The County Administrative Board of Skåne

COORDINATED ACTIONS AGAINST CHYTRIDIOMYCOSIS IN THE NORDIC COUNTRIES

Project Report for 2019

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Preface

Amphibians are in a worldwide decline and among the most threatened organisms, which is also true for the Nordic countries. Up on our latitudes the amphibian's habitats have been destructed and fragmented, they severe from drying out the landscape and from the spread of alien invasive species. The threats are numerous and during the last ten years, one of the most severe is the chytrid fungus Batrachochytrium dendrobatidis (Bd).

Combating invasive alien species that threaten biodiversity is in line with the goals of the UNs Convention of Biodiversity and the Agenda 2030 targets. Therefore, in order to increase knowledge about the chytrid fungi in the Nordic countries and coordinate actions to limit further spread, the County Administrative Board Skåne was granted from the Nordic Council of Ministers in 2019 for coordinated actions against Chytridiomycosis in the Nordic countries.

During 2019, a network focusing on the chytrid fungus was formed among the Nordic countries, knowledge of the prevalence of the chytrid fungus in the Nordic region increased and information was disseminated on a broader scale to people moving between waters in the countryside.

This report summarizes information about the chytrid fungus, the distribution in the Nordic region and what can be done to prevent further spread. The network for actions against chytridiomycosis remains and can be activated by the continued spread of the chytrid fungus and if new waterborne frog diseases enter our countries.

The County Administrative Board Skåne February 2020

Cecilia Backe
Head of the Department of Nature Conservation (Naturskyddsenheten)

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The Nordic Network for Actions Against Chytridiomycosis

During 2019, the Nordic countries Sweden, Norway, Denmark and Finland were awarded a grant from the Nordic Council of Ministers to form a network for actions against the amphibian disease chytridiomycosis and to clarify existing knowledge. The network includes authorities, universities and consultants (participating organisations are listed in Table 1). The purpose of the project was i) to form a network, ii) to get an overall picture of the prevalence of the fungus Batrachochytrium dendrobatidis (Bd) in the Nordic countries (including collecting and analysing new samples), iii) to agree on the most cost-efficient and feasible ways to stop further spread of the fungus and iv) to raise awareness about the disease among different authorities and the public. The
The established network will continue as a Nordic forum for amphibian diseases. It will also be a good foundation to get information across borders in a rapid manner, if the present risk of Bd and/or Bsal (a closely related micro-fungus B. salamandrivorans that infects salamanders) changes (e.g. in accordance to climate change), or if other unknown amphibian diseases will be spread to the Nordic countries.

Photo 1. The network for Actions Against Chytridiomycosis on a fieldtrip outside Oslo in August 2019. Photo Alexander Eiler.

This report is a summary of the project and the results obtained. Since this is not a scientific article and to make it easier to read, we present the background and other information on Bd and Bsal without having too many references in the text. However, at the end of this report we have listed some of the relevant references used and suggested further reading.

Table 1. Participating organisations in the project actions against Chytridiomycosis in the Nordic countries during 2019.

Country	Office
Sweden	County Administrative Board Skåne (coordinator)
Sweden	County Administrative Board Blekinge
Sweden	County Administrative Board Kalmar
Sweden	County Administrative Board Västra Götaland
Sweden	County Administrative Board Uppland
Sweden	National Veterinary Institute
Sweden	Swedish Environmental Protection Agency
Sweden	The Swedish Species Information Centre
Sweden	Uppsala University
Sweden	Nordens Ark
Sweden	Ekoll AB
Sweden	Amplexus
Norway	Miljødirektoratet
Norway	Fylkesmannen in Oslo and Viken
Norway	Norwegian Institute for Nature Research
Norway	eDNA Solutions
Denmark	Amphi Consulte
Finland	Pyhäjärvi Institute

Background

Chytridiomycosis is an infectious disease in amphibians caused by the aquatic chytrid fungi *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal). The pathogens affect frogs, toads and newts and have been linked to the global decline of amphibians and the extinctions of amphibian species (e.g. Stockwell et al. 2004, Lips 2016, Allain et al. 2019). No feasible measure is known for controlling the disease in wild populations and the fungi must be prevented from spreading unintentionally.

Which species are infected?

Bd is regarded as a generalist fungal pathogen and may infect all species of frogs, toads and newts occurring in the Nordic region. There are species-specific differences in sensitivity which affect how serious the disease gets and how severe mortality becomes. However, the amphibians infected by Bd have rarely been observed to die from the disease in the field in Europe (Smith 2014). It is therefore important to remember that several amphibian species can carry Bd infection without showing any visible signs of chytridiomycosis.

Samples based on swabs of amphibians in Sweden indicate that at least 9 out of 13 species may be infected, but also that frequency of infection rate (prevalence) varies between species (Meurling, 2019). Species with a high prevalence are fire-bellied toad (Bombina bombina), green toad (Bufotes variablis) and the natterjack toad (Epidalea calamita). In the UK there are similar observations where common frog (Rana temporaria) seemed to be unaffected by Bd (Smith

2014). Bsal, on the other hand, infects newts and is highly lethal (Stegen et al. 2017). In Norway, risk assessments have been made for Bd and Bsal, showing a moderate risk for Bd to establish and spread, while the same risk was low for Bsal (VKM 2019). However, in Norway it is forbidden to trade amphibians and this downgrade the risk assessment significantly.

