

The following supplement accompanies the article

Qualitative risk analysis of introducing *Batrachochytrium dendrobatidis* to the UK through the importation of live amphibians

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Table S1. Responses to the UK Non-native Organism Risk Assessment (NNRA) (Risk Assessment) scheme regarding the introduction of *Batrachochytrium dendrobatidis* into the UK through the importation of live amphibians. na: not applicable

Stage 1: initiation		
	QUESTION	ANSWER
1	What is the principal reason for performing the Risk Assessment? (Include any other reasons as comments)	The organism has been identified as a risk by scientific research.
2	What is the Risk Assessment Area?	UK
3	What is the name of the organism? This will appear as a heading (other names used for the organism can be entered in the comments box).	<i>Batrachochytrium dendrobatidis</i> (Bd)
4	What is the status of any earlier Risk Assessment?	None exists for the UK.
5	Give details of any earlier Risk Assessment(s).	na
Stage 2, Section A: organism risk assessment		
	QUESTION	ANSWER
6	If you are sure that the organism clearly presents a risk, or that in any case a full Risk Assessment is required, you can omit this section and proceed directly to Section B.	Continue with organism screening.
7	What is the taxonomic group of the organism?	Fungi
8	What is the taxonomic status of the organism?	Single taxonomic entity
9	If not a single taxonomic entity, please give details?	na
10	Is the organism in its present range known to be invasive?	Yes / possible (the organism is considered to be invasive)
12	What is the current distribution status of the organism with respect to the Risk Assessment Area?	Outside effective containment but not widely distributed
13	Are there conditions present in the Risk Assessment Area that would enable the organism to survive and reproduce? Comment on any special conditions required by the species?	Yes / possible
14	Does the known geographical distribution of the organism include ecoclimatic zones comparable with those of the Risk Assessment Area or sufficiently similar for the organism to survive and thrive?	Yes / possible
15	Could the organism establish under protected conditions (such as glasshouses, aquaculture facilities, terraria, zoological gardens) in the Risk Assessment Area?	Yes / possible

16	Has the organism established viable (reproducing) populations anywhere outside of its native range?	Yes / possible
17	Can the organism spread rapidly by natural means or by human assistance?	Yes / possible
18	Could the organism as such, or acting as a vector, cause economic, environmental or social harm in the Risk Assessment Area?	Yes / possible
19	If answers to questions in this section were 'yes' (even if some were only possibilities), then a full assessment is likely to be necessary. Please give an appraisal of whether it is necessary to proceed with a full assessment and briefly give the key reasons in the comment box.	<i>Bd</i> has been identified as a risk to native amphibians elsewhere, and the existence of suitable environmental conditions and host species within the UK necessitates a full risk assessment.
Stage 2, Section B: pathways		
Entry pathways		
	QUESTION	ANSWER
20	How many pathways are relevant to the potential entry of this organism?	Few
21	Please list relevant pathways through which the organism could enter.	<ol style="list-style-type: none"> 1. Legal importation of live amphibians infected with <i>Bd</i> in the pet trade 2. Legal importation of live amphibians infected with <i>Bd</i> in the zoo trade 3. Legal importation of live amphibians infected with <i>Bd</i> in the laboratory trade 4. Legal importation of live amphibians or amphibian products infected with <i>Bd</i> for human consumption 5. Illegal importation of live amphibians infected with <i>Bd</i> 6. Unintentional importation of live amphibians infected with <i>Bd</i> as agricultural stowaways 7. Unintentional introduction via <i>Bd</i>-contaminated water or fomites.

22	Please select the pathway:	Pathway 1: legal importation of live amphibians infected with <i>Bd</i> in the pet trade		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Very likely	High	Amphibians entering the UK for the pet trade may have originated from captive breeding stocks or be wild-caught. Likelihood of presence is variable and depends on the country of origin and the source. There are multiple reports of <i>Bd</i> -infected amphibians in the pet trade (Groff et al. 1991, Berger et al. 1999, Mutschmann et al. 2000, Cunningham et al. 2005, Fisher & Garner 2007, Goka et al. 2009).
24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Very likely	Very high	In 2006, 6 consignments containing pet amphibians entered the UK on average per week, which contained a total of more than 128 000 individual amphibians over the course of the year. Approximately one-quarter of these were imported with reptiles or in amphibian-only consignments. Sampling efforts in 2007 detected <i>Bd</i> infection in 3.2% of amphibians and 20% of consignments. The remaining three-quarters of individuals were aquatic amphibians imported with live fish consignments; however, no individuals positive for <i>Bd</i> were detected in 2007. The likely concentration of <i>Bd</i> in infected amphibians (i.e. infection load) is variable depending on the stage of infection and amphibian species (Carey et al. 2006). Some species (e.g. aquatic species such as <i>Lithobates catesbeianus</i> and <i>Hymenochirus boettgeri</i>) may be infected at low levels in the absence of clinical signs, and infections may be difficult to detect (Daszak et al. 2004, Fisher & Garner 2007). Conversely, amphibian species that are highly susceptible to <i>Bd</i> infection (e.g. many neotropical species) usually develop clinical signs associated with high infection loads (Carey et al. 2006). Under experimental conditions, a high level of exposure in susceptible species results in more rapid demonstration of clinical signs and death (Carey et al. 2006). However, at low levels of exposure, although 100% of amphibians may become infected, time to show clinical signs and to death may

