Risk Assessment on the American bullfrog and the fungus *Batrachochytrium dendrobatidis*

REPTIELEN AMFIBIEËN VISSEN ONDERZOEK NEDERLAND

RAVON

Risk Assessment on the American bullfrog and the fungus *Batrachochytrium dendrobatidis*.

A report by RAVON
for Invasive Alien Species Team (TIE); Ministry of Agriculture, Nature and Food Quality

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CONTENTS

SAMENVATTING

Zowel op wereldwijd, Europees en nationaal niveau hebben amfibieën te maken met grote bedreigingen als het verlies aan kwaliteit en oppervlak van hun leefgebied, als ook van de introductie van invasieve soorten als de Amerikaanse brulkikker (*Rana catesbeiana* or *Lithobates catesbeianus*) en de schimmel (*Batrachochytrium dendrobatidis*) die de ziekte chytridiomycose kan veroorzaken.

Het Team Invasieve Exoten (TIE) van het Ministerie van Landbouw, Natuur en Voedselkwaliteit erkent de potentiële gevaren van zowel de schimmel *B. dendrobatidis* als van de Amerikaanse brulkikker. Om deze reden heeft zij RAVON gevraagd een Plaag Risico Analyse (PRA) op te stellen voor beide soorten.

Voor zowel de brulkikker als *B. dendrobatidis* zijn het risico van binnenkomst, het risico van vestiging en de dispersie capaciteit beoordeeld. Ook is gekeken naar risico gebieden en is de impact van de soort op de biodiversiteit beoordeeld. Al deze overwegingen leiden tot een uiteindelijk oordeel over het risico dat de soorten vormen en gebaseerd op dit oordeel kan een management strategie worden bepaald.

Voor de brulkikker is het risico van binnenkomst en vestiging, alsook de dispersie capaciteit en de ecologische en economische impact groot. Hiermee komt de soort in de hoogste categorie van 'invasiviteit' en voor deze groep geldt dat vestiging ten alle tijden voorkomen dient te worden. Wanneer een enkel individu wordt waargenomen, dient deze direct geëlimineerd te worden. Een 'early warning' systeem moet worden opgezet en een goed uitgewerkt plan moet klaar liggen zodat direct actie kan worden ondernomen wanneer deze soort Nederland binnenkomt.

Het risico van binnenkomst, vestiging en de dispersie capaciteit van *B. dendrobatidis* zijn allen groot. Afhankelijk van het feit of het om gehouden dieren in collecties gaat of om wilde dieren, wordt de ecologische, sociale en economische impact verschillend beoordeeld. Dieren in gevangenschap kunnen afdoende worden genezen door medicatie. De impact van het voorkomen van chytridiomycose onder gehouden dieren is om die reden 'matig'. De ecologische en economische impact is op vrijlevende dieren echter wel groot. In 2009 is over geheel Nederland in bijna alle soorten de schimmel aangetroffen, zowel in vrijlevende als in gehouden dieren. Het succesvol beheersen van deze ziekte behoeft een effectieve methode om de schimmel op grote schaal te detecteren. Nader onderzoek naar o.a. de virulentie van de schimmel die in Nederland is aangetroffen, naar de effecten van een besmetting van de schimmel op individuen (in verschillende levensstadia) en op populatie niveau zouden moeten worden uitgevoerd.

SUMMARY

Both at global, European and national level, amphibian species face rapid declines. Losses result from the loss and degradation of habitat, but also from the introduction if exotic species like the American bullfrog (*Rana catesbeiana* or *Lithobates catesbeianus*) and from the emerging infectious disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*.

Team Invasive Species (TIE; Ministry of Agriculture, Nature and Food Quality) acknowledges the potential risk of *B. dendrobatidis* and the American bullfrog as alien invasive species to Dutch amphibian biodiversity and has asked RAVON (Reptile, Amphibian and Fish Research the Netherlands, Nijmegen) to assess the risk of both threats.

For both the bullfrog and the fungus, the risk of invasion, the risk of settlement, the dispersal capacity of the species and the areas that are at high risk of a invasion are assessed as well as the species' supposed impact on biodiversity are assessed. These considerations lead to a final risk assessment upon which a management strategy can be formulated.

For the bullfrog, the risk of invasion, the risk of settlement, the dispersal capacity and the ecological and economic impact are assessed to be high. For this category of species, settlement should be prevented at all times and upon the earliest detection of only a single individual, eradication should be the standard. Especially with species such as the bullfrog, that has the ability to become invasive from only a small number of founder animals. An 'early warning' system should be set up, as well as a elaborate plan of action for when this species sets foot on Dutch soil.

For *B. dendrobatidis*, the risk of invasion, the risk of settlement and the dispersal capacity all pose large threats. The ecological, social and economic impact depends whether the disease occurs in kept amphibians or in free living amphibians. Since amphibians in collections can be treated with medication, these effects are assessed to be moderate, however, the ecological and economic impact of mass mortalities among free living amphibians are high. In 2009 *B. dendrobatidis* was found in nearly all provinces and in the majority of native species and also in kept amphibians. Successful management of the disease will require effective sampling regimes and detection assays. Further studies on e.g. the virulence of the *B. dendrobatidis* strains, on the effects of *B. dendrobatidis* infection on individual survival of native amphibians at different life stages and on the effects at population level should be conducted.

PREFACE

Current study was conducted by RAVON and was funded by Invasive Alien Species Team (ir. J.W. Lammers, dr. T.M. van der Have), Ministry of Agriculture, Nature and Food Quality. It was carried out in close cooperation with the Faculty of Veterinary Medicine Ghent University, Laboratory of Veterinary Bacteriology and Mycology & Division of Poultry, Exotic Companion and Laboratory Animals Department of Pathology, Bacteriology and Avian Diseases (prof. F. Pasmans), the Institute of Zoology (IOZ, dr. T. W. J. Garner), London and with Dr. Matthew Fisher (Imperial College, Faculty of Medicine, Department of Infectious Disease Epidemiology (London), the Central Bureau of Fungus Cultures (dr. A. de Cock), Radboud University and the amphibian department 'Hyla' of Natuurpunt, a Flemish NGO.

1 INTRODUCTION

Globally, amphibian species face rapid declines and extinctions: 32.5% of 5743 described species are threatened, with at least 9, and perhaps 122, becoming extinct since 1980 (Stuart *et al.,* 2004). Losses result from familiar threats (land-use change, commercial overexploitation) and from the introduction of exotic species as well as from the emerging infectious disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*. Also at a national level amphibians face the above threats. Fifty percent of our native amphibians is listed on the national Red List (Van Delft *et al.,* 2007).

The American bullfrog (*Rana catesbeiana*) is listed on the list of '100 of the world's worst invasive alien species' (Lowe *et al*., 2000) and lives at close distance from the Dutch border in Belgium, where several large reproducing populations occur (Jooris, 2002a; 2002b). In Spitzen – van der Sluijs & Zollinger (2010a) more information on the American bullfrog can be found. Chytridiomycosis is causing amphibian mass mortality and population declines worldwide (Berger *et al*., 1998; Bosch *et al*., 2001; Rachowicz *et al*., 2006). The causative agent, *B. dendrobatidis*, presents low host specificity (Daszak *et al*., 2003). *B. dendrobatidis* seems to disrupt the cutaneous function, and because the skin is critical in maintaining amphibian homeostasis, this may be the mechanism through which *Bd* produces morbidity and mortality across a wide range of amphibian taxa (Voyles *et al*., 2009). Recently, *B. dendrobatidis* has been found in the majority of native amphibians and also in captive amphibians in zoos, with private owners and in laboratories in the Netherlands and in Belgium (Spitzen – van der Sluijs & Pasmans, in prep.; Spitzen – van der Sluijs *et al*., 2010a; 2010b).

Invasive Alien Species Team (Ministry of Agriculture, Nature and Food Quality) acknowledges the potential risk of *B. dendrobatidis* and the American bullfrog as alien invasive species to Dutch amphibian biodiversity and has asked RAVON (Reptile, Amphibian and Fish Research the Netherlands, Nijmegen) to assess the risk of both threats.

Both threats are discussed in this Risk Assessment. In the framework of this study, a scientific report was published (Spitzen – van der Sluijs *et al.,* 2010b), as well as two literature studies (Spitzen – van der Sluijs & Zollinger, 2010a; 2010b) and two scientific manuscripts (Spitzen – van der Sluijs & Pasmans, in prep; Spitzen – van der Sluijs *et al*., 2010a). All the above manuscripts form the base for this Risk Assessment and are in this report referred to regularly in this report.

