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Emerging infectious disease threats to European herpetofauna

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In the past decade, infectious disease threats to European herpetofauna have become better understood. Since the 1990s, three major emerging infections in amphibians have been identified (*Batrachochytrium dendrobatidis*, *B. salamandrivorans*, and ranaviruses) as well as at least one of unknown status (herpesviruses), while two major emerging infections of reptiles (*Ophidiomyces ophiodiicola* and ranaviruses) have been identified in wild European populations. The effects of emerging infections on populations have ranged from non-existent to local extirpation. In this article, we review these major infectious disease threats to European herpetofauna, including descriptions of key mortality and/or morbidity events in Europe of their emergence, and address both the distribution and the host diversity of the agent. Additionally, we direct the reader to newly developed resources that facilitate the study of infectious agents in herpetofauna and again stress the importance of an interdisciplinary approach to examining these infectious diseases.

Keywords: *Batrachochytrium dendrobatids*, *B. salamandrivorans*, herpesviruses, ophidiomycosis, ranaviruses, amphibians, reptiles

INTRODUCTION

Vertebrates are currently experiencing an ongoing mass extinction event (Ceballos et al., 2017). Infectious diseases are contributing to these declines as they are able to cause major population declines, and can contribute to both local and global extirpations (de Castro & Bolker, 2005). Emerging infections have long been known to pose a serious threat to biodiversity (Scott, 1988; Daszak et al., 2000). The link between the decline and extinction of many amphibian populations with the emergence of infectious agents was drawn approximately 20 years ago (Berger et al., 1998; Daszak et al., 1999). While the link between declines in some reptile populations and emerging infections have been less clear, in the past decade growing evidence shows emerging infectious diseases (e.g. snake fungal disease, Lorch et al., 2016) that are clearly able to decimate populations of these animals as well. Infectious disease related declines can even occur in large, seemingly robust populations of wildlife (Daszak et al., 2003). Therefore, the effects in smaller, more fragile, populations are likely to be even more extreme. Unfortunately, populations of both amphibians and reptiles are shrinking around the globe (Gibbons et al., 2000; Sodhi et al., 2008; Stuart et al., 2004; Todd et al., 2010). This will be exacerbated by other factors such as anthropogenic environmental stressors and infectious disease, which negatively affect wildlife health (Acevedo-Whitehouse & Duffus, 2009).

Duffus and Cunningham (2010) reviewed two major disease threats to European amphibians, *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses. Since the publication of that review, other emerging infectious diseases in European amphibian populations have been identified, *B. salamandrivorans* (*Bsal*, Martel et al., 2013), and the potentially emerging amphibian herpesviruses (Franklinos et al., 2018; Origgi et al., 2017). In reptiles, the emergence of two troubling infectious diseases has also been documented in wild populations, snake fungal disease (*Ophidiomyces ophiodiicola*, Franklinos et al., 2017) and ranaviruses (Alves de Matos et al., 2011).

EMERGING INFECTIOUS DISEASE THREATS TO AMPHIBIANS

BATRACHOCHYTRIUM DENDROBATIDIS

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, hereafter *Bd*) is a non-hyphal zoosporic chytridiomycete fungus implicated in the decline of amphibian species globally (Skerratt et al., 2007). *Batrachochytrium dendrobatidis* is the causative agent of amphibian chytridiomycosis (Berger et al., 1998) that was first linked to declines in amphibian communities in Central America and Australia. Since then, *Bd* has been found all over the globe and has been associated with the extinction of at least 90 amphibian species, and the declines of several hundred others (Wake & Vredenburg, 2008; Scheele et al., 2019). *Bd* is considered

Correspondence: Steven Allain (sjra2@kent.ac.uk), Amanda Duffus (aduffus@gordonstate.edu) to be a pandemic pathogen in amphibians (Pasmans et al., 2006) with low genetic diversity among different isolates indicating a rapid spread of the pathogen. Of these strains, the global panzootic lineage (BdGPL) has been attributed to most amphibian declines having likely emerged as a hybrid between less virulent strains (Farrer et al., 2011). For a long time, there was speculation as to where *Bd* had originated, with evidence of America or Africa (Weldon et al., 2004), but recent research shows that the infection originated in east Asia (O'Hanlon et al., 2018).

The standard detection method for *Bd* is to swab an individual with a sterile swab and then test for the presence of *Bd* DNA using real-time PCR (also known as qPCR) after processing the sample (Boyle et al., 2004). Gross clinical signs of *Bd* infection in adult amphibians include, but are not limited to: anorexia, lethargy, unusual skin shedding, reddening of the skin (especially in the vent and upper thigh regions), convulsions and loss of the righting reflex (Fig. 1; Nichols et al., 2011). However, the clinical signs of *Bd* vary among species, their life history stages, and other factors such as environmental conditions. In larval stages, clinical signs of chytridiomycosis are also variable. They include but are not limited to: depigmentation of mouthparts, swimming difficulties, and lethargy (reviewed in van Rooij et al., 2015). Therefore, to properly diagnose chytridiomycosis, histology should be used alongside the standard method of qPCR. The pathogen only infects the keratinized tissues of the body, such as the skin in metamorphosed animals and the mouthparts in larvae (Daszak et al., 1999; Marantelli et al., 2004). There are limited studies on the effects *Bd* has on life history, but Garner et al. (2009a) were able to show that experimental infection decreased the time it took tadpoles (of the common toad, *Bufo bufo*) to reach metamorphosis, but also decreased their weight. At higher doses, there were also higher levels of mortality, but these surprising results show that there may be advantageous effects when tadpoles are infected with *Bd*. For more information on host-fungal interactions of *Bd*, please see Van Rooij et al. (2015), and for further details on the immune response of amphibians to *Bd*, please refer to Grogan et al. (2018).

Figure 1. A common midwife toad (*A. obstetricians*) that died because of *Bd* caused chytridiomycosis. Note the strange position of the legs, due to muscle spasms and the reddish hue of the vent and thighs. Photo by Frank Pasmans.

Figure 2. The distribution of *Batrachochytrium dendrobatidis* (*Bd*) in wild European amphibian populations.

Currently the potential impacts of *Bd* on European amphibian communities are not fully understood, but within the past decade multiple studies have started to help illuminate the scientific community (e.g. Bielby et al., 2015; Rosa et al., 2013; Tobler & Schmidt, 2010). The amphibian chytrid fungus has both a wide geographic range and a wide host range within Europe (Table 1 and Fig. 2), with infection first being identified in wild populations in 2001 (Bosch et al., 2001). So far, the susceptibility of species across Europe is not fully understood, but *Bd* has been attributed to declines seen in species including the common midwife toad (*Alytes obstetricans*), common toad (*B. bufo*) and European fire salamander (*Salamandra salamandra*) across the Iberian Peninsula (Bosch et al., 2001; Bosch & Martínez-Solano, 2006). Elsewhere in Europe, infection does not always lead to the development of disease, which may be why we have not seen the mass-die offs of amphibians such as those elsewhere in the world (Berger et al., 1998). Experimentally, some anurans species have been shown to have a limited immunity to *Bd*, whilst others have exhibited tolerance, such as the water frogs (*Pelophylax* spp.), which may be an important factor for host-disease dynamics (Daum et al., 2012; Woodhams et al., 2012).