				be extended beyond 42 d; i.e. animals may be collected and shipped within a reasonable time period before clinical signs develop.
25	How likely is the organism to survive during passage along the pathway?	Likely	High	<i>Bd</i> survives in a broad temperature range. Temperature and humidity conditions associated with transporting amphibians are generally suitable for survival of <i>Bd</i> .
26	How likely is the organism to enter the UK undetected?	Very likely	Very high	Clinical signs of chytridiomycosis may be absent or non-specific and laboratory testing is required for definitive diagnosis (Daszak et al. 2004, Kriger et al. 2006).
27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Moderately likely	Medium	As little as one zoospore is sufficient to infect amphibians (Carey et al. 2006). An exponential increase in <i>Bd</i> infection load occurs through local re-infection on an individual host (Carey et al. 2006, Briggs et al. 2010, Voyles et al. 2011). Infected amphibians may be shipped within the same packaging as uninfected amphibians, allowing transfer of infection and an increase in prevalence. Further research is required to quantify this.
28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	Very likely	Very high	No specific practices are generally performed to reduce concentration of <i>Bd</i> via treatment or disinfection in the country of origin before shipment or in the UK before or after importation.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Very likely	High	Data from 2006 indicate that the volume of amphibian pet trade imports associated with the fish trade is constant throughout the year. The volume of amphibian pet trade imports associated with the reptile trade was bimodal in data collected from 2006, with peaks in spring and autumn, when environmental conditions are optimal for chytridiomycosis survival and replication. However, the latter component of pet amphibian imports is more likely to involve tropical species, which would be less able to survive in the climate of the UK if release or escape occurs.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Likely	Medium	Intended use of imported amphibians in the pet trade is maintenance in captivity. However, accidental escapes, illegal release or transfer of infection to amphibians in reintroduction programs could aid in transfer to a suitable habitat (Holsbeek et al. 2010). Escaped or released amphibians would need to be released close to suitable habitats for survival of the pathogen and host,

				which is very likely in the case of amphibians kept in backyard ponds.
31	Do other pathways need to be considered?	Yes		

22	Please select the pathway:	Pathway 2: legal importation of live amphibians infected with <i>Bd</i> in the zoo trade		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Very likely	Very high	Amphibians entering the UK for the zoo trade may have originated from captive breeding stocks or be wild-caught. Likelihood of presence is variable and depends on the country of origin and the source. There are multiple reports of chytridiomycosis in zoological collections (Nichols et al. 1998, Pessier et al. 1999, Nichols et al. 2001, Raverty & Reynolds 2001, Banks et al. 2002, Oevermann et al. 2005, Schloegel et al. 2006, Goka et al. 2009). Many amphibian species are being brought into captivity for breeding programs to insure against chytridiomycosis-associated losses in the wild, and the source populations may already contain <i>Bd</i> -infected amphibians.

24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Unlikely	Medium	<p>The volume and frequency of trade is very low when compared with that of the pet trade (in 2006, 35 individuals entered the UK in the zoo trade). A review of testing records conducted at the Institute of Zoology on zoo amphibians indicated a prevalence of infection with <i>Bd</i> of 5.9%.</p> <p>The likely concentration of <i>Bd</i> in infected amphibians (i.e. infection load) is variable depending on stage of infection and amphibian species (Carey et al. 2006). Some species (e.g. aquatic species such as <i>Lithobates catesbeianus</i> and <i>Hymenochirus boettgeri</i>) may be infected at low levels in the absence of clinical signs (Fisher & Garner 2007). Once infected with <i>Bd</i>, highly susceptible amphibian species (e.g. neotropical species) usually develop clinical signs associated with high infection loads (Carey et al. 2006). Zoo trade in amphibians for conservation programs frequently involves transfer of neotropical species highly susceptible to <i>Bd</i>. Under experimental conditions, a high level of exposure in susceptible species results in more rapid demonstration of clinical signs and death (Carey et al. 2006). However, at low levels of exposure, although 100% of amphibians may become infected, time to show clinical signs and to death may be extended beyond 42 d; i.e. animals may be collected and shipped within a reasonable time period before clinical signs develop.</p>
25	How likely is the organism to survive during passage along the pathway?	Likely	High	<i>Bd</i> survives in a broad temperature range. Temperature and humidity conditions associated with transporting amphibians are generally suitable for survival of <i>Bd</i> .
26	How likely is the organism to enter the UK undetected?	Very likely	Very high	Clinical signs of chytridiomycosis may be absent or non-specific and laboratory testing is required for definitive diagnosis (Daszak et al. 2004, Kriger et al. 2006). Quarantine procedures and testing for the presences of <i>Bd</i> should be part of zoo amphibian collection movements. Adequate testing is very likely to detect <i>Bd</i> if it is present.
27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Moderately likely	Medium	As little as one zoospore is sufficient to infect amphibians (Carey et al. 2006). An exponential increase in <i>Bd</i> infection load occurs through local re-infection on an individual host (Carey et al. 2006, Briggs et al. 2010, Voyles et al. 2011). Infected amphibians may be shipped within the same packaging as uninfected amphibians,

				allowing transfer of infection and an increase in prevalence. Further research is required to quantify this.
28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	Moderately likely	Very high	Variable depending on the source. Although quarantine procedures may be put in place, it is likely that no specific practices are performed to reduce concentration of <i>Bd</i> via treatment or disinfection in the country of origin before shipment, particularly for wild-caught specimens. Captive-bred individuals being transferred between zoos with biosecurity protocols in place may have undergone treatment.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Likely	High	Transfers of amphibians between zoos showed no seasonality and are spaced throughout year.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Very likely	Medium	Amphibians imported in the zoo trade may be intended for maintenance in captivity indefinitely, or for conservation breeding programs with intended subsequent release into the wild. Inadequate quarantine, <i>Bd</i> -diagnosis and treatment protocols in reintroduction programs could aid in transfer of <i>Bd</i> to a suitable habitat. Release of <i>Bd</i> -infected amphibians to the wild is known to have occurred for at least one conservation program (Walker et al. 2008). As with any captive colony, accidental escape is also a potential pathway for transfer of <i>Bd</i> to a suitable habitat or host. Escaped or released amphibians would need to be released close to suitable habitats for survival of the pathogen and host.
31	Do other pathways need to be considered?	Yes		