2 METHODS

Multiple factors determine the invasiveness of a species. Depending upon the risk of invasion, a management strategy should be developed. Using a step-by-step approach, the risk of invasion, the risk of settlement, the dispersal capacity of the species and the areas that are at high risk of a invasion are assessed as well as the species' supposed impact on biodiversity. These considerations lead to a final risk assessment upon which a management strategy can be formulated. All the above aspects are summarised by defining them into risk categories: nil – small – moderate – large –high.

2.1 Risk Assessment

Risk of invasion

The risk of invasion is determined by the number and the size of pathways (routes of introduction) that are possible for the entrance of the Netherlands, as well as the likelihood of occurrence.

Risk of settlement

Determining the risk of (definite) settlement involves an analysis of the presence of the necessary habitat and abiotic requirements in relation to species' characteristics such as reproductive strategy, adaptive abilities and propagule pressure.

Dispersal capacity

The invasiveness of a species is also determined by the dispersal capacity of a species, that is also depending on human activities and human alterations of the landscape that may facilitate range enlargement.

Risk areas

Based on the above it will be possible to pinpoint specific areas that are at (high) risk for invasion.

Impact

Looking at areas in which the American bullfrog and the *B. dendrobatidis* fungus are currently present as invasive species, it is possible to estimate the ecological and economic effects of their introduction and extrapolate this to the situation in the Netherlands.

Final Risk Assessment

The final judgement on the risk of a species' entrance is based on the following flow chart (figure 1) in which all of the above points can be answered with either 'yes' or 'no', leading to the final risk category, ranging from 1 (no concern) to 6 (invasive species).

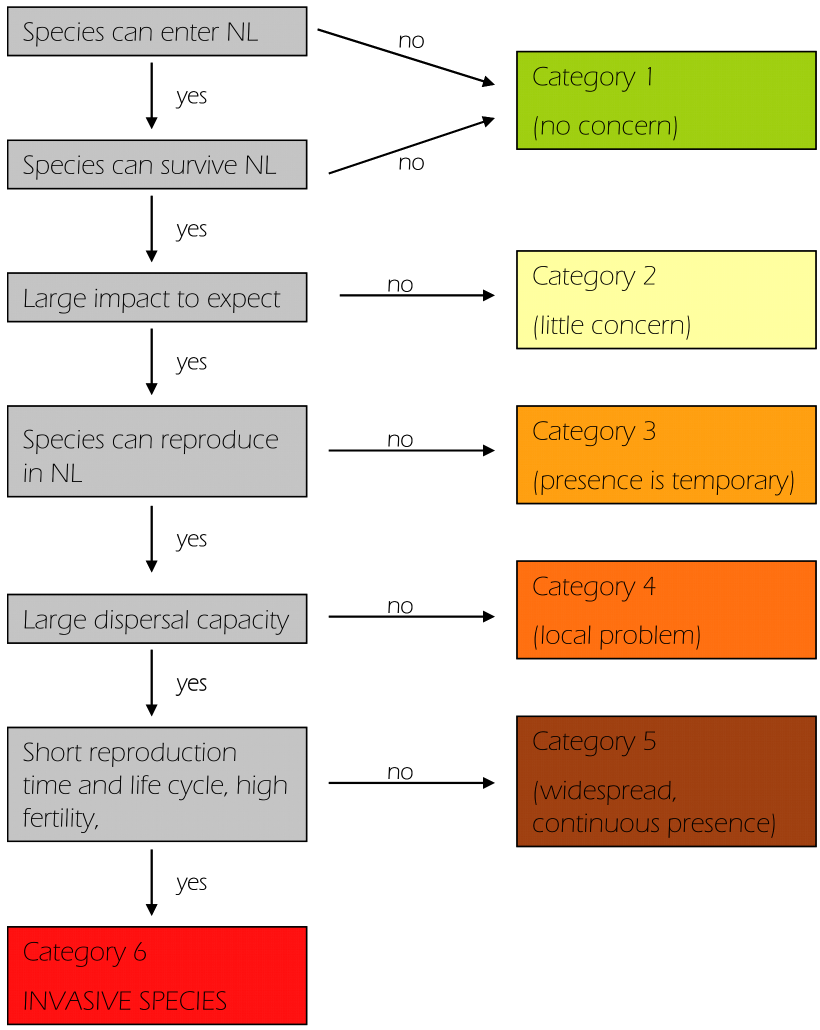


Figure 1. Flow chart as a tool for the assessment of the invasiveness of an exotic species.

2.2 Risk management

Based on the final judgement of the risk analysis (category 1 – 6), measures are proposed to manage the expected problems upon invasion (table 1). For the first category, no management actions are required, since the species won't enter the Netherlands or is unable to settle. Monitoring the distribution and population developments of category 2 and 3 species is necessary to keep a finger on the pulse in case of altering (environmental) conditions that may facilitate settlement and reproduction of the species. When the problem is still local (category 4), eradication should be strived for, especially for species with a low propagule pressure. For species with a high dispersal capacity and the possibility of permanent settlement it should be studied if it is possible to control/eradicate the species at an early stage of invasion, since the larger a species distribution range, the more complex (and expensive) the management of the problem will be. For category 6 species, the true invasive species, settlement should be prevented at all times, since control or eradication will be impossible at later stages of its introduction.

Table 1. Proposed management actions (last column) in relation to the final risk assessment (3rd column) of an invasive species based on the risk of invasion, risk of settlement, dispersal capacity and economic, ecological and social impact upon arrival in a naïve area.

Category		Risk assessment	Proposed management
1	Species not in the NL / unable to settle	No concern	No measurements
2	No impact to be expected	Little concern	Close monitoring
3	Settlement possible, no reproduction	Temporary present	Close monitoring
4	Settlement possible, limited distribution	Local problem	Eradication in early stage
5	Settlement possible, high dispersal capacity	Widespread, continuous presence	Control or extirpation still possible?
6	Settlement possible, high dispersal capacity, short lifecycle and high fertility	Invasive species	Prevent settlement. Control or eradication impossible

3 RISK ASSESSMENT AMERICAN BULLFROG

3.1 Risk of invasion

Bullfrogs can follow two (intentional) routes to enter the Netherlands, either by autonomous distribution from Flanders or facilitated by humans as escaped individuals from the pet trade (or by illegal introduction). Both proposed routes of invasion are likely options. Unintentional introductions, the hitch-hiking in cargos, seems unlikely.

In Belgium the bullfrog seems to have gained a firm foothold both in Flanders and in Walloon regions (figure 2; Jooris, 2002a). Especially in the valley of the river Grote Nete (location 1, figure 2; Jooris, 2002b) the presence of many ponds created for recreational fishing is appreciated by the bullfrogs. The possibility for bullfrogs to expand in these areas (via the river and its contributories, ditches and ponds) poses threats for more vulnerable areas with native amphibians. The bullfrogs are very close to the border of the Netherlands (location 2 - 4, figure 2), and it is very likely that they have entered the Netherlands already or will do so in a short period of time. In 2008 a bullfrog was heard (bullfrogs make a distinctive sound when entering the water upon disturbance) entering the water in the river Mark (location 3, figure 2), which enters the Netherlands south of Breda in the province of Noord-Brabant (Spitzen – van der Sluijs, 2008 pers. obs.), close to the Dutch border (< 2km). Veenvliet (2009) also expects colonisation to come from the Flemish bullfrogs.

From 1997 onwards, it is forbidden to introduce the American bullfrog in Europe. However, in a recent issue of the Belgium magazine 'Aquarium wereld', tips are provided for keeping this species in captivity (Rybnicfan, 2009). The sightings of individuals in the Netherlands in 2002, 2003, 2006 and in 2009 indicate that also in the Netherlands, people are tempted to keep this impressive amphibian either in indoor or outdoor aquaria.

Upon arrival in the Netherlands, bullfrogs are, considering their adaptive biology, likely to find suitable habitat for survival and reproduction easily (see also paragraph 3.2). The species has been known to reproduce successfully in a garden pond in Breda (Stumpel, 1991; 1992) and several other sightings (Veenvliet, 2009) confirm this. A model developed by Ficetola *et al.* (2007a) predicts a medium suitability for the largest part of the Netherlands for the invasion of the American bullfrog. Nonetheless, the southern parts of the Netherlands are at higher risk.

