



VKM Report 2021: 04

Assessment of the risk of negative impact on biodiversity from import and release of eggs or live fish from landlocked Atlantic salmon from Klarälven in Sweden to Trysilelva in Norway

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

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Cover photo: Upper part of Klarälven at Brattmon, downstream of Höljes in Sweden. Photo: Eva B. Thorstad.

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of three VKM members, four external experts and a project leader from the VKM secretariat. Two external referees commented on and reviewed the draft opinion. The VKM Panel on Alien Organisms and Trade in Endangered Species (CITES) evaluated and approved the final opinion. For this assignment, the Panel on Alien Organisms and CITES is supplemented by three members of the Panel on Animal Health and Welfare.

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The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group or an interdisciplinary VKM approval group, appointed specifically for the assignment.

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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

Introduction

Atlantic salmon in the River Klarälven in Sweden live the entire life in freshwater, undertaking feeding migrations to Lake Vänern. The upper part of the watershed is in Norway and comprises the River Trysilelva and associated rivers and lakes. Atlantic salmon previously lived in the Norwegian part of the watershed but were lost due to the construction of 11 hydropower stations that block the upstream migration from Vänern. The power stations also cause a high mortality among downstream migrating fish. Tagging studies showed that there is 71-84% mortality of juveniles (smolts) and 100% mortality of adults during downstream migration past the eight lowermost power stations. Extensive mitigation measures are needed to reduce the mortality of downstream migrants and reestablish a population that can reach areas in Norway naturally without being captured in Sweden and transported to Norway. In 2015, the total costs of establishing fishways bypassing the power stations and securing safe downstream migration was estimated to be 1000 million SEK.

To compensate for a decline of salmon due to lost habitat, hatchery-produced juveniles have been released in the watershed for more than 100 years, and adult salmon have been captured in the lower reaches of Klarälven and released in upstream reaches. After the Höljes power station was built, 80% of the salmon transported upstream were released upstream of Höljes. In 1993, the Norwegian government stopped these releases due to the large mortality of downstream migrating fish at the power stations. The releases had already been stopped from late summer 1988 due to bacterial kidney disease (BKD) outbreaks in salmon populations in the watershed. Since 1988, transported fish have been released upstream of Edsforsen in Sweden, and have not been able to reach Norway.

Aim of report

The Norwegian Environment Agency asked VKM to carry out a risk assessment of three specified methods that can be used to reestablish salmon in the Norwegian part of the watershed. This risk assessment is pertinent because the occurrence of alien organisms and infectious agents have developed differently in the Swedish and Norwegian parts of the watershed after salmon became unable to migrate through the river system. In 2013, the fish parasite *Gyrodactylus salaris* was detected in Klarälven, but has not been recorded in Norwegian parts of the watershed. Here, we assess the risk of negative impacts on native biodiversity by importing Atlantic salmon eggs or live adults from Klarälven to Norway.

Three methods of importing eggs or adults were assessed:

I. Import of fertilised eggs to a local hatchery in Norway, which are planted in the river in the spring or hatched and released as juveniles or smolts.

- II. Import of fertilised eggs that will be used to establish a long-term broodstock in Norway using the gene bank model, from which eggs can be planted into the river, or transferred to a local hatchery with subsequent release of juveniles or smolts.
- III. Import of adult salmon spawners that are captured in the lower parts of Klarälven in Sweden, transported in tanks and released in the Norwegian parts of the watershed.

Methods

The risk assessment was based on a literature review and qualitative assessment of each of the three methods of importing eggs or adults. The risk of impacts on native biodiversity and ecosystems was assessed for infectious agents, including parasites, bacterial pathogens, and viruses, and for other alien species. For each of the infectious agents and alien species, the risk is based on the product of the magnitude of the potential negative impact to native biodiversity and ecosystems, and the likelihood that negative consequences occur. The risk assessment concludes in terms of low, moderate, or high risk.

Results

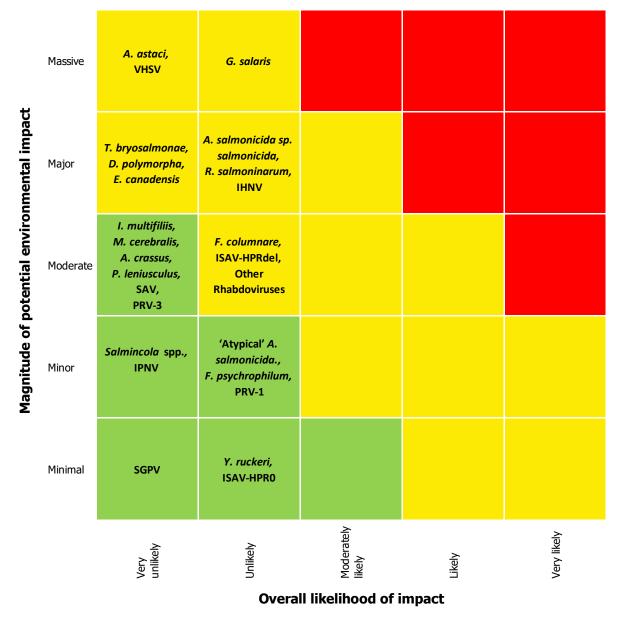
If introduced to the Norwegian part of the watershed, the parasite *G. salaris*, the viral haemorrhagic septicemia virus (VHSV), and *Aphanomyces astaci* (an oomycete causing crayfish plague), are expected to have massive impacts on native biodiversity and ecosystems (Figure 1). The bacteria *Renibacterium salmoninarum* (causing bacterial kidney disease, BKD) and *Aeromonas salmonicida* subspecies *salmonicida* (causing furunculosis), the infectious haematopoietic necrosis virus (IHNV), the myxozoan *Tetracapsuloides bryosalmonae* (causing proliferative kidney disease, PKD), the macrophyte Canadian pondweed *Elodea canadensis*, and the zebra mussel *Dreissena polymorpha* are expected to have major impacts, if introduced. Moreover, nine other assessed disease agents or alien species were assessed to have moderate impact if introduced.

The likelihood that negative consequences occur differs among the three methods of importing eggs or adults (Figure 1) and the organisms and viruses that were assessed. Method I (introduction of eggs to a local hatchery) is associated with an unlikely impact for 12 organisms and viruses, and very unlikely impact for the remaining organisms and viruses. Method II (establishing a gene bank) is associated with a very unlikely impact for all organisms and viruses. Method III (release of adult salmon imported from Sweden) is associated with a very likely or likely negative impact for five organisms and viruses (*T. bryosalmonae, G. salaris, R. salmoninarum*, the myxozoan *Myxobolus cerebralis* causing whirling disease, and infectious pancreatic necrosis virus IPNV). Moreover, method III is associated with a moderately likely negative impact for 14 other organisms and viruses.

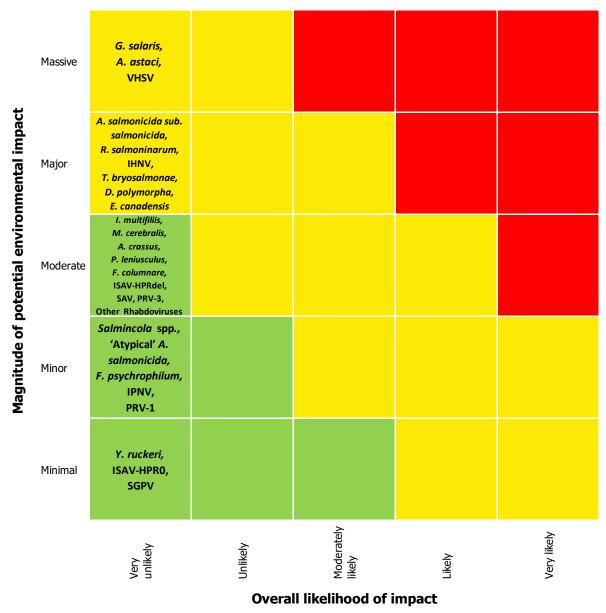
There is at least moderate risk associated with all the three methods of importing eggs or adults to Norway, and high risk associated with one of the methods (Figure 1). For method I, moderate risk is associated with 12 of the organisms and viruses and low risk with the remaining organisms and viruses. For method II, moderate risk is associated with nine of the organisms and viruses, with the highest risk associated with *G. salaris, A. astaci,* and VHSV.

For this method, the remaining organisms and viruses are associated with low risk. For method III, high risk is associated with *G. salaris*, *T. bryosalmonae*, *R. salmoninarum*, *A. astaci*, and VHSV, and moderate risk with most other organisms and viruses.

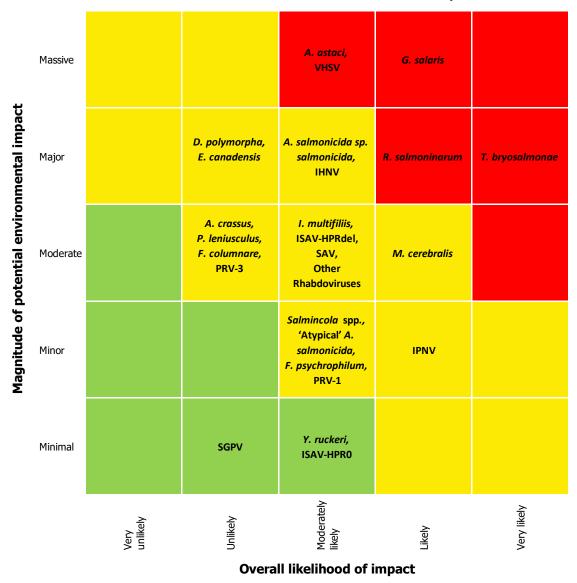
There are additional risks connected to the potential presence of currently unknown and undescribed infectious agents in Vänern or Klarälven, which we have not been able to assess. The risk assessment covers the period until year 2100. We expect future introductions to Vänern and Klarälven of infectious agents or alien organisms through aquaculture, import of European eel, ballast water, fouling organisms on boats, and other human activities.



Risks accociated with method I: introduction of eggs to a local hatchery



Risks accociated with method II: establishment of a gene bank



Risks accociated with method III: release of adult salmon imported from Sweden

Figure 1: The overall risk of introduction of alien species and disease organisms and viruses by using method I (introduction of eggs to a local hatchery, upper panel), method II (establishing a gene bank, middle panel), and method III (release of adult salmon imported from Sweden, lower panel). Red indicates high risk, yellow moderate risk, and green low risk.

Uncertainties

There are uncertainties related to the risk of human error and potential consequences that may occur during treatments and transport of fish and eggs. There are also uncertainties related to the limited monitoring of infectious agents of wild Atlantic salmon, and to the general lack of information on infectious agents from freshwater systems. Much of the knowledge on infectious agents affecting Atlantic salmon comes from marine aquaculture. VKM has not assessed animal welfare and ethical aspects related to import and release of eggs or fish. Ethical concerns are related to the high mortality for fish that pass the power stations during downstream migration. Welfare issues are also related to handling of fish involved in the methods of importing eggs or adults assessed in this report.

The methods assessed here involve planting eggs or releasing salmon in the Norwegian parts of the watershed for a one-way downstream migration to the Swedish parts. VKM notes that these methods will, in isolation, be insufficient for a reestablishment of salmon in the Norwegian part of the watershed. In order to reestablish salmon in Norway, adult salmon must be able to return to their spawning areas. This involves a two-way free movement of fish between Norwegian parts of the watershed and Vänern in Sweden, which will increase the risk compared to our conclusions. Hence, this risk assessment is, according to the terms of reference, focused on three specific methods, and is not an assessment of a reestablishment of salmon in the Norwegian part of the watershed. Assessing the risk related to a reestablishment of freely migrating fish requires a new risk assessment.

Conclusion

The overall risk assessment shows that there is at least a moderate risk of negative impact from infectious agents and alien species on native biodiversity and ecosystems associated with all the three methods of importing eggs or adult salmon to Norway, and a high risk associated with one of the methods (Figure 1). Release of adults imported from Sweden was associated with the highest risk. Establishing a gene bank was associated with the lowest risk, but was nevertheless associated with a moderate risk for nine of the assessed organisms and viruses. If introduced to the Norwegian part of the watershed, the parasite *G. salaris*, the viral haemorrhagic septicemia virus (VHSV), and *A. astaci* causing crayfish plague are expected to have massive impacts on native biodiversity and ecosystems. Several other infectious agents and alien species are expected to have major impacts, if introduced.

VKM notes that these methods will, in isolation, be insufficient for a reestablishment of salmon in the Norwegian part of the watershed, and that they are also associated with fish welfare issues not considered in this report. In order to reestablish salmon to Norway, adult salmon must be freely able to return to their spawning areas, which would require a new risk assessment.

Key words: Pathogens, virus, infectious organisms, disease, *Salmo salar*, Vänaren, hydropower, *Gyrodactylus salaris*

Sammendrag på norsk

Introduksjon

Laksen i Klarälven i Sverige lever hele livet i ferskvann og vandrer fra Klarälven til den store innsjøen Vänern for å spise. De øvre delene av vassdraget, Trysilelva med tilhørende elver og innsjøer, ligger i Norge. Tidligere levde laksen også i de norske delene av vassdraget, men ble utryddet fordi 11 kraftstasjoner hindrer oppvandringen fra Vänern. Kraftstasjonene medfører også høy dødelighet for nedvandrende fisk. Undersøkelser basert på merking av laks viste 71-84 % dødelighet for ungfisk og 100 % dødelighet for voksen fisk satt ut på strekninger ovenfor de åtte nederste kraftstasjonene. Omfattende tiltak er nødvendig for å redusere den høye dødeligheten for nedvandrende laks fra Trysilelva, og for å reetablere en bestand av laks som kan nå områdene på norsk side uten at de må fanges og transporteres opp fra Sverige. De totale kostnadene for å bygge fisketrapper ved kraftstasjonene og sikre trygg nedvandring ble i 2015 beregnet til en milliard svenske kroner.

For å veie opp for den reduserte laksebestanden i vassdraget som følge av tapt habitat, er det produsert og satt ut laksunger fra klekkeri i mer enn 100 år. I tillegg har voksen laks blitt fanget i nedre deler av Klarälven og sluppet ut igjen lenger opp i vassdraget. Etter at Höljes kraftstasjon ble bygd har 80 % av laksen som ble transportert opp blitt satt ut ovenfor Höljes. I 1993 stanset norske myndigheter utsettingen av laks oppstrøms Höljes på grunn av den store dødeligheten av nedvandrende laks ved kraftstasjonene. I praksis var utsettingene stanset allerede i 1988 på grunn av utbrudd av bakteriell nyresyke (BKD) i vassdraget. Siden 1988 har fisk blitt satt ut ovenfor Edsforsen i Sverige, uten mulighet til å vandre til den norske delen av vassdraget.

Formål med rapporten

Miljødirektoratet har bedt VKM vurdere risiko knyttet til tre spesifiserte metoder som kan benyttes til å reetablere laks i den norske delen av vassdraget. En slik risikovurdering er viktig fordi forekomsten av fremmede arter og agens som kan føre til sykdommer, har utviklet seg forskjellig på svensk og norsk side i tiden etter at laksen kunne vandre fritt. I 2013 ble parasitten *Gyrodactylus salaris* påvist på laks i Klarälven, mens den ikke er registrert i den norske delen av vassdraget. Vi vurderer her risiko for negative effekter på biologisk mangfold og økosystemer i Norge knyttet til å importere lakserogn eller voksen laks fra Klarälven i Sverige til den norske delen av vassdraget.

Vi har vurdert tre metoder for å importere rogn eller voksen laks:

- I. Import av befruktet rogn til et lokalt klekkeri i Norge, som plantes i elva om våren eller klekkes og settes ut i elva som yngel eller smolt.
- II. Import av befruktet rogn som brukes til å etablere en stamfiskbestand i et anlegg som drives etter genbankmodellen, der rogn kan hentes fra og plantes direkte i elva, eller overføres til et lokalt klekkeri for klekking og utsetting som yngel eller smolt.
- III. Import av voksen laks fanget i nedre deler av Klarälven i Sverige, som transporteres i tanker og settes ut i norske deler av vassdraget.

Metoder

Risikoanalysen ble basert på litteratursøk og en kvalitativ vurdering av hver av de tre metodene for å importere rogn eller fisk. Risiko for negative effekter på biologisk mangfold og økosystemer i Norge ble vurdert for aktuelle sykdomsagens inkludert parasitter, bakterier og virus, og for andre fremmede arter. Risiko vurderes som en kombinasjon av størrelsen på mulige negative konsekvenser, og sannsynlighet for at negative effekter oppstår. Risikoanalysen konkluderes ut fra dette med lav, moderat eller høy risiko for hver sykdomsagens og fremmed art, for hver av de ulike metodene for å importere rogn eller fisk (Figur 1).

Resultater

Parasitten *Gyrodactylus salaris*, viral hemorrhagisk virusseptikemi virus (VHSV) og *Aphanomyces astaci* (en eggsporesopp som forårsaker krepsepest) forventes å medføre svært store negative konsekvenser for biologisk mangfold og økosystemer dersom de overføres til Norge (Figur 1). Bakteriene *Renibacterium salmoninarum* (som forårsaker bakteriell nyresyke BKD) og *Aeromonas salmonicida* underart *salmonicida* (som forårsaker furunkulose), infeksiøs hematopoetisk nekrose virus (IHNV), parasitten *Tetracapsuloides bryosalmonae* (som forårsaker proliferativ nyresyke PKD) og de invaderende artene vasspest *Elodea canadensis* og sebramusling *Dreissena polymorpha* forventes å medføre store negative konsekvenser dersom de overføres til Norge. I tillegg ble ni andre sykdomsorganismer og invaderende arter vurdert til å medføre medium konsekvenser dersom de overføres.

Konsekvensene for biologisk mangfold og økosystemer av at organismer og virus overføres til Norge, som beskrevet i avsnittet over, vil være de samme uansett hvordan de eventuelt overføres, og er dermed vurdert likt for de tre metodene for import av rogn eller fisk. Sannsynligheten for at de overføres varierer imidlertid mellom de tre metodene for å importere rogn eller fisk, og dermed varierer sannsynligheten for at det vil oppstå negative effekter mellom metodene (Figur 1). Ved bruk av metode I (import av rogn til et lokalt klekkeri) vurderes det som usannsynlig at det vil oppstå negative effekter for 12 av organismene og virusene som ble vurdert, og svært usannsynlig for de øvrige organismene og virusene. Ved bruk av metode II (etablering av genbank) vurderes det som svært usannsynlig at det vil oppstå negative effekter for noen av organismene eller virusene. Ved bruk av metode III (import av voksen laks fra Sverige for utsetting i elva) vurderes det som svært sannsynlig eller sannsynlig at det vil oppstå negative effekter for fem organismer og virus (*T. bryosalmonae, G. salaris, R. salmoninarum*, myxozoen *Myxobolus cerebralis* som forårsaker dreiesyke, og infeksiøs pankreas nekrose virus IPNV). For denne metoden vurderes det i tillegg som moderat sannsynlig at det vil oppstå negative effekter for 14 organismer og virus.

Risiko for negative effekter, basert på en samlet vurdering av konsekvenser og sannsynlighet for at de oppstår, var moderat for mange organismer og virus for alle de tre metodene for import av rogn eller fisk, og høy for en av metodene (Figur 1). For metode I (import av rogn til et lokalt klekkeri) var det moderat risiko for 12 sykdomsagens og invaderende arter og lav risiko for andre organismer. For metode II (etablering av genbank) var det moderat risiko for ni sykdomsagens og fremmede arter, med høyeste risiko for *G. salaris, A. astaci* og VHSV. For denne metoden var det lav risiko for de øvrige organismene. For metode III (import av voksen laks fra Sverige for utsetting i elva) var det høy risiko for *G. salaris, T. bryosalmonae, R. salmoninarum, A. astaci* og VHSV, og moderat risiko for andre organismer.

