Observations on the intensity and prevalence of *Batrachochytridium dendrobatidis* in sympatric and allopatric *Epidalea calamita* (native) and *Discoglossus pictus* (invasive) populations

Albert Montori1,*, Olatz San Sebastian2, Marc Franch3,4, Eudald Pujol-Buxó2, Gustavo A. Llorente2, Andrés Fernández-Loras5, Álex Richter-Boix2, Jaime Bosch5

1 Grup de Recerca de l’Escola de la Natura de Paret del Vallès (GRENP), C/ Galende 12, 08150 Paret del Vallès, Barcelona, Spain.
2 Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 643, 08028 Barcelona, Spain.
3 Departament de Ciències Ambientals, Universitat de Girona, 17003 Girona, Spain.
4 CICGE – Centro de Investigação em Ciências Geo-Espaciais, Universidade do Porto, 4430-146 Vila Nova de Gaia, Portugal.
5 Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain.

*Correspondence: E-mail: amontori@gmail.com

Received: 24 September 2018; returned for review: 14 December 2018; accepted: 11 February 2019.

We analyse if the presence of the invasive *Discoglossus pictus* and the native *Epidalea calamita* in the same geographic area can shape the dynamics of infection by *Batrachochytridium dendrobatidis* (Bd). Both amphibian species share breeding habitat preferences in the area (i.e. ephemeral and temporary ponds) and are common sympatric competitors. We sampled adults of either species in a total of nine breeding localities in Catalonia (NE Iberian Peninsula): six localities within sympatric geographic areas, two localities outside the current invasion range of *D. pictus*, and one locality comprising solely *D. pictus* due to its recent introduction and the ancient extinction of *E. calamita*. We analysed the prevalence and intensity of infection by Bd in 183 individuals (81 *D. pictus* and 101 *E. calamita*). The presence of Bd was detected in eight of the localities, all of which had not been yet confirmed as positive for the fungus. Only in one locality, with presence of *D. pictus*, Bd presence was not detected. Mean prevalence was slightly but non-significantly higher in *E. calamita* (46.5%) than in *D. pictus* (36.6%). Allopatric populations of *E. calamita* showed significantly lower prevalence of Bd infection than sympatric ones, but similar differences were not found for infection intensity. Likewise, no significant differences were found in infection intensity between allopatric and sympatric *D. pictus* populations. The presence of abundant Bd outside the invasion range of *D. pictus* supports the assertion that Bd has not been introduced in the studied area because of *D. pictus* invasion. However, the presence of the two species in sympatry may enhance the infection rates and therefore the prevalence of Bd in the native species.

**Key words:** Chytridiomycosis; *Discoglossus pictus*; *Epidalea calamita*; invasive species.

Infectious diseases are a well-known driver of animal declines worldwide (Fisher et al., 2012). Population declines caused by fungal diseases have been reported in bats, corals, bees, snakes and amphibians (Fisher et al., 2012). Particular-
ly, amphibians have been exceptionally impacted by emerging infectious diseases (EID, Dazak et al., 1999; Skerratt et al., 2007), with observations of massive pathogen-associated amphibian die-offs dating back to the 1970s and 80s (Barrinaga, 1990). Chytridiomycosis is one of those EID (Dazak et al., 2000), caused by the fungal pathogen _Batrachochytrium dendrobatidis_ (Bd). Although globally the number of species affected by habitat loss and degradation is higher than species affected by EID, Bd is considered to be one of the major causes of amphibian declines (Lips et al., 2006; Skerratt et al., 2007). Nowadays, chytridiomycosis is a global threat, related to the mass decline and extinction of amphibian populations worldwide (Berger et al., 1998; Bosch et al., 2001; Rachowicz et al., 2006). This EID is considered as the most devastating ongoing vertebrate disease (Brannelly et al., 2018).

The pathogen infects over 500 species of amphibians (Global Bd Mapping Group, 2018) and is found in all continents except Antarctica. In Europe, Bd was first recorded causing mass amphibian die-offs during the summer of 1997 (Bosch et al., 2001), and is widely distributed nowadays. It has been found in Spain, Portugal, Italy, Germany, Belgium, Netherlands, Great Britain, France, Switzerland, Austria, Hungary, Denmark and Sweden (e.g. Garner et al., 2005; Spitzen-van der Sluijs & Zollinger, 2010). Spain is the European country where Bd distribution is best known (Table 1), with a total of 345 sites sampled so far (Global Bd Mapping Group, 2018). To date, Bd has been found infecting 22 amphibian species in Spain (Table 1), causing mass mortality to at least three of them: the common midwife toad (_Alytes obstetricans_), the fire salamander (_Salamandra salamandra_) and the common toad (_Bufo spinosus_) (Bosch et al., 2001; Bosch & Martinez-Solano, 2006).