Risk of invasion is **high**

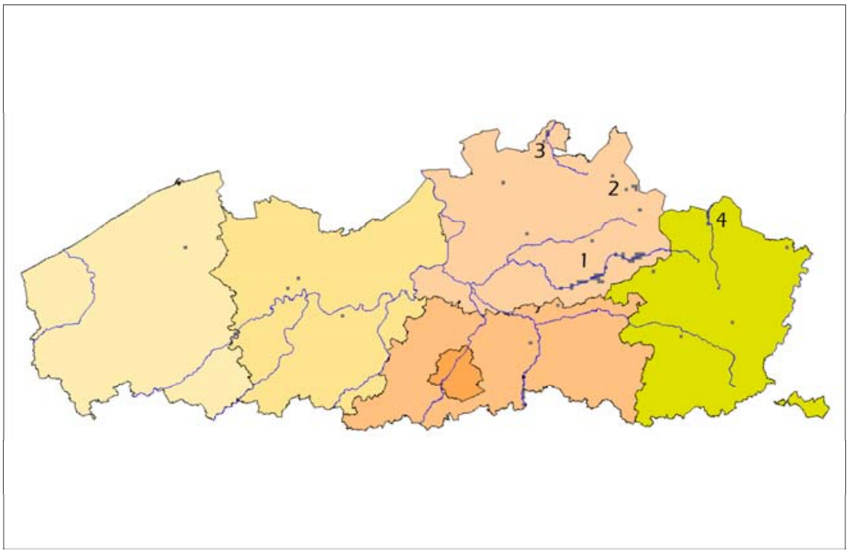


Figure 2. The black dots resemble square kilometers where bullfrogs have been sighted in Flanders from 1995 till now. From: http://www.hylawerkgroep.be/index.php?id=94#verspreiding.
Accessed 11 Jan. 2010
1: Vallei van de Grote Nete (Be)
2: Area around Arendonk (Be) and Reusel (NL)
3: Area around river Mark, near Meerle (Be)
4: Area near 'Het Hageven' (Be)

3.2 Risk of settlement

The American bullfrog is considered a 'warm-adapted species' (Bachmann, 1969), since below 15°C, adults are generally inactive, eggs will not hatch, and larvae will not develop (Viparina and Just, 1975; Harding, 1977), although Mudde (1992) gives a broader thermal range for bullfrog activity: 8 – 37 °C. Developmental thermal limits are considered to have a greater limitative character than the upper and lower thermal limits of adult anurans (Van der Have, 2002) however, winter survival is possible in the Netherlands (Spitzen – van der Sluijs & Zollinger, 2010a; Veenvliet, 2009). More on the favoured temperature range by bullfrogs can be found in paragraph 3.4.4. in Spitzen – van der Sluijs & Zollinger (2010a).

Mudde (1992) does not think that the American bullfrog can settle permanently in The Netherlands, due to the unpredictable winter temperatures (cold, warm, cold) and the relative high temperature needed for successful reproduction. Veenvliet (1996) agrees with this and poses the proposition that a geographic form was introduced that was not resistant to our climate. These insights seem to be out of date, as in Belgium just on the other side of the border, the bullfrog can maintain itself extremely well. Our climate is not an inhibiting factor, which is confirmed by Stumpel (1992) who describes the population at Breda as reproducing very well under our climatic conditions. In a large garden pond five larvae from Belgium were introduced in 1986 and in 1990 160 larvae in several life-stages were caught.

Important factors for successful acclimatisation of bullfrogs in Europe appear to be the presence of several ponds or lakes at close distance to each other and nutrient-rich water with some aquatic vegetation. It is also in these kinds of waters that bullfrogs can be found in Belgium. These are mainly basins, intended for recreational fishing by private owners. These basins are extremely rich in nutrients and have a well developed vegetation on the banks, but lack aquatic vegetation. Contrary to most native amphibians, the presence of (alien) fish species does not seem to have a negative impact on its presence (Veenvliet and Veenvliet, 2002). Ficetola *et al.* (2007a) found that bullfrogs can take advantage from human modifications of land and from the increase of permanent ponds created (Rubbo and Kiesecker, 2005; Maret *et al.*, 2006).

Currently, the bullfrogs in Belgium are restricted to eutrophic ponds on sandy soils and in valleys. Deep and cold water can limit the growth of the larvae increasing the predation rate. This could have influenced the current distribution of the bullfrog in Belgium. In Belgium and in the Netherlands, the tadpoles of bullfrogs require more than one year for metamorphosis, and overwinter in water (Ryan, 1953; Willis *et al.*, 1956; Govindarajulu *et al.*, 2006), so ponds with permanent water are a prerequisite for their longterm survival.

Invasive amphibians are favoured over the native species in sites with hydrological alteration, landscape-level habitat fragmentation and degradation of the habitat (D'Amore *et al.*, 2010). Human altered sites are plentiful in the Netherlands and the American bullfrog will not have problems in finding suitable habitat, especially since bullfrogs are known to survive in garden ponds (Veenvliet, 2009).

Next to suitable habitat, food will not be a limiting factor for settlement of the bullfrog. Young and subadult individuals mainly eat invertebrate prey, but adult bullfrogs eat anything they can manage, from invertebrates and amphibians to fish, small rodents, reptiles and birds (Corse and Metter, 1980; Albertini and Lanza, 1987; Beringer and Johnson, 1995).

Propagule pressure

The bullfrog has the capability to invade an ecosystem from only a very small number of founders (Ficetola *et al.,* 2008). Most non-native populations derive from less than six females. Once bullfrogs colonize a habitat they are difficult to remove and their effects on aquatic systems are long-lasting (Bury and Luckenbach, 1976; Todd, 2001).

The large native range of the bullfrog is indicative of the adaptability and success of the species outside its normal range (Adams and Pearl, 2007). In Kraus (2009) 11 pages list the introductions of bullfrogs per country, the pathways, the date and the successfulness. In a total of 48 countries (excluding 21 USA states) bullfrogs were intentionally or accidentally introduced as food item, for biocontrol, in pet trade or as releases from laboratories. In 30 countries (and in 19 USA states) introduction was successful. The earliest date of introduction goes back to late 1800s (in France) and the most recent was in 2002 (also in France).

In Europe, at least 25 independent introductions of the bullfrog occurred in eight countries (Lanza and Ferri, 1997; Ficetola et al., 2007b).). Italy was the first country where successful introductions occurred and this country suffers the largest number of introductions (Ficetola et al., 2007b).

Current spread of bullfrog in the Netherlands

From an Amsterdam garden pond a reliable sighting in 2002 of a single bullfrog exists. In 2009 it was reported that in 2006 a bullfrog was captured in 'het Wormdal' in the province Limburg. In the period 1971 – 1995 three times successful reproduction has been registered. At least up till 2003 reproducing animals were present in two open-air terrariums in the province Limburg. Even though these are not free ranging specimens, it means that the bullfrog can survive in The Netherlands (Veenvliet, 2009). This year (2009), a single adult bullfrog male was reported from Sint-Oedenrode near the valley of the Belgian-Netherlands small river Dommel (province of Noord-Brabant; figure 3). This animal was shot, but not collected. RAVON will monitor the location the coming years for the presence of more bullfrogs. The origin of this specimen is unknown.

2009

Rooise vogelaar spot een brulkikker

door **Lukas van der Storm**

SINT-OEDENRODE – Het is de kroon op het werk van de ware natuurliefhebber: een zeldzame waarneming in je eigen voor- of achtertuin. Toch was Henk Sierdsema uit Sint-Oedenrode niet onverdeeld blij met de Amerikaanse brulkikker die hij voor zijn huis aantrof. Het dier kan namelijk een ware plaag zijn voor inheemse collega-kikkers.
Sierdsema deed zijn ontdekking – hoe kan het ook anders – omdat hij al een tijdje vreemde geluiden hoorde vanuit zijn huis aan de Hildewarelaan. „Het kwam vanuit het water, we konden het hier door de muren heen horen."
Sierdsema – werkzaam bij Sovon vogelonderzoek Nederland – trof de kikker aan in de sloot voor zijn huis en schakelde Sovons reptielen- en amfibieënbroertje Ravon in. „Het bleek inderdaad om een brulkikker te gaan. Hopelijk zit er maar één, want in België zorgen ze voor flink wat problemen."
Volgens Jeroen van Delft van Ravon brengt de brulkikker de gewone Nederlandse kikkers in gevaar. „Ze eten niet alleen kleine kikkers maar kunnen ook een schimmel overbrengen die voor inheemse kikkers gevaarlijk is. We hebben het dier daarom gevangen. Andere kikkers zijn onderzocht op de schimmel."
De brulkikker kwam dertig jaar geleden naar Nederland als populaire vulling voor de tuinvijver. „Daarvoor bleek hij niet geschikt, waarna er kolonies in de vrije natuur ontstonden. Die zijn in Nederland weer verdwenen. Er zijn wel af en toe meldingen, maar die kunnen zelden – zoals nu – worden geverifieerd."

