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Batrachochytrium salamandrivorans as a threat to British amphibians

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ABSTRACT - Globally, amphibian populations of many species are in critical decline. One major driver of amphibian decline is the emergence of chytridiomycosis. In addition to the initially known causative agent of this disease *Batrachochytrium dendrobatidis*, a novel amphibian pathogen, *Batrachochytrium salamandrivorans*, has recently been discovered. The potential impact this pathogen could have on wildlife health needs to be urgently addressed and assessed. Using amphibian species of the United Kingdom as a case study the present paper is a review of the state of the amphibian trade, current biosecurity measures, as well as the presence of a wild amphibian species in the UK thought to be susceptible to this disease. The review highlights the urgent need for research and bio-control evaluation, in order to ensure wildlife health security for amphibians in the UK.

INTRODUCTION

We are currently in the midst of a biodiversity crisis, which has been termed by many as the sixth mass extinction (Dunn et al., 2009; Barnosky et al., 2011). Amphibians have been one of the hardest hit groups with major declines in a third of species assessed by the IUCN (Beebee & Griffiths, 2005). Many drivers have been suggested for these declines, however disease is continually cited as one of the greatest factors contributing to this loss (Pounds et al., 2006). Chytridiomycosis is one of the most virulent of amphibian diseases, responsible for the highest number of extinctions of any known disease (Skerratt et al., 2007).

Until 2013, chytridiomycosis was thought to be caused by a single species of *Batrachochytrium dendrobatidis*, (Bd) (Daszak et al., 2001; Martel et al., 2013). However, Bd was not found in certain recent mass declines of amphibians that exhibited chytridiomycosis symptoms. These die-offs were eventually explained by the discovery of a novel species, *Batrachochytrium salamandrivorans* (Bsal) (Martel et al., 2013), which appears to target only the Urodelan group (newts and salamanders). Globalisation and trade have been implicated in the spread of both Bd and Bsal. The rate of the spread of Bd, and the geographically isolated nature of the Bsal outbreaks may have only been made possible by anthropogenic means (Martel et al., 2014). Given Britain's limited bio-control of amphibian imports and the mass mortalities that chytridiomycosis can cause, the import of amphibians could present serious epidemiological risks to UK amphibian species. This review focuses on elucidating the potential risks posed by Bsal to British amphibians by assessing the risks of wildlife trade to amphibian epidemiology, reviewing bio-control of amphibian imports to the UK and analyzing threats of Bsal to UK wildlife.

RISKS OF THE AMPHIBIAN TRADE

Scale of amphibian trade

The number of live animals traded each year globally is extremely large with almost 38 million live amphibians, birds, mammals and reptiles legally imported to the US alone every year (Marano et al., 2007). However, accurately quantifying the extent of the trade in animals, including amphibians, is near impossible given the multiple scales of trading from local level transactions to mass international commerce (Karesh et al., 2005). The situation is further complicated by both the illegal wildlife trade (Karesh et al., 2005) and ease of e-commerce (Kikillus et al., 2012). This extensive trade in live animals has been implicated in both amplification and spread of epizootic diseases in amphibians (Fèvre et al., 2006), with serious potential implications for British wildlife (Peel et al., 2012).

Disease amplification through the amphibian trade

The issue of disease amplification was addressed by Karesh et al. (2005) who highlighted how controlling disease at the interface between buyers and sellers is critical, suggesting that markets and shops could be hotspots for spreading and amplifying disease (Karesh et al., 2005; Hartung, 2003). Research into Bd in amphibians supports this assertion, as co-housing studies have shown that common toads (*Bufo bufo*), can cause infection among cohoused animals with increased presence increasing the intensity of Bd infection (Fisher & Garner, 2007; Schmeller et al., 2011). In addition, there are risks associated with long term environmental disease contamination in an area where other frogs may be kept (Peel et al., 2012; Johnson & Speare, 2003). Bsal has been transmitted between adults co-housed on damp toweling (Martel et al., 2013), which highlights how like Bd, Bsal potentially could be spread within the amphibian trade, as amphibians are often co-housed for transport (Peel et al., 2012). However, to determine if co-housing affects intensity of Bsal infection further research is needed.

Links between the amphibian trade and epizootic outbreaks.

These concerns are particularly pressing given the links between disease in the amphibian trade and epizootic outbreaks in wild amphibian populations, as there are multiple examples of how an infection spread by anthropogenic activity, like pet trade, can threaten populations of native species (Schloegel et al., 2010). One example is the Alpine newt, (*Ichthyosaura alpestris*) which has been identified as a spreader of Bd after introduction to the UK (Arntzen et al 2009), and more recently New Zealand (Arntzen et al. 2016). In New Zealand over 70% of individuals caught from the introduced population and screened for disease were found to be infected with Bd, (Artnzen et al. 2016) presenting a serious threat to the survival of native amphibian species.