The composition and the dynamics of the host community can affect host-pathogen dynamics. For instance, it has been noted that a high diversity of amphibians can dilute the effects of Bd (Searle et al., 2011). The dilution effect occurs when biodiversity and disease risk are inversely related, which can occur through a reduction in the encounter rates between susceptible and infectious individuals. By contrast, the near extinction of _A. obstetricans_ by Bd in the Peñalara mountains (central Spain) increased the presence of _B. spinosus_, promoting the spread and maintenance of the fungus to new areas (Bosch et al. 2014). According to these possible scenarios, invasive species may help to dilute the effects of Bd due to the increase in diversity, assuming that those invasive species do not reduce the native species richness. By contrast, when there is a positive relationship between biodiversity and disease risk, the invasive species may help to expand and enhance the presence of the fungus in the invaded areas (Miaud et al., 2016). In that case, the presence of the invasive species has an amplification effect. For example, the invasive North American bullfrog (_Lithobates catesbeianus_) is known to act as a host reservoir of Bd (Mazzoni et al., 2003; Dazak et al., 2004; Miaud et al., 2016; Borzée et al., 2017), while Cunningham & Minting (2008) found a strong association between the presence of non-native amphibian species and Bd infection in the UK.

Species can act differently relative to
the risk of infection, either as reservoir hosts or as resistant species. Reservoir hosts are species that can harbour a pathogen by transmitting it among themselves and to other taxa, thus maintaining the pathogen within the ecosystem in the absence or with a low density of susceptible hosts. Resistant species, in contrast, are refractory to infection or quickly clear infection, and thereby have a limited potential to maintaining a disease within the system (Brannelly et al., 2018). In this way,

Table 1: Prevalence of the amphibian chytrid fungus Batrachochytrium dendrobatidis in Spanish amphibians estimated from published studies compiled by Global Bd Mapping Group (2018). (1) Each entry from the database was considered as a different site. (2) Data from Bosch et al. (2013). (3) Data from the present study.

| Species                  | N samples | N positive samples | Prevalence | N sites (1) | N positive sites | Occurrence |
|--------------------------|-----------|--------------------|------------|-------------|------------------|------------|
| Salamandra salamandra    | 139       | 47                 | 33.81      | 43          | 14               | 32.56      |
| Pleurodeles waltl        | 9         | 9                  | 100.00     | 5           | 5                | 100.00     |
| Calotriton asper         | 21        | 4                  | 19.05      | 7           | 4                | 57.14      |
| Triturus marmoratus      | 21        | 2                  | 9.52       | 8           | 2                | 25.00      |
| Triturus pygmaeus        | 22        | 13                 | 59.09      | 11          | 5                | 45.45      |
| Mesotriton alpestris     | 83        | 23                 | 27.71      | 29          | 14               | 48.28      |
| Lissotriton helveticus   | 19        | 3                  | 15.79      | 7           | 3                | 42.86      |
| Discoglossus jeanneae    | 2         | 1                  | 50.00      | 2           | 1                | 50.00      |
| Alytes dickhilleni       | 36        | 0                  | 0.00       | 10          | 0                | 0.00       |
| Alytes dickhilleni(2)    | 461       | 43                 | 9.33       | 30          | 3                | 10.00      |
| Alytes muletensis        | 589       | 78                 | 13.24      | 48          | 10               | 20.83      |
| Alytes obstetricans      | 1456      | 468                | 32.14      | 174         | 57               | 32.76      |
| Bufo spinosus            | 246       | 74                 | 30.08      | 45          | 15               | 33.33      |
| Epidalea calamita        | 93        | 4                  | 4.30       | 31          | 3                | 9.68       |
| Bufofes balearicus       | 54        | 0                  | 0.00       | 5           | 0                | 0.00       |
| Hyla molleri             | 24        | 3                  | 12.50      | 6           | 2                | 33.33      |
| Hyla meridionalis        | 19        | 17                 | 89.47      | 11          | 10               | 90.91      |
| Pelobates culripes       | 12        | 7                  | 58.33      | 8           | 5                | 62.50      |
| Pelodytes punctatus      | 10        | 0                  | 0.00       | 4           | 0                | 0.00       |
| Rana iberica             | 9         | 2                  | 22.22      | 5           | 2                | 40.00      |
| Rana pyrenaica           | 9         | 4                  | 44.44      | 3           | 2                | 66.67      |
| Rana temporaria          | 72        | 1                  | 1.39       | 27          | 1                | 3.70       |
| Pelophylax perezi        | 67        | 26                 | 38.81      | 31          | 19               | 61.29      |
| Total                    | 3012      | 786                | 26.10      | 520         | 174              | 33.46      |
| Discoglossus pictus(3)   | 82        | 30                 | 36.58      | 8           | 6                | 75.00      |
| Epidalea calamita(3)     | 101       | 47                 | 46.53      | 6           | 6                | 100.00     |
those invasive amphibian species that act as reservoir hosts could multiply their negative impact on native amphibian species by adding increased infection risk to the well-known competition effect.

