Herpetology Notes, volume 11: 451-454 (2018) (published online on 26 May 2018)

# The absence of the amphibian chytrid fungi in the common midwife toad (*Alytes obstetricans*) from an introduced population in Cambridge, UK

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**Abstract.** The amphibian chytrid fungi (*Batrachochytrium dendrobatidis* and *B. salamandrivorans*) threaten amphibian species globally, and the introduction of non-native species is an important pathway via which the pathogen has spread. Here we report the results of disease screening of an introduced population of common midwife toad (*Alytes obstetricans*) in Cambridge, UK. Thirty-three animals were screened for the presence of both *Bd* and *Bsal*, none of which provided positive results. This study is part of an ongoing project to investigate the potential threats of midwife toads to local amphibian populations in Cambridge.

**Keywords:** amphibians, *Batrachochytrium dendrobatidis*, *B. salamandrivorans*, infectious diseases, non-native species

## Introduction

Globally, amphibian populations are declining at an alarming rate. This is due to a number of factors including habitat loss, pollution and over-harvesting (Ceballos et al., 2017). One of the most alarming causes of decline is that of infectious disease. The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) is implicated in amphibian declines, extirpations, and extinctions across the globe, and its spread is considered to have been facilitated by the introduction of non-native species (Fisher & Garner, 2007). There is also evidence that the salamander chytrid fungus (*B. salamandrivorans*), which is believed to have been introduced to Europe via the pet trade may cause similar catastrophic declines (Martel et al., 2014). In recent experiments, *B. salamandrivorans* (*hereafter Bsal*) was shown to be transmitted between anurans and susceptible urodelean hosts (Stegen et al., 2017), which up until now was an overlooked pathway for the spread of the disease. In terms of infectious diseases, such as chytrid, they are the most challenging threats to mitigate against in amphibians (Garner et al., 2016). Disease screening is important to ensure susceptible populations are not at risk of chytridiomycosis, the consequence of infection with the chytrid fungi.

In Northern Europe, *B. dendrobatidis* (hereafter *Bd*) infection has a limited lethal effect in most amphibian species although one of the most susceptible species is the common midwife toad, *Alytes obstetricans* (Duffus & Cunningham, 2010). In recent experiments, it was demonstrated that *A. obstetricans* could act as a reservoir for *Bsal* without showing any clinical signs (Stegen et al., 2017). The increased likelihood of infection by *Bd* may be due to the overwintering of *A. obstetricans* tadpoles acting as a reservoir for an extended period of time, thus increasing the likelihood of infection in conspecifics by repeat exposure (Bosch et al., 2001). It is therefore important to regularly monitor any *A. obstetricans* populations which have been introduced outside of their native range to ensure that they are free from pathogens which could have disastrous impacts on a naïve amphibian fauna (Price et al. 2016; Duffus et al., 2017).

*Alytes obstetricans* has been established in Cambridge, UK, where it is non-native, for at least a decade (Baker, 2007). However, the toads are currently restricted to the back gardens of a small block of parallel Victorian houses, not far from the city centre (Baker, 2007). Due to the extensive mosaic of habitats available, they are able to persist despite being outside of their natural range in mainland Northern Europe (Beebee & Griffiths, 2000). It is unknown from where the toads originated and throughout the UK they have been established for over

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a century (Beebee & Griffiths, 2000). It is not currently clear whether *A. obstetricans* poses a threat to native amphibians.

The study reported herein is part of an ongoing project by the Cambridgeshire and Peterborough Amphibian and Reptile Group to determine where the introduced *A. obstetricans* originate from, to investigate whether they pose a threat as a disease vector and to estimate their population size. As part of a preliminary study, a total of seven individuals were swabbed in 2016, with two swabs from 2017 being fast tracked due to the suspected presence of disease. These nine swabs came back negative for both *Bd* and *Bsal* (Allain & Goodman, 2017a), this report continues directly from that initial study.

**Figure 1.** Cambridge midwife toad being swabbed to test for chytrid.

## Materials and Methods

Between late May and early September 2017, eight residential gardens in central Cambridge were surveyed for *A. obstetricans*. The midwife toads are currently restricted to approximately 35 gardens covering an area of 2000 m$^2$. Surveys involved the use of call playbacks designed to elicit responses from males so that their location could be pinpointed (Allain & Goodman, 2017b). Females and juveniles were located by the active searching of refugia within each garden. Captured toads were held in individual zip-lock bags to prevent cross contamination, and each bag contained sufficient moisture and air in to minimise stress. All appropriate biosecurity measures were taken when handling the toads including the use of nitrile gloves (Mendez et al., 2008), the changing of gloves between each site, and the use of Virkon S when disinfecting field equipment (Young et al., 2007).

