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Assessment of the risk to Norwegian biodiversity from the pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*)

Opinion of the Panel on Alien Organisms and Trade in Endangered Species of the Norwegian Scientific Committee for Food and Environment

Report from the Norwegian Scientific Committee for Food and Environment (VKM) 2019:4 Assessment of the risk to Norwegian biodiversity from the pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*)

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Assessment of the risk to Norwegian biodiversity from the pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*)

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

Key words: VKM, Risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Environment Agency, Amphibians, Chytridiomycosis, infectious emerging disease, alien species.

Introduction:

The Norwegian Environment Agency has requested the Norwegian Scientific Committee for Food and Environment for an assessment of the risk to Norwegian biodiversity from the pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). These fungi can cause amphibian chytridiomycosis, an emerging infectious disease (EID) with severe consequences to global amphibian biodiversity. Both *Bd* and *Bsal* are listed as high-risk "doorstep" species in the Norwegian list of alien species of 2018.

The indigenous amphibian species diversity in Norway is relatively low, consistsing of great creested newt (*Triturus cirstatus*), smooth newt (*Lissotriton vulgaris*), common toad (*Bufo bufo*), common frog (*Rana temporaria*) and moor frog (*R. arvalis*), all widely distributed. In addition, a small native population of the critically endangered Nordic pool frog (*Pelophylax lessonae*) exist in one location. A mixed population of Continental pool frog and edible frog (*P. esculentus*) has been accidentally introduced on the island Finnøy in Western Norway.

Chytridiomycosis caused by *Bd* is associated with population declines with local, regional and even global extinctions of amphibian species since the 1970s, making *Bd* one of the most devastating emerging wildlife pathogens ever known. The global chytridiomycosis panzootic can mainly be attributed to the genetic *Bd*-lineage referred to as the "global panzootic lineage"; *Bd*-GPL. More than 700 species worldwide from all amphibian orders have been reported with *Bd*-infections. However, dramatic declines have occurred mainly in Australia and Latin America. In Africa, Europe and North-America, very few high-mortality outbreaks of chytridiomycosis have occurred despite widespread *Bd* occurrence and spread.

In amphibian species of relevance to Norway, chytridiomycosis with moderate mortality caused by *Bd* has has only been reported twice in Europe. One involved common toad (Spain), the other common frog (France). In the latter case, disease development correlated with early onset of spring. *Bd* is widespread in parts of Sweden and UK with climate comparable to Southern Norway, but there are no reports of population declines or chytridiomycosis outbreaks from these countries. There is some evidence of clinical disease in Sweden in common toad and moor frog, but mass mortalities have never been observed. Disease or mortality caused by chytridiomycosis has not been reported in Norway, but in 2017 DNA of *Bd* was detected in water samples from ponds in South-Eastern Norway.

Different amphibian species seem to possess traits that make them more or less vulnerable to chytridiomycosis. Consequently, the species can be classified as *susceptible* (infection lead to disease), *tolerant* (infected, but do not develop disease) or *resistant* (do not become infected).

Bd infects the amphibian hosts via swimming zoospores, and has a temperature range for growth and infection between 17°C - 25°C. Cool climate thus reduces the risk of disease. Some factors reduce the likelihood of chytridiomycosis caused by *Bd*, e.g. amphibian skin microbiota with protective anti-*Bd* bacteria, and a water microfauna with filtering organisms that eat the zoospores. Co-infections potentially worsen the disease development, and sub-lethal effects of chronic *Bd*-infection is an increasing concern in Europe. With climate change, the amphibians encounter increased temperatures and temperature fluctuations, leading to amphibian acclimation challenges, earlier onset of spring and more humid conditions, factors that are linked to increased risk of disease and mortality due to *Bd*.

Bsal was introduced to Europe from Asia around 2013 as a consequence of exotic pet trade. *Bsal* infects primarity hosts within the family Salamandridae. Chytridiomycosis caused by *Bsal* has resulted in rapid declines and local extinctions of fire salamander in Belgium, the Netherlands and Germany. It has been recorded in a few European countries, but not Scandinavia. Great crested- and smooth newts show some degree of susceptibility in experimental trials, but chytridiomycosis has not been observed in the wild for these species. *Bsal* is able to grow and infect its hosts in the temperature range from 4°C - <24°C. Low temperatures is therefore expected to provide less protection against disease for *Bsal* than for *Bd.* The amphibian skin microbiota may have protective effect when containing anti-*Bsal* bacteria, but the microfauna has not the same preventive effect because *Bsal* in opposite to *Bd* produces floating resting spores in addition to zoospores, and these escape predation from the filter feeders.

Method:

VKM established a working group consisting of experts from Norway and Sweden, representing different fields including herpetology, fungal diseases, wildlife population health, pathology, epidemiology and ecology, to assess the risk of and impact from introduction, establishment and further spread of *Bd* and *Bsal* in Norway. The group has assessed relevant literature and conducted a qualitative risk assessment. The group has also used knowledge from the literature and available data on amphibian distribution throughout Norway to model the distribution and densities of amphibians today, and the potential risks for declines attributed to chytridiomycosis under current- and future climate scenarios (50 years perspective). The group considered the impact on general- and amphibian biodiversity, given that *Bd* and /or *Bsal* establish and spread in Norway, and conducted an evaluation of relevant hazards with respect to animal health and biodiversity loss.

Results:

Positive *Bd* detections in swab samples of great crested newt in South-eastern Norway were recently confirmed as the global panzootic lineage (*Bd*-GPL). Thus, it is very likely that the pathogen already is introduced and established in the country. *Bd* monitoring has only been conducted for in a minor part of Norway. In Sweden, the number of records is increasing in line with increased monitoring, suggesting that Norwegian *Bd*-distribution is not fully uncovered. It is also likely that *Bd* will spread from the known locations, and from unknown or new points of establishment, but the patchy distribution pattern seen in Great Britain and Sweden indicate that there are factors limiting spread between water bodies.

Bsal has not been detected in Norway. It is assessed as unlikely that *Bsal* will be introduced to Norway, and subsequently established. This is based on strong biosecurity measures, that there are very few plausible pathways of entry, and that *Bsal* is not detected in neighbouring countries. Some reports indicate that *Bsal* is a relatively poor disperser, although other observations suggest the opposite. If *Bsal* is introduced, we regard spread between the dispersed newt populations in Norway with low density of susceptible hosts as unlikely.

The main pathways of introduction of *Bd* and *Bsal* are related to human activities, including trade and release of exotic amphibians to the wild, accidental “hitchhikers” organisms or movements of amphibiams across boarders. Migration of infected hosts across boarders, and hitchhiking fungal units on non-host species or mechanical vectors are other pathways of introduction. Once established, the same pathways will contribute to the spread. For *Bd*, freshwater crayfish and reptiles might act as carriers that can contribute to introduction and spread.

Knowledge of *Bd* and *Bsal* abundance and prevalence in Europe for amphibian species present in Norway, combined with the scarcity of reports about severe chytridiomycosis outbreaks, suggest that such outbreaks with severe disease and high mortality are unlikely in Norway. If occurring, it would be more likely associated with *Bsal* than *Bd*, since the newt species might be susceptible and temperature range for *Bsal* is compatible with a Nordic climate. Chytridiomycosis outbreaks would have limited effects on general biodiversity, but locally affect the amphibian species. An outbreak caused by *Bd* would be of special concern the critically endangered pool frog. Our model estimates show, based on changing climatic factors alone, that the risk of a chytridiomycosis outbreak from *Bd* would increase, while from *Bsal* it would remain unchanged.

Mitigation measures to prevent or reduce entry and further spread of *Bd* and *Bsal* should identify sources of infection and spreading pathways. In Norway, import, release, trading, and keeping of amphibians are generally banned, providing important measures against *Bd* and *Bsal* introductions. Further, surveillance of wild population should be established. Public awareness campaigns should inform about the pathogen main pathways of introduction and risk of spread. Once established, *Bd* or *Bsal* will be close to impossible to eradicate. Mitigation measures must then focus on containment of the affected population and minimizing human transmission between populations. Awareness campaigns and preventive measurements are crucial for minimizing transmission risk, such as disinfection of boots and equipment moved between water bodies, and hygiene protocols when handling amphibians.

Discussion:

Although *Bd* seems to pose a limited risk to European amphibians, several factors may contribute to increased disease development and mortality events in the future. Stress factors increase the impact of *Bd* on populations, in particular phenology/temperature mismatch caused by climate change. In addition, factors like co-infections and immunotoxic pollution are also suspected to increase the stress load, which in turn may increase the susceptibility to infection by weakening immune responses or disturbing the protective skin microbiota. Under the prediction that temperatures will increase in the next 50 years, we find an increased risk for chytridiomycosis developement and outbreaks caused by *Bd*. This is because *Bd* is more virulent at higher water temperatures than presently common in Norway, and causes more disease with earlier onset of spring. According to the «thermal mismatch hypothesis», amphibians that are adapted to relatively cold conditions are suspected to be most vulnerable to the combination of increases in mean temperature, increased weather variability and emerging infectious diseases. If Norwegian newts experience reduced immunity and increased vulnerability to infectious diseases as a result of climate change and environmental stress, *Bsal* might, if introduced, also constitute an increased risk to Norwegian newt diversity in a 50-year perspective.

Conclusion:

The overall risk posed by *Bd* to amphibian biodiversity in Norway is moderate. The overall likelihood of establishment and spread is likely since *Bd* already exist in Norway and is widespread in Sweden. The potential impact for the involved amphibian species vary. Common toad may experience moderate impact due to relatively high prevalence in comparable populations in Europe and some susceptibility to disease. Pool frog may experience moderate impact due to relatively high prevalence, some susceptibility to disease and limited distribution. Surveillance and conservation of this small and vulnerable native population should be prioritized. Moor frog may experience minor impact due to low prevalence and few observed effects. Common frog, smooth newt and great crested newt may experience minimal impact due to very low or missing prevalence, wide distribution and very few or none documented effects of *Bd*-infections.

The overall risk posed by *Bsal* to amphibian biodiversity in Norway is minor. The overall likelihood of establishment and spread of *Bsal* is unlikely due to effective mitigation measures and no known presence in neighbouring countries. If introduced, both smooth and great crested newt may experience minor impact due to low or mostly missing prevalence in European populations in countries with *Bsal,* few observed effects and small and patchy populations with relatively low connectivity. This will probably hamper efficient spread.

Complex interactions with other stressors and multifactorial effects like co-infection make the assessment of impact uncertain, in particular in a climate change perspective. The overall risk might keep increasing under ongoing climate change due to potential changes in impact. As long as the risk of mass mortalities from chytridiomycosis remains low, the most effective measures to limit the risk and impact of *Bd* and *Bsal* could be measures to reduce the environmental impact / stress on the amphibian populations (such as habitat protection and measures against environmental toxins and pesticides that can damage amphibians) to compensate for the effects of a new pathogen.

Sammendrag på norsk

Introduksjon:

Miljødirektoratet har bedt Vitenskapskomiteen for mat og miljø om å vurdere hvilken risiko de patogene soppene *Batrachochytrium dendrobatidis* (*Bd*) og *B. salamandrivorans* (*Bsal*) utgjør for norsk biologisk mangfold. Disse patogene soppene kan forårsake chytridiomycose hos amfibier. Chytridiomykose er en fremvoksende sykdom med alvorlige konsekvenser for mangfoldet av amfibier globalt. Både *Bd* og *Bsal* er listet som høyrisiko «dørstokk»-arter på Fremmedartslista 2018.

Det biologiske mangfoldet av stedegne amfibiearter i Norge er relativt lavt. De mest utbredte er stor-, og småsalamander (*Triturus cirstatus* og *Lessitriton vulgaris*), nordpadde (*Bufo bufo*), butt-, og spissnutefrosk (*Rana temporaria* og *R. arvalis*). I tillegg finnes det to froskarter (*Pelophylax lessonae* og *P. escelentus*) i svært begrensede områder. Førstnevnte med ukjent historie, og sistnevnte introdusert fra Polen.

Chytridiomycose forårsaket av *Bd* er forbundet med nedgang i amfibiepopulasjoner med lokal, regional og til og med global utryddelse av enkelte amfibiearter. Det gjør *Bd* til en av de mest ødeleggende sykdomspatogene soppene på ville dyrearter som noen gang kjent. Utbruddene av chytridiomycose skyldes i hovedsak en genetisk linje av *Bd* som kalles "the global panzootic linage"; *Bd*-GPL. Det har blitt rapportert *Bd*-infeksjoner fra mer enn 700 arter i verden fra alle ordner av amfibier. Imidlertid har den dramatiske nedgangen i amfibiebestander og arter primært skjedd i Australia og Latin-Amerika. I Afrika, Europa og Nord-Amerika er det svært få rapporter om chytridiomykoseutbrudd, til tross for utbredt forekomst og spredning av *Bd*.

Chytridiomycose med moderat dødelighet forårsaket av *Bd,* er rapportert to ganger i Europa for amfibiearter som finnes i Norge. Det involverte nordpadde i Spania og buttsnutefrosk i Frankrike. I sistnevnte tilfelle var sykdomsutviklingen korrelert med tidlig start på våren. *Bd* er utbredt i deler av Sverige og Storbritannia som har et klima som er sammenlignbart med Sør-Norge, men det er ingen rapporter om bestandsnedgang eller utbrudd av chytridiomykose i disse landene. Det er noen tegn på klinisk sykdom for padde og damfrosk i Sverige, men det er ikke observert massedød. I Norge er det ikke rapportert sykdom og dødelighet forårsaket av chytridiomykose, men i 2017 ble det påvist DNA av *Bd* i vann fra dammer i Sørøst-Norge.

Ulike amfibiearter ser ut til å ha egenskaper som gjør dem mer eller mindre sårbare for chytridiomycose. Følgelig kan arten klassifiseres som mottakelig (infeksjon fører til sykdom), tolerant (infisert, men utvikler ikke sykdom) eller resistent (blir ikke smittet). *Bd* sprer seg og smitter verten med svømmende zoosporer, og har optimumstemperaturer for infeksjon fra 17°C til 25°C. Kjølig klima reduserer dermed risiko for sykdom. Noen faktorer reduserer sannsynligheten for chytridiomykose forårsaket av *Bd*, som amfibienes hudmikrobiota med beskyttende anti-*Bd* bakterier, og en mikrofauna med filtrerende organismer i vannet som spiser zoosporer. Ko-infeksjoner forverrer sannsynligvis utviklingen av sykdommen, og det er øknende bekymring i Europa for sub-letale effekter av kronisk *Bd*-infeksjon på amfibier. Klimaforandringer fører til høyere temperaturer og mer temperatursvingninger, tidligere start på våren og fuktigere forhold, faktorer som er knyttet til økt risiko for sykdom og dødelighet på grunn av *Bd*.

Chytridiomycosis forårsaket av *Bsal* har ført til rask nedgang og lokal utryddelse av ildsalamander (*Salamandra salamandra*) i Belgia, Nederland og Tyskland etter at patogenet kom til Europa rundt 2013, som følge av handel med eksotiske salamandere. Det har ikke forårsaket utbrudd i stor-, og småsalamander, selv om disse artene viser en viss grad av mottakelighet i smitteforsøk. *Bsal* er registrert i noen europeiske land, men ikke i Skandinavia. Patogenet infiserer primært verter innenfor familien Salamandridae, og kan forårsake sykdom ved temperaturer fra 4°C - <24°C. Lav temperatur forventes derfor å gi mindre beskyttelse mot sykdom for *Bsal* enn for *Bd*. Amfibienes hudmikrobiota har beskyttende effekt mot sykdom når den inneholder anti-*Bsal* bakterier, men mikrofaunaen i vannet har ikke samme preventive effekt som for *Bd*. Det er fordi *Bsal* produserer flytende hvilesporer i tillegg til zoosporer, og disse unnslipper predasjon fra filtrerende organismer.

Metode:

VKM opprettet en arbeidsgruppe bestående av eksperter fra Norge og Sverige, for å vurdere potensiell påvirkning av introduksjon, etablering og spredning av *Bd* og *Bsal* i Norge. Gruppen representerte ulike fagområder, inkludert herpetologi, soppsykdommer, dyrehelse, patologi, epidemiologi og økologi. Gruppen har vurdert relevant litteratur og gjennomført en kvalitativ risikovurdering. Den har også brukt kunnskap fra litteraturen og tilgjengelige data om amfibieutbredelsen i Norge for å modellere fordeling og tetthet av amfibier i dag, og potensiell risiko for nedgang som følge av chytridiomycosis under nåværende og fremtidige klimascenarier (50 års perspektiv). Gruppen vurderte effekten på generelt biologisk mangfold og mangfold av amfibier, gitt at *Bd* og / eller *Bsal* etableres og spres i Norge, og evaluerte relevante farer med hensyn til dyrehelse og tap av biologisk mangfold.

Resultater:

Positive *Bd*-detekteringer i swabprøver av storsalamander i Sørøst-Norge ble nylig bekreftet som den globale panzootiske linjen (Bd-GPL). Det er derfor meget sannsynlig at patogenet allerede er innført og etablert i landet. *Bd*-overvåking har kun blitt gjennomført for en mindre del av Norge. I Sverige øker antall påvisninger i takt med økt overvåking, noe som tyder på at norsk *Bd*-utbredelse ikke er fullt avdekket. Det er også sannsynlig at *Bd* vil spre seg fra kjente lokaliter og fra ukjente eller nye etableringspunkter, men usammenhengende *Bd*-utbredelse, som utbredelsen som er observert i Storbritannia og Sverige, kan tyde på at det er faktorer som begrenser spredningen mellom vann

Bsal er ikke oppdaget i Norge. Det vurderes som usannsynlig at *Bsal* vil bli introdusert og deretter etablert i Norge. Dette baserer seg på sterke biosikkerhetstiltak, veldig få sannsynlige introduksjonsveier, og at *Bsal* ikke er oppdaget i noen av nabolandene våre. Noen rapporter indikerer at *Bsal* spres relativt inneffektivt, selv om andre observasjoner antyder det motsatte. Det er imidlertid lite sannsynlig med spredning mellom de spredte salamanderbestandene i Norge med lav tetthet av mottakelige verter.

Introduksjonsveier for *Bd* og *Bsal* er primært knyttet til menneskelige aktiviteter, inkludert handel med, og utsettelse av, eksotiske amfibier i naturen, tilfeldige "haiker"-organismer eller flytting av smittede amfibier over landegrenser. Andre introduksjonsruter er migrasjon av infiserte verter, «haikende» soppsporer på andre arter eller mekaniske vektorer. Etter etablering vil de samme mekanismene fungere som spredningsveier. For *Bd* kan ferskvannskreps og reptiler være smittebærere som bidrar til introduksjon og spredning.

Kunnskap om utbredelse og prevalens av *Bd* og *Bsal* i Europa for amfibiearter som er tilstede i Norge, kombinert med mangel på rapporter om alvorlige utbrudd av chytridiomykose, tyder på at slike utbrudd med alvorlig sykdom og høy dødelighet er usannsynlig i Norge. Hvis det forekommer vil det være mer sannsynlig forbundet med *Bsal* enn *Bd*, siden våre salamanderarter kan være mottakelige og temperaturområdet for *Bsal* er forenelig med et nordisk klima. Utbrudd av chytridiomykose vil ha begrenset effekt på biologisk mangfold generelt, men påvirke amfibieartene lokalt. Et utbrudd forårsaket av *Bd* ville være spesielt bekymringsfullt for den kritisk truede damfrosken. Våre modellestimater viser at endret klima (e.g. økt temperatur) alene øker risiko for *Bd* utbrudd av chytridiomykose, mens det for *Bsal* forblir uendret.

Tiltak for å forhindre eller redusere innførsel og videre spredning av *Bd* og *Bsal* må identifisere kilder til infeksjon og spredningsveier. I Norge er import, utsettelse, handel og hold av amfibier forbudt. Det er et viktig tiltak mot *Bd*- og *Bsal*-introduksjoner. Videre bør det etableres overvåking av ville amfibiebestander. Kampanjer for å bevisstgjøre allmenheten bør informere om patogenens hovedveier for introduksjon og risiko for spredning. Når *Bd* eller *Bsal* først er etablert, vil de være nær umulig å utrydde. Forebyggende tiltak må da fokusere på inneslutning av den berørte populasjonen, og minimering av menneskemedvirket smitteoverføring mellom populasjoner. Bevissthetskampanjer og forebyggende tiltak er avgjørende for å minimere risiko for smitteoverføring, for eksempel desinfeksjon av støvler og utstyr som flyttes mellom vann, og hygieneprotokoller ved håndtering av amfibier.

Diskusjon:

Selv om *Bd* tilsynelatende utgjør en begrenset risiko for europeiske amfibier, kan flere faktorer bidra til økt sykdomsutvikling og dødelighet i framtiden. Stressfaktorer øker påvirkning av *Bd* på bestander, spesielt fenologi/«termisk mismatch» forårsaket av klimaendringer. I tillegg kan faktorer som ko-infeksjoner og immunotoksisk forurensning bidra til å øke stressbelastningen, som i sin tur vil gi økt mottakelighet for infeksjoner ved å svekke immunresponser eller forstyrre den beskyttende hudmikrobiotaen. Under forutsetning av at temperaturen vil øke i de neste 50 årene, vurderer vi at det er økt risiko for utvikling av chytridiomykose og utbrudd forårsaket av *Bd*. Det er fordi *Bd* er mer virulent ved høyere vanntemperatur enn det som er vanlig i Norge idag, og fordi *Bd* forårsaker mer sykdom ved tidlig vår. Ifølge «termisk mismatch»-hypotesen mistenkes det at amfibier som er tilpasset forholdsvis kalde betingelser vil være mest sårbare for kombinasjonen av økte gjennomsnittstemperaturer, økt variasjon i værforhold, og nye smittsomme sykdommer. Dersom norske salamanderarter opplever redusert immunitet og økt sårbarhet mot smittsomme sykdommer som følge av klimaendringer og miljøbelastning, kan også *Bsal* utgjøre en økt risiko for norsk salamanderdiversitet i et 50 års perspektiv, dersom den introduseres.

Konklusjon:

Overordnet utgjør *Bd* moderat risiko for mangfoldet av amfibier i Norge. Det er sannsynlig at *Bd* etableres og spres i Norge, siden *Bd* allerede eksisterer i Norge og er utbredt i Sverige. Den mulige effekten for de involverte amfibieartene varierer imidlertid ganske mye. Effekten på nordpadde kan være moderat, på grunn av relativt høy prevalens i sammenlignbare populasjoner i Europa og noe mottakelighet for sykdommen. Effekten på damfrosk kan være moderat, på grunn av relativt høy prevalens, noe mottakelighet og meget begrenset utbredelse. Overvåkning og bevaring av den lille populasjonen av denne sårbare stedegne arten bør prioriteres. Effekten på spissnutefrosk anses å være liten, grunnet lav prevalens og få observerte effekter. Effenten på buttsnutefrosk, stor-, og småsalamander anses å være minimal, på grunn av svært lav eller manglende prevalens, bred utbredelse og svært få eller ingen dokumenterte effekter av Bd-infeksjoner.

Overordnet utgjør *Bsal* liten risiko for mangfoldet av amfibier i Norge. Det er usannsynlig at *Bsal* etableres og spres i Norge på grunn av effektive biosikkerhetstiltak og ingen kjent tilstedeværelse i nabolandene. Om den introduseres, vil effekten på både stor-, og småsalamander kunne være liten, på grunn av lav eller manglende prevalens i europeiske populasjoner i land med *Bsal*, få observerte effekter, og små, ujevnt fordelte bestander med liten grad av sammenknytning. Det vil sannsynligvis hindre effektiv spredning av *Bsal*.

Komplekse interaksjoner mellom *Bd*/*Bsal*-infeksjon og miljøindusert stress samt multifaktorielle effekter av ko-infeksjoner, gjør vurdering av effekter usikker, særlig i et klimaendringsperspektiv. Samlet risiko vil kunne øke under pågående klimaendringer på grunn av mulige endringer i effekter. Om risiko for massedødelighet på grunn av chytridiomykose forblir lav, kan de mest effektive tiltakene for å begrense risiko og effekt av *Bd* og *Bsal* være tiltak for å redusere miljøbelastning/stress på amfibiepopulasjonene (som habitatvern og tiltak mot miljøgifter og plantevernmidler som kan skade amfibier) for å kompensere for effekten av et nytt patogen.

Abbreviations

Bd: *Batrachochytrium dendrobatidis*

Bsal: *Batrachochytrium salamandrivorans*

EFSA = European Food Safety Authority

NFSA = The Norwegian Food Safety Authority (In Norwegian: Mattilsynet)

NEA = The Norwegian Environment Agency (In Norwegian: Miljødirektoratet)

OIE = World Organization for Animal Health

VKM = Norwegian Scientific Committee for Food and Environment (In Norwegian: Vitenskapskomiteen for Mat og Miljø)

Background as provided by the Norwegian Environment Agency

Batrachochytrium dendrobatidis (*Bd*)

The pathogen Bd was detected for the first time in Norway in the summer of 2017. *Bd* is a fungus that causes the infectious disease chytridiomycosis on amphibians that reduces osmoregulation and may cause death. Some individuals are severely affected, while others may become carriers without being affected by the disease itself. The disease has led to dramatic declines and extinction of amphibian populations worldwide and is considered one of the major causes of amphibian species loss. Frogs are the main host and more affected than salamanders.

The presence of the fungus was discovered through water samples of environmental DNA (e-DNA). This was carried out by the Norwegian Institute for Nature Research (NINA). It was discovered in five of a total of 34 investigated ponds near Norway's capital Oslo. *Bd* is only found on the east side of the Oslo fjord. Relatively low concentrations of *Bd* have been found in the documented water samples, but the results are consistent. The e-DNA concentration is dependent on many factors, including temperature and activity on the animals, and NINA observed that the DNA concentrations were highest at the last sampling in mid-June.

The geographical spread of *Bd* points to the existence of several infected sites. This can be both ponds that were not sampled in 2017, and sampled ponds that have yielded false negative results. This can happen when the infection level is low, when the main source of infection occurs in other parts of the pond than sampled, or when the samples have been collected at adverse times relative to the zoospore activity. It cannot be ruled out that several of the ponds that were examined have a low or incipient stage of *Bd* infection.

In the summer of 2018, NINA examined the *Bd* situation further. Their project aimed to:
- identify which amphibian species are host to the fungus,
- identify to what extent the different species in these ponds are infected and to what extent this affects the concentration of e-DNA on *Bd*,
- determine wether any of the species has developed Chytridiomycosis caused by the fungus,
- investigate how the e-DNA concentration is in the ponds and evolves for the amphibian species through the season of infected vs. uninfected ponds.
- test other non-tested ponds for *Bd* in Akershus and Østfold counties (now Oslo and Viken county).

Batrachochytrium salamandrivorans (*Bsal*)

Bsal is quite similar to *Bd*, the difference is that *Bsal* has salamander as its main host. *Bsal* has not yet been discovered in Norway, but we know that it has been found on animal in pet stores in Sweden.

15.05.2019

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency requests the Norwegian Scientific Committee for Food and Environment (VKM) to:

1) Describe the status of *Batrachochytrium dendrobatidis* (*Bd*), *Batrachochytrium salamandrivorans* (*Bsal*) and Chytridiomycosis in Norway and other relevant countries as of today.

2) Assess the consequences of
   a) Establishment of *Bd* and *Bsal* in Norway
   b) Spread of *Bd* and *Bsal* in Norway
   c) An outbreak of Chytridiomycosis in Norway in regards to biodiversity

3) Assess the likelihood of:
   a) Establishment of *Bd* and *Bsal* in Norway
   b) Spread of *Bd* and *Bsal* in Norway
   c) An outbreak of Chytridiomycosis in Norway

4) Characterize the risk of:
   a) Establishment of *Bd* and *Bsal* in Norway
   b) Spread of *Bd* and *Bsal* in Norway
   c) An outbreak of Chytridiomycosis in Norway in regards to biodiversity

5) Summarize various mitigation measures initiated around the world in order to:
   a) Prevent or reduce entry and spread of *Bd* and *Bsal*
   b) Prevent or reduce outbreaks of Chytridiomycosis

1 Introduction

1.1 Amphibians in Norway

Compared with other Nordic, and especially European, countries, relatively few amphibian species are found in Norway. Two species of newts (*Triturus cirstatus* and *Lissotriton vulgaris*), one toad (*Bufo bufo*), and three species of frogs (*Rana temporaria*, *R. arvalis and Pelophylax lessonae*) are considered indigenous to Norway. In addition, *Pelophylax escelentus* have been introduced from Poland and is represented with a small populations in a very limited area. Two of the species are listed on the 2015 "Red list" for endangered species by the Norwegian Biodiversity Information Centre (NBIC); the great crested newt (listed as "Nearly threatened") and the pool frog (listed as "Critically endangered"). The introduced edible frog (aka. hybrid frog), and the introduced subspecies of pool frog (*P. lessonae lessonae*) on Finnøy are both listed as species with which pose a very high risk to biodiversity in Norway by NBIC in 2018.

The general text below, on the biology, distribution, and reproduction of the amphibian species builds, first of all, on Dolmen (2018, and pers. comm.), supplemented with Fog et al. (1997), Beebee & Griffiths (2000), and Arnold & Ovenden (2002). Other sources of information are referenced in the text.

1.1.1 Great crested newt (*Triturus cristatus* (storsalamander))

Figure 1.1.1-1: Great crested newt (*T. cristatus*). Photo: Dag Dolmen

The great crested newt (Figure 1.1.1-1) *T. cristatus* (Laurenti, 1768) has three separate distribution sub-areas in Norway: 1) From the Swedish border in Østfold, around the Oslofjord to Skien and Gjerstad. Although it is generally a lowland species, in Telemark it has been recorded at elevations up to 600 m a.s.l. In eastern Norway, it goes as far north as Land, Lillehammer, and Ytre Rendal. 2) The crested newt is also found in western Norway between Stavanger and Bergen, and 3) in central Norway from Nord-Møre and northwards on both sides of the Trondheimsfjord; the northernmost record is at Steinkjer, which represents the northernmost known population in the world. An occurrence map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and density kernels, is shown in Figure 1.1.1-2.

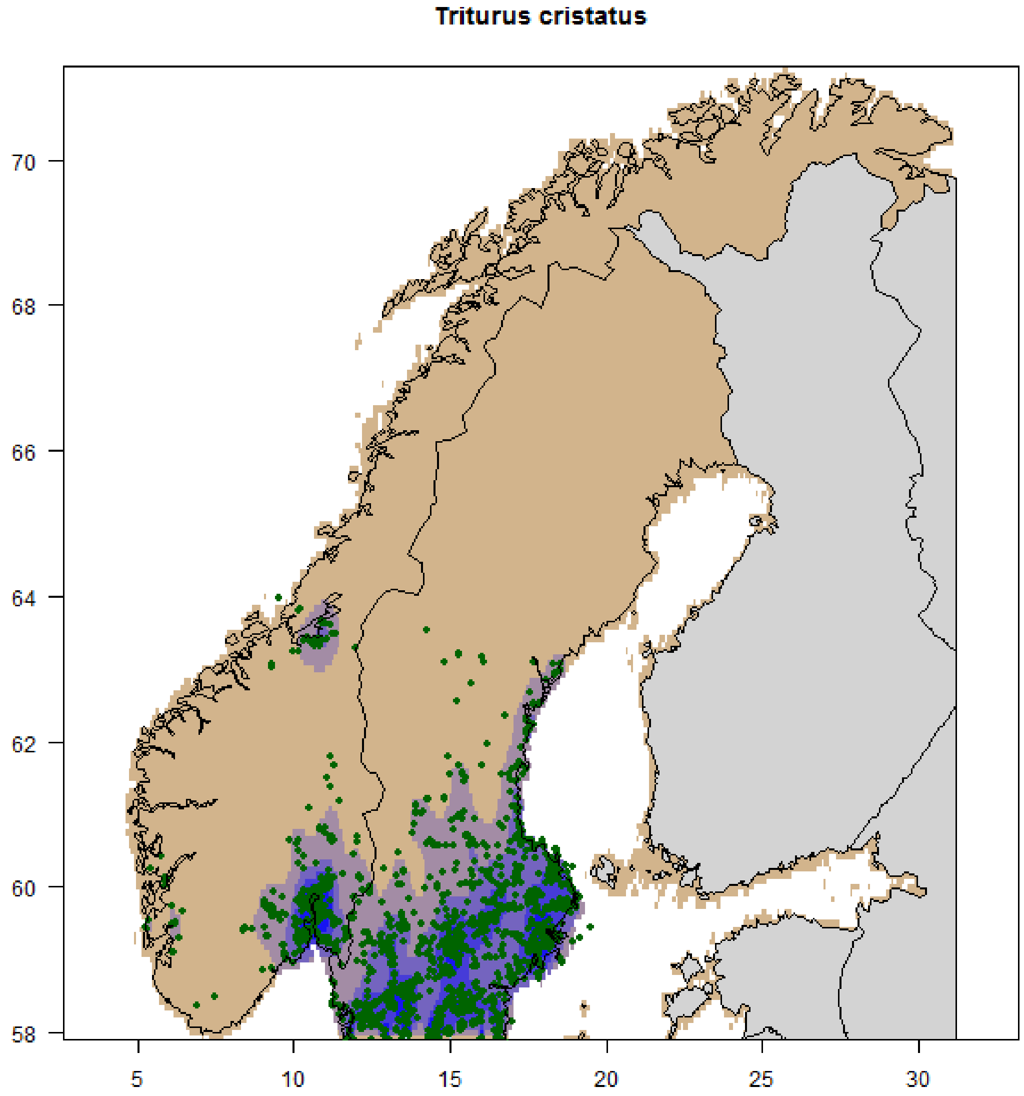


Figure 1.1.1-2: Map of all observations of wild *Triturus cristatus* recorded in Norway and Sweden. Green dots denote point observations, shading indicates the density of observations by allowing overlapping points to give value to surrounding raster cells. See “Spatial points distributions” (2.4.3) for descriptions.

Adult animals are 11-15 cm long, black or dark brown with black spots and white stippling along the flank and with a yellow/orange, black-spotted belly. The male in spring has a tall, serrated dorsal/caudal crest (interrupted at the base of the tail) and a black, swollen cloaca.

Females have a dorsal groove and a saffron yellow cloaca. The skin is rough, and moist even in terrestrial animals.

The crested newt occurs in connection with eutrophic ponds in the cultural landscape and in small lakes in marshes surrounded by woodland. The species is more associated with water and moisture than the smooth newt. Hibernation is usually terrestrial. Courtship and egg-laying take place in the water in May–June, and some individuals then return to land while others stay for another month or more in the water. The species is most active in the twilight/dark, but aquatic animals can often be seen on the pond floor by day, where they hunt for bottom-dwelling invertebrates. The larvae undergo metamorphosis in August–October, when they leave the water. However, some juveniles may be found in the pond the following summer. The crested newt reaches sexually maturity at two- to five-years old. Although the adults have toxic skin and warning colouration (yellow/orange and black), the larvae are not toxic. Because of this and their highly nektonic way of life, the larvae are especially vulnerable to predation by (salmonid) fish. The species may reach up to 15 years of age in nature, and 25 years has been reported from captivity.

Females lay 200-400 eggs, but due to a chromosome anomaly, only half of them hatch (Sessions et al. 1988). From egg to adult, only 0.5% survive (Hedlund 1990). For adult animals, the yearly death rate is about 30%. Larvae are predated upon by invertebrates, especially dragonfly larvae and water beetles, and by fish. Adults have few predators, but may be preyed upon by water birds such as herons and ducks. Although most terrestrial newts stay in the vicinity of the breeding pond (i.e., less than 50 m away), some individuals have been found as much as 1 km away (Kupfer 1998).

The most important threats to the great crested newt are draining/filling-in of ponds, pollution (including acidic precipitation), the release of predatory fish and urbanization with heavy traffic and isolation of breeding ponds (Dolmen 1987).

1.1.2 Smooth newt (*Lissotriton vulgaris* (småsalamander))

The smooth newt (Figure 1.1.2-1) *L. vulgaris* (Linnaeus, 1758) has two main distribution sub-areas in Norway: 1) Southeastern/southern Norway: from the Swedish border in Østfold, around the Oslofjord and along the southern coast to Stavanger. In eastern Norway, it has been found in the valleys as far north as Fron (possibly even further), Ytre Rendal and Tynset. In Telemark it has been recorded at elevations of up to 700 m a.s.l. (In addition, the species has been introduced in several places in western Norway, north of Stavanger.) 2) Central Norway: the smooth newt is also distributed south and east of the Trondheimsfjord and from there, more scarcely, to Vefsn, which represents the northernmost known population in the world. An occurrence map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and density kernels, is shown in Figure 1.1.2-2.

Figure 1.1.2-1: Smooth newt (*L. vulgaris*). Pho to: Dag Dolmen

Adult animals are 7–10 cm long, brown, olive, grey or black with smooth skin, and with a dark line, often pronounced, through the eye. In spring, mature males have a continuous, undulating dorsal/caudal crest, toe webbing, and a red belly with black spots and black, swollen cloaca. In contrast, females in spring have a very low, smooth dorsal crest, orange belly with dark dots and an orange cloaca. In terrestrial animals the skin is usually dry.

The smooth newt lives in and close to various types of stagnant water-bodies, like ponds and small lakes in marshes and woodland or in the cultural landscape. Hibernation is usually terrestrial. Courtship and egg-laying take place in the water in May–June, and most individuals then return to land. The species is most active at twilight, but aquatic animals can often be seen during the day, such as when they are hunting for prey (insects, crustaceans etc.) in surface waters. The larvae undergo metamorphosis in July–September, when they leave the water. When the smooth newt is two to four years old, it is sexually mature and breeds for the first time. The species rarely reaches 7 years of age in nature, but individuals have been reported to live up to 28 years in captivity.

Females lay 200-300 eggs in a season. The metamorphosed young newts have a yearly mortality of 20%, while the adults may suffer a 50% mortality each year. Larvae are preyed upon by invertebrates, such as dragonfly larvae and water beetles, and also fish. Adults may be eaten by fish, herons, ducks and other birds, as well as mammals like shrews, hedgehogs, cats etc. Although the smooth newt is generally quite philopatric, and terrestrial individuals rarely move more than 50–400 m away from the pond, a population may spread 4-5 km within just a few years.

The most important threats to the smooth newt are draining/filling-in of ponds, pollution (including acidic precipitation), the release of predatory fish, and urbanization with heavy traffic and isolation of breeding ponds (Dolmen, 1987).