22 Please select the pathway:		Pathway 3: legal importation of live amphibians infected with <i>Bd</i> in the laboratory trade		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Very likely	High	There are multiple reports of infection with <i>Bd</i> in amphibians traded for laboratory research (Reed et al. 2000, Weldon et al. 2004). Purpose-bred laboratory amphibians entering the UK from reputable establishments are likely to be monitored for clinical signs of disease. The primary species involved in the laboratory trade (<i>Xenopus laevis</i>), however, is an asymptomatic carrier of <i>Bd</i> (Fisher & Garner 2007) and routine testing for <i>Bd</i> infection is not known to take place in this sector of the amphibian trade.
24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Moderately likely	Medium	The number of amphibians imported via this pathway is moderate (an estimated 3200 amphibians in 2006). Species with asymptomatic infections often have low infection loads; however, a high proportion of individuals may be infected. Whilst sampling in 2007 did not detect any infected amphibians, in one UK laboratory facility tested, captive stock housed individually with a recirculating water system had a high prevalence (33%) of <i>Bd</i> infection. Concentration of the organism at the origin is variable depending on the source (disease-free, unknown), and this is information not publicly available.
25	How likely is the organism to survive during passage along the pathway?	Likely	High	<i>Bd</i> survives in a broad temperature range. Temperature and humidity conditions associated with transporting amphibians are generally suitable for survival of <i>Bd</i> .
26	How likely is the organism to enter the UK undetected?	Very likely	Very high	Clinical signs of chytridiomycosis are generally absent in the main species involved in this trade and laboratory testing is required for definitive diagnosis (Daszak et al. 2004, Kriger et al. 2006, Fisher & Garner 2007), yet currently is not routinely performed.
27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Moderately likely	Medium	Several amphibians may be shipped together within one package, allowing potential transfer of infection from infected to non-infected individuals and a potential increase in prevalence. However, it is likely that there is a high prevalence of infected individuals at the origin of the pathways, and any additional increase in prevalence during transport might only be minor.

28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	Very likely	Very high	Variable depending on source and whether specific practices are performed to reduce concentration of <i>Bd</i> via treatment or disinfection in the country of origin before shipment. Routine testing and decontaminations are not generally performed.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Very likely	High	Data from 2006 indicate that the volume of laboratory amphibian trade imports is bimodal, with peaks in spring and autumn when environmental conditions are optimal for chytridiomycosis survival and replication.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Moderately likely	Medium	Intended use of imported amphibians is maintenance in laboratory, with disposal after use. There is a moderate likelihood of escape of live amphibians from research laboratories, as is indicated by the presence of feral <i>Xenopus</i> spp. in most countries where laboratories holding these animals exist. Escaped or released amphibians would need to be released relatively close to suitable habitats for survival of the pathogen and host.
31	Do other pathways need to be considered?	Yes		

22	Please select the pathway:	Pathway 4: legal importation of live amphibians or amphibian products infected with <i>Bd</i> for human consumption		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Very likely	Medium	There are multiple reports of <i>Bd</i> -infected amphibians destined for human consumption (Mazzoni et al. 2003, Hanselmann et al. 2004, Daszak et al. 2006, Garner et al. 2006, Schloegel et al. 2009). Amphibians reared for human consumption are either caught in the wild or reared in conditions without adequate disease control measures. Additionally, <i>Bd</i> -infection is asymptomatic in the main species traded (<i>Lithobates catesbeianus</i>) (Daszak et al. 2004).
24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Very unlikely	High	Species with asymptomatic infections often have low infection loads; however, a high proportion of individuals may be infected. The market for frogs' legs is relatively small in the UK. No shipments into the UK were identified in 2006.

25	How likely is the organism to survive during passage along the pathway?	Very unlikely	High	The importation into the EU of live <i>Lithobates catesbeianus</i> is prohibited. However, other species for human consumption may be imported live in smaller numbers. Imported frogs' legs are skinned, which would result in removal of the chytrid fungus. Frogs' legs are generally transported and stored frozen (FAO 2011). As freezing kills <i>Bd</i> , this processing should result in the absence of any remaining viable chytrid fungus.
26	How likely is the organism to enter the UK undetected?	Very likely	Very high	If viable <i>Bd</i> zoospores did survive the freezing and skinning process, they are extremely unlikely to be detected.
27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Very unlikely	Very high	Frogs' legs are generally transported frozen, and therefore <i>Bd</i> is unlikely to undergo amplification.
28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	na	High	<i>Bd</i> is unlikely to persist after skinning and freezing the host animal.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Unlikely	Low	Unknown; limited data are available to respond to question.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Very unlikely	High	This trade is skinned and frozen amphibian products, which would not come into contact with live hosts. Since processing should result in the absence of viable chytrid fungus, wastewater should also not be an issue.
31	Do other pathways need to be considered?	Yes		