Photo 2. Spadefoot toad (Pelobates fuscus). Photo Per Nyström

The chytrid fungi Bd and Bsal

The chytrid fungus Bd releases zoospores in the water that infect the outermost layer of the amphibian skin, mainly through water exposure. Bd is found in keratin-containing tissues, such as epidermal cells of the terrestrial stages of the amphibians and the mouth parts (labial teeth) of the larvae.

The symptoms of chytridiomycosis are calluses (hyperkeratosis) and cell growth in the skin (epidermal hyperplasia), as well as wounds, impaired oxygen uptake, changes in body water balance (osmotic regulation), shedding of skin and poor condition. Changes in behaviour have also been observed in adult animals. The mouthparts are malformed in infected larvae which can lead to increased mortality due to impaired feeding.

Ecology of the fungi Bd and Bsal

The Bd fungus prefers water temperatures between 17 and 25° C. It has no resting stages and habitats without amphibians are subsequently free from Bd. However, studies have shown that Bd can survive for a long time on feathers, on snakeskin and in the digestive system of crayfish and these finding may affect the spreading and survival of the fungus. The Bsal fungus can, on the other hand, survive longer outside the amphibian host and thrives in cooler water with optimum water temperatures between 4 and 23° C. Bsal has also been found to survive longer periods outside the host.

How to detect the fungus

In Europe, several strains of Bd with differing virulences have been found and there are also variations in the sensitivities of amphibian species; subsequently, some amphibian species can be vectors (reservoir hosts) of Bd and Bsal without showing symptoms. The symptoms on the amphibians may also be caused by other amphibian diseases. In these cases, the presence of Bd and Bsal can only be detected by genetic analyses. The chytrid fungus can be sampled by either swabbing the skin of the terrestrial stages (or mouth parts of late larval stages) or by taking water samples for eDNA analyses.

Geographic distribution of Bd and Bsal

The chytrid fungi Bd and Bsal both originated in East Asia (O'Hanlon et al. 2018). Bd has since been found on all continents with amphibians and become widespread throughout Europe since it was first detected here in 1990s. The distribution of Bsal, on the other hand, is more limited and is only known from wild salamander populations in East Asia and has recently been found in the wild in the Netherlands, Belgium and Germany (Martel et al. 2013) and is today also known from captivity in the UK and Spain.

In the Nordic countries, Bd was detected in Denmark in 2007 (Scalera et al. 2008), in Sweden in 2010 (Kärvemo et al. 2015) and in Norway in 2017 (Taugbøl et al. 2017) but has not yet been detected in Finland. In the literature, Bd was specified from Finland with reference to studies made by Patrelle et.al. (2012), however all the samples from the Finish Lapland in Kilpisjärvi were free from Bd according to this article. In Denmark, Bd is found on the central islands and across to Bornholm in the east, in Sweden, from the south up to the area 150 kilometre north of Stockholm and in Norway, in a small area south of Oslo.

Sampling and analyses

One of the objectives of the project was to get a updated picture of the prevalence of Bd in the Nordic countries. In 2019, the previously known prevalence of Bd in the Nordic countries was supplemented with new sampling and analyses. Most of the locations sampled for Bd were waters with previously known observations of amphibians. The new samples were also chosen to enable a comparison of the sampling methods, i.e. the traditional skin swabbing vs analysis of eDNA from water samples. Water samples were also taken during different periods (i.e. from early April to August) from the same breeding ponds in order to find the best sampling strategy when it comes to probability of detection of Bd.

Permits

All free-living amphibians in the Nordic countries are protected by law and may not be captured, moved or investigated without permission. Before the animals are caught for sampling, one or more separate permits may be needed, partly to catch the animals and partly to take samples, i.e. swab the skin. Permits must always be applied for from the relevant authority. The authorities are listed in Table 2 for each of the Nordic countries.

Table 2. Responsible authority or citizen sciences database for amphibians, animal diseases and reporting observations in the Nordic countries.

Country/Issues	Reporting presence of Bd/Bsal to	Permits to handle amphibians	Permits to take swabs or blood samples	Reporting to global Bd-mapping	Reporting platform for citizen scientists
Sweden	Swedish Board of Agriculture (for further reporting to EU and OIE)	County Administrative Board	(Djurförsöksetiska nämnder) Ethical committees	ArtDatabanken	Artportalen
Norway	Norwegian Food Safety Authority	Norwegian Environment Agency	Norwegian Food Safety Authority	eDNA Solution	Artsobservasjoner
Denmark	Danish Veterinary and Food Administration				Danmarks Miljøportal
Finland	Finnish Food Authority	Centre for Economic Development, Transport and the Environment	Centre for Economic Development, Transport and the Environment	Finnish Food Authority	Finlands Artdatacenter

Sampling protocol

Prior to sampling, the combined knowledge in the network of how to sample for detecting Bd was compiled into a protocol that was distributed among the participants in the network (see protocol in annex 1). The sampling protocol was designed to include relevant information about the sampling site (i.e. pond and/or any amphibians sampled in the terrestrial environment), as well as swabbing of animals and/or whether water samples were taken for eDNA-analysis. The main purpose of the protocol was to standardize how the sampling was done.