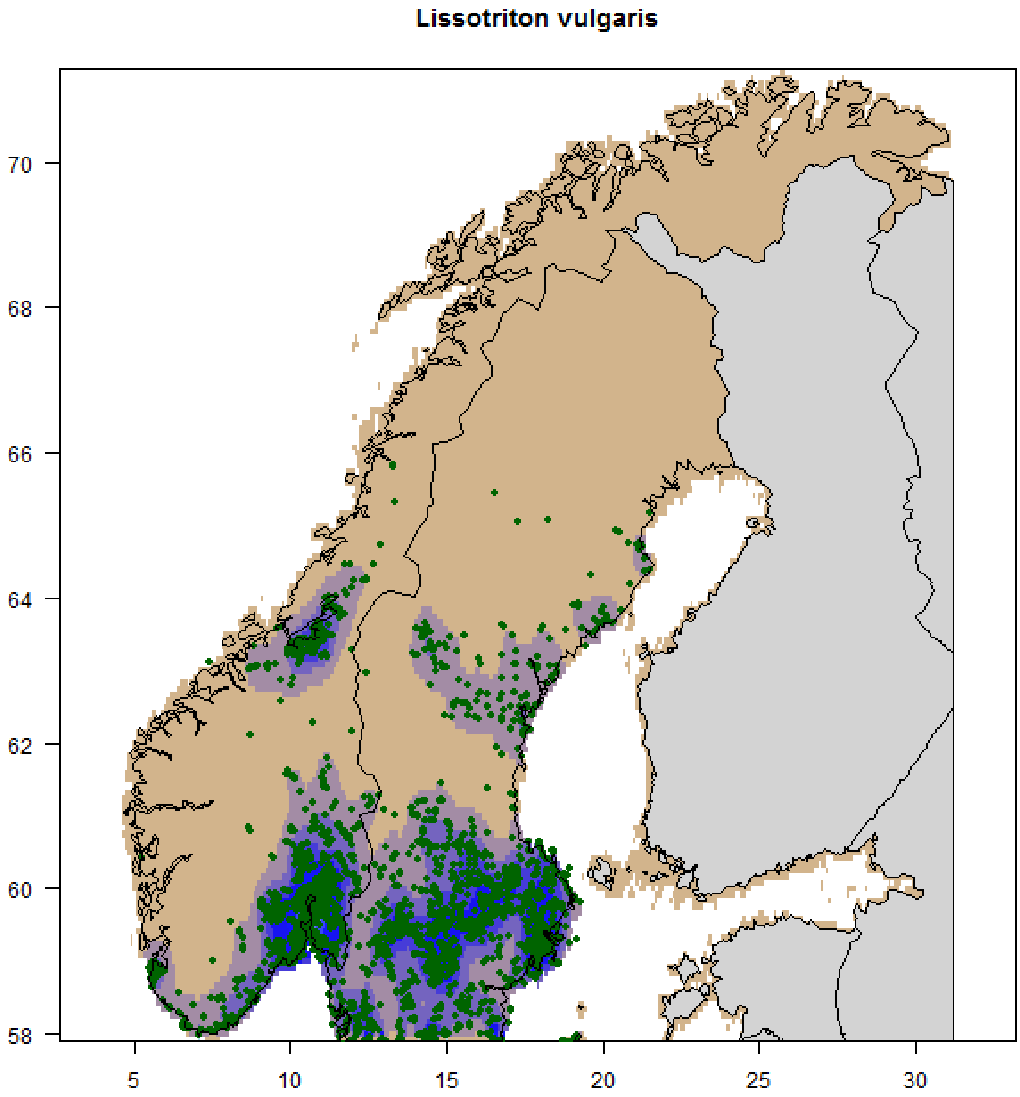


Figure 1.1.2-2: Map of all observations of wild *Lessotriton vulgaris* recorded in Norway and Sweden. Green dots denote point observations, shading indicates the density of observations by allowing overlapping points to give value to surrounding raster cells. See “Spatial points distributions” (2.4.3) for descriptions.

1.1.3 Common toad (*Bufo bufo* (nordpadde))

The common toad (Figure 1.1.3-1) *B. bufo* (Linnaeus, 1758) is distributed over most of the lowlands of southeastern, western, and central Norway, especially along the coast including many islands. North of the Trondheimsfjord, the species is found mainly along the coast up to Dønna. The northernmost record is Hamarøy. Although generally a lowland species, the toad has occasionally been recorded at elevations up to 1000 m a.s.l. An occurrence map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and density kernels, is shown in Figure 1.1.3-2.

The males are usually 6–8 cm long; females are larger, up to 8–10 cm, sometimes as much as 12 cm long. The common toad is grey, brown, reddish or (rarely) yellowish. The iris of the eye is ruby red. The hind legs are considerably shorter than those of frogs, and therefore the toad also jumps less powerfully. The body is also less smooth than that of a frog, and although the warty skin is often dry, it can excrete a strong toxin (bufonin), especially from the large parotoid glands in the neck. The tadpoles also taste unpleasant and are therefore avoided by predatory fish.

Figure 1.1.3-1: Common toad (*Bufo bufo*). Photo: Dag Dolmen

The common toad can be found in both damp and dry habitats throughout most of the summer, in woodland, heaths, or in the cultural landscape, often close to human settlements. During daytime, the toad hides in crevices or under rocks etc., but by night it is fully active, hunting for insects, spiders, and snails. The toad's breeding habitats are small or large lakes and ponds, or slow-running water. The sound most often heard from the common toad, by day and night, when in the water, is the males' "protest" or "release" sound" (against other males). This is a somewhat sad and faint, but nevertheless penetrating, high-pitched "hrrruk, hrrruk", which is repeated at irregular intervals, and has been compared to a whining dog. The actual mating call is a slowly repeated "rrrhuakk, rrrhuakk, rrrhuakk" and may be heard over a distance of 100 meters or more. Breeding takes place during a short period in May–June, and the tadpoles, which usually swim in dense swarms, metamorphose in July or August, and leave the water in large crowds. Hibernation takes place either on land or in the water. Sexual maturity is reached after three years or more. In nature, some toads live for 10 years or more, and in captivity 36 years has been reported.

Females usually lay 2 egg strings containing a total of 1000-7000 eggs, depending on the size of the female. The annual death rate varies greatly between localities; 15–60% is mentioned. The larvae are eaten by dragonfly- and water beetle larvae, and also by ducks and the great crested newt. Despite their toxicity, and although many predators avoid them, the adults may be killed, and are often eaten by badgers and hedgehogs, and also by some birds of prey, crows, and herons. Grass snakes (*Natrix natrix*) are also important predators on toads. The common toad is quite philopatric, but may alternate between closely situated localities. An average population dispersal speed of 0.6 km/yr has been measured. However, migrations may sometimes take place over as much as 1–4 km.

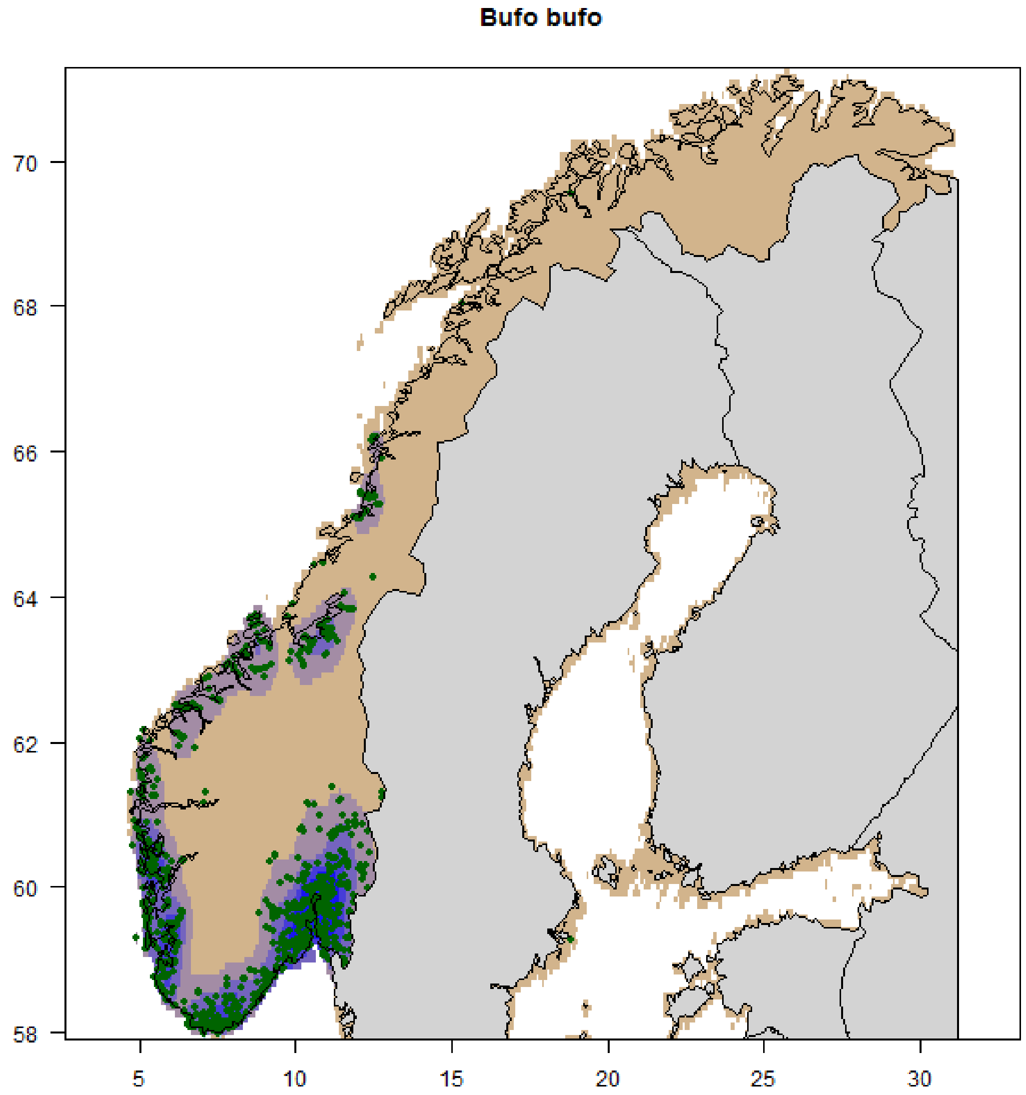


Figure 1.1.3-2: Map of all observations of wild *Bufo bufo* recorded in Norway (data from Sweden were not available). Green dots denote point observations, shading indicates the density of observations by allowing overlapping points to give value to surrounding raster cells. See "Spatial points distributions" (2.4.3) for descriptions.

The most important threats to the common toad are draining/filling-in of oxbow lakes, pollution (including acidic precipitation), and urbanization with heavy traffic and isolation of breeding ponds (Dolmen, 1987).

1.1.4 Common frog (*Rana temporaria* (buttsnutefrosk))

The common frog (Figure 1.1.4-1) *R. temporaria* (Linnaeus, 1758) is distributed over most of Norway, even at high altitudes. Reproduction has been reported up to 1000 m and adults have been observed at 1400 m a.s.l. (Dovre). An occurrence map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and density kernels, is shown in Figure 1.1.4-2.

Figure 1.1.4-1: Common frog (*R. temporaria*). Photo: Dag Dolmen

Common frogs can grow up to a length of 10 cm, but exceed rarely 7–8 cm. The skin colour is very variable, probably depending on habitat, but is usually light brown with dark patterns. However, it can also be, for instance, olive, red, or yellowish. In spring, males may in addition have a weak blue tinge. The belly is marbled, the snout rounded (best seen from the side, but also from above), the light stripe along the upper jaw is relatively marbled, and the metatarsal tubercle is small and soft. The common frog has powerful hind-legs and moves with long jumps.

The species can be observed in marshes in woodland, in the mountains or in the cultural landscape, often in ditches and usually close to freshwater. Frogs tend to come out of their hiding-places at twilight or after rain. Frogs eat all kinds of insects, spiders, and worms etc. The common frog breeds in ponds or in sheltered bays of lakes in April–May (depending on latitude and altitude). The mating call can often be heard during the day, but also at night, and is a low-pitched, snoring “rrruuo”; when several males are calling together, it may sound like a distant chainsaw. The call may be heard at a distance of up to approximately 40 m. The larvae (tadpoles) metamorphose in July–October and then move onto land. The frog hibernates either on land or in the water. Sexual maturity is reached after three years or more. The common frog may live for more than 10 years in nature; maximum longevity is 18 years.

Females usually lay 1 clump of spawn containing 600-4000 eggs. The yearly death rate for metamorphosed frogs is usually between 6% and 30%. The size of local populations may vary greatly from one year to the next. The common frog is hunted and eaten by many different aquatic and terrestrial predators. A population dispersal speed of approximately 0.4 km/yr has been measured, while individual migrations may be up to 1 km or more.

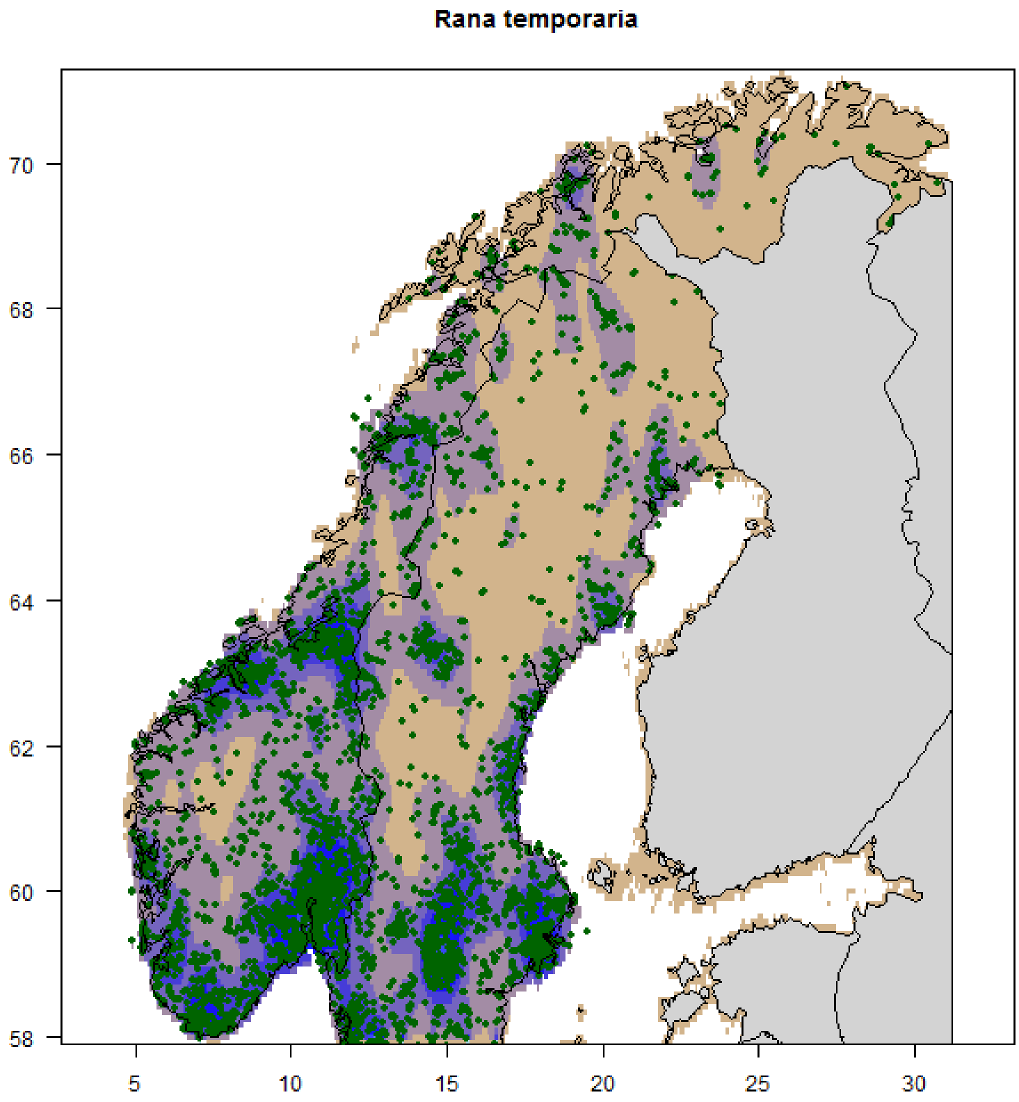


Figure 1.1.4-2: Map of all observations of wild *Rana temporaria* recorded in Norway and Sweden. Green dots denote point observations, shading indicate the density of observations by allowing overlapping points to give value to surrounding raster cells. See "Spatial points distributions" (2.4.3) for descriptions.

The most important threats to the common frog are draining/filling-in of ponds, pollution (including acidic precipitation), and urbanization with heavy traffic and isolation of breeding ponds (Dolmen, 1987).

1.1.5 Moor frog (*Rana arvalis* (spissnutefrosk))

The moor frog (Figure 1.1.5-1) *R. arvalis* (Nilsson, 1842) is found in the lowlands in southeastern and southern parts of Norway: in Østfold, Akershus and Hedmark as far north as Hamar and Rena. West of the Oslofjord its distribution stretches to Skien, and a very few records have been made in Agder (Dolmen 2008). An occurrence map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and distribution kernels, is shown in Figure 1.1.5-2.

Figure 1.1.5-1: Moor frog (*R. arvalis*). Photo: Dag Dolmen

The moor frog is smaller than the common frog; maximum length is 6-7 cm. The color is usually light brown or grey, and the belly white. In spring, males may display vivid, blue coloration. The snout is more pointed ("shark profile") than in the common frog, the light stripe along the upper jaw is a cleaner white, and the metatarsal tubercle is relatively large, flat and hard.

The moor frog prefers damp habitats. Sitting at the edge of a pond or lake, it often escapes a predator by a quick leap into the water. The species tolerates acidic water better than the common frog, and they are often found in acidic bogs, but also in eutrophic ponds and lakes in the cultural landscape. Breeding takes place in April–May, a week later than the common frog. The mating call is a bubbling "hwuk, hwuk, hwuk, hwuk" (increasing in pitch), and can be reminiscent of the sound made by air bubbles when a narrow-necked bottle is being emptied. The larvae metamorphose from mid-July. Hibernation takes place on land or in the water. Sexual maturity is reached after two or three years. The species may live up to 10 years in the wild.

Females usually lay 1 clump of spawn containing 500-2000 eggs, or sometimes more. The yearly death rate is probably similar to that of the common frog. Predators are the same as those of the common frog. The moor frog does not usually move as far away as the common frog from the breeding pond.

The most important threats to the moor frog are draining/filling-in of ponds, pollution (including acidic precipitation), and urbanization with heavy traffic and isolation of breeding ponds (Dolmen, 1987).

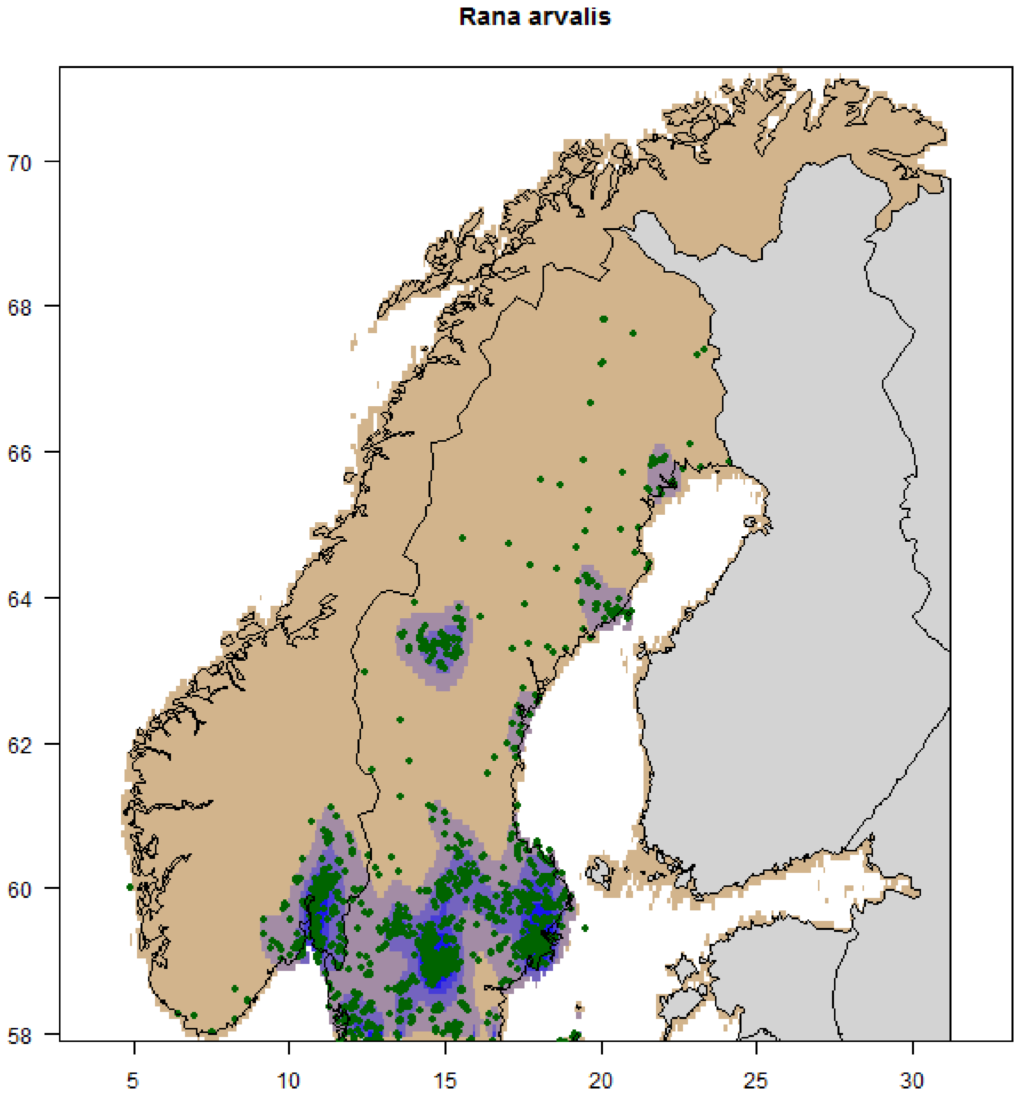


Figure 1.1.5-2: Map of all observations of wild *Rana arvalis* recorded in Norway and Sweden. Green dots denote point observations, shading indicates the density of observations by allowing overlapping points to give value to surrounding raster cells. See “Spatial points distributions” (2.4.3) for descriptions.

1.1.6 Pool frog (*Pelophylax lessonae* (damfrosk))

A small population of the (Nordic) pool frog (Figure 1.1.6-1) *P. lessonae* (Camerano, 1882) in Norway is known only from 2–3 small lakes in Agder. Although this isolated population was first discovered in 1986 (Dolmen 1996), it is believed to be naturally occurring in this area. A separate, introduced, population exists on Finnøy (see 1.1.7). An occurrence map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and density kernels, is shown in Figure 1.1.6-2.

Figure 1.1.6-1: Nordic variant of the pool frog (*P. lessonae*) from Agder. Photo: Dag Dolmen

The pool frog belongs to the “water frogs” or “green frogs”, although in Norway, the individuals are always brownish: females are light brown with darker patterns, males more olive, especially in spring. Maximum size is 6 cm for males and 7 cm for females. Both genders have a narrow, light stripe down the centre of the back. The metatarsal tubercle is large and semi-discoidal. Unlike the two brown frogs, the vocal sacs are external, at the corners of the mouth.

The pool frog is, to a much greater extent than the two other (brown) frogs, associated with water, and rarely moves far from the water’s edge. Typically, it likes to sit and bask in the sunshine at the edge, and when disturbed, makes a long leap and hides in the bottom substrate of the pond. The mating season is no earlier than mid-May or the beginning of June. The mating call consists of intense, loud croaking “woakk, woakk, woakk” and followed by a guttural bleating “wrrrrr” or “wrrrr-rææææææææ”, which, under favourable conditions, can be heard on a distance of a few hundred metres. In Agder, successful development of the larvae takes place only in warm summers, and the tadpoles are very big when they metamorphose in late August or in September. Hibernation is on land. Sexual maturity is reached after three years. Pool frogs can sometimes live for 10–12 years in the wild.

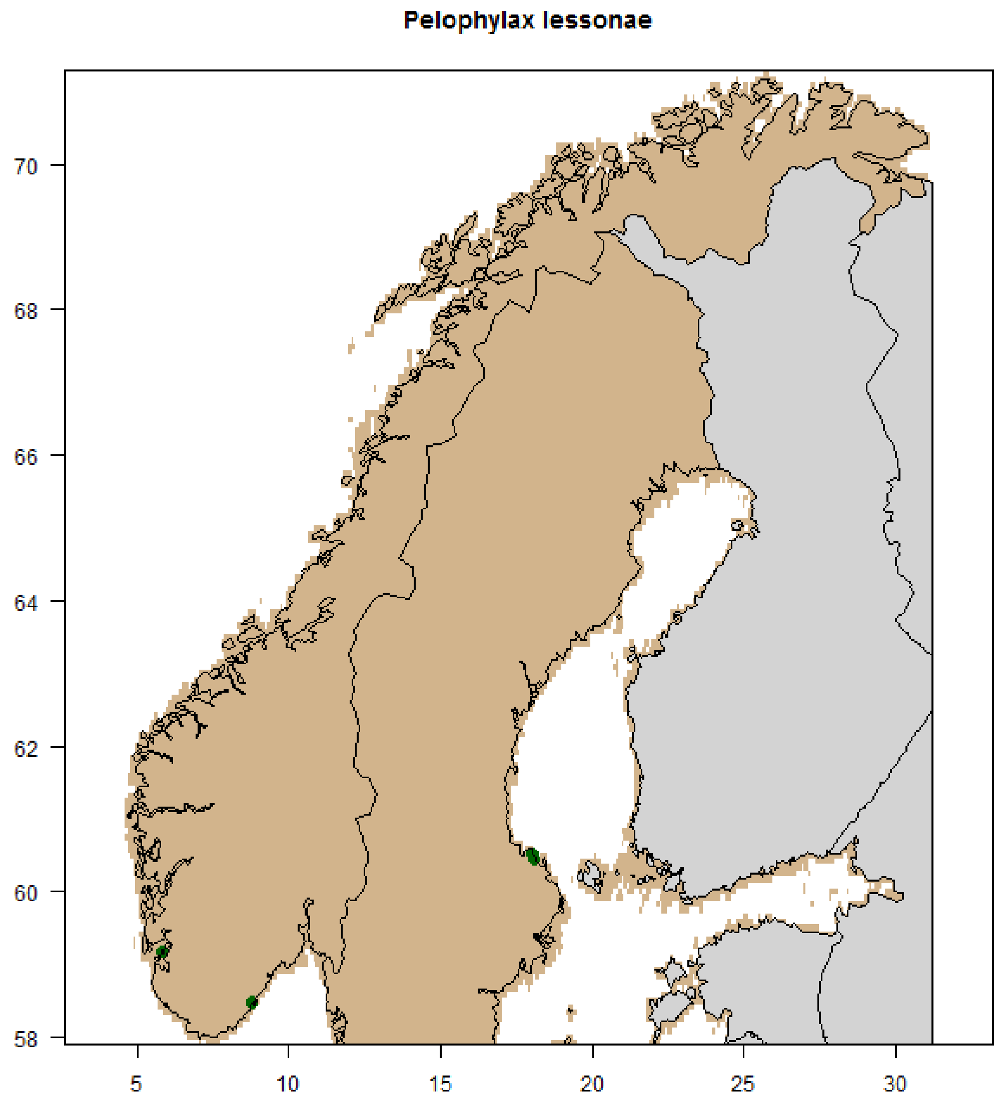


Figure 1.1.6-2: Map of all observations of wild *Pelophylax lessonae* recorded in Norway and Sweden. Green dots denote point observations. There are only two known localities of this species, as indicated on the map. Only the South-East population in Norway is indigenous.

Females lay 500–3000 eggs in a season, separated into several small clumps of spawn containing <50–100 eggs each, or sometimes more. Of newly metamorphosed froglets (in Sweden), only 1-2%, or sometimes up to 6%, reach reproductive age. Thereafter, the yearly death rate is about 55%. Enemies of the larvae are predatory invertebrates like dragonfly larvae and water beetles, and especially fish. Adults are taken by grass snakes, birds of prey and herons, and predatory mammals. The native pool frog in Agder lives at the edge of its climatic tolerance, and in cold summers, reproduction often fails (Dolmen 2012).The frogs seem to be quite strongly philopatric, and do not move far from their localities.

The most important threats to the pool frog, apart from the harsh climatic conditions, is the release of predatory fish.

1.1.7 Edible frog (*Pelophylax esculentus* (hybridfrosk))

In 2003, continental pool frogs, together with edible frogs (Figure 1.1.7-1) *Pelophylax* kl. *esculentus* (Linnaeus, 1758) from Poland, were introduced to Finnøy in Rogaland, southwestern Norway. The frogs thrived in the mild climate, and, after a few years, they had occupied all water bodies of the 7 km-long island (Dolmen 2009a; Dolmen 2009b; Holst 2011). A distribution map, showing the location of reported observations (from the Norwegian Biodiversity Information Centre) and density kernels, is shown in Figure 1.1.7-2.

Figure 1.1.7-1: Continental variant of the pool frog (*R. lessonae*) to the left, and edible frog (*P. esculentus*) on Finnøy. Both of which have been introduced from Poland. Photo: Dag Dolmen

The continental pool frog is for the most part green with black spots, not brown like the native Norwegian pool frogs (Figure 1.1.6-1). The edible frog is also green and may be difficult to distinguish from the pool frog. However, edible frogs have longer hind legs and smaller metatarsal tubercle. This edible frog is a klepton, a “hybrid species” between the pool frog and the marsh frog *Pelophylax ridibundus* (Pallas, 1771). Thus, the edible frog on the island depends on the pool frog for sexual reproduction. The mating season on Finnøy is in mid-May and June. The mating call is very similar to that of the pool frog, but more mechanical, without a tone – and sometimes with a short “laughter” at the end.

The largest recorded male of the edible frog at Finnøy has been 6.3 cm and the largest female 9.6 cm, but larger specimens have been observed. A maximum size of 12 cm has been reported from other parts of Europe. The breeding habitats at Finnøy are mesotrophic lakes and ponds, but single frogs have been found almost everywhere, including in puddles and ditches. Metamorphosis takes place in August and September, and hibernation is either on land or in the water. Sexual maturity is reached after three years. The species has been known to live for up to 14 years in captivity.

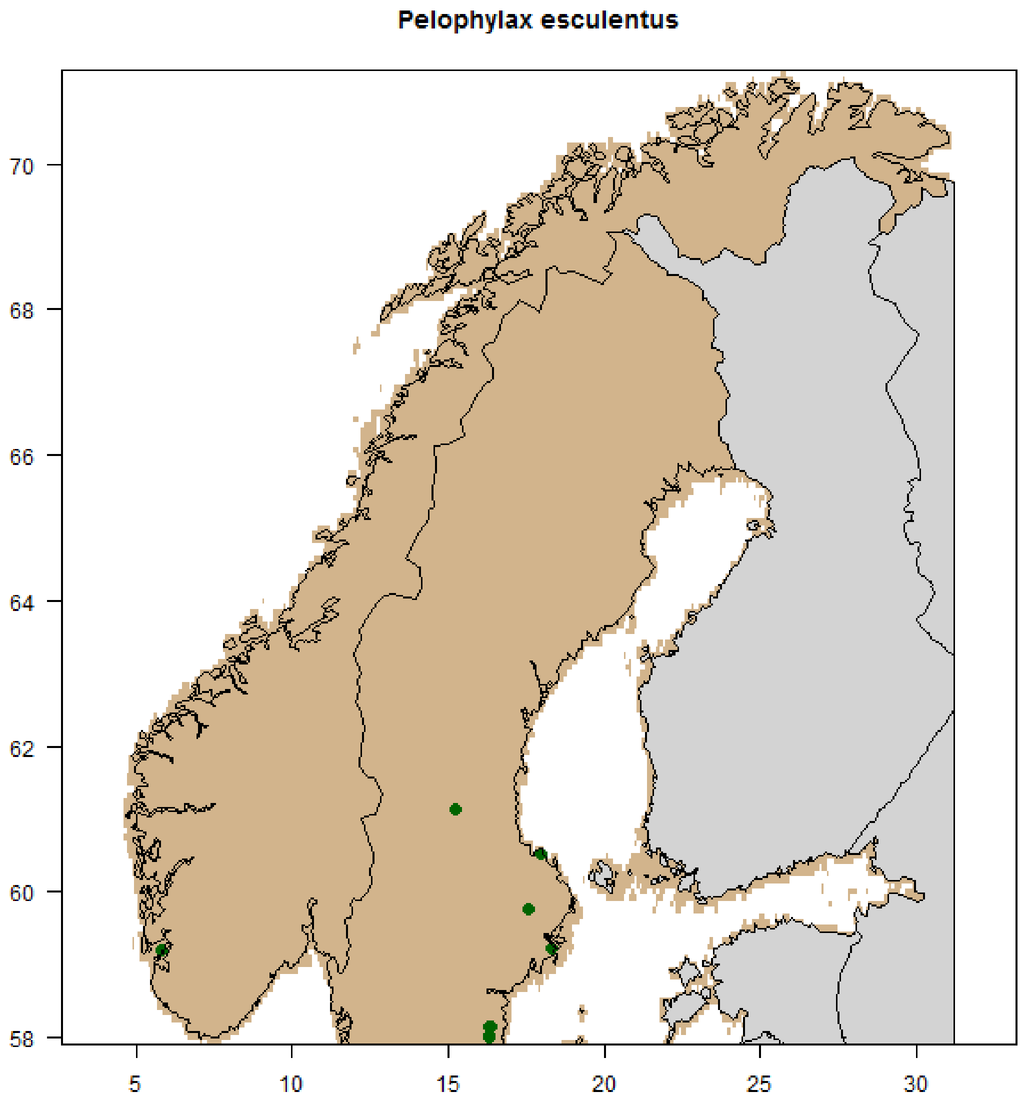


Figure 1.1.7-2: Map of all observations of wild *Pelophylax escelentus* recorded in Norway and Sweden. Green dost denote point observations. In Norway, this species is only represented by the introduced population on Finnøy.

The number of eggs is probably larger for the edible frog than the pool frog, but the death rate supposedly close to that of the pool frog. Predators are also the same. At Finnøy, there are no real threats to the populations for the moment.

1.1.8 Joint distributions

Compared with continental Europe, amphibian species diversity in Norway is low. Figures 1.1.8-1 and 1.1.8-2 show the joint distribution of all the anuran species (frogs and toads), and newts in Norway, respectively.

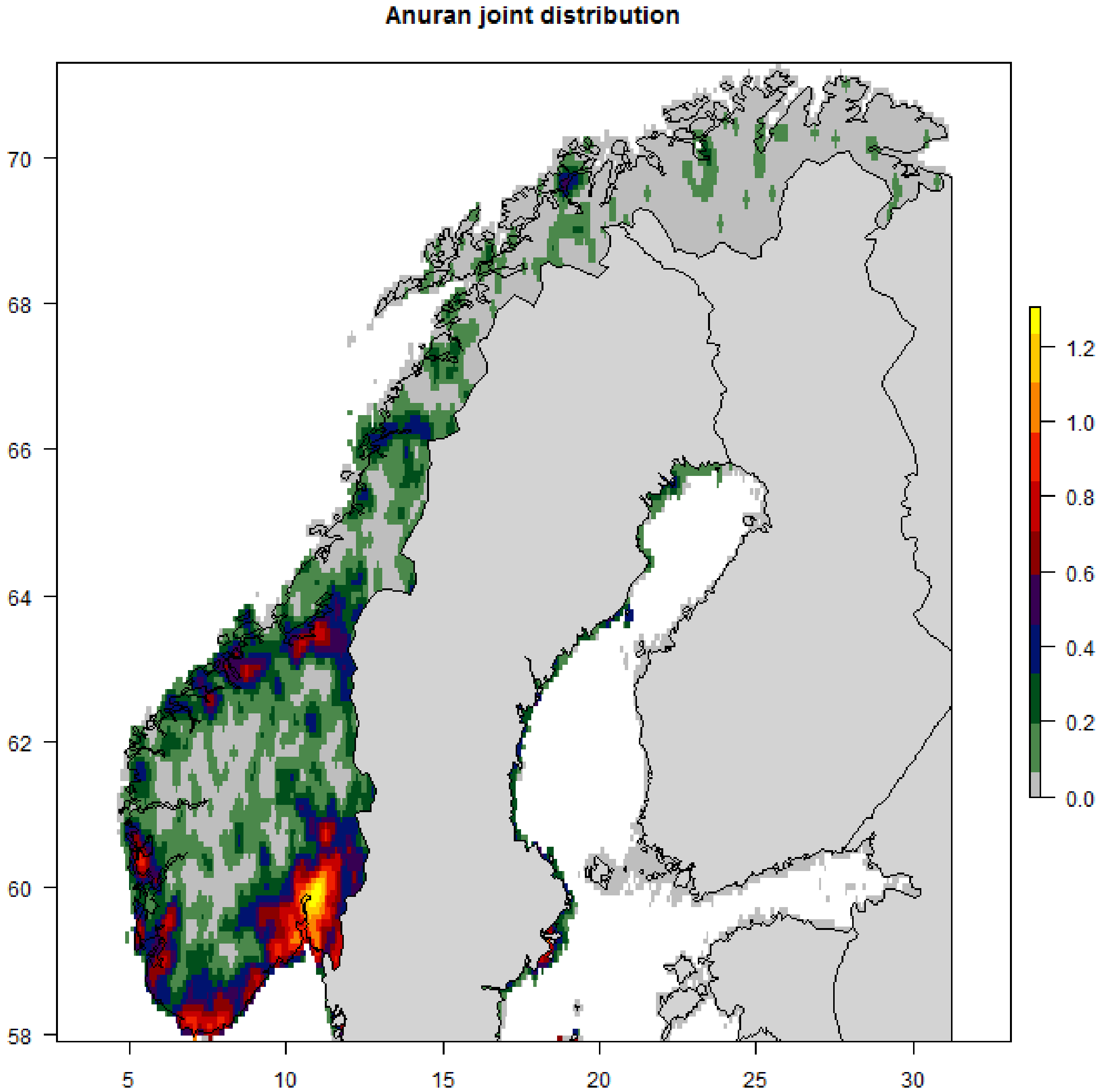


Figure 1.1.8-1: Joint relative density of all anuran species (frogs and toads) in Norway. Individual observations are not shown, spatial density has been smoothed over 5 passes is indicated by colour. See "Spatial point distributions" (2.4.3) for descriptions. This shows that the highest relative density of anurans is found around the Oslofjord, while still low compared to other European countries.

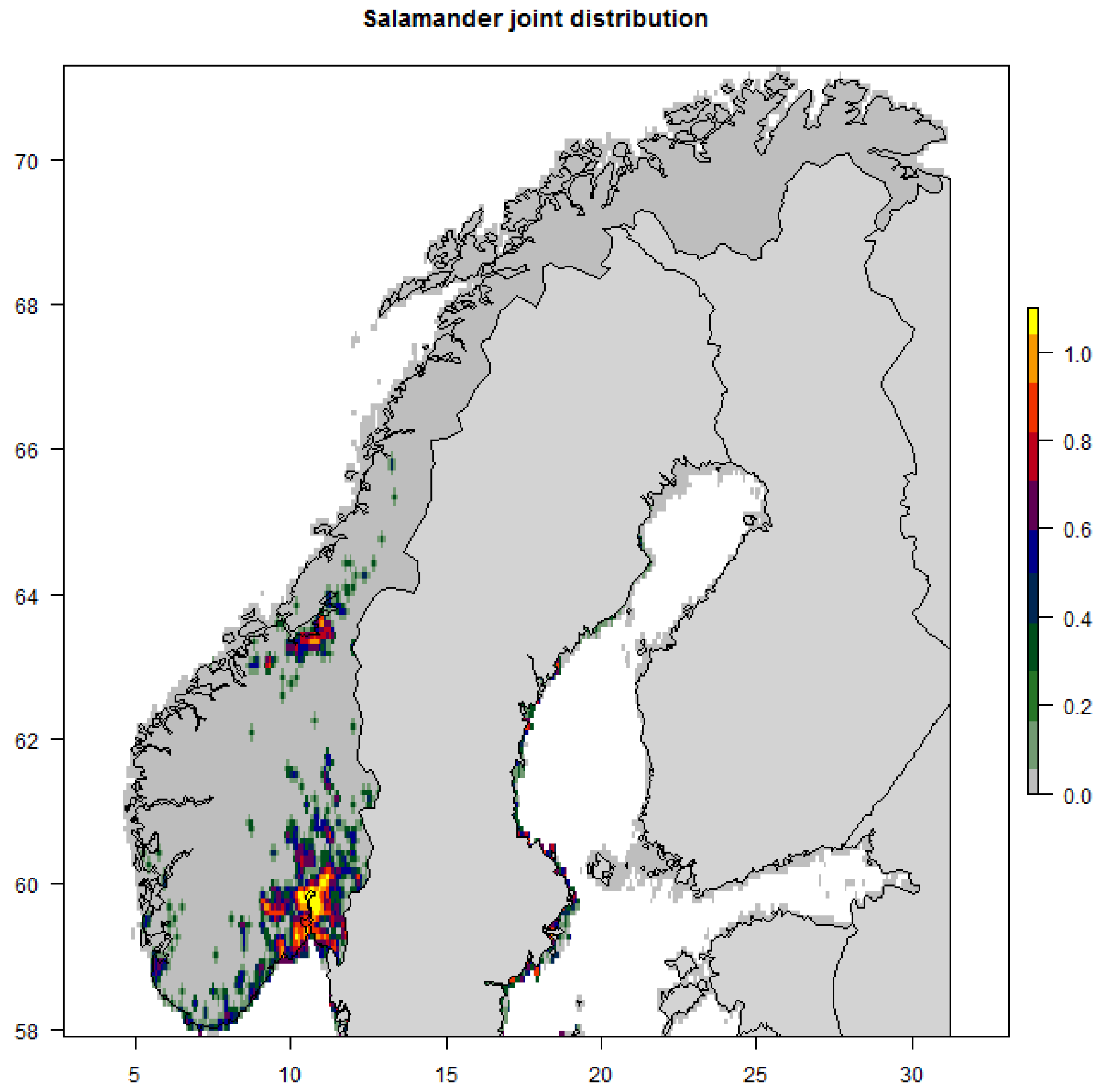


Figure 1.1.8-2: Joint relative densities of the two newts (salamanders) in Norway. Individual observations are not shown, spatial density smoothed over 5 passes is indicated by colour. See “Spatial point distributions” (2.4.3) for descriptions. This shows that the highest relative density of salamanders (newts) is found around the Oslo-, and Trondheim fjords, while still low compared to other European countries.