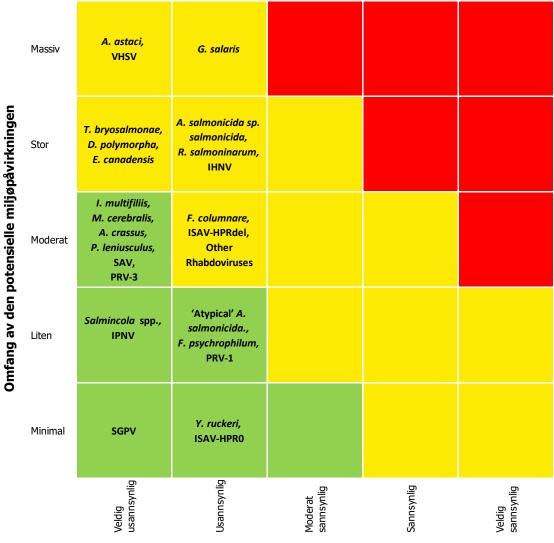
Ytterligere risiko er knyttet til mulig forekomst av ukjente sykdomsagens eller invaderende arter i Vänern eller Klarälven, som allerede finnes, men ikke er påvist, eller som ikke er beskrevet ennå. Risikoanalysen dekker perioden frem til år 2100. I løpet perioden forventer vi at sykdomsagens og fremmede arter kan introduseres til Vänern og Klarälven med akvakultur, utsetting av importert ål, ballastvann, begroing på båter, eller via andre menneskelige aktiviteter.

Usikkerheter

Usikkerheter i vurderingene er knyttet til risiko for menneskelige feil under behandling og transport av rogn og fisk, og konsekvenser av slike feil. Det er også usikkerheter knyttet til at det er begrenset overvåking og manglende kunnskap om sykdomsagens hos villaks, og til at det generelt er mangel på kunnskap om sykdomsagens i ferskvann. Mye av kunnskapen som finnes om agens som kan føre til sykdom hos laks, kommer fra lakseoppdrett i sjøen.

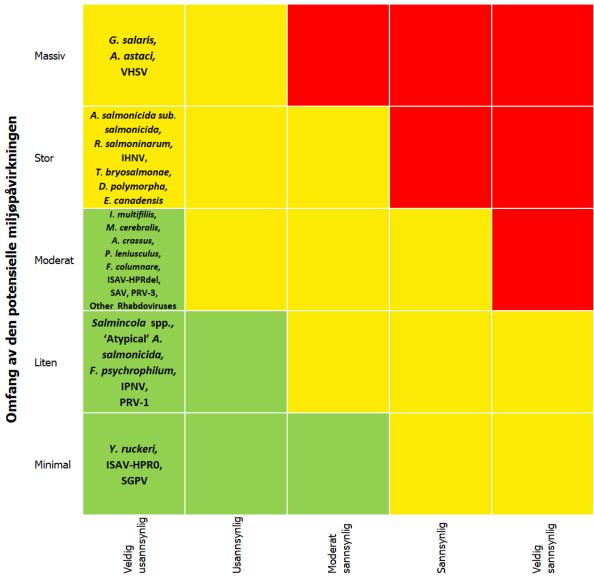
VKM har ikke vurdert dyrevelferd eller etiske aspekter knyttet til import og utsetting av rogn og fisk. Etiske problemer er knyttet til den høye dødeligheten hos fisk som passerer kraftverkene når de vandrer til Vänern. Metodene for import av fisk som er vurdert innebærer også utfordringer knyttet til fiskevelferd ved håndtering av fisk.

Metodene som er vurdert inkluderer rognplanting og utsetting av laks i den norske delen av vassdraget, som kun medfører en enveis nedvandring av fisk til den svenske delen av vassdraget. VKM bemerker at disse metodene alene er ikke tilstrekkelige for å reetablere laks på norsk side. For å kunne reetablere må det åpnes for at voksen laks fritt kan vandre tilbake til gyteområdene. En reetablering vil altså kreve en toveis vandring av fisk mellom den norske delen av vassdraget og Vänern i Sverige, noe som nødvendigvis vil øke risikoen i forhold til våre konklusjoner. Risikovurderingen i denne rapporten er kun knyttet til bruk av de tre metodene for import av rogn og fisk, og er ikke en full risikovurdering av en reetablering av laks i den norske delen av vassdraget. Risiko knyttet til laks som kan vandre fritt må eventuelt vurderes separat.



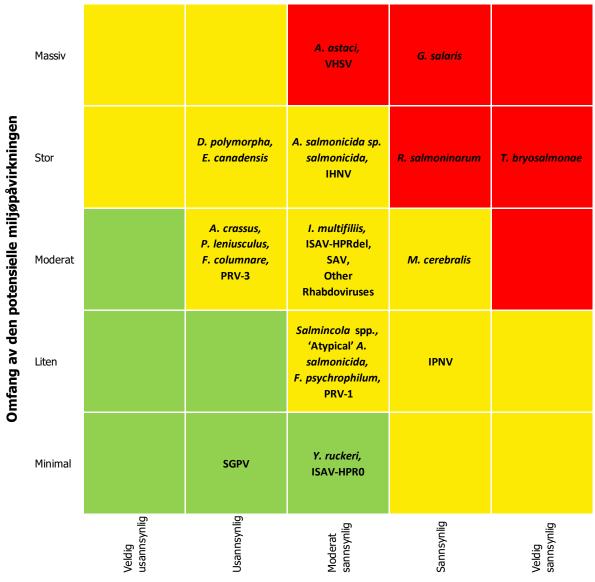
Risiko knyttet til metode I: introduksjon av befruktet rogn til et lokalt klekkeri i Norge

Overordnet sannsynlighet for negativ innvirkning



Risiko knyttet til metode II: stamfiskbestand etter genbankmodellen

Overordnet sannsynlighet for negativ innvirkning



Risiko knyttet til metode III: import av voksen laks fra Sverige

Overordnet sannsynlighet for negativ innvirkning

Figur 1: Risiko knyttet til sykdomsagens og invaderende arter ved bruk av metode I (import av rogn til lokalt klekkeri, øverste figur), metode II (etablering av genbank, midterste figur), og metode III (utsetting av voksen laks importert fra Sverige, nederste figur). Rød viser høy risiko, gul moderat risiko og grønn lav risiko.

Konklusjon

Vurderingen viser at det er minst moderat risiko for negative effekter av sykdomsagens og invaderende arter på biologisk mangfold og økosystemer i Norge knyttet til metodene for import av rogn eller voksen laks, og høy risiko for en av metodene (Figur 1). Import av voksen laks fra Sverige for utsetting i norsk del av vassdraget er forbundet med høy risiko. Etablering av en genbank er metoden det er knyttet lavest risiko til, men selv denne metoden er forbundet med moderat risiko for ni sykdomsagens og fremmede arter. Parasitten *Gyrodactylus salaris*, viral hemorrhagisk virusseptikemi virus (VHSV) og *Aphanomyces astaci* (som forårsaker krepsepest) forventes å medføre svært store negative konsekvenser for biologisk mangfold og økosystemer dersom de overføres til norsk del av vassdraget. Flere andre sykdomsagens og fremmede arter forventes å medføre store negative konsekvenser dersom de innføres.

VKM bemerker at bruk av de vurderte metodene for import av rogn eller fisk ikke alene vil være tilstrekkelig til å reetablere laks i den norske delen av vassdraget. Metodene er i tillegg forbundet med utfordringer knyttet til fiskevelferd, som ikke er vurdert. For å reetablere laks på norsk side må laksen være i stand til å vandre fritt til gyteområdene, noe som vil kreve en ny risikovurdering.

Key words: Patogener, virus, sykdomsagens, Salmo salar, Vänern, Gyrodactylus salaris

Abbreviations and glossary

Abbreviations

- ADNS Animal disease notification system
- BKD Bacterial kidney disease
- EC European Comission
- EEA European Economic Area
- EEC European Economic Community
- EIBS Erythrocytic inclusion body syndrome
- EFTA European Free Trade Association
- ERM enteric redmouth disease
- ESA EFTA Surveillance Authority
- EU European Union
- EVEX- Eel virus European X
- HMSI Heart and muscle inflammation
- IHNV Infectious haematopoietic necrosis virus
- IPNV Infectious pancreatic necrosis virus
- ISA Infectious salmon anemia
- ISAV Infectious salmon anaemia virus
- IUCN International Union for Conservation of Nature
- NASCO North Atlantic Salmon Conservation Organisation
- OIE World Organisation for Animal Health
- PD Pancreas disease
- PKD Proliferative kidney disease
- PRV Piscine orthoreovirus

- RAS Recirculating aquaculture systems
- RCP Representative concentration pathways
- SAV Salmonid alpha virus
- SGPV Salmon gill poxvirus
- SPF specific-pathogen free
- VHSV Viral hemorrhagic septicemia virus
- WSSV White spot syndrome virus
- WTO World Trade Organization

Glossary

Anadromous salmon: Salmon that spawn in freshwater and perform feeding migrations to the ocean.

Endemic: Regarding infectious agents, indicates a constant presence within a defined (e.g., geographically) host population. An infection that is able to maintain at a similar baseline level in a geographical area over a prolonged time without external input (basic reproduction number will, on average, equal 1 (R_0 =1)).

Eyed eggs: Fish eggs containing an embryo that has developed enough so that the black spot of the eyes is visible through the egg membrane.

Landlocked salmon: Salmon that use a freshwater lake for feeding and growing instead of migrating to the sea, or live the entire life in the river habitat.

Horizontal transmission: The lateral transfer of an infectious agent between individuals or populations (OIE). Transmission can occur either directly between fish or indirectly, by living or inanimate vectors, water, or anthropogenic activity.

Infectious agent: Cellular organisms (e.g., bacteria and parasites) or non-cellular agents (e.g., viruses) capable of colonising/invading and multiplying in or on susceptible host species (i.e., causing an infection).

Infectious disease: Refers to the biological disorders (and/or manifestations thereof) that may arise within an organism as a result of infection.

Invasive species: A species that is not native to a location (has been introduced) and that tends to spread to a degree believed to cause damage to the environment, human economy, or human health.

Isostatic rebound: Rising of the land masses after the removal of the huge weight of ice sheets during the last glacial period.

Listing (of diseases): EU/Norwegian legislative framework listing infectious fish diseases considered to be of particular significance, and which for instance trigger specified regulative measures upon detection. This includes List 1 (exotic diseases), List 2 (non-exotic diseases) and List 3 (national diseases).

Parasite: An organism that lives on or in a host organism and obtains its nutrition from, or at the expense of, its host. Classically, a parasite is a eukaryote (thereby excluding bacteria).

Pathogen: Infectious agent capable of causing disease.

Sea ranching: Release of captive-bred fish (usually as smolts) that are captured on their return migration as adults, either in a fishery or for use as broodstock for the next captive-bred generation.

Smolt: Smolt is the life-stage in salmon when they finish the juvenile stage and start to migrate downstream for feeding in the sea, or in the case of this report, the lake Vänern.

Vertical transmission: The transmission of an infectious agent from one generation to the next through eggs or milt. True vertical transmission refers to the agent being within the content of gametes (egg/milt). Alternatively, passive vertical transmission occurs by contamination, where the agent is present at the surface of gametes, in the ovarian fluid or in mucus.

Background as provided by the Norwegian Environment Agency

Following up an initiative from the ministers of the Environment in Norway and Sweden, an Interreg project was started in 2010 with the intention of investigating whether, among other things, it is possible to re-establish the salmon stock on the Norwegian side. The project "Vänerlaxens Fria Gång" was established in 2010. The first period of the project was started up in January 2011 and ended in August 2014, reported in 2015. The second period, " Två länder - én elv ", started in March 2017 and will end during 2020. Project owners have in both periods been the County Administrative Board of Värmland and the County Governor of Hedmark (now Innlandet County).

The goal of the Interreg project is to examine the possibilities of strengthening (Swedish side) and re-establishing (Norwegian side) the Väner-salmon population in the Klarälv/Trysil/Femund river system. Three methods are proposed to be used to re-establish the salmon on Norwegian side:

- I) Introduction of fertilized salmon eggs, which are kept in a local cultivation facility until being either planted in the river as eyed eggs or released as fry or smolts.
- II) Follow the Norwegian gene bank model, over a number of years establish a broodstock with a documented origin in a fishing facility operated according to the gene bank model. From the broodstock, it is then possible to either transfer fertilized eggs that are planted directly in the river, or eggs are transferred to a local hatchery for hatching. The fish then are kept in the hatchery until eleased as fry and smolts. Over time, the broodstock must be supplemented with new material.
- III) Migrating spawning fish are caught in the lower part of the watercourse and, after treatment, transported in tanks, to the Norwegian side of the border and released in the river.

In the years that have passed since Femund/Trysil river were "separated" from Lake Vänern, the occurrence of alien organisms and infectious agents has developed differently in the Swedish and Norwegian sides of the watercourse. This applies to several infectious substances and alien species. As an example, *Gyrodactylus salaris* has been confirmed on salmon in Lake Vänern and in the river Klarälv, a parasite not found on the Norwegian side of the watercourse.

There will always be uncertainty about the risk associated with moving wild fish. In Norway, the Norwegian Environment Agency is responsible according introduction of alien organisms. The Norwegian Food Safety Authority has the administrative responsibility regarding disease in wild fish, and they follow up, in collaboration with the Norwegian Environment Agency. Cooperation has also been established between the Norwegian Food Safety Authority and the National Food Administration on the Swedish side.

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency, in consultation with the Norwegian Food Safety Authority, asks VKM to assess the risk associated with the introduction of alien species and disease organisms (including *G. salaris*) when moving fertilized eggs or adult Vänersalmon, from Sweden to Norway.

The assessment shall include the three above-mentioned methods for introduction, and it is assumed that the individuals have undergone treatment in accordance with current Norwegian regulations before and during relocation. The assessment is based on annual transfers up until 2100.

1 Introduction

In this report, we assess the risk of negative impact on biodiversity by importing Atlantic salmon *Salmo salar* eggs or live adults from the Swedish river Klarälven to Norway, by planting eggs or releasing fish from these imports into the river Trysilelva. Trysilelva, and associated rivers and lakes in Norway, constitute the upper parts of the Klarälven watershed. Salmon from Klarälven and the lake Vänern could reach these areas before hydropower stations were built along Klarälven during 1904-1961. The hydropower dams have blocked upstream fish migration, resulting in the loss of salmon from the Norwegian part of the watershed.

The possibility of reestablishing Atlantic salmon to the Norwegian parts of the watershed has been investigated (Hedenskog et al. 2015, Olstad et al. 2020). The Norwegian Environment Agency asked VKM to carry out a risk assessment of specific methods that can be used for such reestablishment. This risk assessment is important because the occurrence of alien organisms and infectious agents has developed differently in the Swedish and Norwegian parts of the watershed after salmon and other fish became unable to migrate freely through the river system, including the detection of the parasite *Gyrodactylus salaris* in Klarälven and Vänern.

The methods we have been asked to assess are: I) import of fertilised eggs to a local hatchery in Norway that are planted in the river in the spring or hatched and released as juveniles or smolts, II) import of fertilised eggs that will be used to establish a long-term broodstock in Norway using the gene bank model, from which eggs can be planted into the river, or transferred to a local hatchery with subsequent release of juveniles or smolts, and III) import of adult salmon spawners that are captured in the lower parts of Klarälven in Sweden, transported in tanks and released in the Norwegian parts of the watershed. (chapter 1.4).

All these methods involve planting eggs or releasing salmon in the Norwegian parts of the watershed, for a one-way downstream migration to the Swedish parts. Hence, these methods will, in isolation, be insufficient for reestablishment of salmon stocks in the Norwegian part of the watershed. In order to reestablish a viable salmon population in this area, fish from this population must be able to return to their spawning areas subsequent to downstream migration, which involves a two-way movement of fish from the population. The risks to biodiversity connected to a two-way movement of fish would necessarily increase compared with the risks associated with the specific import-and-release methods assessed here. Hence, this risk assessment is restricted to the use of these specific methods, and is not a full risk assessment of a fully fledged reestablishment of salmon in the Norwegian parts of the watershed.

In this report, we describe the watershed, including the Atlantic salmon population, other fish species, red-listed species, alien species, hydropower development, aquaculture, and

infectious agents of considered relevance. This is followed by an outline of the methods used in the risk assessment. The risk assessment itself is performed by identifying and characterising each of the hazards, evaluating likelihoods of negative impact, and assessing the risks. The last part of the report constitutes a conclusion, and a discussion of riskreducing measures and data gaps.

Shortly after the publication of this opinion, a new 'Animal Health Law' will be implemented in EU (1.5.5). As detailed information regarding the consequences of new regulations are not available for the panel, all assessments are based on current regulations in Sweden and Norway.

1.1 Atlantic salmon

Atlantic salmon is native to the temperate and subarctic regions of the North Atlantic Ocean. The species has a large variety of life-histories, but most forms are anadromous, which means individuals have a juvenile phase in fresh water, followed by a long ocean migration for feeding and growth, and a return migration to fresh water to spawn (Webb et al. 2007; Thorstad et al. 2011). Migrations to marine habitats are thought to have evolved because of better food availability in the ocean than in freshwater habitats (Gross et al. 1988). Most individuals spawn one or two times during their adult life, but, on rare occasions, they can spawn up to 5-6 times. Between each spawning, they perform a new marine migration (Webb et al. 2007; Thorstad et al. 2011).

A few Atlantic salmon populations are landlocked and use a freshwater lake for feeding and growing instead of migrating to the sea, or live their entire lives in the river habitat. A nonanadromous life cycle, without migrations to the sea, is common among Atlantic salmon in parts of North America (Klemetsen et al. 2003; Webb et al. 2007), but only few populations are known in Europe. The landlocked Atlantic salmon in northwestern Europe originate from colonization of watercourses around 11,000 years ago, when the watercourses became accessible in early postglacial times. Arctic charr, Salvelinus alpinus, and brown trout, Salmo trutta, colonized these areas in the same period and readily established landlocked populations, while Atlantic salmon rarely did so. Eight lakes with nonanadromous populations utilizing river-lake systems are known in Russia, one in Sweden (Vänern), two in Norway (Byglandsfjord and Nelaug, the latter being extinct), and one in Finland (Saimaa) (Dahl 1928, Kazakov 1992, Nilsson et al. 2001, Barlaup et al. 2005, Säisä et al. 2005). In Norway, there is also a landlocked freshwater salmon, in the Namsen watershed, where individuals only stay in the river and do not migrate to lakes (Sandlund et al. 2014). A large fraction of landlocked populations has declined due to anthropogenic impacts, and some populations have become extinct (Ozerov et al. 2010; Hutchings et al. 2019). The greatest threats to the persistence of landlocked salmon are found in Europe, where many of the populations are negatively impacted by habitat degradation and development for hydropower production (Hutchings et al. 2019).

In general, Atlantic salmon return with high precision to their home river after the feeding migration, and some even to the same area of the river where they spent their juvenile period. Precise homing may generate and maintain local adaptations through natural selection, and salmon populations in different rivers differ both ecologically and genetically (Garcia de Leaniz et al. 2007). Ecological and genetic differences among subpopulations within rivers have also been documented. Salmon is therefore managed on the river or watercourse level, both in Norway and other countries, assuming that each river holds a unique population that should be conserved. Freshwater populations often show a particularly high degree of genetic divergence compared to other anadromous salmon populations (Bourret et al. 2012).

1.2 History, biology and description of Vänern and Trysilelva/Klarälven

1.2.1 Väneren and Trysilelva/Klaraälven

Lake Vänern and the river Trysilelva/Klarälven are part of the longest watershed in Scandinavia, which is shared between Norway and Sweden (Hedeskog et al. 2015, Figure 1.2.1-1). The watershed origins in Lake Rogen in Sweden and continues into Norway to Lake Femunden, and thereafter through several smaller lakes and river stretches, including Lake Isteren, to Femundselva and Trysilelva. Trysilelva flows back into Sweden, where its name is Klarälven. Klarälven enters Vänern, which is the largest lake in Sweden and the third-largest lake in Europe. Vänern drains to Kattegat, through the river Göta älv, entering the sea at Gothenburg on the Swedish west coast. Vänern also became connected to the Baltic Sea after the construction of the Göta and Trollhätte canals (chapter 1.2.2). The watershed is thoroughly described by Hedenskog et al. (2015).

The main rivers between Femunden and Vänern have several names, either because they are separate river stretches with different names between lakes, or because the river has different names in different areas. For practical reasons, we use the name Trysilelva/Klarälven in this report, although it includes river stretches with other names between Femunden and Trysilelva.

The difference in elevation between Femunden and Vänern is 617 m, and the distance is about 400 km (Hedeskog et al. 2015). The mean annual water discharge at the outlet of Femunden is 26/36 m³/s, at the border between Norway and Sweden 84 m³/s, and at the mouth of Klarälven at Vänern 162/171 m³/s (Hedeskog et al. 2015; Mean discharge at the outlet of Femunden and Klarälven is given as different values in different chapters of that report, hence we refer to two different values. One reason for this could be that there are different calculations of mean discharge based on different years).