Swabbing

Swab sampling was performed carefully by using a light hand and repeatedly swiping a swab over the skin of the amphibian, preferably on the underside (ventrum) of adults or metamorphs. The amphibian's toes should also be swabbed. The sampled swabs were thereafter air-dried and stored in ethanol (or preferably in a freezer) before being sent to the laboratory for analyses. The Norwegian analyses worked better when the samples were stored in an ATL buffer. It was recommended that all samples were kept individually and stored in separate tubes. Based on previous studies on prevalence it was recommended to sample (swab) at least 20-30 individuals from the same population in order to be able to detect if Bd was present.

The animals should preferably be captured during the breeding period (or for froglets/toadlets when leaving the ponds in late summer). Depending on the purpose of the sampling, some recommendations can be given when selecting species for sampling, it is advisable to sample species that are relatively easy to catch/handle and species previously known to have a greater susceptibility to the infection. Species breeding later in the year (when water temperatures become higher) seem to have an increased risk of infection (Meurling, 2019).

Informative instructions (and film clip) for swabbing can be found at Amphibia Web (https://amphibiaweb.org/chytrid/swab_protocol.html) and in the project-films at https://www.youtube.com/watch?v=myRc6GwUBIo&feature=youtu.be (in English) and at https://www.youtube.com/watch?v=nP3DRKgbsd4&feature=youtu.be (in Swedish).

Photo 3. Swabbing a Fire-bellied toad (Bombina bombina) at Högestad in Skåne. Photo Per Nyström.

Water samples for eDNA

Presence of Bd in a pond may be detected by analysing eDNA in water samples. Preferably, water samples should be taken from ponds with a known presence of amphibians and during the breeding season, when the animals spend much time in the water and when Bd is likely to be spread in the water.

Initially, it was not totally clear how the sampling for detection of Bd by eDNA-analyses should be done. Based on our discussions, we decided to take five water samples along the shoreline from smaller ponds (maximum 2 500 m2) and then pooled these subsamples to get the desired water volume. We also decided to sample 300-500 ml water per pond for waterbodies 100-2500 m2, but that 100 ml could be enough if the water body was smaller (e.g. rock pools or temporary ponds with a total water volume of less than 5 m3). It was recommended that the water samples be kept somewhere cool and dark until frozen or be filtered directly in the field after which the filters were frozen.

Information can also be found at http://www.nrm.se/forskningochsamlingar/centrumforgenetiskidentifiering/miljoovervakarensdnaskola.9004349.html (in Swedish) and in the project-films (see links under Swabbing above).

Photo 4. Preparing for taking water samples at a pond outside Oslo in August 2019. Photo Gabrielle Rosquist.

Analyses for Bd

For scientific studies for which prevalence is needed the swab samples must be analysed individually, but if only occurrence/non-occurrence of Bd is of importance, the samples can be pooled at the laboratory to keep the costs down (e.g. pooling three samples). This will however also decrease the likelihood of Bd-detection if Bd is present as less samples are being processed. The samples in this project were sent for Bd-analyses at accredited laboratories in Sweden (for samples from Sweden, Denmark and Finland) and in Norway. In Sweden, the swabs were sent to the Swedish Veterinary Institute (SVA) and the water samples were sent to the Natural History Museum for eDNA analyses, while the eDNA analyses of the Norwegian water samples were analysed by eDNA Solutions. The protocols for the analyses in Norway and Sweden will also be published at www.protocols.io by the laboratories to be available and reproducible for others. Swabs analysed in Sweden followed the protocol by Boyle et al. (2004).

Prevalence of Bd in the Nordic countries

Results from the sampling and analyses

During 2019, 175 water samples were collected for eDNA-analyses (66 in Sweden, 100 in Norway, 3 in Denmark, 6 in Finland) and 185 amphibian individuals were swabbed (153 in Sweden, 30 in Denmark, 2 in Finland) (Table 3; figure 1). No animals were swabbed in Norway in 2019.

In Sweden, the total sampling number was supplemented with 100 water samples taken during the years 2017 and 2018 that showed positive eDNA for amphibians (in most cases Rana temporaria and Rana arvalis) but that had not yet been analysed for Bd.

Table 3. The numbers of water samples for eDNA-analyses and swabbed animals for analyses of the presence of the micro-fungus Batrachochytrium dendrobatidis (Bd) in Sweden, Norway, Denmark and Finland during 2019.

		eDNA				Swab			
Country	County	Waters sampled in 2019 (n)	Waters with in Bd sampled in 2019 (n)	Waters sampled in 2017-2018 (n)	Waters with Bd sampled in 2017-2018 (n)	Animals swabbed in 2019 (n)	Swabbed animals with Bd in 2019 (n)	Animals swabbed in 2017-2018 (n)	Swabbed animals with Bd in 2017-2018 (n)
Sweden	Skåne	0	-	40	6	0	-	0	-
Sweden	Halland	1	0	0	-	0	-	0	-
Sweden	Blekinge	14	0	5	0	106	9*	12	0
Sweden	Kalmar	13	0	0	-	25	0	10	0
Sweden	V Götaland	4	1	34	0	0	-	0	-
Sweden	Uppsala	34	0	21	0	0	-	0	-
Norway		100	3	-	-	0	-	0	-
Denmark		3	0	0	-	30	1	0	-
Finland		6	0	0	-	2	0	0	-
SUMMARY		**175**	**4**	**100**	**6**	**163**	**10**	**22**	**0**