1.1.9 The role of amphibians in the ecosystem

Amphibians can affect ecosystem structure through soil burrowing and aquatic bioturbation, and ecosystem functions such as decomposition and nutrient cycling through waste excretion and, indirectly, through predatory changes in the food web. Thus, amphibians can influence primary production in aquatic ecosystems through direct consumption and nutrient cycling.

Amphibians also contribute to regulating disease vectors and agricultural pests (Hocking & Babbitt 2014; Khatiwada 2016).

1.2 Pathogenic fungi infecting animals

The estimated number of fungal species exceeds 5 million, but the majority have not yet been described. Fungi are major decomposers in ecosystems and essential symbionts of many organisms, but are also pathogens on a multitude of plants and animals (Blackwell 2011). Oomycetes (water moulds) are phylogenetically distant from fungi and classified as Straminopiles, together with brown algae and diatoms (Lee et al. 2012). However, they are often referred to as fungi due to their similar ecological roles and modes of nutrition, and are included in the examples of emerging fungal diseases in the subsequent text. More recent classification of fungi also includes microsporidia (Burki 2014), which are single-celled endosymbionts, parasites, or pathogens in animals.

Numerous pathogenic fungi infect animals worldwide, and many of these might have the potential to regulate and control host population densities. For example, about 1000 species of fungi are pathogens that infect and kill insects, and these have sometimes been exploited in biological pest control (Shang et al. 2015).

Some fungal pathogens of wild mammals and birds, are currently associated with emerging infectious diseases (EIDs) in humans and domestic animals (Refai et al. 2017). In many cases, the vast majority of the patients are immunocompromised individuals, but there are also examples of emerging fungal pathogens causing severe diseases and sometimes mortalities also in immunocompetent individuals (R. Wake & Govender 2016).

Fungi and fungal-like pathogens causing wildlife diseases with high case-fatality rates are less common as a cause of epizootics than pathogenic bacteria and viruses. Nevertheless, they have been associated with some of the most devastating examples of epizootics ever seen, such as amphibian chytridiomycosis, crayfish plague, and bat white-nose syndrome (WNS) (see below) (Fisher et al. 2012; Fisher et al. 2016). A major driver behind the emergence of these high-impact epizootics is anthropogenic spread of pathogenic fungi outside their natural geographical and host range, into new environments with susceptible hosts that are immunologically naïve and have insufficient innate protection.

1.2.1 Animal diseases caused by pathogenic fungi

Infectious fungi are involved in a wide variety of host associations, from mutualistic host-beneficial infections, commensal infections that normally do not cause harm to the host, and parasitic infections where the fungi exert some degree of harm, from mild, subclinical effects to clinical disease, and even death. However, the population impact can result from any infection affecting either reproduction or survival, and is not necessarily linked to dramatic signs of disease, such as severe clinical signs or high mortality. A pathogenic fungus is a fungus that can cause damage or disease in a susceptible host.

EIDs caused by fungi and oomycetes with a very high negative impact on wildlife species include chytridiomycosis in amphibians worldwide, crayfish plague in European freshwater crayfish, and WNS in American bat species, (Fisher et al, 2012 and references therein). Chytridiomycosis is covered in detail in the next section, while some other examples are briefly outlined below.

The oomycete *Aphanomyces astaci* causes mass-mortalities and drastic decline of European freshwater crayfish (*Astacus astacus*). The pathogen was accidentally introduced to Europe in the mid-1880s, and the origin was unknown for more than a century (Söderhall & Cerenius 1999). When Unestam (1972) discovered that American freshwater crayfish, specifically signal crayfish (*Pacifastacus leniusculus*), were clinically healthy carriers of *A. astaci*, Europe had already introduced numerous populations of American signal crayfish and two other *A. astaci*-carrying American crayfish species into the wild (Grandjean et al. 2014). The only strain of *A. astaci* (the A-genotype) that is now avirulent to European crayfish was the first strain that arrived Europe, and the only strain that is absent from its natural American host in European waters. The remaining four genotypes are all associated with American crayfish invasive in Europe (Grandjean et al., 2014). These remain highly virulent and have caused mass mortalities and drastic declines of European freshwater crayfish populations.

The fungus *Pseudogymnoascus destructans* has caused WNS and mass mortalities in a number of North American bat species since its emergence in 2006 (Cryan et al. 2013) (Zukal et al. 2016). The psychrophilic fungus is believed to infect bats during hibernation, but whereas WNS is responsible for an unprecedented decline in North American bat populations, skin lesions in bats caused by *P. destructans* have been confirmed in many European countries without any reports of mass mortalities. Thus, European bat species might be the natural reservoir of the fungus, supported by tests confirming that European *P. destructans* isolates are pathogenic for North American bats (Cryan et al. 2013). Zukal et al. (2016) found high prevelances of *P. destructans* infections in bat populations not only in Europe, but also on the West Siberian Plain in Asia, and concluded that the natural reservoirs of the fungus include the whole Palearctic region.

Snake fungal disease has been spreading rapidly among snakes in North America since its discovery in 2006. It is caused by *Ophidiomyces ophiodiicola,* and characterized by a necrotizing skin disease, which is often fatal, in a broad range of snake species over large areas of North America. It typically manifests when they emerge from hibernation in the spring (Lorch et al. 2016). The reason for emergence of this disease is unknown, but the distribution pattern does not indicate that the pathogen has been recently introduced. On the contrary, previous reports about “hibernation sores” might indicate that the pathogen was present in North America before 2006, and that the increased disease incidence is associated with environmental change and/or changes in host susceptibility (Lorch et al. 2016).

Examples of severe mammalian and avian fungal diseases include coccidioidomycosis and cryptococcosis. The former is caused by the fungi *Coccidioides immitis* and *Coccidioides posadasii* (Saubolle et al. 2007). In humans, the disease is referred to as valley fever and involves potentially fatal pneumonia. It is considered emerging and endemic to semi-arid regions in south-western US and northern Mexico. *Coccidioides* species also infect and cause disease in terrestrial and aquatic wild and domestic mammals, reptiles and birds (del Rocío Reyes-Montes et al. 2016). Cryptococcosis, caused by the pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*, is a lethal fungal disease reported to be emerging in immunocompromised humans, but is also found to cause disease in domestic and wild mammals and birds (Refai et al. 2017).

1.2.2 Definition of, and distinction between, disease and outbreak

Disease can be defined as any impairment that interferes with or modifies the performance of normal functions and/or length of life (Wobeser 2007). The same fungal species may infect a resistant, non-susceptible host species where no clinical disease or mortality is observed. It is therefore important in the context of this report, to distinguish between the pathogen itself and the disease it causes. For example, *Bd* and *Bsal* can infect a number of amphibian species without causing clinical signs of the disease chytridiomycosis. Detection of the fungus itself is therefore not proof of disease nor of a negative impact in the associated population. Confirmation of a disease diagnosis requires more data, including the combination of detection of *Bd* or *Bsal* DNA in the skin of the amphibian combined with observation of clinical disease, death of animals with pathological lesions consistent with disease, and/or indirect evidence (e.g., population decline) (OIE, 2018).

Current diagnostic methods of *Bd* are covered in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2018). Unless there is a high mortality rate, there are few, if any, reliable field diagnostic methods. Clinical signs are absent in most animals until shortly before mortality occurs, although some behavioral changes might be observed (see 1.4.1.2). There are numerous diagnostic methods, but the gold standard that is routinely used, and regarded as the best method fit for purpose (i.e., targeted surveillance, presumptive diagnosis, and confirmatory diagnosis), is a well-described and validated TaqMan qPCR method (Boyle et al. 2004; Hyatt et al. 2007). For *Bsal*, OIE has not (yet) made a diagnostic manual. Here, several qPCR protocols have been described. However, non-invasive sampling protocols that have been developed for *Bd* diagnosis (Hyatt et al. 2007; Skerratt et al. 2008) are currently used to collect samples from live wild and captive amphibians for testing for the presence of *Bsal*..

The disease chytridiomycosis is an infectious disease in amphibians caused by the chytrid fungi *Bd* and *Bsal*. The factors that determine whether the infectious agents (*Bd* and *Bsal*) will cause disease development within an animal include and the strain virulence, the host immunity or susceptibility, and environmental factors (climate, abiotic and biotic factors) that might suppress or promote virulence in the fungi and/or immunity in the hosts. Thus, species of amphibians may resist or tolerate the infection, or the environment or other factors may prevent sub-clinical infection resulting in disease.

The term outbreak is used as a synonym of the term epizootic, and refers to the occurrence of more cases of disease than expected in a given area or among a specific group of animals over a particular period of time (the same definition as for «epidemic» in humans) (https://www.cdc.gov/reproductivehealth/data_stats/glossary.html). When an epizootic spread over a wide area involving several countries, it is, by definition, a panzootic (equivalent to pandemic in humans). The term *Bd*-GPL (Bd-global panzootic lineage) reflects that isolates from this phylogenetic intraspecific lineage have been involved in a number of chytridimycosis outbreaks worldwide (O'Hanlon et al. 2018).

It is important to distinguish between a disease outbreak in a population and detected prevalence (*Bd*- or *Bsal*-positive individuals) without clinical signs of disease. In the first case, a large number of individuals in a particular period and area will die or show severe sign of chytridiomycosis. In the latter case, diagnostic tests will detect *Bd* or *Bsal* in amphibian populations in the absence of visual disease or long-term declines; this seems to be common in amphibian populations in UK and Sweden (Smith 2014; Höglund*,* pers. comm.), and is also the situation that has been briefly observed in Norway (Taugbøl et al. 2017).

1.3 Pathogenic fungi of amphibians

With the exception of chytridiomycosis caused by *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*)*,* there are no pathogenic fungi of amphibians that are associated with high morbidity, mortality, or population impact, although a few examples of moderate impact have been reported. Oomycetes are associated with amphibian egg mortality worldwide (Rutig 2009). For example, *Saprolegnia* infections causing egg mortality affected a local *Bufo boreas* population in northwestern United States (Berger et al. 2009). Furthermore, infection trials showed that *Saprolegnia* and *Leptolegnia* isolates acquired from *Rana catesbeiana* and *Pseudacris crucifer* eggs caused elevated mortality rates (up to 40%) in eggs of these species (Ruthig 2009) The fungus *Mucor amphibiorum* causes a fatal disseminated disease in amphibians, referred to as mucormycosis, causing granuloma formation in most organs. Outbreaks of mucormycosis have been reported in captive amphibians, but not in the wild where it appears to only cause sporadic infections (Berger et al. 2009).

1.3.1 *Batrachochytrium dendrobatidis* (*Bd*)

Batrahcochytrium dedrobaditis (*Bd*) is a chytrid fungus that can cause fatal chytridiomycosis in freshwater amphibian species. This disease was first observed in Australia in the 1970s, and has later been associated with global population decline. Local, and even regional and global, extinctions of amphibian species, demonstrate that *Bd* is one of the most devastating emerging wildlife pathogens ever known (Olson et al. 2013; Brannelly et al. 2015; Skerratt et al. 2016; Scheele et al. 2019). The source of *Bd* was unknown for a long time, but a global genome-wide approach has traced the origin of *Bd* to East Asia, probably the Korean peninsula (O'Hanlon et al. 2018). The authors date the emergence of *Bd* to the early 20th century, which corresponds well with the global expansion of commercial trade in amphibians and the occurrence of known global amphibian declines (Scheele et al. 2019). East Asia seems to be the geographic hotspot for biodiversity of *Bd* strains, and the original source of the pandemic lineages that today are decimating amphibian populations worldwide (O'Hanlon et al. 2018). Dramatic amphibian declines resulting from chytridiomycosis have occurred mainly in the tropics of Australia, Mesoamerica, and South America, while other parts of the world including the warmest (Asia and Africa) and the coolest (Europe and North America) have, despite widespread occurrence of *Bd,* very low numbers of declines to date that can be attributed to chytridiomycosis (Scheele et al. 2019), although lack of data from regions such as Africa has been noted.

1.3.1.1 Taxonomy and strains

The current classification of *Batrachochytrium dendrobatidis* according to MycoBank database (http://www.mycobank.org): Fungi, Chytridiomyceta, Chytridiomycota, Chytridiomycotina, Rhizophydiomycetes, Rhizophydiales, *Batrachochytrium*

Batrachochytrium dendrobatidis (Bd) was first isolated by Berger et al. (1998), and taxonomically described in 1999 by Longcore et al. (1999). This established not only a new species, but also a new genus within the order Chytridiales (now Rhizophydiales) that differed markedly from other chytrid fungi within this order on the basis of characters visualized by transmission electron microscopy (Longcore et al. 1999). Berger (1998) had proposed this fungus as the causal agent of the emerging disease in amphibians, and the pure culture strain used to confirm this in experimentally exposed frogs (and also fulfilling Koch's postulates about causality between microorganism and disease), was originally isolated from a captive collection of frogs within the genus *Dendrobates* at the US National Zoo (Berger et al. 1998). This is the reason for the species name 'dendrobatidis', while "batracho" means frog in Greek (Longcore et al. 1999; Smith 2014).

From the disease emerging in the early the 1970s in Australia and America (Berger et al. 2009), the geographic origin has been heavily discussed in the literature, with suggestions including Africa, North America, South America, Japan, and East Asia (O'Hanlon et al. 2018). Farrer et al. (2011) established that the global chytridiomycosis panzootic can be attributed to the single *Bd*-GPL-lineage (global panzootic lineage). They found that isolates belonging to the phylogenetic clade *Bd*-GPL emerged globally during the 20th century, and are associated with amphibian epizootics in North America, Central America, the Caribbean, Australia, and Europe. Farrer et al. (2011) further demonstrated two other lineages of *Bd* on the basis of genome analyses: *Bd*-CAPE including two strains form South Africa and Mallorca, and *Bd*-CH including only one strain originating from Switzerland. Both *Bd*-Cape and *Bd*-CH were described as hypovirulent, since *Bd*-CAPE resulted in very low mortality rates in amphibian-exposure experiments compared with *Bd*-GPL. For *Bd*-CH, there was a lack of association between infection status and population decline (Farrer et al. 2011). In a whole-genome sequencing (WGS) study on *Bd*, O'Hanlon et al. (2018) analyzed a panel of more than 200 isolates, revealing a total of five *Bd* lineages. These were: *Bd*-GPL, *Bd*-CAPE, *Bd*-CH, *Bd*-ASIA-1, *Bd*-ASIA-2 / *Bd*-BRAZIL. In addition to these, a few hybrids were also recognized. The authors traced the origin of *Bd* to a hyperdiverse hotspot in the Korean peninsula. Here, according to the authors, the lineage *Bd*-ASIA-1 exhibits the genetic hallmarks of an ancestral population that seeded the panzootic. They dated the emergence of *Bd* to the early 20th century, which is coincident with the global expansion of commercial trade in amphibians (O'Hanlon et al. 2018) and losses of amphibians ascribed to chytridiomycosis.

In the study from O'Hanlon and co-authors (2018), *Bd*-GPL massively dominates and constitutes 187 of the 200 isolates analysed. The vast majority of European isolates are *Bd*-GPL, although a few European isolates were *Bd*-CAPE. In Sweden, the few isolates examined belonged to *Bd*-GPL (O'Hanlon et al. 2018). In UK, eight *Bd*-isolates have been sequenced and identified as *Bd*-GPL. This is consistent with the *Bd*-introduction to UK in the 20th century (Smith 2014). However, these isolates were collected either from natterjack toads (*Epidalea calamita*) bred for reintroductions, or from sites of non-native amphibian introductions, both of which represent populations associated with anthropogenic activity (Smith 2014) . Thus, Smith (2014) do not rule out the possibility that an endemic genotype of *Bd* in UK could be associated with the native amphibian species with low *Bd*-prevalence. Valenzuela-Sanchez et al. (2018) have noted the high similarity between isolates of *Bd*-GPL and those found in Chile, suggesting recent transmission between UK and South America (Valenzuela-Sánchez et al. 2018).

1.3.1.2 Host specificity

With the ability to infect hundreds of amphibian species worldwide, *Bd* is regarded as a generalist fungal pathogen (Skerratt et al. 2007; Olson et al. 2013; A. R. Ellison et al. 2017; Scheele et al. 2019). The host species range from highly susceptible to highly resistant, and plausible indications of *Bd*-infections without any clinical signs of chytridoimycosis have also been reported in reptiles (Kilburn et al. 2011) and crustaceans (McMahon et al. 2013).

According to the World Organization for Animal Health (OIE) Manual of Diagnostic Tests for Aquatic Animals (OIE 2018), *Bd* has been identified on six continents, from two amphibian orders, 14 families, and in over 350 species. However, Olson et al. (2013) report that *Bd* has been detected in a total of 516 out of 1240 (42%) investigated amphibian species globally, and, according to Lips et al*.* (2016), has been detected in more than 700 amphibian species (Lips 2016). Scheele et al. (2019) report that chytridiomycosis has contributed to the decline of at least 501 amphibian species. This is the greatest documented biodiversity loss attributable to a single pathogen. The OIE manual states that most anurans and urodeles are susceptible to *Bd* infection, but morbidity and mortality vary among species. Mortality in tadpoles has not been reported, and viable *Bd* has not been detected on eggs (OIE, 2018). As only the oral discs of tadpoles and larval stages of anurans are keratinized (Pessier et al. 1999; Smith 2014), *Bd* only infects the mouth during these life stages, presumably causing less damage.

1.3.1.3 Mode of infection

The infective units of *Bd* are small, flagellated zoospores (see section 1.3.1.5 Lifecycle below). The zoospores seem to find a suitable host by chemotaxis (Van Rooij et al. 2015). There is evidence indicating that keratin or components thereof act as chemotactic factors, but it has also been suggested that sugar components of amphibian mucus could play a role (Van Rooij et al. 2015). How zoospores of *Bd* attach to a host and establish infection in the amphibian skin has yet to be definitively determined. The zoospores form thick-walled cysts that adhere to the superficial epidermal cells with fine fibrillar projections and rhizoids (Van Rooij et al.

2015). From the cysts, a tubular structure develops that penetrates the epidermal cells, allowing transfer of genetic material from the cyst into the cell. The infecting germtube forms a swelling at its end and develops into a thallus (Van Rooij et al. 2012). In some susceptible hosts, rhizoid-like projections are formed from this first thallus. These penetrate cells in deeper layers, swell up, and finally seed off new intracellular thalli. The epidermal cells are pushed towards the surface, and the thalli mature into several zoosporangia, and finally release new zoospores through discharge tubes when the cells cornify on the epidermal surface (Longcore et al. 1999). Bd seem to be dependent on keratinized stratified squamous epithelium to establish an infection. Consequently, while adult amphibians can get the infection over large parts of their body surface, the infection in tadpoles is restricted to the only part of their body that has an external keratinized layer, the mouthparts, or oral discs (Pessier et al. 1999; Smith 2014). Notably, in less susceptible hosts, like *Xenopus laevis*, there is some evidence indicating that the thalli are not seeded intracellularly in deeper cell layers of epidermis, but that the whole host-dependent part of the lifecycle takes place on the surface of stratum corneum (Voyles et al. 2011). In highly susceptible species, incubation times during exposure experiments have been found to vary from 9 to 83 days, with most frogs dying between 18 and 70 days post-exposure (Berger et al. 2009). In addition, time until death varies with fungal dose and fungal strain. However, laboratory conditions with constant temperatures and small volumes of still water and microbial populations of reduced diversity may result in higher mortality rates than in the wild (Berger et al. 2009; Bates et al. 2018).

1.3.1.4 Lifecycle / persistence

The majority of chytrid fungi reproduce by forming asexual reproductive motile zoospores with a single, posterior flagellum. For *Bd*, the lifecycle consists of two stages; 1) the motile, waterborne short-lived zoospores for dispersal, and 2) the stationary zoosporangium producing the asexual zoospores. The lifecycle is illustrated in Figure 1.3.1.4-1. If a zoospore attaches to a suitable substrate, it starts forming a simple thallus anchoring the fungus to the substrate by root-like rhizoids for nutrient absorption (Voyles et al. 2011). The fungal thallus expands by mitotic divisions, creating a multinucleate fungal body that, at maturity, develops the zoosporangium. Here, zoospores at variable amounts - from several to hundreds - are produced, depending on the sporangium size. After release of zoospores through discharge papillae (1 to 6 have been observed), only the walls of the empty, clear sporangia remain. Those of the discharged zoospores that encounter a suitable substrate will start a new cycle (Berger et al. 2011, and references therein).

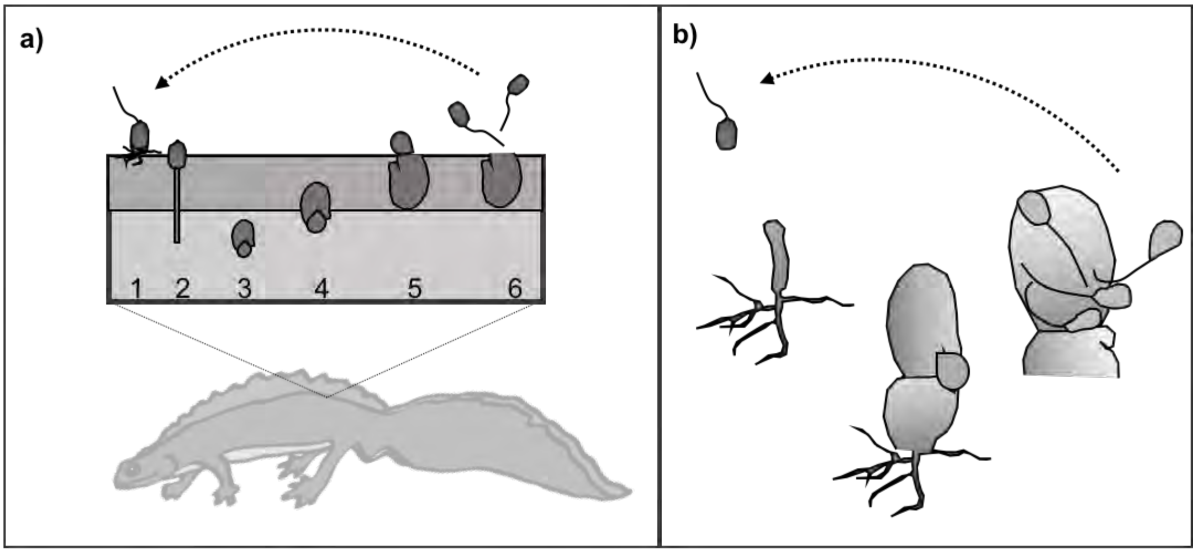


Figure 1.3.1.4-1: asexual life cycle for Bd on a) amphibian hosts and b) outside the hosts. Figure a illustrates 1) a waterborne, short lived zoospore that has found and attached itself to a host in the epidermic layer (stratum corneum which is containing keratin, represented by dark grey color) and reabsorbs the flagellum, 2) it penetrates the skin, until it reaches the deeper layers (stratum granulosum and stratum mucosum, represented by light grey color), where it 4) develops at the same speed as the cell layers are being pushed to the outer layer of epidermis and are represented at the skin surface as it 5) releases a plug that again 6) releases new mobile zoospores. The zoospores can infect novel amphibians or re-infect the same host (Berger et.al. 2005). Figure b) illustrates the life cycle of *Bd* as it is known from laboratory cultures, where it has been found that the attached zoospore can reabsorbs its flagella and develop in to fully grown zoosporangia within 4-5 days at 20°C (Berger et. al. 2005).

The cycle from dispersed zoospores, via formation of zoosporangium to production of new zoospores, takes about 4-5 days at 23°C in culture (Longcore et al. 1999). In natural environments, a mature zoosporangium may stay dormant for longer periods under dry conditions unfavourable for zoospore release (Voyles et al. 2011). The small *Bd* zoospores (3-4 µm in diameter with 19-20 µm flagellum) are motile, but with a short dispersal distance. It is assumed that large-scale dispersal occurs by passive water transport or by vectors moving thalli-containing substrates. Zoospores remain motile for hours, maybe even days, depending on temperature, before they encyst (Voyles et al. 2011).

In contrast with other close relatives of chytrid fungi, a single *Bd* zoospore does not form a single zoosporangium, but several abutting zoosporangia where each has a separate discharge papilla – so called "colonial sporangia" (Longcore et al. 1999). Colonial sporangia are one of the diagnostic features, aiding identification of *Bd* in fresh skin or in stained sections (Voyles et al. 2011). The *Bd* fungus seems well adapted to living in amphibian skin, where the sporangium initially lives inside deeper epidermal cells, then developing in parallel with the maturing amphibian cells as they are pushed outwards (Voyles et al. 2011). Thus, the sporangium initially grows in living cells, but completes its development in keratinized dead amphibian cells, soon shed from the surface. Here, the discharge tubes penetrate the epidermal cell membranes and open onto the surface of the skin, facilitating release of zoospores into the ambient water. These specialized adaptations suggest that *Bd* has a long evolutionary history with amphibian hosts (Voyles et al. 2011).

Sexual reproduction is rarely observed in chytrid fungi. It may occur if zoospores fuse with each other, or with sporangia or rhizoids. This can result in formation of thick-walled resting spores, which are not only long-lived, but also resistant to high temperatures and may survive for decades. However, studies in culture along with molecular evidence from multi-locus and genome sequencing, support *Bd* as a fungal species of primarily asexually producing clones (Berger et al. 2011 and references therein). The persistence of *Bd* in the absence of live hosts is therefore believed to be limited.

1.3.1.5 Virulence

A pathogen is defined as a microorganism that causes, or can cause, disease or damage to a host. Pathogenicity is the capacity of a microbe to cause disease or damage in a susceptible host, while virulence describes the degree or quantitative measure of pathogenicity. Importantly, while the capacity of pathogenicity and virulence are attributes of the microorganisms, the manifestations of disease or damage are also highly host-dependent. Thus, pathogenicity and virulence are microbial variables that only can be expressed in susceptible hosts (Pirofski & Casadevall 2012).

Virulence factors refer to the properties of a microorganism that enable it to harm a host and enhance its potential to cause disease. Examples include mechanisms for adhesion, colonization, and invasion of host tissues, host immune response inhibitors, and toxins for cell damage and immunotoxicity. The virulence factors of bacterial pathogens have been described in detail at the molecular and cellular level (Cross 2008; Chen et al. 2011), while virulence factors in fungal pathogens, such as *Bd* and *Bsal,* have been less studied and therefore our understanding is less complete (Fisher et al. 2016). Traits of *Bd* linked to virulence include zoospore production (Langhammer et al. 2013), zoosporangium size (Fisher et al. 2009), ability to inhibit growth of host immune cells (Fites et al. 2013), and production of immunotoxic compounds (Piovia-Scott et al. 2015).

The impact of a disease can be viewed as an interaction between characteristics of host, pathogen, and environment (the ‘epidemiologic triangle’), and epizootic events are often driven by the emergence of hyper-virulent strains of a pathogen (Piovia-Scott et al. 2015). This is also the case for *Bd*, where the global chytridiomycosis panzootic is attributed to the single *Bd*-GPL-lineage that contains the most deadly and high-virulence *Bd* isolates (Farrer et al. 2011; Rosenblum et al. 2013). In this lineage, there is genetic evidence for loss-of-heterozygosity events compared with other less-virulent *Bd*-lineages (Farrer et al. 2011), and other identified gene families might also have a role in *Bd* virulence (Joneson et al. 2011; Rosenblum et al. 2012; Farrer et al. 2013).

The virulence of *Bd* varies among isolates and phylogenetic lineages (Fisher et al. 2009; Farrer et al. 2011). Even the within-strain virulence may change rapidly during serial passages through artificial culture conditions (Ellison et al. 2017). The virulence of *Bd*-GPL strains is markedly affected by environmental factors, such as temperature, skin microbiota and water microfauna (see 1.3.1.9). Also, the virulence of different *Bd*-GPL isolates varies greatly, even within the same amphibian host. Piovia-Scott et al. (2015) conducted a field survey during a massive amphibian epizootic in Cascades frog (*Rana cascadae*), a montane species that ranges from southern Canada to northern California. Previous studies had shown that, despite dramatic *R. cascadae* declines in California's Cascades mountains thought to be caused by *Bd*, the pathogen was widespread in the whole range of *R. cascadae* without evidence for universal declines (Piovia-Scott et al. 2015 and references therein). The large variation in the effect on *Bd* in populations of this species could involve environmental effects on *Bd* virulence. However, Piovia-Scott et al. (2015) were able to document a link between a dramatic decline in juveniles of *R. cascadae* in northern California and the emergence of a hypervirulent and highly immunotoxic strain of *Bd* isolated in the middle of the decline in an affected lake. Another *Bd*-isolate with much lower virulence was obtained in the same year from the same species, but in a population that did not experience a dramatic decline during the same period. The hypervirulent isolate showed higher immunotoxicity, i.e., inhibition of lymphocyte proliferation and induction of apoptosis, and higher optical density in liquid culture than the less virulent isolate. Both isolates were within the *Bd*-GPL lineage, but the more virulent isolate seemed to experience more rapid genomic evolution, showing high rates of polysomy in specific regions of the genome and elevated LOH, compared with other genome-sequenced *Bd*-GPL isolates. The findings underline that even within the *Bd*-GPL lineage, the virulence between strains can vary drastically. The results also support the theory that the lethal effect of *Bd* on host organisms is linked to the pathogen's ability to inhibit critical immune functions, and suggests that emergent phenotypes expressing enhanced immunotoxicity may drive amphibian declines (Piovia-Scott et al. 2015).

Several factors are believed to contribute to variation in the pathogens virulence, but also the host responses. For infection-tolerant host species, which seem to dominate in Norway, as based on other European studies, there is evidence supporting that innate immune responses are involved (Smith 2014). More specifically, this appears to be associated with species-specific anti-microbial secretions produced by granular glands present in the amphibian skin (Rollins-Smith & Conlon 2004) (Woodhams et al. 2007). Also, *Bd*-antagonistic symbiotic bacteria that are found within normal amphibian skin microbiota appears to mediate a defense barrier against the pathogen (Woodhams et al. 2007; Bletz et al. 2018; S. Ellison et al. 2018), which can keep the *Bd*-prevalence low and contribute to suppression of disease development.

1.3.1.6 Prevalence and effects on different amphibian species

Bd is known primarily for its association with devastating amphibian declines, and even total eradications of amphibian species globally, for example in the Neotropical genera *Atelopus*, *Craugastor*, and *Telmatobius* (Scheele et al. 2019).However, it is important to address that several amphibian species can carry *Bd* infection, without showing signs of from chytridiomycosis. The terms "resistant" and "tolerant" are used in the literature, but some authors use "*Bd*-resistant" when *Bd* has not been observed to infect the species, and "*Bd*-tolerant" has been used when *Bd* infects, but there is no progression to a clinical state of chytridiomycosis (Van Rooij et al. 2015). The distinction between "tolerant" and "resistant" is important when predicting ecological outcomes of host-pathogen co-evolution. This is exemplified by the discovery of tolerance to WNS in certain bat populations, suggesting that tolerance to even extremely virulent fungal diseases may emerge (Langwig et al. 2017), giving these populations the ability to act as reservoirs and carriers.

The most common examples of *Bd*-tolerant species are North American bullfrog (*Lithobates catesbeianus*) and the African clawed frog (*Xenopus laevis*), which have both been demonstrated to be healthy carriers of the infection without developing sign of the disease (Daszak et al. 2004). When introduced to areas outside their natural range, these species have been involved in spreading and maintenance of *Bd*, serving as non-affected reservoirs of the fungus (Schloegel et al. 2012). A number of European amphibian species also seem generally unaffected by *Bd* infection, e.g. common frog (*R. temporaria*) in UK (Smith 2014). The absence of reports of severe disease in European species, in particular those found in Norway, might indicate that these species also show some degree of tolerance or resistance to *Bd*. Below, we cover specifically what is known regarding the prevalence and effects of *Bd* on amphibian species found in Norway. Most of the information originates from a PhD-thesis (Smith 2014), that presents extensive cross-sectional surveys of *Bd*-prevalence and epidemiology in UK (England, Scotland, and Wales) in 2008 and 2011.

- *Lissotriton vulgaris* (smooth newt) was found to carry the *Bd* at very low prevalence in nature in UK, with only 2.9 % and 1.7 % of 1824 and 814 individuals screened in 2008 and 2011, respectively (Smith 2014). A follow-up infection trial demonstrated that even at high doses of *Bd*-GPL zoospores, only a very low prevalence occurred in the exposed individuals, and no *Bd*-associated mortality could be demonstrated (Smith 2014).

- *Triturus cristatus* (great crested newt) was found to carry the pathogen at extremely low prevalence in nature in UK. Only 0.2 % (= 1 individual) was *Bd*-positive out of 577 individuals screened in 2008, and none of the 315 individuals screened were *Bd*-positive in 2011 (Smith 2014).

- *Bufo bufo* (common toad) was found to carry the pathogen at low to very low prevalence in nature in UK, with only 4 % and 2 % *Bd*-positive individuals of 1182 and 503 screened in 2008 and 2011, respectively (Smith 2014). In southern Sweden, a recent study also observed low *Bd* prevalence, from 1.5% - 3.5%, in the common toad (Kärvemo et al. 2018). *Bd*-infection leading to lethal chytridiomycosis has been reported in the common toad in a national park in Spain (Bosch & Martínez-Solano 2006). However, there was no mass mortality, and dead individual common toads were only found at one location, despite several locations being infected. Here, less than 50 post-metamorphic toads were found dead or ill each year of the study, and these individuals shared refuges with hundreds of healthy animals (Bosch & Martínez-Solano 2006). In Sweden, body condition index of *B. bufo* was negatively associated with *Bd* infection (Kärvemo et al. 2019). In laboratory experiments, *B. bufo* were unaffected by exposure to *Bd* as larvae, but experienced substantial mortality (<40% survival) when exposed to the fungus after metamorphosis as juveniles (Clare et al. 2016; Meurling, Bengtsson, Höglund, Laurila; pers. comm.)

- *Rana temporaria* (common frog) was found to carry the pathogen at very low prevalence in nature in UK. Only 0.5 % (= 2 individuals) were *Bd*-positive out of 394 individuals screened in 2008, and none of the 15 individuals screened were *Bd*-positive in 2011. The low number of individual frogs screened relates to two factors: 1) the species is the earliest of UK amphibians to emerge from hibernation, and the breeding peak was outside the survey period, and 2) a previous study (Baláž et al. 2013) had demonstrated that *Bd*-infection in common frog was very uncommon. Thus, maximizing the sample size was not prioritized in the Smith (2014) survey. In Sweden, the prevalence of *Bd* in common frog is also low (Kärvemo et al. 2018, Meurling and Höglund; pers. comm., Fig. 1.3.1.6-1).

- *Rana arvalis* (moor frog) has only been studied in Sweden regarding *Bd*-prevalence. Here the prevalence was moderate and above 10% (Kärvemo et al. 2018). Experimental infections showed no effects on moor frogs from southern Sweden, but limited effects (90% survival) on frogs from the northern part of the country (Meurling, Bengtsson, Höglund, Laurila; pers. comm.)

- *Pelophylax lessonae* (pool frog) was found to carry the pathogen at moderate to very high prevalence in nature in UK, with 15.8 % and 100 % *Bd*-positive individuals found from a total of 19 and 5 individuals screened in 2008 and 2011, respectively (Smith 2014). The low number of screened individuals prevents general conclusions being reached, but a prevalence close to 40% in studies from Sweden (Fig. 1.3.1.6-1) indicates a much higher prevalence in this species than in the other native Norwegian amphibian species. *Bd* had negative impact on the movement patterns in pool frog, with infected individuals moving shorter distances from their breeding ponds to their hibernation sites (Kärvemo, Laurelia and Höglund; pers. comm.)

- *Pelophylax esculentus* (edible frog) was only represented by three individuals in the UK study, and no *Bd* was detected in these few samples (Smith 2014). However, in Sweden, *Bd*-detections were close to 40% (Fig. 1.3.1.6-1). This indicates a relatively high prevalence of *Bd* in this species, which is an introduced species to Norway.

Studies across southern and central Sweden (2014 - 2018) found *Bd* in eight anuran species, its prevalence varying between 1.9 % and 61.5 % (Kärvemo et al. 2018; Meurling et al. in prep., Fig. 1.3.1.6-1). In Skåne, *Bd*-infected anurans were detected in 13 of 31 ponds surveyed (42 %), and 156 out of 947 individuals examined were infected (16 %). At least one individual of each sampled species was infected (Kärvemo et al. 2018).

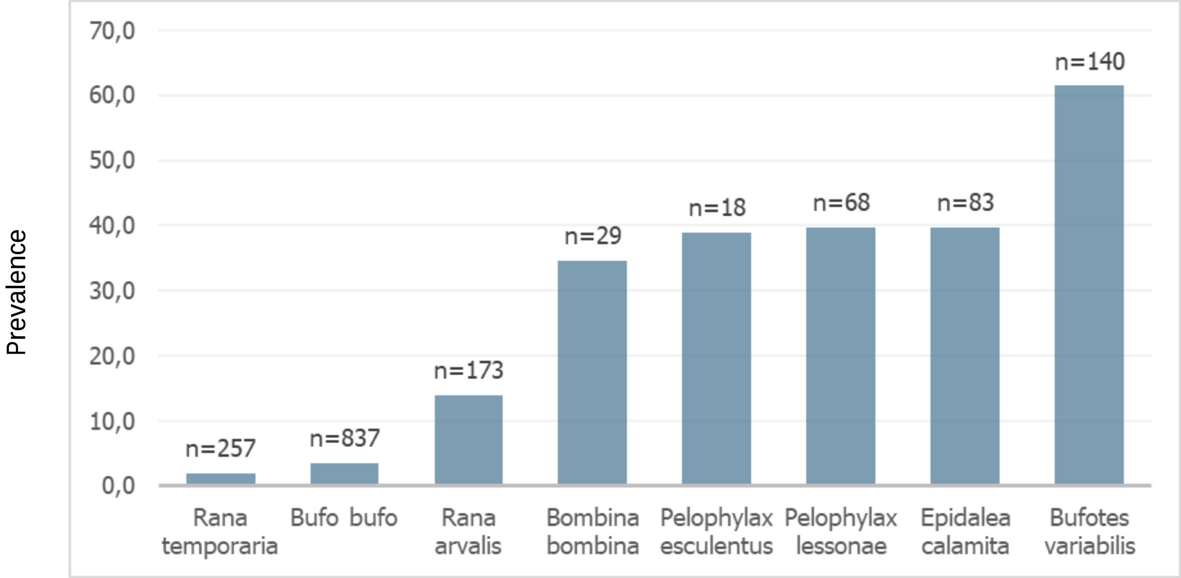


Figure 1.3.1.6-1: Average prevalence of *Bd* (as quantified via qPCR of body swabs) among eight anuran species studied in Sweden 2014-2018 (Meurling, Höglund, Laurila; *pers. comm.*), n indicates sample size (i.e. number of individuals screened).

