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SHORT NOTE

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No detection of the chytrid fungus (*Batrachochytrium dendrobatidis*) in a multi-species survey of Ireland's native amphibians

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We present the results of the first survey undertaken to determine the status of *Batrachochytrium dendrobatidis* (*Bd*) on the island of Ireland. All three species of Ireland's native amphibian fauna were sampled, resulting in 195 skin swabs obtained from 22 locations in eight counties across the island. Using *Bd*-specific DNA primers in quantitative real-time PCR (qPCR) analysis, all samples tested negative for the presence of chytrid. These results may be considered good news for Ireland's amphibians, however we advise that the establishment of a long-term monitoring system will be essential for future management.

Key words: chytrid, *Epidalea calamita*, Ireland, qPCR, *Rana temporaria*

Various factors can interact to negatively impact amphibian populations, including disease, increased solar ultraviolet radiation, agrochemical use, invasive species, habitat modification and climate change (for examples see St-Amour et al., 2008; Blaustein et al., 2010; D'Amore et al., 2010; Hof et al., 2011; Mitchell et al., 2012; Baker et al., 2013). Of these, the emergent amphibian disease chytridiomycosis (caused by the pathogenic chytrid fungus *Batrachochytrium dendrobatidis*; *Bd* hereafter) has been identified as a major factor in recent extinctions and declines of amphibian populations worldwide (e.g., Berger et al., 1998; Daszak et al., 2003; Lips et al., 2006; Skerratt et al., 2007; Voyles et al., 2009). Europe has not escaped these declines (Garner et al., 2005; Duffus & Cunningham, 2010) yet the island of Ireland is assumed to be one of the last remaining areas of western Europe to be free of *Bd* (Inns, 2009). To date there have been no reports of mass die-offs or noticeable declines in native amphibian populations, both of which can indicate the presence of chytrid. However the lack of any empirical scientific study to determine the status of *Bd* on the island means that Ireland's "chytrid free" status is purely an assumption.

Three amphibian species are native to Ireland: the common frog (*Rana temporaria*), the smooth newt (*Lissotriton vulgaris*) and the natterjack toad (*Epidalea calamita*). Of these species, the common frog and the natterjack toad have been shown to possess unique genetic lineages (Rowe et al., 2006; Teacher et al., 2009; May & Beebee, 2010). Ireland's natterjack toad populations exist on the very edge of the species distribution in north-western Europe, making them valuable both scientifically and from a conservation perspective. Therefore it was of utmost importance that an island-wide survey was conducted to determine if *Bd* is present.

The Irish Amphibian Chytrid Survey 2012 (IACS) was an initiative by the Herpetological Society of Ireland (HSI) to utilize teams of licensed volunteers to obtain amphibian skin swabs from all three species of amphibians across the island of Ireland. The IACS was executed by the HSI in concert with the National Parks and Wildlife Service and the Northern Ireland Environment Agency. The IACS was conceived in order to address the following questions: (i) Is *Bd* present in populations of amphibians on the island of Ireland? (ii) If *Bd* is present, how prevalent is its occurrence? (iii) If *Bd* is not present, what are the future directions that need to be taken in order to maintain this status?

We standardized our field protocols with those of Smith (2011). We assumed each amphibian species was equally likely to be infected with the chytrid fungus and that, if present, the infection would be randomly distributed amongst the amphibians present, regardless of life-stage and geographic proximity. Volunteers were recruited via a call for assistance on a popular national radio wildlife show and through an online and social media advertising campaign. Sampling, by way of non-invasive dermal swabbing, was conducted from May to October 2012. Samples were collected by swabbing (with MW100 medical swabs) the ventral and femoral skin surfaces and the webbing between the digits of the hind feet (and the tail of newts) of each amphibian

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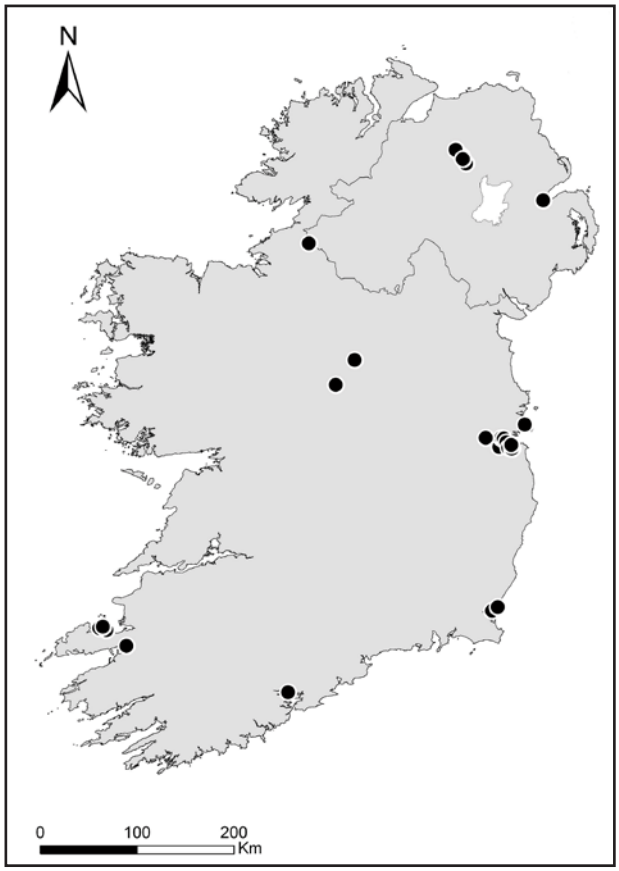