22 Please select the pathway:		Pathway 5: illegal importation of live amphibians infected with <i>Bd</i>		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Moderately likely	Medium	Amphibians illegally entering the UK are primarily likely to be CITES-listed species for the pet trade. These may have originated from captive breeding stocks or, more likely, be wild-caught. Likelihood of presence of <i>Bd</i> is variable and depends on the country of origin and the species. There are multiple reports of <i>Bd</i> -infected amphibians in the pet trade (Groff et al. 1991, Berger et al. 1999, Mutschmann et al. 2000, Cunningham et al. 2005, Fisher & Garner 2007, Goka et al. 2009).
24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Unlikely	Low	The volume and frequency of individuals being imported into the UK via this pathway is unknown. The likely concentration of <i>Bd</i> in infected amphibians (i.e. infection load) is variable depending on stage of infection and amphibian species (Carey et al. 2006). Once infected with <i>Bd</i> , highly susceptible amphibian species (e.g. neotropical species) usually develop clinical signs associated with high infection loads (Carey et al. 2006).
25	How likely is the organism to survive during passage along the pathway?	Likely	Medium	<i>Bd</i> survives in a broad temperature range. Temperature and humidity conditions associated with transporting amphibians are generally suitable for survival of <i>Bd</i> . However, the nature of illegal trade means that environmental conditions may be highly variable and not necessarily suitable for the amphibian or <i>Bd</i> .
26	How likely is the organism to enter the UK undetected?	Very likely	Very high	Clinical signs of chytridiomycosis may be absent, slow to develop, or non-specific and laboratory testing is required for definitive diagnosis. The underground nature of illegal trade means the individual amphibians are entering the UK undetected, whether they are <i>Bd</i> -infected or not.
27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Moderately likely	Medium	As little as one zoospore is sufficient to infect amphibians (Carey et al. 2006). An exponential increase in <i>Bd</i> infection load occurs through local re-infection on an individual host (Carey et al. 2006, Briggs et al. 2010, Voyles et al. 2011). Infected amphibians may be shipped within the same packaging as uninfected amphibians, allowing transfer of infection and an increase in prevalence.

28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	Very likely	Very high	It is very unlikely that specific practices are performed to reduce concentration of <i>Bd</i> via treatment or disinfection in the country of origin before or during shipment.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Likely	Medium	Unknown temporal distribution of trade; however, conditions in the UK are favourable for survival of <i>Bd</i> during much of the year.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Moderately likely	Medium	Intended use of imported amphibians is maintenance in captivity. However, illegal animals may be intentionally released into suitable habitats to avoid prosecution for possession. Escaped or released amphibians would need to be released very close to suitable habitats for survival of the pathogen and host.
31	Do other pathways need to be considered?	Yes		

22	Please select the pathway:	Pathway 6: unintentional importation of live amphibians infected with <i>Bd</i> as agricultural stowaways		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Unlikely	Low	Likelihood of presence is highly variable and depends on the country of origin and the source.
24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Moderately likely	Low	This pathway has been demonstrated to exist in Australia (Marantelli & Hobbs 2000). An estimated 500000 frogs are transported annually into Melbourne, Australia, in produce. Figures for the UK are unknown.
25	How likely is the organism to survive during passage along the pathway?	Likely	Medium	Temperature and humidity conditions associated with transport of fresh agricultural produce are also likely to be generally suitable for survival of <i>Bd</i> . Amphibians have previously been documented to survive transport via this pathway in Australia (Marantelli & Hobbs 2000).
26	How likely is the organism to enter the UK undetected?	Very likely	High	Detection often occurs after importation and transportation, e.g. when banana boxes are opened at their point of sale.

27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Unlikely	Medium	It is unlikely that multiple amphibians are present and in contact within the same shipment to allow increase in prevalence.
28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	Unlikely	Low	Detection is likely to occur at the point of sale of the produce. Transfer to a suitable habitat depends on whether the person finding the <i>Bd</i> -infected stowaway destroys the individual, transfers it to captivity or releases it.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Likely	Medium	Agriculture produce is imported into the UK from many international locations all year round.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Unlikely	Low	Release of a discovered <i>Bd</i> -infected stowaway could aid transfer to a suitable habitat.
31	Do other pathways need to be considered?	Yes		

22	Please select the pathway:	Pathway 7: unintentional introduction via <i>Bd</i>-contaminated water or fomites		
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
23	How likely is it that the organism is strongly associated with the pathway at the point(s) of origin?	Moderately likely	Low	Likelihood varies greatly depending on where the water or fomite was transferred from and the degree of <i>Bd</i> -contamination at the source, e.g. natural watercourses, amphibian-rearing farms or captive establishments. Limited data are available.
24	How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin?	Unlikely	Low	The volume of water and fomites along this pathway is unknown. Concentration of the organism in water or on fomites is likely to be highly variable depending on source. Where <i>Bd</i> is present in amphibian species, water is readily contaminated.
25	How likely is the organism to survive during passage along the pathway?	Likely	High	<i>Bd</i> can survive within a wide temperature range (4–28°C) (Longcore et al. 1999, Piotrowski et al. 2004). It can survive in lake water at 23°C for up to 7 wk (Johnson & Speare 2003). Growth ceases at 28°C and zoospores experience 100% mortality within 4 h at 37°C (Johnson et al. 2003).