The river Trysilelva/Klarälven is heavily impacted by hydropower production. There are 11 power stations along the river. Of these, 9 are situated in Klarälven in Sweden, and two in

Trysilelva in Norway. The hydropower development and power stations are described in chapter 1.2.9.

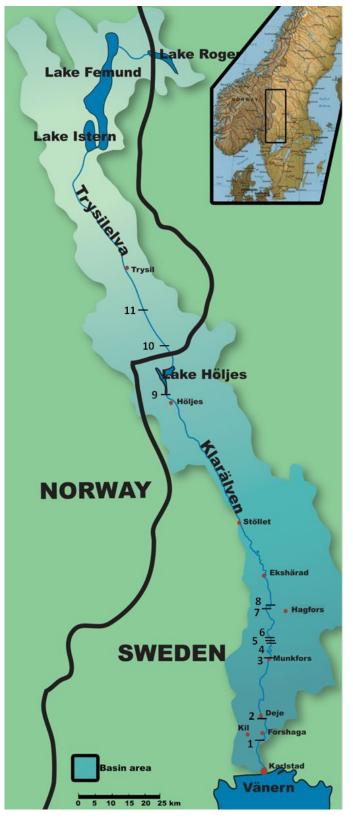


Figure 1.2.1-1.

Map of Trysilelva/Klarälven from lakes Rogen and Femunden to Vänern. The numbers 1 to 11 indicate the location of the power stations 1: Forshaga, 2: Deje, 3: Munkfors, 4: Skymnäs, 5: Forshult, 6: Krakerud, 7: Skoga, 8: Edsforsen, 9 Höljes, 10: Lutufallet and 11: Sagnfossen. Map modified after sv.wikipedia.org and previously published by Olstad et al. 2020.



Isteren, a lake close to Femunden in the upper part of the watershed in Norway. Photos: Eva B. Thorstad



Klarälven at Karlstad, close to Vänern in Sweden. Photos: Eva B. Thorstad

1.2.2 Trollhätte and Göta Canals connecting Kattegat, Vänern, Vättern, and the Baltic Sea

The Trollhätte Canal (82 km) and the Göta Canal (190.5 km) are parts of a 390 km long waterway, linking a number of lakes and rivers to provide a route from Gothenburg (Göteborg) on the west coast to Söderköping at the Baltic Sea. The waterway includes the large lakes Vänern (44 m.a.s.l.) and Vättern (89 m.a.s.l.). The highest point of the Göta Canal is Lake Viken (92 m.a.s.l.) between Vänern and Vättern. The Göta Canal was officially opened in 1832, after 22 years of construction of minor canals combining several lakes. The Göta Canal was made for goods traffic by boats, but, upon the arrival of the railways in the mid-19th century, the goods traffic ceased. Today, the canal is used for tourism. The Göta Canal has 64 locks. There are six locks in the canal from Kattegat to Vänern, while the remaining locks are located east of Vänern to the Baltic Sea.

The canal between Kattegat and Vänern is named Trollhätte Canal. The first lock from Kattegat, at Lilla Edet, was made in 1607, but not until 1800 was a boat able to travel all the way to Vänern. The locks at Trollhättan were improved in 1844 and 1916.

The Trollhätte Canal (1800) and the Göta Canal (1832) provided a route for immigration and introduction of different organisms to Vänern from the Kattegat and the Baltic Sea. It is not known whether Atlantic salmon in river Göta älv have migrated to Vänern, but other species have probably done so. Some years after the opening of the Trollhätte and Göta Canals, dams and other constructions were established in Klarälven that stopped the upstream migration of salmon and other species to areas of the water course located in Norway. Thus, there are potentially many alien species in Vänern today that have never occurred in the Norwegian part of Trysilelva/Klarälven.

1.2.3 Timber flumes connecting Klarälven and Glomma watersheds

The Glomma watershed in Norway, including the River Glomma, Norway's longest river, has no natural connection to the Klarälven watershed. However, a canal, including four timber flumes, was built in the upper parts of the watersheds, between Lakes Femunden and Feragen, which now connects the two watersheds.

Before the floatway was built, water from Femunden flowed to the east into Sweden, while the water from Feragen flowed westward into the Glomma watershed. There was no exchange of water or fish between the two watersheds. When water from Femunden started flowing into Feragen through the canal, Northern pike, *Esox lucius*, European perch, *Perca fluviatilis*, common whitefish, *Coregonus lavaretus*, grayling, *Thymallus thymallus*, burbot, *Lota lota*, and common minnow, *Phoxinus phoxinus*, spread from Femunden to Feragen and Glomma, where they had not previously been reported.



Timber flumes between Femunden and Feragen in Norway, connecting the Klarälven and Glomma watersheds. Photos: Eva B. Thorstad (upper left, lower left and right) and Odd Terje Sandlund (upper right).

The construction of the canal from Femunden via Langtjønna to Ferangen was started in 1714 and opened in 1715, because there was a need to transport timber from the forests around Femunden to the copper works in Røros. In the 19th century, the copper works shifted from using timber to coal. The forest companies continued to use the canal and timber flumes until 1973. The flumes fell into decay, but were restored in the 1990s. Since 2010, they have been part of the UNESCO World Heritage Site "Røros Mining Town and the Circumference".

1.2.4 Fish communities

There are 34 fish species in Vänern, of which about 24 are recorded in the lower Klarälven (Hedenskog et al. 2015). The species diversity in the watershed is lower in the upstream parts. There are eight fish species in Femunden: brown trout, Arctic char, grayling, common whitefish, Northern pike, European perch, burbot, and common minnow (Hedenskog et al. 2015). In total, 14 naturally occurring fish species are recorded in the Norwegian part of the watershed. In addition to those recorded in Femunden, fish species on the Norwegian side are common bleak, *Alburnus alburnus*, common roach, *Rutilus rutilus*, common dace,

Leuciscus leuciscus, Alpine bullhead, *Cottus poecilopus,* and brook lamprey, *Lampetra planeri* (Hedenskog et al. 2015). The introduced species, brook trout, *Salvelinus fontinalis,* has also been recorded in the Norwegian part of the watershed (Hesthagen and Kleiven 2013). Atlantic salmon occurs in the watershed, which is described below (chapter 1.2.5.).



Isterfossen at the outlet of Lake Isteren in Norway. Photo: Odd Terje Sandlund.



Elvedalen in Trysilelva in Norway. Photo: Odd Terje Sandlund.

1.2.5 Origin, biology, and status of the Atlantic salmon

1.1.1.1 Immigration history and origin

Freshwater bodies in Scandinavia are relatively species-poor in fish. Since the last deglaciation, and the subsequent rising of the land masses after the removal of the huge weight of ice sheets during the glacial period (isostatic rebound), topography and climate have restricted immigration opportunities. Atlantic salmon increased its distribution area and started to colonize watersheds in early postglacial times, about 11,000 years ago.

Atlantic salmon can be grouped into Western Atlantic, Eastern Atlantic, Baltic salmon, and Barents and White Seas salmon according to their origin and genetics (Nilsson et al. 2001, Bourret et al. 2013). The Atlantic salmon in Vänern seems to be of Baltic origin, which means they likely immigrated from the east (e.g., Nilsson et al. 2001, Palm et al. 2012), even though the watershed now drains to the west coast of Sweden.

In the early period of the deglaciation, a large freshwater lake called the Baltic Ice Lake evolved in the Baltic Sea basin as glaciers retreated. About 10,000 years ago, the water level of the lake rose and it connected to the sea. For a period, the water turned brackish and became the Yoldia Sea. Atlantic salmon and brown trout entered the Yoldia Sea in the Baltic area during this period, and migrated upstream in the watersheds. As the ice continued to melt, the landmass was rising. The area again turned into a large freshwater lake, called the Ancylus Lake, when the saltwater influx stopped because of the uplift of the land. The Ancylus Lake covered what is now the Baltic Sea and parts of Finland and Sweden. Atlantic salmon in Vänern became isolated from the sea during this period, when Vänern was part of the Ancylus Lake. When the landmass rose further, the Vänern was cut off from the Baltic. Hence, Atlantic salmon have been isolated in Vänern for some thousand years after the latest glaciation period (Nilsson et al. 2001).

There are also salmon in Göta älv, in the same watershed downstream of Vänern, which connects to the sea in Kattegat on the Swedish west coast. Salmon in Göta älv are likely of Atlantic origin, similar to the Norwegian anadromous salmon populations (Nilsson et al. 2001, Palm et al. 2012).

1.1.1.2 Distribution in Trysilelva/Klarälven

Atlantic salmon spawn and live as juveniles in the river habitats and perform feeding migrations to Vänern. Atlantic salmon used to be present in Klarälven, Trysilelva, and Femundselva to Femunden, including tributaries as far up as they could reach before they encountered impassable waterfalls or other natural migration barriers (Hedenskog et al. 2015). The distribution area in Norway included Grøna, Tannåa, Lutua, Sølna, Sømåa and the lakes Engeren, Lille Engeren, Sølensjøen, Isteren, Langsjøen and Hodalssjøene.

The distribution of Atlantic salmon is now restricted to the lower part of Klarälven due to impassable man-made dams at the power stations (chapter 1.2.9). Atlantic salmon have, due to these migration barriers, disappeared from the Norwegian part of the watershed.

At least four other rivers draining to Vänern have hosted Atlantic salmon populations, in addition to Trysilelva/Klarälven (Hedenskog et al. 2015). Atlantic salmon is extinct in three of these, and only the population in Gullspångälven remains, which is genetically different from the salmon in Trysilelva/Klarälven (Hedenskog et al. 2015).

1.1.1.3 Biological characteristics

Atlantic salmon in Trysilelva/Klarälven are relatively large in size. The average body weight of adult salmon captured at Forshaga during the 2000s was 3.8 kg, and individuals up to 8 kg were regularly captured (Hedenskog et al. 2015). This is similar to the average body weight from reported catches during early 1900s, which was 3.5-4.0 kg. The average body weight decreased during the 1900s, to about 2 kg during the 1970s, but has since increased again (Hedenskog et al. 2015).

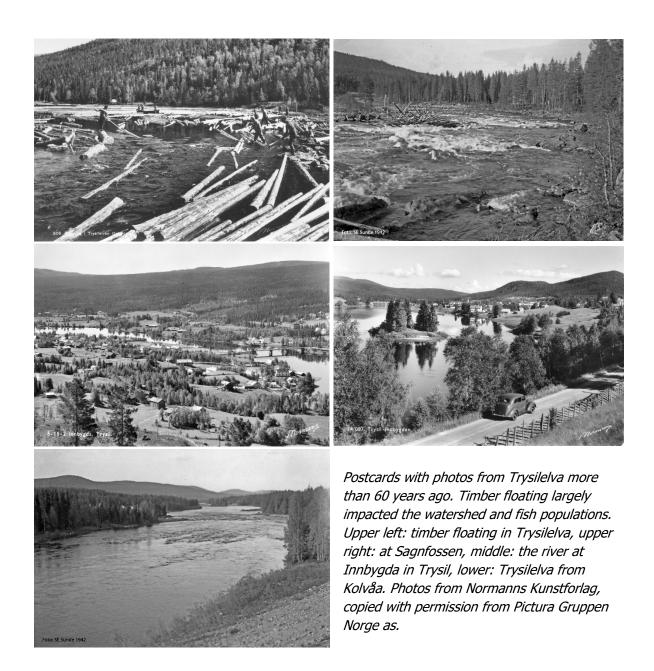
The main upstream migration period of adult salmon from Vänern to Klarälven is in July and August (Hedenskog et al. 2015). The spawning period is in October-November. After spawning, adult salmon migrate downstream from November and onwards, and some individuals remain in the river during the entire winter and migrate downstream in the spring. Atlantic salmon can survive spawning and spawn several times during their lifetime, but, considering the large mortality at the power stations during downstream migration, it is unlikely that individuals survive for another spawning in Trysilelva/Klarälven watershed (Hedenskog et al. 2015).

Salmon eggs hatch in the spring, and the juveniles live in the river for 2-3 years before they migrate downstream to Vänern. Most of the juveniles stay in the river for 3 years, but a larger proportion seem to migrate downstream as 2-year-olds than was reported in 1940 (Hedenskog et al. 2015). In a study from 1940, the juveniles had an average length of 184 mm, compared with 150-160 mm in recent years during downstream migration to Vänern (Hedenskog et al. 2015). In Vänern, Atlantic salmon largely feed on other fish, such as three-spined stickleback, *Gasterosterus aculeatus*, vendace, *Coregonus albula*, and European smelt, *Osmerus eperlanus*. In the past, the majority of the salmon used to stay in Vänern for three years before they returned to the river, while a small proportion stayed for two or four years, or even five years (Hedenskog et al. 2015). In recent years, the salmon tend to stay for a shorter period in Vänern, and the majority return to the river for spawning as 2-year-olds, and a few even as 1-year-olds (Hedenskog et al. 2015). It is not known if the differences in life history characteristics of Atlantic salmon between recent studies and studies in the past are mainly due to changes over time, or to a difference between wild and hatchery-produced fish (Hedenskog et al. 2015).

1.1.1.4 Population status

The number of salmon in Trysilelva/Klarälven declined hugely during the last part of the 1800s and the first part of the 1900s (Hedenskog et al. 2015). The size of the present spawning population of Atlantic salmon in the watershed is likely below 5% of the original population size (Hedenskog et al. 2015, Olstad et al. 2015). The decline was due to overexploitation, particularly by net fishing in Vänern during the last part of 1800s, building of dams and installation of power stations, timber floating, and industrial activities (Hedenskog et al. 2015). When the Höljes power station was built in the 1960s, this ended the migration of Atlantic salmon and trout to upstream areas, including Trysilelva and all stretches that used to be accessible to migrating salmonids in the Norwegian part of the watershed (Hedenskog et al. 2015, see chapter 1.2.9 for description of the hydropower development).

The areas available to Atlantic salmon spawning and juveniles are highly reduced in Trysilelva/Klarälven, mainly due to habitat degradation related to power production, but also to timber floating (Hedenskog et al. 2015). The total area classified as good spawning and juvenile area in Klarälven is estimated at about 77 ha, whereas a similar estimate between Sagnforsen and Femunden in Norway is about 560 ha (Hedenskog et al. 2015). This means that 88% of the good area for salmon production is on the Norwegian side. If the areas classified as medium are included, the total area on the Norwegian side is 750 ha and on the Swedish side 300 ha (Hedenskog et al. 2015). The spawning target, which defines the number of female salmon spawners needed to utilise the reproductive potential of the river, is provisionally estimated at 5900 females; 4550 females on the Norwegian side and 1350 females on the Swedish side (Museth et al. 2015).



1.1.1.5 Fishing

Atlantic salmon are exploited through recreational angling in Klarälven, recreational fishing from boats in Vänern (mainly fishing by trolling), and commercial fishing in Vänern. Hedenskog et al. (2015) estimated a mean annual catch of Atlantic salmon of 65 tonnes in total in Vänern at present. Fishers can only keep hatchery-reared salmon (recognised by the adipose fin having been removed), and when wild salmon are caught, they should be released (Hedenskog et al. 2015).

Angling in Klarälven mainly occurs on a 2 km stretch below Forshaga, and between Deje and Forshaga power stations. According to Hedenskog et al. (2015), Atlantic salmon are also fished on the river stretches between Vingängsjön and Höljes, between Edsforsen and Skoga and between Almar and Karlstad. During 2011-2013, on average 305 salmon and trout were

fished on the stretch below Forshaga each year (Hedenskog et al. 2015). Wild salmon caught during angling should be released. For the other river stretches, catch statistics are not available (Hedenskog et al. 2015).

We are not aware of any assessments of the injury and mortality rates of wild salmon that are caught and released in the fisheries in Vänern and Klarälven. Also, we have not found any information on potential misclassification of wild and hatchery-reared salmon in the fisheries, or if any illegal fishing is suspected. Hence, an overview of catch and mortality rates of wild salmon in the fisheries in Vänern and Klarälven seems to be lacking. Typical mortality after catch-and-release during angling of Atlantic salmon in other rivers is 7% if the salmon are handled carefully by anglers (Lennox et al. 2017), but increases with water temperature to, typically, 16% mortality at 18-20 °C (van Leeuwen et al. 2020). These studies are partly based on data from rivers where the most injured salmon were not released for welfare reasons because they were regarded as too injured to survive anyway, which were up to 10% of the angled salmon (Havn et al. 2015). Hence, a catch-and-release only fishery may, in total, imply a loss of 10-20% of the angled salmon, or more if the salmon are not handled carefully by the anglers. **Red-listed species**

1.2.6.1 Freshwater pearl mussel (Margaritifera margaritifera)

The freshwater pearl mussel (red list status in Norway: vulnerable VU) occurs in many localities in the Trysilelva/Klarälven main river and tributaries. The occurrence in the Norwegian part (Trysilelva) is sparce (Sandås and Enerud, 2018). The freshwater pearl mussel has a parasitic stage (glochidium) in the gills of salmonids where they develop for several months before release and establishment in the river bottom. Most freshwater pearl mussel populations are host specific and use either Atlantic salmon or brown trout in the lifecycle, and the two mussel types can be identified genetically (Karlsson et al. 2014). Genetic analysis of three mussels in Trysileva found that they use brown trout as host for the glochidium (Sten Karlsson pers. comm.).

In the period 2011-2017, increased mortality, up to 100%, in freshwater pearl mussel populations were observed in many Swedish rivers, including Värån tributary to Klarälven and two other rivers draining to Vänern (Wengströmt et al., 2019). The cause of death has not been identified, but there are no obvious infectious diseases or parasites (Wengströmt et al. 2019).

1.2.6.2 Noble crayfish (Astacus astacus)

The noble crayfish is the only indigenous species of freshwater crayfish in Norway and Sweden (Souty-Grosset et al. 2006). There are about 470 populations of noble crayfish in Norway (Johnsen and Vrålstad 2017), most of which are located in south-eastern Norway (Figure 1.2.6-1). Noble crayfish is not present in Trysilelva. However, the timber flumes between Femunden and Feragen ensure a connection to crayfish populations in the Glomma watershed, suggesting that pathogens and species that are released in Trysilelva may potentially influence noble crayfish in Glomma. Due to significant declines in the number of populations of noble crayfish during the last few decades, it is listed as a vulnerable (VU) on the International Union for Conservation of Nature (IUCN) Red List (Edsman et al. 2010) and endangered (EN) on the Norwegian red list (www.artsdatabanken.no).

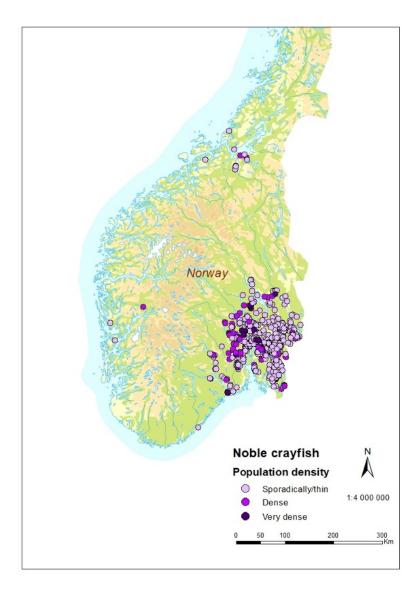


Figure 1.2-1: Distribution of noble crayfish in Norway. Produced by Stein I. Johnsen and reprinted from VKM et al. 2021 with permission of the authors.

1.2.6.3 European eel (Anguilla anguilla)

The European eel is classified as a critically endangered (CR) in the IUCN Red List¹. European eel has been recorded in four lakes in the catchment of Trysilelva (Foldvik et al.

¹ <u>http://www.iucnredlist.org/details/60344/0</u>

2019). Hedenskog et al. (2015) also refer to catches in the Norwegian part of the watershed during the early 1900s. European eels did not occur naturally in Klarälven and upstream areas, but access to Vänern and Klarälven was achieved through the building of locks at Trollhättan in Göta älv, and eels were regularly captured in Klarälven from around 1820 (Hedenskog et al. 2015 and references therein).

