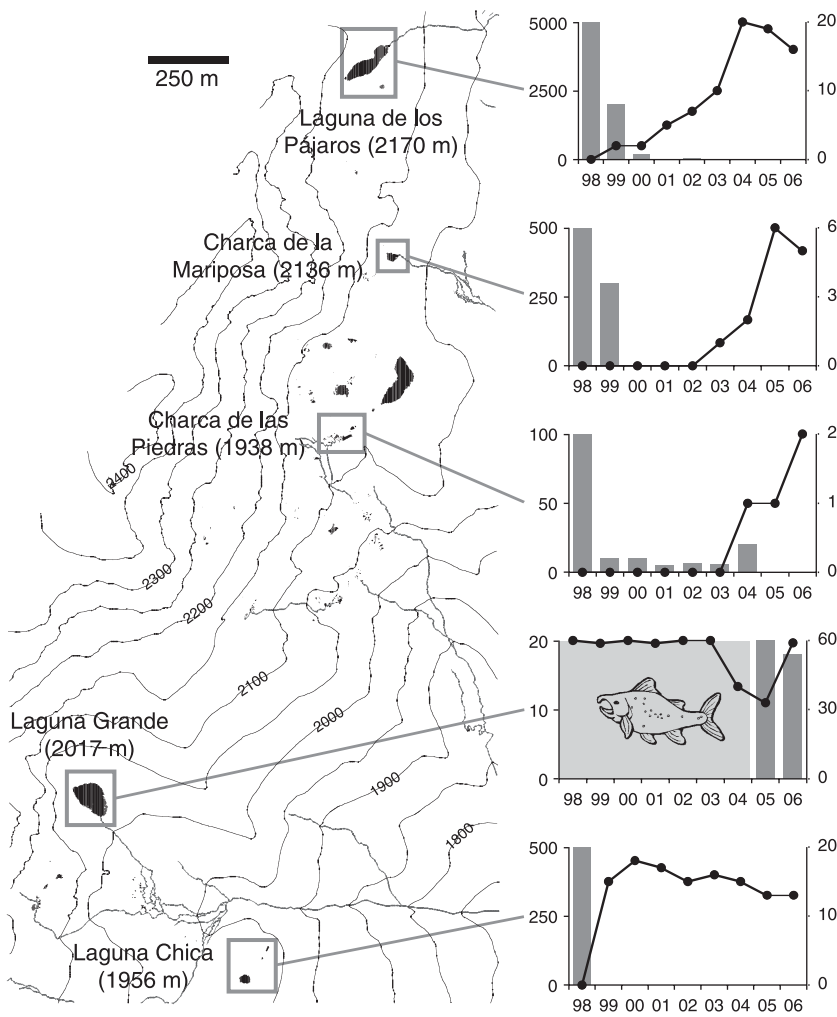


Figure 1 Location of ponds where *Bufo bufo* currently breeds in Peñalara Natural Park and temporal changes in the number of over-wintering *Alytes obstetricans* tadpoles (bars, left axis) and of *B. bufo* clutches (line, right axis) in them from 1998 to 2006. Note the differences in the scale of vertical axes across sites. In Laguna Grande, *A. obstetricans* was absent up to 2005, when the non-native brook trout (*Salvelinus tontinalis*) was finally extirpated (Bosch *et al.* 2006).

common and represent a more substantial impact than typically thought (Bowers & Turner, 1997; Tompkins *et al.*, 2003). Additionally, our results illustrate an indirect effect of chytridiomycosis of a kind (disruption of biotic relationships influencing assemblage structure) not documented previously. Thus, they add to a still limited, but quickly growing, body of evidence indicating that the ongoing, worldwide extirpation of amphibian populations by chytridiomycosis will unleash a host of diverse ecological consequences at many levels of organization (Ranvestel *et al.*, 2004; Hunte-Brown, 2006; Whiles *et al.*, 2006). Finally, as far as we know, all field evidence on indirect effects chytridiomycosis has so far come from the Neotropical region (Hunte-Brown, 2006; Whiles *et al.*, 2006). Our results demonstrate the existence of those effects in the temperate zone as well and, consequently, suggest that they may be widespread geographically and possibly present in any regions where outbreaks of the disease have occurred.