The Mediterranean Painted Frog (*Discoglossus pictus*) is an invasive species that has spread in the northeast of the Iberian Peninsula. This species is a north African amphibian introduced to southern France in the early 20th century that became invasive and spread up to 170 km southwards and northwards along the Mediterranean coastal plains (Montori et al., 2007; Llorente et al., 2015). The species usually co-occurs with native anurans that breed in ephemeral and temporary ponds, sharing these breeding sites mostly with natterjack toads (*Epidalea calamita*) and common parsley frogs (*Pelodrytes punctatus*) (Escoriza & Boix, 2012; Richter-Boix et al., 2013). Because the invasive species represents an addition, in terms of diversity and biomass, to the community of many ephemeral and temporary ponds from the area, the aims of this study were (i) to investigate Bd presence in the invasive species *D. pictus* and the native species *E. calamita* from the same geographic area, and (ii) to analyse if the presence of *D. pictus* in sympatry modifies the prevalence or intensity of Bd infection in *E. calamita*. Given that, during the many years we have been prospecting the study area, we have not observed any *D. pictus* dead because of Bd infection, we assume that the invasive species is a tolerant host. According to previous literature on tolerant host invasive species, like *L. catesbeianus* and the results obtained by Cunningham & Minting (2008) in the UK, we hypothesized that Bd infection prevalence in the native species should increase in the presence of the invasive *D. pictus*, acting the invasive species as a reservoir host of the fungal pathogen.

**Materials and Methods**

**Sample collection and processing**

From February 2010 to May 2013, we carried out a survey on selected aquatic environments previously identified as suitable breeding sites of *D. pictus* and/or *E. calamita* in the north-eastern region of the Iberian Peninsula. We sampled adults of either species from a total of nine ephemeral and temporary ponds (Fig. 1). Six of these sites were located in geographic areas that comprised populations of both species; we obtained samples from both species at one of the sites (05), samples from *D. pictus* only at three sites (01, 02 and 03), and samples from *E. calamita* only at two sites (06 and 09) (Table 2). Two additional localities were outside the invasion range of *D. pictus* and had the regular presence of *E. calamita* only, and another one had only *D. pictus* due to the extinction of *E. calamita* from the area during the third quarter of the last century (Montori et al., 2009) (Fig. 1, Table 2). Minting (2012) found that detection of Bd in adult amphibians is increased when animals are in the aquatic phase, and so all individuals sampled in the present study were captured from aquatic habitats during the reproductive period. In total, we captured 198 individuals, 84 *D. pictus* and 114 *E. calamita*.

We collected a sample of skin tissue by rubbing a sterile cotton swab (MW100-100; Medical Wire & Equipment Co, Cors-
CHYTRIDIOMYCOSIS IN *EPIDALEA CALAMITA* AND *DISCOGLOSSUS PICTUS*

...over the ventral side of the body and thighs (20 strokes) and the webbing of the hind feet (10 strokes), consistent with standard swabbing protocols. Samples were stored at 4°C until processing and DNA was extracted with PrepMan® Ultra (Thermo Fisher, Waltham, Massachusetts, USA) following Boyle et al. (2004). Extractions were diluted 1:10 in Milli-Q-grade water before

---

**Figure 1:** Location of sampling sites and current distribution of *Discoglossus pictus* in the Iberian Peninsula (shadowed area). Black circle represent sites where both species occur in sympatry, grey circles represent sites where only *E. calamita* allopatric populations occur and the white circle represents the site where only the *D. pictus* allopatric population occurs. For details on names and coordinates of the sites, see Table 2.

**Table 2:** Detail on sampling sites and Bd prevalence (in %) and intensity (mean and range, excluding zeros, of zoospore genome equivalents) in *Discoglossus pictus* (DP) and *Epidalea calamita* (EP) individuals sampled in Catalonia. S/A: sympatric (S) or allopatric (A) population with the other species. (*) Population introduced in the Llobregat River delta from an unknown northernmost population.