There have been introductions of European eel in Vänern since about 1900. These were eels that had entered the watershed naturally, but were captured in Göta älv at Trollhättan for assisted transport past dams and hydropower stations. Later, there have been introductions of European eel—from either the lower part of the watershed or imported from France and the United Kingdom—to several parts of the watershed in Sweden (H. Wickström, SLU, pers. comm. referred to in Foldvik et al. 2019). Imported eels are quarantined and examined for infectious pancreatic necrosis prior to release.

1.2.7 Alien and invasive species

Ecological interactions of sympatric species in their native range are often difficult to define and measure (Harrison & Cornell 2008; Ricklefs 2008), suggesting that it is even more difficult to predict ecological impacts of alien (non-native or introduced) species (Jeschke et al. 2014). We still know that many intentional or unintentional introductions have had profound effects on ecosystems (Ehrenfeld 2010). Alien species that have become naturalized and negatively alters their new environments are considered as invasive (Davis and Thompson 2000). Invasive species are increasingly recognized as one of the greatest threats to biodiversity (Clavero and García-Berthou 2005, Early et al 2016). Climate and landuse changes will likely cause drastic range shifts in species distribution in the future and can dramatically influence the future of biodiversity (Bellard et al 2013).

Many alien species have become established in Vänern. Intentional introductions include several fish species (Degerman et al. 2001), the signal crayfish (*Pacifastacus leniusculus*) from North America, and ornamental plants. Unintentional introductions include the zebra mussel (*Dreissena polymorpha*) and Chinese mitten crab (*Eriocheir sinensis*), which were introduced with ballast water (Josefsson & Andersson 2001). In addition, the distribution of small organisms and pathogens that are difficult to identify, such as microorganisms (microalgae, bacteria, fungi, etc.) and viruses, are not known (Josefsson and Andersson, 2001). In particular, disease-causing organisms and the hosts that can be infected are not sufficiently known, meaning there is a risk that any alien species may be infected with a pathogen that is new to the area. Here, we consider only known pathogens and species.

1.2.7.1 Signal crayfish (Pacifastacus leniusculus)

There are two species of crayfish in Swedish waters, the noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*). The signal crayfish is a North American species that was introduced to Europe in the 1960s to supplement the North European *A. astacus* fisheries, which were being damaged by crayfish plague (caused by the oomycte *Aphanomyces*)

astaci). The signal crayfish is now considered an invasive species across Europe. There are about 5000 sites with signal crayfish in Sweden (Bohman 2021). In Norway, it has been found in about ten sites from 2006 to 2021 (Velle et al. 2021), including a finding in the river Glomma in 2020 of specimens infected with *A. astaci*².

There are four major concerns associated with the introduction of signal crayfish to Norway. First, signal crayfish are chronic and healthy carriers of *A. astaci*, which can cause crayfish plague (see chapter 1.6.5.7). Second, signal crayfish may significantly influence the population of noble crayfish due to superior competitive abilities and reproductive interference (Westman et al. 2002). Third, predation by signal crayfish may have negative consequences to local populations of diverse native taxa, such as leeches (Olsen et al. 1991, Stenroth and Nystrøm 2003), salamanders (*Lissitriton vulgaris* and *Triturus cristatus*) (Nyström et al. 1997), the freshwater pearl mussel (*M. margaritafera*) (Sousa et al. 2019), macrophytes (Nyström 1999), benthic fish (Guan and Wils 1997), Atlantic salmon (Griffiths et al. 2007), and brown trout (Peay et al. 2009). Fourth, signal crayfish dig burrows, which can reach high densities (14 per m²) and cause collapse of river banks (Guan 1994, Sibley 2000).

Signal crayfish are spread by intentional and illegal introductions as a food source. They can also be spread by accidental release through fish movements and transport on equipment, such as fishing nets and boats. Once at a new site, signal crayfish can migrate up and down rivers, and over land. Their colonization rate is relatively slow (about 1 km per year) (Stanton, 2004).

1.2.7.2 Chinese mitten crab (Eriocheir sinensis)

The Chinese mitten crab is an important commercial species in Asia that has become invasive in North America and Europe (Veilleux and De Lafontaine 2007). It has a high potential for spreading and is considered one of the worst invasive species worldwide (Global Invasive Species Database 2020), being responsible for dramatic ecological and economic consequences. Of major concern, the Chinese mitten crab can be carriers of *A. astaci* (Svoboda et al. 2014).

The Chinese mitten crab has been found sporadically in Norway (Norling and Jelmert 2010, Wergeland Krog et al. 2009), for example in the Glomma estuary, suggesting development to the adult life stage in the river Glomma (Johnsen et al. 2009). At present, it is uncertain whether it can complete its life cycle in Norway. In Sweden, the Chinese mitten crab has been regularly observed along the western and eastern coasts since the 1930s (Drotzal 2010). It is now widely present in Vänern (Drotz et al. 2012).

Human-mediated transport has an important role in the spread of the Chinese mitten crab (Herborg et al. 2007). Freight ship ballast tanks can act as invasion vectors, distributing the

² https://www.vetinst.no/nyheter/krepsepestsmitte-pavist-hos-signalkreps-funnet-i-glomma

crab to new freshwater areas (Drotz et al. 2012), suggesting that re-location of water, e.g., with salmon eggs or fry, can potentially facilitate its spread to the Norwegian side of the border. However, the adult usually migrates to estuarine habitats to spawn and tolerance of the larvae toward very low salinities is weak (Anger, 1991). Thus, adaptation to freshwater environments is restricted to benthic juvenile and adult life-cycle stages.

1.2.7.3 Round goby (Neogobius melanostomus)

The round goby (*Neogobius melanostomus*) is a euryhaline bottom-dwelling goby of the family Gobiidae, native to central Eurasia including the Black Sea and the Caspian Sea. It is considered an invasive species, with significant ecological and economic impact (Corkum et al. 2004). Round gobies have established large populations in the Baltic Sea, after an introduction to the Gulf of Gdańsk in the southern Baltic Sea in 1990. Later, the distribution of the round goby expanded along the Swedish west coast of Kattegat, and is now commonly found in the river mouth of Göta älv. To date, it has not been found in Vänern. The primary diet of round gobies includes mollusks, crustaceans, worms, fish eggs (including salmonid eggs), zebra mussels, small fish, and insect larvae.

In the Gulf of Gdańsk, the parasite fauna of the invasive round goby includes at least 12 species (Kvach and Skóra, 2006). The core of the parasite fauna comprises two species of trematode metacercariae: *Cryptocotyle concavum* and *Diplostomum spathaceum*. In the Baltic Sea, the round goby is also paratenic host of the invasive nematode *Anguillicoloides crassus* (Kvach, 2004).

1.2.7.4 Zebra mussel (Dreissena polymorpha)

The zebra mussel (*Dreissena polymorpha*) is a small freshwater mussel. To date, the zebra mussel is among the most aggressive invasive species in freshwaters worldwide due to its wide niche, rapid population growth, and negative impacts on the ecology, economy, and ecosystem services (Lowe et al. 2000, Mckindsey et al. 2007, Strayer 2009). It can be an effective ecosystem engineer by changing existing habitats and providing new habitats for other organisms, and can affect trophic interactions and the availability of foods for both pelagic species and other benthic species (Karatayev 2002, Strayer 2009). Among other ecological effects, zebra mussels can outcompete native freshwater pearl mussels (Ricciardi et al. 1998).

A female zebra mussel is estimated to produce 30,000 to 1,000,000 eggs in one year (Sprung 1992). The eggs develop into free-floating, microscopic larvae that can disperse by water current, wind, and wave action. Zebra mussels are presently found in lakes in south eastern Sweden, but not in Vänern or other parts of western Sweden (von Proschwitz and Wengström 2020). They require hard-water lakes with a high concentration of ions, especially magnesium (Hallstan et al. 2020). Currently, the populations of zebra mussels in eastern Sweden are separated from Vänern by a natural barrier of soft-water boreal lakes.

The pH of the water sheds around Trysilelva is above 7, but can drop below 7 during snow melt (Hindar and Sckanke 2013).

1.2.7.5 Canadian pondweed (Elodea canadensis)

Canadian pondweed (*Elodea canadensis*) is a submerged macrophyte native to North America. It was introduced to Ireland in the mid-1880s and has now spread throughout Europe (Josefsson 2011). Its spread is facilitated by a wide ecological tolerance and asexual reproduction, including vigorous re-growth of small fragments (Redekop et al. 2016). Although seed formation is rare, dispersal of overwintering dormant apices and stem fragments by water and by waterfowl can result in rapid spread (Spicer and Catling 1988). Small fragments can also spread by human activities, such as boating and angling (Owens et al. 2001, Anderson et al. 2014). *E. canadensis* can form dense mats that interfere with human uses of waters, such as infrastructure, fishing, boating, and swimming. In addition to this, dense stands reduce water movement, cut off light, produce anoxic conditions, and trap sediments in the system (Simpson 1984; Barrat-Segretain, 2005). Dense stands deteriorate the water quality and habitats, which can affect the entire ecosystem (Josefsson and Andersson 2001), including the composition of native plant communities (Mjelde et al. 2012) and causing a decline in crayfish populations (Hessen et al. 2004).

Canadian pondweed was introduced to Norway in the 1920s. In 2013, it was present in 101 sites, and the number of sites where it occurs increases annually (Anglès d'Auriac et al. 2019). According to the Norwegian biodiversity information centre, it is associated with a very high risk for environmental impact in Norway (Elven et al. 2018). In Sweden, it is widely distributed, including in the lake Vänern area (Palmgren 2005).

1.2.8 Aquaculture in Väneren

Two aquaculture licenses have been issued for rainbow-trout farming in cages in Vänern. Fish are held in open net-pen farms from spring to autumn, as weather and ice conditions hamper farming during the winter months. Rainbow trout from Vänern are delivered to putand-take ponds and to processing plants for consumption. We do not know the origin of the rainbow trout used in the fish farms, but the Swedish production of rainbow trout is not selfsufficient with eggs and fry, and therefore rely on imports.

Escapes from fish farms have been recorded, but there are no self-reproducing rainbow trout stocks in Vänern. All registered fish farms in Sweden participate in the official health control programme, regulated in accordance with SJVFS2014:4 and by Council Directive 2006/88/EG. According to the Swedish Veterinary Institute, none of the diseases listed in Sweden or EU have been detected in connection with the registered disease outbreaks in fish farms in Vänern (listing of diseases is described in Chapter 1.5). The aquaculture activity in Vänern, with annual inputs of new fish, represent an important route of entry for infectious agents.

1.2.9 Hydropower development

1.2.9.1 Power stations in Trysilelva/Klarälven

Eleven power stations were built along Trysilelva/Klarälven from early 1900s to the 1960s, utilising 32% (194 m) of the total drop in elevation of the river. The first power stations in Klarälven were built at Deje, Munkfors, Forshult, Forshaga, and Krakerud. These were later followed by power stations at Skymnäs, Skoga, and Edsforsen. The last power station that was built was at Höljes, which was operational from 1962. On the Norwegian side, in Trysilelva, two power stations were built. These were Sagnfossen (1946) and Lutufallet (1964) power stations.

Forshaga and Deje are the two power stations located furthest downstream, situated 24 km and 37 km upstream of Vänern, respectively (Hedenskog et al. 2015). Munkfors is the next power station, 75 km from Vänern. Then there are five power stations (Skymnäs, Forshult, Krakerud, Skoga, and Edsforsen) in the same area, along a 17.5 km long river stretch, 94-111 km from Vänern. Höljes is situated much further upstream, close to the Norwegian border, i.e., 140 km from Edsforsen power station, and 251 km from Vänern. The two power stations on the Norwegian side are situated 15 and 30 km from Höljes, and 266 and 281 km from Vänern (Hedenskog et al. 2015).

Höljes is by far the biggest power station in Trysilelva/Klarälven, and the only one with a reservoir upstream of the dam, where water is stored and the water level can be regulated according to the need for water to power production (Hedenskog et al. 2015). All the other power stations are run-of-the-river power stations without reservoirs that store water, and they can only use the river flow rate of water to generate power (Hedenskog et al. 2015). However, the run-of-the-river power stations also have dams built across the river, connected to their water intakes. Although the area above is not a reservoir, the dam creates an area of slow-flowing water above each dam (Hedenskog et al. 2015).



Höljes power station in Sweden including the dam, the old Francis turbine, and the reservoir upstream of the dam. Photos: Eva B. Thorstad.

1.2.9.2 Impacts of power stations on Atlantic salmon and other fish

Power stations greatly impact fish populations and migrations, and Atlantic salmon became extinct in Trysilelva because the dams block upstream fish migration. In addition, the power stations cause high mortalities among downstream-migrating fish, hatched or released upstream of them (Hedenskog et al. 2015).

Atlantic salmon migrate upstream from feeding areas, in this case from Vänern, to the spawning areas in the river. The power station dams block this migration, and, at present, there are no fishways to facilitate passage of the dams by upstream migrating fish, except at the two uppermost power station in Trysilelva in Norway (Hedenskog et al. 2015). Hence, fish migrating upstream from Vänern are blocked at the lowermost dam at Forshaga (Hedenskog et al. 2015). If salmon are transported past the lowermost power stations and released above Edsforsen, they can move freely 140 km to Höljes. If they are transported and released above Höljes, they can access all the stretches that are naturally available to migrating Atlantic salmon in the Norwegian part of the watershed (Hedenskog et al. 2015).

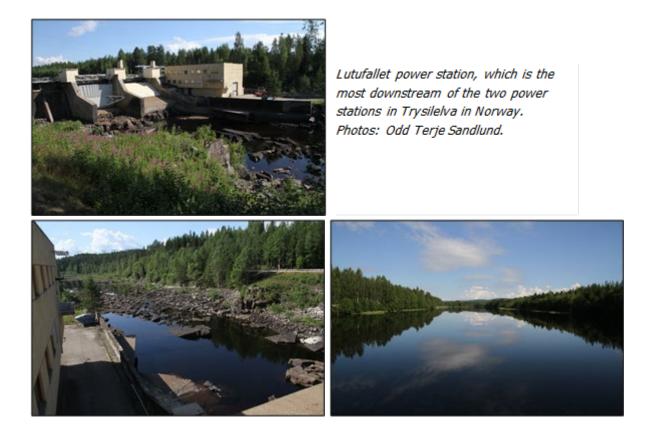
Atlantic salmon migrate downstream as juveniles (usually termed smolts at this life stage), and as adults (termed kelts) if surviving spawning. A large proportion of downstream migrating fish may become injured and die when passing power stations, particularly when there are no alternative routes other than following the water through the turbines (Ruggles 1992; Larinier 2008; Calles & Greenberg 2009; Kärgenberg et al. 2020). Fish can be cut by the moving parts of the turbines, or they may be injured and die from other physical forces through the turbines (Montén 1985; Ruggles 1992; Larinier 2008). If there are alternative

routes, such as when water is released over dams, passage via these routes can also lead to mortality, for instance if the fish falls down onto something hard, or because of increased predation due to migration delays if the fish hesitate (Ruggles 1992; Larinier & Travade 2002; Larinier 2008). Even where purpose-built bypass routes are built, there may be elevated mortality related to the use of these routes, for instance due to fish being stuck in bar racks supposed to guide the fish, or due to increased predation (Havn et al. 2018; 2020).

The mortality of downstream-migrating Atlantic salmon in Klarälven is high. Mortalities among downstream-migrating juveniles (smolts) and adults (kelts) were assessed by tagging individual fish with acoustic transmitters, which enabled their movements to be followed. In a dry year, when most of the water, and hence the fish, were passing the power stations through the turbines, 84% of the juveniles died between where they were released upstream of Edsforsen and Vänern (passage of eight power stations) (Norrgård et al. 2009). In a wetter year, when there was some spill of water past the power stations, in addition to the water feeding the turbines, the mortality of juveniles on the same stretch was 71% (Bergman & Norrgård 2015). Although the mortality was low on the river stretches between the power stations, there was elevated mortality when passing the power stations. All the adults that migrated downstream after spawning died (Nyqvist et al. 2016). A higher mortality rate is expected for larger fish than for small fish, because they have a greater probability of being hit by the moving parts of the turbine, and usually suffer a higher mortality through power station turbines (Montén 1985; Ruggles 1992). None of the power stations in Trysilelva/Klarälven have purpose-built fish passages for downstream-migrating fish. None of the power stations have bar racks in front of the water intakes to prevent the fish from entering the turbines. And none of the power stations have procedures for directing the water through alternative routes at the power stations and dams during periods of more intensive downstream fish migration to attempt to reduce the mortality.

On river stretches above the dams, where the water velocity is slowed down, or in reservoirs, such as above the Höljes power station, the altered habitat is less suitable for Atlantic salmon than the original river stretches. This is because Atlantic salmon are adapted to rivers with a fast current. These slow-flowing areas instead favour other fish species, such as northern pike, *Esox lucius*. Slower migration makes juvenile salmon more vulnerable to predators, thereby increasing their mortality (Jepsen et al. 1998, Havn et al. 2018, 2020).

We focus on Atlantic salmon here, but take the opportunity to note that power stations have similar impacts also on other migrating fish, such as brown trout and grayling.



1.2.9.3 Mitigating the loss of salmon – transport of adult Atlantic salmon past the power stations

Blocking fish migration with hydropower dams, such as those built in Klarälven, will quickly result in the loss of Atlantic salmon upstream of the dams if no fish can pass. Fish passes were initially built in some of the power stations to facilitate upstream fish migration and prevent the loss of Atlantic salmon. However, they did not function very well as few salmon managed to pass (Hedenskog et al. 2015). Since the early 1930s, adult Atlantic salmon have therefore been captured during their spawning migration below the lowermost power stations, transported by truck, and released further upstream to help them to pass the power stations and reach the upstream spawning areas (Hedenskog et al. 2015). The first transport was performed in 1931, with capture of adult salmon downstream of the Deje power station and then release upstream of Edsforsen power station. From here, the salmon could access large parts of the watershed by swimming, including the areas accessible to salmon in the Norwegian part of the watershed (Hedenskog et al. 2015). After the Höljes power station was built, totally blocking upstream migration at that site also, 80% of salmon transported upstream were released upstream of Höljes, according to an agreement made between Norway and Sweden in 1969 (Hedenskog et al. 2015).

In 1993, the Norwegian government stopped releasing adult salmon upstream of Höljes due to the large mortality of downstream-migrating fish at the power stations (Hedenskog et al. 2015). Releases had already been stopped from late summer 1988 to 1992 due to outbreaks

of bacterial kidney disease (BKD) in the Klarälven watershed (Hedenskog et al. 2015). Hence, the last release of adult Atlantic salmon upstream of Höljes, which could reach the Norwegian part of the watershed, occurred in 1988. Since 1988, transported fish have been released upstream of Edsforsen power station in Sweden, and have not been able to reach the Norwegian parts (Hedenskog et al. 2015). Some egg planting on Norwegian river stretches was performed in 2012 and 2013, and in 2012 there was release of juveniles as part of an experiment (Sundet & Dokk 2015).

Today, none of the Swedish power stations have operating fish passes, and upstream migrating salmon from Vänern to Klarälven are captured in a trap at Forshaga, which is the lowermost power station (Hedenskog et al. 2015). The salmon are transported by truck and either released on the river stretches between Deje and Höljes, released immediately above the dam at Forshaga, or taken to the hatchery to be used in the production of juveniles (Hedenskog et al. 2015, Olstad et al. 2020). At Forshaga, the Atlantic salmon seem to have problems finding and entering the trap, which is built in the old fish passage, and salmon may remain on the river stretches below for several weeks, particularly in years with a high water discharge (Hagelin et al. 2020). There might be some natural reproduction of Atlantic salmon on the river stretches between Vänern and Forshaga (Olstad et al. 2020).

Capture, handling, and transport of adult Atlantic salmon often affect their post-release behaviour as demonstrated by stress-related delays and/or downstream movements after release (Thorstad et al. 2008, Frechette et al. 2020). Tagging studies have shown that this is also the case for Atlantic salmon transported in River Klarälven, where 12% of the wild Atlantic salmon released upstream of Edsforsen moved back to Edsforsen (Hagelin et al. 2016a, 2016b). An even higher proportion of hatchery-reared salmon, compared to wild specimens, moved downstream again.

1.2.9.4 Mitigating the loss of salmon – hatchery-production of juveniles and genetic impacts on the Atlantic salmon

To compensate for the declining salmon population, hatchery-produced juveniles or smolts have been released in the watershed for more than 100 years (Hedenskog et al. 2015, Olstad 2020). Juveniles were released during the first years, whereas smolts have been released from the 1960s until today.