Bsal in the amphibian trade

Bsal also has links with the international amphibian trade. Bsal was discovered in the Netherlands, following dramatic mortality of the native fire salamanders (*Salamandra salamandra*) (Martel et al., 2014). 96% of the Netherlands fire salamander population was lost between 2010 and 2013 (Spitzen et al., 2013), and subsequent ex-situ conservation efforts were compromised by 49% mortality of the captive population (Martel et al., 2013). After initial screening for known amphibian pathogens including Bd (Spitzen et al., 2013) returned negative results, further investigation by Martel et al., (2013) identified the presence of Bsal in fire salamander specimens from the 2010 mortality, and demonstrated that it causes mortality under lab conditions. Discovery that Bsal DNA showed 100% identity to samples found in Thailand, Vietnam and Japan, and the absence of overt disease in other parts of Europe, suggests that the pathogen was previously limited to East Asia (Martel et al., 2014). However, a recent large scale study to assess Bsal in Chinese amphibians showed no incidence of this disease, yet despite the lack of disease, infection may be widespread. This is because it is difficult to tell the level of infection from the Zhu et al., (2014) work as the long-term formalin storage of museum specimens used could have interfered the genetic analysis, and therefore detection of infection in carriers. Additionally, as noted by the authors many samples were taken in locations which exceeded the thermal tolerance of Bsal, so may not reflect disease prevalence at higher elevation. Therefore, despite disease not being found in the Zhu et al. (2014) study, it remains probable that Bsal recently emerged from Eastern Asia. Therefore, given the distance between Eastern Asia and the Netherlands, and other European countries like Germany (Pinto et al., 2015), it is likely that Bsal was spread by anthropogenic means, probably via the pet trade (Martel et al., 2014). Importantly Eastern Asia is one of the regions with the highest levels of indigenous live newt exports, the scale of which was demonstrated in a recent report which estimated that 2.3 million individuals of *Cynops orientalis* (native to China) were traded in the United States between 2001 and 2009, 44.1% of which were estimated to be wild caught (Herrel & Meijden, 2014). Further to this Bsal was recently discovered in three Vietnamese crocodile newts (*Tylototriton vietnamensis*) traded in Europe, two of which were imported into Europe in 2010 (Martel et al., 2014). All of this indicates that Bsal can probably spread from the amphibian trade to wild populations of urodela with catastrophic effects, and there is evidence to suggest that there is still prevalence of Bsal in the European and international amphibian trade. The seriousness of the risks posed by Bsal has been further highlighted by the alarming recent spread of this disease through Belgium and Germany (Spitzen-van der Sluijs et al., 2016). The US Fish and Wildlife Service has responded to the threat of Bsal by banning of the importation of 201 species of salamander (Injurious Wildlife Species; Listing Salamanders Due to Risk of Salamander Chytrid Fungus, 2016), while the Bern Convention and Council of Europe have issued strong recommendations for disease screening and trade restrictions (Council of Europe, 2015). However, there is still a pressing need for similarly progressive steps to be taken to ensure biosecurity of amphibians in Britain.

BIOCONTROL OF AMPHIBIANS IN THE UK

The current state of biocontrol of amphibians in the UK

In 2006, 131 599 amphibians from outside the EU were transported into the UK through the Heathrow Animal Reception Area as imports for the UK amphibian trade (Peel et al., 2012). However, numbers are likely to be far higher as this figure does not include amphibians transported via Manchester or Gatwick, those transported as pets, or those from within the EU (Peel et al., 2012).

In Britain, the importation of live amphibians is regulated by the International Animal Health division of the Department for Environment, Food and Rural Affairs (DEFRA) (Fisher & Garner, 2007). DEFRA is guided by the European Commission's General Animal Health and Welfare commission, as well as the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) (Fisher & Garner, 2007). Despite strict regulation to prevent disease in livestock (Waage & Mumford, 2008) there is limited legislation in place to counter the spread of disease in amphibian populations. Animals entering from outside the EU may be subject to veterinary examination (Department for Environment, Food & Rural Affairs and Animal and Plant Health Agency, 2015); yet PCR-based screening needed to detect disease in carriers is not required (Peel et al., 2012). Within the EU, imports of live animals are allowed with a certificate of health (McGrann & Wiseman, 2001). However, widespread e-commerce, makes this an area which is challenging to regulate (Lincoln et al., 2012; Kikillus et al., 2012).