De brulkikker onderscheidt zich door een platte rug. foto **H. Sierdsema**

Figure 3. Newspaper article on the most recent sighting of a bullfrog in the Netherlands, St. Oedenrode, 2009.

Not all sightings of bullfrogs are mapped in figure 4 (from: Veenvliet, 2009). Several locations are not mentioned, such as historical sightings by Van Diepenbeek (pers. comm.) in Veghel (eastern part province Noord-Brabant) in 1991 and by Van Rijsewijk in June 1997 (pers. comm.) in the

'Wilhelminapark' in Tilburg, suffocated in a duckling (figure 5; map from www.telmee.nl; accessed 26March2010).

The presence of other (amphibian) species do not form an obstruction for the colonisation of new areas. Population invasiveness (Ficetola *et al.*, 2007b) is positively related to the number of amphibian species recorded in the community. The larvae of the bullfrog are highly competitive with native amphibian larvae, inhibiting their growth and development (Jooris, 2005).

The overall conclusion is that the risk of settlement is **high**.

Figure 4. All sightings of American bullfrogs in the Netherlands from: Veenvliet (2009).
■ *1971 - 1995*
● *1996 - 2007*

Figure 5. Additional validated sightings of the American bullfrog in 1991 and in 1997 (green squares). From: www.telmee.nl

3.3 Dispersal capacities

Post-metamorphic stages are capable of dispersing long distances and are adapted to colonize new sites (>1200 m; Willis *et al.*, 1956). In Smith and Green (2005) an overview is given of the dispersal distances of many amphibian species, among which the bullfrog. Three studies are quoted that give maximum distances recorded of 1600, 914 and 966 meter, tracked with mark-recapture studies by adult individuals (Ingram and Raney, 1943; Raney, 1940; Willis *et al.*, 1956).

Obviously, the spread of bullfrogs can be facilitated by human activities. It is known that bullfrogs are facilitated by human altered landscapes with permanent water holding ponds. Besides these facilitations in habitat requirements, intentional direct transport poses a threat as well. Nonetheless, it must be noted that this does not seem to be a large contributor to the spread of the species. Bullfrogs are not highly attractive species for garden ponds or indoor aquaria and it is expected that this route of dispersal is not likely to be the most important way of dispersal.

Reducing the natural spreading of the species over the country, once it has set foot on our side of the border, is an uncontrollable factor due to its possibility to survive in artificial landscapes. In Flanders (Belgium) an eradication project is started in 2010 to eliminate bullfrogs living at close distance from our border (pers. comm. G. Louette & R. Jooris). This action will hopefully increase the time bullfrogs will enter the Netherlands from that side. Stimulating this culling of bullfrogs in Flanders is in both Belgium and the Netherlands interest.

The overall conclusion is that the risk of fast dispersal through the Netherlands is **high**

3.4 Risk areas

Based on the permanent presence of bullfrogs close to the Dutch border (paragraph 3.1) and the analysis of Ron (2005), the main focus for the Netherlands should be firstly aimed at the southern provinces Zeeland, Noord-Brabant and Limburg, all adjacent to the Belgian border. Additionally, risk areas are the sites where recently the presence of bullfrogs has been reported, such as St. Oedenrode.

3.5 Impact

The American bullfrog is listed on the list of '100 of the world's worst invasive alien species' (Lowe *et al.*, 2000). These species were selected using two criteria: their serious impact on biological diversity and/or human activities, and their illustration of important issues of biological invasion. Bullfrogs are considered to be among the most harmful alien invasive species around the world (Lowe *et al.,* 2000). Plans to halt their expansion and/or new introductions are a priority for amphibian conservation.

Bullfrogs can have negative impacts on native amphibian populations and on numerous fresh water taxa (see also Spitzen – van der Sluijs & Zollinger, 2010a; § 3.4.6 and § 4.1). The larvae of the bullfrog are highly competitive with native amphibian larvae, inhibiting their growth and development (Jooris, 2005). In North America its introduction to new areas has, in combination with habitat changes and the introduction of non-native fish (Adams *et al.* 2003), been linked with declines in other amphibian species. In California, examples include declines of red-legged frog *R. aurora* and yellow-legged frog *R. boylii* (Moyle, 1973). Bullfrog tadpoles reduced the percentage survival and body mass at metamorphosis of both species (Kupferberg, 1997; Kiesecker and Blaustein, 1998). Bullfrogs have also been linked with declines of the plains leopard frog *R. blairi* and the northern leopard frog *R. pipiens* in Colorado, and the spotted frog *R. pretiosa* in the Pacific north-west of America (Hammerson, 1982).

Since bullfrogs are vectors for *B. dendrobatidis* (Spitzen – van der Sluijs & Zollinger, 2010a; § 3.5), disease, predation and competition all influence native species after a bullfrog introduction. Until now, no European evidence is presented that, due to the presence of the American bullfrog, native amphibian populations decrease in population size. However, the predation pressure on native species can not be underestimated (Jooris, 2005).

The costs of bullfrog eradication will be limited when executed in an early stage, but will increase later on. Nonetheless, the economic costs of the loss of native amphibians and/or restoring damaged ecosystems are always higher.

Expected ecological and economic damage in the Netherlands
The expected ecological impact of the bullfrog in the Netherlands in especially the risk areas is substantial, since bullfrogs are known to prey on endangered species (table 3.1 in Kraus, 2009). In the province of Limburg, the only remaining natural, and therefore threatened populations of midwife toads (*Alytes obstetricans*) and yellow bellied toads (*Bombina variegata*), will not be able to cope with direct predation, indirect competition and habitat alteration by bullfrogs. Upon further spread of the bullfrog over the Netherlands, the alteration of ecosystems will be detrimental to not only other amphibian species, but will also influence other taxa. The negative influences will therefore be considerable.

Economically, all money spent on habitat restoration in earlier years will be in vain and the financial input required for the elimination of settled bullfrog populations will be substantial (see also chapter 4 and Spitzen – van der Sluijs & Zollinger, 2010a; § 5.2).

Overall, the ecological an economic impact of permanent bullfrog presence is **high**

3.6 Final Risk Assessment

The American bullfrog is able to reach the Netherlands within a short period of time, and is able to survive and reproduce. The species has a large dispersal capacity, a high fecundity and a strong competition advantage over native species, therefore a large impact on native species is expected and the final assessment places the bullfrog in category 6 (figure 1): invasive species. It's expected impact is summarised in table 2. Branquart *et al* (2007) also give the bullfrog the highest 'invasion' score for Belgium, using the ISEIA-protocol (http://ias.biodiversity.be/ias/documents/ISEIA_protocol.pdf).

Table 2. Summary of the final judgements of each assessment category on the invasiveness of the American bullfrog

assessment categories	impact
risk of invasion	high
risk of settlement	high
dispersal capacity	high
ecological and economic impact	high
Final assessment	hgh

4 RISK MANAGEMENT AMERICAN BULLFROG

For category 6 species, settlement should be prevented at all times and upon the earliest detection of only a single individual, eradication should be the standard. Especially with species such as the bullfrog, that has the ability to become invasive from only a small number of founder animals.

Adams and Pearl (2007) suggest two management options: 1) direct removal and 2) habitat manipulation. Depending on location and habitat, different management techniques are feasible. Draining livestock grazing ponds is possible, but draining larger wetlands is often too detrimental for other organisms. Shooting is an option in those cases (Schwalbe and Rosen 1988; Rosen and Schwalbe 1995). In their model simulations Doubledee *et al.* (2003) calculated that the combined management strategy of pond draining and the shooting of adults (once a year), will drive populations extinct within 10 years. Combining these management techniques was much more successful than either strategy independently. Govindarajulu *et al.*, (2005) state that killing metamorphs in fall is the most effective method of decreasing bullfrog population growth. The partial removal of tadpoles may lead to higher tadpole survival and development rates and higher postmetamorphic survival due to decreased density-dependent competition. Removal of adults leads to higher survival of early metamorphic stages through reduced cannibalism. More information on the possible management strategies can be found in Spitzen – van der Sluijs & Zollinger, 2010a; chapter 5.

An 'early warning system' should be set up to make sure that bullfrogs are observed in an early stage. Then, an exhaustive plan of action should be put into action based on the information in this report and in Spitzen – van der Sluijs & Zollinger (2010a).