| Sp | Code | Locality                  | UTM x | UTM y | S/A | N | Prev | GE       |
|----|------|----------------------------|-------|-------|-----|---|------|----------|
| DP | 01   | Solius                     | 497511| 4630028| S   | 21| 0    | 0        |
| DP | 02   | Vidreres                   | 480427| 4626272| S   | 19| 89.5 | 80.4 (4-420) |
| DP | 03   | Portbou                    | 510841| 4697190| S   | 4 | 75.0 | 83.3 (10-200) |
| DP | 04   | El Prat de Llobregat       | 424754| 4571162| A* | 11| 54.5 | 66.51 (1-370) |
| DP | 05   | Riudarenes                 | 476581| 4630824| S  | 25| 12.0 | 1323.5 (0.5-3930) |
| EC | 06   | St. Feliu de Buixalleu     | 466582| 4620440| S  | 20| 75.0 | 60.13 (3.7-500) |
| EC | 07   | Sant Çeloni                | 459557| 4616686| A  | 21| 4.8  | 0.4 (0.4-0.4) |
| EC | 08   | Llinars del Vallès         | 453001| 4612353| A  | 14| 28.6 | 1.5 (0.2-3.2) |
| EC | 09   | Sils                       | 476935| 4629315| S  | 18| 77.8 | 20.8 (3.5-110) |
| EC | 05   | Riudarenes                 | 476581| 4630824| S  | 28| 46.4 | 3653.3 (0.5-18280) |
real-time PCR amplification, performed in duplicate with a CFX96 thermocycler (Bio-Rad, Hercules, California, USA) following Boyle et al. (2004). Each 96-well assay plate included samples, a negative control and standards of 100, 10, 1, and 0.1 Bd zoospore genome equivalents (GE) in duplicate. Samples were considered positive when both replicates were ≥ 0.1 GE and the amplification curves had the typical sigmoidal shape. When only one of the replicates of a sample amplified, we ran this sample a third time. If the third run did not result in an amplification profile, we considered the sample as negative for infection. Samples that showed signs of inhibition (non-sigmoidal amplification) were further diluted to 1:100 and re-analysed. If signs of inhibition remained, the samples were excluded from the analyses.

**Statistical analysis**

Prevalence of Bd-infected animals was calculated by dividing the number of individuals positive for Bd by the corresponding number of individuals that we swabbed in the population. We fitted binomial models for the number of infected and uninfected individuals per population (1: infected individual, 0: uninfected individual) to detect differences in prevalence between species and among areas. Using the package nlme (Pinheiro et al., 2018), we fitted a global model including species and geographic status (sympatric vs. allopatric), and all possible nested simpler models, including a null model. We ordered all models according to the corrected Akaike Information Criterion (AICc) and tested the relevant pairs of models against each other using likelihood ratio tests (LRTs). Since interactions between species and distribution status were significant (see Results), we explored the differences between distribution statuses separately for each species by fitting a binomial model that included distribution status as explanatory variable. This model was tested using a LRT against a null model. Prevalence analyses were conducted using R (R Core Team, 2018).

Intensity analyses were performed using QP 3.0 software (Quantitative Parasitology 3.0, Budapest; Rózsa et al., 2000; Reiczigel & Rózsa, 2005), comparing the following pairs of groups: (i) *E. calamita* total vs. *D. pictus* total, (ii) *E. calamita* allopatric vs. sympatric and (iii) *D. pictus* allopatric vs. sympatric. We use de Mood’s median test to compare median intensities and the test of two-sample comparison of mean crowding of 97.5% of confidence limits with 2000 bootstrap replications.

**Results**

Of the 198 adults captured, 183 yielded conclusive analyses (81 *D. pictus* and 101 *E. calamita*). Fifteen samples continued showing signs of inhibition after having been diluted 1:100, and were therefore eliminated from the analyses. Only one of the sampled populations, that of *D. pictus* from Solius (site 01, Table 2), did not give any positive result for Bd presence, whereas all the analysed *E. calamita* populations in both sympathy and allopatry tested positive for Bd (Table 2).

The best model explaining the prevalence of infection in a sampled population included the species, distribution status (allopatric or sympatric), and their interac-
tion, signalling that populations of each species react differently to their distribution status (Table 3). This model was the best according to AICc and was significantly better than any other tested option, as corroborated by LRTs (Table 3). Mean prevalence of Bd infection was higher in *E. calamita* than in *D. pictus* (46.5% vs. 36.6%).

Table 3: Selection of the best-fit model, according to the corrected Akaike Information Criterion (AICc), to explain the prevalence of Bd infection in sampled populations as a function of the species and distribution status. (*) Refers to interaction between terms and (+) refers to additive effects.

| Model                           | df | AICc | Δ(AICc) |
|--------------------------------|----|------|--------|
| prevalence ~ distribution status * species | 4  | 103.3 | 0      |
| prevalence ~ distribution status + species | 3  | 111.8 | 8.55   |
| prevalence ~ distribution status     | 2  | 112.3 | 9.06   |
| null model                        | 1  | 117.8 | 14.52  |
| prevalence ~ species              | 2  | 119.1 | 15.79  |

Although this difference cannot be considered as statistically significant because the model including only the species was worse, according to AICc, than the null model, and its LRT against the null model was above the significance threshold (*P* = 0.163). Differences between allopatric and sympatric populations of a given species were found for *E. calamita* (*P* < 0.001) but not for *D. pictus* (*P* = 0.182).