The apparent lack of mass die-offs within many areas Europe may be attributed to the fact that *Bd* has multiple strains of varying virulence (Fisher et al., 2009). Research in the Netherlands has shown over a seven-year time period, populations of the yellow-bellied toad (*Bombina variegata*) are able to coexist with *Bd* (Spitzen-van der Sluijs et al., 2017). Although for now the toads seem to be safe, this may change in the future due to the effects of climate change and other such environmental effects. For more information on the environmental impact of host-pathogen interactions, which can be hugely deterministic in some cases, see Blaustein et al. (2018) for an in-depth discussion. Experiments have also shown that some salamanders such as those in the genus *Speleomantes* also have some immunity to *Bd* (Pasmans et al., 2013). This combined effect of peptide defence and varying *Bd* strains show that at least for now, some species of European amphibians are protected against the potentially negative effects of *Bd* outbreaks (Fisher et al., 2009).

BATRACHOCHYTRIUM SALAMANDRIVORANS

Table 1. Summary of the countries and amphibian species affected by *Batrachochytrium dendrobatidis* infections in wild European populations.

Location	Common Name	Species	First Report
Albania	Macedonian crested newt	*Triturus macedonicus*	Vojar et al., 2017
	Yellow-bellied toad	*Bombina variegata*	Vojar et al., 2017
Austria	Alpine newt	*Ichthyosaura alpestris*	Sztatecsny & Glaser, 2011
	Smooth newt	*Lissotriton vulgaris*	Sztatecsny & Glaser, 2011
	European water frog complex	*Pelophylax* spp.	Sztatecsny & Glaser, 2011
	Fire-bellied toad	*B. bombina*	Sztatecsny & Glaser, 2011
	Great crested newt	*T. cristatus*	Sztatecsny & Glaser, 2011
	Italian crested newt	*T. carnifex*	Sztatecsny & Glaser, 2011
	Yellow-bellied toad	*B. variegata*	Sztatecsny & Glaser, 2011
Belgium	African clawed frog	*Xenopus laevis*	Spitzen-van der Sluijs et al., 2014
	American bullfrog	*Lithobates catesbeianus*	Spitzen-van der Sluijs et al., 2014
	Common midwife toad	*Alytes obstetricans*	Pasmans et al., 2010
	Common toad	*Bufo bufo*	Martel et al., 2012
	Marbled newt	*T. marmoratus*	Spitzen-van der Sluijs et al., 2014
Czech Republic	Alpine newt	*Ichthyosaura alpestris*	Civiš et al., 2012
	Eurasian toads	*Bufo* sp.	Baláž et al., 2014
	European water frog complex	*Pelophylax* spp.	Baláž et al., 2014
	Fire-bellied toad	*B. bombina*	Civiš et al., 2012
	Great crested newt	*T. cristatus*	Civiš et al., 2012
	Yellow-bellied toad	*B. variegata*	Civiš et al., 2012
Denmark	Common frog	*Rana temporaria*	Scalera et al., 2008
	Edible frog	*P. esculentus*	Scalera et al., 2008
Finland	Common frog	*R. temporaria*	Patrelle et al., 2012
France	American bullfrog	*L. catesbeianus*	Garner et al., 2006
	Common midwife toad	*A. obstetricans*	Garner et al., 2005
	European water frog complex	*Pelophylax* spp.	Ouellet et al., 2012
Germany	Agile frog	*R. dalmatina*	Ohst et al., 2011
	Alpine newt	*I. alpestris*	Ohst et al., 2011
	Common frog	*R. temporaria*	Ohst et al., 2011
	Common midwife toad	*A. obstetricans*	Ohst et al., 2011
	Smooth newt	*L. vulgaris*	Ohst et al., 2011
	Common toad	*B. bufo*	Ohst et al., 2011
	European water frog complex	*Pelophylax* spp.	Ohst et al., 2011
	European spadefoot toad	*Pelobates fuscus*	Ohst et al., 2011
	European tree frog	*Hyla arborea*	Ohst et al., 2011
	Fire-bellied toad	*B. bombina*	Ohst et al., 2011
	Fire salamander	*Salamandra salamandra*	Ohst et al., 2011
	Great crested newt	*T. cristatus*	Ohst et al., 2011
	Green toad	*Bufotes viridis*	Ohst et al., 2013
	Moor frog	*R. arvalis*	Ohst et al., 2011
	Natterjack toad	*Epidalea calamita*	Ohst et al., 2011
	Palmate newt	*L. helveticus*	Ohst et al., 2011
	Yellow-bellied toad	*B. variegata*	Ohst et al., 2011
Greece	Epirus water frog	*P. epeiroticus*	Azmanis et al., 2016
	Green frog	*B. viridis*	Azmanis et al., 2016
	Marsh frog	*P. ridibundus*	Azmanis et al., 2016
Hungary	Alpine newt	*I. alpestris*	Vörös et al., 2018
	Edible frog	*P. esculentus*	Vörös et al., 2018
	European water frog complex	*Pelophylax* spp.	Tünde et al., 2012
	Fire-bellied toad	*B. bombina*	Vörös et al., 2018
	Green toad	*B. viridis*	Vörös et al., 2018
	Marsh frog	*P. ridibundus*	Vörös et al., 2018
	Yellow-bellied toad	*B. variegata*	Tünde et al., 2012
Italy	American bullfrog	*Lithobates catesbeianus*	Garner et al., 2006
	Apennine toad	*B. pachypus*	Stagni et al., 2004
	Edible frog	*P. esculentus*	Adams et al., 2008
	Edible frog complex	*Pelophylax* spp.	Simoncelli et al., 2005
	Fire-bellied toad	*B. variegata*	Stagni et al., 2002 (in Bovero et al., 2008)
	Italian agile frog	*R. latastei*	Garner et al., 2004 (in Bovero et al., 2008)
	Italian alpine newt	*I. alpestris apuanus*	Zampiglia et al., 2013
	Italian crested newt	*T. carnifex*	Grasseli et al., 2019
	Italian fire salamander	*S. salamandra gigliolii*	Zampiglia et al., 2013
	Italian stream frog	*R. italica*	Zampiglia et al., 2013
	Sardinian brook salamander	*Euproctus platycephalus*	Bovero et al., 2008
	Tyrrhenian painted frog	*Discoglossus sardus*	Bielby et al., 2009
Luxembourg	Alpine newt	*I. alpestris*	Wood et al., 2009
	Edible frog complex	*Pelophylax* spp.	Wood et al., 2009
	Palmate newt	*L. helveticus*	Wood et al., 2009
Macedonia	European water frog complex	*Pelophylax* spp.	Vojar et al., 2017
Montenegro	Common or smooth newt	*L. vulgaris*	Vojar et al., 2017
	Edible frog complex	*Pelophylax* spp.	Vojar et al., 2017
	Great crested newt	*T. cristatus*	González et al., 2019