4.1 Cases

Successful eradication was performed in at least three cases in Europe, while in 11 further cases bullfrogs disappeared after the introduction (Ficetola *et al.*, 2007b). Two of the successful eradications (UK and Germany) included killing individuals (adults and tadpoles) and complete drainage of ponds where the population was breeding. In the third successful eradication (Germany) a complete fencing of the breeding pond was performed in addition to the killing of individuals (Thiesmeier *et al.*, 1994). The three successful attempts have been performed at early stages of invasion and by means of strenuous destruction or fencing of all wetland breeding sites (Ficetola *et al.*, 2007b).

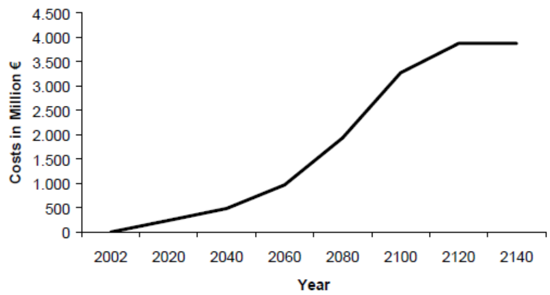


Figure 6. Costs of eradication of the bullfrog, when starting in the future, from: Reinhardt et al (2003).

The costs of bullfrog eradication will be limited when executed in an early stage, but will increase later on. Nonetheless, the economic costs of the loss of native amphibians and/or restoring damaged ecosystems are always higher.

In Germany, five infested ponds were pumped out twice, with the help of

20 volunteers and the local fire department. Adults bullfrogs and tadpoles were removed. In addition, these ponds were electronically fished twice (Weizmann, 2002). Costs for these measures were: 20 volunteers, working occasionally over the course of a year (roughly the equivalent of one full-time employee, hence € 50,000,-). Costs to pump out and electrofish was € 500,- and € 1,200,- per day, respectively. This predicts an annual cost of € 53,000,- per pond per year (Reinhardt *et al.,* 2003). Reinhardt *et al.* (2003) calculated that eradicating bullfrogs in Germany would grow exponentially (figure 6) with time. Starting control management early upon establishment is most cost efficient.

Direct removal: Banks *et al.* (2000) installed fences around the main ponds to limit dispersal and used lamps to collect adult frogs at dusk. They then drained the ponds and excavated the sediment to remove remaining frogs and larvae. This effort apparently did not result in complete eradication: limited breeding was detected the following summer, and post metamorphic bullfrogs were found in the vicinity two years after management. Another direct removal effort that has been partially documented in the literature is in ponds that are relatively isolated in a desert landscape in Arizona, USA (Schwalbe and Rosen, 1988; Rosen and Schwalbe, 1995). They used funnel traps, gigs, guns, and hand capture to remove bullfrogs annually. Reductions in bullfrog densities were said to be small and short-lived.

Habitat management/manipulation: Maret *et al.* (2006) found that the drainage of ponds could be used to eliminate bullfrogs in some livestock watering ponds. Pond drying was also effective for local elimination of non-indigenous fish (Maret *et al.*, 2006) as was also successfully done in a Dutch moorland pool to eradicate pumkinseed (Bosman, 2004). Non-native fish such as bluegill sunfish can interact with bullfrogs in ways detrimental to indigenous amphibians (Kiesecker and Blaustein, 1998; Adams *et al.*, 2003).

In Britain a large eradication project was undertaken in 1999 in an attempt to eradicate the population of American bullfrogs that was still fairly localised. The ponds were surrounded by a 1 meter-high, plastic frog-proof fence and visited at dusk to capture bullfrogs. Captured individuals were anaesthetised using Benzocaine and promptly dispatched. Terrestrial activity of froglets was greatest on mild (> 10°C) damp evenings. They congregated around the inner perimeter of the frog-proof fence and were easy capture by hand, with 477 animals caught in one night. Bottle traps placed in the pond, and carpet sections placed on land as refugia however caught relatively few frogs. The ponds were drained in December 1999 in order to eliminate the remaining tadpoles and allow a significant number of the remaining frogs to be captured by hand in the mud. The frogs did not accumulate in the central hollow, but remained in burrows in the mud throughout the pond, among stands of emergent vegetation, under collapsed mats of green algae and water plants lying over deep silt and litter. The bullfrogs were most frequent in deeper 'mudslide' areas of silt below steeper rocky banks. Finally, the ponds silt was excavated, buried and covered in compacted soil. Small numbers of bullfrogs were found hibernating on land around the margin of the enclosure fence. By the end of 1999, 4,744 tadpoles, 2,269 froglets and an adult female bullfrog had been captured (Banks *et al.*, 2000).

When breeding is suspected, ponds may need to be enclosed promptly in a frog-proof fence, the ponds drained (preferably before the tadpoles are able to metamorphose) and any frogs or tadpoles collected. This is most feasible on small water bodies. Should bullfrogs become established in more extensive wetlands, control is likely to be very difficult (Banks *et al.*, 2000).

5 RISK ASSESSMENT BATRACHOCHYTRIUM DENDROBATIDIS

5.1 Risk of invasion

In contrast with the American bullfrog, intentional distribution seems an unlikely pathway for *B. dendrobatidis* to enter the Netherlands. Unintentional distribution by the unnoticed presence of these microorganisms in legally or illegally imported amphibians as scientific and medical material as well as pets (Goka *et al.*, 2009) is a proven pathway. Birds may also distribute the fungus during migration, or when foraging at different sites. Current evidence suggests that the world trade in amphibians is implicated in the emergence of chytridiomycosis. Fisher & Garner (2007) supply strong evidence that the amphibian trade is contributing to the spread of *Bd.* Amphibian trade is driving the emergence of chytridiomycosis by (1) spreading infected animals worldwide, (2) introducing non-native infected animals into naïve populations and (3) amplifying infection of amphibians by co-housing, followed by untreated discharge of infectious zoospores into water supplies. The OIE (Aquatic Animal Health Standards Commission) *ad hoc* Group on Amphibian Diseases agreed that chytridiomycosis causes mortality in amphibians being traded and that infected animals in the international trade are a risk to amphibian populations in the importing country (OIE, 2006).

A second unintentional route is by the natural spread of asymptomatically infected amphibians from surrounding countries. Obviously, the effects of this pathway seem to be smaller (e.g. on a smaller scale) than large scale introductions of infected amphibians, but this route is also less verifiable. Also, dispersal due to well intended conservation actions is possible (Walker *et al.,* 2008). In this latter study, unintendedly infected amphibians from a breeding program were introduced in their native habitat.

Once established, *B. dendrobatidis* may spread as an epidemic wave into uninfected populations (Lips *et al.*, 2006). All native amphibians can be carriers of the fungus, some will be so asymptomatically while others may be faced with difficulties. Upon invasion, the fungus will be able to find hosts easily and is able to survive in our climatic zone (Garner *et al.,* 2005).

Conclusion: the risk of invasion of *Batrachochytrium dendrobatidis* is **high**

5.2 Risk of settlement

In an extensive study (Spitzen – van der Sluijs & Pasmans, in prep.; Spitzen – van der Sluijs *et al.*, 2010b) the spread of *B. dendrobatidis* in the Netherlands was studied. In that study it was found that *B. dendrobatidis* is widespread (figure 7) and present in the majority of native amphibians Spitzen – van der Sluijs & Pasmans, in prep.; Spitzen – van der Sluijs *et al.*, 2010b).

B. dendrobatidis in Europe

B. dendrobatidis is broadly but patchily distributed in the EU (Garner *et al.*, 2005). It has been found in Spain, Italy, Germany, the Netherlands, Belgium, UK, France, Switzerland, Hungary, Denmark and in Portugal (e.g. Garner *et al.*, 2006; Spitzen – van der Sluijs & Pasmans, in prep).

In France, *B. dendrobatidis* was detected in American bullfrogs (Garner *et al.*, 2006). At least 3 species of amphibians (*Alytes obstetricans, Salamandra salamandra* and *Bufo bufo*) are currently experiencing mass mortality due to chytridiomycosis in Spain (Bosch *et al.*, 2001; Bosch & Martinez-Solano, 2006). Furthermore, *B. dendrobatidis* has been detected in *B. calamita, Hyla arborea, Rana iberica, R. perezi, Triturus alpestris* and *T. marmoratus* (http://www.spatialepidemiology.net/bd).