In another study from central and southern Sweden, *Bd* prevalence was low (1.5% - 3.5%) in the common toad, *B. bufo*, but was significantly higher in the moor frog, *R. arvalis* (12.9**-**13.9 % in southern Sweden and 19.0 % in Uppland) (Kärvemo et al. 2019).

In the study from UK, the highest prevalence and infection loads (interpreted in terms of DNA quantities in the samples) were found in sites with non-native amphibians, which, for various reasons, been introduced illegally into the wild. The site with the highest prevalence (30 %) contained multiple introduced amphibian species. The native species with the highest prevalence (at most 23%) was natterjack toad (*Epidalea calamita*) that had been re-introduced to their native habitat after local extinction. The breeding for re-introduction was conducted in close proximity to non-native amphibians, which might explain the high prevalence.

1.3.1.7 Bd in Europe

On a global and regional scale, the pathogen has been detected in 52 of 82 countries in which sampling has been reported, and has been detected in 516 of 1240 (42 %) amphibian species. In Europe, up until 2013 it been detected in 10 countries (Garner et al. 2005; Olson et al. 2013). However, this number has risen, and in an updated distribution map based on several sources (Figure 1.3.1.7-1.), 15 countries are listed as having the pathogen present.

Figure 1.3.1.7-1: Map of countries in Europe where *Bd* has been detected in wild amphibian populations. Some of the countries where *Bd* is not detected, the pathogen was not surveyed.

Dramatic amphibian declines resulting from chytridiomycosis have mainly occurred in the tropics of Australia, Mesoamerica, and South America. Despite the widespread occurrence of *Bd* in Europe, here only minor species declines that can be attributed to chytridiomycosis have been reported (Scheele et al. 2019), placing Europe as the continent that has experienced the least impact from the disease worldwide.

In UK, which is relatively similar to Norway in terms of climate and native amphibian species, *Bd* is widespread, but with a patchy distribution all over England, Scotland, and Wales, without an obvious spatial clustering that would be expected for a single point of introduction or direction of spread (Smith 2014). During the thorough national cross-sectional surveys conducted by Smith (2014), the epidemiology *Bd* covering most of UK was investigated. Swab samples were collected from 5776 amphibians at 125 sites in 2008, and from 3106 amphibians at 122 sites in 2011, and yielded 20% and 11% *Bd*–positive sites, respectively. The sites were scattered all over UK, demonstrating that *Bd* is widespread, but often at very low prevalence and with low infection load, particularly within the native species. There was no sign of *Bd*-associated mortalities during the surveys. Furthermore, the *Bd*-status - in terms of infected sites and prevalence - did not expand from 2008 to 2011, but instead seemed to diminish slightly (Smith 2014).

1.3.1.8 Bd in Scandinavia

In Sweden*, Bd* was first detected in the southernmost part of the country in 2010 (Hallengren 2013), followed by records from several amphibian species in southernmost Sweden and the Stockholm area, providing the northernmost records of *Bd* in Europe (Meurling and Höglund; pers. comm.). No *Bd* was found in an examination of 197 Swedish samples of museum specimens collected between 1994 and 2004 (Garner et al. 2005), raising the possibility that *Bd* has colonized Sweden relatively recently. Currently, *Bd* has been found in 46 % of 101 investigated localities, with an overall prevalence of 14 % in southern and central Sweden, but ponds in the north of Sweden (near Luleå) did not contain *Bd* (Meurling, Laurila, and Höglund; pers. comm.). The environmental factors affecting the occurrence of *Bd* in northern Europe and at higher latitudes remain largely unexplored (Spitzen van der Sluijs et al. 2016). However, Kärvemo et al. (2018) reported that in the southern province of Sweden, *Bd* infection prevalence was positively associated with pond pH, and negatively associated with areas of mature forest and/or wetlands in the surroundings for four species: *B. bufo, R. arvalis, R. temporaria and Epidalea calamitata.*

In Denmark, *Bd* has been discovered on *Rana temporaria* and *Pelophylax* kl. *esculentus* in three populations studied, one on each of the islands Fyn, Zealand, and Bornholm (Scalera et al. 2008).

In Norway, *Bd* was detected in e-DNA samples from five ponds in Akershus county in 2017 (Taugbøl et al. 2017) (See chapter 3.1 for additional information).

1.3.1.9 Factors influencing prevalence and disease development

a) *Temperature and climate*

Climate and temperature are key factors for how the disease is expressed or suppressed (Fisher et al. 2012). *Bd* virulence is reduced at temperatures above 26°C (OIE (World Organisation For Animal Health) 2018), and observations also indicate lower virulence in terms of slow growth and reduced zoospore production below the optimum temperature range (17°C - 25°C) of *Bd* (Woodhams et al. 2003; Kriger & Hero 2006; Smith 2014). In a seven-year study, Clare et al. (2016) found that early onset of the spring was strongly correlated with high prevalence of *Bd*-infection in *Bufo spinosus* and *Rana temporaria*. Although *R. temporaria* is rarely found to be infected, both in this and other European studies, Clare et al. detected widespread infection in the species and mortality due to chytridiomycosis in years with early onset of the spring (Clare et al. 2016). Further, *B. spinosus* became locally extinct in 2013 – likely due to chytridiomycosis driven by the high *Bd* prevalence and mortality detected in metamorphs in previous years with early spring. As climate is changing more rapidly in higher latitudes, the trend towards earlier spring is predicted to keep increasing, and this seems likely to lead to higher prevalence and mortalities in amphibian populations, even those that currently seem tolerant, such as *R. temporaria.* The recently proposed "thermal mismatch hypothesis" predicts that cool-, and warm-adapted hosts should be vulnerable to disease at unusually warm and cool temperatures, respectively. Cohen et al. conducted experiments on the critically endangered frog *Atelopus zeteki* to test this hypothesis, and obtained results suggesting that hosts adapted to relatively cool conditions will be most vulnerable to the combination of increases in mean temperature and emerging infectious diseases (EIDs) such as *Bd* (Cohen et al. 2018).

b) *Environmental stress and co-infections*

Different factors, such as temperature volatility and infections with other diseases (e.g., Ranavirus) might also affect the susceptibility of the anurans to *Bd*.

Environmental stress, for example unpredictable temperature fluctuations which is the expected situation with climate change, decrease frog resistance to *Bd* (Raffel et al. 2013). Acclimation status has been found to significantly affect Bd loads on newts, where unacclimated newts acquire higher *Bd* loads than the controls (Raffel et al. 2015). For amphibians adapted to rather high temperatures, it seems that exposure to low temperatures combined with high moisture increase *Bd* prevalence and mortality due to infection (Raffel et al. 2015). This could, at least in part, be explained with that amphibian immune responses are stronger at higher temperatures (Raffel et al. 2015).

Interactions between one or several co-infecting parasites or pathogens can be antagonistic or facilitative. Antagonistic interactions can appear as a result of resource competition or induction of cross-effective immune responses within the host, while facilitative interactions may increase the degree of infection and disease in the host via immunosuppression and resource depletion (Warne et al. 2016 and references therein). Chytridiomycisis can be accompanied with co-infections that potentially reduce (e.g. *Bd*-antagonistic skin bacteria) or increase the disease condition and mortality rates. The antagonistic interactions have been covered under “skin-microbiota” below. Little is known about facilitative co-infections involving *Bd*. In captive amphibians *Bd* has been found in co-infections with Ranavirus, *Chlamydia pneumoniae*, *Aeromonas hydrophila* and *Mycobaterium* spp (van Rooji et al. 2015 and references therein). There are also reports of co-infections with *Bd* and Ranavirus from wild amphibians. In any case, it is difficult to determine which pathogen contributes the most to morbidity and mortality, if they interact, or which is the primary and secondary pathogen. Whether the interactions between co-occurring pathogens affect the disease severity is largely unknown. However, a positive correlation has been found between infection by Ranavirus and *Bd* in some neotropical amphibian species, such as Craugaster fitzingeri, where the odds of finding Ranavirus were significantly higher in individuals infected with *Bd* (van Rooji et al. 2015 and references therein). In addition, a study from South America reported co-infections with *Bd* and Ranavirus as rather common in stream-dwelling frogs (30% co-infections; Warne et al. 2016). The study raise the concern that *Bd*-interactions increase amphibian vulnerability to secondary infections across differing life stages, but more studies are needed to test if this is a problem leading to increased declines in amphibian populations (Warne et al. 2016).

c) *Skin microbiota*

The mucus coating layer of amphibians consists of a microenvironment of symbiotic bacteria and host defence compounds. There is accumulating evidence that symbiotic bacteria play a functional role in amphibian skin defence against *Bd* (Catenazzi et al. 2018; S. Ellison et al. 2018). Amphibian populations that successfully persist in the presence of *Bd* have a higher occurrence of skin bacterial isolates that produce antifungal compounds than amphibian populations experiencing major *Bd*-induced declines (McCoy & Peralta 2018 and references therein). Burkart et al. (2017) found that *Bd*-resistant *Gastrotheca excubitor* frogs harbour a greater proportion (40 %) of anti-*Bd* skin bacteria than the related *Bd*-susceptible *G. nebulanastes* that only harboured 9% anti-*Bd* skin bacteria (Burkart et al. 2017). These findings are supported by Catenaszzi et al. (2018), who demonstrated that the proportion of anti-*Bd* bacterial isolates ranged from 0 to 14 % in six *Bd*-susceptible host species from a wide range of habitats, whereas two non-susceptible host species harboured much larger proportions of anti-*Bd* bacterial isolates in their skin (40% and 45%). Importantly, Bletz et al. (2017) also found in experimental mesocosms that newt skin microbe fluctuations were not correlated with that of pond microbiota; however, a portion of community variation was explained by environmental temperature. They also found that the proportion of ‘potentially’ *Bd*-inhibitory groups of bacteria did not vary temporally for two of three newt species, suggesting that protective function may be maintained despite temporal variation in community structure (Bletz et al. 2018). However, Bates et al. (2018) report on an association between bacterial diversity and die offs in the Pyrenean lake system, suggesting that this is not straight forward (Bates et al. 2018).

Kueneman et al. (2019) recently documented that global amphibian skin bacterial richness was consistently correlated with temperature-associated factors, where environments with colder winters and variable thermal conditions gave more diverse skin microbiomes than environments with warm winters and less annual temperature variation. Thus, temporal and spatial characteristics of the hosts’ macro-environment mediate microbial diversity, which, again, potentially mediates *Bd*-protection (Kueneman et al. 2019). In this context, Norway seems a good candidate country for rich amphibian skin microbiota.

d) *Water microfauna*

Mesocosm experiments have demonstrated that *Daphnia,* a keystone of zooplankton in freshwater systems, is a highly efficient grazer of *Bd* zoospores (Hamilton et al. 2012) and, if present, can drastically reduce the *Bd*-infection pressure in freshwater habitats. Schmeller et al. (2014) demonstrated a strong site-specific component regarding the success or failure of *Bd*. They demonstrated that *Bd*-zoospore abundance rapidly decreased when exposed to water from “low-prevalent” sites, but remained present and vital for days when exposed to water from “high-prevalent” sites. This was strongly correlated with the amount of microfauna (protozooans and microscopic metazoans) from the water; low-prevalent sites hosted a much broader and larger microfauna than the high-prevalent sites. They also demonstrated that both ciliates and rotifers were efficient consumers of *Bd*-zoospores. Thus, many grazing microorganisms can be expected to act as *Bd*-zoospore predators that reduce the infection pressure in natural freshwater habitats with rich microfauna (Schmeller et al. 2014).

e) *Species composition*
Data from Sweden have shown that both *Bd* presence and *Bd* prevalence were positively correlated with amphibian species richness (Kärvemo et al. 2018). Multi-species ponds are characterised by the presence of species that breed at warmer water temperatures, when *Bd* is more active. This suggests that *Bd* would be more prevalent in such ponds, and thus *Bd* zoospores are likely to be more prevalent.

1.3.1.10 Hosts and vectors influencing/facilitating the spread of Bd

a) *Introductions of non-native amphibian species*
There is no doubt that international trade in amphibians has contributed directly to the transmission and spread of *Bd* worldwide (O'Hanlon et al. 2018). In many European countries, introductions/release of non-native amphibians is the single most important cause of the introduction and transmission of *Bd* to native amphibian populations (O'Hanlon et al. 2018). In UK, Smith (2014) found that out of a total of 30 *Bd*-positive sites, 12 were linked to non-native introductions, with either a current presence of non-native amphibians or historical records of non-native introductions.

b) *Spread via infected native amphibian species*
When introduced to a country, *Bd* will easily spread by infected native (and introduced) amphibians, both as a consequence of local movements between ponds and water systems, and also during more active migration periods. In this respect, migrating amphibians crossing the border from Sweden to Norway will constitute a likely source for *Bd*-introduction from Sweden.

c) *Humans*
In addition to the (in Norway illegal) release of exotic amphibians into the wild, humans may contribute to the spread of *Bd* in several ways, including accidental transport of *Bd* zoospores as “hitchhikers” on boots, fishing gear, nets, traps, or boats between waterbodies. The zoospores might survive for several hours under dry conditions, and even longer on humid surfaces and in small sites of water accumulation, *e.g.*, in the bottom of a canoe. Movement of frog eggs, tadpoles, or adult amphibians between sites, which is a popular activity for children, can also contribute to the spread of *Bd*. In the perspective of introduction to Norway, this is one of the possible routes in areas bordering between Sweden and Norway.

d) *Birds*
Waterfowl might transport *Bd* between waterbodies, as the zoospores can attach to their legs. It has been shown that the zoospores can attach, adhere, proliferate, and

survive on the keratin of bird legs (Garmyn et al. 2012), though for how long is unclear. In this study, 397 wild geese in Belgium were also screened, and 15 % were found to have *Bd*-positive feet based on qPCR screening. Using molecular screening, Hanlon et al. found that the feet of three different duck species all were *Bd*-positive (Hanlon et al. 2017). Thus, waterfowl might serve as mechanical *Bd*-vector across country borders, as well as between local populations.

e) *Crustaceans*

American freshwater crayfish species (*Procambarus* spp. and *Orconectes* spp.) can carry and transmit *Bd*. McMahon et al. (2013) demonstrated zoosporangia of *Bd* to occur within freshwater crayfish gastrointestinal tracts and field studies indicated a *Bd* prevalence in crayfish of up to 29 %. The presence of crayfish in Colorado wetlands was a positive predictor of *Bd* infections in sympatric amphibians. Experimental studies showed that *Bd* could infect crayfish and that the infection could be maintained and transmitted to amphibians (McMahon et al. 2013). Additional studies have demonstrated a low prevalence of *Bd* in both farmed and wild stocks of American freshwater crayfish species, indicating that crayfish could be an important vector in the spread of *Bd* in those areas where these are present (Brannelly et al. 2015).

f) *Reptiles*

Reptiles commonly live in close proximity to amphibians and share similar ecological traits. Killburn et al. (2011) screened a large number of lizards and snakes in Panama in habitats where sympatric amphibians either were at pre-epizootic, epizootic, or post-epizootic stages of chytridiomycosis (Kilburn et al. 2011). They used the same diagnostic swab-method followed by qPCR as recommended for *Bd*-diagnostics in amphibians and found a prevalence of 32 % and 9 % of *Bd*-positive individuals in the lizard species *Anolis humilis* and *A. lionotus*, respectively. Also, three snake species were found to harbour one *Bd*-positive individual each. Since the prevalence of *Bd*-positive reptiles was positively correlated with the infection prevalence in cohabiting anuran amphibians, Killburn et al. (2011) propose that reptiles serve as mechanical vectors or even reservoir hosts for *Bd*, and consequently also serve as disease transmission agents.

g) *Other vectors*

Any animal inhabiting or visiting a waterbody with *Bd*-zoospores can, in theory, serve as vector, including fish, mammals, birds (already covered above), and even insects. However, the impact and likelihood for different animals are unknown.

1.3.2 *Batrachochytrium salamandrivorans* (*Bsal*)

Batrachochytrium salamandrivorans (*Bsal*) is the closest known sister taxon to *Bd*, and can cause fatal chytridiomycosis in salamanders, including newts (Caudata). The Netherlands might be the first entry point of *Bsal* to European wild salamander populations as *Bsal* was first reported to be emerging here in a rapidly declining population of fire salamander (Spitzen van der Sluijs et al. 2013; Martel et al. 2014; European Food Safety Authority (EFSA) 2017). There are more positive cases of *Bsal* reported from salamanders in captivity in Europe (e.g., Belgium, Germany, Spain, the Netherlands, UK, and Sweden) than from wild populations; the latter currently only involves populations in The Netherlands, Belgium, and Germany (EFSA Panel on Animal Health and Welfare (AHAW) 2018; Sabino-Pinto et al. 2018). Martel et al. (2014) found evidence both from exposure trials and screenings of natural amphibian populations from five continents that only species within Caudata (urodeles) were infected by *Bsal*, of which many were found to be susceptible. Some *Bsal* strains have also been shown to infect anurans (e.g., midwife toad), which are not susceptible to disease, but can transmit the disease to salamanders (Stegen et al. 2017) *Bsal*-tolerant salamander species exist in East Asia, and apart from the recent introductions to Europe, only wild salamander populations in East Asia appeared to host the pathogen (Martel et al. 2014).

1.3.2.1 Taxonomy and strains

The current classification of *Bsal* according to MycoBank database (http://www.mycobank.org): Kingdom - Fungi, Phylum - Chytridiomycota, Order - Rhizophydiales, Family - Incertae sedis, Genus - *Batrachochytrium*

Martel et al. (2013) isolated and described *Bsal* from diseased individuals of *Salamandra salamandra* (fire salamander) from the Netherlands. This was the second species of *Batrachochytrium* ever described. Although *Bsal* is closely related to *Bd*, the two species diverged more than 50 million years ago, in the Late Cretaceous or early Paleogene (Martel et al. 2014).

Martel et al. (2013) investigated mass mortalities of fire salamander, and found pathological signs similar to *Bd*-infections and chytridiomycosis, but PCR analysis for *Bd* were negative. Fungal strains were isolated from the affected fire salamanders, and, based on sequencing, culture studies, microscopy, and ultrastructure, it was concluded that this was a new *Batrachochytrium* species. Salamanders experimentally exposed to zoospores from pure culture strains of *Bsal* all died after 12-18 days after exposure (thus fulfilling Koch's postulates about causality between microorganism and disease). In the species epithet “salamandrivorans”, vorans (eating, devouring) refers to the extensive skin destruction and rapid mortality observed in infected salamanders (Martel et al. 2013).

Experimentally exposed *Alytes obstetricans* (midwife toads), the most highly susceptible species to *Bd* in Europe (Bosch et al. 2001), remained uninfected, which was the first proof of a different and narrower amphibian species range for *Bsal* than for *Bd* (Martel et al. 2013).

Bsal has not yet been included into the OIE Manual of Diagnostic Tests for Aquatic Animals, but is listed in OIE with its own disease card in 2018). To our knowledge, there are no studies of intraspecific genetic lineages of *Bsal* in the literature.

1.3.2.2 Host specificity

Bsal infects a wide range of species within the family Salamandridae, but shows higher degree of host specialisation than *Bd*. Martel et al. (2014) screened more than 5000 amphibians from across four continents. Combined with exposure studies of a corresponding selection of relevant taxa, they provided convincing evidence that *Bsal* is restricted to salamanders and newts (Urodela), to which it is highly virulent and lethal. Anurans are not affected, but can harbor transient infections (Stegen et al. 2017).

According to Martel et al. (2014), *Bsal* originated in Asia, and coexisted with a clade of salamander hosts for millions of years on this continent. Pet trade and lack of biosecurity measures resulted in the recent introduction into susceptible salamander populations in the Netherlands (Martel et al. 2014). Thus, in contrast to *Bd*, which infects a wide range of amphibian species within two amphibian orders, *Bsal* pathogenicity seems restricted to the order Urodela (Caudata), where mortalities have most often been reported in species within the family Salamandridae (Martel et al. 2014). Several other species in other urodelan families are tolerant (infected but not affected) or resistant (not infected) (Martel et al. 2014).

1.3.2.3 Mode of infection

Unlike *Bd*, *Bsal* produces two types of infective spores, both the short-lived motile zoospores, but also persistent thick-walled encysted spores (Martel et al. 2013; Stegen et al. 2017). Similar to *Bd*, the flagellated zoospores actively swim in the water searching for a suitable host skin to infect, but are only infective for a short period. In contrast, the encysted non-motile spores float passively with the water flow and remains infective for a longer period (Stegen et al. 2017). Both types of spores infect the salamander skin. In susceptible fire salamanders, infection with *Bsal* causes multifocal superficial erosions and deep ulcerations in the skin all over the body. Infected keratinocytes (the predominant cell type in the epidermis) contain one centrally located thallus. Lesions caused by *Bsal* are characterized by marked skin ulceration, opposed to those caused by *Bd*, which typically induces epidermal hyperplasia and hyperkeratosis (Martel et al. 2013).

1.3.2.4 Lifecycle / persistence

The lifecycle of *Bsal* involves the same stages as *Bd*, but with the additional production of encysted non-motile spores with longer viability than the motile zoospores. Thus, *Bsal* is probably more persistent in nature than *Bd* (Stegen et al. 2017). Both the motile and non-motile zoospores for dispersal and host-infection are produced in zoosporangia. In pure culture, it forms predominantly monocentric thalli, although some are colonial, whereas in the epidermis of amphibians inside keratinocytes, *Bsal* form predominantly colonial thalli (similar to *Bd*) that contain several walled sporangia (Martel et al. 2013) .

In culture, *Bsal* grows at test temperatures ranging from 5°C - 22°C, but no growth was observed above 24°C, and the thalli died after 5 days at 25 °C. The zoospores were slightly larger than *Bd* (4.0– 5.5 µm), with highly irregular surface and cell surface projections. The cycle (or generation) time was 5 days at 15°C. (Martel et al. 2013). Exposure experiments reported by Stegen et al. (2017) found that *Bsal* caused 100% mortality in fire salamanders, regardless of temperature, in a test range from 4°C to 22°C.

The encysted non-motile spores are much more resilient than the zoospores. They can float at the water–air interface, and are capable of quickly adhering to salamander skin and to scales on the feet of waterfowl. They remain infective for at least one month in water, and are more resistant to predation by zooplankton than the zoospores. Further, *Bsal* present in wet soil has been found to remain infective for up to 48 hours after the soil was contaminated by an infected salamander (Stegen et al. 2017). *Bsal* can also survive on tolerant amphibian hosts (including some anurans) for several months, and these can act as active or passive carriers (Martel et al. 2014; Stegen et al. 2017).

1.3.2.5 Virulence

The terms virulence and impact of disease have been defined and discussed previously (see 1.3.1.5). Traits of *Bsal* linked to virulence include not only zoospore production, but also production of persistent infective non-motile spores (Stegen et al. 2017). The severe disruptions of the skin in susceptible species suggest that *Bsal* has efficient enzyme capacity and toxin production for disruptions of amphibian epithelial cells and immune cell inhibition, although specific literature on the subject appears to be lacking.

Unlike *Bd*, no hypervirulent global emerging clone has been identified. So far, it is assumed that *Bsal*-induced chytridiomycosis in salamander species in Europe results from the introduction of exotic, *Bsal*-tolerant salamanders from Asia. The Asian salamanders have adapted to the fungal pathogen over millions of years, but the naive salamander species of European ecosystems are highly susceptible (Stegen et al. 2017; EFSA Panel on Animal Health and Welfare (AHAW) et al. 2018).

Bsal is highly virulent in fire salamander, causing mortality rates up to 100% in both exposure trials and in nature (Martel et al. 2013; Martel et al. 2014; Stegen et al. 2017). The fungus remained virulent, with the ability to cause lethal chytridiomycosis, both at low doses of zoospores and at low temperature (4°C), with only a slightly slower buildup of the infection (Stegen et al. 2017). Thus, in contrast with *Bd*, *Bsal* virulence seems unaffected by low temperatures and is able to kill in the temperature range from 4°C - <24°C, with an optimum at 15 °C (Martel et al. 2013; Stegen et al. 2017).

1.3.2.6 Prevalence and effect on different amphibian species

Shortly after the discovery of *Bsal* (Martel et al. 2013), Martel et al. (2014) predicted the potential impact of *Bsal* on amphibian diversity by conducting infection trials on 35 species from the three amphibian orders (anurans, urodelans, and caecilians). They found that *Bsal* only infected urodelans, and none of the anuran and caecilian species. For the urodelans, alarmingly high mortality rates were observed, with 41 of the 44 tested Western Palearctic salamanders in the families Salamandridae and Plethodontidae dying rapidly after *Bsal* infection (Martel et al. 2014).

The current range of *Bsal* was estimated by qPCR screening of more than 5000 wild amphibian individuals from four continents. Again, positive results were restricted to urodelans in Asia and Europe. The absence of disease in Asian salamanders suggests *Bsal*-tolerance and long-term endemism in Asia, whereas the high mortality rates in European salamanders confirm these as highly susceptible species (Martel et al. 2014), resulting from the recent introduction of this exotic pathogen.

Lacking et al (2017), found that *Bsal* was more widespread than *Bd* in Vietnam, and that it occurred at higher temperatures than reported to be tolerated by the *Bsal* type strain. They screened a large number of individuals from eight salamander species across 11 provinces in northern Vietnam (Laking et al. 2017). The species belonged to the genera *Paramesotriton* and *Tylototriton*. In total, five species were *Bsal* positive with an overall low prevalence (2.9 %). There are no known declines or reports of chytridiomycosis from the area, supporting the hypothesis that *Bsal* is endemic to Asia, and that Asian amphibians may act as a disease reservoir (Yuan et al. 2018).

Martel et al. (2014) found that common frog was resistant to *Bsal*, but great crested newt was susceptible and died from *Bsal* infection. Interestingly, *Lissotriton helveticus* was the only tested species within the Salamandridae family that was not identified as being susceptible (Martel et al. 2014). Below, we address what is known for the prevalence and effects of *Bsal* on amphibian species found in Norway. Most information originates from Martel et al. (2014), and Bates et al. (preprint online 2018).

- *Lissotriton vulgaris* (smooth newt) has not yet been reported to be infected or die from *Bsal* infections in the wild. However, in infection trials, 40% (four individuals) of ten *L. vulgaris* test animals became infected (Bates et al. 2018). Of these, three individuals developed skin lesions and one died. The other three animals that had been *Bsal*-positive cleared the infection after a period. The authors emphasize that they saw reduced microbial diversity in the captive populations and suggest that these experiments may not reflect the reality of a wild-infection.

- *Triturus cristatus* (great crested newt) has not been reported to be infected or to die from *Bsal* infections in nature, but is susceptible to *Bsal* and died from the infection in the Martel et al. (2014) study. For this species, Bates et al. (2018) found that 60% (six of 10 individuals) became infected during infection trials, of which three developed skin lesions and died. Only one out of six infected individuals cleared the infection after a period.

From the limited number of studies available, it seems likely that Norwegian salamander species are susceptible to *Bsal* infection, at least under experimental conditions.

1.3.2.7 Bsal in Europe

Bsal has been identified in wild populations of salamanders in the Netherlands, Belgium, and Germany (Figure 1.3.2.7-1), and in kept salamander populations in the Netherlands, Belgium, Germany, Spain, and UK (EFSA Panel on Animal Health and Welfare (AHAW) et al. 2018). In Belgium, the Netherlands, and Germany, the fungus has been implicated in rapid declines and local extinctions of the rare and threatened fire salamander, *Salamandra salamandra* (Martel et al. 2013; Spitzen van der Sluijs et al. 2016; Stegen et al. 2017). Both great crested newt (*Triturus cristatus*) and smooth newt (*Lissotron vulgaris*) were found to be susceptible to infection in infection trials (Martel et al. 2014; Bates et al. 2018).

Figure 1.3.2.7-1: Map of countries in Europe where *Bsal* has been detected in wild amphibian populations. In some of the countries where *Bsal* is not detected, the pathogen was not surveyed.

1.3.2.8 Bsal in Scandinavia

To our knowledge *Bsal* has never been documented in Scandinavia. However, according to EFSA (2018), there is no active surveillance of *Bsal* in Scandinavia.

In Norway, screening for *Bsal* was performed on e-DNA samples from five ponds in Akershus county in 2017, where *Bd* was detected (Taugbøl et al. 2017), but no positive results were found (Taugbøl, Dervo, Sivertsgård, et al. 2018). In 2018, also swab samples from great crested newt tested positive (Taugbøl et al. 2019)

1.3.2.9 Factors influencing the prevalence and disease development

a) *Temperature and climate*

Unlike *Bd*, *Bsal* virulence is not reduced at low temperatures, and the pathogen has been shown to be highly virulent in a temperature range from 4°C - <24°C, with an optimum at 15°C (Martel et al. 2013; Stegen et al. 2017). Above 24°C, the fungus does not grow in pure culture and sporangia die off within a few days (Martel et al. 2013). Thus, a cooler Northern European climate seems to offer little protection to *Bsal* outbreaks if the pathogen is introduced. Lacking et al (2017) found that *Bsal*-infected salamanders in Vietnam occurred in ponds or streams with water temperatures between 20–25°C (even reaching 26.4°C), indicating that the thermal preferences and tolerances in *Bsal* might be wider in Asian strains than in the strain(s) currently characterized in Europe.

b) *Dispersal capacity*

Unlike *Bd*, *Bsal* develop resting spores that can serve as more robust dispersal units than the short-lived zoospores, both for passive dispersal (water flow) and vector dispersal. It seems therefore probable that *Bsal* would have a higher capacity for long distance spread than *Bd* (Stegen et al. 2017). In contrast with this assumption, Spitzen et al. (2018) demonstrated that *Bsal* may be a poorer disperser than previously hypothesized. In terrarium experiments, *Bsal* transmission did not occur between infected and non-infected salamander groups separated only by a mesh. In nature, *Bsal* shows little ability to spread over rather short distances even in the absence of obvious physical barriers; no sign of spread of *Bsal* from an infected fire salamander population to another subpopulation connected by a stream and only 800 m apart was reported (Spitzen et al., 2018). Both alpine newts (*Ichthyosaura alpestris*) and fire salamanders were present in the landscape between the two interconnected sites. Given the short distance between the sites and the long surveillance period, it seemed that dispersal failed. Although the reasons for dispersal failure remain unclear, these results may explain why *Bsal* , unlike *Bd*, has not yet shown any sign of becoming a global pandemic (Spitzen van der Sluijs et al. 2018). However, the available literature provides rather different and contrasting views and future predictions.

c) *Skin microbiota*

Although the role of amphibian skin microbiota in relation to possible protection against *Bsal* is still largely unresolved, the topic is being given increasing attention. Bletz et al. (2018) found that wild healthy fire salamanders had *Bsal*-inhibitory bacteria in their skin. In an experimental setting, addition of more of the *Bsal*-inhibitory bacteria slowed down disease progression in the fire salamanders (Bletz et al. 2018). Bates et al. (2018) characterised the impact of captivity and exposure to *Bsal* on the skin microbiota of smooth newts and great crested newts, and found indications for both newt species that *Bsal* infection and subsequent mortality was associated with perturbation of the skin microbiome and possible dysbiosis. They also showed that the microbiome community rapidly decreased in species richness and changed its species composition rapidly after the transition from wild to captivity, potentially leading to increased risk of infection in salamanders kept in captivity.

d) *Water microfauna*

Similar to *Bd*, *Bsal* zoospores are predated on by zooplanktonic organisms. Stegen et al. (2017) conducted mesocosm experiments, where the predator microfauna was represented by copepods, ciliates, rotifers, ostracods, heliozoans, and water fleas. Here, the survival (or content) of *Bsal* zoospores was reduced by 50% in two hours, while the encysted *Bsal* resting spores were hardly affected by the predators over the experimental period. This is probably because zoospores swim within the water column, whereas the resting spores float on the water surface and thereby evade the filtering micro-predators (Stegen et al. 2017). Thus, water microfauna probably provide less reduction in the *Bsal* infection pressure than for *Bd*.

e) *Species composition*

To our knowledge, studies on the effect of amphibian species composition on *Bsal* prevalence have not been conducted.

1.3.2.10 Hosts and vectors influencing/facilitating the spread of Bsal

a) *Introductions of non-native amphibian species*

Martel et al. (2014) assumed that pet trade of amphibians from Asia and lack of biosecurity measures caused the introduction of *Bsal* into susceptible salamander populations in the Netherlands. Accidental releases from private collections originating in the pet trade are considered the predominant mode of transmission of the disease to wild populations (Fitzpatrick et al. 2018).

b) *Spread via infected native amphibian species*

Bsal can also spread by infected native (and introduced) amphibians, but as the host range is considerably narrower than for *Bd*, the risk might be lower. As *Bsal* is currently not known to be present in the wild in Sweden, the Swedish-Norwegian border is not (yet) a likely source for *Bsal*-introduction to Norway.

c) *Humans*

Similar to *Bd*, anthropogenic activity is a major cause of *Bsal* spread. In addition to the (in Norway illegal) release of exotic amphibians into the wild, routes of transmission and spread may involve humans accidentally transporting *Bsal* zoospores and resting spores as “hitchhikers” on boots, fishing gear, or boats between waterbodies. Whereas the zoospores might survive for some hours under dry conditions and even longer on humid surfaces and small water accumulations, e.g., in the bottom of a canoe, the resting spores will also be likely to survive mechanical spread (Stegen et al. 2017). In contrast with *Bd*-spread, it is currently less likely that movement of frog eggs, tadpoles, or adult amphibians between sites will contribute to *Bsal* introduction and spread in

Norway because *Bsal* generally has a narrower host range, and currently has a limited distribution in Europe.

d) *Other amphibians*
It is assumed in the literature that other amphibian species can act as carriers of *Bsal*, either if they are *Bsal*-tolerant and carry low amounts of the pathogen, or if they are resistant, and serve as mechanical carriers of zoospores and resting spores between sites. Stegen et al. (2017) found that the urodelan midwife toads could host a non-fatal *Bsal* infection for weeks at low infection intensity, and that the number of zoospores released from them was sufficient to establish infection in susceptible salamanders.

e) *Birds*
Stegen et al. (2017) demonstrated that non-motile encysted spores of *Bsal* are capable of quickly adhering to scales of the feet of waterfowl (specifically goose feet). Waterfowl are therefore likely to act as mechanical carriers of *Bsal* across physical barriers that would hinder amphibian dispersal and over larger distances. However, despite possible dispersal pathways, such as birds, infected hosts, and interconnected water streams, *Bsal* has not yet spread from the first *Bsal*-outbreak area and to a small population 800 m away (Spitzen van der Sluijs et al. 2018).

f) *Crustaceans and reptiles*
In contrast with *Bd*, to our knowledge there are no reports of *Bsal*-infections associated with reptiles and crustaceans. However, it cannot be excluded that reptiles and crustaceans could serve as mechanical carriers of “hitchhiking” spores.

g) *Other carriers*
Similar to *Bd*, any animal inhabiting or visiting a waterbody containing *Bsal* zoospores or *Bsal* resting spores could, theoretically, serve as vector, including fish, reptiles, mammals, birds (already covered above), and even insects. However, the impact and likelihood are unknown. Based on the study by Spitzen et al. (2018), mechanical transmission of *Bsal* does not happen readily in nature.

1.4 Chytridiomycosis

Chytridiomycosis is an infectious disease in amphibians that is caused by the chytrid fungi *Bd* and *Bsal*. Chytridiomycosis is listed as a notifiable disease in Australia’s National List of Reportable Diseases of Aquatic Animals (AHC) and by the World Organization for Animal Health (OIE) in the Aquatic Animal Health Code. It was included as a notifiable disease in Norway in previous years, but was omitted from the list in around 2014. The mortality rate of chytridiomycosis can vary greatly between different amphibian species, and some species may have subclinical chytridiomycosis with no obvious signs of disease. The clinical signs of severe chytridiomycosis are non-specific, thus a diagnosis can only be provisional and needs confirmation by laboratory tests. Affected animals might have skin lesions but tend to show lethargy, reduced appetite, and unprotected sitting during daytime with hind legs slightly abducted (Berger et al. 2009). In an experimental setting, within two to five days of exhibiting lethargy, frogs usually become moribund. Regarding skin lesions caused by *Bd*, these range from subtle to more obvious changes, and can include skin darkening and patchy discolouration of skin, reddened toe tips, presence of excessive sloughed skin, erosions or, although rather rare, ulcerations (Berger et al. 2009; Voyles et al. 2011) Clinical skin lesions caused by *Bsal* are characterized by marked skin ulceration in fire salamanders (Martel et al. 2013), while milder lesions have been described in other species (Fitzpatrick et al. 2018). More common clinical signs of disease are disturbed locomotion and emaciation (Mutschmann 2015).

1.4.1.1 Pathological effects

The main effects of *Bd* and *Bsal* infection on individual amphibians are loss of skin integrity, which results in loss of control of the transport of water, oxygen, and electrolytes necessary for osmoregulation. Work by Campbell et al. (2012) indicates that this does not occur by physical destruction of the epidermis, but mainly by specific inhibition of active sodium transport in the stratum granulosum of the epidermis (Campbell et al. 2012). Electrolyte disturbances, such as reduced plasma osmolality and lowering of plasma sodium, potassium, and chloride ion concentrations, will subsequently impair cardiac function and lead to cardiac arrest (Voyles et al. 2009; Salla et al. 2018). Lesions induced by *Bsal* can be characterized by marked skin ulceration, unlike those caused by *Bd*, which typically induces epidermal hyperplasia and hyperkeratosis (Martel et al. 2013), but mortality without obvious skin lesions is observed also in case of *Bsal* infection (Fitzpatrick et al. 2018).

Amphibian eggs do not seem to be infected by *Bd* (OIE (World Organisation For Animal Health) 2018), and probably not *Bsal*. However, the chitinous mouth parts of larvae can become infected and experimental infections of larvae with different strains of *Bd* on Swedish common toads, green toads, and moor frogs have shown increased larval mortality in green toads, whereas common toads appear to suffer from increased mortality as post metamorphs. Moor frogs appear not to suffer from increased mortality (Meurling and Höglund*,* pers. comm.)

Experimental studies on Swedish populations have shown that mortality of *B. bufo* was as high as 60% among juveniles infected with a *Bd*-strain isolated from Swedish *B. variabilis*, but there was no significant mortality among *R. arvalis* juveniles (Bengtsson 2018; Meurling and Höglund*,* pers. comm.). As *B. bufo* and *R. arvalis* commonly occur in the same ponds in Sweden, these results suggest that *R. arvalis* may act as a *Bd*-reservoir species for *B. bufo*.

1.4.1.2 Subclinical effects

Laboratory experiments and field studies have shown that *Bd* infections can reduce body condition of the host (Retallick & Miera 2007; Deguise & Richardson 2009; Pearl et al. 2009; Voyles et al. 2012; Iglesias-Carrasco et al. 2017; Kärvemo et al. 2019). Radio-telemetry studies of Swedish pool frogs (*Pelophylax lessonae*) have shown that *Bd* infection status affects the distance frogs move from their breeding ponds to winter hibernation sites (Kärvemo and Höglund, pers. comm.).

1.4.2 Global impact

The chytridiomycosis panzootic has recently been identified to be responsible for "the greatest recorded loss of biodiversity attributable to a disease" by Scheele et al., (2019). The authors demonstrate that this disease has played a role in the decline of at least 501 amphibian species, and presumably caused extinctions of 90 or these.