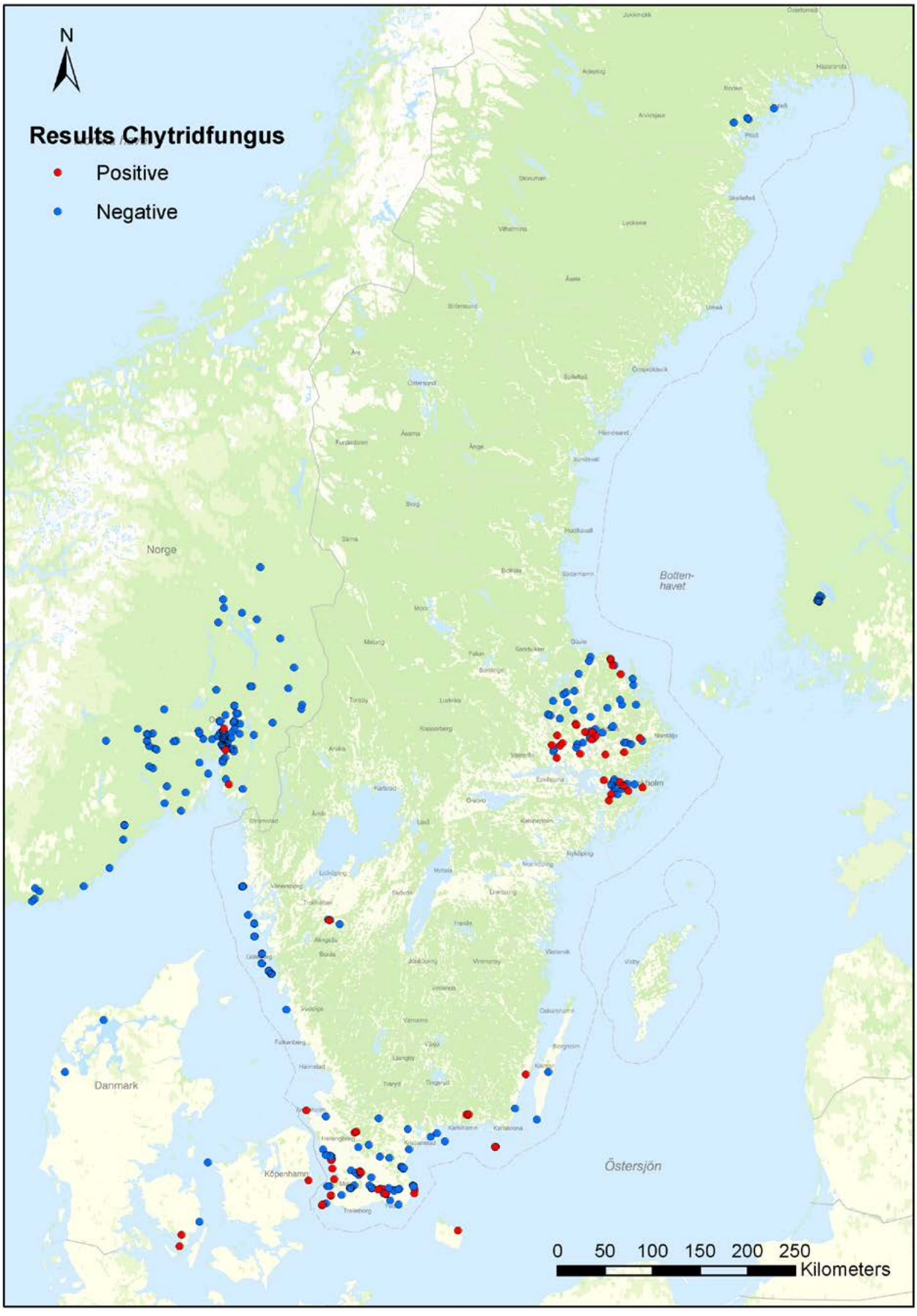
In Sweden, all 166 water samples were negative for Bd, except six sites in Skåne (from 2017-2018, three ponds in Frihult, in south-eastern Skåne) and one site in Västra Götaland (from 2019, a sample at Vårgårda east of Gothenburg with Bombina bombina). In the counties of Kalmar and Blekinge, 153 individuals were swabbed (of which 131 in 2019). Two sites were sampled for Bd in Blekinge and the results showed that Bd was present at both the island of Utklippan (Bufotes variabilis) and the mainland site in Kallinge (Pelophylax lessonae). The results also showed that sampling season affected detection. Positive samples were found during the breeding season but not in autumn. Similar patterns were found when analysing water samples from five ponds in Skåne (Frihult). Bd was confirmed in three water samples mainly during the breeding season of Rana-species. Furthermore, at the sites with positive swabs in Blekinge, eDNA of water samples could not confirm Bd. In total for Sweden, the analyses of water samples or swabbing for Bd during the period 2010-2019 have resulted in 70 positive and 207 negative samples for Bd (i.e. 25 % positive).