The first record in Italian wild populations of *B. dendrobatidis* has been reported in 2001 in *Bombina pachypus* (Stagni *et al.,* 2004). Furthermore chytridiomycosis has been detected in *Rana latastei* and in American bullfrog populations in the north (Garner *et al.*, 2006) and in *Rana* kl. e*sculenta*/*R. lessonae* populations in Umbria (Simoncelli *et al.*, 2005; Di Rosa *et al.,* 2007) and in the Turin province (Federici *et al*, 2008). Simoncelli *et al.* (2005) did not find mortality in the water frogs, but noted that all infected individuals were from areas with intensive anthropogenic pressure. *B. dendrobatidis* has also been detected in Sardinia in endemic populations of *Euproctus platycephalus* (Bovero *et al.*, 2008).

Figure 7. The blue dots resemble all sites where samples were collected and all red dots represent the sites that tested positive for the presence of B. dendrobatidis *in the Netherlands and Flanders (from: Spitzen – van der Sluijs* et al.*, 2010b).*

In Germany infected *Rana arvalis*, *R. esculenta* and *R. esculenta* synklepton have been found (www.spatialepidemiology.net/bd) and a recent, unpublished study states that *B. dendrobatidis* is widespread in Germany (Kielgast, pers. comm.). In Switzerland, *A. obstetricans* is infected and in Portugal *Pleurodeles waltl.* Scalera and colleagues (Scalera *et al.*, 2008) conducted a survey on the presence of *Bd* in Danish common frogs (*R. temporaria*) and water frogs (*Rana* kl. *esculenta*). In both species the fungus was found, and further studies are necessary to determine the extent of the pathogen in Denmark.

Concluding from the widespread and continuing presence of *B. dendrobatidis* in Western Europe, *B. dendrobatidis* can sustainably survive in this climatic zone with the native amphibian host species. Additionally, the widespread presence of *B. dendrobatidis* in the Netherlands cautiously suggests a historic introduction. This latter still needs to be studied.

Risk of settlement is **high** (the species is already present and possibly settled)

5.3 Dispersal capacities

As mentioned in § 5.1, once established, *B. dendrobatidis* may spread as an epidemic wave travelling up to 100 km per year into uninfected populations (Lips *et al.*, 2006). *B. dendrobatidis* is already occurring widespread in the Netherlands, and has invaded even vulnerable populations (Spitzen – van der Sluijs & Pasmans, in prep).

Dispersal of the fungus is possible via several pathways, either by amphibians and other organisms or by human transfer.

The larval stage of amphibians breeding in permanent waterbodies can last up to several years and as such, they have ample opportunity to become infected with the waterborne zoospores of *B. dendrobatidis*. Furthermore, tadpoles serve as ideal reservoir hosts for *B. dendrobatidis* (Woodhams & Alford, 2005), and can transmit infection to uninfected adults (Rachowicz & Vredenburg, 2004) and so, congregations of tadpoles may become extremely large reservoirs for the fungus, especially the species with long larval stages. They may even increase the likelihood of an outbreak, by releasing zoospores over extending periods (Rachowicz & Vredenburg, 2004). Adult amphibians that act as asymptotic carriers of the fungus, may spread the fungus to naive ponds. This rate of distribution is relatively limited.

B. dendrobatidis can survive up to 7 weeks in sterile lake water and can survive in sterile moist river sand for up to 12 weeks (Johnson & Speare 2003; 2005). Dissemination of the fungus is greatly assisted by flowing water (Kriger & Hero, 2007). Additionally, potential means of translocation may be moist soil and bird feathers. In laboratory studies by Johnson & Speare (2005), *B. dendrobatidis* attached to and grew on sterile feathers and, even after 1 - 3 hours of drying the fungus could even establish new cultures. These pathways: streaming water, birds and the displacement of contaminated soil present a much higher rate of distribution.

Yearly, many live amphibians are imported in the Netherlands. It has proved to be hard to obtain figures on the size of amphibian trade. Customs, Schiphol Airport, the Food and Consumer Product Safety Authority (VWA) and Statistics Netherlands (CBS) could not provide data on the number of amphibians that are imported. Only from the CITES website (Convention in International Trade in Endangered Species; www.cites.org) data could be obtained, but only from species that are on the CITES list (table 3). This information indicates that in the period from 2004 – 2009 at least approximately 6000 individuals were imported, excluding the nearly 10,000 kilo's of amphibian meat. These amphibians are mainly for the pet trade. In the pet trade, the joining of infected amphibians with naïve animals may cause substantial mortality and through intensive trade practices, infection may spread rapidly among collections.

The rate of dispersal of *B. dendrobatidis* zoospores by human activities in the field (polluted dipnets, soiled boots and car tires) is of a much greater magnitude than caused by natural dispersal by amphibians.

Prevention

Prevention of the spread of *B. dendrobatidis* by natural dispersal of amphibians, birds and other organisms is impossible. Prevention of the spread of the fungus between collections of private owners, zoos and museums is possible by the regular screening of the collection on the presence of the fungus, the introduction of quarantine measures and the obligatory screening for the presence of *B. dendrobatidis* in imported animals at the airport. These tests can be done quickly and reliably at the Faculty of Veterinary Medicine of Ghent University. The prevention of the spread of the pathogen by human field activities can be further, but only partly, prevented by obliging fieldworkers dealing with amphibians to take hygienic measurements to prevent infection of naïve sites.

Since, *B. dendrobatidis* is already widely occurring in the Netherlands, the risk of dispersal through the Netherlands is estimated to be **high.**

5.4 Risk areas

Figure 7 illustrates the wide distribution of *B. dendrobatidis* in the Netherlands. Presence seems unrelated to habitat type, species distribution or geographic region (Spitzen – van der Sluijs *et al.,* 2010b). As currently the impact *B. dendrobatidis* infections on Dutch native amphibians is unknown, the whole of the country should be regarded as a risk area. Special emphasis should however go out to those areas inhabiting high risk species, such as the common midwife toad (*Alytes obstetricans*; e.g. Bosch *et al.*, 2001). This species is highly impacted on by the fungus in Spain and in Switzerland. Natural midwife toad populations only occur in the southern part of the province Limburg.

5.5 Impact

Impact ecology

As mentioned in chapter 1, both at global and national level, amphibians face large declines in numbers due to pollution, the deterioration and reduction of suitable habitat as well as due to amphibian diseases. The impact of chytridiomycosis on frogs is the most spectacular loss of vertebrate biodiversity due to disease in recorded history (Skerratt *et al.,* 2007). In Spain, the fire salamander (*Salamandra salamandra*), the midwife toad and the common toad (*Bufo bufo*) face substantial population declines by the disease. Thus it is clear that the impact may be enormous on both common and rare species.

Currently, no substantial population declines have been reported in the Netherlands which can indicate either a recent (effects are yet to come) or a historic (effects on amphibian populations occurred in previous years, or are negligible) introduction. Therefore continuing studies should be executed with haste, since many factors are yet unknown, such as the virulence of the *B. dendrobatidis* strain in the Netherlands, the date of introduction and the possible co-existence of the fungus with amphibian communities. For more information on necessary studies that should be conducted if the true impact on biodiversity is to be assessed, see § 4.1 in Spitzen – van der Sluijs *et al.* (2010b).

Table 3. Number of amphibians imported in the Netherlands from various countries in the period 2004 – 2009. Reported import (the number of animals registered to enter the Netherlands) and export numbers (the number of animals registered to have left their home country) are given. Data obtained from www.cites.org (accessed 23 March 2010).

species	from	reported import	reported export		purpose	source
Allobates						
Allobates femoralis	Guyana	50	50	live	commercial trade	specimens from the wild
Cryptophyllobates						
Cryptophyllobates azureinventris	United States of America	30	30	live	commercial trade	animals born in captivity
Phyllobates						
Phyllobates vittatus	Canada	40	40	live	commercial trade	animals bred in captivity
Phyllobates terribilis	United States of America	50	50	live	commercial trade	animals born in captivity
Mantella						
Mantella betsileo	Madagascar/Canada	1390	1899	live	commercial trade	specimens from the wild/bred in captivity
Mantella aurantiaca	Canada	75	135	live	commercial trade	animals bred in captivity
Mantella laevigata	Canada	12	12	live	commercial trade	animals bred in captivity
Mantella milotympanum	Canada	20	20	live	commercial trade	animals bred in captivity
Mantella pulchra	Madagascar/Canada	115	70	live	commercial trade	specimens from the wild/ bred in captivity
Mantella viridis	Canada	170	195	live	commercial trade	animals bred in captivity
Mantella baroni	Madagascar	0	43	live	commercial trade	specimens from the wild
Mantella crocea	Canada	75	0	live	commercial trade	animals bred in captivity
Mantella nigricans	Madagascar	0	25	live	commercial trade	specimens from the wild
Dendrobates						
Dendrobates auratus	Canada/United States of America	1378	1488	live	commercial trade	animals bred in captivity
Dendrobates pumilio	Canada	65	45	live	commercial trade	animals bred in captivity
Dendrobates azureus	Canada/United States of America	230	110	live	commercial trade	animals bred in captivity
Dendrobates reticulatus	Canada	165	130	live	commercial trade	animals bred in captivity
Dendrobates tinctorius	Canada	515	20	live	commercial trade	animals bred in captivity
Dendrobates ventrimaculatus	Canada	670	405	live	commercial trade	animals bred in captivity
Dendrobates spp	Canada	0	40	live	commercial trade	animals bred in captivity
Dendronates fantasticus	Canada	155	110	live	commercial trade	animals bred in captivity
Dendrobates imitator	Canada	233	70	live	commercial trade	animals bred in captivity
Dendrobates variabilis	Canada	65	20	live	commercial trade	animals bred in captivity
Dendrobates duellmani	Canada	55	0	live	commercial trade	animals bred in captivity
Dendrobates flavovittatus	Canada	55	0	live	commercial trade	animals bred in captivity