Infection intensity was not significantly different between the two species (mean ± SD GE: *E. calamita* = 482.1 ± 2178.3, *D. pictus* = 77.9 ± 438.2; *P* = 0.147). The same results were obtained by comparing allopatric and sympatric populations of *E. calamita*. De Mood’s median test comparing median intensities was not significant (*P* = 0.060) despite mean GE in allopatric *E. calamita* populations (1.40 ± 0.89) being much lower than in sympatric ones (1159.26 ± 3281.69). By contrast, the two-sample comparison of mean crowding of distributions between *E. calamita* allopatric and sympatric populations is highly significant (*P* = 0.001).

Figure 2: Prevalence (proportion of infected individuals) of Bd in *Epidalea calamita* and *Discoglossus pictus* populations sampled in the present study. Vertical lines indicate the 95% confidence intervals. For details on site characteristics and location, see Figure 1 and Table 2.
patric populations indicated that the 97.5% confidence intervals did not overlap, and therefore the difference between the two samples is significant ($P < 0.05$) (Fig. 2).

No significant differences were found in intensities between both allopatric and sympatric $D. pictus$ populations according to either the de Mood’s median test (mean ± SD GE: allopatric = 66.7 ± 148.7, sympatric = 7212.9 ± 711.8; $P = 0.182$) or the mean crowding 97.5% confidence intervals ($P > 0.05$).

**Discussion**

The results of our sampling show eight new localities with confirmed presence of Bd in northeast Iberian Peninsula. Hitherto, only a positive detection of Bd in $D. pictus$ in Aiguamolls de l’Empordà (SAURAMAS et al., 2016) and eight in Iberian green frog ($Pelophylax perezi$) had been reported (FERNÁNDEZ-GUIBERTEAU et al., 2016; MIRAS et al., 2017) in Catalonia. These new localizations indicate that the Bd presence in Catalonia could be more spread than currently assumed.

The anthropogenic spread of pathogens has been responsible for the emergence of the known agents of amphibian chytridiomycosis, $B. dendrobatidis$ and $B. salamandrivorans$ (CUNNINGHAM et al., 2017). Species invasions and commercial trade of amphibians have been demonstrated to provide a vehicle for the global movement of amphibian hosts and their associated pathogens (PICCO & COLLINS, 2008). It has even been hypothesized that the invasive bullfrog is responsible for the dispersal of the hypervirulent Bd-GPL lineage globally (SCHLOEGEL et al., 2012). The alien nature and captive origins of the $D. pictus$ populations in southern Europe could suggest a certain role of the species as a reservoir or enhancer of the presence of Bd in the invaded areas. Despite the numerous cases of the so-called “pathogen pollution” via invasive species, our data indicate that Bd was present in the area before contact between the two analysed species because both allopatric and sympatric populations of $E. calamita$ are infected. This result is consistent with various positive detections of Bd in other species and populations from the area (FERNÁNDEZ-GUIBERTEAU et al., 2016), and the detection of Bd in many other parts of the Iberian Peninsula where the introduced species $D. pictus$ is not present (GARNER et al., 2005; WALKER et al., 2010). Unfortunately, while there is a consensus in the Algerian origin of the $D. pictus$ populations introduced in Europe (ZANGARI et al., 2006; MONTORI et al., 2007; LLORENTE et al., 2015), there are no existing data of Bd in Algeria. Data on a nearby and similar species ($Discoglossus scovazzi$, in Morocco), reflect a level of infection prevalence (8.3%) clearly lower than that found in our study (36.6%), but with similar values of intensity (mean: 77.9 GE, range: 10-100 GE) (EL MOUDEN et al., 2011), suggesting that Discoglossus from northern Africa are not a reservoir of Bd. In the same genus, unusual mortalities of the Tyrrhenian painted frog ($Discoglossus sardus$) were found at three sites in the Limbara Mountains of northern Sardinia (BIELBY et al., 2009). These authors indicated that infection and mortality occurred at locations with characteristics that are not consistent with the published habitat requirements of the pathogen (Bd), and that $D. sardus$
would be a primary host for Bd, unlike *D. pictus* in NE Iberian Peninsula.