26	How likely is the organism to enter the UK undetected?	Very likely	High	Water cannot be detected as being contaminated with <i>Bd</i> without specific, time-consuming testing (Hyatt et al. 2007). Swabbing of research equipment to detect contamination with <i>Bd</i> is not routinely performed.
27	How likely is the organism to multiply/increase in prevalence during transport /storage?	Very unlikely	High	Although <i>Bd</i> zoospores may persist in water and on fomites under the right conditions, amphibian keratinised skin is required for multiplication (Berger et al. 2005).
28	How likely is the organism to survive existing management practices within the pathway (answer na for intentional introductions)?	Likely	Medium	No specific practices are performed routinely to decontaminate water used for transporting aquatic plants or animals. Thorough cleaning and decontamination of research equipment and clothing should, but may not always, occur after visiting a <i>Bd</i> -infected site.
29	How likely is the organism to arrive during the months of the year most appropriate for establishment (if intentional introduction answer na)?	Likely	High	<i>Bd</i> grows well in the laboratory between 17 and 25°C and is most virulent to amphibians between 12 and 23°C (Berger et al. 2004). It is able to survive in temperatures as low as 4°C and therefore has the potential to overwinter in hosts (Piotrowski et al. 2004). Growth ceases at 28°C and zoospores experience 100% mortality within 4 h at 37°C (Johnson et al. 2003). There is only a remote likelihood of temperatures in the UK reaching 37°C for long enough to kill <i>Bd</i> zoospores.
30	How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Moderately likely	Low	More information is required on the potential pathways of disposed water and the likelihood of survival of <i>Bd</i> along those pathways. Transfer of aquatic plants into garden ponds visited by wild amphibians is a potential pathway for transfer to a suitable habitat.
31	Do other pathways need to be considered?	No		
Overall likelihood of entry				
32	Please estimate the overall likelihood of entry into the Risk Assessment Area for this organism (please comment on the key issues that lead to this conclusion).	Very likely	Very high	Presence of <i>Bd</i> has been reported in the pet trade, laboratory trade, and trade for zoological collections (OIE, 2006). The volume of these trades into the UK is sufficiently large to make entry of <i>Bd</i> very likely, and <i>Bd</i> -infected individuals were detected at Heathrow Animal Reception Centre in 2007.

Potential to establish				
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
33	How likely is it that the organism will be able to establish in the UK based on the similarity between climatic conditions in the UK and the area of the organism's current distribution?	Very likely	High	Environmental conditions associated with chytridiomycosis outbreaks often include cool temperature, high altitude and wet climate (Drew et al. 2006). Bioclimatic modelling predicting the potential distribution of chytridiomycosis has repeatedly included the UK as an area of high suitability for survival and spread of <i>Bd</i> (Ron 2005, Lötters et al. 2009). Two sites in the UK have already supported establishment of <i>Bd</i> . However, there is large variability in the similarity of climatic conditions in the UK and in species imported.
34	How likely is it that the organism will be able to establish in the UK based on the similarity between other abiotic conditions in the UK and the area of current distribution to be similar?	Likely	High	Amphibians breeding in permanent water bodies, and particularly in permanent streams, are at the highest risk of becoming infected with <i>Bd</i> (Kriger & Hero 2007). A range of water temperatures is suitable, although salinity can prevent establishment.
35	How many species or suitable habitats vital for the survival, development and multiplication of the organism species are present in the UK? Please specify in the comment box the species or habitats.	Moderate number	High	Native: <i>Bufo bufo</i> , <i>Epidalea calamita</i> , <i>Rana temporaria</i> , <i>Triturus cristatus</i> , <i>Lisotriton helveticus</i> , <i>L. vulgaris</i> . Reintroduced: <i>Pelophylax lessonae</i> . Introduced: <i>Bombina bombina</i> , <i>Alytes obstetricians</i> , <i>Hyla arborea</i> , <i>Xenopus laevis</i> , <i>Lithobates catesbeianus</i> , <i>P. esculenta</i> , <i>P. perezi</i> , <i>P. ridibunda</i> , <i>M. alpestris</i> , <i>Triturus carnifex</i> .
36	How widespread are the species or suitable habitats necessary for the survival, development and multiplication of the organism in the UK?	Widespread	High	All amphibians must be considered susceptible to chytridiomycosis. Amphibians that inhabit <i>Bd</i> -preferred ecoclimatic zones are widely distributed across the UK.
37	If the organism requires another species for critical stages in its life cycle, then how likely is the organism to become associated with such species in the UK?	na	Very high	na

38	How likely is it that establishment will occur despite competition from existing species in the UK?	Very likely	Medium	Establishment of <i>Bd</i> -infected amphibians depends on the species, its invasiveness and its ability to adapt to the UK's climate. There have been no reports of <i>Bd</i> fungus being prevented from becoming established in an environment owing to competition with other saprophytic fungi. However, infection of individual amphibian species may be prevented or reduced by the presence of skin peptide secretions. The presence of such secretions in UK native amphibians has not been investigated.
39	How likely is it that establishment will occur despite predators, parasites or pathogens already present in the UK?	Moderately likely	Medium	As above
40	How likely are management practices in the UK to favour establishment?	Likely	Medium	The practice of placing introduced amphibians in garden ponds is likely to increase the risk of establishment.
41	How likely is the organism to establish despite existing management practices in the UK?	Likely	Medium	No existing control or husbandry measures are in place to routinely detect accidental release of <i>Bd</i> . Once release of <i>Bd</i> is detected, establishment of <i>Bd</i> in wild populations may have already occurred.
42	How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the UK?	Very likely	High	Eradication success would be highly dependent on the stage of establishment. Treatment of individual amphibians infected with chytridiomycosis has been achieved with several methods (heat, antifungal drugs, disinfectants); however, containment and control within a natural environment is much more difficult and eradication efforts have proven unsuccessful in natural environments (Lubick 2010).
43	Is establishment likely to be aided by the biological characteristics of the organism?	Likely	High	<i>Bd</i> has the potential to survive across a wide temperature range, including the ability to overwinter in its hosts. It is extremely infectious; as little as one zoospore is sufficient to infect amphibians (Carey et al. 2006). <i>Bd</i> is able to persist in biotic and abiotic reservoirs (Johnson & Speare 2003).