species	from	reported import	reported export		purpose	source
Dendrobates						
Dendrobates leucomelas	Guyana	100	0	live	commercial trade	specimens from the wild
Epipedobates						
Epipedobates bassleria	Canada	355	225	live	commercial trade	animals bred in captivity
Epipedobates pictus	Canada	12	47	live	commercial trade	animals bred in captivity
Epipedobates trivittatus	Canada/Suriname	456	390	live	commercial trade	specimens from the wild/ bred in captivity
Epipedobates hahneli	Canada	90	40	live	commercial trade	animals bred in captivity
Epipedobates rubriventris	Canada	10	0	live	commercial trade	animals bred in captivity
Hoplobatrachus						
Hoplobatrachus tigerinus	Vietnam	6000	13200	kg (meat)	commercial trade	animals bred in captivity
Scaphiophryne						
Scaphiophryne gottlebei	Madagascar	0	40	live	commercial trade	specimens from the wild

Financial impact
Currently, testing imported and traded amphibians for the presence of *B. dendrobatidis* is not compulsory. As whole collections may die due to chytridiomycosis, this should become standard practice. When testing is mandatory, the impact on amphibian trade will be negligible, but when refrained from preventive testing, (inter)national trade should be restricted and then the impact will be substantial, also for zoos participating in breeding programs.

The economic costs of restoring disrupted of ecosystems are higher than the costs of preventive or curative measures.

Social impact
The idea that the human use of amphibian habitat (not only nature reserves, but also ditches and garden ponds) could extirpate species, is a thought that is likely to cause much distress among people with nature affection, which are plentiful considering the number of people with membership of nature organisations (in 2009: 3,987,694 – Vroege Vogels website). It is possible, that when *B. dendrobatidis* negatively influences amphibians, hypothetically, restrictions on entering nature reserves could be enforced.

A restriction or ban on amphibian trade will affect owners of private collections, as well as zoos and other public organisations.

Ecological impact of presence of *B. dendrobatidis* in native amphibians, when proven lethal, is **substantial**, also for amphibians in breeding programs.
Economic impact on amphibian trade may be **small** once compulsory testing is done on imported amphibians.
Social impact is **substantial** when restrictions are enforced on the entering of specific areas, if not, the social impact will be **small**. The social impact on private owners and zoo visitors of a restriction on amphibian trade is **large**.

Summarising, the economic, ecological and social impact of the presence of *B. dendrobatidis* in native and kept amphibians is **moderate to high**.

5.6 Final Risk Assessment

The fungus *B. dendrobatidis* has entered the Netherlands, is widespread and has infected the majority of native species. It has proven to be able to survive and the species has a high dispersal capacity due to its diversity of hosts. The impact on native species is yet to be assessed, but data on the effects on rare and common species in Europe from other studies, indicate a possible major effect on population dynamics. The final assessment places the fungus in the highest risk category 6 (figure 1). It is expected impact is summarised in table 4.

Table 4. Summary of the final judgements of each assessment category on the invasiveness of B. *dendrobatidis*

assessment categories	impact
risk of invasion	high
risk of settlement	high
dispersal capacity	high
ecological , social and economic impact	moderate/high*
Final assessment	high

** depending on the virulence of the present strain and on the effects on native amphibians*

6 RISK MANAGEMENT BATRACHOCHYTRIUM DENDROBATIDIS

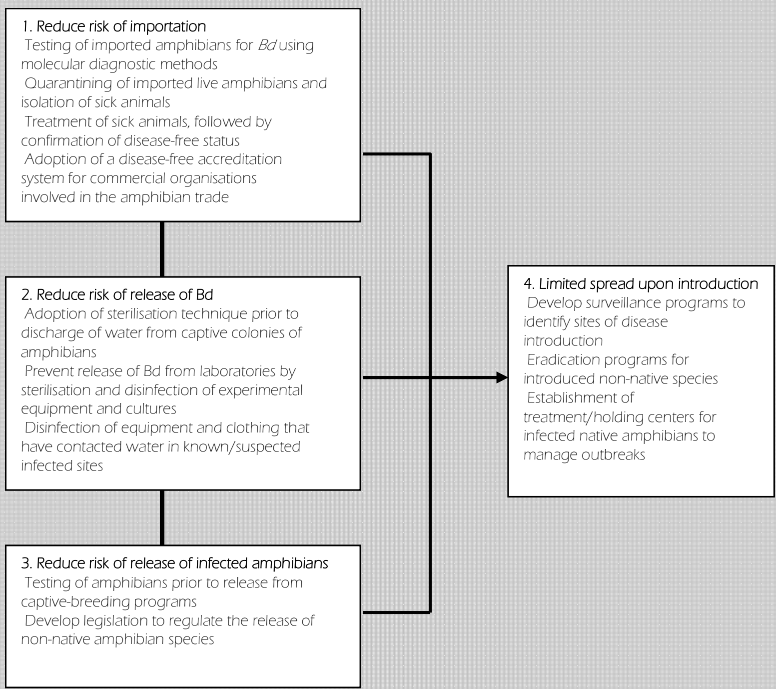
Successful management of the disease will require effective sampling regimes and detection assays (Hyatt *et al.*, 2007). The effective management of chytridiomycosis depends on the recognition of all countries, including the Netherlands, that the disease is as a 'threatening process' (a disease that threatens, or may threaten the survival, abundance, or evolutionary development of a native species or ecological community) and starts implementing strategies for its control. Managerial strategies will involve the detection of infected populations of both laboratory-housed and free-ranging animals, identification of infected geographical areas and control of human-mediated movement of animals from one location to another (Hyatt *et al.,* 2007).

RAVON is partner in the European research project on biodiversity: RACE (Risk Assessment of Chytridiomycosis to European amphibian biodiversity) and has an extensive network of volunteers and (inter)national colleagues, as well as in-depth knowledge of the problem, and is as such a key partner in the management and control of the fungus *B. dendrobatidis* in the Netherlands (and Europe). It is important to start a dialogue between all stakeholders, being the national government, researchers, conservation organisations, zoos, traders and breeders in order to take measures to control the current situation. Resources and energy should be focussed towards research on the origin of the fungus, the virulence of the strain, the effects of the disease on native amphibians as well as on the control and management of chytridiomycosis. In Australia, *B. dendrobatidis* has been accepted as a 'key threatening process and has as such, generated the deserved resources for management and control (see box 1).

As for the American bullfrog, also a high risk category species, settlement should be prevented at all times and upon the earliest detection of only a single individual, eradication should be the standard. Since *B. dendrobatidis* has been found in nearly all provinces and in the majority of native species and also in kept amphibians (Spitzen – van der Sluijs & Pasmans, in prep.; Spitzen – van der Sluijs *et al.*, 2010a; 2010b), we have passed the stage of the detection of a single infection. Eradication has, so far, not been feasible in large scale infected regions (Garner *et al*, 2006). The fungus can however being killed in ex-situ situations.

B. dendrobatidis has be been accepted by the federal government as a "Key Threatening Process" in 2002. It has led to major fund funding from the Australian Government and the Australian Research Council for research and for the development of guidelines on determining the distribution, preventing the spread, and und understanding the epidemiology and pathogenesis of chytridiomycosis (Skerratt *et al.*, 2007; www.deh.gov.au/biodiversity/invasive/diseases/).