In Norway, water samples were collected from 100 waters and analysed for Bd. Bd was found in 3 ponds southeast of Oslo.

In Denmark, water samples were taken from three ponds with B. bombina and analysed for eDNA and 10 animals per water were swabbed in 2019. All the water samples were negative, and one out of the 30 swabbed animals were tested positive for Bd. This new site for Bd was located on a small island south of Fyn.

In Finland, 6 water samples and 2 common frogs (Rana temporaria) were swabbed during 2019. All samples tested negative for Bd. The area investigated for Bd is situated in the Satakunta region north of Åbo.

Figure 1. Map of known distribution of Bd in the Nordic countries (red circles) and negative samples for Bd (blue circles). Data were collected during the years 2010-2019 and the sampling includes both eDNA and swabbing. Previous data of prevalence were taken from Scalera et al. (2012) in Denmark and from Meurling (2019) in Sweden.



Evaluation of method and time

Comparing methods

Two different methods were used to detect Bd in the project; swabbing adult animals and filtering water samples for eDNA-analysis. The advantage of swabbing animals is that the results show both the number of infected animals and which species are infected, but the method is time-consuming and assumes that one must sample a sufficient number of animals in order to be able to detect any infection in the population. Water samples are easy to take but do not provide information on the number of individuals infected and what species are infected. eDNA samples can also be taken during the day, while swabbing is usually done at night.

Sampling using both methods were conducted at a total of 16 sites in Sweden, Denmark and Finland, and even though the number of sites were limited, the two methods could be compared. Statistical analyses (Chi2, p = 0,003, figure 2) showed that the number of positive Bd findings was dependent on method used. At seven breeding sites at least one individual tested positive for Bd but all eDNA-analyses of the pond water at these sites were negative for Bd.

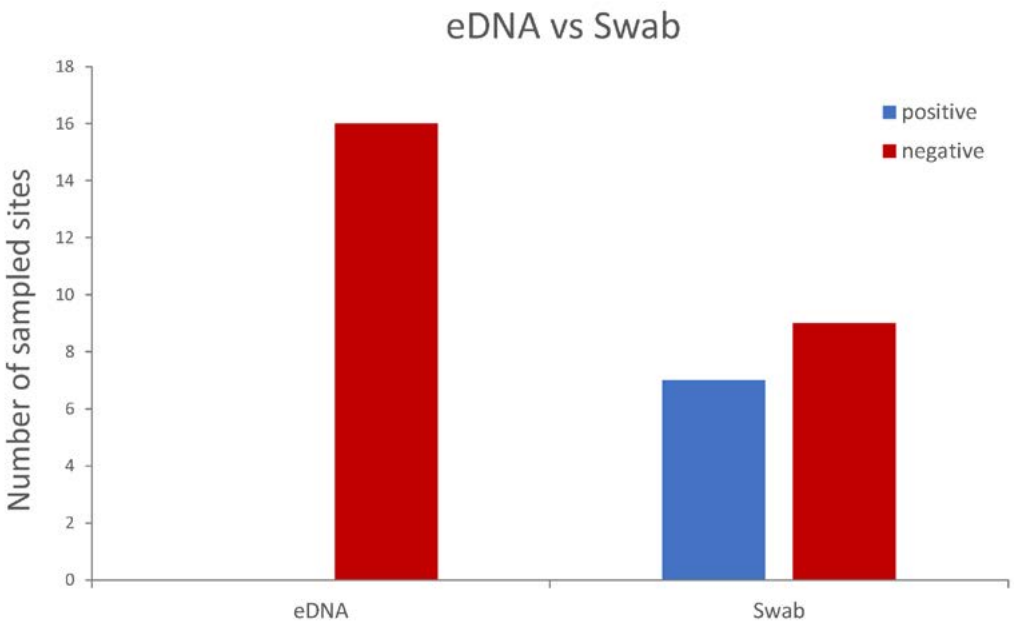


Figure 2. Number of sampled sites in Sweden (11), Denmark (3) and Finland (2) and detection results of Bd (positive or negative) by either swabbing or by taking water samples (eDNA-analyses).

Seasonal variation

Results from Skåne (Frihult), Blekinge (Utklippan, Kallinge) and Norway (south of Oslo) also showed that the detectability was highest during the breeding season when the amphibians spent time in the water. Therefore, detectability of Bd may vary depending on the amphibian species present in the pond, but also if the breeding period for a species varies along a climatic gradient in a country.

How to prevent further spreading

What is allowed?

All free-living amphibians in the Nordic countries are protected by law and may not be captured or moved without permission. Even if permission has been granted, sampling for possible infections should be done prior to moving animals to avoid the introduction of unwanted diseases. Exotic amphibians and amphibians from other countries must not be released into the wild. In Norway, it is forbidden to privately keep or trade amphibians.

How to stop future dispersal?

The trade of exotic species is believed to have contributed to the spread of the disease to new countries and continents as imported amphibians carry the fungus without showing signs of infection or illness. When trading and releasing exotic amphibians or non-host aquatic species into the wild, the fungi can then accidentally be introduced in nature.