Location	Common Name	Species	First Report
Netherlands	Alpine newt	*I. alpestris*	Spitzen-van der Sluijs et al., 2014
	Common midwife toad	*A. obstetricans*	Spitzen-van der Sluijs et al., 2014
	Common frog	*R. temporaria*	Spitzen-van der Sluijs et al., 2014
	Common or smooth newt	*L. vulgaris*	Spitzen-van der Sluijs et al., 2014
	Common toad	*B. bufo*	Spitzen-van der Sluijs et al., 2014
	Edible frog	*P. esculentus*	Spitzen-van der Sluijs et al., 2014
	European water frog complex	*Pelophylax* sp.	Spitzen-van der Sluijs et al., 2014
	European tree frog	*Hyla arborea*	Spitzen-van der Sluijs et al., 2014
	Pool frog	*P. lessonae*	Spitzen-van der Sluijs et al., 2014
	Natterjack toad	*Epidalea calamita*	Spitzen-van der Sluijs et al., 2014
	Yellow-bellied toad	*B. variegata*	Spitzen-van der Sluijs et al., 2014
Poland	Edible frog	*P. esculentus*	Kolenda et al., 2017
	European water frog complex	*Pelophylax* sp.	Sura et al., 2010
	Pool frog	*P. lessonae*	Kolenda et al., 2017
	Yellow-bellied toad	*B. variegata*	Kolenda et al., 2017
Portugal	Bosca's newt	*L. boscai*	Rosa et al., 2017
	Common midwife toad	*A. obstetricans*	Rosa et al., 2013
	Fire salamander	*S. salamandra*	Rosa et al., 2017
	Iberian green frog	*P. perezi*	Rosa et al., 2017
	Iberian tree frog	*Hyla molleri*	Rosa et al., 2017
	Marbled newt	*Triturus marmoratus*	Rosa et al., 2017
	Spiny toad	*B. spinosus*	Rosa et al., 2017
Romania	Common Frog	*R. temporaria*	Vörös et al., 2013
	Smooth newt	*L. vulgaris*	Vörös et al., 2013
	Yellow-bellied toad	*B. variegata*	Vörös et al., 2013
Serbia	Edible frog	*P. esculentus*	Mali et al., 2017
	Marsh frog	*P. ridibundus*	Mali et al., 2017
	Pool frog	*P. lessonae*	Mali et al., 2017
Spain	Betic midwife toad	*A. dickhilleni*	Bosch et al., 2013
	Bosca's newt	*L. boscai*	Hidalgo-Vila et al., 2012
	Common midwife toad	*A. obstetricans*	Bosch et al., 2001
	Common toad	*B. bufo*	Bosch & Martínez-Solano, 2006
	Fire salamander	*S. salamandra*	Bosch & Martínez-Solano, 2006
	Iberian green frog	*P. perezi*	Hidalgo-Vila et al., 2012
	Iberian painted frog	*Discoglossus galganoi*	Hidalgo-Vila et al., 2012
	Majorcan midwife toad	*A. muletensis*	Garner et al., 2009a
	Mediterranean tree frog	*H. meridionalis*	Hidalgo-Vila et al., 2012
	Natterjack toad	*E. calamita*	Martínez-Solano et al., 2003
	Spanish ribbed newt	*Pleurodeles waltl*	Hidalgo-Vila et al., 2012
	Southern marbled newt	*T. pygmaeus*	Hidalgo-Vila et al., 2012
	Western spadefoot toad	*Pelobates cultripes*	Hidalgo-Vila et al., 2012
Sweden	Common frog	*R. temporaria*	Kärvemo et al., 2018
	Common toad	*B. bufo*	Kärvemo et al., 2018
	Fire-bellied toad	*B. bombina*	Kärvemo et al., 2018
	Moor frog	*R. arvalis*	Kärvemo et al., 2018
	Natterjack toad	*E. calamita*	Kärvemo et al., 2018
	Variable toad	*Bufotes variabilis*	Kärvemo et al., 2018
Switzerland	Alpine newt	*I. alpestris*	Tobler et al., 2012
	Common midwife toad	*A. obstetricans*	Tobler et al., 2012
	European water frog complex	*Pelophylax* sp.	Tobler et al., 2012
	Palmate newt	*L. helveticus*	Tobler et al., 2012
UK	African clawed frog	*Xenopus laevis*	Tinsley et al., 2015
	American bullfrog	*Lithobates catesbeianus*	Garner et al., 2005
	Natterjack toad	*E. calamita*	May et al., 2011

Table 2. Summary of the countries and amphibian species affected by *Batrachochytrium salamandrivorans* infections in wild European populations.

Location	Common Name	Species	First Report
Belgium	Alpine newt	*Ichthyosaura alpestris*	Spitzen-van der Sluijs et al., 2016a
	Fire salamander	*Salamandra salamandra*	Spitzen-van der Sluijs et al., 2016a
Germany	Fire salamander	*S. salamandra*	Spitzen-van der Sluijs et al., 2016a
Netherlands	Alpine newt	*I. alpestris*	Spitzen-van der Sluijs et al., 2016a
	Smooth newt	*Lissotriton vulgaris*	Spitzen-van der Sluijs et al., 2016a
	Fire salamander	*S. salamandra*	Martel et al., 2013
Spain	Palmate newt	*L. helveticus*	González et al., 2019

Table 3. Summary of countries and amphibian species affected by herpesvirus infections in wild European populations.