In our study, we observed lower Bd prevalence in areas where there is only one of the two analysed species (Fig. 2) and a trend towards lower intensities in allopatric populations of both species compared with sympatric ones. These results could point to cross infection when both species coexist in the same geographic area. This would agree with the results of Fernández-Beaskoetxea *et al.* (2016), who experimentally found that *B. spinosus* larvae had a significantly higher infection burden when raised in the presence of *A. obstetricans* larvae than when raised in the absence of *A. obstetricans*. On the other hand, our results also concur with those of Cunningham & Minting (2008), who found a strong association between Bd infection and presence of allochthonous amphibians in aquatic habitats. This finding supports the hypothesis that some non-native species, like *D. pictus*, might be linked to the spread of Bd and could increase the infection level of autochthonous species.

*Discoglossus pictus* coincides with *E. calamita* in the use of temporary and ephemeral aquatic environments as breeding habitats (Bosch & Martínez-Solano, 2003). These habitats are generally shallow and sun-exposed, and are therefore prone to drying up in the early summer, which may help to kill or remove chytrid fungi because the sporangia and zoospores are not resistant to desiccation (Johnson *et al.*, 2003). These observations agree with our data, as the only studied population negative for Bd presence was sampled in autumn, after pond desiccation. These results are in line with those of Minting (2012), who demonstrated that adults of *E. calamita* showed higher infection rate in wet conditions during the breeding season than when they had a completely terrestrial activity. The same results have also been found in *B. spinosus* and the Alpine newt (*Ichthyosaura alpestris*), where infections of terrestrial individuals are rare, suggesting that terrestrial habitats can be less suitable for Bd than aquatic ones (Davera, *et al.*, 2018a,b). As Bd detection was performed from swabs on the surface of the skin, the lack of autumnal detections of Bd might not represent a real absence of infection but a change in the activity of the pathogen. In the current study, detection was based on the presence of the infectious stage of Bd zoospores, which may not continuously emerge from infected animals. Bd zoospores are aquatic and killed by drying (Johnson *et al.*, 2003), so their emergence in dry conditions is probably maladaptive (Minting, 2012). In this sense, some studies have evidenced the existence of cryptic but persistent infection in terrestrial hosts (Minting, 2012).

Up to date, no symptomatology or mortality due to Bd infection has been detected in the surveyed area. However, parasite infection differs dramatically across years and habitats, and severe epidemics occur infrequently and unpredictably in a relatively small subset of habitats (Hite *et al.*, 2016). The study conducted by Hite *et al.* (2016) found that the structure of habitat and the existence of permanent waterbodies were linked to the density of Bd. Our results show great differences among populations, possibly caused by important differences in structure among ephemeral
or temporary ponds. All the individuals analysed in our study were adults and use different terrestrial habitats, so it is probably during the larval period when cross infection can happen.

*Discoglossus pictus* is a dominant species in ephemeral ponds within their distribution area. Our findings indicate that their occupancy of these aquatic environments can be an important contribution as a reservoir host of Bd to the maintenance of the fungal pathogen in sympatric species using ephemeral ponds as breeding habitats.

**Acknowledgement**

Permission for capture was granted by the Departament de Territori i Sostenibilitat, Generalitat de Catalunya. AFL was funded by Fundación General CSIC and Banco Santander.

**References**

**Barrinaga, N.** (1990). Where have all the frogs gone. *Science* 247: 1033-1034.

**Berger, L.; Speare, R.; Daszak, P.; Green, D.E.; Cunningham, A.A.; Goggin, C.L.; Slocombe, R.; Ragan, M.A.; Hyatt, A.D.; McDonald, K.R.; Hines, H.B.; Lips, K.R.; Marantelli, G. & Parkes, H.** (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences* 95: 9031-9036.

**Biely, J.; Bovero, S.; Sotgiu, G.; Tessa, G.; Favelli, M.; Angelini, C.; Doglio, S.; Clare, F.C.; Gazzaniga, E.; Lapietra, F. & Garner, T.W.J.** (2009). Fatal chytridiomycosis in the Tyrrhenian painted frog. *EcoHealth* 6: 27-32.

**Borzée, A.; Kosch, T.A.; Kim, M. & Jang, Y.** (2017). Introduced bullfrogs are associated with increased *Batrachochytrium dendrobatidis* prevalence and reduced occurrence of Korean treefrogs. *PLoS One* 12: e0190551.

**Bosch, J. & Martínez-Solano, I.** (2003). Factors influencing occupancy of breeding ponds in a montane amphibian assemblage. *Journal of Herpetology* 37: 410-413.

**Bosch, J. & Martínez-Solano, I.** (2006). Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park (Central Spain). *Oryx* 40: 84-89.

**Bosch, J.; Martínez-Solano, I. & García-París, M.** (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* 97: 331-337.

**Bosch, J.; Fernández-Beaskoetxea, S.; Scherer, R.D.; Amburgey, S.M. & Muths, E.** (2014). Demography of common toads after local extirpation of co-occurring midwife toads. *Amphibia-Reptilia* 35: 293-303.