Bd infections appear to be the primary cause of chytridiomycosis as this pathogen has been detected in more than 700 amphibian species (Lips 2016), and has caused mortality and population declines all over the world (Stuart et al. 2004; D. B. Wake & Vredenburg 2008; Kilpatrick et al. 2010; Bower et al. 2017). *Bsal,* on the other hand, has only caused population declines in a few species in central Europe and seems to have limited, or no effect on salamander species in Asia, where the pathogen originates. *Bsal* has not been detected beyond Eurasia.

1.4.3 Status of chytridiomycosis in Europe

Although *Bd* is widespread in Europe (1.3.1.8) and *Bsal* has been found in central Europe (1.3.2.8), outbreaks of severe chytridiomycosis with associated mass mortality are limited to a few species in Europe. The most well-documented cases are the midwife toad (*Alytes obstetricians*) in the Iberian peninsula (France and Spain), where mass mortalities and population extinction due to chytridiomycosis have been observed (Bosch et al. 2001). BdCAPE has also been found on the midwife toad on the island of Mallorca (Doddington et al. 2013). *Bsal* has been identified as the cause of the mass extinction of the European fire salamander (*Salamandra salamandra*) in Belgium, the Netherlands, and northern Germany (Martel et al. 2013). Chytrid fungus infection has also been related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park (Central Spain) (Bosch & Martínez-Solano 2006).

In UK and other northern European countries, infection seems to be widespread. However, although *Bd*-associated mortalities have been documented (Bosch et al. 2001; Bosch & Martínez-Solano 2006; Bielby et al., 2009; Pasmans et al., 2010; Walker et al., 2010), there are no reports on chytridiomycosis-driven population declines to date (Smith 2014).

1.5 Other important amphibian diseases

Bd and *Bsal* have received considerable attention over the last 30 years, especially the last 10 years, due to the confirmation of the devastating effect that these pathogens have had on amphibian populations. However, several other pathogens cause serious declines in various amphibian species (Duffus 2009), and two of these have been detected in Nordic countries in recent years and are thus worth mentioning here.

1.5.1 Ranavirus

Ranaviruses pose another serious threat to amphibian populations globally. These double-stranded DNA viruses belong to the family *Irodiviridae* and primarily infect fish and amphibians, but also some reptile species (Stöhr et al. 2015). In contrast to *Bd* and *Bsal*, these viruses also infect the larval stages of amphibians, and the same strain can persist in both fish and amphibian hosts (Gray et al. 2009). Since 1990, ranavirus has been recognized as the cause of mass di-offs in amphibian populations in North America, South America, and Japan, and outbreaks in several European countries, including Denmark, have been reported in the last 20 years (See Miaud et al. 2016 and references therein). Ranavirus has never been reported from Sweden, but surveys are lacking.

The pathogen is transmitted through both direct (contact with, or ingestion of, contaminated animals), and indirect (i.e., contaminated water) routes, and can persist for several weeks outside a host (Gray et al. 2009). As with *Bd* and *Bsal*, amphibian species differ greatly in the susceptibility to ranavirus, with some species showing 100% mortality in a matter of days, while other species can function as vectors without developing symptoms. Several of the amphibian species of Norway are highly susceptible to this pathogen, including *Bufo bufo*, *Rana temporaria*, *Lissotriton vulgaris,* and *Pelophylax* sp*.* (Miaud et al. 2016).

1.5.2 Bloat

“Bloating” is a term used on many web pages dedicated to amphibia, describing fluid accumulation in body cavities and tissues. According to Mitchell and Tully (2009), a disease called “oedema syndrome”, characterized by marked excess fluid subcutaneously and in the coelom, is caused by “bacterial septicaemia (especially *Flavobacterium* spp.); renal, cardiac, or hepatic failure; toxic insult; poor water quality” (Michell & Tully 2009). However, we have not identified any scientific description of the phenomenon and its aetiology.

An outbreak of “bloat” was reported from a substantial number of frogs in a pond close to Oslo (Norway) in April 2010, in which the frogs swelled up, exploded, and died. Diagnostic investigations at the Norwegian Veterinary Institute revealed growth of various potentially pathogenic bacteria, but no specific cause of the outbreak could be found (B. Ytterhus, pers. comm.).

1.5.3 Severe Perkinsea infection (SPI)

Severe Perkinsea infection (SPI) causes mass mortalities in tadpoles across USA, and is regarded an emerging disease in frogs. It is caused by a pathogenic lineage of protozoa linked to a Novel Alveolate Group 01 of the phylum Perkinsozoa (superphylum Alveolata), tentatively named pathogenic Perkinsea clade (PPC). This disease recently caused mass mortality of green frog (*Rana clamitans*) tadpoles in Wisconsin, which represented the first detection of PPC in the midwestern US (Isidoro-Ayza et al. 2019). To our knowledge, SPI has not been reported in Europe.

1.6 Norwegian regulations concerning amphibians

In Norway, import, release, trading, and keeping of amphibians and reptiles are generally banned through "FOR-2015-06-19-716 - Regulation on foreign organisms" and "FOR-2017-05-11-597 - Regulations on the prohibition of introducing, trading and keeping exotic animals". Here, it is explicitly stated that "*It is forbidden to introduce, trade, and keep exotic mammals, reptiles and amphibians. Exotic animals are animal species that do not live wild in Norway, and which are not traditional production animals, sports animals or family animals in Norway*". Exemption from this ban involves 19 reptile species that were legalized by the Food Safety Authority (NSFA) in 2017, and that can be imported from any country of the world "if the import is considered non-commercial", according to NFSA (https://www.mattilsynet.no/dyr_og_dyrehold/kjaledyr_og_konkurransedyr/eksotiske_dyr/reptilene_som_er_lovlige_etter_15_august_2017.27196)

Protection measures against *Bsal* are covered in "FOR-2018-03-15-356 - Regulations on special protection measures against the fungus *Batachrochytrium salamandrivorians* in connection with the import and export of salamanders". Here, regulations involving health certificates and quarantine are included. The regulations apply to animal health conditions for the import of salamanders from EEA states and third countries to Norway. The regulations also apply to animal health conditions for the export of salamanders from Norway to EEA states. Given that any exotic amphibian import/trade is prohibited according to FOR-2017-05-11-597, and that import of live mammals, reptiles and amphibians from third countries and Svalbard are prohibited by "FOR-2004-02-20-464 - Regulations on animal trade", the *Bsal* regulation seems of little practical applicability in Norway. It would cover the unlikely event of import/export of salamanders native to Norway (smooth newt, *L. vulgaris* and great crested newt, *T. cristatus*) from EEA to Norway and vice versa, which would require a permit from the Norwegian Environment Agency (NEA).

2 Methods and data

2.1 Methodology for risk assessment

The panel used a qualitative risk assessment method that comprises addressing a range of questions covering all aspects requested in the Terms of Reference of this report. The questions cover the organisms' probability of entry and the pathways of entry, establishment and spread, and the potential impact the organisms may have on biodiversity and ecosystem services.

2.1.1 GB-NNRA

The project group used a modified version of the Non-native Species Secretariat for Great Britain form (GB Non-native Risk Assessment scheme, or GB-NNRA, (http://www.nonnativespecies.org/home/index.cfm), with permission to adapt the template granted by the GB-NNRA. The form was developed by a consortium of risk analysis experts in 2005, and has since been improved and refined, and then tested and peer-reviewed by risk analysis experts operating with similar forms in Australia and New Zealand (Roy et al. 2013). The GB-NNRA form complies with the Convention on Biological Diversity and reflects standards used by other forms, such as the Intergovernmental Panel on Climate Change, the European Plant Protection Organization, and the European Food Safety Authority.

2.1.2 Rating and definitions

For each of the questions in the form, the assessor ranks the uncertainty of their response, and also can add further comments. For the taxa assessed in the current assignment, assessors could indicate the level of uncertainty behind a particular response and add further comments to clarify.

Based on the assessment of the overall probability of establishment (based on the probability of entry, establishment, and spread), and potential for environmental impact on Norwegian biodiversity, the project group ended the assessment with a "Conclusion of the risk assessment". This over all summary concludes whether each of the two pathogenic fungi present a low, moderate, or high risk to Norwegian biodiversity, as illustrated in Figure 2.1.2-1.

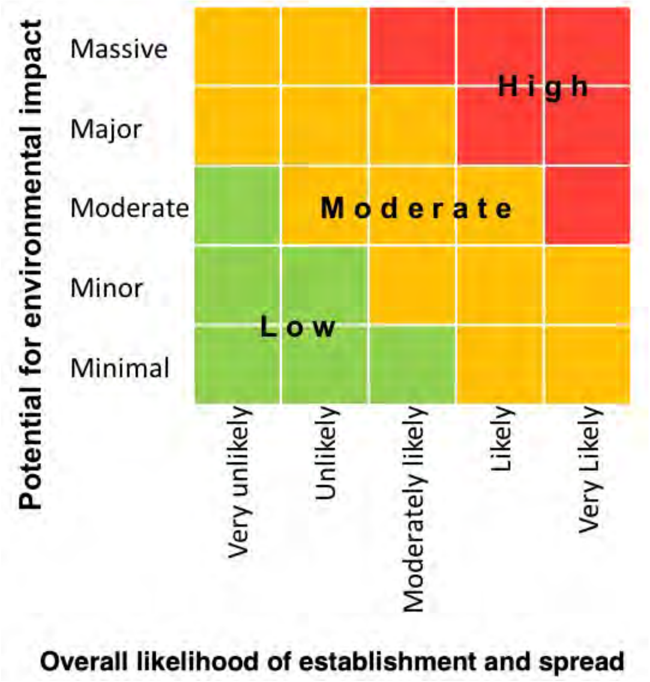


Figure 2.1.2-1: The conclusions of the risk assessments (Low, Moderate, or High) are based on the overall probability of establishment (which includes entry, establishment, and spread) and the potential for environmental impact on Norwegian biodiversity.

In order to provide clear justification when a rating is given in the risk assessment template, the Panel used ratings and adapted versions of the descriptors from Appendix E in (EFSA Panel on Plant Health (PLH) 2015) A description of the ratings used can be found in Tables 2.1.4-2 – 2.1.2-6 below.

Table 2.1.2-1 Rating of the likelihood of entry.

Rating	Descriptors
Very unlikely	The likelihood of entry would be very low because the species: • is undocumented in Europe • is host specific • cannot survive outside its hosts
Unlikely	The likelihood of entry would be low because the species: • is rare in Europe • can only infect to or three species • is difficult to keep alive outside its hosts
Moderately likely	The likelihood of entry would be moderate because the species: • is established in some parts of Europe • mostly host specific, but can also infect a few other species • can survive outside its host for short periods
Likely	The likelihood of entry would be high because the species: • is established in several areas of Europe • can infect a small range of species • can survive for several hours outside its hosts
Very likely	The likelihood of entry would be very high because the species: • is common in Europe • is a generalist pathogen • can survive for longer periods outside its hosts

Table 2.1.2-2 Rating of the likelihood of establishment.

Rating	Descriptors
Very unlikely	The likelihood of establishment would be very low because: • of unsuitable environmental conditions • of the absence or very limited availability of required hosts • the occurrence of other considerable obstacles prevents establishment
Unlikely	The likelihood of establishment would be low because: • of the unsuitable environmental conditions in most parts of Norway • of the limited availability of required hosts • the occurrence of other obstacles that hinders establishment
Moderately likely	The likelihood of establishment would be moderate because: • environmental conditions are suitable in few parts of Norway • required hosts are abundant in only a few areas of Norway • there are only minor obstacles to establishment occur
Likely	The likelihood of establishment would be high because: • environmental conditions are suitable in some parts of Norway • required hosts are widely distributed in some areas of Norway • no obstacles to establishment occur
Very likely	The likelihood of establishment would be very high because: • environmental conditions are suitable in most parts of Norway • required hosts are widely distributed in Norway • no obstacles to establishment occur

Table 2.1.2-3 Rating of the likelihood of spread.

Rating	Descriptors
Very unlikely	The likelihood of spread would be very low because: • the species can only spread through specific infected hosts • highly effective barriers to spread exist (e.g. patchy distributed habitats) • required hosts are not, or very rarely, present in the area of possible spread
Unlikely	The likelihood of spread would be low because: • the species can only spread through a limited range of infected hosts • effective barriers to spread exist (e.g. patchy distributed habitats) • required hosts are only occasionally present
Moderately likely	The likelihood of spread would be moderate because: • the pathogen can spread through a wide range of hosts, but not by human activity • partly effective barriers to spread exist (mosaic landscape of suitable habitats) • required hosts are usually present, but at a low abundance
Likely	The likelihood of spread would be high because: • the pathogen spreads easily through a wide range of hosts and can to some degree be spread by human activity • no effective barriers to spread exist • required hosts are always present, but at a low abundance
Very likely	The likelihood of spread would be very high because: • the pathogen spreads easily through a wide range of hosts and can easily be spread by human activity • no effective barriers to spread exist • required hosts are always present, and with high abundance

Table 2.1.2-4 Rating of the assessment of impact.

Rating	Descriptors
Minimal	No known impact on local biodiversity
Minor	Potential impact on local biodiversity, but only occasional deaths of individuals
Moderate	Impact may cause moderate reduction in native populations
Major	Impact may cause severe reductions in local populations with consequences for local biodiversity and ecosystem functions and services
Massive	Impact may cause severe reductions in local biodiversity (local extinctions), with severe consequences for ecosystem functions and services

Table 2.1.2-5 Ratings used for describing the level of confidence

Rating	Descriptors
Very low	There is very little or no published data on the topic. Only expert judgement used.
Low	Available information on the topic is limited, and mostly expert judgements are used.
Medium	Some published information exists on the topic, but expert judgements are still used.
High	There is sufficient published information, and expert judgements are in concurrence.
Very high	The topic is very well debated in peer-reviewed journals, and international reports. Expert judgements are in concurrence.

2.2 Literature search

A series of searches in Web of Science (Science Citation Index Expanded, Social Sciences Citation Index og Arts & Humanities Citation Index), Wiley (STM collection (Science, Technical, Medical)) and Science Direct (Elsevier, ink. Academic Press, Harcourt Health Sciences, ESME and Urban & Fisher imprints) was performed using the following search terms:

Search term(s)	# Retrieved publications
Batrachochytrium	1504
Chytridiomycosis	1299
Batrachochytrium AND chytridiomycosis	1083
Batrachochytrium AND dendrobatidis	1477
Batrachochytrium AND salamandrivorans	76
Batrachochytrium AND Europe	71
Batrachochytrium AND Sweden	2
Batrachochytrium AND Denmark	1
Batrachochytrium AND Norway	1
Batrachochytrium AND Finland	0
Batrachochytrium AND Bufo	102
Batrachochytrium AND Bufo bufo	102

Search term(s)	# Retrieved publications
Batrachochytrium AND Rana	421
Batrachochytrium AND Rana temporaria	28
Batrachochytrium AND Rana arvalis	4
Batrachochytrium AND Pelophylax	26
Batrachochytrium AND Pelophylax esculentus	9
Batrachochytrium AND Pelophylax lessonae	5
Batrachochytrium AND Lissotriton	10
Batrachochytrium AND Lissotriton vulgaris	4
Batrachochytrium AND Triturus	10
Batrachochytrium AND Triturus cristatus	2

Not all of the articles were examined, but these, and references therein, form the scientific basis for this report.

2.3 Other literature

In addition to published scientific papers, important sources of information for this project have been four reports on the subject from the Norwegian Institute for Nature Research (Taugbøl et al. 2017; Taugbøl et al. 2018a; Taugbøl et al. 2018b; Taugbøl et al. 2019). Also, the risk assessment of *Bathrachocytrium salamandrivorans* in EU (EFSA Panel on Animal Health and Welfare (AHAW) et al. 2018), commissioned by EFSA, and the doctoral thesis of Freya Smith (Smith 2014) provided useful data and insights on these organisms.

2.4 Modelling

2.4.1 Data

Occurrence data for Norwegian amphibians were obtained from The Norwegian Biodiversity Information Centre (Artsdatabanken, https://www.biodiversity.no/), the Global Biodiversity Information Fability (GBIF, https://www.gbif.org/).

Temperature and precipitation data were taken as monthly gridded reanalysis data from the ERA5 dataset from the Copernicus Climate Change Service (https://climate.copernicus.eu/climate-reanalysis), sub-setted and aggregated to monthly values by the Norwegian Meteorological Institute. Thus, minimum (T_{min}), maximum (T_{max}), and mean (T), temperatures below refers to the respective annual values of these.

Land cover and land use (LCLU) data, as well as a digital elevation model (DEM), were provided by the Norwegian Mapping Authority (Kartverket) as a merge of the AR250 database (see description in English at https://kartkatalog.geonorge.no/metadata/norsk-institutt-for-biookonomi/arealressurskart-ar250-arealtyper/de72929c-b250-461a-85d8-2557a2597ab4). Information on *Bd/Bsal* was systematized from literature referenced in this publication.

2.4.2 Software

R (v3.5.2) was used, with the packages chron, RColorBrewer, lattice, chron, RColorBrewer, ncdf4, mgcv, nlme, lattice, ggplot2, rgbif, dismo, rgdal, maptools, devtools, raster, sp, sf, spatialEco, fasterize, rasterVis, oce, smoothr, lwgeom, rgeos, and rmapshaper.

2.4.3 Spatial point distributions

Spatial point distribution were made for each amphibian species by merging the data sources into latitude-longitude coordinates of the same projections, adding and subtracting a few (< 20) observations known to be erroneous or lacking.

The point observations were also converted to raster density maps by merging all observations within a raster cell to one count value and capping outliers at the 0.99 percentile. To counter artificial effects of arbitrary raster edges and show connectivity the raster was smoothed, so that values on the edge of the matrix were unaltered. For interior points, the result was defined in terms of the original as follows: r_[i,j] = (2 m_[i,j] + m_[i-1,j] + m_[i+1,j] + m_[i,j-1] + m_[i,j+1])/6. Here r[i,j] is the focl raster cell and *i,j* the matrix coordinates.

While some studies reported a local abundance of ponds to be associated with lower prevalence of *Bd* (Kärvemo et al. 2018), others found increasing host density to significantly increase the likelihood of *Bd* infection, irrespective of diversity or susceptibility (Bielby et al. 2015). As local spread is facilitated by higher densities and shorter distances between habitats, and a higher number of species gives both a higher probability of *Bd* being present (Höglund, pers. comm. 2019), as well as indicating a more important habitat for amphibians (and thus higher impact on biodiversity), joint density is a positive contributor to the risk/impact maps. One species, *Rana arvalis,* has been shown to act as a reservoir species in Sweden (Kärvemo et al. 2018) and thus given extra weight. As *Rana temporaria* is generally resistant, but has been shown to be infected under strong temperature stress, its occurrence is downweighted inversely to the strength of the estimated temperature mismatch.

Bsal, however, is likely to be a poorer natural disperser, as lack of *Bsal* dispersal has been found between neighbouring salamander populations (Spitzen van der Sluijs et al. 2018). Thus, the observation density does not carry over into neighbouring raster cells to increase local risk, as is the case for *Bd*.

2.4.4 Temperature and LCLU

The ERA5 monthly temperature data, as well as the elevation raster, were resampled to match the resolution of the occurrence data, and the AR250 LCLU polygons were rasterized by cell centre point on the same resolution for each map layer. No smoothing procedures were performed on temperature, elevation, or LCLU data, and the LCLU values were treated as factorial for further analysis. Coast and border polygons were smoothed for visibility, and countries for which data are lacking were masked out in grey. Mean and minimum temperatures, together with elevations, are shown in figures A3-1 – A3-6 (Appendix III).

2.4.5 Temperature increase and temperature mismatch potentials

Being at high latitudes, Norway is currently experiencing climate changes significantly faster than the global average. Thus, an attempt at assessing risk or impact on amphibian biodiversity must attempt to address how this is impacted by ongoing climate processes. However, too little is known about the biology of the pathogens and their interactions with host community, phenology, microbial community, and the wider environment to parameterize predictive models for Norway. Thus, due to the paucity of data, a qualitative assessment is the only option available.

We measured the rate of temperature increase as the linear long-term trend for monthly average temperature, T, in each raster cell *ij* after controlling for monthly variation, so that

$$T_{t,i,j}=a_{0,i,j}+a_{1,i,j}Y_t+f(M_t)+\varepsilon_{t,i,j}$$

where $\varepsilon_{t,i,j}$ are normally distributed errors, and $f(M_t)$ a non-parametric smoothing spline through monthly mean values M. Thus, the trend is found as the maximum likelihood $\Delta T_{i,j}$ estimate for $a_{1,i,j}$. As Norway is still far from experiencing upward temperature limitation, and *Bd* have a decreased growth rate at low temperatures, we believe the weight of the evidence suggests that mean temperature, temperature increase, and, in particular, increasing temperature variance are stressors positively associated with risk of establishment and impact on populations. Several studies have suggested that the effect of temperature mismatch is important because it affects the skin microbiota and/or stresses the immune system of potential hosts, leading to a greater mortality rate for the host (and, presumably, a higher replication rate for the pathogen). Stress due to climate change impacts the immune system unpredictably (Rollins-Smith 2017), but infection risk in ectotherms is generally predicted to increase as the difference between host and pathogen environmental tolerances decreases (Nowakowski et al. 2016; Cohen et al. 2019). Changes in temperature-dependent immune parameters lag behind short-term temperature changes, and the seasonal acclimation hypothesis, which predicts that immune cell production declines during long-term temperature decreases until amphibians can fully acclimatise to winter conditions (Raffel et al. 2006). This supports the hypothesis that increased variance may be a factor in increasing *Bd* virulence and/or prevalence.

While disease-induced extinction is generally considered rare, diseases with load-dependent pathology have led to extinction in wildlife populations. Wilber et al (2017) found that *Bd*-induced extinction dynamics are likely to be far more sensitive to host resistance and tolerance than to changes in *Bd* transmission, as non-linear resistance and tolerance functions may interact such that small changes in these functions lead to drastic changes in extinction dynamics. Thus, changes in host resistance or tolerance due to environmental stress may play a disproportionate role in the population effect and extinction risk following Bd/Bsal spread.

Studies of *Bd* in culture suggest that optimal growth occurs between 17 and 23°C, and death of the fungus occurs above 29°C or below 0°C (Sonn et al. 2017). However, *Bd* strains differ in thermal response, and studies of multiple strains have shown several to be viable after freezing (24 h at -12°C) and heat shock (28 °C) treatments, with widely differing optimum growth conditions and thermal sensitivity (Voyles et al. 2017) Amphibian immune systems, however, are also temperature dependent and adapted to work optimally within a certain range.

Thus, a study of *Bd*-infected northern cricket frog (*Acris crepitans*) native to temperate north-eastern Mexico and eastern US under optimal and colder regimes, found pathogen load and mortality to be inversely related to temperature (Sonn et al. 2017). Survival of infected hosts was greatest between 20 and 26°C, temperatures at which *Bd* grows well in culture. This suggests that the conditions under which a pathogen grows best in culture do not necessarily reflect patterns of pathogenicity in the wild.

Northern or isolated populations may be more vulnerable to *Bd* infection due to temperature mismatch, but also because of lower genetic diversity (Cortazar-Chinarro et al. 2019). A study in the Pyrenees investigated seasonality (the timing of spring ice-thaw) vs. susceptibility to *Bd* infection in a montane amphibian community that is suffering declines and extirpations. They found a robust temporal association between the timing of the spring thaw and *Bd* infection in two host species, and showed that an early onset of spring forced high prevalence of infection (Clare et al. 2016). The susceptibility of larger hosts and hosts from lower latitudes to *Bd* may be particularly prone to be influenced by thermal mismatches (Cohen et al. 2019). A recent study suggests that only the combination of rapid increases in temperature and infectious disease could account for the patterns of amphibian declines, especially in species adapted to relatively cool environments. This is because hosts adapted to relatively cool conditions are most vulnerable to the combination of increases in mean temperature and EIDs (Cohen et al. 2018).

In addition to the rate of temperature increase, the frequency and magnitude of expected temperature mismatch for each raster cell was expressed in three different ways for this analysis: If the temperature difference between seasons for each year, y, so that:

$$T_{\Delta S,y} = T_{\text{summer max, y}} - T_{\text{winter min,y}}$$

where $T_{\text{summer max, y}}$ is the highest monthly mean May-August and $T_{\text{winter min,y}}$ the lowest monthly mean of late winter January-March, then the mean seasonal difference T_S is simply:

$$T_S = \sum(T_{\text{summer max, y}} - T_{\text{winter min,y}})N_Y^{-1}$$

Where N_Y is the number of years. Similarly, the maximum likelihood estimate of $T_{\Delta s}$ in:

$$T_{\Delta s} = (a_0 + T_{\Delta S,y} + \varepsilon_t)\ Y^{-1}$$

where Y is year and ε a normally distributed set of residuals, gives a measure of the rate of change in seasonal difference.

To quantify increasing anomalies, we also used a measure A_v where if for each spring month m{3,4,5}, year y the anomaly A is measured as the deviation from the 1979-2000 mean:

$$A_{m,y} = T_{m,y} - \frac{1}{N_Y}\sum\nolimits_{y=1979}^{y=2000} T_{m,y}$$

Then A_V is the difference between the mean monthly anomaly before and after the year 2000:

$$\mathrm{A}_V = \frac{1}{18}\sum\nolimits_{y=2001}^{y=2018} A_{m,y} - \frac{1}{22}\sum\nolimits_{y=1979}^{y=2000} A_{m,y}$$

Similarly, the anomaly measure A_G based on the monthly temperature expectation for the whole year predicted by:

$$T_{G,m}=f(m) + \varepsilon_t$$

where $f(m)$ is a non-parametric smoothing spline based on all monthly temperatures 1979-1990. Then the mean squared difference between $f(m)$ and the observed values 2001-2018 gives us:

$$A_G = \frac{1}{18}\sum_{y=2018}^{y=2001} (T_{m,y} - f(\mathrm{m}))^2$$

thus, capturing anomalies throughout the year. As this measure seemed to be the least unbiased towards seasons and elevations, it was used as the primary measure of temperature anomalies and mismatch potential. As a measure of raw variance V we have:

$$V_\Delta = (a_0 + V_{\Delta,y} + \varepsilon_t)\, Y^{-1}$$

obtained as maximum likelihood estimates of rate of change in within-year monthly temperature variance $V_{\Delta,y}$ and the corresponding regression.

2.4.6 Longer-term risk

Sub-lethal, longer-term effects are not well known for the species under consideration, but should be expected to correlate only to some extent with the direct risk estimates. Both lethal and sub-lethal costs have been found in individuals that are able to resist *Bd* infection, indicating that successful immune response to infection comes at a cost. As increasing host density significantly increased the likelihood of susceptible individuals becoming infected with *Bd*, even populations of resistant species are likely to suffer ill-effects of exposure to *Bd* (Bielby et al. 2015). Tolerating *Bd* infection may alter the thermal thresholds of hosts (Greenspan et al. 2017) and erode the capacity of populations to withstand periodic recruitment failure; a common challenge for species reproducing in uncertain environments. Reduced capacity to tolerate other sources of mortality may explain variations in decline severity among other *Bd*-challenged species and highlights the need to mitigate disease impacts through minimizing other sources of mortality to avoid population declines and biodiversity loss (Scheele et al. 2016).

2.4.7 Distribution models

As point observations are necessarily biased towards areas with more observers, and not all populations are noted for each species, a simple spatial occurrence model was conducted for each species' occurrence, so that the observation density, D, of each cell i,j is expressed as:

$$D_{i,j} = a_0 + f(LCLU_{i,j,g}) + f(MOH_{i,j}|k_1) + f(T_{mean,i,j}|k_1) + f(T_{min,i,j}|k_1) + f(P_{mean,i,j}|k_1) + f(T_{S,i,j}|k_1) + f(La_{,i,j}|k_1) + \varepsilon_{i,j}$$

Here, LCLU refers to a set of factorial variables denoting g layers of land use/land cover data for each cell, MOH is elevation in meters above sea level (raster center), T_{mean} and T_{min} being temperature mean and minimum, P_{mean} mean precipitation, T_S the mean seasonal temperature range described above, La the latitude, and ε a set of quasi-Poisson distributed errors to allow for overdispersion. $f(X|k_1)$ denotes a non-parametric penalized smoothing function with $k_1=3$ maximum degrees of freedom to achieve non-linear responses where applicable while retaining unimodality. The fitted values E(D) were then used as a measure of habitat suitability and expected density of unobserved populations to be combined with climate suitability for the risk/impact estimates (see below). The maps are shown on log scale.

2.4.8 Risk/Impact assessment

Here, we use available data to map a qualitative assessment of how the likelihood of *Bd* and *Bsal* outbreaks with a negative impact on amphibian populations vary over Norway. These are presented in Figures 9.2-2 and 9.3-2.

2.4.8.1 Assumptions

The model incorporates the following literature-based assumptions and limitations:

- The total number of host populations recorded in each map raster cell of roughly 6x6 km is positively correlated with risk.
- Our databases of amphibian populations are incomplete. In order to partially address this, we modelled their occurrence density using climate and land use/land cover data to get a smoothed and spatially complete estimate of potential observation density.
- *Bsal* has a wide temperature range, and is thus independent of mean temperatures.
- *Bd* has a wide temperature range, but may be more favoured by warm springs than its hosts at Norwegian latitudes. There is, therefore, some positive correlation with temperature and temperature increase.

- For both *Bd* and *Bsal*, rapid changes/increased variance in climate makes host populations more susceptible through stress and/or perturbations oin protective skin microbiota.
- Some species (*Rana arvalis*) are potential amplifiers/reservoir species and are weighted extra.
- Some species (*Rana temporaria*) are fairly resistant and are weighted less except when temperature mismatch is high.
- Some species (*Bufo bufo*) are of low to intermediate susceptibility, but northern and/or cold adapted populations may be more susceptible and are weighted according to temperature.

These factors combined, as described in the main text, allows estimation of the most likely parts of Norway to suffer negative consequences should *Bd* or *Bsal* occur there, and the most likely regions of spread, should this occur naturally and locally. Long-distance (human-driven) transmission is not possible to predict in this context, and no reliable information about the frequency of natural leptokurtic (bird-driven) spread is available.

2.4.8.2 Scale

The risk is scaled from 0 (no risk/impact due to no amphibian hosts present, or few and resistant hosts in climate conditions not expected to favour the disease), to 5 (highest risk areas within Norway with high host densities, amplifying species present, and climate conditions suspected of facilitating disease outbreaks).

2.4.8.3 Other factors

Other risk factors are expected to be present, such as differences in environmental microbiota, water chemistry, genetic differences in hosts and pathogens, unknown vectors/reservoirs, and landscape effects. However, the data and/or knowledge are not currently available for their incorporation. Thus, this assessment is, at best, a best estimate from the factors that we have, and its accuracy will depend on the extent to which these correlate with the factors that we lack information about and their respective effect sizes.

2.4.8.4 Climate parameters for risk/impact maps

The climate parameters considered most likely to impact the potential for *Bd/Bsal* persistence, prevalence, and mortality were feature scaled so that:

$$F(X) = \frac{X - \min(X)}{\max(X) - \min(X)}$$

Where X is the temperature feature in question (see below).

And combined with the joint occurrence models for qualitative spatial risk/impact estimates so that:

$$\mathrm{C}_{Bsal,i,j} = \mathrm{c}_0\mathrm{F}\left(A_{G,i,j}\right) + \frac{1}{1 + e^{\mathrm{c}_1(\mathrm{F}(V_{i,j}) + \mathrm{c}_1\mathrm{F}(\Delta\mathrm{Ti,j}\,) + \mathrm{c}_2\mathrm{F}(A_G,\mathrm{i,j})}}$$

Where c_{0-2} are constants weighing the contributions chosen to scale the maximum risk to 5 and minimum to 0, and giving a contribution consisting of mismatch and a logistic increase in probability from the lesser factors. Thus, the final measure of estimated spatial risk distribution becomes:

$$\mathrm{R}_{\mathrm{Bsal,i,j}} = \ln(\mathrm{D}_{\mathrm{i,j}}\mathrm{C}_{Bsal,i,j} + 1)$$

For anurans, *Bufo bufo* was scaled inversely to minimum temperature as it is generally resistant, but has shown more susceptibility further north. *Rana arvalis* was weighted double, as it has been seen to function as a reservoir species, and *Rana temporaria* scaled according to the main measure of temperature mismatch so that:

$$\mathrm{D}_{\mathrm{anur,i,j}} = \mathrm{PL} + \mathrm{PL} + \mathrm{F}(1/\mathrm{T}_{\mathrm{min}})\ \mathrm{BB} + 2\mathrm{RA} + \mathrm{F}(A_{G,i,j})\mathrm{RT}$$

Otherwise, the process was identical, except the addition of a temperature dependency in the susceptibility part as *Bd* is suspected to respond more favourably to increasing temperatures than *Bsal* (see above):

$$\mathrm{C}_{Bd,i,j} = \mathrm{c}_0\mathrm{F}\left(A_{G,i,j}\right) + \frac{1}{1 + e^{\mathrm{c}_1(\mathrm{F}(V_{i,j}) + \mathrm{c}_1\mathrm{F}(\Delta\mathrm{Ti,j}\,) + \mathrm{c}_2\mathrm{F}(A_G,\mathrm{i,j})}} + \frac{1}{1 + e^{\mathrm{c}_3(\mathrm{F}(T_{mean,i,j}) + \mathrm{c}_4\mathrm{F}(T_{min,i,j})}}$$

So:

$$\mathrm{R}_{\mathrm{Bd,i,j}} = \ln(\mathrm{D}_{\mathrm{i,j}}\mathrm{C}_{Bd,i,j} + 1)$$

The maps are thus known to incorporate a number of qualitative assessments where data are non-existent, and thus represent concrete visualizations of the underlying assumptions rather than qualitative statistics.

3 Hazard identification

3.1 *Batrachochytrium dendrobatidis* (*Bd*) in Norway

As of January 2019, *Bd* has been identified in six ponds in Akershus county from analysis of filtered water samples (environmental DNA; e-DNA). Within these six ponds, *Bd* DNA has been detected on crested newts using swab samples in four of the five ponds where animals were swabbed. The prevalence of infected crested newts varied between the ponds, and in total 18 of 54 animals had positive swab samples (Taugbøl et al. 2019). DNA from the positive swabs were recently tested at the Norwegian Veterinary Institute with a new qPCR method specifically amplifying *Bd*-GPL, and kindly provided by P. Ghosh and M. Fisher (unpublished). All sample yielded positive results, strongly suggesting that it is the global panzootic lineage (GPL) of *Bd* that has been introduced to Norway (D. Strand, T. Vrålstad, A. Taugbøl and F. Fossøy, unpublished results). None of the 36 swabbed smooth newts from the same infected ponds were found to be positive for *Bd*, and neither were three swabbed frogs and three swabbed tadpoles. As the number of swabbed individuals, especially for frogs, is low, the negative result could be false negatives. Figure 3.1-1 illustrates the sites for sampling and the results of *Bd*-screening in Norway.

Figure 3.1-1: Areas in Norway where ponds have been screened for *Bd*; from the left, Lier in Buskerud (green rectangle, 14 ponds screened), Akershus (red rectangle, 17 ponds screened) ,and Østfold (green rectangle, 25 ponds screened). Akershus and the sampled ponds are shown to the right with the six infected ponds in red and ponds not infected in green.

Water samples from ponds inhabited by crested newts in Østfold in 2018 (25 ponds, sampled in July), Lier (15 ponds, sampled in June), Hordaland (five ponds, sampled in late June), Møre and Romsdal (four ponds, sampled in July) and Telemark (two ponds, sampled in June) have also been tested for *Bd* with negative results. Although these results might indicate that the occurrence *of Bd* in Norway is restricted to the six ponds in Akershus, this is probably an underestimate. The ponds were sampled in June and July 2018 due to e-DNA results from 2017 that indicated the *Bd* concentration in an infected pond to increase through the season. However, the results from 2018 identify the opposite trend, with the concentration of *Bd* decreasing with season in the infected ponds, and the samples from Akershus being negative for *Bd* in July.

3.2 *Batrachochytrium salamandrivorans* (*Bsal*) in Norway

As of January 2019, *Bsal* has not been detected in Norway. In 2017, water samples from a total of 15 ponds in Lier and 15 ponds in Akershus were screened for *Bsal* and were found to be negative.

3.3 Chytridiomycosis in Norway

As of January 2019, there have been no reports or sightings of sick animals, or any detection of large numbers of dead animals, associated with presence of *Bd* or *Bsal* in Norway.

In order for the pathogenic fungi to pose a risk to biodiversity in Norway, they need to enter the country through one of the pathways listed below (Appendix I, Table A1-1, and Appendix II - Table A2-1). A successful entry is required, but not sufficient, for establishment of the organism. The potential pathways of entry for *Bd* and *Bsal* are discussed below. The viability of *Bd* and *Bsal* under sub-optimal conditions, as is the case in some of the various pathways of entry, are unclear.

3.3.1 Anthropogenic – pet trade

Trade in exotic amphibians for private keeping, is probably the most prominent factor that has promoted spread of *Bd* across the world (O´Hanlon 2018), and the introduction of *Bsal* to Europe from Asia (Martel et al. 2014). As private keeping of amphibians remains illegal in Norway, although keeping of some reptile species has recently been legalized, the trade in exotic amphibians in Norway is currently very limited (less than 20 individuals per year).

Overall, entry of *Bd* through this pathway is considered to be unlikely with medium confidence (See Appendix I – Table A1-2), whereas entry of *Bsal* through this pathway is considered to be very unlikely with medium confidence (See Appendix II – Table A2-2).

3.3.2 Anthropogenic – other live animals

Second to pet trade, export of live amphibians for other uses (e.g., research and food), is believed to be the major contributor to spread of *Bd*. As very few (or no) frogs are currently used in research in Norway, and live frogs are not imported for food, this otherwise important pathway of entry appears to be of lesser importance, especially for *Bsal*. However, humans catching and transporting amphibians (*i.e.,* tadpoles) from neighbouring countries could be another plausible mode of entry, but more so for *Bd* than *Bsal*.

Overall, entry of *Bd* through this pathway is considered to be moderately likely with low confidence (See Appendix I – Table A1-3), whereas entry of *Bsal* through this pathway is considered to be very unlikely with medium confidence (See Appendix II – Table A2-3).

3.3.3 Anthropogenic – “hitchhiking” organisms

Bd and *Bsal* could enter the country with hosts that are not intentionally imported, but that are accidental “hitchhikers” on other organisms. This has been the case in Sweden, where imported plants used in nurseries or in sewage-treatment facilities have contained alien frog species. Anecdotal information from researchers involved in screening programmes for introduction of plant and insect species, suggests that amphibians also can “hitchhike” in containers used for terrestrial garden plant import.

Overall, entry of *Bd* through this pathway is considered to be unlikely with low confidence (See Appendix I – Table A1-4), whereas entry of *Bsal* through this pathway is considered to be very unlikely with medium confidence (See Appendix II – Table A2-4).

3.3.4 Anthropogenic – mechanical

Both *Bd* and *Bsal* can both survive for shorter periods on recreational and other gear used by people, such as boots, boats, canoes, traps, and fishing equipment (e.g., nets) (Stegen et al. 2017). As many Norwegians undertake outdoor activities in Sweden, this is a potential pathway of entry from Sweden to Norway.

Overall, entry of *Bd* through this pathway is considered to be moderately likely with low confidence (See Appendix I – Table A1-5), whereas entry of *Bsal* through this pathway is considered to be very unlikely with medium confidence (See Appendix II – Table A2-5).

3.3.5 Non-host animals

Bd and *Bsal* can potentially be transported by different organisms that are not infected, but only function as vectors. These include crustaceans (e.g., crayfish) and reptiles (e.g., *Natrix natrix*), and, most prominently, different species of waterfowl.

Overall, entry of *Bd* through this pathway is considered to be moderately likely with low confidence (See Appendix I – Table A1-6), whereas entry of *Bsal* through this pathway is considered to be unlikely with medium confidence (See Appendix II – Table A2-6).