UK legislation is in place to mitigate spread of disease once animals have been imported. Importers who notice disease in the animals must report them to local authorities which may require slaughter of the animals without compensation under Council Directive 90/425/EEC. Despite this ruling it is possible that lack of compensation may act as a disincentive to report disease.

Recognition of risks resulting from current state of amphibian biocontrol in the UK

The potential risks caused by lack of strict controls on the amphibian trade have been recognized by the British Veterinary Association and the British Veterinary Zoological Society which issued a joint statement calling for an EU ban on the trade in wild caught amphibians and reptiles (British Veterinary Association, 2003). In addition to this, a large NGO coalition which include members from the UK have written to the European Commission demanding that Europe takes similar steps as the US in banning imports of salamander into Europe (Steenwegen & Funcken, 2016). DEFRA is aware of these concerns as highlighted by the funding of research into amphibian disease (Martel et al., 2014). However, no significant regulatory action appears to have been taken on the research findings. These concerns become even more pressing given the recent finding of Bsal in three species of imported urodele acquired from a UK breeder (Cunningham et al. 2015), which has led to calls for amphibian breeders, keepers and veterinarians to ensure that recommended biosecurity measures are strictly followed (Cunningham et al. 2015).

RISKS OF BSAL TO BRITISH WILDLIFE

Potential vulnerability of British newts

Britain has three native species of Urodelaes (Arnold, 1995): the palmate newt (*Lissotriton helveticus*), the common newt (*Lissotriton vulgaris*), and the great crested newt (*Triturus cristatus*). Martel et al. (2014) carried out a suite of infection studies with Bsal which showed the great crested newt to be very susceptible to this disease, with mortality in all animals experimentally infected after 25-57 days (Martel et al., 2014). Interestingly the palmate newt was found to be resistant to the disease, showing no mortality on infection. The common newt was not tested. However, there are currently no published studies on the passage of Bsal from a resistant individual to a susceptible individual, or any data on how long after infection the resistant species will be infectious to susceptible species. Furthermore, it must be noted that these studies have been carried out under laboratory conditions using small numbers of captive bred individuals and consequently there may be differences in the field.

Potential for emergence and spread of Bsal in the UK

There are also serious gaps in knowledge as to how this pathogen spreads environmentally. Bd has shown potential for transmission via zoospores in water as well as by direct contact (Johnson & Speare, 2003) and potentially even wind-blown during rainstorms (Kolby et al., 2015). The finding that Bsal zoospores are highly motile may suggest transfer by water, while infection by co-housing could suggest transmission by direct contact or contact to a contaminated environment (Martel et al., 2013). However, these speculations need to be addressed in order for an accurate assessment of likely disease spread in Britain, especially given the finding that of Bsal is can grow at 5˚C with an optimum 10-15˚C (Martel et al., 2013). March to August is reported to be the six-month period with the highest great crested newt activity in the UK (Arnold, 1995), the majority of which is confined to England and Wales (Arnold, 1995). Between 1910-2016, the average maximum and minimum temperature recorded for March to August in England was 15.9˚C -7.2˚C, and for Wales, 14.7-6.7˚C (MET office, 2016). This overlap of UK temperatures during peak periods of salamander activity, and temperatures compatible with Bsal growth raises serious concerns about the great crested salamander, with the possibility that Bsal spread is unlikely to be limited by temperature across much of the UK range of the great crested newt.

In addition to addressing questions regarding the environmental spread of this disease, there are also questions regarding the risks of infected captive animals or infected material from captive animals coming in contact with the environment. There are currently unknown levels of disease in captive amphibians in the UK, as well as unknown rates of escape or release, making it difficult to quantify risks (Peel et al., 2012). However, examples like the introduction of North American bullfrogs to the UK, and with them chytridiomycosis (Cunningham et al., 2005; Fisher & Garner, 2007) show that there is precedent for escape (Manchester & Bullock, 2000).

CONCLUDING REMARKS

In conclusion, Bsal can cause mortality in urodeles, including the great crested newt native to Britain, and is able to survive British temperatures (Martel et al., 2013). In addition to these two risk factors, Bsal has been found in the European pet trade, and furthermore has had devastating effects on several wildlife species in Europe (Martel et al, 2014.). There are still gaps in data that need to be addressed before risk can be accurately quantified, like risk of environmental contamination with Bsal, levels of amphibian escape in Britain, and the potential of resistant individuals to act as carriers. However, while current lack of data prevents complete analysis of what risk Bsal poses to Britain, examples of mass mortalities in the Netherlands (Martel et al., 2013) should act as a warning, emphasising the need for both more research into this disease, as well as re-evaluation and enhancement of the bio-controls in place for amphibian disease in both the UK and the EU.

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