Location	Common Name	Species	First Report
Germany	Common frog	*Rana temporaria*	Mutschmann & Scheenweiss, 2008
	Moor frog	*R. arvalis*	Mutschmann & Scheenweiss, 2008
	European spadefoot toad	*Pelobates fuscus*	Mutschmann & Scheenweiss, 2008
Italy	Agile frog	*R. dalmatina*	Bennati et al., 1994
Switzerland	Common frog	*R. temporaria*	Origgi et al., 2017
	Common toad	*Bufo bufo*	Origgi et al., 2018
UK	Common frog	*R. temporaria*	Franklinos et al., 2018

The salamander chytrid fungus (*Batrachochytrium salamandrivorans*, hereafter *Bsal*) was first identified in 2013 after a dramatic loss of European fire salamanders (*Salamandra salamandra*) in the Netherlands (Martel et al., 2013; Spitzen–van der Sluijs et al., 2013). The fungus itself is closely related to *B. dendrobatidis*, having diverged sometime in the late Cretaceous or early Paleogene (Martel et al., 2014). The introduction pathway of the pathogen to Europe is believed to have been via the trade in Asian newt species, with *Bsal* being the predominant chytrid fungus affecting Vietnamese salamanders (Laking et al., 2017). There is also alternative evidence to support the introduction of *Bsal* to Europe through the trade in small-webbed fire-bellied toads (*B. microdeladigitora*) from Vietnam (Nguyen et al., 2017). In lab experiments, Asian newts (*Cynops cyanurus*, *C. pyrrhogaster* & *Paramesotriton deloustali*) were discovered to be potential reservoirs of *Bsal*, and worryingly, most European newts died shortly after infection with *Bsal* (Martel et al., 2014). Recent evidence suggests that one of the factors that makes *Bsal* such a threat to urodelean diversity is the fact that infection does not elicit immunity and anurans, such as the common midwife toad (*A. obstetricans*), may act as intermediary hosts (Stegen et al., 2017). More worryingly, alpine newts (*Ichthyosaura alpestris*) can survive for extended periods of time whilst infected with low doses of *Bsal*, with the ability to even clear infection. This is a concern as alpine newts co-occur with *S. salamandra* whilst *A. obstetricans* does not (Stegen et al., 2017).

The clinical signs of *Bsal* are varied (like those of *Bd*) but are usually seen as anorexia, lethargy, ataxia, and skin lesions (Martel et al., 2013). The skin lesions (Fig. 3) are quite diagnostic and easily identified on adult individuals (although this varies by species); however, they are not always present and tend to occur at the end of pathogenesis when the animal is nearly dead (Martel et al., 2013). This means that detailed histological and genetic analyses are needed to confirm the presence of *Bsal* in a suspected infected individual. Again, the standard technique for detection, like *Bd*, is the use of swabs and qPCR.

In the wild, *Bsal* has been only found in a small number of European countries (Table 2) although the presence of the fungus is known to be more widespread in private collections (Fitzpatrick et al., 2018; Sabino-Pinto et al., 2015). It is vital that private collectors take the necessary biosecurity protocols when disinfecting wastewater and enclosures. More information can be found in Van Rooij et al. (2017). Despite the potentially devastating effects of *Bsal* on naive populations, *Bsal* has a poor dispersal potential which allows some sub-populations to persist in areas where the pathogen is known to be present (Spitzen-van der Sluijs et al., 2018). As highlighted by Spitzen-van der Sluijs et al. (2018), this provides potential for in-the-field mitigation strategies and also shows the importance of biosecurity to ensure that researchers are not accidentally transferring *Bsal* zoospores between sites.

At this time, *Bsal* is limited to wild populations in Germany, Belgium, Spain and the Netherlands (Fig. 4). In *Bsal*'s range the species of focus is the European fire salamander (*S. salamandra*) although other species have also tested positive for *Bsal* (Table 2). In order to track the pathogen's spread through Europe, ongoing surveillance needs to carried out in areas that may be susceptible to spillover, as well as monitoring vulnerable species. Limited screening in the Czech Republic revealed that *Bsal* was not present in wild or captive salamanders (Baláz et al., 2018), although more samples are needed

Figure 3. Close up of the skink of a fire salamander (*S. salamandra*) that died because of *Bsal* caused chytridiomycosis. Note the skin erosion with black margins indicated by the white arrows. Photo By: Frank Pasmans.

Figure 4. The distribution of *Batrachochytrium salamandrivorans* (*Bsal*) in wild European amphibian populations.

Figure 5. The distribution of amphibian herpesviruses in wild European amphibian populations.

to ensure that *Bsal* is not present within the country. Ongoing surveillance is needed to ensure the emergence of *Bsal* is not missed in areas where it has not yet been recorded in the wild. This surveillance can be either active or passive, depending on the time and resources available. Recently in the UK, 2,409 swabs collected from wild newts in 2011 and 43 newts submitted for post-mortem examination (between March 2013–December 2017) all tested negative for *Bsal* (Cunningham et al., 2019). However, there is experimental evidence to suggest that salamanders infected with low loads of *Bsal* may go undetected due to latency in detection via qPCR (Thomas et al., 2018). Due to the nature of the pathogen, screening should be completed regularly, especially of those populations that are deemed most vulnerable to ensure that there are no false negatives. In addition to this, to prevent the spread of the pathogen within Europe from captive collections, we advocate the trade in captive bred, certified disease-free salamanders only. Furthermore, the use of the early warning system developed for *Bsal* in Europe should be used (see below for more information).

HERPESVIRUSES

It is thought that amphibian herpesviruses are widely distributed in Europe (Garner et al., 2013); however, they have received relatively little attention in the literature and therefore we cannot determine its status as emergent, although reports appear to be on the rise. The first disease associated with herpesvirus-like particles in an amphibian species reported in Europe occurred in the early 1990s and was published by Benatti et al. (1994). They reported herpesvirus-like particles in agile frogs (*Rana dalmatina*) from Italy suffering from herpes-like lesions. However, the outbreak was not accompanied by a mortality event (Benatti et al., 1994). Subsequently, amphibian herpesviruses have been reported in Germany, Switzerland, and the UK in five different species, one of conservation concern (*Pelobates fuscus*, Mutschmann & Scheenweiss, 2008, and see Table 3 and Fig. 5).

The gross clinical signs of amphibian herpesvirus infections appear to be conserved across affected species. Typically, lesions are present on the skin that may be tan (more terrestrial species), grey, or grey-blue in colour (typical of more aquatic species; see Origgi et al., 2017; Franklinos et al., 2018; Origgi et al., 2018). These are areas where the skin has become thickened and they may appear on any region of the body (Origgi et al., 2017; Franklinos et al., 2018; Origgi et al., 2018). The number of lesions that appear on the skin of affected animals is often variable, ranging from one to covering most of the skin (Origgi et al., 2017; Franklinos et al., 2018; Origgi et al., 2018; Fig. 6).

There are two species of amphibian herpesvirus known to be present in Europe and both were first described in Swiss amphibians. Origgi et al. (2017) identified and characterised *Ranid Herpesvirus 3* (RaHV3) from common frogs (*R. temporaria*) that were suffering from a proliferative dermatitis. Subsequently, Origgi et al. (2018) described *Bufonid Herpesvirus 1* (BfHV1) in common toads (*B. bufo*) that were also suffering from dermatitis. These are the first amphibian herpesviruses outside of North America to be fully characterised and described in wild caught amphibians. Additionally, Franklinos et al. (2018) found herpesvirus-associated dermatitis in common toads. However, the virus(es) present in UK amphibians was/were not fully characterised, so it is unknown if two different virus species are present.