3.3.6 Natural spread by infected hosts

Infected hosts can introduce the pathogenic fungi to new habitats. In Norway, the only natural introduction following this pathway, would be from Sweden, and then would be mainly restricted to the areas close to the border. It is, however, unclear whether these infections occur in the areas of Sweden that border Norway.

Overall, entry of *Bd* through this pathway is considered to be likely with high confidence (See Appendix I – Table A1-7), while entry of *Bsal* through this pathway is considered to be very unlikely with medium confidence (See Appendix II – Table A2-7).

3.3.7 Summarized likelihood of entry of *Bd*

In summary, the likelihood of *Bd* entering Norway, through any of the pathways outlined above, is considered to be moderately likely with medium confidence. The most likely pathway of entry includes infected animals from Sweden by dispersal in areas close to the border.

3.3.8 Summarized likelihood of entry of *Bsal*

In summary, the likelihood of *Bsal* entering Norway through any of the pathways outlined above, is considered to be very unlikely with medium confidence. The only scenario that appears to be anything more than very unlikely is entry via waterfowls transporting the pathogenic fungal spores on their feet. However, this would require rapid translocation from high-risk areas, such as the Netherlands, without exposure to seawater.

4 Hazard characterisation

4.1 Consequences of establishment of *Bd* in Norway

The potential consequences of establishment of *Bd* in Norway are directly linked to whether such an introduction would cause chytridiomycosis. Infected animals may succumb to the pathological effects (See 1.4.1.1), or experience only subclinical effects (See 1.4.1.2).

The overall likelihood of negative environmental impact on biodiversity in Norway from the establishment of *Bd* is considered to be minor with medium (bordering to high) confidence (See Appendix I – Table A1-8).

When assessing the consequences of *Bd* establishment, the prevalence of *Bd* (as reported from other comparable countries) was considered (See 1.3.1.5). Thus, for each of the indigenous amphibian species in Norway, we assessed the consequences of establishment of *Bd* as:

- minimal with medium confidence for *Triturus cristatus*
- minimal with medium confidence for *Lissotriton vulgaris*
- moderate with low confidence for *Bufo bufo*
- minimal with medium confidence for *Rana temporaria*
- minor with low confidence for *Rana arvalis*
- moderate with medium confidence for *Pelophylax lessonae*

For the introduced frog species on Finnøy, we assessed the consequences of establishment of *Bd* in Norway as:

- moderate with medium confidence for *Pelophylax esculentus*

4.2 Consequences of establishment of *Bsal* in Norway

The overall likelihood of negative environmental impact on biodiversity in Norway from the establishment of *Bsal* is considered to be moderate with medium (bordering to high) confidence (See Appendix I – Table A2-8).

When assessing the consequences of *Bsal* establishment, the prevalence of *Bsal* (as reported from other comparable countries) was considered. (See 1.3.1.5). Thus, for each of the indigenous amphibian species in Norway we assessed the consequences of establishment of *Bsal* as:

- minor with medium confidence for *Triturus cristatus*
- minor with medium confidence for *Lissotriton vulgaris*

4.3 Consequences of spread of *Bd* within Norway

Should *Bd* spread widely in Norway, the consequences would be of similar magnitude as described for establishment, but over a broader geographic area. The only additional consequence related to spreading of the disease would be an increased likelihood of transmission to the small ingenious Nordic pool frog (*P. lessonae*) population in Agder.

4.4 Consequences of spread of *Bsal* within Norway

Should *Bsal* spread widely in Norway, the consequences would be of similar magnitude as described for establishment, but over a broader geographic area. The project group cannot envisage any additional consequences related to spreading of the disease.

4.5 Consequences of a chytridiomycosis outbreak in Norway with regards to biodiversity

A chytridiomycosis outbreak in Norway may reduce local population sizes of certain amphibian species, in particular, the two newt species and the common toad. If mass mortality due to chytridiomycosis were to occur in Norway, its main ecological effect would be on the infected species themselves and on different trophic levels:

1. Tadpoles filter-feed on single-celled organisms and chew on plant and animal debris, thereby contributing to decomposition and nutrient cycling. Reduced predation pressure on the plankton community and a reduction in decomposition rates in the waterbodies in which the amphibians currently breed might cause eutrophication through alterations in the food web.
2. Adult amphibians feed on invertebrates and small fish, and a reduction in their population sizes might have positive effects on their prey species. However, the amphibian species found in Norway are generalist feeders, so it is unlikely that a particular prey species will gain a significant advantage.
3. Reduced amphibian population sizes will reduce the availability of food for the amphibians' main predators. Amphibians usually breed in waters where few predators exist, whereas reptiles, carnivores, and birds of prey might feed on the adults. However, as none of these are amphibian specialists, they would be able to survive on other prey.

We assess the potential ecosystem effects of a chytridiomycosis outbreak in Norway to be limited.

5 Exposure / Probability

5.1 Likelihood of establishment of *Bd* in Norway

Bd has already been identified in Norway (See 3.1) and we therefore conclude that it is very likely, with high confidence, that *Bd* will establish (or re-establish following new entries) in Norway (See Appendix I, Table A1-9).

5.2 Likelihood of establishment of *Bsal* in Norway

Neither climatic, nor other abiotic factors are likely to be of hindrance for establishment for *Bsal* in Norway. However, as this pathogen is not yet detected in any neighbouring countries, and with the absence of likely pathways of entry, the project group concludes that establishment of *Bsal* in Norway is very unlikely with medium confidence (See Appendix II, Table A2-9). Strong biosecurity measures and the existing management practices already in place in Norway (See 1.7) and other European countries (European Food Safety Authority (EFSA) et al. 2017) underpin this conclusion.

5.3 Likelihood of spread of *Bd* within Norway

Bd has been found in a restricted area in Norway, with a patchy distribution (See 3.1), similar to the distributions reported from Sweden and UK. This illustrates that although the infection may spread among water bodies, there are factors limiting the spread throughout a water system, as uninfected ponds are found in close vicinity of infected ponds. Importantly, we observe a patchy distribution, and not an infection pattern consistent with a wave of spread, neither in Norway nor other comparable countries (e.g., Sweden and UK). We therefore conclude that *Bd* is likely to spread in Norway over the next 10 years, with high confidence (Appendix I, Table A1-10). Lack of knowledge on dispersal capability outside its hosts makes it difficult to assess whether this is likely or very likely.

The pathways for spread within Norway are the same as for entry into the country, and we assess the likelihood of further spread within Norway along these pathways to be the same as for entry (See 3.4.1 – 3.4.5).

5.4 Likelihood of spread of *Bsal* within Norway

Recent but few reports suggest that *Bsal* is a poor disperser, even in suitable climate with an adequate density of very susceptible hosts (Spitzen van der Sluijs et al. 2018). In Norway, where susceptible hosts are both fewer in numbers, and more patchily distributed, the project group is of the opinion that spread of *Bsal* is unlikely (in the rare event that it would enter the country and become established), with medium confidence (See Appendix II, Table A2-10).

The pathways for spread within Norway are the same as those for entry into the country, and we assess the likelihood of further spread within Norway along these pathways to be the same as for entry (See 3.4.1 – 3.4.5).

5.5 Likelihood of an outbreak of chytridiomycosis in Norway

The only registered chytridiomycosis outbreak in Europe caused by *Bd*, was in Spain in 2005 (Bosch & Martínez-Solano 2006) and appeared to have a limited effect on the population size, as the mortality rate due to the disease was moderate. In other European countries (e.g., UK, Sweden, Germany), where *Bd* has been studied extensively, no chytridiomycosis outbreaks have been detected.

For *Bsal*, a few outbreaks have been registered in Europe, more specifically in Belgium, the Netherlands, and Germany where *Bsal* has caused rapid declines and local extinctions of the fire salamander, *Salamandra salamandra* (Martel et al. 2013; Spitzen van der Sluijs et al. 2016; Stegen et al. 2017). The Norwegian species great crested newt (*Triturus cristatus*) and (*Lissotron vulgaris*) have been demonstrated susceptible to *Bsal* in infection trials (Martel et al. 2014; Bates et al. 2018), but outbreaks in the wild has not been reported.

We therefore assess that the overall likelihood of a future chytridiomycosis outbreak in Norway is unlikely, but should one occur it would be more likely to be associated with *Bsal* than *Bd*, based on susceptibility of the Great crested newt and smooth newt, and the temperature optimum for *Bsal* that is overlapping with a Northern European climate.

6 Risk characterisation

6.1 Risk of negative impact on biodiversity following establishment of *Bd* in Norway

For the indigenous amphibian species in Norway, we assess that the total risk of negative impact following establishment of *Bd* in Norway as:

- low with high confidence for *Triturus cristatus*
- low with high confidence for *Lissotriton vulgaris*
- moderate with medium confidence for *Bufo bufo*
- low with medium confidence for *Rana temporaria*
- moderate with medium confidence for *Rana arvalis*
- moderate with medium confidence for *Pelophylax lessonae*

For the introduced frog species on Finnøy, we assess the consequences of establishment of *Bd* in Norway as:

- moderate with medium uncertainty for *Pelophylax esculentus*

6.2 Risk of negative impact on biodiversity following establishment of *Bsal* in Norway

For the two indigenous species of newts in Norway, we assess the total risk of negative impact following establishment of *Bsal* in Norway as:

- low with medium confidence for *Triturus cristatus*
- low with medium confidence for *Lissotriton vulgaris*

6.3 Risk of negative impact on biodiversity following spread of *Bd* in Norway

Based on the low prevalence of *Bd* infections in the amphibian species found in Norway, the risk of negative impact on the biodiversity would not increase significantly with spread. The only change would be a minor increase in the risk of negative impact on the small indigenous (Nordic) pool frog (*P. lessonae*) population in Agder, but not enough to increase the risk category from moderate to high.

6.4 Risk of negative impact on biodiversity following spread of *Bsal* in Norway

Given the patchy distribution of the two host species, and relatively low prevalence compared with other species in Europe, the risk posed to biodiversity in Norway is not expected to increase following spread of *Bsal*.

6.5 Risk of negative effects on Norwegian biodiversity from an outbreak of chytridiomycosis

If a chytridiomycosis outbreak were to happen and result in mass mortality of amphibians in Norway, we assess that it would have limited effects on Norwegian biodiversity in general. Norwegian amphibians are not keystone species and they have naturally large among-year variations in population sizes. The main concern of chytridiomycosis relates to the amphibian species themselves, in particular the (Nordic) pool frog, which is listed as “Critically endangered” on the Norwegian Redlist of Species. Special attention should therefore be directed toward surveillance and conservation of this species.

6.6 Risk posed by *Bd* and *Bsal* in a 50-year perspective

Expected climate changes includes an increase in temperature, earlier onset of spring, and increased precipitation. This is based on climate date from the period 1960-1990 and towards year 2068 under the CO_2 emission scenarios RCP4.5 (emission peak 2040-2050, then decline) and RCP8.5 (business as usual). These scenarios indicate a general temperature increase in Norway at 2.2°C (RCP4.5) to 3. °C (RPC8.5) in the next 50 years. Given the preferred climatic niche of the two pathogenic fungi, the project group predict that these changes will affect the possible risk posed by *Bd* and *Bsal* differently.

6.6.1 *Bd* in a warmer climate

Increased temperature and especially earlier onset of spring is predicted to impact the likelihood of establishment and spread, and thus increase the overall risk of *Bd* infection, for amphibians in Norway. This is because it has been reported that there is a correlation between early onset of spring and an increased prevalence of *Bd* and mortality resulting from chytridiomycosis in European amphibian species. It could be assumed that increased temperatures could possibly lead to higher *Bd* abundance and increased host susceptibility, thus slightly increased likelihood of chytridiomycosis outbreaks resulting in higher mortality rates of frogs and toads in Norway (See Appendix I, Table A1-11). However, the risk associated with a chytridiomycosis outbreak remains low.

6.6.2 *Bsal* in a warmer climate

For *Bsal*, which has been reported to have optimum growth at 10-15°C, it is expected that an increased average temperature of 2-3°C will have a negative effect on the likelihood of establishment, spread, and impact. The project group predicts that increased temperatures will not increase the prevalence of *Bsal* and thus the risk of chytridiomycosis outbreaks in Norway due to this pathogen will remain low (See Appendix II, Table A2-11). However, if Norwegian newts experience reduced immunity and increased vulnerability to infectious diseases as a result of climate change and environmental stress, *Bsal* might, if introduced, also constitute an increased risk to Norwegian newt diversity in a 50-year perspective.

7 Mitigation measures

7.1 Measures tested/used to reduce entry of *Bd* and *Bsal*

Bd is already detected, and presumed to be established in Norway, but even in infected areas, control measures are important to prevent spread of new strains, which may be of higher virulence. As *Bd* and *Bsal* probably have similar entry pathways, the measures extend to both species.

- Identify route(s) for spread and stop the pathway
- Establish surveillance in the wild and in human-held aquariums
- Public awareness campaigns

In order to reduce entry, we need to identify the most likely routes for the species (See 3.3.1-3.3.6), establish surveillance and biosecurity strategies in the wild, in the pet trade for pets that are potential vectors, and for zoological facilities. This could be achieved by implementing diagnostic assays, reference laboratories, and approaches for confirmation of positive samples. Further, to increase public awareness to reduce the risk of the release of illegally housed pet amphibians (non-native or native) there need to be developed an information portal for communication, outreach, and education.

7.2 Measures tested/used to reduce spread of *Bd* and *Bsal*

Once a pest pathogen is present in a water system, it is usually impossible to eradicate. Passive surveiliance is considered the most suitable approach for detecting of Bd and Bsal emergence in wild host populations (EFSA Panel on Animal Health and Welfare (AHAW), 2018). Also, to reduce spread of *Bd* and *Bsal* there is need to establish a surveillance programme to define the extent of the infection, to detect new outbreaks in currently uninfected areas, and to monitor whether the disease is impacting upon amphibian populations. With knowledge of the distribution of *Bd* and *Bsal*, it is possible to establish restricted sites and control areas to which quarantine and movement restrictions can be applied (e.g., schools should avoid certain areas for education) and with more accurate knowledge on distribution it will be easier to obtain appropriate data to monitor the progress and success of any control strategy.

Freshwater pests and pathogens spread to new areas when contaminated water, mud, gravel, soil, plant material, or infected animals are moved between sites. Contaminated materials and animals are commonly transported by humans on boots, equipment, vehicle tyres, and during road construction and maintenance activities. Detailed information is available on how to disinfect all types of equipment after contact with water to prevent the spread of waterborne pests (e.g., Allan & Gartenstein 2010)

In the field, care is needed in order to avoid; 1) increasing transmission between animals at the site, and 2) spreading disease agents between sites. A detailed risk assessment and appropriate hygiene protocols to mitigate risks associated with amphibian fieldwork have been published (Phillott et al. 2010). For example, when handling each new amphibian a new pair of gloves must be worn, and separate containers should be used for holding individual animals. If gloves for handling animals are unavailable, hands can be disinfected with 70% ethanol and rinsed in water, or at the very least hands should be rinsed in the water to which the amphibian is normally exposed (EFSA Panel on Animal Health and Welfare (AHAW). When moving to a new site, boots and equipment such as nets may need to be cleaned and disinfected depending on the level of risk of spread.

Table 7.2-1: Potential action categories considered limiting spread for *Bd* and *Bsal* between sites (Grant et al. 2015, EFSA Panel on Animal Health and Welfare (AHAW, 2018). Combining measures will improve effectiveness.

Potential action category	Expected relative effectiveness	Relative confidence in effectiveness
Containment of infected sites	Low	Low
Alter host species composition	Low	Low
Apply anti-fungal agents to amphibian	Low	High
Remove susceptible and tolerant amphibian from infected sites	Low	High
Limit site access (by humans and other vertebrates)	Low	High
Quarantine amphibians	Moderate	Low
Require health certificate	Moderate	Low
Apply anti-fungal agents to habitats	Moderate	Low
Vaccinate amphibians	Moderate	Low
Apply probiotics to amphibians	Moderate	Low
Physical modification of habitat	Moderate	Moderate
Enforce fieldwork biosecurity - **Care in handling animals and ensure that individuals do not have elevated exposure levels to pathogens when being handled** - **Cleaning and disinfection of all field equipment** - **Disposable gloves/hands disinfection**	Moderate	Moderate-High
Create assurance colonies	Moderate	High
Avoid wild amphibian capture and handling	Moderate-High	Low-High
Prevent translocation of wild animals and return of captive animals and their larvae	Moderate-High	Moderate-High
Breed amphibians for resistance and/or tolerance	High	Low
Deploy Bd/Bsal zoospores removal method	High	Low
Enact legislation that authorizes action on wildlife pahogens	High	Low
Ban all import on amphibians	High	Moderate
Restrict amphibian trade	High	Moderate
Destroy habitats of infected sites	High	Moderate

Table 7.2-1 lists up potential action categories considered for *Bd* and *Bsal* management, with their expected level of effectiveness and level of confidence in efficiency across participants. The expectations were established using expert groups in a workshop led by the Amphibian Research and Monitoring Initiative (ARMI) in 2015 (Grant et al. 2015). As can be seen from

Table 7.2-1, there are only a few options with a high expectation of having a relatively high confidence of preventing *Bd* and *Bsal* from being spread or eradicated from infected sites.

7.3 Measures tested/used to prevent or reduce outbreaks of chytridiomycosis

If mass mortalities are observed in Norway, the Norwegian Food Safety Authority should be notified. Dead animals should be collected and sent for diagnostics.

The feasibility of controlling an outbreak of chytridiomycosis depends on the nature and location of the outbreak and the management strategy adopted. Essentially, there are three response options; eradication, containment, control and zoning or control and mitigation.

Eradication: disinfection of the waterbody may involve desiccation or application of antifungal compounds. There is one example where this has been tested and found efficient (Bosch et al. 2015), but as this example involves only rocky pools it is unknown how the method would function in a more complex habitat. It is suspected, but unproven, that leaving a site devoid of hosts for an extended time will eliminate the fungus as it does not have a resistant resting phase and does not grow in the environment. The duration of treatment after cure or time until restocking will depend on the time required for environmental reservoirs to senesce, which is currently unknown.

Containment, control and zoning: this is a priority for isolated populations, such as those on islands or in habitats where natural spread is unlikely to, or could not, occur. In moist wilderness areas with abundant wildlife, attempts to stop natural spread appear to be unlikely to succeed. Also, if the main route for spread is unknown, the efficacy of reducing the risk of spread by focussing on humans can be low to negligible.

Control and mitigation: currently there are no proven methods to the control the disease in the wild. It is important to investigate a broad range of potential solutions. Experimental ideas include culling, barrier fencing and control of reservoir species, treatment and vaccination of hosts, disinfection of water bodies, bio-augmentation of hosts with inhibitory bacteria, and habitat modification (EFSA Panel on Animal Health and Welfare (AHAW, 2018), such as increased salinity (Woodhams et al. 2011).

8 Uncertainties

8.1 Uncertainty regarding the impact of *Bd* and *Bsal*

There are contradictory reports on the severity of the pathogens, both regarding the probability of the pathogens causing chytridiomycosis and the impact the disease might have on amphibian populations, especially for *Bsal*. Whether *Bd* might have sub lethal-effects on Norwegian amphibians is unknown. There are also uncertainties regarding the interactions between *Bd/Bsal* and the skin microbiome and to what extent the microbiome could make the amphibians less susceptible to infection. Furthermore, there are limited information on how *Bd/Bsal* might interact with other infections, e.g. Ranavirus.

8.2 Uncertainty regarding the presence of *Bd* and *Bsal* in neighbouring countries

Bd has been detected in Sweden, but no surveillance have been conducted towards the Norwegian border. It is therefore uncertain whether *Bd* is present in these areas at all.

8.3 Uncertainties relating to eDNA data

The first detection of *Bd* in Norway relied on qPCR from water samples (Taugbøl et al. 2017). Later, *Bd* has also been detected by qPCR from skin swabs of great crested newt (Taugbøl et al. 2019) using the diagnostic qPCR method that is not discriminating between different *Bd* lineages. Unpublished results confirms positive detection of *Bd*-GPL from these swab-samples using a specific qPCR that solely amplify the *Bd*-GPL lineage (se 3.1).

8.4 Uncertainties relating to strains of *Bd* and *Bsal*

There is a strong suspicion that there are yet-unidentified lineages of Bd in SE Asia (perhaps elsewhere) that are not detected by the current molecular diagnostics. These are referred to as unknown lineages / species *Bx*. Testing for these is expensive and would require a broad-spectrum test such as mycobiome profiling. Unpublished results from a *Bd*-GPL specific qPCR screening have however, confirmed that the samples collected in Norway in 2017 contained the *Bd*-GPL strain.

8.5 Uncertainties relating to modelling

We do not have a data quality that allows a formal analysis of density, and there are obvious distortions in observation density, thus it is not an amphibian population estimate we are aiming for. What we have done is to take LCLU and climate types and probabilistically model observation density to provide a more nuanced and realistic picture of the likely situation than based on recorded point observations alone. Then we would have an even much stronger bias towards populated areas with more observers. The areas with high observation density are still over-represented, but on the other hand this means we weigh observations that are actually made heavier than hypothetical populations that have not been observed.

Since we have not had any outbreaks of *Bd* / *Bsal* in Norway, it is not possible to model the outbreak distribution anyway, so small to moderate biases in the observation data of amphibians thus makes no great error. But should such a situation occur, efforts would need to be made to overcome observational basis for Scandinavian amphibians.

8.6 Uncertainties related to the spatial distribution and spread

Although *Bd* has been detected in Norway it is uncertain how widespread it is and when it arrived. Extensive sampling in the UK and the limited sampling conducted in Norway in 2017 shows that *Bd* is patchy distributed. There is limited knowledge on the actual processes facilitating, or hampering, spread among waterbodies, although it seems that *Bd* does not move as a wave of infection when it is spreading to new areas. Also uncertain to what extent saltwater act as a barrier to *Bd*-spreading.

8.7 Uncertainty related to anthropologic import and spread

There is no data on how many amphibians that are transported by humans across the border from Sweden and from Continental Europe. These imports include exotic animals pet trade (pers. comm. data only), and wild caught specimens of species indigenous to Norway (or Northern Europe). We assume however that these imports are not very common, although it surely occurs every now and then.

9 Conclusions with answers to the terms of reference

9.1 Current status of *Bd*, *Bsal,* and chytridiomycosis in Norway and Scandinavia today

Both *Bd* and *Bsal* are associated with cythridiomycosis-related declines in Europe. However, these declines are minor (<20%; Sheele et al. 2019), and no documented declines are reported from the Scandinavian countries. However, most studies focus on species with a small geographic distribution, and there is a lack of good studies on the more common species, such as those occurring in Norway.

Bd appear to be widespread in Southern and central Sweden and has been detected in three different areas in Denmark (see 1.3.1.8). It has been detected in six ponds in a limited geographic area Norway in 2017 (see Figure 3.1-1), and in 2019 swab samples from great crested newts from these ponds were confirmed *Bd*-GPL positive (see 3.1).

To date, *Bsal* has not been found in wild amphibian populations in any of the Scandinavian countries (see 1.3.2.8 and 3.2)

9.2 The overall risk associated with establishment and spread of *Bd* in Norway

Although the amphibian species affected by *Bd* are not keystone species of the overall ecosystem in Norway, they may play a vital role in specific ecosystems. Thus, potential negative impacts on biodiversity in Norway not only relate to the effect on the amphibian species themselves, but may also have local effects on decomposition rates in pond environments and on both predator and prey species. The overall risk associated with *Bd* for the species found in Norway is summarized in Figure 9.2-1. The figure summarizes the likelihood of establishment (see 5.1) and spread (see 5.3) and relates this to the potential for environmental impact (see 4.1 and 6.1). Importantly, these risks pre-suppose that the animals develop chytridiomycosis (not necessarily an outbreak), as the risk associated with only the presence and spread of *Bd,* without causing illness, is minimal.

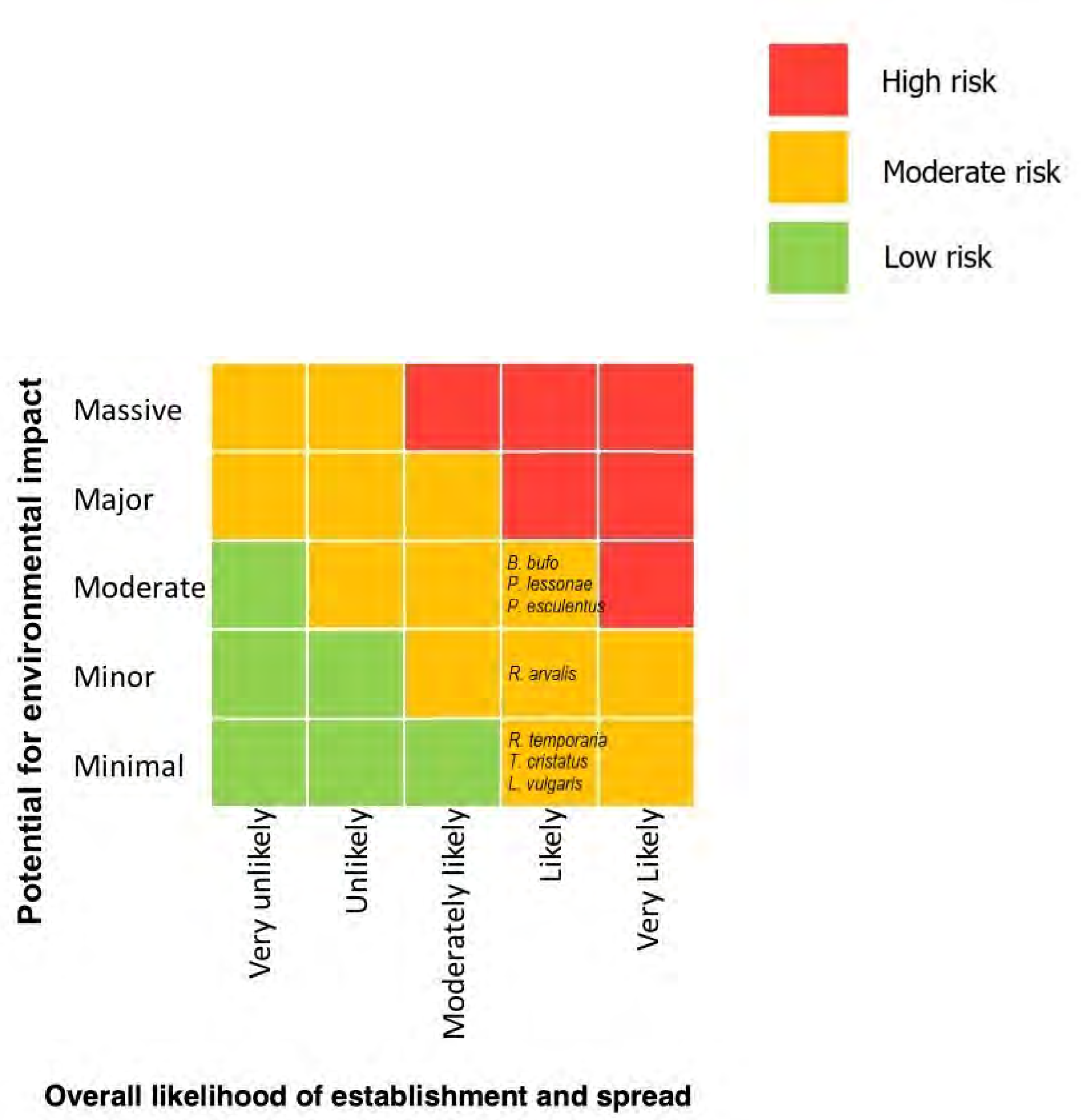


Figure 9.2-1: Conclusion of the overall risk posed by *Bd* to biodiversity (i.e., the potential anuran hosts) in Norway

A risk/impact map is shown for Norway in Figure 6.3.1. This figure shows those areas in Norway that will have the highest risk of negative impact, following spread of *Bd*. The risk/impact is scaled from 0 (no risk/impact due to no amphibian hosts present, or few and resistant hosts in climate conditions not expected to favour the disease), to 5 (highest risk areas within Norway with higher host densities, amplifying species present, and climate conditions suspected of facilitating disease outbreaks). See 2.4.8 for details.

Bd is on the list of non-indigenous species that have been evaluated by the Norwegian Biodiversity Information Centre (NBIC) in 2018. It was then categorized as a doorstep species posing a "very high risk" to Norwegian biodiversity. Based on new information regarding prevalence and mortality rate (both *in-situ* and *in-vitro*) of the indigenous species in Norway, the project group has concluded differently. It is also important to note that *Bd* is present in Norway, and thus not a "doorstep" species.

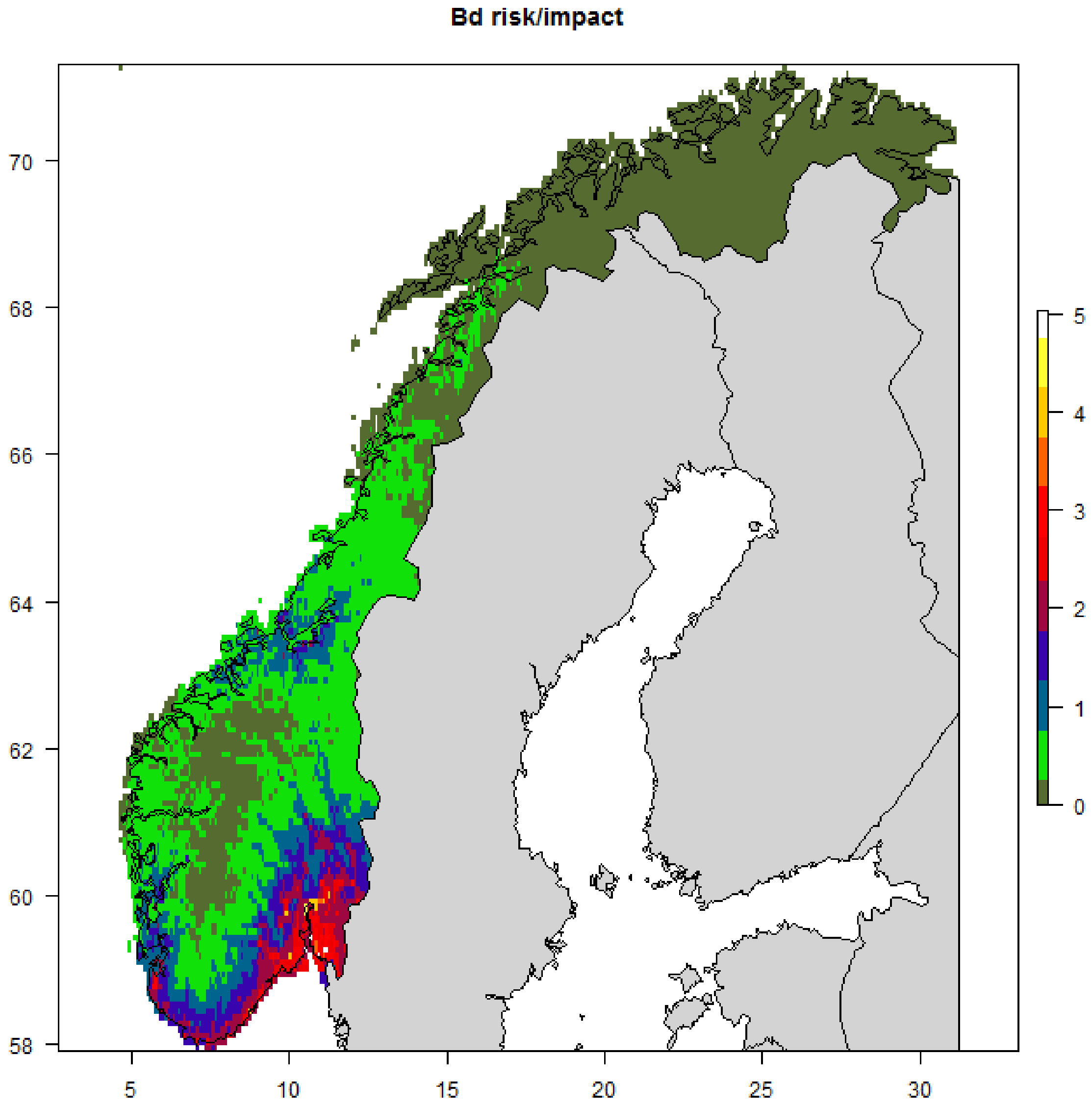
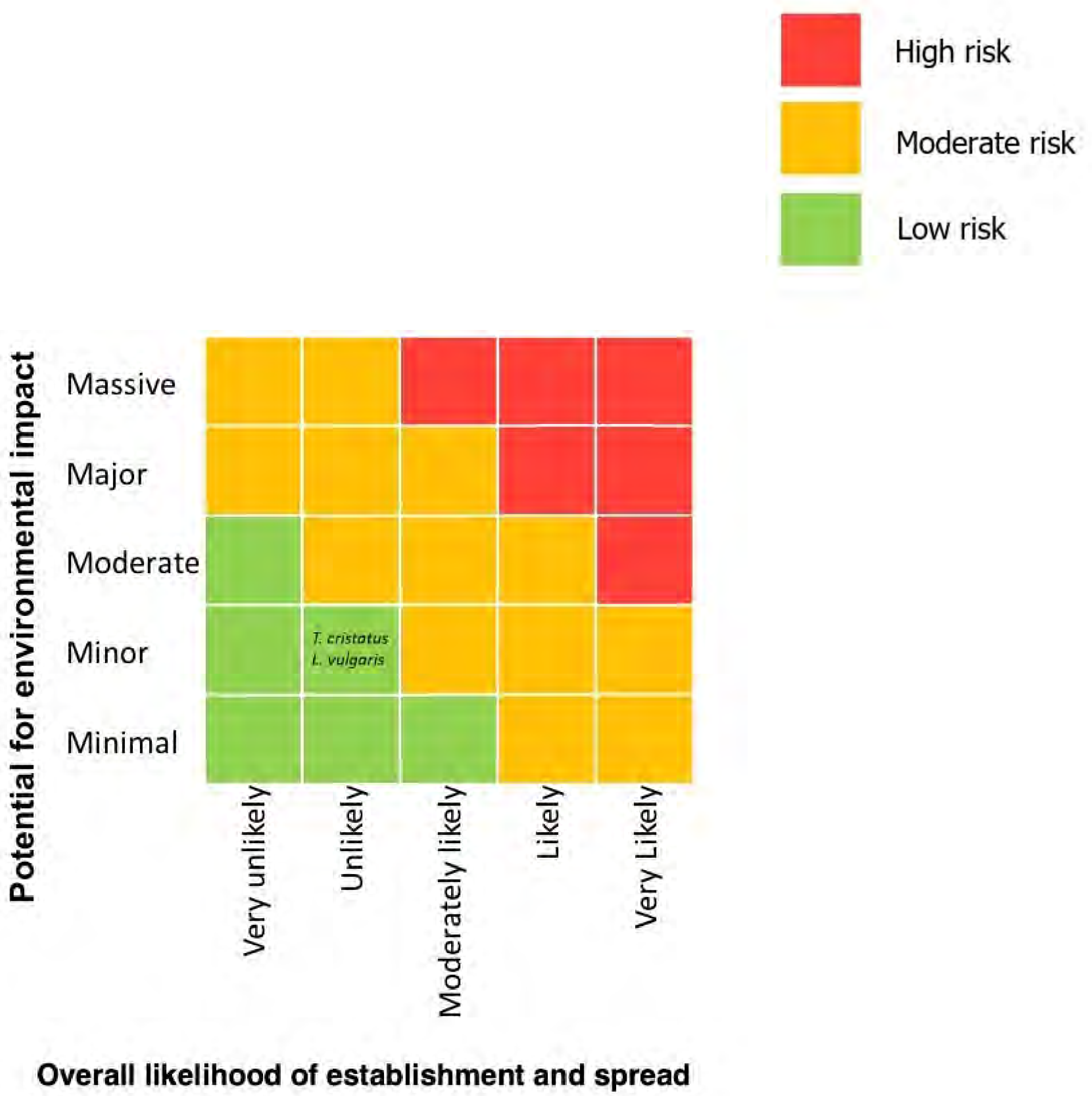


Figure 9.2-2: Map of Norway with modelled qualitative variation in risk/impact of *Bsal* following spread. The scale ranges from 0 ("no impact possible") to 5 ("potentially moderate risk") See 2.4.8 for additional information.

9.3 The overall risk associated with establishment and spread of *Bsal* in Norway

As with *Bd*, the urodelan species affected by *Bsal* are not keystone species of the Norwegian ecosystem. As the two Norwegian newt species are less numerous and produce much fewer larvae than the anurans, which are also affected by *Bd*, the main potential negative impact on biodiversity in Norway relates to the effect on the newt species themselves. The overall risk associated with *Bsal* for the species found in Norway is summarized in Figure 9.2-1. The figure summarizes the likelihood of establishment (see 5.2) and spread (see 5.4) and relates this to the potential for environmental impact (see 4.2 and 6.2). Importantly, these risks pre-suppose that the animals develop chytridiomycosis (not necessarily an outbreak), as the risk associated with the presence and spread of *Bsal,* without causing illness, is minimal.



🟥 High risk
🟧 Moderate risk
🟩 Low risk

Potential for environmental impact	Very unlikely	Unlikely	Moderately likely	Likely	Very Likely
Massive	🟧	🟧	🟥	🟥	🟥
Major	🟧	🟧	🟧	🟥	🟥
Moderate	🟩	🟧	🟧	🟧	🟥
Minor	🟩	🟩 *T. cristatus* *L. vulgaris*	🟧	🟧	🟧
Minimal	🟩	🟩	🟩	🟧	🟧

Figure 9.3-1: Conclusion of the overall risk posed by *Bsal* to biodiversity (i.e., the indigenous newt species) in Norway

A risk/impact map is shown for Norway in Figure 9.3.2. This figure shows those areas in Norway that will have the highest risk of negative impact, following spread of *Bsal*. The risk/impact is scaled from 0 (no risk/impact due to no amphibian hosts present, or few and resistant hosts in climate conditions not expected to favour the disease), to 5 (highest risk areas within Norway with higher host densities, amplifying species present and climate conditions suspected of facilitating disease outbreaks). See 2.4.8 for details.

Bsal is on the list of non-indigenous species that have been evaluated by the Norwegian Biodiversity Information Centre (NBIC) in 2018. It was then categorized as a “doorstep” species posing a “very high risk” to Norwegian biodiversity. Based on new information regarding prevalence and mortality rate (both *in-situ* and *in-vitro*) of the indigenous species in Norway, as well as the evaluation of the invasive potential of this pathogen, the project group has concluded differently.