Our results suggest that through the near extirpation of the *A. obstetricans* population, chytridiomycosis in Peñalara would have ended an exclusion of *B. bufo* from potential breeding sites caused by the presence of large *A. obstetricans* larvae in them. *Bufo bufo* shows a clear preference for breeding in permanent, relatively large and deep ponds throughout its range (Van

Buskirk, 2003). The four new ponds colonized during its expansion in Peñalara match well that pattern (Toro & Granados, 1999; Bosch & Martínez-Solano, 2003), but were only used after the chytridiomycosis-induced collapse of the large number of *A. obstetricans* tadpoles previously present. In laboratory tanks, *A. obstetricans* larvae produced significant reductions in growth and survival, of *B. bufo* tadpoles, greater than those induced by conspecifics (Richter-Boix *et al.*, 2007). Conditions in the oligotrophic ponds of Peñalara (Toro & Granados, 1999) seem unlikely to ameliorate those negative effects as *Bufo bufo* tadpoles have low filtering and ingestion rates at low food concentrations (Viertel, 1990). Moreover, *B. bufo* tadpoles seem to be generally weak competitors and hence more prone to be 'losers' in asymmetric interactions than other species (Richter-Boix *et al.*, 2007). Hence, *A. obstetricans* tadpoles appeared likely to exclude *B. bufo* from otherwise suitable breeding sites and the emergence of chytridiomycosis would have released the latter species from that excluding factor.

Accordingly, experiment 1 showed that breeding *B. bufo* adults avoided laying eggs in water containing large *A. obstetricans* tadpoles and thus, provided evidence of a causal mechanism for the pattern we observed in the field. Our results agree well with

previous reports of adult anurans selecting against water bodies containing potential predators or competitors of their offspring as oviposition sites. For example, *Bufo americanus* avoided breeding in ponds containing *Rana sylvatica* tadpoles who prey on the former's eggs and early hatchlings (Petranka *et al.*, 1994) and diverse adult anurans have shown a similar avoidance of sites previously occupied by hetero- or conspecific larvae (Resetarits & Wilbur, 1991; Spieler & Linsenmair, 1997; Marsh & Borrell, 2001; Murphy, 2003).

It does not seem likely that environmental factors, namely, favourable climatic conditions, rather than the disappearance of *A. obstetricans* larvae, had driven the colonization events. The green frog, *Rana perezi* and the tree frog *Hyla arborea* have shown recent expansions in Peñalara that may be mediated by climate change (Martínez-Solano *et al.*, 2003; Bosch, 2007). However, unlike *Bufo bufo*, in those cases occupation of new ponds has shown no relation to prior *A. obstetricans* presence (J.B., unpublished data) and the results of experiment 1 show that presence of *A. obstetricans* larvae can be a determinant of breeding site occupancy by *B. bufo*.

Experiment 2 failed to show predation by *A. obstetricans* tadpoles on *B. bufo* eggs. This agrees with the notion that *B. bufo* eggs are generally unpalatable for most vertebrate predators (Duellman & Trueb 1986), although tadpoles of *R. dalmatina* and *R. temporaria* have been attested feeding on them (Heusser, 1970; Heusser *et al.*, 2002). This seems to suggest that, in Peñalara, *A. obstetricans* larvae may negatively affect *B. bufo* tadpoles through the strong asymmetric competition identified by Richter-Boix (2005) in laboratory settings.

We propose that the recent expansion of *B. bufo* in our study area represents the first field evidence of an indirect effect of chytridiomycosis through disruption of biotic interactions among amphibians. Although *B. bufo* has also been affected by the disease in Peñalara (Bosch & Martínez-Solano 2006), this has occurred later in time and the consequences up to now has been far less severe than for *A. obstetricans* (Walker *et al.* unpublished data). As reported in other species (Kriger & Hero, 2006), it seems that chytrid-induced mortality on *B. bufo* is restricted to metamorphs (Bosch & Martínez-Solano, 2006) and high numbers of healthy *B. bufo* metamorphs are still recorded yearly in the area (J.B., unpublished data). *B. bufo* spends a relatively short time in the water (about 4 months in the study area) and has high fecundity (up to 10,000 eggs in the Iberian Peninsula; Campeny & Montory, 1988). Both are traits claimed to lessen the impact of chytridiomycosis (Williams & Hero, 1998; Lips *et al.*, 2003). In contrast, *A. obstetricans* has low fecundity (120–185 eggs per season; Reading & Clarke, 1988) and its aquatic phase lasts more than 3 years in Peñalara. Hence, inter-specific differences in the severity of the effects of an agent potentially harmful for two anuran species may modify their biotic interaction indirectly favouring one of them.

Our findings agree with other recent work in suggesting that this kind of indirect effect may be a relatively frequent consequence of EIDs (Bowers & Turner, 1997; Tompkins *et al.*, 2003). Considering the major role that biotic interactions among amphibian larvae can play (Morin, 1983; Morin & Johnson, 1987;

Petranka *et al.*, 1994; Werner & Anholt, 1996) and the scale of the ongoing spread of chytridiomycosis (Mendelson *et al.*, 2006), the impact of the disease upon amphibian biotic relationships seems an area worthy of further attention.

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