44	Is the organism's capacity to spread likely to aid establishment?	Very likely	High	<i>Bd</i> is known to be highly transmissible and is able to spread via natural watercourses at rates of up to 100 km yr ⁻¹ , or via anthropogenic mechanisms of spread such as translocation and release of infected amphibians occurred (Aplin & Kirkpatrick 2000, Johnson & Speare 2005). This capacity for spread increases its exposure to suitable hosts and environments over the minimum threshold required for establishment.
45	How likely is the adaptability of the organism to aid its establishment?	Moderately likely	High	<i>Bd</i> occurs in a wide range temperature range, and its capacity for global spread demonstrates its ability to adapt to new environments.
46	How likely is it that the organism could establish despite low genetic diversity in the founder population?	Moderately likely	Medium	Global genetic isolates of <i>Bd</i> are known to have an extremely low diversity generally (James et al. 2009).
47	How likely is the organism to be established in protected conditions (in which the environment is artificially maintained, such as wildlife parks, glasshouses, aquaculture facilities, terraria, zoological gardens) in the UK?	Very likely	Very high	<i>Bd</i> infected amphibians have commonly been recorded in pristine environments around the world (Berger et al. 1998, Fisher et al. 2009).
48	Based on the history of invasion by this organism elsewhere, how likely is it to establish in the UK? (If possible, specify the instances of invasion in the comments box.)	Very likely	Very high	Chytridiomycosis is an emerging infectious disease that has recently increased in incidence, geography and host range (the novel pathogen hypothesis) (Berger et al. 1999, Daszak et al. 1999, Rachowicz et al. 2005). Rapid global spread of chytridiomycosis is likely to be a result of human assisted amphibian movements.
49	If the organism does not establish, then how likely is it that transient populations will continue to occur?	Likely	Medium	Establishment has already proved possible in at least one county, Kent County, in the UK (Cunningham et al. 2005). Reintroduction via tadpoles is possible.
Overall likelihood of establishment				
50	Please estimate the overall likelihood of establishment (mention any key issues in the comment box)	Likely	High	<i>Bd</i> has previously established in the UK as a result of release of non-native species. Although legislation exists to prohibit and regulate release and translocation of amphibians, illegal and unintentional introductions are likely to continue. This provides a pathway from presence of <i>Bd</i> within captive populations in the UK to establishment in native or non-native populations in the wild.

Potential for spread				
	QUESTION	LIKELIHOOD	CERTAINTY	COMMENT
51	How rapidly is the organism liable to spread in the UK by natural means?	Rapidly	High	Maximum spread of <i>Bd</i> is likely to occur at its optimal temperature range (12–27°C) along areas of uninterrupted suitable natural habitat/watercourses. <i>Bd</i> is able to spread via natural watercourses at rates of up to 100 km yr ⁻¹ (Aplin & Kirkpatrick 2000) and at rates of up to 282 km yr ⁻¹ at continental scales (Lips et al. 2008). Predictions are that within 4 to 6 mo of <i>Bd</i> arrival at a site where it has not previously been present, ~50% of amphibian species and ~80% of individuals may disappear (Lips et al. 2006).
52	How rapidly is the organism liable to spread in the UK by human assistance?	Rapidly	High	Anthropogenic mechanisms of spread such as translocation of infected amphibians, moist substrate or on other contaminated fomites enable <i>Bd</i> to move rapidly over long distances or past natural barriers. Common practices such as collection of tadpoles by school classes and release of adults at sites other than the collection point could aid spread throughout the country.
53	Within the UK, how difficult would it be to contain the organism?	Difficult	Medium	Difficulty arises in detecting infected populations within a reasonable time to erect amphibian-proof fencing to contain the infected population and habitat. Once contained, the populations must be exterminated and the habitat left fallow for a period of time, as treatment of the habitat is not feasible at this stage (DEH 2005).
54	Based on the answers to questions on the potential for establishment and spread in the UK, define the area endangered by the organism.	UK		Many parts of the UK are probably suitable for establishment of this pathogen, specifically, areas with permanent watercourses and especially flowing watercourses with temperatures consistently below 27°C. It could be introduced into these areas via intentional or unintentional release of amphibians.

Overall likelihood of spread				
55	Please estimate overall potential for spread (using the comment box to indicate any key issues).	Moderately	Medium	Spread will depend on availability of suitable habitat adjoining the site of establishment (i.e. watercourses), seasonal factors (more rapid spread in <i>Bd</i> optimal temperatures) and human activities resulting in dispersal (i.e. collecting and re-releasing amphibians).

Assessment of potential impacts				
	QUESTION	IMPACT	CERTAINTY	COMMENT
56	How great is the economic loss caused by the organism within its existing geographic range, including the cost of any current management?	Major	High	There are significant economic costs involved with mortality, monitoring and surveillance, biosecurity, eradication and control programmes for species conservation efforts. The Australian Government Department of Environment and Heritage has contributed several million dollars to research and management plans for chytridiomycosis (Skerratt et al. 2007), where it has caused long-term widespread irreversible ecosystem change.
57	How great a loss of production is the organism likely to cause in the UK? For example, how serious is the direct negative economic effect of the organism likely to be on crop yield and/or quality, livestock or fish health and production? (Describe the nature and extent of expected losses in the comment box.)	Minimal	Medium	Direct production costs are likely to be incurred by amphibian breeders, the pet trade, zoos and conservation programmes through morbidity and mortality of infected animals, the detection and treatment of infection and the implementation of enhanced biosecurity measures.
58	How great are the additional economic costs associated with managing this organism likely to be?	Moderate	Medium	Economic losses in the UK are likely to be suffered by government and others owing to the costs of control and eradication, surveillance and monitoring, and the possible introduction of enhanced biosecurity requirements.
59	How great a reduction in consumer demand is the organism likely to cause in the Risk Assessment Area?	Minimal	High	Consumer demand is unlikely to be greatly reduced. With increased stock mortalities, demand could actually increase owing to the demand for replacement stock.