Fig. 1. Map of Ireland displaying the locations (black circles) where amphibians were surveyed for *Bd*.

caught. Only post-metamorphic individuals were sampled. Priority was given to sampling animals found in water, as there have been some indications that the chytrid fungus is harder to detect in amphibians in their terrestrial phase (Cunningham & Minting, 2008). All volunteers were instructed to adhere to biosecurity and good practice guidelines in order to minimize the risk of spreading the fungus between sites. We endeavoured to sample a minimum of 30 individuals per location in order to meet suggested thresholds for the detection of *Bd* (Cunningham & Minting, 2008). In the case that this threshold could not be met at a particular site the samples were still considered to be informative, though the results of these sites would be interpreted with a degree of consideration.

Irish natterjack toads occur in twelve populations in the counties of Kerry and Wexford (see Beebee, 2002) and were grouped into five distinct population categories for the purpose of this study; i) large native meta-population, ii) small native meta-population, iii) isolated native, iv) isolated native reintroduction and v) introduced. Every effort was made to adequately sample known natterjack toad breeding sites in Kerry and Wexford due to the unique characteristics and status of this species in Ireland.

Following collection, the swabs were frozen while awaiting analysis in order to minimize degradation of the sample. Real-time quantitative PCR (qPCR) was used to analyze the samples and test for prevalence (presence/absence). The analysis was performed at the Institute of Zoology, London (IoZ) following the protocol described by Boyle et al. (2004). This laboratory technique utilizes *Bd*-specific PCR primers to isolate and amplify any *Bd* DNA present in the swab samples. This approach allows not only the detection of the fungus, but quantification of the number of zoospores (and therefore infection level) in the case of samples that are positive. Any samples that yielded inconclusive results in the qPCR were retested in order to minimize the chance of obtaining a false positive or false negative result.

In total, 195 swabs were analyzed from 28 locations, representing 22 distinct sampling sites, in eight counties across the island of Ireland (Fig. 1). A full summary of the results of this analysis broken down by county and by species is presented in Table 1. Representative samples for all three native species of amphibian were acquired. All 195 samples (comprising 151 common frogs, 30 natterjack toads and 14 smooth newts) tested negative for the presence of *Bd* as determined by qPCR analysis (Table 1).

The widespread negative results from the analysis are welcome, although this was not the expected outcome of the survey, given the history and prevalence of *Bd* elsewhere in the UK and mainland Europe (Bosch et al., 2001; Cunningham et al., 2005; Garner et al., 2005; Cunningham & Minting, 2008; Scalera et al., 2008; Ohst et al., 2011; Sztatecsny & Glaser, 2011; Civiš et al., 2012). While these results are encouraging and tentatively hopeful for the future of Ireland's native amphibians, it should be taken into account that only three locations

Table 1. Number of sampling locations, species, number of individuals sampled and results of qPCR for each county. Species abbreviations are as follows; *Rt*=*Rana temporaria*, *Lv*=*Lissotriton vulgaris* and *Ec*=*Epidalea calamita.*

Species	County	No. of distinct sampling sites	No. of specimens sampled	No. infected with *Bd*
Rt	Antrim	1	30	0
Rt	Cork	1	15	0
Rt	Derry	4	26	0
Rt, Lv	Dublin	10	34	0
Ec	Kerry	2	7	0
Rt	Leitrim	1	12	0
Rt, Lv	Longford	2	41	0
Ec, Rt, Lv	Wexford	1	30	0
Total		22	195	0

achieved the target of 30 samples per sample site, therefore it is possible that false-negative results could have been due to inadequate sampling. However, it should be noted that small sample sizes may still be representative in cases where the number of animals sampled at each location actually comprised a large proportion of the population at these sites.

It seems highly likely that if the chytrid fungus is not already present in Ireland at low levels (and thus not detected by this study) then many risk factors are in place for a future establishment. The two primary risk factors could be considered to be the presence of transmission routes for the fungus to reach the island, and ecological factors that might make subsequent establishment possible. Both of these factors are present in this case, as Ireland shares a number of waterfowl species with the island of Great Britain (which has confirmed chytrid-positive amphibian populations) and migrating waterfowl have recently been identified as potential vectors of *Bd* (Garmyn et al., 2012). Invasive species may also introduce the fungus, and since the sampling of this study was completed a small population of non-native alpine newts (*Icthyosaura alpestris*) has been identified in Co. Galway (K. French, pers comm). This may be noteworthy as their presence in Great Britain has been associated with the presence of *Bd* (Cunningham & Minting, 2008). In terms of ecological viability, niche models based on bio-climatic variables have already determined that the island of Ireland should provide a highly suitable environment for the chytrid fungus (Ron, 2005).

The IACS was successful in terms of generating essential baseline data on the status of *Batrachochytrium dendrobatidis* on the island of Ireland. Future monitoring of amphibian populations in Ireland is crucial and we recommend that a follow up study be undertaken.

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