The threat of amphibian herpesviruses to European amphibian populations is unknown. Their ability to cause infection and subsequent mortality in *P. fuscus* is a cause for concern (Garner et al., 2013). More research is needed to fully understand the effects of viral

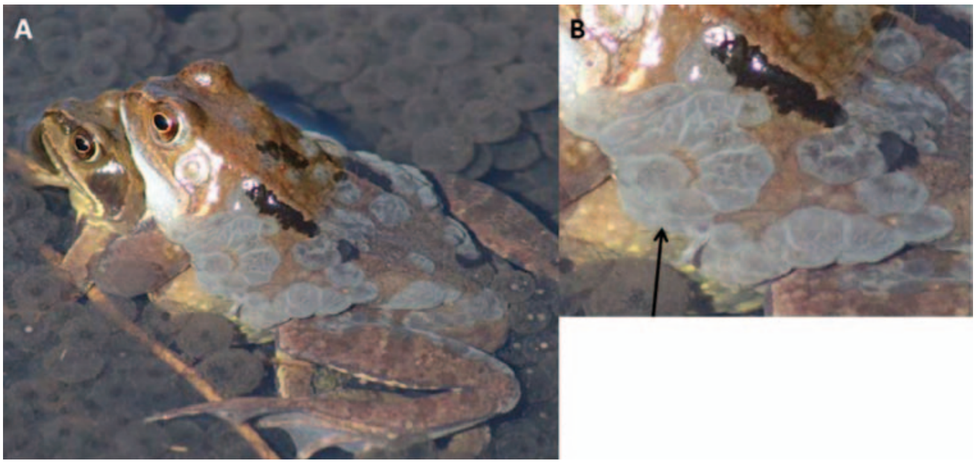

Figure 6. (A) Herpesvirus infection in a male common frog (*R. temporaria*) from the UK. Note the grayish nature of the lesions that are distributed on the dorsal aspect of the frog. **(B)** Close up of the herpesvirus lesions on the dorsum of the same male common frog. Note the 'fluid filled' appearance of the lesions. Photo by Matthew Chester.

persistence in populations of affected amphibians. It will be important for interdisciplinary teams to investigate amphibian herpesviruses because of the difficulties that are often encountered with proper identification and diagnosis (see Garner et al., 2013 for further discussion).

RANAVIRUSES

Amphibian ranaviruses are widely distributed in Europe. They are currently known to be present in over ten countries and in over ten amphibian species (see Table 4 and Fig. 7). Amphibian ranaviruses belong to the viral family *Iridoviridae*, which are large, double stranded DNA viruses (Chinchar et al., 2017). In fact, the first documented cases of what were likely to be *Ranavirus* infections were broadly identified as iridovirus-like agents. Fijan et al. (1991) found iridovirus-like particles in edible frogs (*Pelophylax esculentus*) from a population that experienced a mortality event in Croatia. This discovery was quickly followed by the identification of an iridovirus-like agent associated with morbidity and mortality events in UK common frogs (*Rana temporaria*), that was eventually classified as a *Ranavirus* (Cunningham et al., 1993; Cunningham et al., 1996; Drury et al., 1995). Despite the early report of an iridovirus-like agent in Croatia, no subsequent reports of amphibian ranaviruses can be found in the literature for continental Europe until a new *Ranavirus* species emerged in 2007 (see below for further discussion).

The gross clinical signs of ranaviral disease are conserved across species and life history stages. It is important to note that not all ranaviral infections will result in disease, and therefore the absence of gross clinical signs should not be taken as a clean bill of health (see Rijks et al., 2016). Additionally, in most cases, the gross signs of ranavirosis are considered to be non-specific to the disease, and therefore it is imperative that a full and proper pathological investigation be performed when infection or disease is thought to be a contributing factor to a morbidity and/or mortality event. Gross clinical signs of ranavirosis of juvenile and adult amphibians include, but are not limited to: lethargy, skin ulcerations, haemorrhages in the skin, reddening of the skin, necrosis of digits, and internal haemorrhages of multiple organ systems (predominantly the gastrointestinal tract and reproductive tracts; Cunningham et al., 1996; Cunningham et al., 2007a; Cunningham et al., 2007b; Price et al., 2014; see Figure 8 and images in Duffus & Cunningham, 2010). The signs of disease in affected tadpoles/larvae included systemic external and internal haemorrhages (Balseiro et al., 2009; Balseiro et al., 2010). External haemorrhages occur predominantly in the tissues surrounding the eyes of the tadpoles and in the tail, including the musculature (Balseiro et al. 2009; Balseiro et al., 2010) and edema has also been observed (Rosa et al., 2017). Infection, disease, and mortality can occur at any life history stage in amphibians, except for the egg stage. However, the life history stage affected seems to be species specific. In some species, it is the adults that are predominantly affected [e.g. common frogs (*R. temporaria*, Cunningham et al., 1996)] whereas in other species, the larvae/tadpoles are affected [e.g. common midwife toads (*A. obstetricans*) and Alpine newts (*I. alpestris*) (Balseiro et al., 2009; Balseiro et al., 2010)].

There are several different types of *Ranavirus* that are present in Europe. *Frog virus 3* (FV3), *Common midwife toad virus* (CMTV), and their derivatives appear to be the most common types of ranaviruses present in amphibians. FV3 is the type virus of the genus *Ranavirus* (Tan et al., 2004) and is perhaps the most common species of *Ranavirus* around the globe in amphibian populations (see Duffus et al., 2015). However, CMTV-like viruses are known to recombine with FV3-like strains and can create extremely virulent chimeric viruses (Claytor et al., 2017). Experimental infection of smooth newts (*Lissotriton vulgaris*) with different strains of CMTV *Ranavirus* from the Netherlands clearly demonstrates distinct strain-associated pathogenicity within closely related strains of the virus (Saucedo et al., 2019).

The emergence of amphibian ranaviruses and likely misdiagnosis as 'red-leg syndrome' in Europe has had a measurable negative effect on some, but not all, populations. The first well-documented morbidity and mortality event in continental Europe occurred in 2007

Table 4. Summary of countries and amphibian species known to harbour *Ranavirus* infections in wild European populations.