**Boyle, D.G.; Boyle, D.B.; Olsen, V.; Morgan, J.A.T. & Hyatt, A.D.** (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60: 141-148.

**Brannelly, L.A.; Webb, R.J.; Hunter, D.A.; Cleemann, N.; Howard, K.; Skerratt, L.F.; Berger, L. & Scheele, B.C.** (2018). Non-declining amphibians can be important reservoir host for amphibian chytrid fungus. *Animal Conservation* 21: 91-101.

**Cunningham, A.A. & Minting, P.** (2008). *National Survey of Batrachochytrium dendrobatidis Infection in UK Amphibians,* 2008. Scottish Natural Heritage Countryside, Council for Wales, States of Jersey, Institute of Zoology, London, UK.

**Cunningham, A.A.; Daszak, P. & Wood, J.L.N.** (2017). One health, emerging infectious diseases and wildlife: two decades of progress? *Philosophical Transactions of the Royal Society B* 372: 20160167.

**Daszak, P.; Berger, L.; Cunningham, A.A.; Hyatt, A.D.; Green, D.E. & Speare, R.** (1999).
Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5: 735-748.

Daszak, P.; Cunningham, A.A. & Hyatt, A.D. (2000). Emerging infectious diseases of wildlife-- threats to biodiversity and human health. *Science* 287: 443-449.

Daszak, P.; Streeby, A.; Cunningham, A.A.; Longcore, J.; Brown, C. & Porter, D. (2004). Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. *The Herpetological Journal* 14: 201-207.

Daversa, D.R.; Manica, A.; Bosch, J., Jolles, J.W. & Garner, T.W.J. (2018a). Routine habitat switching alters the likelihood and persistence of infection with a pathogenic parasite. *Functional Ecology* 32: 1262-1270.

Daversa, D.R.; Monsalve-Carcano, C.; Carrascal, L.M. & Bosch, J. (2018b). Seasonal migrations, body temperature fluctuations, and infection dynamics in adult amphibians. *PeerJ* 6: e4698.

El Mouden, E.H.; Slimani, T.; Donaire-Barroso, D. & Bosch, J. (2011). First record of the chytrid fungus *Batrachochytrium dendrobatidis* in North Africa. *Herpetological Review* 42: 71-75.

Escoriza, D. & Boix, D. (2012). Assessing the potential impact of an invasive species on a Mediterranean amphibian assemblage: a morphological and ecological approach. *Hydrobiologia* 680: 233-245.

Fernández-Beascoetxea, S.; Bosch, J. & Bielby, J. (2016). Infection and transmission heterogeneity of a multi-host pathogen (*Batrachochytrium dendrobatidis*) within an amphibian community. 7

Fernández-Guiberteau, D.; Bargalló, F.; Martínez-Silvestre, A.; Miras, M.; Blanco, O. & Mompart, J.M. (2016). Projecte Grafi: aportacions al coneixement sobre biopatologia de les poblacions catalanes de granotes verdes. *Butlletí de la Societat Catalana d’Herpetologia* 23: 39-43.

Fisher, M.C.; Henk, D.A.; Briggs, C.J.; Browstein, J.S.; Madoff, L.C.; McCraw, S.L. & Gurr, S.J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186-194.

Garner, T.W.J.; Walker, S.; Bosch, J.; Hyatt, A.D.; Cunningham, A.A. & Fisher, M.C. (2005). Widespread European distribution of a global amphibian pathogen. *Emerging Infectious Diseases*, 11: 1639-1641.

Global Bd Mapping Group (2018). Bd-Maps. Imperial College London - The Wellcome Trust, London, UK. Available at https://www.bd-maps.net/. Retrieved on 7 February 2019.

Hite, J.L.; Bosch, J.; Fernández-Beascoetxea, S.; Medina, D. & Hall, S.R. (2016). Joint effects of habitat, zooplankton, host stage structure and diversity on amphibian chytrid. *Proceedings of the Royal Society B* 283: 20160832.

Johnson, M.L.; Berger, L.; Philips, L. & Speare, R. (2003). Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57: 255-260.

Llorente, G.A.; Montori, A. & Pujol-Buxó, E. (2015). El sapillo pintojo mediterráneo (*Discoglossus pictus*) en la península ibérica. *Boletín de la Asociación Herpetológica Española* 26: 15-19.

Lips, K.R.; Brem, F.; Brenes, R.; Reeve, J.D.; Alford, R.A.; Voyles, J.; Carey, C.; Livo, L.; Pessier, A.P. & Collins, J.P. (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences* 103: 3165-3170.

Mazzoni, R.; Cunningham, A.A.; Daszak, P.; Apolo, A.; Perdomo, E. & Speranza, G. (2003). Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. *Emerging Infectious Diseases* 9: 995-998.

