

## Absence of chytrid fungus (*Batrachochytrium dendrobatidis*) in an introduced population of the common midwife toad (*Alytes obstetricans*) in Cambridge, UK

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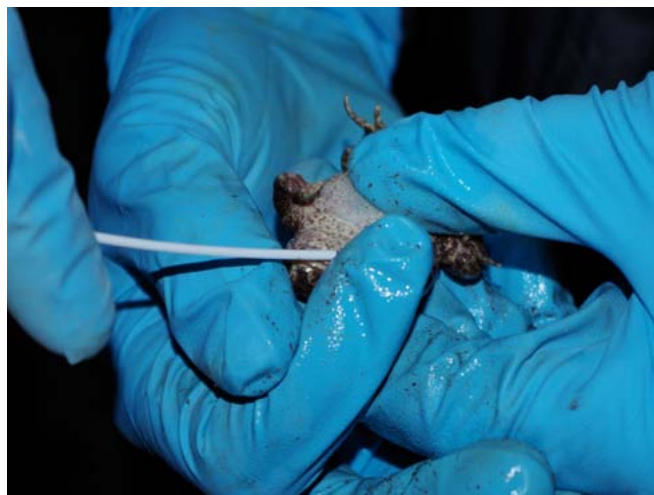
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Amphibian populations globally are currently declining at an alarming rate due to a number of factors, with disease being one of the most troublesome (Stuart et al., 2004). The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) has been implicated in the decline of amphibian species globally and is thought to have been spread through the introduction of non-native species (Fisher & Garner, 2007). Recently a new chytrid fungus (*B. salamandrivorans*) affecting salamanders was discovered (Martel et al., 2014). In recent experiments it was demonstrated that the common midwife toad (*Alytes obstetricans*) was able to act as a reservoir of this new amphibian chytrid fungus without showing any clinical signs (Stegen et al., 2017). In Europe, *B. dendrobatidis* (hereafter *Bd*) has a limited effect on most amphibian species (Duffus & Cunningham, 2010) although *A. obstetricans* is one of the more susceptible species; it is capable of spreading a number of infectious diseases to other amphibians. It is therefore important to monitor and screen any populations which may have an impact on further amphibian species. *Alytes obstetricans* has been established in Cambridge, UK for at least a decade (Baker, 2007) with very little monitoring have taken place before our study.

It is currently unknown where the toads originated from or the detail around their release but it is likely that they originated as pets which escaped into the local environment. The toads are currently restricted to the back gardens of a small block of parallel Victorian houses not far from Cambridge city centre (Baker, 2007). Due to the mosaic of habitats available to the toads, they are able to persist despite being outside of their natural range in northern Europe. The species has been part of the British landscape for over a century (Beebee & Griffiths, 2000) and it is not clear whether or not they pose a threat to our native amphibian species. This study aimed to determine whether or not the toads could be a potential threat as a disease vector in terms of being infected with the amphibian chytrid fungi.

Between June 2016 and May 2017, five gardens were investigated for the presence of midwife toads. This involved the use of call playbacks which were designed to elicit responses from males so that their location could be pinpointed (Allain & Goodman, 2017). Females and juveniles were located by actively searching potential refugia sites within each of the gardens. When midwife

toads were located, they were placed in separate zip-lock bags for a short duration until the search had been completed; this was on average between twenty and thirty minutes. 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 after use (Young et al., 2007). Before the toads were swabbed, data such as their sex, weight and snout to vent length were taken. A set of digital scales and plastic Vernier callipers were used to collect this data. As part of the swabbing process, each individual was sprayed with a small amount of water in order to free their ventral surface of any detritus which may interfere with the analysis process (Kosch & Summers, 2013). Sterile cotton tipped swabs (Medical Wire & Equipment, MW-100) were used to swab the abdomen, thighs, groin and feet of each individual between 10 and 15 times (Fig. 1). Each of the midwife toads were photographed for future reference before being released at the point of capture.



**Figure 1.** One of the midwife toads is swabbed before being released

Nine individuals were caught and screened; of which 4 were male, 2 female and 3 sub-adults. Midwife toads were found in all of the gardens surveyed and could be heard calling from others in the surrounding area. The snout to vent length of the *A. obstetricans* sampled averaged 3.43 cm (SD = 0.91 cm) and their mean weight was 5.49 g (SD

= 3.05 g). All of the swabs were sent off for analysis using qPCR to test for the presence of both amphibian chytrid fungi. The swabs were tested at the Institute of Zoology at the Zoological Society of London in July 2017. All samples were qPCR negative for both *Bd* and *Bsal*. The samples were refrigerated at 5 °C before they were sent to the lab for analysis. Our initial results are promising but further sampling is required to rule out infection in the rest of the population. The individuals sampled make up approximately 10 to 20% of the suspected post-metamorphic population. We are yet to sample any tadpoles, mainly due to difficulty in identifying the breeding pond(s). While the toads are cryptic in nature (Beebee & Griffiths, 2000), determining their potential location became easier as we developed our search image for the species. It is unlikely that the toads will spread further without anthropogenic intervention due to barriers that have been built in the environment, such as walls and especially roads, which have been shown to limit amphibian dispersal (Carr & Fahrig, 2001).

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