Hatchery-reared smolts from both the Klarälven and Gullspångälven populations have been released in Klarälven below Forshaga (Olstad et al. 2020). Gullspångälven is another large river draining to Vänern, and the population is genetically different from the salmon from Klarälven (Olstad et al. 2020). In some years during the 1970s, hybrids between the two populations were intentionally released in Klarälven (Olstad et al. 2020). Later, the intention has been to separate the two populations and release salmon from the Gullspång population below Forshaga only, or on the stretches between Forshaga and the next power station at Deje (Olstad et al. 2020). River stretches between Forshaga and Deje are regarded as

unsuitable for Atlantic salmon spawning and juvenile production, but releases of salmon on this stretch are performed to support a fishery (Olstad et al. 2020).

Morphology, body size, and migration timing were used to identify individuals of the Klarälven versus Gullspång populations at Forshaga before 1980 (Olstad et al. 2020). Using these methods rather than genetic or tagging methods, may have resulted in some unintentional hybridisation due to misclassification (Olstad et al. 2020). From 1980, released smolts of the Gullspång population were tagged by fin clipping (Olstad et al. 2020). From 1991, fin clipping was also required to separate hatchery-produced fish of the Klarälven population from wild fish (Olstad et al. 2020). Although this tagging system has been introduced, human mistakes are likely to happen, and it seems to have resulted in unintentional introduction of genetic material from the Gullspång population to the salmon released in Klarälven (Palm et al. 2012).

Genetic studies have shown that 80-95% of the original genetic material was left in the Klarälven population in 2009, which corresponds to an influx of genes from the Gullspångälven population of 1.3% per generation (Palm et al. 2012). The relative impact of unintentional and intentional hybridisation is not known. Olstad et al. (2020) concluded that there is a significant genetic contribution from the Gullspång population to the Klarälven population, but also that there is still a large genetic difference between the populations.

In addition to unintentional and intentional hybridisation between salmon from Klarälven and Gullspångälven, there are also other practices that have likely contributed to the present Klarälven population being genetically different from the salmon residing in the watershed before human activities greatly altered habitats and blocked fish migrations. The genetic variation is lower among salmon in Klarälven than Gullspångälven, which is likely due to too few individuals being used as parental fish in the hatchery production (Palm et al. 2012, Palm & Prestegaard 2015a). The trap at Forshaga is not functioning well, particularly under some water discharge conditions, and is closed at high water temperatures. The trap may therefore be selective in terms of capturing lower proportions of large fish and of fish migrating at certain times (Olstad 2020). According to Olstad (2020), there have likely been several genetically differentiated populations within Klarälven, but if so, the salmon originating from the lower parts of the watershed probably now dominate in the remaining population.

Until 2012, adult salmon of both wild and hatchery-reared origin that were captured at Forshaga were transported and released upstream, if they were identified as belonging to the Klarälven population (Hedenskog et al. 2015, Olstad 2020). There was a larger genetic contribution from the Gullspång population in the hatchery-reared than wild fish (Palm & Prestegaard 2015a). According to both genetic and behavioural studies, hatchery-reared salmon were less successful at reproducing than wild salmon (Palm & Prestegard 2015b, Hagelin et al. 2016). From 2012, adult Klarälven salmon of hatchery origin captured at Forshaga have therefore only been released below Forshaga, or above Forshaga to support the fishery between Forshaga and Deje (Hedenskog et al. 2015, Olstad 2020).

1.3 Reestablishment of fish populations

Transfer of eggs or fish from Sweden to Norway will not result in reestablishment of a selfsustainable salmon population in the Norwegian part of the watershed, because fish from these releases will not be able to return to the spawning areas and reproduce later in life. Thus, the methods assessed are more similar to "sea-ranching", which refers to situations where captive-bred fish are released (usually as smolts) and then captured on their return migration as adults, either in a fishery or for use as broodstock for the next captive-bred generation (O'Sullivan et al. 2020). The reestablishment of a salmon population will require extensive supplementary mitigation actions, in addition to the measures evaluated here.

Reestablishment would require the introduced fish to be able to return to the Norwegian parts for spawning, following their migration to Vänern. This could, in turn, result in an even greater risk of introducing alien organisms and infectious agents than the scenarios analysed here. This report should not be regarded a risk analysis of reestablishing a salmon population in the Norwegian part of the watershed. To clarify these points, we outline below steps that would be required in order to reestablish a salmon population.

1.3.1 What is a reestablished salmon population?

The first step towards reestablishing an Atlantic salmon population is ensuring that offspring from fish that matured to smolts in a specific area are able to return to that area as adults for spawning. Hence, reestablishment of a salmon population in Trysilelva would require that adult salmon from this area, which have been feeding in Vänern, are able to migrate back upstream, cross the border from Sweden to Norway, and spawn in those areas where they developed into smolts. Return to these river stretches would either require the installation of functioning fishways at all the power stations, and hence open waterways for fish movements and migrations, or the implementation of a system in which salmon are caught and transported past power stations by humans. If the aim is to reestablish a reproducing population in that area, then for spawners caught on downstream stretches, transported and released upstream, it will be necessary that they can be identified as having matured in to smolts on the stretches in Norway. However, a salmon population dependent on upstream transport in tanks is not self-sustainable, and therefore, arguably, should not be defined as a viable population. This would require the possibility for free migration by the fish themselves (Hedenskog et al. 2015).

The next step towards reestablishing a salmon population is to ensure that the size is sufficient for successful conservation. The North Atlantic Salmon Conservation Organisation (NASCO) has defined this as maintaining populations above their conservation limits with a certain probability (NASCO 1998). The conservation limits demarcate the population size at which recruitment would begin to decline significantly. Conservation limits are defined for Norwegian salmon rivers, as the minimum number of salmon eggs that should be spawned in each river each year. The conservation limit can be recalculated from number of eggs to number of female spawners, according to the size distribution of the females in the river. For Norwegian rivers, the conservation limits are termed "gytebestandsmål", or spawning

targets. The spawning target for the Norwegian part of Trysilelva has not been formally established, but Museth et al. (2015) suggested this to be about 4550 female spawners. According to Norwegian legislation (Nature Diversity Act), fishing is only permitted if there is a harvestable surplus of spawners exceeding the conservation limits.

An important principle in Norwegian legislation, which forms the basis for salmon management, is that populations should be managed such that they are in a good enough state to ensure both conservation and a harvestable surplus of salmon. A quality norm sanctioned by the Nature Diversity Act was adopted by the Norwegian government in 2013. The quality norm is a standard that all salmon populations should attain; which includes the population must not be genetically impacted by escaped farmed salmon or other anthropogenic activities, it must have a large enough spawning population to be maintained above the conservation limits, and it must provide a normal harvestable surplus³.

A definition has been developed to determine when a salmon population is reestablished in limed rivers, where acid rain had severely reduced or eradicated salmon populations, or after eradication of the parasite *Gyrodactylus salaris* from infested rivers (Vitenskapelig råd for lakseforvaltning 2020). According to this definition, a population is reestablished when: 1) the population is recruited from natural spawning of wild fish in the river and not from egg planting or releases of hatchery-produced fish, and 2) the naturally reproduced population is large enough to reach the conservation limit and has a harvestable surplus that is larger than 60% of a normal harvestable surplus. This definition was used to develop catch recommendations for salmon populations in sea fisheries. The background was that the Norwegian Ministry of Climate and Environment asked the Environment Agency to consider not opening for fisheries on populations that were not reestablished, and where the government had spent resources on mitigation measures to attempt to reestablish these populations.

1.3.2 The need for extensive mitigation measures to reestablish the salmon population in Trysilelva/Klarälven

At present, most of Trysilelva/Klarälven cannot be reached by Atlantic salmon from Vänern without being transported in tanks, and a large proportion of downstream migrants do not reach Vänern due to the high mortality at power stations. Hence extensive mitigation measures would be necessary if there is an intention to: 1) reduce the high mortality of downstream migrating salmon from Trysilelva, 2) reestablish a population of Atlantic salmon

³ Salmon populations that are not negatively impacted by human activities, are normally large enough to exceed their conservation limits and have a harvestable surplus. For anadromous populations, the normal harvestable surplus for non-impacted population vary among year-classes, because of a variable survival during the ocean phase. For Norwegian anadromous populations, the normal harvestable surplus has been estimated at 54-79% of the number of salmon returning from the ocean, for the years 2010-2019.

that can reach these areas without being captured and transported by humans, and 3) build up a population that is large enough to reach the conservation limits and utilise the reproductive potential of the river, and perhaps even produce a surplus that can be harvested in fisheries. The need for mitigation measures has been described and evaluated through the projects "Vänerlaxens Fria Gång" and "Två länder – én elv" (Hedenskog et al. 2015, Olstad et al. 2020). For upstream migrants, there is need to establish fishways that bypass the power stations in Klarälven, and for downstream migrations there is need to secure safe passage solutions at the power stations, which in 2015 was estimated at a total cost of 1000 MSEK.

Plans for fish passages and solutions at power stations in Klarälven and Trysilelva are in only the initial phases, and the estimates of total costs are uncertain (Hedenskog et al. 2015). When solutions are constructed and installed in the first place, extensive evaluation studies and adjustments over many years will be required in order to reach the goals of safe downstream fish migration in terms of low fish mortality and high passage success for upstream migrating fish (Greenberg et al. 2015, Kraabøl 2015). Those solutions currently used to facilitate downstream fish migration at power stations elsewhere in Europe have been developed and tested in small- and medium-sized rivers only. At power stations where the water discharge through the turbines is larger than 90-100 m³/s, existing solutions are either not installed, or if installed, they function poorly (Forseth & Museth 2020). Hence, there are no existing solutions that have been tested elsewhere and shown to be efficient at such large power stations, so development of solutions for the power stations in Klarälven will require thorough evaluation and new innovations. Furthermore, due to the large number of power stations in Klarälven, the total cumulative mortality in passing them is likely to be high, even if the individual mortality at passing each of them is low. For instance, even with a survival of 90% of the fish at each power station, less than 40% of the fish will survive the passage of nine power stations in Klarälven and reach Vänern. Fish released in the upper parts, will, in addition, have to pass two power stations in Trysilelva, and only about 30% will survive the migration to Vänern.

1.4 Three methods for transferring eggs or fish to Norway covered by this risk analysis

We have been asked to evaluate the risks related to transfer of eggs or fish from the Swedish part of the watershed to Norway. Three specific methods are evaluated according to the terms of reference. These are: I) direct import of fertilized eggs, II) following the Norwegian gene-bank model, by establishing a broodstock from which fertilized eggs either can be planted directly in the river or transferred to a local hatchery for hatching and subsequent release of live fish, and III) transfer of adult spawners (broodfish) by capturing them in Sweden and transporting and releasing them in the Norwegian part of the watershed. Each of these methods are further described below.

1.4.1 Direct import of fertilized eggs (Method I)

The first method is transfer of fertilized eggs from Sweden. The eggs can either be planted in the river as eyed eggs in the spring (April/May) or hatched in the hatchery and released into the river as juveniles or smolts. The development of eggs and juveniles before they initiate feeding in a hatchery should follow the natural development that they would otherwise have experienced in the river, and water temperatures in the hatchery should therefore approximate the temperatures in the river.

According to Olstad et al. (2020), the unpredictable water discharge and ice conditions in the Norwegian part of the watershed may mean that egg planting will not be possible to accomplish each year. Thus, if planning for egg planting, a back-up plan enabling production of juveniles in the local hatchery would be an advantage so that the risk of wasting the entire year-class of eggs is minimized.

Juveniles can be released before or after they initiate feeding. Due to unpredictable climate conditions and a short time-window for releasing juveniles before they have initiated feeding, it is necessary to be prepared for initiating feeding of juveniles in the hatchery, which has a greater demand for infrastructure (Olstad et al. 2020).

If the aim is to produce and release smolts, the juveniles must be kept and fed in the hatchery for one or two more years before being released. It should be noted that producing smolts in a hatchery and releasing them on river stretches in Norway will not contribute to full utilisation of areas or feeding opportunities on Norwegian stretches, as these are fish that will start their downstream migration soon after release. Considering the large mortality-risk that fish moving downstream face when passing the power stations, this does not appear to be an appropriate strategy unless for specific investigation of potential future mitigation measures.

1.4.1.1 Procedures in place to prevent spread of infectious agents

According to Commission Regulation (EC) No 1251/2008, imported eggs must be accompanied by a health certificate (Annex II Part A) issued by an official inspector that certifies that:

- The eggs have been inspected within the 24 hours prior loading and showed no signs of clinical disease.
- The eggs come from an aquaculture facility where, according to the records of the facility, there are no indications of disease problems.
- The eggs have been disinfected with a method that has been proven effective against *G. salaris.*

In Norway, the egg disinfection method of choice is a 100 mg/l iodophor solution, with a contact time of at least 10 minutes, and at pH between 6.0 and 8.0.

As disinfection of eggs does not eliminate infectious agents capable of exhibiting true vertical transmission, target tissue from broodfish can be tested for vertically transmissible agents with subsequent rejection of gametes of test-positive fish.

In Sweden, testing of female anadromous salmon for *Renibacterium salmoninarum* is required according to Appendix 3 of SJVFS 2014:4 (Statens jordbruksverks föreskrifter om djurhälsokrav för djur och produkter från vattenbruk). In addition, organs from up to 10 salmon are pooled and tested for virus in cell cultures. The same legal act does not describe any requirements for testing non-anadromous salmon, but, in practice, testing of female broodfish of Vänersalmon for *R. salmoninarum* and virus (cell cultures) are conducted routinely (Garseth et al. 2020, Charlotte Axen, SVA personal communication).

In Norway, the Aquaculture Operation Regulation (Forskrift om drift av akvakulturanlegg) requires that relevant examinations are carried out depending on the health status of the area where the wild fish are caught. Wild-caught anadromous fish that are stripped must at least be tested for BKD (*R. salmoninarum*). Testing of non-anadromous salmonid broodfish for *R. salmoninarum* is not required.

For this risk assessment, it is assumed that eggs are disinfected as described above, and that kidney tissue from female broodfish are tested for *R. salmoninarum* by a validated method with high sensitivity. We assume that a potential holding period in a hatchery does not influence the risk.

1.4.2 The Norwegian gene-bank model (Method II)

The second method follows the Norwegian gene-bank model and involves establishment of a Klarälven salmon broodstock in Norway. This would be achieved by importing a limited number of fertilized eggs (300) from each of about 10 selected salmon families yearly, for a limited number of years. The establishment phase (i.e., the period when fertilized eggs are collected), will at least last the full generation length, which is approximately 5-7 years. Here we envisage a collection period of 10 years. In accordance with the gene-bank model, a biorepository for cryopreserved milt would be established simultaneously. The gene bank has a pedigree-based structure, in which all founder fish (F0) go through genetic testing to avoid inbreeding and secure a broadest possible genetic basis. All offspring (F1) are individually tagged, and have a pedigree, which means their ancestry is recorded. Approximately 4-5 years after the first eggs are imported, the broodstock will be used to start production of eggs and milt, hence making it possible to transfer fertilized eggs and plant them in the river, or, alternatively, transfer eggs to a local hatchery for hatching, with subsequent release of live fish into the river.

The established broodstock must be supplemented with new genetic material (eggs from wild fish) during the nearly 80-year period covered by this risk assessment. Nevertheless, application of the gene-bank model will significantly reduce the total number and duration of imports, the number of eggs per import, and, therefore, the total number of eggs translocated to Norway, compared with the direct import of eggs (Method I).

The Directorate for Nature Management (now the Norwegian Environment Agency) established the Norwegian gene-bank programme for wild Atlantic salmon in 1986. The model consists of a gene bank for cryo-preserved milt (long-term measure) and five gene-bank stations (hereafter called gene banks) with live fish. Four of the gene banks are safeguarding a genetically representative sample of live fish, pending the elimination of the parasite *G. salaris* from infested watercourses (short-term measure). The fifth safeguards salmon and sea trout from the Hardanger region, where escaped farmed salmon and salmon lice are identified as the largest threats.

Introduction of infectious agents into the live gene bank or the sperm bank, have several negative implications in the gene bank programme. Introduction of infectious agents may lead to disease outbreaks and mortality, with subsequent loss of valuable salmon populations, loss of genetic diversity within salmon populations, and even disease-induced genetic selection. It is important that the gene bank is kept free from vertically transmitted diseases such that the gene bank programme does not contribute to spread of vertically transmissible infectious agents during reestablishment of salmon stocks. Thus, the gene bank concept has implemented a strict biosecurity strategy to avoid introduction, propagation, and spread of infectious agents. The most important routes of entry for infectious agents are the entry of fish, which, in the gene banks are therefore land-based facilities where salmon are held in freshwater throughout their lifespan. Introduction of fish is assessed to represent the highest likelihood of introducing infectious agents. To reduce this likelihood, only disinfected eggs from founder fish that are subject to a thorough health control and testing for specific infectious agents are introduced to the gene banks.

Wild-caught founder fish (F0) are kept in tanks until stripping. After stripping, they are killed and undergo a health control by an authorized fish-health professional. The health control consists of necropsy, sampling of target tissue, and analyses for pathogens that are transmitted vertically (from parent fish to offspring via gametes).

Today, the gene bank activity only comprises anadromous salmonids. Accordingly, the infectious agents included in the brood fish test programme are those that are most relevant for anadromous salmonids. Today, all broodfish (male and female) are tested for *R. salmoninarum* and infectious pancreatic necrosis virus (IPNV), and detection of these infectious agents results in rejection of gametes. In addition, all salmon are tested for piscine orthoreovirus-1 (PRV-1), and, from 2016 to 2020, also for piscine myocarditis virus (PMCV). The fate of gametes from PRV-1 and PRV-3-positive fish is subject to an assessment of the risk of transmission and the genetic impact of rejecting the positive fish. In parallel with gene bank operations, research is carried out to assess which pathogens are relevant for wild salmonids, and whether these are vertically transmissible. If a gene bank for non-anadromous salmon from Klarälven is established, the test programme and the parallel research on fish diseases would need to be adjusted to accommodate the risks in freshwater salmonids.

The gene-bank model enables a higher standard of health control of both founder fish and broodstock than in traditional hatchery-practices, including the possibility to perform lethal sampling of target tissues optimal for analyses for selected vertically transmissible

pathogens. Finally, the holding period of fish in a live gene bank facilitates health monitoring throughout the lifespan of the broodfish. Important prerequisites for realisation of the objectives in the gene-bank model are thorough health control of the founder fish and a safe water source.

1.4.2.1 Procedures in place to prevent spread of infectious agents

Import of eyed eggs from Sweden to a Norwegian gene bank in a *Gyrodactylus*-free zone is subject to the same requirements concerning the health certificate and disinfection as described in chapter 1.4.1.1.

In this risk assessment, we have assumed that the gene bank is located in Norway and is dedicated to non-anadromous salmonids only. Furthermore, we assume that the same procedures and practices as in other Norwegian gene banks are used.

In this risk assessment, we therefore assume that imported eggs are disinfected according to the procedure described in chapter 1.4.1.1., and that all broodfish (male and female) are subject to a thorough postmortem examination, sampling of suitable target tissue (head kidney) and tested for IPNV, *R. salmoninarum* and PRV-1. Upon detection of any of these agents, gametes would be rejected. We further assume that reestablishment is based on planting of eggs from the gene bank in Trysilelva directly, or that eggs from the gene bank are moved to a local hatchery, where they are hatched and subsequently released as fry or smolts. We assume that a potential holding period in a hatchery does not influence the risk.

1.4.3 Transfer of broodfish (Method III)

The third method is the transfer of adult spawners (broodfish) from Sweden to Norway. This involves catching adult salmon in the lower parts of Klarälven during their upstream migration, transporting them in tanks, and releasing them in the Norwegian part of the watershed so that they can spawn there.

1.4.3.1 Procedures in place to prevent spread of infectious agents and hitchhiking organisms

According to the Commission Regulation (EC) No 1251/2008, all imports of fish must be accompanied by an animal health certificate (Part A of Annex II) issued by an official inspector. The official inspector certifies that the salmon have been examined within the last 24 hours before loading and that they showed no clinical signs of disease.

Both Norway and Sweden have free status for IHN and VHS, hence no specific requirements are applicable concerning these pathogens. Due to the fish health status in the Norwegian marine-based aquaculture, Norway's fish health status is considered worse than Sweden's status for the diseases ISA, SAV, IPN, and BKD. Accordingly, none of the requirements concerning these diseases are applicable when importing broodfish.