Although Bd and Bsal are found in Europe and Bd also in the Nordic countries, further spread must be prevented. Since Bsal occurs only in parts of Europe it is important to take all precautions to prevent further spread and introduction into the Nordic countries. The same applies if more virulent strains of Bd than those that already exist in Europe (including the Nordic countries) develop and spread. Bd-GPL is currently the most virulent strain in Europe and the Nordic countries.

The chytrid fungus Bd spreads naturally between bodies of water via migrating animals such as amphibians, birds and insects. It can also be spread by humans on boots, boats and equipment or by moving amphibians. It is very important to have good disinfection routines when working in and around water with wild amphibians, during which equipment and boots are cleaned and disinfected (Table 4). These routines also prevent Bsal and other waterborne diseases from spreading. See further information under section “Dissemination of project results” below.

Photo 5. Cleaning boots before entering a new water. Photo Marika Stenberg.

There are many ways to avoid spreading waterborne infectious diseases and the most important are listed below:

1. You should always follow national legislation in the event of any trade and import of amphibians. In case of import, trade or purchase, always demand documentation showing that the animals are free from infectious diseases.
2. You should never release amphibians, or substrates/water in which amphibians have been held in captivity, into the wild. For example, never empty an aquarium with water or plants into the wild.
3. You should never move amphibians between waters without permission.
4. Urge all personnel visiting or sampling freshwater environments to follow the precautions given below. This will reduce the risk of spreading not only amphibian diseases (chytridiomycosis, rana-viruses and herpes) but also other waterborne infectious diseases such as the fungus causing crayfish plague.

In case you need to move between waters you should always:

- Clean and disinfect boots and other equipment that have been in contact with water. You can use a brush and water to clean off dirt, mud etc. before disinfection.
- For other gear that cannot be cleaned and disinfected in the field, use completely dry and clean equipment before visiting new sites.
- Make sure your hands and skin are dry or use a refreshing tissue (containing ethanol).

Table 4. The following disinfection methods should be used depending on what is feasible for each type of gear or equipment.

Method	Concentration/temperature	Time
Virkon S® (biodegradable disinfectant)	10 mg/l of water	at least 7 minutes
NaCl	10% salinity	10 minutes
Ethanol	70% concentration	1 minute
Heat (in air or water)	at least 37° C	4 hours
	at least 60° C	5 minutes
Washing of clothes with washing detergent	at least 40° C	

Dissemination of project results

One of the objectives of the project was to raise awareness among authorities, municipalities, NGOs, landowners and users as well as the public about chytridiomycosis, its effects on amphibians and what precautions need to be taken to take to prevent further spread.

Information was provided through a folder, an information sheet, a film on

YouTube, a seminar, lectures and in this summarizing report on how to handle chytrid fungus in the Nordic countries. Besides lectures, the information was spread through emails to target groups, through websites and social media, both within the participating organizations and externally to various target groups.

A final seminar about chytridiomycosis was held in Malmö on 04/12/2019, in which the project results were presented to the 55 participants from Sweden, Norway, Denmark and Finland. In conjunction with the seminar a pressrelease was completed and news spread through websites, for example https://www.lansstyrelsen.se/skane/om-oss/nyheter-och-press/nyheter---skane/2019-12-05-sa-ska-grodsjukdomen-hejdas-fran-spridning.html. The information is also available on the common website for the county administrative boards in Sweden at https://www.lansstyrelsen.se/skane/natur-och-landsbygd/invasiva-framman-de-arter.html.

The following information materials produced within the project are available:

- Folder in English (https://www.lansstyrelsen.se/download/18.4a4eb7416faedec12567f8/1579620191512/Folder%20chytridiomycosis%202019%20eng.pdf) and Swedish (https://www.lansstyrelsen.se/download/18.4a4eb7416faedec12567f9/1579620191625/Folder%20chytridio-mycosis%202019%20sv.pdf) with general information about chytridio-mycosis that is targeted to the general public. The folder will also be translated into Danish and Norwegian.
- Information sheet in English (https://www.lansstyrelsen.se/download/18.4a4eb7416faedec12567e9/1579619919856/Info%20sheet%20-%20Avoid%20spreading%20infectious%20amphibian%20diseases%20-%20eng.pdf) and Swedish (https://www.lansstyrelsen.se/download/18.331fe7f916e44e55ff3177cc/1575531531558/Informationsblad%20-%20Undvik%20att%20sprida%20amfibiesjukdomar.pdf) with information about how to avoid spreading the chytrid fungus that is targeted to all persons moving between water bodies and who are at risk of spreading fungi or other waterborne diseases. The information sheet will also be translated into Danish and Norwegian.
- Films in English (https://www.youtube.com/watch?v=myRc6GwUBIo&feature=youtu.be) and Swedish (https://www.youtube.com/watch?v=nP3DRKgbsd4&feature=youtu.be) with information about chytridiomy-cosis and how to avoid spreading the chytrid fungus. The film is targeted to all persons moving between water bodies and who are at risk of spreading fungi or other waterborne diseases. The film will also be translated into Danish ad Norwegian.
- Information was also put on the participating organizations' websites and shared on (their) social media. For links to websites see below.