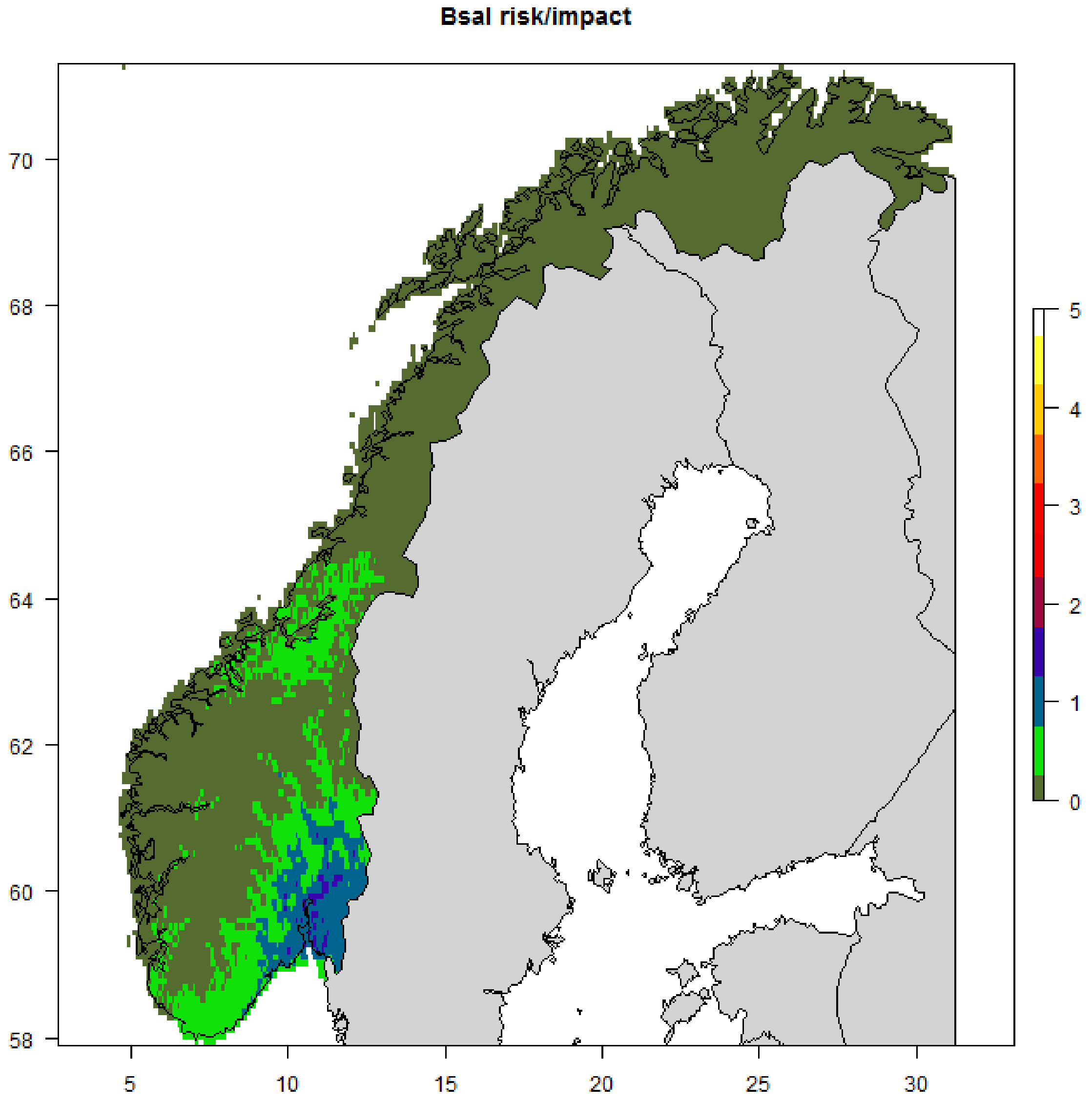


Figure 9.3-2: Map of Norway with modelled qualitative variation in risk/impact of *Bsal* following spread. The scale ranges from 0 (“no impact possible”) to 5 (“potentially moderate risk”) See 2.4.8 for additional information.

9.4 The overall risk associated with an outbreak of chytridiomycosis in Norway

Although some individual amphibians die from chytridiomycosis, this does not necessarily equate to an outbreak of the disease (see 1.6). There has been only a handful of documented chytridiomycosis outbreaks in Europe, of which one is *Bd*-related, and none of which have involved the amphibian species found in Norway. Thus, we conclude that there very unlikely/minor risk for an outbreak of chytridiomycosis in Norway. If an outbreak should emerge – this will of course by definition have serious concequences for the involved amphibian populations.

9.5 Mitigation measures that can be used to prevent or reduce entry and spread of *Bd* and *Bsal*

Entry and spread of *Bd* and *Bsal* are largely mediated by various human activities. Although transport by infected hosts or waterfowl may be more likely, these factors are impossible to influence. Mitigation measures that can be implemented include:

- Information campaigns regarding hygiene procedures. All equipment (boats, canoes, fishing gear, boots etc.) that are in contact with areas known to host amphibians should be appropriately cleaned and disinfected.

- Ban all import of amphibians, including for research and zoos. If import is allowed, all animals should be quarantined and tested for both *Bd* and *Bsal* before entry.

- According to EFSA Panel on Animal Health and Welfare (AHAW) (2018), passive surveilliance is considered the most suitable approach for detection of Bd and Bsal emergence and an active surveilliance approach of pathogens, combined with knowledge of population sizes and species distributions of amphibiands are data that will provide valuable tools for future modelling of occupancy and also be valuable if an outbreak of chytridiomycosis should be registered. It is very important to establish a monitoring programme from the start that employs the very best method to detect the precence of pathogens, as false negatives are highly destructive for modeling approaches. However, demonstrating that the pathogens are absent from any wild population with high confidence is currently not considered feasible.

9.6 Mitigation measures that can be used to prevent or reduce outbreaks of chytridiomycosis

Measures that can be taken to prevent and reduce outbreak should include active surveillance and monitoring of infected ponds and ponds in the vicinity (see 9.5). By monitoring infected ponds, it is possible to keep track of infection pressure and potential changes in infection pressure over time. By including sampling in a larger area, any spread of *Bd* and *Bsal* can be documented. Results over time from the monitoring programme of *Bd* and *Bsal* dynamics can then be used for appropriate conservation decisions.

Currently, we do not find any examples in the literature of measures taken to reduce outbreaks of chytridiomycosis once the disease is actively reducing amphibian populations in the wild. Using a modeling approach, Canessa et. al. (2018) found that mitigating actions against Bsal infections on S. Salamandre during an epidemic event are very unlikely to be effective if the treatment are not almost completely effective at very high coverage, with over 80% of the population being treated within three days. Also, if the host cannot acquire immunity (which is the case for S. salamandre infected with Bsal) and the pathogen is not fully eradicated, the treatment will likely only slow the growth of pathogen, resulting in a larger pool of infected individuals that can spread the pathogen to other populations (Canessa et. al.,2018). This is likely a probable outcome also by using habitat destruction as a mitigating measure (Woodhams et.al. 2011); if the habitat is not eradicated in a highly effective way, infected animals could escape and spread the infection to other nearby ponds. Also, se table 7. 2-1 for a list of measures and methods for on-site protection of wild amphibians.

10 Data gaps

While writing this report the project group has identified the following data gaps relevant for this topic:

- The geographic distribution of *Bd* in Norway is largely unknown and not yet mapped
- The occurrence of *Bd* on both sides of the Swedish boarder is unknown and not yet mapped
- The occurrence of *Bsal* in Scandinavia is unknown and not yet mapped
- The factors preventing chytridiomycosis in Norwegian species is not known and could either be a result of host immunity or environmental factors such as temperature, skin microbiome, pond microfauna etc – or a combination.
- The diversity of *Bd* strains / lineages in Norway is largely unknown and not yet mapped
- Data on the effects of stressors, such as co-infections, and how this influence Bd/Bsal lethality are still largely unknown.
- There is a lack of population estimates and abundance of the Norwegian amphibian populations, and we don’t know much about the connectivity of different populations in Norway.

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Appendix I

Forms used in risk assessment of *Bd*

Table A1-1

LIKELIHOOD OF ENTRY			
Important instructions: • **Entry is the introduction of an organism into Norway. Not to be confused with spread, the movement of an organism within Norway.** • **For organisms which are already present in Norway, only complete the entry section for current active pathways of entry or if relevant potential future pathways. The entry section need not be completed for organisms which have entered in the past and have no current pathways of entry.**			
Question	**Response**	**Confidence**	**Comment**
1.1. How many active pathways are relevant to the potential entry of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	none very few (1-3) **few (4-6)** moderate number (7-10) many (11-20) very many (20+)	high	
1.2. List relevant pathways through which the organism could enter.Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 1.3 to 1.10 (copy and paste additional rows at the end of this section as necessary).	Anthropogenic – pet trade Anthropogenic – other live animals Anthropogenic – mechanical Anthropogenic–hitchhiking organisms Non-host animals Natural spread with infected hosts		Table A1-2 Table A1-3 Table A1-4 Table A1-5 Table A1-6 Table A1-7

Table A1-2

Pathway name:	Anthropogenic – pet trade		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	Always accidental
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	unlikely	low	Very likely that *Bd* will get on to the pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	The organism can survive and multiply on the host following this pathway.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	There is no applied management practices for *Bd* in Norway. The trade in hosts is prohibited (with some exceptions), but there might still be a limited influx of animals.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	Unless the animals shows clinical signs of cythridiomycosis, it would not be detected.

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	low	Trade (either illegal or for Zoos would predominantly happen in spring, summer or early autumn.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely	low	Release of "pet" amphibians has never been documented in Norway. Also unlikely due to the effort put in to getting hold of these in the first place.
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	unlikely	moderate	Strict Norwegian regulations considerably reduce the likelihood of entry of *Bd* through this pathway. Also, likelihood of escape or release is very unlikely.

Table A1-3

Pathway name:	Anthropogenic – other live animals		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	Always accidental.
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	moderately likely	low	Moderately likely that *Bd* will get on to the pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	The organism would survive, and potentially replicate along this pathway.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	There is no applied management practices for *Bd* in Norway. However, the import of frogs for food and or laboratory use is restricted.
1.7. How likely is the organism to enter Norway undetected?	likely	high	If it happens, it would probably be undetected.

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	medium	If the host is captured and transported by humans, it would have to be in an appropriate time of year. If the host is intended for food or research, this is very unlikely.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	unlikely	medium	If the host is released in the wild it is likely to be in a suitable habitat for the host. However, it is not as likely that there will be other hosts present.
1.10 All-over likelihood of the organism entering a suitable habitat in Norway through this pathway	unlikely	medium	The main source would be the transport of tadpoles from neighboring countries which do occasionally happen.

Table A1-4

Pathway name:	Anthropogenic – hitchhiking organism (Plant nurseries and sewage treating facilities etc.)		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	Always accidental.
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	moderately likely	low	It is moderately likely that *Bd* will get on to the pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	low	*Bd* could be transported with suitable hosts hitchhiking with other imported goods, or in the water used to transport plants and goods. It is likely that *Bd* would survive, but rarely multiply along this pathway.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	low	The existing management practices for these types of import (e.g. plants used in sewage treating facilities etc) would not prevent entry of *Bd*.

1.7. How likely is the organism to enter Norway undetected?	very likely	high	It would be next to impossible to detect.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	If hitchhiking with outdoor plants, this would be likely, but unlikely for other types of imports.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	unlikely	low	The organism which it is hitchhiking on is unlikely to be destined for a suitable habitat for *Bd*, especially with suitable hosts.
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	unlikely	low	Predominantly import from Netherlands and other exporting hotspots for plants is of concern.

Table A1-5

Pathway name:	Anthropogenic - mechanical		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	Always accidental.
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	unlikely	low	It is moderately likely that Bd will get on to this pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	*Bd* will potentially survive, but not multiply along this pathway.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	high	Current management practices implemented to inhibit spread of other pathogens (e.g., Gyrodactylus and crayfish plague) will to some degree hamper survival of the organism on this pathway.

1.7. How likely is the organism to enter Norway undetected?	very likely	very high	There is no practice for detecting it.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	The transport of gear / equipment happens in a wider time window than what is optimal for Bd.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	low	The habitat where boats and fishing gear is not 100% overlapping with the small ponds that is good habitat for anurans.
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	moderately likely	low	Mainly transport of boats with engines containing water, and fishing gear (nets and traps) that do not dry out completely.

Table A1-6

Pathway name:	Non-host animals (e.g. waterfowl, crustaceans and reptiles)		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	Always accidental.
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	moderately likely	medium	It is moderately likely that *Bd* will get on to this pathway in the first place
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	Bd can survive for shorter periods (maximum 4-6h), but not multiply along this pathway.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	Not common practice to monitor or manage.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	Not possible to detect on this pathway.

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	high	Spring would be the best time for establishment, and this is when migrating birds are returning from the south.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	medium	Both birds, Grass snakes (*Natrix natrix*), and crayfish utilize the same habitat as the hosts.
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	moderately likely	low	Mainly waterfowl is believed to have the highest likelihood of getting infected (on their feet), but the survival from infected areas to Norway depends on no exposure to salt water, which is unlikely following transit from continental Europe. American crayfish can be carriers of Bd – and could transmit the pathogen.

Table A1.7

Pathway name:	Natural spread by infected hosts		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	Always accidental.
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very likely	low	It is very likely that *Bd* will enter this pathway in the first place. However, the status of *Bd* in bordering areas in Sweden is currently unknown.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	very high	*Bd* would be able to both survive and multiply along this pathway.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	No management practices for this pathway.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	Not possible to detect.

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	very high	It would be the same species as we have in Norway, so they would migrate at a suitable time.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	high	It would be the same species as we have in Norway, so they would migrate to a suitable habitat.
1.10 All-over likelihood of the organism entering a suitable habitat in Norway through this pathway	likely	high	Given that *Bd* is established in the bordering areas in Sweden.

Table A1-8

LIKELIHOOD OF ENVIRONMENTAL IMPACT			
Important instructions: • When assessing potential future environmental impacts, climate change should not be taken into account. This is done in later questions at the end of the assessment. • Each set of questions starts with the impact elsewhere in the world, then considers impacts in Norway separating known impacts to date (i.e. past and current impacts) from potential future impacts.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
4.1. How much environmental harm is caused by the organism within its existing geographic range, excluding Norway?	massive	very high	In some areas the pathogen causes mass mortality and wipes out entire populations. However, there is a huge variation in susceptibility to the disease, and it seems most of the amphibians found in Europe and Norway handle the infection fairly well without developing the disease. Mass mortality only seen outside Europe, while high mortality rate (one of 50 metamorphs) only seen once in Southern Europe (Spain).
4.2. How much impact would there be, if genetic traits of the organism were to be transmitted to other species, modifying their genetic makeup and making their environmental effects more serious?	minimal	low	Taxonomy of these organisms is complex. This response covers only the event of a new *Bd*-strain evolving in Norway. Likely due to high mutation rate.
4.3. How much impact does the organism have, as food, as a host, or as a symbiont or a vector for other damaging organisms (e.g. diseases)?	minimal	high	Zoospores of *Bd* is eaten by filter feeders in freshwater habitats, such as *Daphnia*
4.4. How much impact do other factors have, factors which are not covered by previous questions (specify in the comment box)	moderate	high	Most importantly, *Bd* has different effects on different species.

			Temperature, co-infections with other pathogens and other stressors are important, as these factors may result in higher host susceptibility and thus higher negative impact on amphibians.
4.5. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in Norway?	minor	high	Microbiota in the water and on the amphibians reduce the impact. Also, water microfauna (filter feeders) feed on *Bd* and reduce the infection pressure.
4.6. Indicate any parts of Norway where environmental impacts are particularly likely to occur (provide as much detail as possible).	See Figure 1.1.8.1.	low	Related to the distribution of the different Norwegian species and their differences in susceptibility. See map(s).
4.7. Estimate the expected impacts of the organism if it is able to establish and spread in Norway (despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present).	minor	medium	The species found in Norway is to a high degree tolerant to *Bd* in their natural habitat. The most susceptible species is *Bufo Bufo* and this has a very widespread distribution in Norway, and would not be very affected by even local outbreaks of chytridiomycosis.

Table A1-9

LIKELIHOOD OF ESTABLISHMENT			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
2.1. How likely is it that the organism will be able to establish in Norway based on the similarity between climatic conditions in Norway and the organism's current distribution?	very likely	high	*Bd* is already established in Norway. It has been detected in water in 2017, and on great crested newt in 2018. This strongly suggest local establishment in the surveilled area in Norway. It is also established in Sweden under climate conditions comparable to southern Norway.
2.2. How likely is it that the organism will be able to establish in Norway based on the similarity between other abiotic conditions in Norway and the organism's current distribution?	very likely	high	The same host species are found in Norway as in Sweden, where the pathogen is established. There are however several other species in Sweden, most of which show higher prevalence for *Bd*, than the species found in Norway. Further, *Bd* has been detected on great crested newt in Norway in 2018
2.3. How likely is it that the organism will become established in protected conditions (in which the environment is artificially maintained, such as wildlife parks, glasshouses, aquaculture facilities, terraria, zoological gardens) in Norway?	moderately likely	medium	In zoological gardens this would be unlikely, due to the precautions taken prior to, and during, captive keeping, including monitoring. In private collections (terraria), this would be moderately likely. Both zoos and private collections are few and far between. Other protected conditions are not relevant.

Subnote: gardens are not considered protected conditions			
2.4. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in Norway?	widespread	high	We know the distribution of potential hosts. The distribution is related to water bodies, which creates a somewhat patchy distribution.
2.5. How likely is it that establishment will occur despite management practices (including eradication campaigns), competition from existing species or predators, parasites or pathogens in Norway?	very likely	high	We do not know much about the organism's ability to survive without its host. Small ponds can be desiccated, but this has a very limited effect on a larger scale. Several studies suggests survival on non-host animals such as reptiles and crustaceans.
2.6. How likely are the biological characteristics (including adaptability and capacity of spread) of the organism to facilitate its establishment?	very likely	high	It is a global species, inhabiting a wide range of habitats and climatic conditions. Its distribution is driven by the distribution of its hosts – which are more than 700 amphibian species.
2.7. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very likely	high	The Bd-GPL lineage is a separate intraspecific phylogenetic lineage of *Bd* with low genetic diversity that has spread from Asia to all other continents in the world.
2.8. Based on the history of invasion by this organism elsewhere in the world, how likely is to establish in Norway? (If possible, specify the instances in the comments box.)	very likely	high	It has spread globally, fast. It was detected in water samples in Norway in 2017 (ddPCR), and on skin swabs of live animals in 2018 (qPCR). These were confirmed to be *Bd*-GPL in 2019.

2.9. Estimate the over-all likelihood of establishment in Norway (mention any key issues in the comment box).	very likely	high	It has been identified in a limited number of ponds in one particular area.

Table A1-10

LIKELIHOOD OF SPREAD

Important notes:

- Spread is defined as the expansion of the geographical distribution of an alien species within an area.

QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
3.1. How likely is it that this organism will spread widely in Norway by *natural means*? (Please list and comment on the mechanisms for natural spread.)	moderately likely	medium	Limited knowledge on spread, but both infected amphibians moving between ponds and fungal spores hitchhiking on fish, crustaceans, birds and other animals visiting an infected water system might cause spread. Time and distance is of essence. Although it is more likely that *Bd* will spread by natural means, we find it moderately likely that it will be widely distributed.
3.2. How likely is it that this organism will spread widely in Norway by *human assistance*? (Please list and comment on the mechanisms for human-assisted spread.)	moderately likely	medium	Hobby herpetologists moving among waterbodies and infected animals released from captivity might spread the disease. The use of fishing gear and boats etc. will also contribute to spreading the pathogen. See pathways of entry (3.3.1 – 3.3.7)
3.3. How likely is it that spread of the organism within Norway can be completely contained?	very unlikely	high	Reducing or delaying spread might be possible, through information campaigns and disinfection of gear (Virkon S), but completely containment is very unlikely.
3.4. Based on the answers to questions on the potential for establishment and spread in Norway, define the area endangered by the organism.	More or less the same as the distributional range of its hosts	medium	Possibly hampered by lower temperatures in the northernmost distribution areas in Norway.
3.5. Estimate the over-all potential for future spread	likely	high	Based on studies from Sweden and the UK, we find it likely that *Bd* will

for this organism in Norway (using the comment box to indicate any key issues).			spread in Norway, but maybe not become widely distributed. Lack of knowledge on dispersal capability outside its hosts makes this difficult to assess.

Table A1-11

ADDITIONAL QUESTIONS - CLIMATE CHANGE			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
5.1. What aspects of climate change (in a 50 years perspective), if any, are most likely to affect the risk assessment for this organism?	Increased temperature, shorter winters and earlier onset of spring.	medium	Mainly in the spring when the hosts are in the ponds, and all life stages are present. Also spring is when *Bd* is actively sporulating (not detected in samples from summer (by qPCR) in more than one study).
5.2. What aspects of the risk assessment are most likely to change as a result of climate change? • Establishment • Spread • Impact on biodiversity • Impact on ecosystem functions	Establishment, spread and impact on biodiversity	medium	Higher *Bd*-virulence leading to increased mortality rates and negative impact on amphibian biodiversity. Increased risk for actual outbreaks of chytridiomycosis, both due to higher *Bd*-virulence and due to possibly reduced immunity/increased susceptibility in stressed amphibians that have to adapt to a changing and fluctuating climate

Appendix II

Forms used in risk assessment of *Bsal*

Table A2.1

Table A2.2

LIKELIHOOD OF ENTRY			
Important instructions: - **Entry is the introduction of an organism into Norway. Not to be confused with spread, the movement of an organism within Norway.** - **For organisms which are already present in Norway, only complete the entry section for current active pathways of entry or if relevant potential future pathways. The entry section need not be completed for organisms which have entered in the past and have no current pathways of entry.**			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
1.1. How many active pathways are relevant to the potential entry of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	none very few (1-3) **few (4-6)** moderate number (7-10) many (11-20) very many (20+)	high	
1.2. List relevant pathways through which the organism could enter.Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 1.3 to 1.10 (copy and paste additional rows at the end of this section as necessary).	Anthropogenic – pet trade Anthropogenic – other live animals Anthropogenic – mechanical Anthropogenic – hitchhiking organisms Non-host animals Natural spread with infected hosts		Table A2-2 Table A2-3 Table A2-4 Table A2-5 Table A2-6 Table A2-7

Pathway name:	Anthropogenic – pet trade		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very unlikely	medium	Likely that *Bsal* will get onto the pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	Yes
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	There is National legislation demanding health certificate of imported salamanders from EU and third countries. However, keeping amphibians is illegal in Norway, and existing management practices would then not apply.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	low	

1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely	moderate	The number of host species, their range and density are much lower than for *Bd*.
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	very unlikely	moderate	Strict Norwegian regulations considerably reduce the likelihood of entry of *Bsal* through this pathway.

Table A2-3

Pathway name:	Anthropogenic – other live animals		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very unlikely	low	Very unlikely that *Bsal* will get onto the pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	Yes
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	There is National legislation demanding health certificate of imported salamanders from EU and third countries.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	low	

1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely	low	
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	very unlikely	high	Salamanders and newts are not used for research or food in Norway.

Table A2-4

Pathway name:	Anthropogenic – hitchhiking organism (Plant nurseries and sewage treating facilities etc.)		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	unlikely	medium	It is unlikely that *Bsal* will get onto the pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	low	Yes
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	low	There is National legislation demanding health certificate of imported salamanders from EU and third countries.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	unlikely	low	
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	very unlikely	medium	Predominantly import from Netherlands and other exporting hotspots for plants is of concern.

Table A2-5

Pathway name:	Anthropogenic - mechanical		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very unlikely	medium	It is unlikely that *Bsal* will get onto this pathway in the first place.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	No
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	high	Current management practices implemented to inhibit spread of other pathogens (e.g., Gyrodactylus and crayfish plague) will to some degree hamper survival of the organism on this pathway.
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely	medium	
1.10 over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	very unlikely	medium	*Bsal* is not reported in neighboring countries in the wild.

Table A2-6

Pathway name:	Non-host animals (e.g. waterfowl, crustaceans and reptiles)		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very unlikely	medium	It is moderately likely that *Bsal* will get onto this pathway in the first place
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	No
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	very high	
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	medium	
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	unlikely	medium	Mainly waterfowl is believed to be the problem.

Table A2-7

Pathway name:	Natural spread by infected hosts		
Question	Response	Conf.	Comments
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	very high	
1.4. How likely is it that the organism will travel along this pathway from the point(s) of origin, multiple times (>10) over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very unlikely	medium	It is very unlikely that *Bsal* will enter this pathway in the first place. However, the status of *Bsal* in bordering areas in Sweden is currently unknown.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	very high	Yes
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	
1.7. How likely is the organism to enter Norway undetected?	very likely	very high	

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	very high	
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very likely	high	
1.10 Over-all likelihood of the organism entering a suitable habitat in Norway through this pathway	very unlikely	medium	*Bsal* has not been reported in Sweden in the wild.

Table A2-8

LIKELIHOOD OF ENVIRONMENTAL IMPACT

Important instructions:

- When assessing potential future environmental impacts, climate change should not be taken into account. This is done in later questions at the end of the assessment.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in Norway separating known impacts to date (i.e. past and current impacts) from potential future impacts.

QUESTION	RESPONSE	UNCERTAINTY	COMMENTS
4.1. How much environmental harm is caused by the organism within its existing geographic range, excluding Norway?	moderate	high	Bsal was recently introduced to Europe from Asia, and is not present elsewhere. The known distribution is limited to a few European countries. In some areas the pathogen can causes mass mortality in fire salamander. Only urodelan amphibian species are susceptible, and only a few of these species die from the infection. Only outbreaks in fire salamander populations are known from the wild in Europe.
4.2. How much impact would there be, if genetic traits of the organism were to be transmitted to other species, modifying their genetic makeup and making their environmental effects more serious?	minimal	low	Taxonomy of these organisms is complex. This response covers only the event of a new Bsal-strain evolving in Norway. Likely due to high mutation rate.
4.3. How much impact does the organism have, as food, as a host, or as a symbiont or a vector for other damaging organisms (e.g. diseases)?	minimal	high	
4.4. How much impact do other factors have, factors which are not covered by previous questions (specify in the comment box)	minor	high	

4.5. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in Norway?	minor	high	Microbiota in the water and on the amphibians reduce the impact.
4.6. Indicate any parts of Norway where environmental impacts are particularly likely to occur (provide as much detail as possible).	See map.	low	Related to the distribution of the two Norwegian species and their differences in susceptibility. See map(s).
4.7. Estimate the expected impacts of the organism if it is able to establish and spread in Norway (despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present).	minor	medium	

Table A2-9

LIKELIHOOD OF ESTABLISHMENT			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
2.1. How likely is it that the organism will be able to establish in Norway based on the similarity between climatic conditions in Norway and the organism's current distribution?	likely	medium	Not found in neighboring countries with climate conditions comparable to Norway (eg. Sweden and UK). But it is found in several European countries.
2.2. How likely is it that the organism will be able to establish in Norway based on the similarity between other abiotic conditions in Norway and the organism's current distribution?	very likely	high	Bsal thrives at water temperatures common for Northern Europe, optimum 10-15C
2.3. How likely is it that the organism will become established in protected conditions (in which the environment is artificially maintained, such as wildlife parks, glasshouses, aquaculture facilities, terraria, zoological gardens) in Norway? Subnote: gardens are not considered protected conditions	moderately likely	medium	In zoological gardens this would be unlikely, due to the precautions taken prior to, and during, captive keeping, including monitoring. In private collections (terraria), this would be moderately likely. Both zoos and private collections are few and far between. Other protected conditions are not relevant.

2.4. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in Norway?	Corresponds to the distribution of newts in Norway (see map).	high	We know the distribution of potential hosts. The distribution is related to water bodies, which creates a somewhat patchy distribution.
2.5. How likely is it that establishment will occur despite management practices (including eradication campaigns), competition from existing species or predators, parasites or pathogens in Norway?	unlikely	medium	We do not know much about the organism's ability to survive without its host. Trade in live salamanders is prohibited. Small ponds can be desiccated, but this has a very limited effect on a larger scale.
2.6. How likely are the biological characteristics (including adaptability and capacity of spread) of the organism to facilitate its establishment?	unlikely	high	Appears to rely on human activity to spread over larger distances. "Poor dispersal characteristics" is indicated in one study (Spitzen, 2018).
2.7. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very likely	medium	
2.8. Based on the history of invasion by this organism elsewhere in the world, how likely is to establish in Norway? (If possible, specify the instances in the comments box.)	very unlikely	medium	Biosecurity measures, prohibition of trade, poor dispersal on its own.
2.9. Estimate the overall likelihood of establishment in Norway (mention any key issues in the comment box).	very unlikely	medium	Biosecurity measures, prohibition of trade, poor dispersal on its own.

Table A2-10

LIKELIHOOD OF SPREAD

Important notes:

- Spread is defined as the expansion of the geographical distribution of an alien species within an area.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
3.1. How likely is it that this organism will spread widely in Norway by *natural means*? (Please list and comment on the mechanisms for natural spread.)	unlikely	medium	Reported low spread between neighbouring populations of Salamandra salamandra, which is a highly susceptible species.
3.2. How likely is it that this organism will spread widely in Norway by *human assistance*? (Please list and comment on the mechanisms for human-assisted spread.)	unlikely	medium	Hobby herpetologists moving among waterbodies and infected animals released from captivity might spread the disease.
3.3. How likely is it that spread of the organism within Norway can be completely contained?	moderately likely	medium	Containment and reduced spread should be possible, through information campaigns and disinfection of gear (Virkon S).
3.4. Based on the answers to questions on the potential for establishment and spread in Norway, define the area endangered by the organism.	More or less the same as the distributional range of its hosts	medium	Thrives in colder climate than Bd. Optimum 10-15C
3.5. Estimate the overall potential for future spread for this organism in Norway (using the comment box to indicate any key issues).	unlikely	medium	

Table A2-11

ADDITIONAL QUESTIONS - CLIMATE CHANGE			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
5.1. What aspects of climate change (in a 50 years perspective), if any, are most likely to affect the risk assessment for this organism?	Increased temperature	medium	The optimal temperature of Bsal is reported to be 10-15C.
5.2. What aspects of the risk assessment are most likely to change as a result of climate change? • Establishment • Spread • Impact on biodiversity • Impact on ecosystem functions	Establishment, spread and impact on biodiversity	medium	Lower *Bsal*-virulence leading to reduced mortality rates. However, other factors related to the newt immune responses and potentially increased susceptibility as a result of climate change and environmental stress could lead to negative consequences of *Bsal* and increased risk of chytridiomycosis outbreaks.

Appendix III

Background data for risk-impact maps

Maps of raw temperature and precipitation data, as well as elevation and LCLU data as described under 2.4.4. Measures of temperature distributions, variance and trajectories as described under "Temperature increase and temperature mismatch potential" (2.4.5). All climate data are based on the gridded monthly averages described in 2.4.1, with minimum (T_{min}), maximum (T_{max}), and mean (T), temperatures referring to the annual values of these.

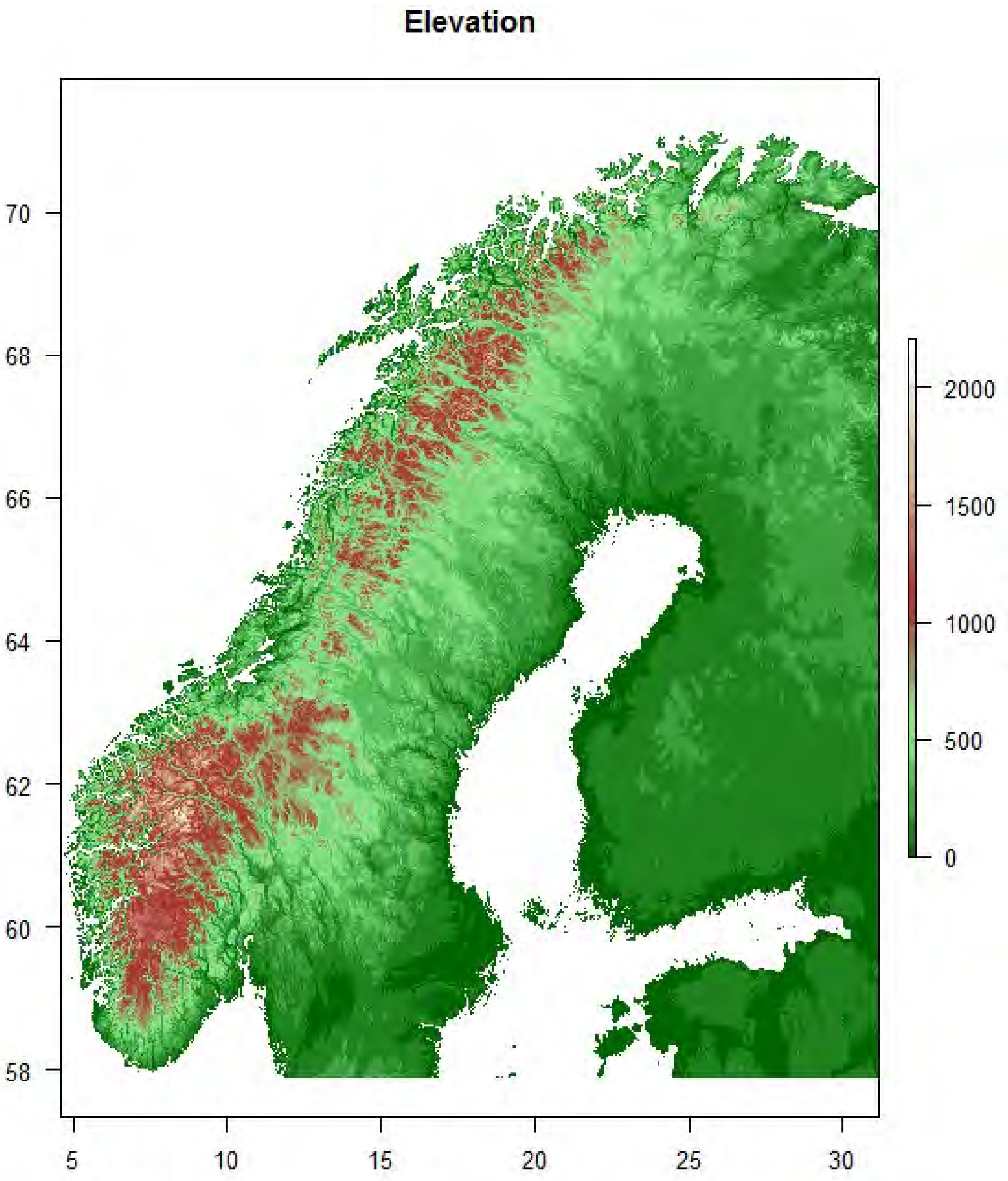

Figure A3-1: Elevation data for Scandinavia

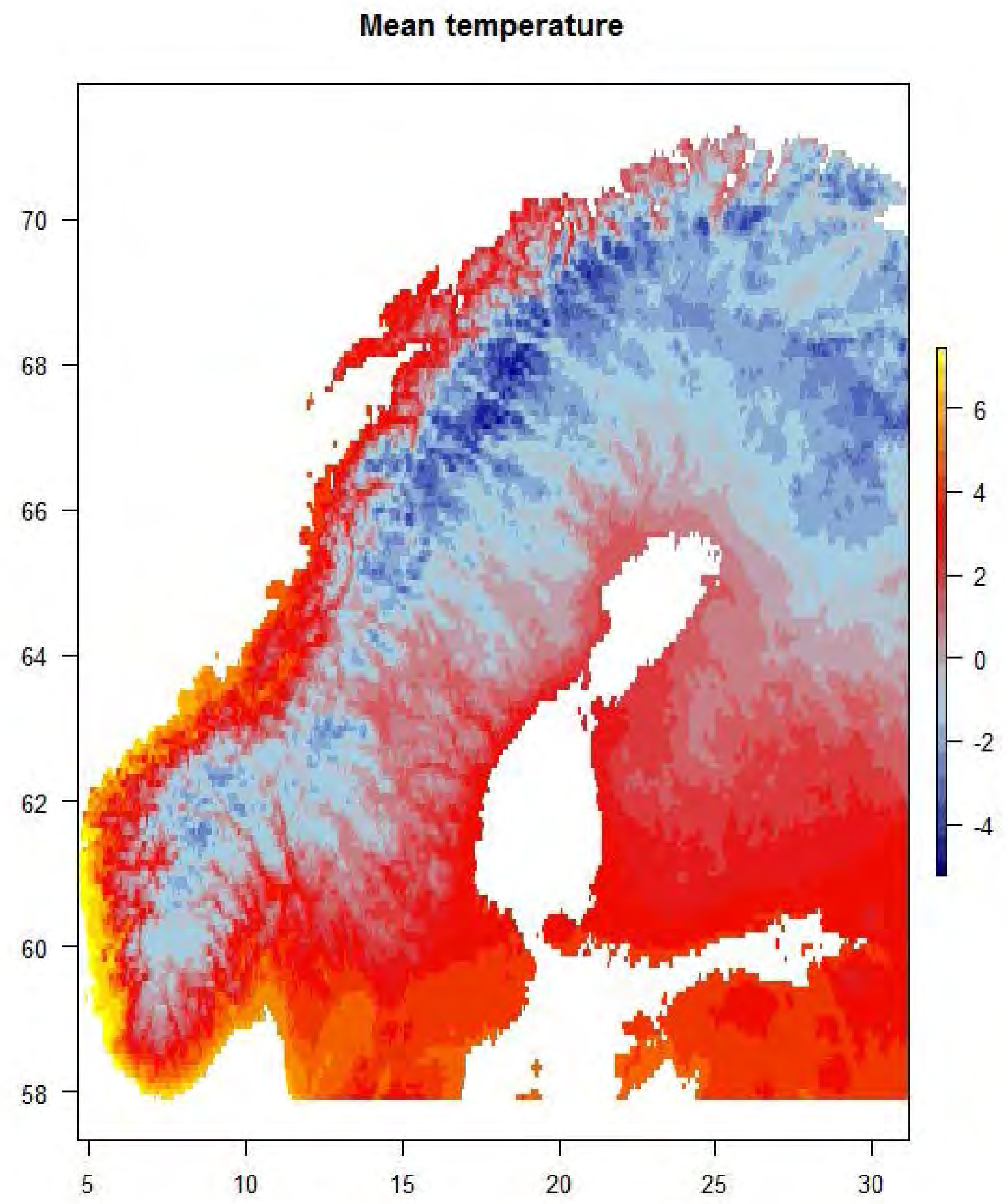

Figure A3-2: Data on Mean temperature in Scandinavia

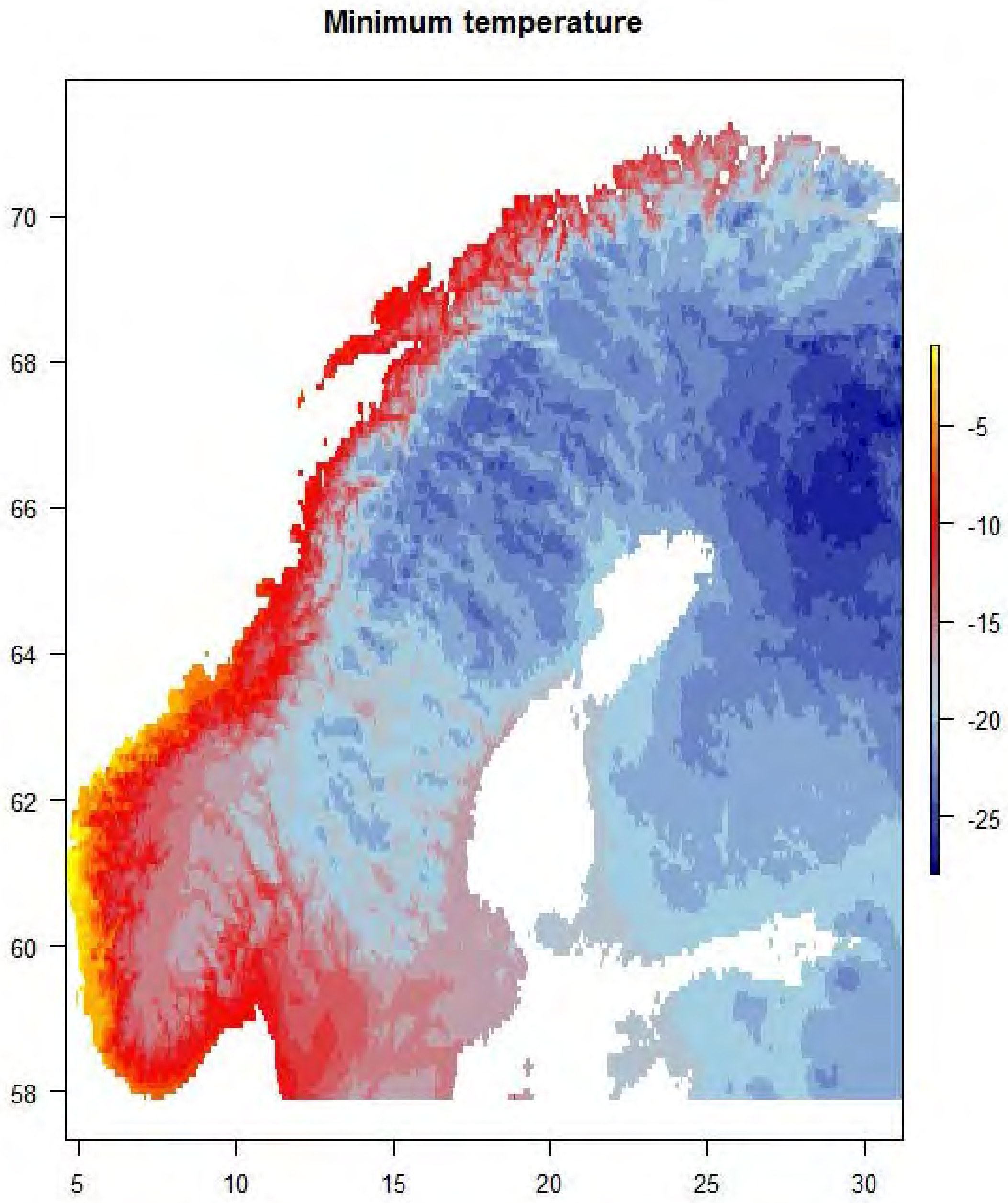

Figure A3-3: Data on minimum temperature in Scandinavia

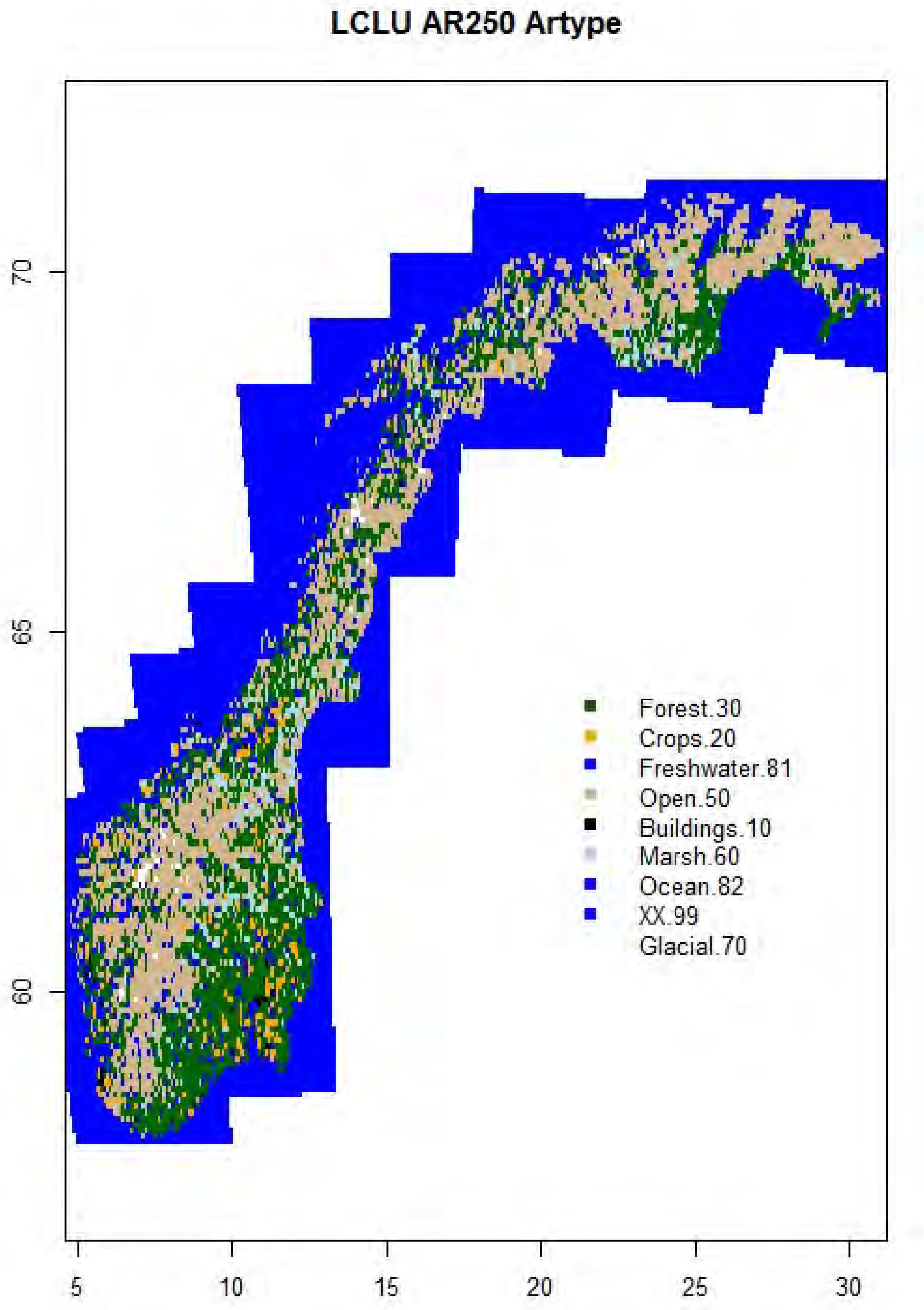

Figure A3-4: Data on habitat types in Norway.