Trysilelva is a disease-free zone for *G. salaris*, and Norway has an approved eradication programme in place. Accordingly, in addition to examining and certifying the absence of clinical disease, the official inspector must certify that, immediately before transfer, the imported fish have been held continuously in a tank with at least 25 parts per thousand (ppt) salinity for 14 days and that, during this period, new fish have not been added to the tank.

All aquaculture animals of species susceptible to the above-mentioned diseases that are imported to the export area must meet the requirements laid down in point II.6 of the health certificate. The inspector must certify that the transport container has been cleaned and disinfected before loading of fish, and that during transport, the fish are held in conditions that do not compromise fish health.

In this risk assessment, we assume that broodfish are captured in the trap at Forshaga and subsequently transported to a holding place near the Höljes dam. We further assume that all broodfish are held continuously in a tank with at least 25 ppt salinity for 14 days and that, during this period, new fish are not added to the tank. We also assume that the broodfish are held in water from the river upstream of the Höljes dam or in groundwater, such that reinfection is avoided.

Over an 80-year period, which is the time span covered by this risk analysis, there is a possibility that human errors will be made in the implementation of the salinity treatment. Further, documentation that this method is 100% efficient in killing all *G. salaris* individuals seems to be lacking.

Reference is often made to the use of a similar procedure when sea trout, as a conservation measure, are translocated above fish barriers in watercourses that are treated against *G. salaris* (Olstad et al., 2013). However, it is the genetic species test that is the actual control measure here, ensuring that susceptible hosts are not translocated, while the treatment in saline water is merely an additional prophylactic measure.

1.5 Listing of infectious diseases in aquatic animals

Fish diseases are listed both internationally and nationally to establish standards in the control of fish diseases. Both the World Organisation for Animal Health (OIE) and EU list diseases. One disease may have different status in these two organizations, because they operate at different geographical levels. EU/Norwegian legislative framework listing infectious fish diseases is of particular significance, and for instance trigger specified regulative measures upon detection.

OIE publishes health standards for international trade in animal and animal products. In the EU/EEA area, Lists 1 and 2 were originally declared by the European Commission (Council Directive 91/67/EEC, Annex A) and are included in Part II of Annex IV of the Council Directive 2006/88/EU (Fiskehelsedirektivet). The Norwegian national List 3 has been adopted by the Ministry of Trade, Industry and Fisheries in the Annex I in Regulation 17 June 2008 on the placing on the market of aquaculture animals and products thereof, and on the

prevention and control of certain diseases in aquatic animals (FOR-2008-06-17-819, omsetnings- og sykdomsforskriften, Norwegian Ministry of Trade, Industry and Fisheries).

A further description is given in Appendix 9 of the disease list of the OIE, relevant regulations of the European Community, listing of diseases in Norwegian regulations, relevant regulations regarding import of aquatic animals to Norway, and implementation of the new Animal Health Law in EU. See Appendix I in this report for further details.

1.6 Infectious agents in fish and crayfish in Norway and Sweden

1.6.1 Surveillance in Sweden

Swedish fish farming comprises food production and production of salmonids for restocking. In addition, European eel is imported for restocking. Rainbow trout is the most important species in aquaculture and Arctic char the second most important. A few production sites farm other species including carp, tilapia, African catfish, sturgeon, and Atlantic salmon. The most common production systems are land-based flow-through systems for production of parr, with grow-out in cages in lakes. Warm-water species, sturgeon, and salmon are held in land-based recirculating aquaculture systems (RAS) (Anonymous 2019).

It is compulsory for all registered aquaculture sites in Sweden to participate in the Official Health Control Programme. The Board of Agriculture Farms categorizes farms into risk classes, which regulate the number of health controls: RC 1 = two health controls per year, including sampling for virus and BKD every year; RC 2 = one health control per year, sampling every second year; RC 3 = one health control every second year, and sampling only upon suspicion; RC 4 = no health controls or sampling unless there is suspicion.

The surveillance programme includes the following infectious agents (associated disease in parentheses); infectious pancreatic necrosis virus/IPNV (IPN) other than serotype ab, *Renibacterium salmoninarum* (bacterial kidney disease/BKD), viral haemorrhagic septicaemia virus/VHSV (VHS), infectious haematopoietic necrosis virus/IHNV (IHN), cyprinid herpesvirus 3/CyHV-3 (koi herpes virus disease/KHVD) in imported koi, and *Aphanomyces astaci* (crayfish plague). Surveillance for viruses is based on cell culture (according to EU 2015/1554).

Official health status: Sweden is a disease-free zone for VHS and IHN, and has additional guaranties for spring viremia of carp (SVC) (the whole country) and IPN (in the inland zone). The inland zone has an approved eradication programme for *R. salmoninarum*/BKD and the coastal zone for IPN (Anonymous 2020). The eradication programme for BKD enables Sweden to set requirements for import, and there has been some criticism of the eradication programme for BKD because a time limit for the duration of the programme has not been set, and the programme has lasted for several years without being successful.

The National Veterinary Institute in Sweden (SVA) indicates that BKD is the most problematic disease to control due to vertical transmission and the prolonged period from diagnosis to

slaughter, indicating that the presence of *R. salmoninarum* in a farm does not lead to stamping out, i.e., sanitary slaughter of all susceptible fish at site. SVA expects that control of BKD will improve by introduction of non-lethal sampling (i.e., sampling of target tissues that does not require the fish to be killed, rather than sampling postmortem (Anonymous 2020).

Other notifiable diseases (national list) are furunculosis (caused by *Aeromonas salmonicida* subspecies *salmonicida*) and yersiniosis/enteric redmouth disease (ERM) (caused by *Yersinia ruckeri*). These are not included in the active surveillance programme. Flavobacteriosis (including rainbow trout fry syndrome) caused by *Flavobacterium psychrophilum* is the most common disease in farmed rainbow trout in Sweden, and, in contrast to the Norwegian situation, this disease is not notifiable.

In their annual report on health surveillance in animals and humans, SVA states that Sweden has a very good health status in aquaculture, as well as in wild populations of fish and shellfish, and, furthermore, that none of the serious diseases that occur throughout Europe are prevalent in Sweden (Anonymous 2020).

SVA highlights that the presence of hydroelectric dams (migration barriers) in rivers contribute to maintaining a good health status and that the presence of dams result in a different health status at the coast than in the more disease-free continental zone. Transport of live fish from the coast to the inland zone is therefore forbidden, and a national restocking programme compensates for the lack of natural migration and production of salmonids.

Surveillance in wild fish is based on observations reported from the public via SVA's reporting system for disease and mortality in wild fish (https://rapporterafisk.sva.se).

Tourist fishing in put-and-take facilities is common in Sweden. This industry is based on tourists being willing to pay for fishing of farmed fish that have been released into ponds and lakes. Common species are rainbow trout, brown trout, and brook trout.

1.6.2 Surveillance in Norway

Norwegian fish farm categories include farms for food production, production of cleaner fish, and stock enhancement hatcheries. Unlike in Sweden, tourist fishing in put-and-take ponds has not become a widespread activity in Norway. Most salmonid food production cycles include an initial phase in land-based hatcheries followed by a marine grow-out phase in open net pens at the sea sites (hereafter called marine production). Production of brown trout, Arctic char, and a minor portion of the production of rainbow trout takes place in freshwater-based grow-out sites inland in Norway where fish are held in tanks, ponds, or net pens in lakes (hereafter called inland farming).

Disease surveillance is primarily risk based and performed by the Norwegian Food Safety Authority (NFSA) and private fish health services. Fish health controls performed by licensed fish health personnel are mandatory in all Norwegian fish farms. The Aquaculture Operations Regulation (FOR-2008-06-17-822 Akvakulturdriftsforskriften) regulates the content, number, and frequency of obligatory controls. Knowledge about fish diseases in Atlantic salmon and rainbow trout in the marine farming environment is extensive compared with knowledge about inland farming and fish diseases relevant for wild fish species.

The NSFA targeted health-monitoring programme for farmed fish includes VHSV, IHNV, ISAV, BKD, *G. salaris* in hatcheries, and drug resistance in salmon lice in marine sites. The VHS/IHN programme is risk-based and includes mandatory controls at fish farms where authorized fish health personnel are obliged to notify NFSA and submit specimens to the Norwegian Veterinary Institute when fish with suspicious clinical signs are observed or there are other reasons to suspect IHN or VHS at the site. In the absence of such submissions, material submitted to the Norwegian Veterinary Institute for routine diagnostics will be examined for VHSV/IHNV (PCR-based). NFSA's targeted health-monitoring programme for wild fish and crayfish includes surveillance for *G. salaris*, salmon louse (*Lepeophtheirus salmonis*), and crayfish plague (*Aphanomyces astaci*). According to the Aquaculture Operations Regulations, stock enhancement hatcheries and the gene bank for wild Atlantic salmon are obliged to test all wild-caught anadromous broodfish for BKD (*R. salmoninarum*), and other relevant pathogens, depending on the health status in the area where the fish was captured.

Since 2012, NFSA has instigated monitoring programmes of wild anadromous salmonids in both the marine phase, conducted by the Institute of Marine Research, and the freshwater phase, conducted by the Norwegian Veterinary Institute. The programme monitors the presence of selected pathogens in wild fish, mainly viruses that are common and cause disease in the aquaculture industry. The material included in the programme comprises both anadromous salmonids and landlocked salmonids such as "bleke" in Byglandsfjorden, "småblank" in Namsen, brown trout, Arctic char, and whitefish. Some of the findings may be relevant for this risk assessment.

Disease surveillance of wild fish is also based on observations reported by people to NFSA. A regulation in Norway is § 6 in the Norwegian Food Act (LOV-2003-12-19-124 Lov om matproduksjon og mattrygghet mv. (matloven)) states that businesses and everyone else have a duty to notify NFSA immediately if there is reason to suspect a contagious animal disease that may have significant consequences for society. The Norwegian Veterinary Institute has received few submissions of wild fish or samples from wild fish, and the health state in wild fish populations is not well known. The largest number of samples has so far been from anadromous salmonids, wild-caught cod that are held in cages pending slaughter, and wild-caught wrasse and lumpsucker.

In 2020, NFSA and the Norwegian Veterinary Institute launched a national reporting system for disease and mortality in wild fish that is based on the Swedish system (https://rapporterafisk.sva.se). Fish pathologists at Norwegian Veterinary Institute continuously assess all reported cases and provide feedback to the people reporting. When necessary and possible, diseased fish that are sampled in the field are examined as a followup. In recent years, the relevant geographical areas, Femunden and the Trysilvassdraget on the Norwegian side, have not been included in the active or targeted disease surveillance programme.

1.6.3 Viral pathogens

1.6.3.1 Infectious haematopoietic necrosis virus - IHNV

Infectious haematopoietic necrosis virus (IHNV) belongs to the Rhabdoviridae family and is the causative agent of infectious haematopoietic necrosis (IHN). IHN is listed as a non-exotic disease in the Fish Health Directive (EU Directive 2006/88/EC), and is also notifiable to OIE (OIE, 2019). In the new Animal Health Law in EU (Regulation (EU) 2016/429), IHN is a category C disease. IHN is thus notifiable to the national competent authority (e.g., NFSA and the EU (EU reference laboratory)).

IHNV has been divided into five main phylogeographic groups based on sequence analyses, U, M, and L, representing the upper-, middle- and lower-parts of the North American west coast (Kurath et al. 2003), and furthermore genotype J (Japan) and E (Europe), both having their origins in North America (reviewed by Dixon et al. 2016).

According to the European Commission animal disease notification system (ADNS), IHN was detected in several European countries during the period 2019 to 2021, including Austria, Croatia, the Czech Republic, France, Germany, Italy, Poland, Republic of North Macedonia, Slovenia and Switzerland. In 2017, the Finnish surveillance programme for IHNV and VHSV detected IHNV infection in a marine cage production of rainbow trout on the Finnish side of the Gulf of Bothnia.

IHN has never been diagnosed in Norway, and active surveillance has documented Norway as being free from IHNV since 1994, with the exception of the Norwegian parts of the Grense Jakobselv catchment, the Pasvik watercourse, and watercourses between these rivers and associated coastal area outside, which has an unresolved status. Sweden is also free from IHNV and has an active monitoring programme based on routine sampling. Sampling frequency in the individual fish farm depends on the farm's or area's risk category (Lindberg, A. Ed. 2018). To our knowledge, IHNV is thus currently not present in the Vänern, Klarälv, or Trysilelva ecosystems.

Susceptible species relevant for this risk assessment are Atlantic salmon, rainbow trout, Arctic char, grayling, European eel, brown trout, and pike (OIE 2019, Dixon et al. 2016). Juvenile fish are more susceptible than adult fish, and disease outbreaks typically occur in juvenile fish during spring or autumn at between 8 and 15 $^{\circ}$ C (Dixon et al. 2016).

IHNV transmits horizontally, and vertical transmission cannot be ruled out. Whether suspected cases indicate true vertical transmission or contamination of the egg surface is uncertain (Dixon et al., 2016). IHNV can survive in freshwater for over one month, but movement of eggs and live fish are considered the most important risk factor for the spread

of virus, with subsequent emergence of disease in salmonid fish (Peeler et al., 2009; Dixon et al., 2016).

In summary, IHNV is not present in Vänern now, but the ongoing imports of susceptible species, including imports of European eels for release in Vänern and its catchments, and rainbow trout for aquaculture and put-and-take fisheries, represent potential routes of entry to Vänern, Klarälv, and tributaries. Further spread to Trysilelva by imports of either eggs or broodfish is possible. After introduction to Trysilelva/Femunden, IHNV could spread horizontally to other fish, directly by fish-to-fish contact (mating, fighting, or predation), or after shedding of virus into the water. Given the range of susceptible species, several fish communities could be affected by the introduction of IHNV.

1.6.3.2 Viral haemorrhagic septicaemia virus - VHSV

Viral haemorrhagic septicemia virus (VHSV) belongs to the Rhabdoviridae family. Viral haemorrhagic septicemia (VHS) is listed as a non-exotic disease in the Fish Health Directive (EU Directive 2006/88/EC), since the disease is present in EU. VHS is also notifiable to the OIE (OIE, 2019). In the new Animal Health Law in EU (Regulation (EU) 2016/429), VHS is a category C disease. The disease is notifiable to the national competent authority (NFSA) and the EU (EU reference laboratory).

VHSV has been shown to infect more than 80 marine and freshwater fish species, and is probably endemic in marine areas in the northern hemisphere (OIE 2019). VHSV is divided into four genotypes (I-IV) with subtypes.

According to the European Commission ADNS, VHSV was detected in Austria, Belgium, Czech Republic, France, Germany, Italy, Poland, and Switzerland during the period 2019 to 2021. Both Sweden and Norway are now free from VHS in farmed fish, with the exception of Norwegian parts of the Grense Jakobselv catchment, the Pasvik watercourse, and watercourses between these rivers and the associated coastal area, which have an unresolved status. VHSV has been detected in wild marine fish in the Baltic Sea, Skagerak, and Kattegat, and was found to be highly prevalent in Norwegian spring-spawning herring, *Clupea harengus,* along the Norwegian coast (Genotype Ib) (Johansen et al. 2013). In 2007 and 2008, a VHS disease outbreak occurred in marine farming of rainbow trout in Storfjord, Norway. This outbreak was due to a virus belonging to genotype III, which, prior to this detection, was linked to marine fish (Dale et al. 2009). VHS was endemic in Denmark for several years, but has not been detected since 2009.

Disease and mortality in farmed fish have primarily been recorded in rainbow trout, turbot and Japanese flounder (*Paralichthys olivaceus*). During VHS outbreaks in the North American Great Lakes area (United States and Canada), disease and mortality were recorded in more than 28 species of wild fish.

The incubation period in rainbow trout is between 5 and 12 days, but is temperature dependent. VHS in rainbow trout causes a serious disease with high mortality.

Atlantic salmon is not considered a natural host for VHSV, but is susceptible to the virus. In experimental infection studies (cohabitant), Atlantic salmon developed clinical signs that correspond to VHS infection and shed virus, but mortality was low (Lovy et al. 2013). Whitefish, pike, brown trout, burbot (*Lota lota*) and grayling are also considered susceptible. Perch and Arctic char are also probably susceptible (OIE 2019). Data concerning the susceptibility of European eel to VHSV is iinsufficient (OIE 2019).

VHSV is excreted in urine, milt and ovarian fluid, and transmitted horizontally. There are no indications of true vertical transmission of the virus.

Survival of VHSV in water depends on water temperature, salinity, and organic load, with longer survival at low temperatures and in fresh water than at high temperatures and in saltwater. Survival is also longer in filtered freshwater (249-489 days at 4 °C) than in raw water (40 days at 4 °C) (Hawley & Garver 2008).

In summary, VHSV is not present in Vänern now, but the ongoing imports of susceptible rainbow trout for aquaculture and put-and-take fisheries, represent potential routes of entry to Vänern, Klarälv, and tributaries. Further spread to Trysilelva by imports of broodfish is possible. After introduction to Trysilelva/Femunden, VHSV could spread horizontally to other fish, directly by fish-to-fish contact (mating, fighting, or predation), or after shedding of virus into the water. Given the range of susceptible species, several fish communities could be affected by the introduction of VHSV.

1.6.3.3 Infectious salmon anaemia virus – ISAV

Infectious salmon anaemia virus (ISAV) belongs to the Orthomyxoviridae family and has caused epidemic outbreaks in Atlantic salmon farming since the mid-1980s. Like IHNV and VHSV, ISAV is a list II non-exotic disease, notifiable in OIE, and is categorized as a type C disease in the new EU system.

Disease spreads slowly through a salmon farm with 0.05-0.1% daily mortality, resulting in 10-90% mortality, depending on viral strain, management, and fish genetics (Mjaaland 2002). Infection of endothelial cells leads to circulatory distress, bleeding, and, finally, anaemia (Evensen 1991). Pathogenic ISAV strains (ISAV-HPR Δ) are characterized by a truncated haemagglutinin-esterase (HE) stalk domain and mutations in the cleavage site of the fusion protein (Rimstad 2020). These deletions separate the pathogenic strains from the ISAV-HPR0 strains. The HPR0 viruses (with no deletions in HE) cause asymptomatic localized epithelial infections and have widespread distribution in the fresh- and salt-water phase of both farmed and wild salmon populations. It is a widely accepted hypothesis that virulent ISAV-HPR Δ is derived from ISAV-HPR0 through deletions, and that this is a stepwise process (Christiansen et al. 2011). Mutation to virulent ILAV-HPR Δ , with subsequent spread to other salmon and establishment of the infection in a population, is considered a rare event. Disease often develops slowly at the population level. The main drivers of the change from HPR0 to HPR Δ are presently unknown.

Phylogenetic analyses of the ISAV HE gene support a distinction between two monophyletic clusters, called the North American (NA) and the European (EU) clusters, based on the origin of the isolates. The EU clusters can be subdivided into four sub-clusters and descendants from these groups have been found in North and South America, causing outbreaks in salmon farms (Godoy 2008). Most countries farming Atlantic salmon (Canada Chile, Faroes, UK, USA) have experienced outbreaks, but the virus have not been reported in Sweden. ISAV can be carried by multiple fish species, including wild Atlantic salmon, brown trout, and rainbow trout. Experimental infections have been established in brown trout, rainbow trout, Arctic char, herring, and Atlantic cod. Saithe, *Pollachius virens,* has also been suggested as a vector, carrying the virus between farms (Dempster 2009). Salmon louse can also act as a mechanical vector, spreading the virus between fish (Nylund 1993). After a peak of more than 80 outbreaks in the early 1990s, between 2-20 outbreaks have occurred annually. This reduction has been achieved through changes in management practices.

1.6.3.4 Salmonid alpha virus - SAV

Salmonid alpha virus (SAV), previously named salmon pancreas disease virus, is an enveloped, positive-strand RNA virus belonging to the Togaviridae family. The genome encodes 8 proteins (4 structural and 4 nonstructural), with the envelope E1-E2 proteins as the main antigenic epitopes (McLouglin 2007). Depending on strain, it causes pancreatic disease (PD) or sleeping disease in salmonid fishes in both fresh- and salt-water in many European countries (Rodger et al. 2017). PD is a list III non-exotic disease, notifiable in OIE, and not categorized in the new EU system.