To communicate knowledge about amphibians and their diseases to the public and those not familiar with the species, a table with the names in the Nordic

languages, English as well as the scientific names were compiled into a table (Table 5).

Table 5. Amphibian names in different languages.

Scientific name	English	Swedish	Danish	Norwegian	Finish
Triturus cristatus	Great crested newt	större vattensala-mader	stor vandsala-mander	storsalamander	rupimanteri (rupi-lisko)
Lissotriton vulgaris (Triturus vulgaris)	Smooth newt	mindre vattensa-lamander	lille vandsala-mander	småsalamander	manteri (vesilisko)
Bombina bombina	Fire-bellied toad	klockgroda	klokkefrø	klokkefrosk	kellosammakko
Pelobates fuscus	Spadefoot toad	lökgroda	løgfrø	løkfrosk	kaivajasammakko
Bufo bufo	Common toad	vanlig padda	skrubtudse	nordpadde	rupikonna
Epidalea calamita (Bufo calamita)	Natterjack toad	strandpadda (stinkpadda)	strandtudse	strandpadde	haisukonna
Bufotes variabilis (Bufo viridis)	Green toad	grönfläckig padda	grønbroget tudse	grønnflekkpadde	viherkonna
Hyla arborea	European tree frog	lövgroda	løvfrø	løvfrosk	euroopanlehti-sammakko (lehti-sammakko)
Rana dalmatina	Agile frog	långbensgroda	springfrø	springfrosk	hyppysammakko
Rana temporaria	Common frog	vanlig groda	butsnudet frø	buttsnutefrosk	ruskosammakko (tavallinen sam-makko)
Rana arvalis	Moor frog	åkergroda	spidssnudet frø	spissnutefrosk	viitasammakko
Pelophylax esculentus (Rana esculenta)	Edible frog	ätlig groda	grøn frø	hybridfrosk	ruokasammakko (syötävä sam-makko)
Pelophylax lessonae (Rana lessonae)	Pool frog	gölgroda	kortbenet grøn frø	damfrosk	pikkuvihersam-makko (lessonan-sammakko)

Reporting

If sick animals are observed

If an outbreak of Bd, Bsal or any other infectious disease is suspected, for example when masses of tadpoles are found dead, the responsible authority should be contacted immediately for advice and analyses. See the responsible authority for each country under the Contact-part below.

Reporting observations

Observations of Bd can be reported to the citizen science databases Artportalen www.artportalen.se in Sweden, Artsportalen www.artsobservasjoner.no in Norway, Danmarks Miljøportal in Denmark and the Finnish Artdatacenter in Finland, as well as on the Amphibian Disease Portal www.amphibiandisease.org (replaces the old portal www.bd-maps.net) where the contact person is d.aanen-sen@imperial.ac.uk. However, until 2019 very limited information could be seen at those sites for the Nordic countries. At the end of the project the results have been, or will be, reported on the citizen science data-bases and the organiza-tions in each country responsible for reporting the results on the local Bd-map were pointed out for both national and global reporting.

Contacts

All wild amphibians in the Nordic countries are protected by law and may not be caught or moved without permission. Even if permits have been granted for the movement of animals, sampling for possible infections should be done to avoid the spread of unwanted diseases. Amphibians from other countries must not be released into the wild and are not allowed to be kept in private in Norway.

Responsible authorities for animal diseases in the Nordic countries are:

- **Sweden** – the Swedish Board of Agriculture – www.jordbruksverket.se
- **Norway** – Norwegian Food Safety Authority – www.mattilsynet.no
- **Denmark** – the Danish Veterinary and Food Administration – www.foedevarestyrelsen.dk
- **Finland** – the Finnish Food Authority – www.ruokavirasto.fi

Who to contact in Sweden:

- The National Veterinary Institute (SVA) for disease and mortality in amphibians, https://rapporteravilt.sva.se (see also folder about Chytridiomycosis from SVA 2019)
- The Swedish Board of Agriculture for reporting diseases in amphibians, www.jordbruksverket.se
- Animal research ethics committees for permission to take samples from amphibians, www.djurforsok.info
- The County Administrative Boards for trapping and handling of amphibians, www.lansstyrelsen.se
- The Swedish Environmental Protection Agency conservation measures for Swedish amphibians, www.naturvardsverket.se

The future

It is almost impossible to predict what the future will look like for the Nordic amphibians and chytridiomycosis. Climate changes will certainly provide both opportunities and challenges for the amphibians, as well as for the amphibian diseases. A more favourable climate for amphibians may also favour Bd, which may spread further north. New and more virulent strains of Bd may be introduced into the Nordic countries, as well as other species of micro-fungi (like Bsal) or bacteria that affect the amphibians negatively.

Questions that were raised within the project about effects of climate changes:

- A warmer climate may affect host-parasite interactions, for example, some of the amphibian species may choose to hibernate in ponds instead of on land. They will then get exposed to Bd for a longer period each year and Bd may then be favoured.

- A warmer climate may favour the spread of vector species (e.g. edible frog, fire-bellied toad, green toad) and if they carry Bd, the fungus may become more common in the Nordic countries with warmer temperatures.
- More precipitation may favour Bd if temporary ponds become permanent, but this may also disfavour the amphibians that breed in temporary waters.