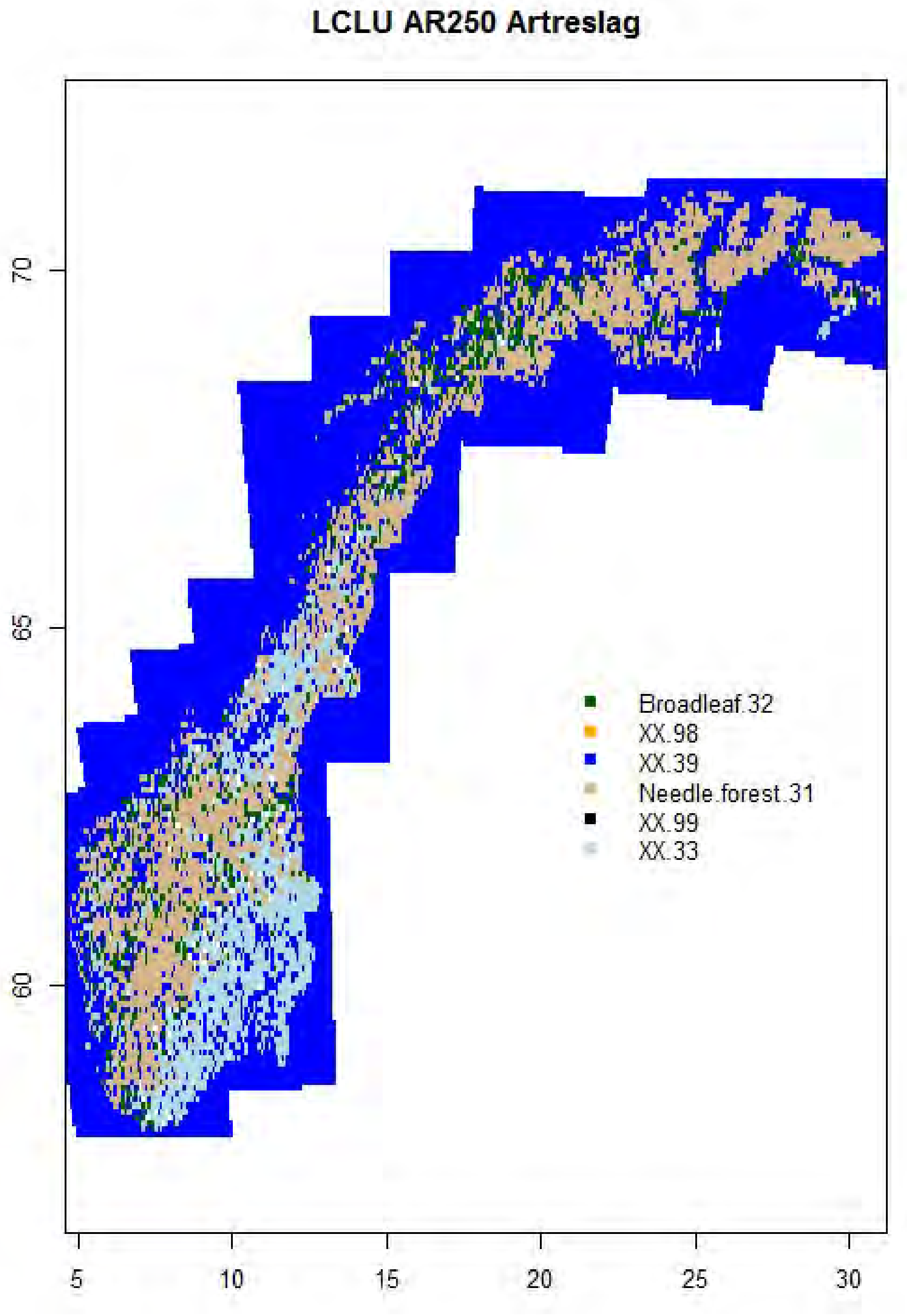

Figure A3-5: Data on forest (tree) types in Norway

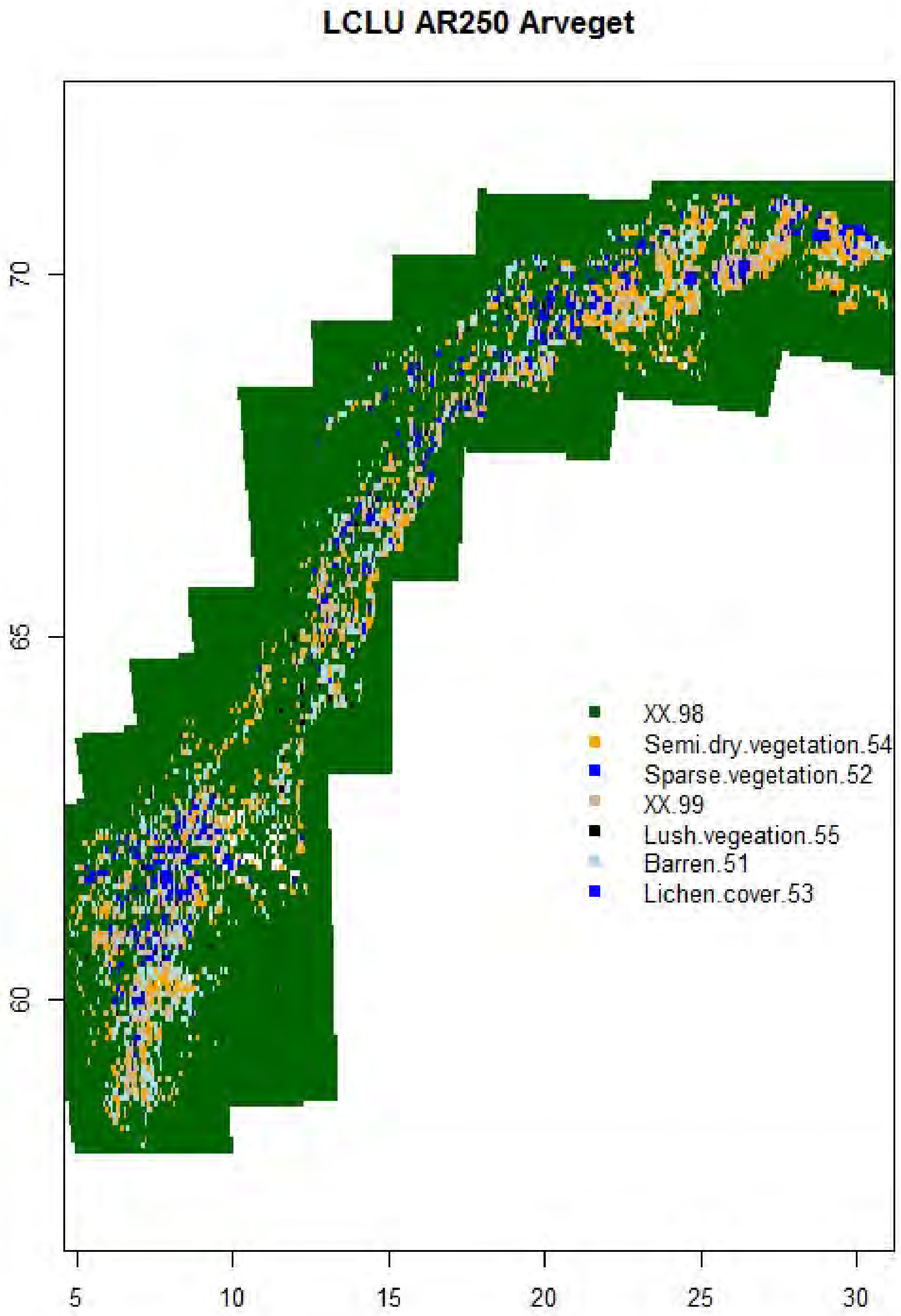

Figure A3-6: Data on vegetation types in Norway

Appendix IV

Distribution of parameters used for risk-impact maps

For each of the amphibian species the point values from the georeferenced observations were extracted from the underlying raster map layers, and a frequency distribution for the number of observation thus generated (black line). This is contrasted against the underlying total frequency distribution (light blue histograms) of each data interval (variables).

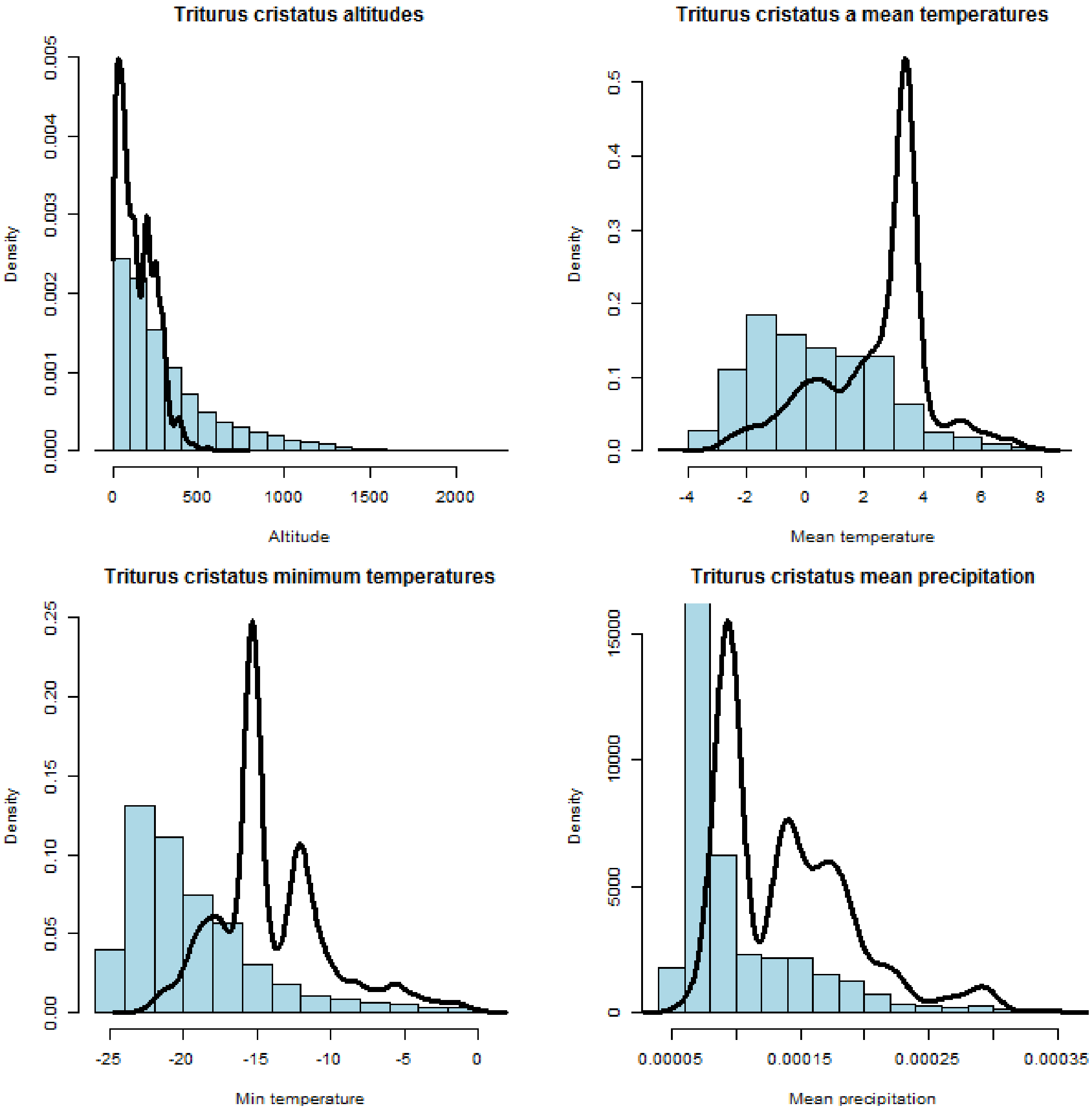

Figure A4-1: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Triturus cristatus*. Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.

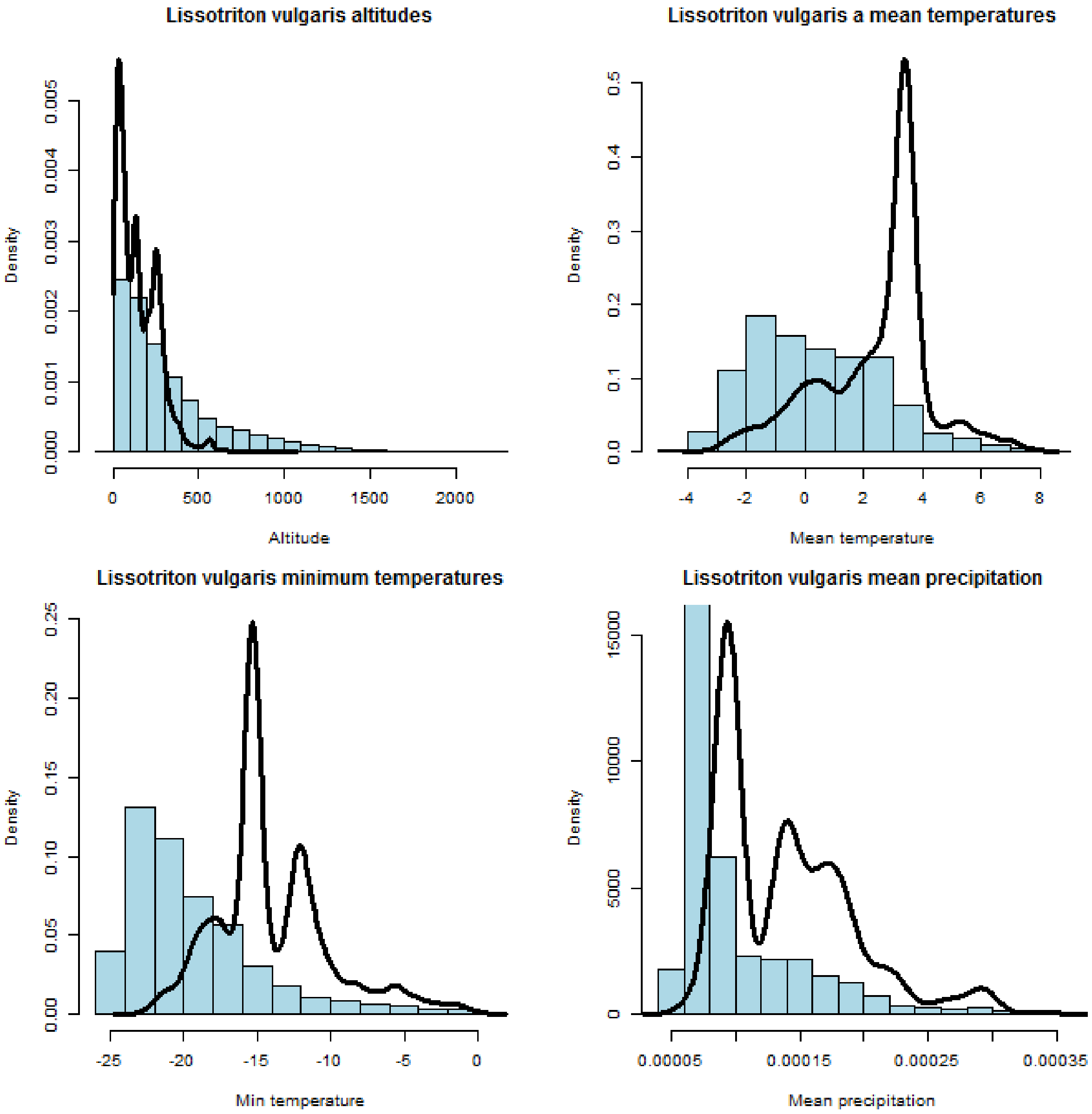

Figure A4-2: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Lissotriton vulgaris*. Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.

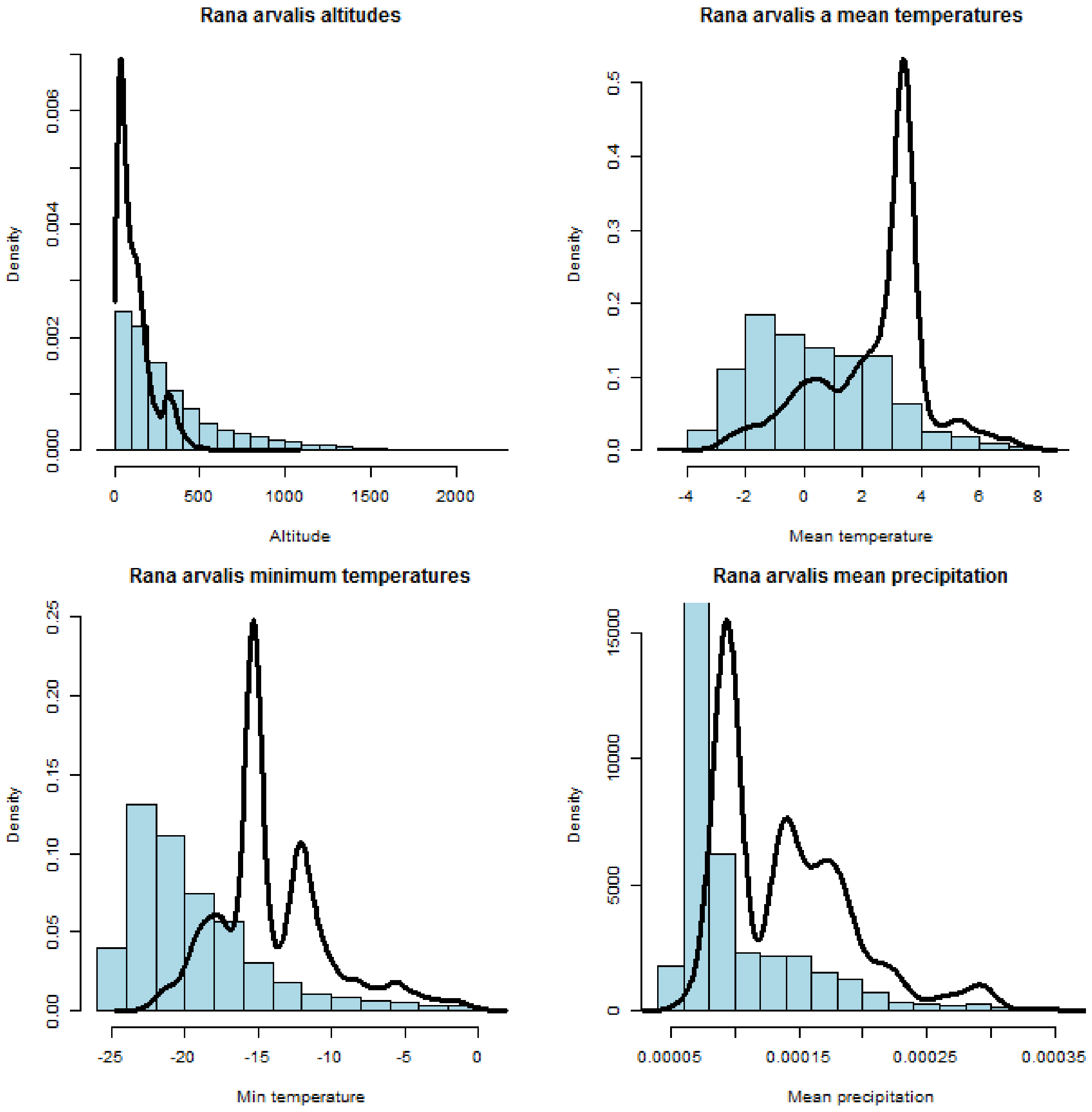

Figure A4-3: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Rana arvalis*. Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.

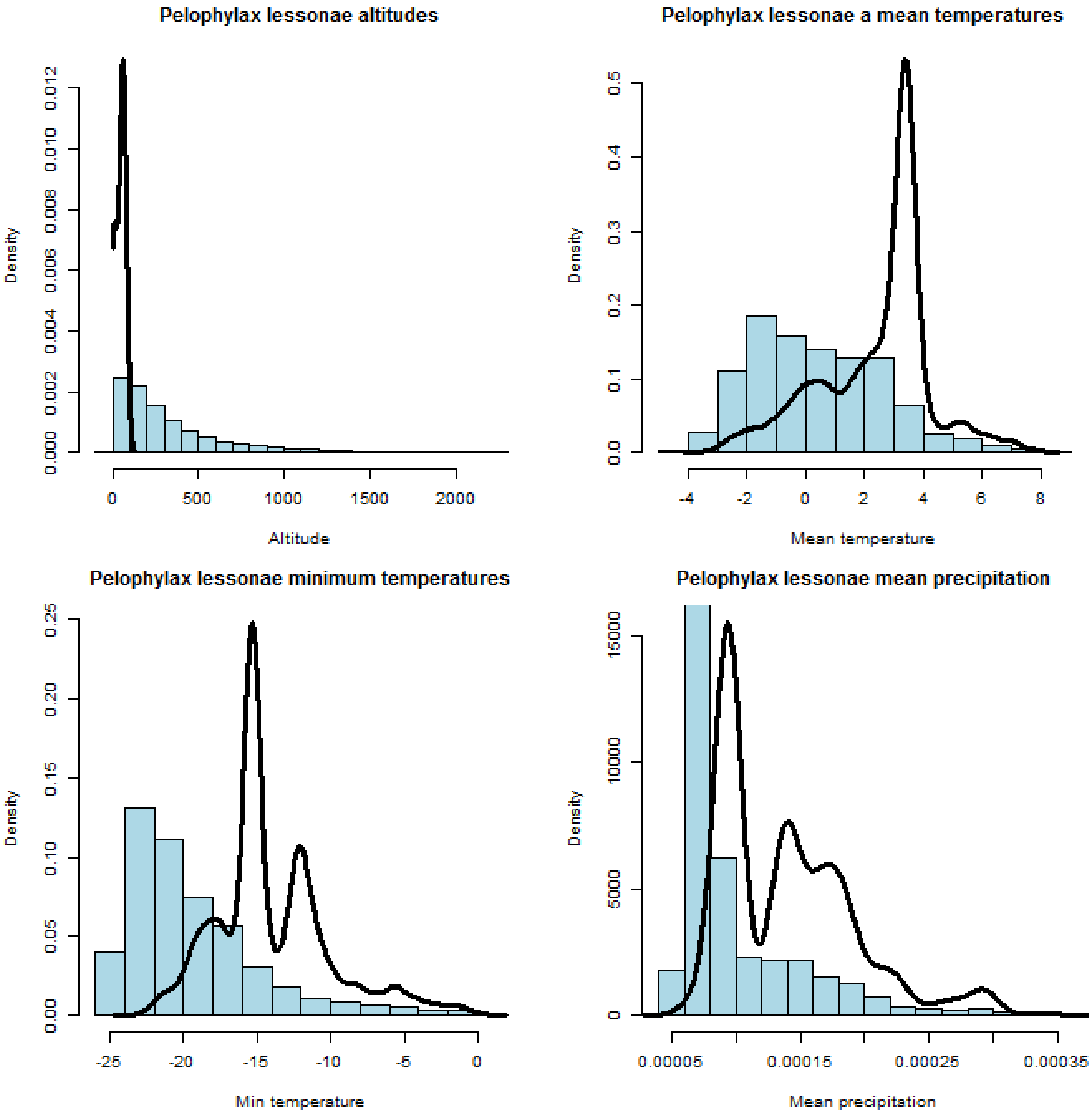

Figure A4-4: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Pelophylax lessonae*. Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.

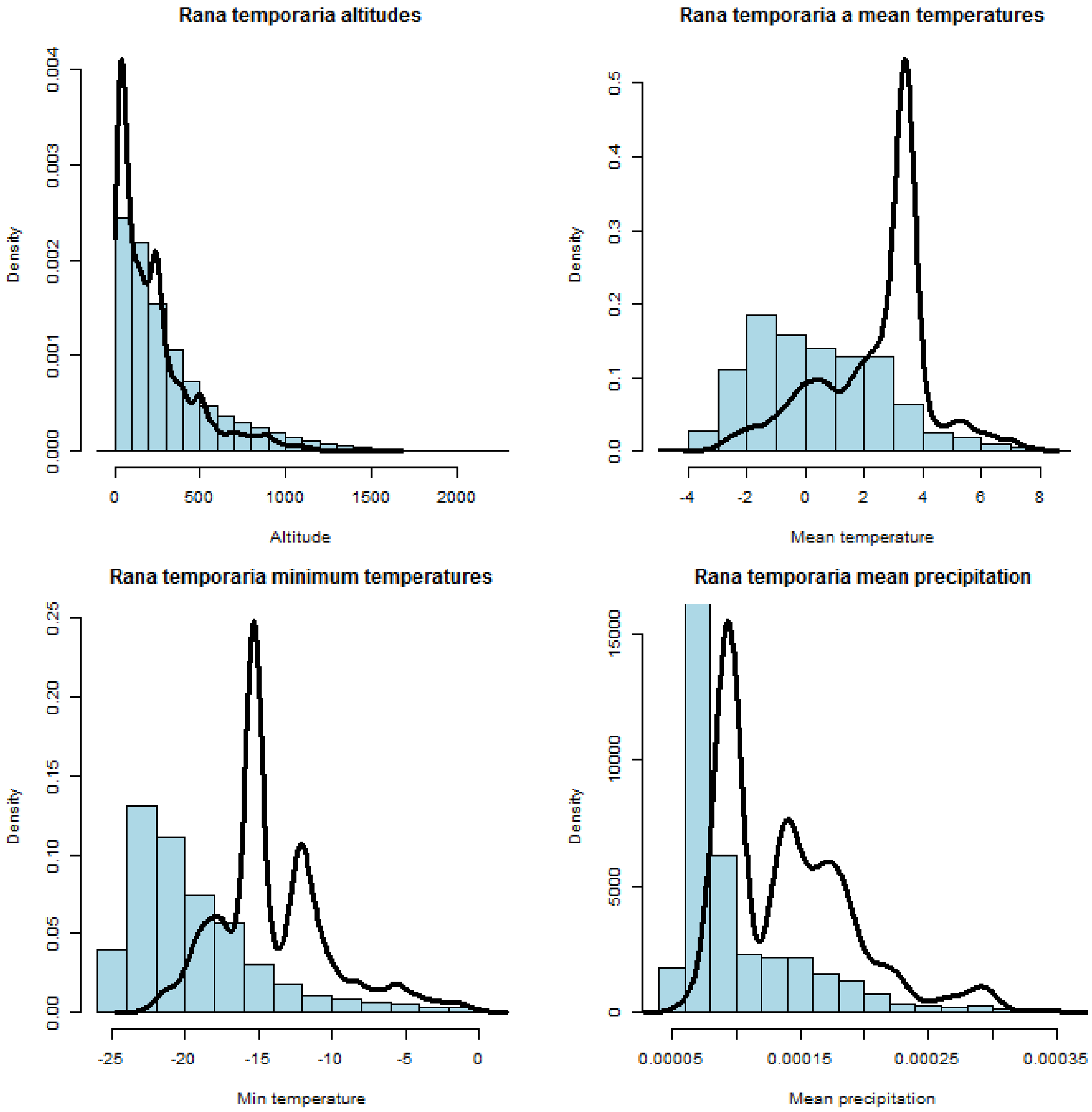

Figure A4-5: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Rana temporaria.* Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.

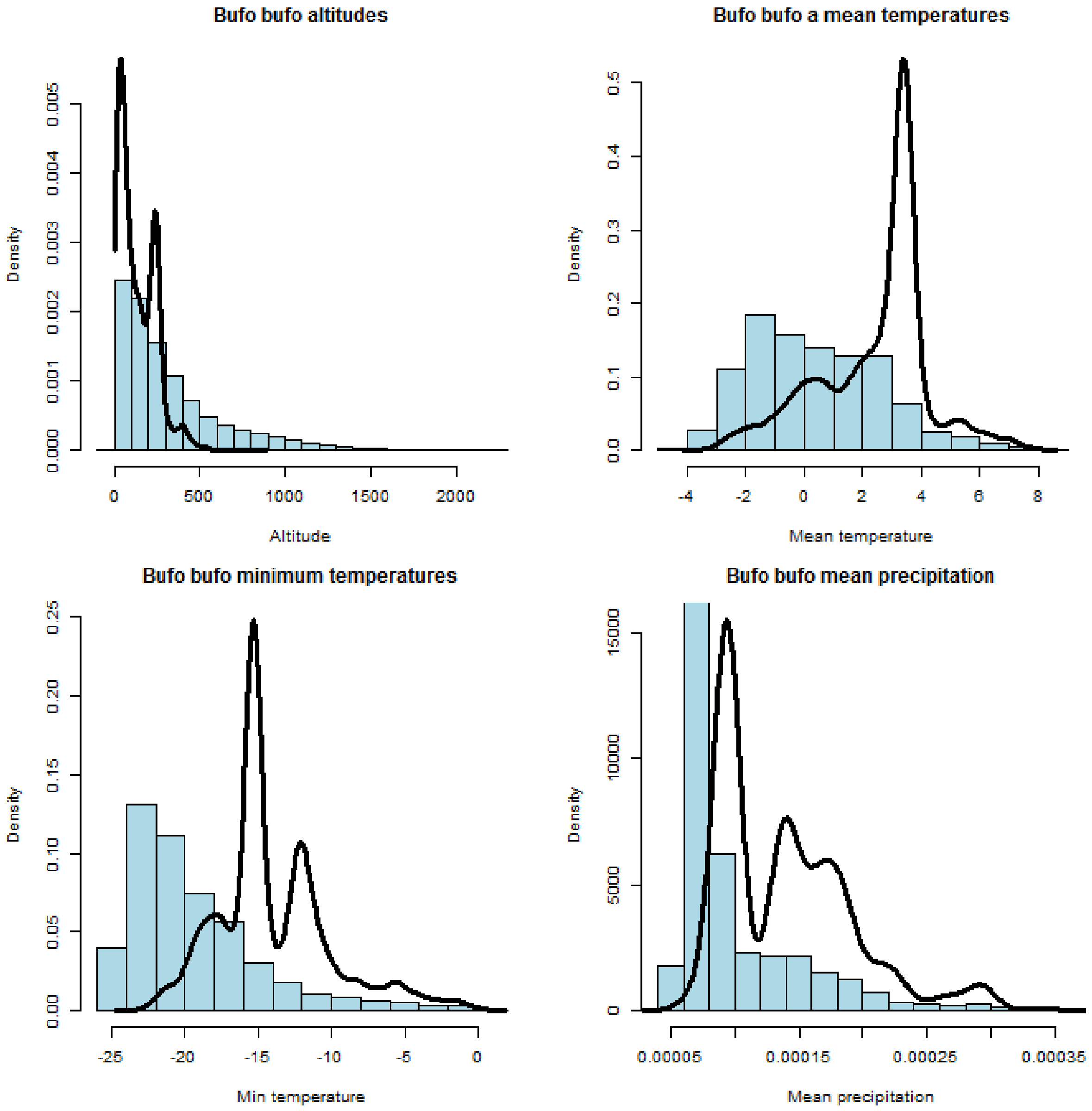

Figure A4-6: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Bufo bufo*. Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.

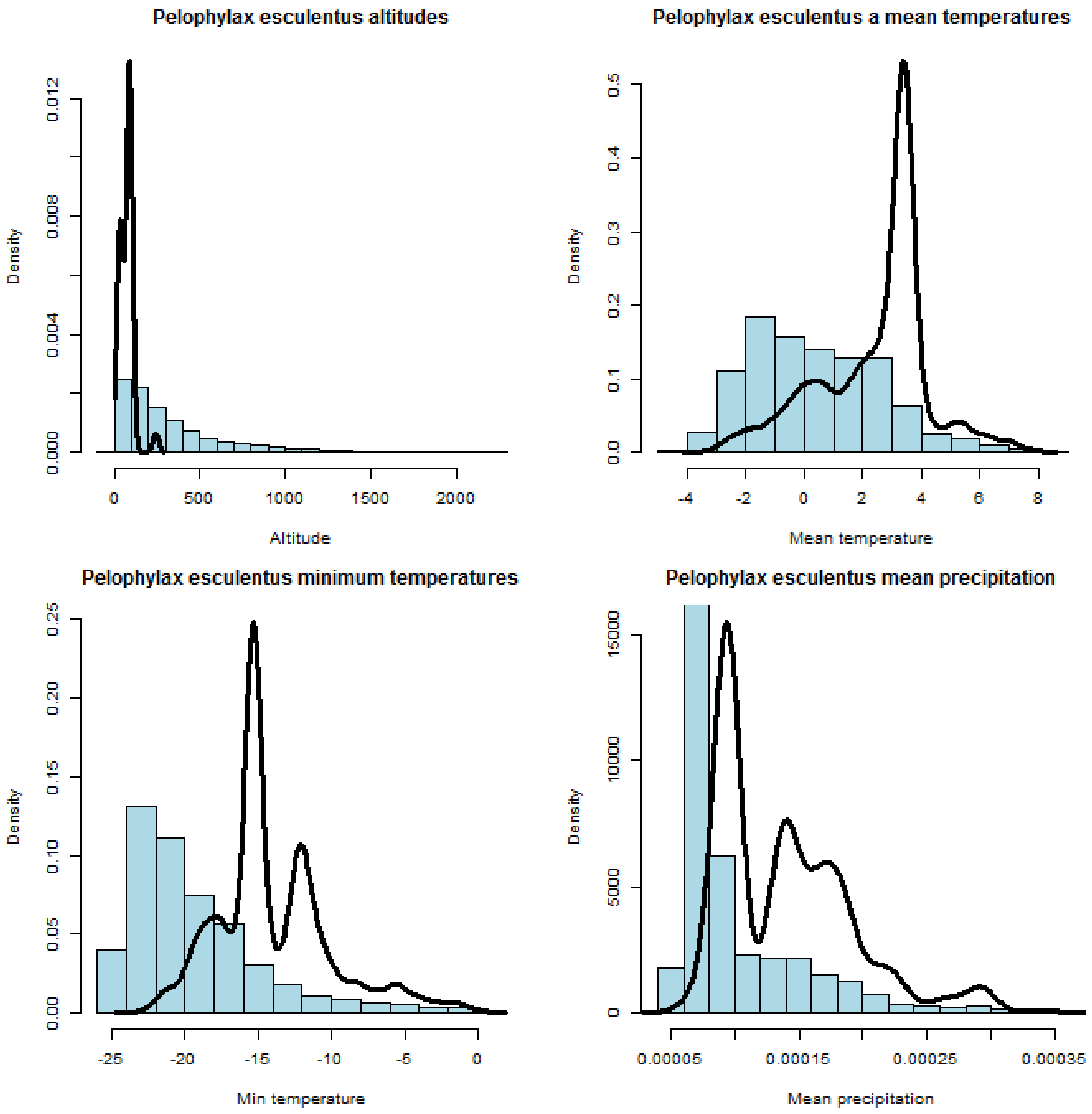

Figure A4-7: Species specific preferences of altitude, max/min temperature and mean percipitation (observed distribution) for *Pelophylax esculentus.* Total frequency distribution in Norway, for each parameter, is shown as light blue histograms.