60	How significant might the losses in export markets be due to the presence of the organism in the Risk Assessment area?	Minimal	High	There is currently very little export market from the UK.
61	How important might other economic costs be resulting from introduction of the organism? (Specify in the comment box)	Moderate	Medium	Additional costs include allocation of funds for scientific research.
62	How important is environmental harm caused by the organism within its existing geographic range under any current management regime?	Massive	Very high	The impact of chytridiomycosis on frogs has been described as ‘the most spectacular loss of vertebrate biodiversity due to disease in recorded history’ (Skerratt et al. 2007). Predictions are that within 4 to 6 mo of <i>Bd</i> arrival at a site where it has not previously been present, ~50% of amphibian species and ~80% of individuals may disappear (Lips et al. 2006). The potential exists for widespread, long-term population loss and extinction, affecting several species with serious ecosystem effects.
63	How important is environmental harm likely to be in the UK, taking into account any management interventions that might be implemented?	Major	High	Although the susceptibility of many UK species is unknown, there is potential for widespread, long-term population loss and extinction, affecting several species with serious ecosystem effects. Management interventions are available; however, there is currently little political motivation for these to be enforced
64	How important is social, health or other harm (not directly included in economic and environmental categories) caused by the organism within its existing geographic range under any current management regime?	Moderate	Medium	Education programs involving amphibians may be affected.
65	How important is the social, health or other harm likely to be in the UK taking into account any management interventions that might be implemented?	Moderate	Medium	Maintenance of ecosystem health is critical (UK National Ecosystem Assessment 2011) and viable amphibian populations play a role in this. Current management options to reduce spread are limited.

66	How important is it that genetic traits of the organism could be carried to native species, modifying their genetic nature and making their economic, environmental or social effects more serious?	Minimal	Medium	This is unlikely.
67	How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the UK?	Major	Medium	The potential for natural control by other organisms is poorly understood. It has been reported that microcrustacean zooplankton consume zoospores of some chytrid fungi (Kagami et al. 2004); however, this is unlikely to have a major effect on overall environmental or economic impacts.
68	How difficult is it likely to be to control the organism in the UK?	Difficult	High	Treatment of <i>Bd</i> -infected individual amphibians has been achieved using several methods (heat, antifungal drugs, disinfectants); however, control within a natural environment is much more difficult and has proved impossible in other areas.
69	How likely are control measures introduced for this new organism to disrupt existing biological or integrated systems used to control other organisms in the UK?	Very likely	Medium	Control measures would involve isolating the area and eradicating amphibian populations from this area. Treatment of the environment with antifungal drugs is possible, but would have severe effects on natural environmental fungi
70	How likely is the organism to act as food, a host, a symbiont or a vector for other damaging organisms?	Very unlikely	High	<i>Bd</i> can result in apparent competition between susceptible and carrier species, enhancing the invasiveness of the latter. Species such as <i>Lithobates catesbeianus</i> , which can be infected with <i>Bd</i> at low intensities of infection without causing mortality, can act as effective reservoirs and vectors for transmission locally and internationally (Daszak et al. 2003, Hanselmann et al. 2004, Retallick et al. 2004, Weldon et al. 2004).
71	Indicate any parts of the UK where economic, environmental and social impacts are particularly likely to occur (provide as much detail as possible).	Environmental impacts across the country		Economic impacts are likely to be felt by amphibian breeders, pet shops and zoos and by institutions funding amphibian disease research. Environmental impacts are likely to be widespread across the UK; however, the greatest effects will probably occur in areas where amphibian conservation and reintroduction programs are in place.

Overall impact rating				
72	Overall impact rating (please comment on the main reasons for this rating).	Major	High	Economic impacts are difficult to quantify; however, the potential impact on biodiversity is major and long lasting. Under the Convention on Biological Diversity, the Bern Convention and EU Wildlife Trade Regulations, the UK has a responsibility to prevent the introduction of, to control or to eradicate those non-native species that threaten ecosystems, habitats or species.
Overall risk				
	QUESTION	OVERALL RISK	COMMENT	
73	Give an overall assessment of the risk, taking into account the likelihood of entry and establishment, the expected level of spread and the potential impact.	High	The presence of <i>Bd</i> in all aspects of the amphibian trade and the sufficiently large volume of this trade into the UK makes entry of <i>Bd</i> very likely under current systems. Notable entry and exposure pathways with high likelihoods include release of amphibians from the pet or illegal trades, release of contaminated water and through the reintroduction of amphibians from captive breeding and rearing programs with inadequate <i>Bd</i> -screening and eradication protocols. Establishment and spread within natural habitats will depend on the availability of suitable habitat adjoining the site of establishment, seasonal factors and human activities resulting in dispersal, but overall, it is likely that <i>Bd</i> will be able to establish and spread in UK. Economic and environmental impacts are expected and the overall impact is likely to be major. Using a risk estimation matrix, the overall risk was determined to be high.	