Other questions or knowledge raised during the project:

- Studies have shown that the infection loads of Bd were negatively correlated with salt concentration of the aquatic habitat and the degree of water level fluctuations and positively correlated with fish abundance (Stockwell et.al. 2015). This could be due to salt negatively affecting Bd zoospores and fish reducing the abundance of the zooplankton which in turn may consume zoospores.
- More studies on food web structures and Bd across environmental gradients are needed
- Some studies suggest that it is better to detect Bd in September, when the infection has time to proliferate, while others say that it is better to detect Bd in spring because of zooplankton grazing.
- Bd is most likely to be found in waters with a high amphibian diversity (green toad, natterjack toad, green frogs).
- Some species are more resistant to Bd than others, for example some amphibian species in South America have been shown to survive from the Bd and develop resistance.

Results from studies by Uppsala University (Meurling, 2019):

- So far, we have never seen massive death of amphibians in Europe, only one individual each in Spain and France. In the laboratory, mortality increases in experimentally infected individuals, but this has not yet been shown in the field.
- Infected common toads have been observed to have lower body conditions (i.e. they move more slowly) in the field (Kärvemo et al. 2019).
- Furthermore, an infection experiment took place for 30 days, and showed that survival rate and growth decreased in young common toads and moor frogs.
- There is a positive correlation between Bd prevalence and water temperature at spawning at species level.

In the future, the network may be used to spread new information about chytrid fungi and chytridiomycosis. It may also be activated if Bd is discovered to expand its known distribution and if Bsal is found within the borders of the Nordic countries.

Photo 6. Skoghusets enefälad, an amphibian rich site in Skåne. Photo Per Nyström.

Summary

Chytridiomycosis is caused by the micro-fungus Batrachochytrium dendrobatidis (Bd). It is spread all over the world and has caused extinction of amphibian species in the tropics. The fungus is well-spread over Europe, however knowledge of the distribution of the chytrid fungus in the Nordic countries was insufficient and consensus was needed to prevent further spread. Another chytrid fungus Batrachochytrium salamandrivorans (Bsal) has been observed to cause mortality in salamanders in Belgium, the Netherlands and Germany.
A project on Actions Against Chytridiomycosis in the Nordic Countries was granted by the Nordic Council of Ministers in 2019 with the objectives to establish a Nordic network for future work with chytridiomycosis, establish the best practice for sampling, develop a common policy for dealing with the chytrid fungi Bd and Bsal, find cost effective ways to stop chytridiomycosis from spreading and raise awareness about chytridiomycosis.

A Nordic network for future work with chytrid fungi and other amphibian diseases has been established. Participants in the project included authorities, municipalities, universities and consultants in Sweden, Denmark, Norway and Finland. Current information about the chytrid fungi and best practice to prevent further spread, as well as responsible authority for various issues about water spread diseases and threatened amphibians were discussed during project meetings and through ongoing contacts during 2019.

Additional sampling and analyses were made in all four countries to update the knowledge of the distribution of Bd. Prior to the sampling a common protocol was developed to standardize the sampling procedure. Analyses for Bd were

made at accredited laboratories in Sweden and in Norway. The protocols for the analyses will also be published at www.protocols.io to be available and transparent for others. Bd was found in 17 out of 315 water samples (11 in Sweden, 3 in Norway, none in Denmark and Finland) and in 10 out of 186 samples from swabbed amphibians, some samples were analysed by pooling individuals to reduce costs (9 in Sweden, one in Denmark and none in Finland, no animals were swabbed in Norway). In summary, Bd is still found in Denmark, Sweden and Norway, but not yet in Finland.

The results have been reported on the citizen science data bases Artportalen in Sweden, Artsportalen in Norway, Danmarks Miljøportal in Denmark and the Finnish Artdatacenter in Finland. The organizations in each country responsible for reporting the results on the local Bd map were pointed out.

Information materials have been developed (folder, information sheet and film) in English and Swedish and were disseminated during the project final seminar in 05/12/2019 and through email to various target groups. The information material will also be translated into Danish and Norwegian in 2020. A press release about the results was held at the seminar and the information was provided through websites and Facebook. Information about chytrid fungi was also given in talks and newsletters.

The project ended in 2019, but the established network will continue to keep an eye on amphibian diseases.

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AMPHIBIANS ARE IN A WORLDWIDE DECLINE and among the most threatened organisms. The threats are numerous and one of the most severe is the chytrid fungus Batrachochytrium dendrobatidis (Bd). A Bd infection may cause the disease chytridiomycosis in amphibians and Bd has been detected in over 700 different amphibian species all over the world. The fungus is waterborne and infects the skin of the amphibian.

The chytrid fungus is spread over Europe and was detected in Denmark in 2007, the first finding in the Nordic countries. To increase knowledge about the chytrid fungus in the Nordic countries and coordinate actions to limit further spread, a network was formed in 2019 supported with grants from the Nordic Council of Ministers.

This report summarizes the outcome from the work within the network in terms of knowledge about the chytrid fungus, updated prevalence in the Nordic countries and what can be done to prevent further spread. The network formed for actions against chytridiomycosis can be used to share future information about chytrid fungi or other waterborne amphibian diseases.

The County Administrative Board of Skåne
www.lansstyrelsen.se/skane