Additionally, the Australian Government has designed a strategic plan with the two broad goals of preventing introduction of *B. dendrobatidis* into disease free areas, and decreasing the impact of the disease on populations that are currently infected (Department of the Environment and Heritage, Commonwealth of Australia 1996). This threat abatement plan has the potential to be modified for countries that have, as yet, minimal establishment of *B. dendrobatidis* and/or lack appropriate legislation addressing the threat posed by this wildlife pathogen. The figure below summarises the sections of the Australian threat abatement plan that are necessary to action in order to control the potential for introduction of *B. dendrobatidis* into disease-free countries and regions, and to limit spread if introduction has occurred (Fisher & Garner, 2007).



6.1 Ex-situ

Clearing *B. dendrobatidis* infections from live amphibians is possible ex-situ, using heat or medication. Since *B. dendrobatidis* can not survive desiccation or high temperatures Retallick & Miera (2007) placed infected animals in an incubator for 5 days at 32°C (Johnson *et al.* 2003). This method was successful and all infected frogs regained weight after their infection was cleared, to the point of becoming indistinguishable from controls (Retallick & Miera, 2007). Not all amphibian species will be able to survive these high temperatures and this method will therefore not be successful for all species

Treating amphibians with itraconazole was proven to be successful by Nichols & Lamirande (2001), Forzán *et al.*, (2008) and by Garner *et al.* (2009) however, larval depigmentation was noticed by the latter authors. Martel *et al.* (submitted) suggest a voriconazole treatment as a successful way for clearing *B. dendrobatidis* infections.

6.1.2 Preventive screening

Spitzen – van der Sluijs *et al.* (2010a) recommend a mandatory testing of amphibians destined for the pet trade for the presence of *B. dendrobatidis*. As mentioned in § 5.3, the introduction of quarantine measures and the obligatory screening for the presence of *B. dendrobatidis* in imported animals at the airport is a prerequisite in controlling the human induced introduction of the fungus in the Netherlands. Not only in collections, but also in free living amphibians. Animals involved in (re-)introduction programs should be proven fungus-free before being released in their new habitat. Currently, this is not mandatory.

6.2 In-situ

Clearing *B. dendrobatidis* infections in the field is yet impossible. No medication or other technique that can be applied easily in open water without negative side effects for other aquatic life is available. In isolated ponds with limited amphibian numbers, it is possible to catch and treat individuals, but this is extremely time consuming and expensive. For now, the emphasis should be on prevention and simultaneously on increasing the knowledge of the *B. dendrobatidis* strains to assess virulence and impact.

6.2.1 Hygienic measures in the field

To prevent the dispersal of *B. dendrobatidis* to naïve sites, hygienic measurements should be prerequisite for fieldworkers. Several hygiene protocols exist. RAVON (supplement 1) has one and other useful examples are those formulated by RACE (2010) and by Froglife and others (2008). St. Hilaire *et al.* (2009) developed a useful tool for assessing the risk of anthropogenic spread of *B. dendrobatidis* between water bodies.

All field equipment should be disinfected or dried between water bodies to prevent infection. Disinfection can be done by using a salt solution (10%) or by a bleach solution (1% is sufficient) or by using a 1% Virkon® solution. When bleach is used as a disinfectant, it must not get in contact with amphibians. Virkon® appears to be a disinfectant that can be used against *B. dendrobatidis* with no detectable negative effects on tadpoles and zooplankton (Schmidt *et al.*, 2009). The complete drying (> 3 hours) of dipnets and boots is also sufficient. 70% Ethanol kills the zoosporangia after 20 seconds (Johnson *et al.*, 2003).

6.3 Further studies

In Spitzen – van der Sluijs *et al. (*2010b), § 4.1, suggestions for further studies are given. Since the presence of *B. dendrobatidis* in the Netherlands is only discovered in 2009, it is essential to obtain more information on short notice.

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SUPPLEMENT 1 FIELD PROTOCOL RAVON

RAVON ADVIES 1 - HYGIENE PROTOCOL VOOR VELDWERKERS

Achtergrond

De huidziekte chytridiomycose, veroorzaakt door de schimmel *Batrachochytrium dendrobatidis,* veroorzaakt wereldwijd massale sterfte onder amfibieën, ook in Europa. In Spanje en in Frankrijk is sterfte opgetreden die direct verband houdt met deze ziekte. Onderzoek van RAVON in 2009 heeft aangetoond dat de schimmel in Nederland is. Wat de effecten zijn op onze soorten is vooralsnog onbekend. Er is geen reden om aan te nemen dat chytridiomycose schadelijk is voor mensen.

Er is nog geen sluitende verklaring over de manier waarop de schimmel zich verspreidt tussen locaties. Desalniettemin is het evident dat de schimmel verspreid kan worden door verplaatsingen van materialen die in contact zijn gekomen met de schimmel, door het verspreiden van besmet water of door bewegingen van besmette amfibieën zelf. Menselijke activiteiten vormen een zeer grote bron van verspreiding van de zoosporen van de schimmel en daarom vormen activiteiten in het veld een potentieel risico voor amfibieën.

Individuele dieren kunnen succesvol behandeld worden, maar besmette 'wilde' amfibieën kunnen tot op heden niet behandeld worden. Echter, eenvoudige desinfectiemaatregelen die kleding en veldmaterialen reinigen, zijn zeer effectief in het verminderen van de verspreiding van de schimmel naar nu nog 'schone' gebieden. In dit advies treft u eenvoudige, preventieve maatregelen aan die u kunt nemen om niet alleen de verspreiding van deze schimmel tegen te gaan, maar ook om de verspreiding van andere ziekteverwekkers en invasieve (micro)organismen tegen te gaan.

Het onderstaande advies richt zich alleen op de 'standaard' veldonderzoeksmethoden. Wanneer herintroducties, verplaatsingen van dieren etc. uitgevoerd worden gelden strengere hygiëne-eisen.

Advies

- Hanteer amfibieën alleen als het echt noodzakelijk is. Er zijn geen beperkingen in het veld, zolang u voorzorgsmaatregelen neemt.
- Neem ook hygiënemaatregelen in acht als u met vissen werkt (en de amfibieën ongemoeid laat).
- Amfibieën moeten altijd weer op de exacte vangslocatie worden losgelaten.
- Als uw handen in contact komen met water of met amfibieën moeten wegwerphandschoenen (poederloos) worden gedragen. Neem ook handzeep mee.
- Alle materialen die tussen verschillende locaties gebruikt worden, moeten worden gedesinfecteerd.
- Als u het water in bent gelopen, of contact hebt gemaakt met het water (of modder), moeten schoenen/laarzen/waadpak worden gedesinfecteerd.
- Er is nog geen bewijs dat de schimmel verspreid wordt door autobanden, maar het is wel goed om de auto iets verderop op een verhard pad te zetten en niet op (zachte modderige) vegetatie.
- De schimmel overleeft droogte niet. Het laten drogen van je veldmaterialen tussen veldbezoeken door is dus ook effectief (materialen moeten dan 4 uur of langer droog zijn).
- Dode en/of zieke amfibieën zijn 'high risk'. Hanteer ze enkel met handschoenen, rapporteer zieke dieren en neem dode dieren mee (in dubbele plastic zak, in vriezer of in alcohol). Neem bij het aantreffen van dode dieren contact op met het DWHC (voor contactgegevens zie website RAVON), meld het aantreffen van massale sterfte bij RAVON en meld ook vondsten van ziek uitziende dieren (a.spitzen@ravon.nl).

Desinfecteren

- Borstel plantenresten, modderkluiten etc. af
- Spoel met water. Water uit een poel of vijver is prima
- Desinfecteer op 1 van onderstaande manieren, op ruime afstand van het oppervlaktewater:
    - Maak een 1% Virkon® oplossing en spuit deze oplossing op alle veldmaterialen, wacht 5 minuten alvorens u de materialen weer gebruikt, maar het liefst tot de materialen volledig gedroogd zijn.
- Bent u niet in staat om uw materiaal op de locatie schoon te maken, neem het dan mee, van elkaar gescheiden in plastic zakken en doe het thuis.
- Was uw handen met een desinfectant. Alle drogisten verkopen zeep waarbij geen water nodig is.
- Optie: neem 2 sets materialen mee

<u>Gooi uw oplossing niet in de natuur!</u>

Checklist benodigdheden

- borstel
- emmer
- spons
- Virkon ®
- plastic zakken
- handzeep
- wegwerphandschoenen
- spuitfles


1


3

2


4


5


6

Virkon S is te verkrijgen bij

www.virkons.nl

Let op: ook bij visseninventarisaties is het noodzakelijk bovenstaande hygiënemaatregelen in acht te nemen.

Voor meer informatie, of bij vragen: Annemarieke Spitzen (a.spitzen@ravon.nl)