Infected fish display reduced appetite, circulatory disturbance, and loss of exocrine pancreatic tissue in the late phase (Jansen 2017). Six genotypes have been classified based on sequence data (SAV1-6), where SAV2 occurs in both freshwater and seawater. Freshwater SAV2 can cause sleeping disease in salmon, rainbow trout and Arctic char, but this virus has not been observed in Sweden or Norway. Marine SAV2 and SAV3 are prevalent in Norwegian salmon farming and cause more than 100 outbreaks annually (Sommerset et al. 2020). SAV infections have been documented from most places where salmon or rainbow trout are farmed in Europe (Croatia, France, Germany, Ireland, Italy, Norway, Poland, Spain, Switzerland, and the United Kingdom).

Fish are infected via uptake in gills or intestine and, in the acute stage, virus can be isolated from organs throughout the animal. Viral particles are shed from infected fish via excretions and mucus over extended periods, thereby facilitating horizontal transmission (Graham 2012). Subclinical infections may also develop into disease upon changes in environmental factors like temperature (Stene 2014) or stress from coinfections like sea lice, but there is no evidence of vector transmission. Infection spreads through water and cohabitation, and there is no strong evidence for vertical transmission (VKM 2011).

The documented host range for SAV includes Arctic char, Atlantic salmon, common dab (*Limanda limanda*), and rainbow trout at all life stages. Most natural infections occur in

seawater, but experimental infections in the freshwater parr stage of Atlantic salmon have been documented (McVicar 1990). In addition, positive PCR samples have been obtained from a range of saltwater fish species, but without evidence of infection or viral replication. The prevalence of SAV in farmed salmon may be as high as 100% in certain areas but serological surveys of wild salmon in Ireland found no evidence of previous infections. Despite several extensive investigations and large studies, SAV has only once been detected in wild Atlantic salmon and has not been documented in samples from Vänern.

1.6.3.5 Infectious pancreatic necrosis virus - IPNV

Infectious pancreatic necrosis virus (IPNV) was the first fish virus to be isolated in vitro and is an RNA virus belonging to the Birnaviridae family (Rodriguez 2003). Members of the genus are found in aquatic habitats worldwide and infect numerous fish species, molluscs, and crustaceans. However, the name IPNV is reserved for members causing acute catarrhal enteritis disease in salmonids (Rodriguez 2003). The viral genome consists of two segments encoding a total of 5 proteins (VP1-VP5). The main antigenic epitopes are on the VP2 capsid protein. IPNV is not an OIE-listed disease and there is no concerted public eradication programme in Norway for this disease. IPN genogroup 2 is present in coastal areas in Sweden and is notifiable. The IPN virus causes acute disease in juvenile salmonids and transmits both horizontally and vertically, via roe. In Norway, this virus is mainly found in farmed Atlantic salmon (in both freshwater and marine phase) where it causes about 20 outbreaks per year (Sommerset et al. 2020). The frequency has been declining, mainly due to selection of genetically resistant fish. Mortalities during outbreaks vary from very low to 90%, but survivors often develop lifelong persistent-carrier status. Infected fish display distended abdomens, darkened pigmentation, and erratic swimming behaviour (Hill 1982). Diagnosis is based on histopathology (necrotic exocrine pancreas tissue) and immunostaining for viral antigens (Evensen 2008).

The virus itself is resistant to disinfectants and can maintain infectivity for long periods in both seawater and freshwater. IPNV genogroup 5 has been detected a few times and the first report of IPNV-genogroup 6 in Sweden came from Vänern in 2016 during harvest of salmon for hatcheries production (SVA 2017)

1.6.3.6 Salmon gill poxvirus - SGPV

Salmon gill poxvirus (SGPV) belongs to the *Orthopoxviridae* subfamily causing salmon gill poxvirus disease in farmed Atlantic salmon (Gjessing et al. 2015). The virus is widely distributed in both farmed and wild Atlantic salmon but has not been verified in samples from other fish species. Infections are seen at all stages but occur most often in the transition from fresh to salt water and may lead to high mortalities in the affected farms. Infected fish display respiratory distress, reduced appetite, and often contract secondary infections. Diagnosis is most often by PCR but characteristic histopathologial changes in gill tissues with apoptotic epithelia is another marker (Sommerset et al. 2019).

The virus is distributed in farms along the Norwegian coast and has also been confirmed in Canada, Faroe Islands, Iceland, and UK (Sommerset et al. 2020, LeBlanc 2019). PCR analysis of various salmonids has confirmed a high prevalence of the virus in wild-caught salmon in stock enhancement hatcheries, but not in sea trout or Arctic char (low amounts of viral-DNA was found in anadromous trout cohabitating with infected salmon) (Garseth et al. 2018, Gåsnes et al. 2019). The prevalence in returning Atlantic salmon captured in coastal fisheries was lower (~3.9%) than in returning salmon captured in rivers (15.5%) (Sommerset et al. 2021). SGVP DNA was detected in one fish with skin lesions from Mørrumsån) during a survey (Axen et al 2017). SGPV has not been detected in landlocked salmon in Norway (småblank and byglandsbleke). These waterbodies are more isolated from external influences, including imported fish, ballast water, and aquaculture than Vänern. SGPV is transmitted horizontally, but vertical transmission remains under investigation, although one study was able to exclude this possibility in two suspected cases (Gulla et al. 2020).

There are no systematic studies of SGPV regarding environmental stability or susceptibility to disinfection measures, but, given their similar physiochemical properties, data from other poxviruses are probably a good guideline. Like other enveloped viruses, poxviruses are sensitive to most chemical and virucidal disinfectants. However, poxviruses are much more resistant to drying than other enveloped viruses (Rheinbaben 2007). These viruses stay infective for months in material that has been in contact with infected tissue. In addition, poxviruses are more resistant to extreme pH, organic solvents, and high temperatures than most enveloped viruses. One recent study indicated that SGPV has a potential for establishing and prevailing as 'house-strains' within freshwater salmon farms across production cycles (Gulla et al. 2020).

1.6.3.7 Piscine othoreovirus - PRV

Piscine orthoreovirus (PRV) is a naked, RNA virus belonging to the *Reoviridae* family. PRV- 1 causes heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (Palacios et al. 2010) and PRV-3 causes disease resembling HSMI in rainbow trout (Olsen et al. 2015). PRV-1 has also been associated with jaundice syndrome in Chinook salmon (*Oncorhynchus tshawytscha*) (Di Cicco et al., 2018). A third genotype, PRV-2, has been found in coho salmon (*Oncorhynchus kisutch*) in Japan and causes erythrocytic inclusion body syndrome (EIBS) (Takano et al., 2016). PRV-1 from salmon and PRV-3 from rainbow trout have a total genetic similarity of approximately 90%, although some parts of the viral genome display only 80% similarity (Sommerset et al. 2020).

HSMI in salmon has not been notifiable in Norway since 2014, and HSMI-like disease in rainbow trout has never been notifiable (Sommerset et al., 2020).

PRV-1 is common in farmed salmon (Sommerset et al., 2021) and occur in wild salmon and seatrout in Norway and several other countries (Garseth et al. 2012). Many genetically different PRV-1 strains have been detected, and it is suggested that some strains may be more pathogenic than others.

Occurrence of PRV-1 in wild Atlantic salmon captured in the sea along the coast of Norway in 2019 was 1.3% (Garseth et al. 2020), while Madhun et al. (2018) found that 8% of wild salmon in the sea in Northern parts of Norway were PRV-1 positive. Garseth & Biering (2018) detected PRV-1 in two of nine brown trout stock-enhancement hatcheries, but the viral load, as depicted by Ct-values, was low. The study included a total of 60 brown trout from two hatcheries in former County of Hedmark, and none of these were PRV-1 positive. In the same study, PRV-1 was not detected in the landlocked salmon småblank and byglandsbleke in Norway, although the sample sizes were small (24 and 28 fish, respectively). A limited number of anadromous wild Atlantic salmon at several localities in Sweden have been tested for PRV-1, and one of 24 wild salmon in Mörrum (south coast) were virus positive (Vendramin et al. 2019).

HSMI (caused by PRV-1) is characterized by abnormal swimming behaviour, anorexia, and up to 20% mortality. Salmon dying of HSMI often display signs of circulatory disturbances.

PRV-3 is a common virus in wild seatrout, but has thus far not been detected in landlocked Atlantic salmon or brown trout in Norway (Garseth et al. 2017). PRV-3 is present in inland farming of rainbow trout and brown trout on the European continent (Sorensen et al. 2020). Corresponding surveys of inland farming in Norway and Sweden have not been conducted. PRV-3 has only been detected in a few wild Atlantic salmon, and experimental studies suggest that the virus is poorly adapted to this species (Hauge et al., 2017).

The occurrence of PRV-1 and PRV-3 in Vänern and in the Femund and Trysil watercourses is unknown. It is likely that PRV-3 is present in rainbow trout in Vänern based on experiences from Denmark, where Sorensen and co-workers (2020) found that 72% of examined farms were infected.

Atlantic salmon (Palacio el al 2010), brown trout (Garseth et al. 2012), coho salmon *Oncorhynchus kisutch*, Chinook salmon *Oncorhynchus tshawytscha*, and pink salmon *Oncorhynchus gorbuscha* (Purcell et al. 2018, Garseth et al. 2020) are susceptible for PRV-1. The susceptibility of other species that are present in Trysilelva is not known.

PRV-1 and 3 is transmitted horizontally. Vertical transmission is not confirmed.

1.6.3.8 Rhabdoviruses (other than VHSV and IHNV)

Fish rhabdoviruses are assigned to the genera Sprivivirus, Novirhabdovirus, and, the recently described Perhabdovirus (Hoffmann et al., 2005; Stone et al., 2013). Several rhabdoviruses cause serious disease in fish, including the above described VHSV and IHNV (see sections 1.6.3.1 and 1.6.3.2), both belonging to the Novirhabdovirus and spring viremia of carp virus (SVCV) belonging to Spirivivirus.

However, more than ten other rhabdoviruses have been described in a wide range of wild and farmed fish species, both in marine and freshwater environments. These include grass carp rhabdovirus (GrCRV), tench rhabdovirus (TenRV), pike fry rhabdovirus (PFRV), *Siniperca* *chuatsi* rhabdovirus (SCRV), Eel rhabdovirus Eurpoean X (EVEX), Eel virus American (EVA) perch rhabdovirus (PRV), Swedish sea trout virus (STRV), European lake trout rhabdovirus (LTRV), snakehead rhabdovirus (SHRV), hirame rhabdovirus (HIRRV), and eelpout rhabdovirus (EpRV).

Several of these rhabdoviruses have caused losses and mortalities in farmed and wild percids and other freshwater fish species in Europe since the 1980s and earlier (De Kinkelin et al. 1973, Dorson et al. 1984, Nougayrède et al. 1992, Jorgensen et al. 1993, Bjørklund et al. 1994, Dannevig et al. 2001). Rhabdoviruses have received more attention after the intensification of aquaculture of percids (perch and pikeperch *Sander lucioperca*) and are considered emerging diseases in Europe (Ruane et al. 2014, Wahli et al. 2015, Bigarré et al. 2017, Caruso et al. 2019, Betts et al. 2003, Pallandre et al. 2020).

Infected fish may exhibit serious disease with abnormal swimming behaviour, lethargy, haemorrhaging, nervous symptoms, and high mortality. The biological properties of these viruses are largely unknown because they have not been studied, but some seem prone to host switching (Pallandre et al. 2020). For instance, genetically related viruses have been found in percids and non-percid species, such as brown trout and grayling (Gadd et al. 2013), and sea trout rhabdovirus has been identified in perch and black-bass, *Micropterus salmoides* (Talbi et al. 2011).

Eel Virus European X (EVEX) is a Rhabdovirus that causes disease in European eel and rainbow trout. The disease occurs in Asia and several European countries, and was detected in Sweden in 2017 in eels that were quarantined after import from France. The eels were intended for release in several watersystems including Vänern, but were destroyed after detection of EVEX.

A Rhabdovirus was shown to cause mass mortality in eelpout, *Zoarces viviparous*, along the Swedish Baltic Sea coastline in 2014. The virus was tentatively given the name eelpout rhabdovirus (EpRV genus Perhabdovirus) (Axen et al. 2017). Lack of genetic variation between the described outbreaks indicate that the virus was new to the Baltic Sea, but the origin is unknown. The authors speculated that the virus could have travelled there in ballast water or in an invasive species acting as a passive virus carrier (Axen et al 2017). The Sea trout rhabdovirus was also first described after detection in sea trout in the Baltic Sea (Stockholm archipelago). A rhabdovirus has also been detected in perch from Lake Årungen near Oslo, Norway, in 1997/98 when wild perch were translocated to an experimental facility for research purposes (Dannevig et al. 2001).

Whether the rhabdoviruses mentioned above are present on either side of the border in the Trysilelv/Klarälv/Vänern watershed is uncertain as there is no active surveillance for them. It is, however, considered likely that known and yet unknown genotypes will emerge in Europe during the next decades, and, due to their host-switching capacity, they will likely affect various hosts (Pallandre et al. 2020).

1.6.4 Bacterial pathogens

1.6.4.1 Renibacterium salmoninarum

Bacterial kidney disease (BKD) is a fish disease of worldwide relevance that is notifiable in Norway (national list 3). BKD is caused by *Renibacterium salmoninarum*, and the clinical disease affects only salmonid fish species, farmed and wild. However, rare detections have also been made in non-salmonid fish (Kent et al., 1998). While BKD outbreaks may be associated with high mortality, particularly in juvenile fish, lifelong subclinical carrier status is not uncommon amongst wild specimens (Jónsdóttir et al., 1998).

R. salmoninarum is a facultatively intra-cellular bacterium that may transmit both horizontally and vertically (Austin and Austin, 2012). Genetic evidence suggests that trade with live fish, feed, and/or ova has played a historical role in disseminating the bacterium (Brynildsrud *et al.*, 2014). While the possibility for vertical transmission of *R. salmoninarum* via ova is well-established (Evelyn et al. 1985), the potential role of milt in transmission remains uncertain (Daly and Stevenson, 1989).

No commercial vaccine exists against BKD and effective treatments are also lacking, largely due to the potential for intra-cellular infection that makes the bacterium inaccessible to many antibiotic treatments. Iodine is ineffective for disinfection of *R. salmoninarum*-infected ova (Evelyn et al. 1984). Preventive measures and slaughtering upon detection thus remain the only viable options available for mitigating the disease today, although a combination of these, together with administrating antibiotic injections to spawning adults, has resulted in some success in farming of Chinook salmon (Munson et al. 2010).

Although *R. salmoninarum* has, historically, been a significant problem in re-stocking hatcheries for Atlantic salmon in Norway, extensive screening of broodstocks have resulted in its decline and it is now rarely detected in such sites. It is currently considered to be low-grade endemic amongst wild anadromous salmonids in some river systems along the west coast. In recent years, zero to one detection, exclusively from marine salmon in western Norway, have been made annually at the Norwegian Veterinary Institute (Sommerset et al., 2020). The *R. salmoninarum* infection status in Femund- and Trysilvassdraget is unknown, but it has never been detected in landlocked Atlantic salmon or brown trout in Norway (Garseth et al. 2020).

In Sweden, *R. salmoninarum* was first documented in 1985, before spreading both inland and along the coast over the following years. A few BKD outbreaks occurred in rainbow trout stocks in Vänern/Klarälven's drainage basins between the late 1980s and early 1990s. Today, Sweden follows an EU-certified eradication programme against BKD in inland areas (2010/221/EU), and several detections have been made in rivers in northern Sweden in recent years. The possibility of undetected, minor BKD outbreaks occurring sporadically amongst wild salmonid populations cannot ruled out. Of note, the current broodstock screening programme for *R. salmoninarum* in Sweden, as opposed to that in Norway, focuses solely on the vertical transmission potential of ova, and thus disregards the yetundetermined possibility for spread via milt (Daly and Stevenson, 1989; VKM, 2019; Garseth et al., 2020). A separate screening initiative in 2015 targeted wild specimens of several salmonid species, captured in a Swedish river system (not connected to Vänern/Klarälven), where BKD had been diagnosed in a cultured fish stock (Axén, 2016). This study detected, by ELISA, approximately 10% positive kidney samples from fish caught in certain downstream areas. However, this method is not conclusive regarding confirmation of a live *R. salmoninarum* infection, and only a single sample could be verified as positive by PCR.

In 2019, BKD was diagnosed (ELISA + PCR on kidney) in farmed fish in Sweden at six (unspecified) locations, and in a single wild Arctic char captured in lake Vättern (Ståhl, 2020).

1.6.4.2 Aeromonas salmonicida

Aeromonas salmonicida subsp. salmonicida

Furunculosis, or classical furunculosis, in fish is a term used primarily to describe the detrimental clinical disease in salmonid fish species caused by *Aeromonas salmonicida* subsp. *salmonicida*. Only this particular subspecies of *A. salmonicida* is notifiable (national list 3) in Norway, and it is also often termed 'typical' *A. salmonicida*. Although primarily occurring in salmonids, it can also cause disease in several other fish species. Furunculosis is effectively controlled through vaccination in Norwegian salmonid aquaculture, but the disease still represents a significant threat to salmonid aquaculture throughout the northern hemisphere.

While the primary route of transmission for *A. salmonicida* subsp. *salmonicida* is horizontal, the potential for passive vertical transmission via sexual products cannot be ruled out. Nevertheless, the evidence indicates that standard disinfection procedures should eliminate this latter possibility (Rimstad et al., 2019; Garseth et al., 2020).

Although farmed Atlantic salmon are vaccinated against *A. salmonicida* subsp. *salmonicida* in Norway today, the bacterium is considered endemic amongst wild salmonid populations in rivers draining to Namsfjorden in mid-Norway. Here, sporadic detections have occurred during recent years in farmed and wild salmon, as well as in lumpsucker used for salmon delousing (Sommerset et al., 2020). The infection status in Femund- and Trysilvassdraget is unknown. Furunculosis is occasionally reported from salmonid aquaculture industries in Sweden, including four cases in 2019, but no detections have been reported in association with Vänern (Garseth, Adolfson and Hansen, 2020).

Other Aeromonas salmonicida lineages

Aeromonas salmonicida, as a whole, has a near global distribution and may cause serious disease in an extremely wide range of fish species (Austin and Austin, 2012), but a disproportional historical focus has been upon the subspecies *salmonicida*, which has dominated in salmonids. Strains of *A. salmonicida* that are not identified as belonging to subsp. *salmonicida* have often been collectively termed 'atypical' *A. salmonicida*, and four validly described subspecies (*achromogenes, masoucida, smithia* and *pectinolytica*) are included within this group (Martin-Carnahan and Joseph, 2005). A recent study, involving 675 clinical *A. salmonicida* isolates, recovered over 59 years from 50 different fish species in 26 countries, has highlighted the existence of multiple discrete genetic lineages that cannot

be assigned to any of the subspecies named to date (Gulla et al., 2019). Importantly, most of these *A. salmonicida* lineages/genotypes display a strong predilection for particular fish species, indicating host specificity, and a few genotypes outside of subsp. *salmonicida* show complete or partial preference for salmonid hosts. Amongst 26 Swedish isolates examined from various fish species, two out of five detected genotypes (excl. subsp. *salmonicida*) displayed salmonid host predominance. However, all *A. salmonicda* genotypes detected in Sweden (with the exception of one 'non-salmonid') have also been recorded at some point somewhere in Norway, albeit not in Femund- and Trysilvassdraget. *A. salmonicida* has been detected in at least one river draining into Vänern (Garseth et al., 2020), but it is unlikely that this isolate was genotyped.

1.6.4.3 Flavobacterium psychrophilum

Flavobacterium psychrophilum may cause severe disease, primarily, but not exclusively, in salmonid fry and parr (e.g. rainbow trout fry syndrome), and constitutes a significant threat to salmonid aquaculture industries worldwide. In Norway, sporadic detections in Atlantic salmon occur, but only systemic *F. psychrophilum* infections in rainbow trout are notifiable (national list 3). Strain differences in terms of virulence and host specificity exist, and the bacterium is commonly present in temperate freshwater environments (Nilsen et al., 2014; Rimstad et al., 2019). Evidence from genotyping studies has indicate the spread of some virulent *F. psychrophilum* strains via anthropogenic activities (Nicolas et al., 2008). Vertical transmission via both milt and ova are likely, and the potential for locating within egg cells could render it resistant to some standard disinfection procedures, such as iodine (Brown et al., 1997; Ekman et al., 1999; Taylor, 2004; Cipriano, 2005; Rimstad et al., 2019).