Location	Common Name	Species	First Report
Belgium	Common toad	*Bufo bufo*	Martel et al., 2012
	American bullfrog	*Lithobates catesbeianus*	Sharifian-Fard et al., 2011
Croatia	Edible frog	*Pelophylax esculentus*	Fijan et al., 1991
Denmark	Edible frog	*P. esculentus*	Ariel et al., 2009
France	Common frog	*Rana temporaria*	Miaud et al., 2016
Germany	Edible frog	*P. esculentus*	Stöhr et al., 2013
Italy	Edible frog	*P. esculentus*	Holopainen et al., 2009
Netherlands	European water frog complex	*Pelophylax spp.*	Kik et al., 2011
	Smooth newt	*Lissotriton vulgaris*	Kik et al., 2011
	Common frog	*Rana temporaria*	Rijks et al., 2016
	Common toad	*B. bufo*	Rijks et al., 2016
	European spadefoot toad	*Pelobates fuscus*	Spitzen-van der Sluijs et al., 2016c
	Great crested newt	*Triturus cristatus*	Spitzen-van der Sluijs et al., 2016c
Portugal	Common midwife toad	*Alytes obstetricans*	Stöhr et al., 2015
	Bosca's newt	*L. boscai*	de Matos et al., 2008
	Spiny toad	*B. spinosus*	Rosa et al., 2017
	Fire salamander	*Salamandra salamandra*	Rosa et al., 2017
	Iberian tree frog	*Hyla molleri*	Rosa et al., 2017
	Marbled newt	*T. marmoratus*	de Matos et al., 2008
Spain	Common midwife toad	*A. obstetricans*	Balseiro et al., 2009
	Common frog	*R. temporaria*	Price et al., 2014
	Alpine newt	*Ichthyosaura alpestris*	Balseiro et al., 2010
	Bosca's newt	*Lissotriton boscai*	Price et al., 2014
	Marbled newt	*T. marmoratus*	Price et al., 2014
Switzerland	Marsh frog	*P. ridibunda*	Stöhr et al., 2015
UK	Common frog	*R. temporaria*	Drury et al., 1995
	Common toad	*B. bufo*	Hyatt et al., 2000
	Common midwife toad	*A. obstetricans*	Duffus et al., 2014
	Smooth newt	*L. vulgaris*	Duffus et al., 2014

Figure 7. The distribution of *Ranavirus* spp. in wild European amphibian populations.

in 'Picos de Europa' National Park, Spain (Balseiro et al., 2009). The mortality event involved common midwife toad (*A. obstetricans*) tadpoles that showed classic signs of ranavirosis (Balseiro et al., 2009). Investigations into the cause of the mortality event revealed that it was caused by a novel *Ranavirus*, then tentatively called the common midwife toad virus (CMTV, Balseiro et al., 2009) and CMTV was only recently designated as its own species in the genus *Ranavirus* (Chinchar et al., 2017). The following year, CMTV was responsible for another morbidity and mortality event. This time not only did it affect common midwife toad tadpoles, but also alpine newt (*I. alpestris cyreni*) larvae (Balseiro et al., 2010). Since then, declines and local extirpations of several species have been documented in association with the emergence of ranaviruses in the same park. Price et al. (2014) found that at several locations, common midwife toads (*A. obstetricans*) were extirpated and both alpine newts (*I. alpestris*) and common toads (*B. bufo*) experienced severe declines. Unfortunately, it does not appear that the populations are recovering from the effects the emergence of ranaviral infection and disease at this time (Price et al., 2014). Many more outbreaks of ranavirosis have now been documented across continental Europe. Perhaps the largest scale morbidity and mortality event involving a CMTV-like virus occurred in 2010 in the Netherlands (Kik et al., 2011; van Beurden et al., 2014). The event involved more than 1000 individuals, mostly

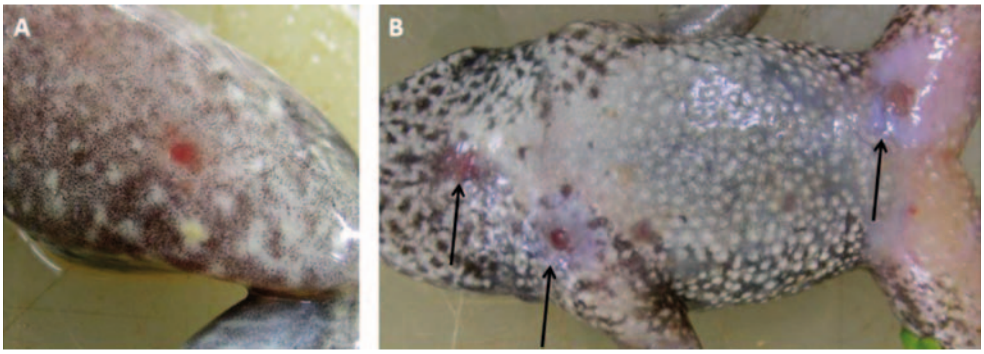

Figure 8. (A) Ranavirus-associated ulceration on the upper portion of the leg of a common frog (*R. temporaria*) from the UK. **(B)** Ranavirus-associated ulcerations in a common midwife toad (*A. obstetricans*) from the UK. Arrows indicate the lesions. Note the greyish area around the ulcerations. Photos by Zoological Society of London/Amanda L.J. Duffus

Figure 9. A barred grass snake (*N. helvetica*) in from the UK with suspected ophidiomycosis. **(A)** Arrow indicates the 'crusty' scales that are common in snakes suffering from ophidiomycosis. **(B)** Arrows indicate some of the deformed scales frequently seen in snakes with ophidiomycosis. **(C)** The arrow indicates the crusty scales on the snout. Photos by Silviu Petrovan.

Pelophylax spp. but some common newts (*L. vulgaris*; Kik et al., 2011). Subsequent investigations into amphibian morbidity and mortality events in the Netherlands has revealed that the CMTV-like *Ranavirus* is spreading and is the causative agent of many of the observed events (Rijks et al., 2016; Spitzen-van der Sluijs et al., 2016b; Saucedo et al., 2018). It is also important to note that these outbreaks of disease are affecting the common spadefoot (*P. fuscus*), which is a threatened species (Spitzen-van der Sluijs et al., 2016c). In the UK, common frog populations where ranaviruses have emerged have significantly decreased in size. Teacher et al. (2010) found that on average, common frog populations where *Ranavirus* was found declined by approximately 81 % when compared to populations where the virus was absent. Furthermore, Teacher et al. (2010) report that larger populations were disproportionately affected, with larger populations losing a greater number of animals to disease emergence than those that were initially smaller when *Ranavirus* infections emerged. The emergence of amphibian ranaviruses has also changed the population structure of common frogs in the UK, making them more susceptible to stochastic events (Campbell et al., 2018). This is cause for great concern with the ever-increasing random events that climate change will cause. Thus, the emergence of ranaviruses in European amphibians is a cause for great conservation concern. They have the potential to be lethal pathogens and are now known to drive populations to decline or even local extinction (e.g. Teacher et al., 2010, Price et al., 2014, Rosa et al., 2017), as a single responsible agent or in co-occurrence with for instance *Bd* and/or *Bsal*. Ranaviruses are persistent pathogens, meaning that they remain on field gear far longer than *Bd* or *Bsal* (Nazir et al., 2012; Van Rooji et al., 2017); therefore, proper disinfection methods should always be used on field gear and enhanced biosecurity in facilities where the pathogen may be found. Investigating amphibian health and disease should be made a priority for many species, even common ones, and include an interdisciplinary approach to ensure that these animals have a future.