Miaud, C.; Dejean, T.; Savard, K.; Millery-
Vigues, A.; Valentini, A.; Gaudin, N.C.G. & Garner, T.W.J. (2016). Invasive North American bullfrogs transmit lethal fungus Batrachochytrium dendrobatidis infections to native amphibian host species. Biological Invasions 18: 2299-2308.

Minting, P. (2012). An Investigation into the Effects of Batrachochytrium dendrobatidis (Bd) on natterjack toad (Bufo calamita) populations in the UK. PhD. Dissertation, University of Sussex, Brighton, UK.

Miras, M.; Fernández-Güibertau, D.; García, X.; Bargalló, F.; Espuny, A.; Baena, O.; García, A. & Maluquer-Margalef, J. (2017). Noves aportacions en la distribució de Batrachochytrium dendrobatidis a Catalunya. Butlletí de la Societat Catalana d’Herpetologia 24: 66-72.

Montori, A.; Llorente, G.A.; Richter-Boix, A.; Villero, D.; Franch, M. & Garriga, N. (2007). Colonización y efectos potenciales de la especie invasora Discoglossus pictus sobre las especies nativas. Munibe 25: 14-27.

Montori, A.; Franch, M.; Llorente, G.A.; Richter, À.; Sansebastian, O., Garriga, N. & Pascual, G. (2009). Declivi de les poblacions d’amfibis al delta del Llobregat. Materials del Baix Llobregat 15: 65-70.

Picco, A.M. & Collins, J.P. (2008). Amphibian commerce as a likely source of pathogen pollution. Conservation Biology 22: 1582-1589.

Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D. & R Core Team (2018). nlme: Linear and Nonlinear Mixed Effects Models, Version 3.1-137. R Foundation for Statistical Computing, Vienna, Austria. Available at https://CRAN.R-project.org/package=nlme/. Retrieved on 23 September 2018.

R Core Team (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at https://www.R-project.org/. Retrieved on 23 September 2018.

Rachowicz, L.J.; Knapp, R.A.; Morgan, J.A.T.; Stice, M.J.; Vredenburg, V.T.; Parker, J.M. & Briggs, C.J. (2006). Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87: 1671-1683.

Reiczigel, J. & Rózsa, L. (2005). Quantitative Parasitology 3.0. J. Reiczigel & L. Rózsa, Budapest, Hungary.

Richter-Boix, A.; Garriga, N.; Montori, A.; Franch, M.; San Sebastián, O.; Villero, D. & Llorente, G.A. (2013). Effects of the non-native amphibian species Discoglossus pictus on the recipient amphibian community: niche overlap, competition and community organization. Biological Invasions 15: 799-815.

Rózsa, L.; Reiczigel, J. & Majoros, G. (2000). Quantifying parasites in samples of hosts. Journal of Parasitology 86: 228-232.

Saura-Mas, S.; Pifarré, M. & Pedernera, C. (2016). Diagnosi de la diversitat i distribució d’amfibis al Parc Natural dels Aiguamolls de l’Empordà. Annals de l’Institut d’Estudis Empordaneses 47: 237-266.

Schloegel, L.M.; Toledo, L.F.; Longcore, J.E.; Greenspan, S.E.; Vierira, C.A.; Lee, M.; Zhao, S.; Wangen, C.; Ferreira, C.M.; Hipolito, M.; Davies, A.J.; Cuomo, C.A.; Daszak, P. & James, T.Y. (2012). Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Molecular Ecology 21: 5162-5177.

Searle, C.L.; Bígà, L.M.; Spatafora, J.W. & Blaustein, A.R. (2011). A dilution effect in the emerging amphibian pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences 108:16322-16326.

Skerratt, L.F.; Berger, L.; Speare, R.; Cashins, S.; McDonald, K.R.; , Phillott, A.D.; Hines H.B. & Kenyonet, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4: 125-134.

Spitzen-van der Sluijs, A.M. & Zollinger, R. (2010). Literature review on Batrachochytrium dendrobatidis. Stichting RAVON, Nijmegen, The Netherlands.
Walker, S.F.; Bosch, J.; Gomez, V.; Garner, T.W.J.; Cunningham, A.A.; Schmeller, D.S.; Ninyerola, M.; Henk, D.A.; Ginestet, C.; Arthur, C.P. & Fisher, M.C. (2010). Factors driving pathogenicity vs. prevalence of amphibian panzootic chytridiomycosis in Iberia. Ecology Letters 13: 372-382.

Zangari, F.; Cimmaruta, R. & Nascetti, G. (2006). Genetic relationships of the eastern Mediterranean painted frogs based on allozymes and mitochondrial markers: evolutionary and taxonomic inferences (Amphibia, Anura, Discoglossidae). Biological Journal of the Linnean Society 87: 515-536.