Sterile cotton tipped swabs (Medical Wire & Equipment, MW-100) were used to swab for chytrids using standardised protocols. Tadpoles were captured using a small aquarium net and returned to the pond after being sampled, the swabs were gently spun for 10 s between the fingers whilst in contact with their mouthparts (Retallick et al., 2006). As part of the swabbing process, each toad was sprayed with a small amount of water in order to free their skin of any dirt or detritus which may inhibit the PCR assay (Kosch & Summers, 2013). In adults, the abdomen, thighs, groin, and feet of each individual was swabbed 15 times (Fig. 1). After toads were swabbed, data such as their sex, weight and snout to vent length were recorded. All toads caught were also photographed in order to allow the recognition of individuals (using software such as Wild-ID and manual comparisons) and to aid in population estimates before being released at the point of capture.

The swabs containing the samples were refrigerated at 5°C until the time when they were sent to the Institute of Zoology at the Zoological Society of London for analysis. The samples were analysed following the protocol described by Boyle et al. (2004).

## Results

Twenty-nine post-metamorphic individuals and four tadpoles were caught and screened; of which 16 were male, five female, and eight undetermined post-metamorphic toads. All samples were negative for both *Bd* and *Bsal*. A deceased juvenile *A. obstetricans* was submitted for post-mortem evaluation at the end of June 2017. In appearance the toad was slightly desiccated so we were unable to determine the cause of death without further investigation. The juvenile toad was negative for *Bd* and *Bsal* (as well as *Ranavirus*).

## Discussion

Thirty-three individuals were captured and swabbed during the search period described above for *A. obstetricans*, of which twenty-nine were post-metamorph, all of which were negative for amphibian chytrids. Although our results are negative for both amphibian chytrid fungi, a greater sample size is required to rule out infection in the rest of the population (estimated at 80 – 100 post-metamorphic individuals). Skerratt et al. (2008), estimated the need to test at least 60 individuals per population to achieve 95% certainty of detecting one *Bd*-positive frog if the infection prevalence in the population was >5%. If *Bd* is present, it is likely at a prevalence below 10% and some modification of our protocols may be needed in the future (Gray et al., 2017). If infected animals are found then further investigation would be needed to identify the strain of *Bd* and link it to a possible dispersal/introduction pathway. We estimate that the number of individuals sampled so far (including samples used in Allain and Goodman, 2017a) account for 37 – 46% of the suspected post-metamorphic population.

With the exception of the deceased *A. obstetricans,* the study population is yet to be tested for the presence of a *Ranavirus*, while swabs can be used for this; liver samples are a more reliable source for analysis (Gray et al., 2012). The toads may be infected with *Ranavirus* but the tell-tale signs of a mass die-off have yet to be seen in the local naïve amphibian populations. The deceased juvenile had a deformed hind limb which was also observed in three individuals earlier in June 2017. It is not clear as to whether these deformities were caused by disease, predation, inbreeding or another factor. Recently a new *Ranavirus* was isolated from *A. obstetricans* from the Iberian Peninsula (Balseiro et al., 2009) which has since caused declines in native populations of amphibians within continental Europe (Balseiro et al., 2010; Kik et al., 2011, Price et al. 2014). If mortality events are observed in the future, whole carcasses will be collected for necropsy and a more detailed investigation into the cause of the mortality event, such as molecular screening for the presence of *Ranavirus* DNA, will take place.

*Alytes obstetricans* typically shares the gardens with *Bufo bufo*, *Rana temporaria*, *Lissotriton helveticus* and *L. vulgaris,* all of which are seen regularly on surveys at the study site but in low numbers; *A. obstetricans* is the most frequently encountered amphibian species. All five species breed in the same ponds and infection transmission between them is likely to occur. These breeding ponds were only identified during 2017 due to an expansion in the search area mediated by the cooperation of local residents. The other amphibian species were not swabbed for infectious diseases, as *A. obstetricans* is the most susceptible species in the assemblage these were targeted for cost effectiveness due to the higher chance of them being infected if the disease is present in the area being studied. Other amphibian species will also be swabbed in future surveys.

**Acknowledgements.** We would like to thank the local residents for allowing us access to their gardens so that surveys could be undertaken, thanks must also be paid to our small but dedicated team of survey volunteers. We would also like to thank Matthew Perkins of the Institute of Zoology, Zoological Society of London for carrying out the qPCR analysis and the Garden Wildlife Health project for analysing the deceased midwife toad collected in June. The project would not be possible without our generous backers from our successful crowdfunding campaign, thank you everyone. All work was carried out under Natural England licence 2016-26495-SPM-NNR-2. We would also like to thank Jim Labisko for useful and informative reviews, which helped improve the manuscript.

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*Accepted by Simon Maddock*