Table S2. Evaluation of options for sanitary measures aimed at reducing the risk associated with the importation of *Bd* into the UK

Objective	Sanitary measure	Comments
Reduce risk of importation of <i>Bd</i>	Utilize Wildlife Trade Regulations (EC Council Regulation No.338/97) to prohibit importation of invasive species other than <i>Lithobates catesbeianus</i>	The potential for introduction of non-native amphibians into uninfected regions needs to be minimized, in particular, those species known to be globally invasive and in addition to being asymptomatic carriers of <i>Bd</i> . (Table 1 in Fisher & Garner 2007). However, the emphasis of EC Wildlife Trade Regulations is on prohibiting the import of live specimens of species based on their impact on native species rather than their disease-carrying capacity (species in this context refers to species of animals, not pathogens).
	Provide guidelines to stakeholders regarding handling and packaging of live amphibians for shipment	This measure aims to reduce mixing individuals of different species and of different origins to reduce pathogen transfer from infected to uninfected individuals and populations.
	Impose pre-export quarantine requirements for live amphibians in country of origin	The incubation time in experimentally infected frogs varies from 9 to 76 d, and most frogs die 13 to 31 d after exposure (Nichols et al. 2001). A 30 d pre-export quarantine and observation period would enable expression of clinical signs in susceptible species and would reduce the likelihood that <i>Bd</i> remains undetected. Housing amphibians at an optimal temperature for <i>Bd</i> pathogenicity (12–23°C) would enhance expression of clinical signs. This measure relies on staff being capable of recognising a sick amphibian and would fail to detect amphibians with asymptomatic chytridiomycosis.
	Adopt a <i>Bd</i> -free testing and accreditation system for commercial organizations involved in the aquatic trade	Sourcing animals from government- or industry-accredited establishments that adhere to monitoring, testing and quarantine protocols for <i>Bd</i> would reduce the risk of release. For example, amphibians from such establishments should have been held there continuously for at least 6 mo or since birth, and the introduction of new animals, e.g. wild-captured animals, should occur infrequently and be regulated. The facility should not have had any cases of <i>Bd</i> infection during the 12 mo before shipment.
	Introduce requirements for health certification of live amphibian imports and allocate an amphibian commodity code	Legal and practical infrastructure regarding quarantine documentation of disease status and additional screening at the veterinarian's discretion is necessary for importation of domesticated animals and could be extended for use in traded amphibians. Subsequent to regulatory changes, amphibian trade monitoring by TRACES would be improved by removing amphibians from the 'Other live animals' commodity code and allocate an amphibian-specific commodity code, requiring a separate Common Veterinary Entrance Document for Animals (CVEDA).
	Implement post-arrival veterinary	The benefits and limitations of post-arrival quarantine are similar to pre-export

	inspection and quarantine of live amphibians, including PCR testing protocols	quarantine. PCR testing is very sensitive and is highly likely to reduce risk to an acceptable level, but it is expensive. In consideration of the potential for false positive results, amphibians testing positive for <i>Bd</i> could be retested before rejection of the consignment or treatment of all cohorts in the consignment.
	Treatment of sick or high risk animals, followed by confirmation of disease-free status	Various treatment methods for chytridiomycosis have the potential to reduce risk by reduction of pathogen load but vary in efficacy and cost (Taylor et al. 1999, Nichols & Lamirande 2000, Taylor 2001, Parker et al. 2002, Woodhams et al. 2003, Garner et al. 2009a, Chatfield & Richards-Zawacki 2011).
Reduce risk of transfer of <i>Bd</i> via release of infected amphibians	<i>Bd</i> -testing of amphibians before release from captive breeding programs	Strict adherence to quarantine and health screening protocols before translocation and release of amphibians into the wild is a priority, and the expense of a series of PCR testing is warranted (Cunningham et al. 2001).
	Educate stakeholders and general public about <i>Bd</i> risks and relevant legislation	Much of the general population is unaware of <i>Bd</i> and the risks that it poses to amphibian populations. Pet owners, schools and others may also be unaware of existing legislation regarding the release of non-native amphibian species. Collection and re-release of native species after collection from the wild, as is commonly performed by schools, which could be targeted to promote returning amphibians to the same pond from which spawn was collected to minimise human-assisted spread.
	Refine and supplement existing guidelines on sale of surplus amphibians from laboratories and zoos	Appendix 4 to the Secretary of State's Standards of Modern Zoo Practice states that surplus stock bred in a zoo may be sold as pets to the general public, although a licence may be required from the local authority under the Pet Animals Act 1951. No such legislation could be found to apply to research laboratory stock. Banning invasive native species from this pathway and applying quarantine and testing restrictions is appropriate.
Reduce risk of transfer of <i>Bd</i> via release of contaminated fomites	Stakeholders to adopt standard disinfection protocols before release of waste water from captive collections	This could be based on existing protocols 1 (Lynch 2001) and is highly likely to reduce risk at a low cost. Transferring responsibility to stakeholders via codes of conduct and self-regulating guidelines is preferable over creating new legislation.
	Prevent release of <i>Bd</i> from research laboratories by sterilization or disinfection of experimental equipment and cultures	Optimal disinfectants are highly likely to reduce risk at a low cost (Webb et al. 2007). Transferring responsibility to stakeholders via codes of conduct and self-regulating guidelines is preferable over creating new legislation
	Disinfection of equipment and clothing that have contacted water in known and suspected infection sites	Disinfection protocols to prevent transmission from infected to uninfected field sites are available (Johnson et al. 2003, Speare et al. 2004) This requires training of field workers and education of the general public and hikers as it must be performed thoroughly to reduce risk.

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