The effects of the emergence of ranaviruses may be compounded when another disease, such as *Bd*, has emerged previously in a population. In the 'Serra da Estrela' Natural Park, *Bd* related declines of amphibian populations were occurring for many years before ranavirosis emerged (Rosa et al., 2017). Ranavirosis was first documented in 2011 and is now known to occur in

Table 5. Summary of the countries and snake species affected by snake fungal disease (*Ophidiomyces ophiodiicola*) infections in wild European populations.

Location	Common Name	Species	First Report
Czech Republic	Dice snake	*Natrix tessellata*	Franklinos et al., 2017
Switzerland	Grass snake	*N. natrix*	Meier et al., 2018
UK	Barred grass snake	*N. helvetica*	Franklinos et al., 2017
	Adder	*Vipera berus*	Franklinos et al., 2017

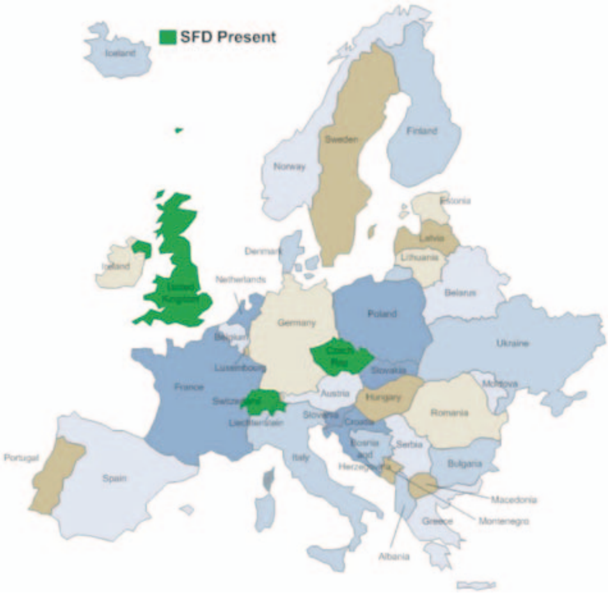

Figure 10. The distribution of ophidiomycosis cases in wild European snake populations.

several species (*T. marmoratus*, *A. obstetricans*, *L. boscai*, *S. salamandra*, and *B. spinusus*, Rosa et al., 2017). The emergence of ranavirosis was correlated with sharp declines in two species (*L. boscai* and *A. obstetricans*) at one of the sites within Serra da Estrela Natural Park (Rosa et al., 2017). The ability of *Ranavirus* emergence in amphibians to change the species assemblages at a site and alter host community composition and structure is a huge threat to these animals (Rosa et al., 2017). Therefore, surveillance efforts must not just be centred around one potential pathogen or one potential host; a community-based approach for pathogen surveillance should be used whenever possible.

EMERGING INFECTIOUS DISEASE THREATS TO REPTILES

OPHIDIOMYCOSIS/SNAKE FUNGAL DISEASE

In recent years it has become apparent that amphibians are not the only herptiles to suffer from declines caused by emerging fungal pathogens. There are a number of minor fungal diseases that infect reptiles (Paré & Sigler, 2016) but the one that has recently caught international attention is snake fungal disease. The causative agent was first identified in 2009 as *Chrysosporium ophiodiicola* (Rajeev et al., 2009) but subsequent research has seen the fungus moved to its own genus of *Ophidiomyces* (Sigler et al., 2013). Commonly known as snake fungal disease (hereafter SFD) but more correctly know as ophidiomycosis, is troubling for many reasons, one of which is that the clinical signs are not pathognomonic and so histological evidence, as well as genetic, is needed to confirm infection. SFD was first identified from snakes in the US (Dolinski et al., 2014; Rajeev et al., 2009) and it has recently been found to be present in snake populations in Europe (Franklinos et al., 2017; Meier et al., 2018).

The clinical signs of ophidiomycosis vary slightly between species but include skin lesions, dermatitis, scale deformity and yellow-brown crusty areas (Lorch et al., 2016). Abrasion may create opportunities for infection. Once the stratum corneum has been compromised, *O. ophiodiicola* can then quickly penetrate the epidermis, at which point the hosts immune response leads to the aforementioned clinical signs developing (see Fig. 9; Lorch et al., 2015). As a response to the disease, snakes tend to increase their sloughing frequency in order to try to cast off the pathogen. The new skin underneath is clinically normal and this behaviour may lead to snakes successfully curing themselves of ophidiomycosis. However, if old skin is stuck to the new skin during a shed, then there is the potential for reinfection (Lorch et al., 2015). All of this is very worrying for snake species that may already be suffering from the effects of fragmentation, persecution, and inbreeding depression (Madsen et al., 1996; Ursenbacher et al., 2009). The current evidence suggests that *O. ophiodiicola* only has the ability to infect snakes, although this is an area that needs further investigation.

So far, investigations into the presence of ophidiomycosis in the Europe are limited, but the presence has been confirmed in a number of species in a small number of countries (Table 5, Fig. 10). The first evidence of ophidiomycosis being present in Europe came from a screening of archived specimens collected by the Garden Wildlife Health project between 2010-2016 (Franklinos et al., 2017). This effort identified that wild European snakes in two countries (the UK and Czech Republic) were positive for *O. ophiodiicola* using a combination of histological and genetic diagnostic techniques. Since then, snakes from Switzerland have also tested positive for the causative agent of ophidiomycosis (Meier et al., 2018). At this stage it is not known how virulent the disease is to European snake species. Franklinos et al. (2017) noted that skin lesions were mild in most of the cases they examined but in some, these lesions were quite severe and would have contributed to the animals mortality. At this time, we do not have a clear picture of how widespread ophidiomycosis is in Europe or what that means for its snake species. More research is needed to help understand the status of this pathogen. In particular, it is not known whether or not ophidiomycosis is an

Table 6. Summary of countries and reptile species known to harbour *Ranavirus* infections in wild European populations.

Location	Common Name	Species	First Report
Portugal	Iberian mountain lizard	*Iberolacerta monticola*	Alves de Matos et al., 2011
Spain	Viperine snake	*Natrix maura*	Price et al., 2014
UK	Slow worm	*Anguis fragilis*	Price et al., 2017

Figure 11. The distribution of *Ranavirus* spp. in wild European reptile species.

endemic disease that has become more virulent due to recent changes or whether it is introduced. Genetic work conducted by Franklinos et al. (2017) demonstrates that the strains of ophidiomycosis in Europe are different to those in the US, but again further research is needed to fully understand this relationship.

RANAVIRUSES

Amphibians are not the only group of poikilothermic vertebrates to be affected by ranaviruses; both fish and reptiles are also susceptible. In Europe, most cases of *Ranavirus* infections in reptiles have been reported in captive populations (see Duffus et al., 2015). There are only three reports of *Ranavirus* infections in wild European reptiles in three different species; however, it is extremely likely that this is not reflective of the actual situation (see Table 6 and Fig. 11).

Signs of ranavirosis in reptilians are diverse and non-specific, including but not limited to: lethargy, anorexia, upper respiratory tract necrosis, edema (especially in the region of the neck), dermatitis, and nasal discharge (Marschang, 2011). Most reptilian *Ranavirus* infections have actually been reported in testudinids, with comparatively few in squamates (see Duffus et al., 2015). Since the gross signs of ranavirosis in reptiles are diverse, it is important for a full pathological investigation to be undertaken when disease is thought to be the cause of mortality. The first confirmed report of an iridovirus-like virus was documented in an Iberian mountain lizard (*Lacerta monticola*) from 'Serra da Estrela', Portugal (Alves de Matos et al., 2011). The animal was asymptomatic and released after a blood sample was taken (Alves de Matos et al., 2011; Stöhr et al., 2015). A *Ranavirus* spp. was isolated from this blood sample and it was shown to be an FV3-like virus (Alves de Matos et al., 2011; Stöhr et al., 2015). A report of a *Ranavirus* infection in a slow worm (*Anguis fragilis*) is made in Price et al. (2017) but very little information about the affected animal was provided. However, in 2014, a more concerning report of a *Ranavirus* infection in a reptile was published. Price et al. (2014) report the first Ranavirus-associated mortality in a wild European reptile. A CMTV-like *Ranavirus* was isolated from a dead viperine snake (*Natrix maura*), from 'Picos de Europa' National Park, Spain, that had been scavenging on amphibians from a Ranavirus-associated mortality event (Price et al., 2014). This underscores the ability of ranaviruses to infect multiple hosts from different taxa and is a further demonstration of how severe a threat to herpetofauna that this group of pathogens is.

GENERAL RESOURCES FOR STUDYING HERPETOFAUNAL INFECTIOUS DISEASES

In the past decade, there have been a number of important resources published that examine different aspects of the study of herpetofaunal infectious diseases. Gray et al. (2017) provides an in-depth methodology on pathogen surveillance specifically for herpetofauna. They examine how to design a surveillance study, how to collect samples to perform the study, biosecurity, and even intervention strategies for when an emerging infectious disease has been detected. Gray et al. (2017) is a valuable resource for those seeking to improve study design and limit the spread of infectious diseases in herpetofauna. Langwig et al. (2015) provide an excellent resource for examining the emergence of infectious diseases in wildlife. They provide a framework for decision making and necessary actions pre-and post-infection detection.

Few resources for wildlife professionals have been developed that deal with necropsy techniques, as most have been written for veterinary professionals. While we encourage multidisciplinary teams that include veterinary pathologists, necropsies of herpetofauna may also be carried out by trained wildlife professionals. Duffus et al. (2017) provides a guide for investigating herpetofaunal mortality events. The manuscript is geared towards wildlife professionals and provides methods for necropsies, tissue sample collection, and sample storage and shipping. There are also publications that specifically deal with the detection and diagnosis of specific pathogens. For example, Thomas et al. (2018) examines the current diagnostic tools available for *Bsal*. They provide guidelines not only for the use of these tools, but also for interpreting the results that these tools provide (Thomas et al., 2018). It is incredibly important to standardise both the methodologies and the interpretation of their results to ensure that results from different studies can be easily and consistently compared. Unfortunately, not all studies follow these guidelines and this leads problems in comparing results. For example, Dalbeck et al. (2018) uses a significance threshold of 0.05 GE when testing for *Bsal*, whereas a threshold of 0.1 GE is recommended by Thomas et al. (2018), when diluting extracted samples prior to qPCR. This of course, will lead to confusion and a difference in results when comparing studies unless there is a universally agreed standard, such is recommended in the case of *Bd* (Boyle et al., 2004; Blooi et al., 2013).

In addition to print resources, there are online databases that have been developed to track some emerging infections of herpetofauna. The Amphibian Disease Portal (https://amphibiandisease.org) is a database that is used to track reports of *Bd* and *Bsal* around the globe. Additionally, there is a *Bsal* reporting system for Europe (www.BsalEurope.com) and an associated early warning system. The *Bsal* reporting system and the early warning system for Europe will be extremely important in tracking infections and mitigating the effects of *Bsal* once it is found in an area. The Global Ranavirus Reporting System (https://mantle.io/grrs) is another online database that tracks reports of *Ranavirus* infection around the globe. These are both relatively new online databases and rely on researchers to upload their data into the system. To ensure that these resources are viable, we encourage researchers to upload their published data to these sites. An older resource that has been previously been used to track the global emergence of *Bd* is www.bd-maps.net (Olson et al., 2013). There are over 40,000 entries from over 80 countries in this database.

Ranaviruses, *Bsal*, and *Bd* are all reportable infections in amphibians (see http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2019/ for a list of reportable infections). The OIE (http://www.oie.int/) has specific guidelines for reporting these infections and they should be reported to the appropriate agency in the country that they are discovered in. Additionally, risk analyses may be performed and there are several sources for guidelines, including Jackob-Hoff et al. (2014) and one jointly published by the OIE and IUCN (OIE and IUCN 2014). These are invaluable resources for anyone who deals with infectious diseases in wildlife.

CONCLUDING REMARKS

With the global declines of both amphibians and reptiles, it is now more important than ever to understand the threats to these unique and important animals. Although we have only focused on five emerging infectious disease threats to these animals in Europe, there are likely more (e.g. Amphibiocystidium, identified in Duffus & Cunningham, 2010), that are understudied and poorly understood, or even unknown infections that are taking a toll on the populations of these animals. Unfortunately, as stated above, the emergence of several of these infectious diseases has resulted in population declines and local extirpations of several species, some of which were rare, but others, more worryingly, were considered to be common.

An important and necessary difference between the infection status of an animal and the appearance of clinical signs of disease is still often overlooked in many studies in herpetofauna. Infection is the presence of the potentially pathogenic or disease-causing agent in an organism or population, whereas disease is a measurable negative effect that the infection process has imposed in an animal (Scott, 1988). The effects of an infection may or may not result in the development of clinical signs of disease (Scott, 1988). Usually, disease is measured by the appearance of clinical signs. However, the presence/absence of these signs are often dependent upon the scale at which one is examining the organism (Scott, 1988).

Recent research has focused on both the imperfect detectability of infectious diseases (such as *Bd*) when using swabs and qPCR (DiRenzo et al., 2018) as well as the costs involved to complete the analysis (Sabino-Pinto et al., 2018). As previously mentioned, qPCR isn't infallible and false-positive or false-negative results are always possible. We therefore recommend that multiple detection techniques be used in order to be certain when screening suspected infected individuals. In order to fully understand the effects that emerging infectious diseases have on populations, an interdisciplinary team, as stressed in Duffus and Cunningham (2010), should be used. Ideally, such a team would include ecologists, herpetologists, population biologists, and veterinary pathologists. The team should investigate the entire situation, sometimes including multiple species in an area, to garner a deeper understanding of the issues that are potentially being caused by the emergence of an infectious agent.

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