Many different genotypes of *F. psychrophilum* have been documented in the Nordic countries (Nilsen et al., 2014) and it is a common cause of disease in farmed rainbow trout in Sweden (Garseth et al., 2020). As this bacterial species is common to freshwater environments, it is likely to be present in Vänern and/or connected waterways, but it is uncertain whether or not those strains would be capable of infecting and/or cause disease in Atlantic salmon.

1.6.4.4 Flavobacterium columnare

Flavobacterium columnare is the aetiological agent responsible for columnaris disease in a wide range of freshwater fish species (including salmonids). It is not uncommon for virulent strains of the bacterium to be associated with extremely high mortalities, in the range of 60-90% (Austin and Austin, 2012). The disease is mainly limited to fish inhabiting warmer waters, primarily presenting at water temperatures around 18-22°C, and seldom below 15°C (Austin and Austin, 2012), but it does occur in salmonid fish farms in Sweden (Ståhl, 2020). As far as we can ascertain, *F. columnare* has never been detected in Norway.

As with many other members of the *Flavobacterium* genus, *F. columnare* likely occurs naturally in many freshwater environments, although they thrive at higher temperatures than what is commonly experienced in Trysilvassdraget. In the face of ongoing global warming, however, the relevance of *F. columnare* in Northern Europe may increase over time, e.g., by allowing its establishment in areas that remain yet too cold.

1.6.4.5 Yersinia ruckeri

Yersiniosis (also often termed enteric redmouth disease), caused by *Yersinia ruckeri*, constitutes a significant disease problem in salmonid aquaculture worldwide. While primarily occurring in farmed rainbow trout internationally, in Norway the disease is regularly diagnosed only in farmed Atlantic salmon (Sommerset et al., 2020). However, recent studies have shown that only relatively few specific strains are associated with disease outbreaks, whereas a diverse array of environmental and seemingly avirulent strains occur naturally in freshwater environments (Gulla et al., 2018). While detections in ovarian fluid indicates the possibility of vertical transmission (Glenn et al., 2015), routine disinfection measures are probably sufficient to prevent such spread (Rimstad et al., 2019).

In Atlantic salmon farming in Norway, yersiniosis was a growing problem until widespread implementation of vaccination against the disease, which resulted in a steep decline in the number of annual outbreaks (Sommerset et al., 2020). A single *Y. ruckeri* clone exclusive to Norway has been found responsible for almost all major yersiniosis outbreaks in Norwegian farmed Atlantic salmon. In addition, a distinct clone, specifically virulent to rainbow trout, has been spread intercontinentally, likely through anthropogenic activity, and now dominates internationally amongst disease outbreaks in this fish species (Gulla et al., 2018). Although this rainbow trout-clone of *Y. ruckeri* has never been detected in Norway, it was verified from farmed rainbow trout in Sweden (not Vänern) in 2017. The status of Vänern and/or connected waterways regarding the potential presence of virulent *Y. ruckeri* strains is uncertain, but no detections have yet been reported.

1.6.5 Parasites

A large number of parasite species have been found in Atlantic salmon in freshwater in Norway and Sweden (Bakke and Harris 1998, Sterud 1999). Apart from *Gyrodactylus salaris*, the presence of parasites in Atlantic salmon in Vänern and its tributaries is almost unknown. This also applies to the occurrence of parasites in other animal species that could potentially migrate upstream Klarälven to Norway. Despite all eukaryotic parasites having, per definition, a negative impact on their hosts, relatively few parasites found in wild Atlantic salmon or other migrating species are known to cause disease or severe pathology. Here we focus on a few parasites with known pathogenic potential, or which may affect the migration capacity of Atlantic salmon or other migrating species.

1.6.5.1 Gyrodactylus salaris

The monogenean *Gyrodactylus salaris* has caused severe disease outbreaks in Atlantic salmon parr in 51 Norwegian rivers, with an 86% yearly mean mortality. *G. salaris* is considered to be among the largest threats to Atlantic salmon populations in Norwegian rivers (Vitenskapelig råd for lakseforvaltning, 2020). More than 1 billion NOK has been used in eradication and surveillance of this invasive parasite to date. *G. salaris* is alien to Norway and was probably introduced several times to Norwegian fish farms and rivers with imports

of live salmonids from Sweden (Mo, 2020). Gyrodactylosis caused by *G. salaris* has been listed as a notifiable disease in Norway since 1983.

G. salaris probably occurs naturally in lakes and rivers draining to the Baltic Sea (Malmberg and Malmberg, 1993; Kudersky er al., 2003; Meinilä et al., 2004; Kuusela et al., 2007, 2009). This assumption is based on laboratory experiments that demonstrated that Baltic salmon stocks generally have an innate and acquired resistance against *G. salaris* (Bakke et al., 1990a, 2002; Dalgaard et al., 2003), although Baltic salmon in the River Indalsälven, Västernorrland County, is an exception (Bakke et al., 2004). Furthermore, clinical outbreaks of gyrodactylosis have not been reported in wild or farmed salmon parr in the Baltic area (Rintamäki, 1989; Rintamäki-Kinnunen and Valtonen, 1996; Anttila et al., 2008), although high intensities of *G. salaris* have occasionally been observed in farmed Baltic salmon (Rintamäki, 1989; Ozerov et al., 2010). The presence of *G. salaris* on farmed and wild Baltic salmon is confirmed in Sweden (Malmberg, 1957; Malmberg and Malmberg, 1993), Finland (Rintamäki, 1989; Rintamäki-Kinnunen and Valtonen, 1996; Anttila et al., 2008; Kuusela et al., 2009), Russia (Ergens, 1983; Kudersky et al., 2003; Ieshko et al., 2016), Estonia (Ozerov et al., 2010), and Latvia (Hansen et al., 2003).

The natural distribution of *G. salaris* on Atlantic salmon is likely to include rivers draining to the Kattegat area (Malmberg et al., 1995; Degerman et al., 2012). Kattegat is located north of the outlet of the Baltic Sea, and salmon in the rivers draining to Kattegat are east Atlantic salmon, which are genetically different from Baltic salmon (Ståhl, 1987; Bourret et al., 2013). Several haplotypes of G. salaris have been found in Swedish rivers draining to Kattegat (Hansen et al., 2003). The origin of the *G. salaris* haplotypes in rivers of the Swedish west coast is unknown, but were likely spread with salmonids migrating through brackish water from the Baltic Sea drainage, as G. salaris is not present in any of the rivers north of Kattegat except a recent spread of another G. salaris haplotype to three rivers in the Oslo Fjord (see below). However, the haplotypes that occur in Göta älv are similar to those in Vänern (see below) and may have been spread downstream from this lake (Olstad et al., 2013). Alternatively, G. salaris could have spread with migration of Baltic salmon from the freshwater Ancylus Lake through the opening in mid Sweden to the west coast after the last glacial period. However, if this is so, closely related haplotypes of G. salaris in Vänern would be expected to occur in several rivers on the Swedish west coast but have not been found in the many examinations of salmon parr from these rivers. Despite the Atlantic salmon in the rivers draining to Kattegat being genetically close to the Atlantic salmon from Norwegian and Scottish rivers that have been shown experimentally to be susceptible to G. salaris (Bakke et al., 1990a; Bakke and Mackenzie, 1993), no severe disease outbreaks due to G. salaris have been reported in the Swedish rivers draining to Kattegat (Malmberg et al., 1995), although hundreds of *G. salaris* specimens may be found on salmon parr in some of the rivers (Malmberg, 1993; Malmberg and Malmberg, 1993). In Denmark, several non-pathogenic strains of G. salaris have been found on wild and farmed Atlantic salmon and rainbow trout in watercourses draining to Kattegat and the North Sea (Lindenstrøm et al., 2003; Jørgensen et al., 2007, 2008), which could also be natural occurrences of *G. salaris*.

All three haplotypes of *G. salaris* found in Atlantic salmon rivers in Norway have caused mass mortality of salmon parr. However, one of these haplotypes, with a minor genetic difference in the ribosomal internal transcribed spacer, is also found on Arctic charr in several lakes, and this genotype is non-pathogenic to Atlantic salmon. Thus, haplotype alone cannot be used as a proxy for pathogenicity.

The first detection of *G. salaris* in Vänern watersheds was on rainbow trout at Källefalls fishfarm close to Tidaholm in 1972 (Malmberg and Malmberg, 1993), and the first observation on Atlantic salmon in Vänern and Klarälven was in 2013 (Olstad et al. 2013). So far, three closely related haplotypes of *G. salaris* have been found on salmon in Klarälven and Vänern (Garseth et al. 2020), the origin of which is unknown. They may have been present since the establishment of salmon in Vänern and the rivers draining to the lake, which means for several thousand years (Olstad et al. 2013). However, if so, it would be expected that *G. salaris* has not been found on salmon from Gullspångälven as it is in river Klarälven. *G. salaris* has not been found on salmon from Gullspångälven in several studies (Degerman pers. komm. in Olstad et al. 2013). Alternatively, the occurrence of *G. salaris* in Vänern could be a relatively recent introduction, either as a consequence of the establishment of the Göta Canal, or due to transport and stocking of salmonids from other areas in Sweden to fish farms or rivers in Vänern.

In other reports (Olstad et al. 2013, Hedenskog et al. 2015), it is claimed that salmon in Vänern is likely resistant to *G. salaris* because of its close relation to Baltic salmon, but, as far as we know, this has not been experimentally tested in any experiments. Furthermore, Bakke et al. (2004) demonstrated that Baltic salmon in the river Indalsälven is not resistant, and therefore the general assumption that the Baltic salmon is resistant to *G. salaris* is incorrect. Josefsson and Andersson (2001) claimed that *G. salaris* was introduced to Swedish waters in the 1950s with fry or roe imported for aquaculture from the Baltic states, and, furthermore, that mortality in Swedish salmon populations in aquaculture due to *G. salaris* is 60%, but the authors provide no reference or source for this statement and likely, such high mortality is not correct (C. Axén, pers. comm.). At present, there is no indication of disease outbreaks due to *G. salaris* in Klarälven. However, it is not known whether this is due to salmon in Klarälven/Vänern being resistant to *G. salaris*, or whether the *G. salaris* haplotypes in Klarälven/Vänern to have the same pathogenic potential in Norwegian Atlantic salmon populations as the strains previously introduced to Norway from Sweden.

1.6.5.2 Ichthyophthirius multifiliis

The parasitic ciliate *Ichthyophthirius multifiliis* causes white spot disease, also termed Ich, in many freshwater fish species, including salmonids. Although the trophonts that cause the white spots are macroscopically visible, they are endoparasites and located below the epidermis. *I. multifiliis* uses only one host during its lifecycle but has multiplication stages (tomonts) in the water or attached to the substratum that produce infective theronts (Dickerson 2012). Outbreaks of white spot disease can occur at water temperatures down to

6 °C but are most frequent in the warmest period of the year (Dickerson 2012). Outbreaks of Ich disease have occurred in spawning wild salmonids (Traxler et al. 2011) and recently (2018-2020) in European eel in River Ätran and other rivers on the Swedish west coast (C. Axén, SVA, pers. comm.).

1.6.5.3 Tetracapsuloides bryosalmonae

The myxozoan Tetracapsuloides bryosalmonae causes proliferative kidney disease (PKD) in salmonids, which mostly occurs when the water temperature is above 15 °C for at least two weeks (Okamura et al. 2011). T. bryosalmonae is considered to be a "climate parasite", because the frequency and severity of PKD outbreaks seem to increase with longer periods with water temperatures above 15 °C (Okamura and Feist 2011). PKD mainly affects kidney and spleen of yearlings, and anaemia is commonly observed. Survivors usually have acquired immunity against future infections, however, older naïve salmonids may suffer from disease. The mortality in wild salmonid populations can be very high. Surviving salmonids may be disease-free carriers of *T. bryosalmonae* for many years (Soliman et al. 2018). Sterud et al. (2007) estimated the mortality due to PKD to be around 60-70%. T. bryosalmonae use bryozoans as their definitive host, and salmonids as an intermediate host. Thus, the occurrence and abundance of this parasite are also dependent of the size of the bryozoan host populations and the production of *T. bryosalmonae* spores (Tops et al. 2006). In addition, T. bryosalmonae can affect growth and survival of its bryozoan hosts (Tops et al. 2009). T. bryosalmonae occurs in Atlantic salmon and brown trout in many Norwegian rivers (Mo and Jørgensen 2017). We are not aware of a similar mapping or surveillance in Swedish rivers.

1.6.5.4 Myxobolus cerebralis

The myxozoan Myxobolus cerebralis causes whirling disease in salmonids worldwide (Hoffman 1990). This parasite uses oligochaetes in the genus *Tubifex* as the definitive host and salmonids as an intermediate host (El-Matbouli et al. 1999). M. cerebralis spores released from *Tubifex*, which are morphologically very different from spores in the fish host, attach to young salmonids and attack the cartilage, mainly in the head, before ossification (Hallett and Bartholomew 2012). The damage causes nervous disorders resulting a whirling swimming behaviour and, commonly, a dark, almost black, tail region and may cause significant mortality in salmonid populations, especially among yearlings (Thompson et al. 1999). Whereas brown trout is considered partly resistant to *M. cerebralis*, Atlantic salmon is considered susceptible (Hallett and Batholomew 2012). Co-infection with Tetracapsuloides bryosalmonae (PKD) can exacerbate the pathological changes of both diseases and elicit higher mortality rates (Kotob et al. 2017). In addition, *M. cerebralis* can negatively affect populations of the definitive host including reproduction and feeding (Stevens et al. 2001, Shirakashi and El-Matbouli 2009). *M. cerebralis* has been reported from salmonids in both Norway and Sweden (Alexander and Bartholonew 2020), but the geographical distribution in wild salmonids is not known.

1.6.5.5 Salmincola spp.

Parasitic crustaceans of the genus *Salmincola*, sometimes named gill maggots, attach mostly to the gills of freshwater fish, mainly salmonids, and feed on blood and tissue. These parasites have one host during their life cycle and are considered host specific, and each *Salmincola* species uses only one host species (White et al. 2020). When numerous, these parasites may cause severe damage to the gills and reduce the oxygen uptake (White et al. 2020). This can affect the host behaviour and potentially reduce the migration capacity of adults and juveniles (Herron et al 2018). In larger fish, *Salmincola* spp. attach primarily to the gills causing extensive damage, with serious epizootics causing mortality, particularly in summer when the temperature is high and oxygen levels low (Kabata and Cousens 1977, Black 1982, Sutherland and Wittrock 1985). Species of the genus *Salmincola* are known from salmonids in Norway (Amundsen et al. 1997, Mo et al. 1998, Kusterle et al. 2012) and Sweden (Boxshall 2020), but the occurrence of these parasites seems to be unstudied in the Trysilelva/Klarälven/Vänern watercourse.

1.6.5.6 Anguillicoloides crassus

The invasive parasitic nematode *Anguillicoloides crassus* was introduced to Europe with imports of live Japanese eel (*Anguilla japonica*) from Asia in the early 1980s. *A. crassus* uses eel, also European eel, as a definitive host in a complicated lifecycle including several invertebrates and vertebrates as intermediate and paratenic (transport) hosts, respectively (Lefebvre et al. 2012). *A. crassus* feeds on blood and develops in the eel swim bladder. Eggs released from adult females, hatch in the swim bladder. Larvae migrate via the pneumatic canal to the gut and are released to water with host faeces. Completion of the life cycle depends upon predator-prey interactions. *A. crassus* may cause severe bleeding and pathology in the eel swim bladder and may affect the behaviour and survival of eel (Lefebvre et al. 2012). According to Sjöberg et al. (2009), *A. crassus* affects the migration capacity of European silver eel. As the invasive *A. crassus* uses hosts at several trophic levels, it may negatively affect paratenic hosts, such as small fish, and copepods, which act as first intermediate hosts (Ashworth et al. 1996).

The first record of *A. crassus* in Sweden was in 1988, and in the first study it was only found in coastal waters of the Baltic Sea and the south-west coast (Höglund et al. 1992). A few years later, *A. crassus* was found in several Swedish lakes, most likely as a result of stocking with infected wild eels caught on the Swedish west coast (Wickström et al. 1998). So far, *A. crassus* has not been reported from Vänern. However, as European eel has repeatedly been stocked in this lake, *A. crassus* may also have been introduced to Vänern. In Norway, *A. crassus* was first observed in a fish farm (Mo & Steien 1994) and has later been observed in eel in the lower part of a few rivers (Mo 2009). We are not aware of any studies of European eel or potential intermediate hosts in Norwegian lakes for the presence of *A. crassus*.

1.6.5.7 Aphanomyces astaci

Crayfish plague is caused by infection with the oomycete (a fungus-like eukaryote) *A. astaci*, a notifiable list 3 (national) disease. *A. astaci* was first detected in Norway in 1971 and has been detected in several Norwegian watercourses and lakes (Vrålstad et al. 2014). Crayfish plague can lead to 100% mortality in susceptible species, such as the noble crayfish. All stages of European crayfish species are highly susceptible. Crayfish plague, resulted in eradication of 98% of all crayfish populations in Sweden between 1900 and 2020 (Bohman 2021, Jussila and Edsman 2020). Crayfish plague has also eradicated several populations of noble crayfish in Norway.

A. astaci is native to North America and co-evolved as a parasite of North American crayfish species. North American crayfish are healthy carriers of *A. astaci*, with an immune defense that controls the *A. astaci* (Söderhäll and Cerenius 1999). These defense mechanisms are absent in freshwater crayfish species of other continents, resulting in rapid death following infection.

A. astaci can spread with its crayfish host, including dead hosts. It remains viable for 5 days, and possibly longer, in crayfish kept in water at 21 °C after dying of crayfish plague (Oidtmann et al. 2002). In addition to direct spread by crayfish, *A. astaci* can also spread by free-living zoospores in the water and by mechanical vectors (birds, water-active mammals, and human equipment) of the infective zoospores (Oidtmann et al. 2002, Oidtmann et al. 2005, Vrålstad et al. 2006). *A. astaci* fed to fish with infected abdominal cuticle were still viable after passage through the gastrointestinal tract (Oidtmann et al. 2002). Thus, fish that feed on infected crayfish can potentially spread crayfish plague.

1.6.5.8 White spot syndrome virus

White spot syndrome virus (WSSV) which causes white spot disease is listed as a notifiable list 2 (non-exotic diseases) disease in Norway. It is regarded as the most serious viral pathogen in cultured in prawn aquaculture. WSSV can cause mass mortality within 3-10 days following an initial outbreak in normal culture conditions (Dey et al. 2019).

WSSV can infect a wide range of crustaceans, including decapods (marine, brackish, and freshwater prawns, crabs, crayfish, and lobsters; Maeda et al. 1998, OIE 2019). It has been detected in Australian crayfish, *Cherax quadricarinatus*, in the aquarium trade in Europe (Mrugala et al. 2015), but not the wild in Europe. Signal crayfish and noble crayfish can be healthy carriers of WSSV at water temperatures between 4 °C and 12 °C, however, 100% mortality occurs at 22 °C (Jiravanichpaisal et al. 2004). This suggests that WSSV can cause mass mortality in noble crayfish and other freshwater crustaceans in Norway at temperatures above 20 °C. In the case of suspected disease outbreaks in Sweden, noble crayfish are tested for WSSV if they test negative for *A. astaci*. Signal crayfish are also tested for WSSV.

1.7 Effects of climate change

1.7.1 Future climates

The globally averaged combined land and ocean surface temperature shows a warming of 0.85 °C (0.65 °C to 1.06 °C) over the period 1880 to 2012, for which multiple and independently produced datasets exist (IPCC 2013). The rate of the warming has accelerated towards the present. Future climate change is expected to vary between and within regions and according to season. For the climate period 1971-2000, the mean annual air temperature of the area near Trysilelva varied between 0.1 °C and 2.0 °C. Given the CO₂ emission scenarios (representative concentration pathways) RCP 4.5 and RCP 8.5, the area can expect an annual temperature increases of about 2.6 and 4.6 °C, respectively, in the climate period 2071-2100 (Source: klimaservicesenter.no). The increase is expected to be highest during the winter months. Maximum summer temperatures will increase by about 2.6 °C and 3.6 °C given the scenarios RCP 4.5 and RCP 8.5, respectively (Figure 1.8-1).

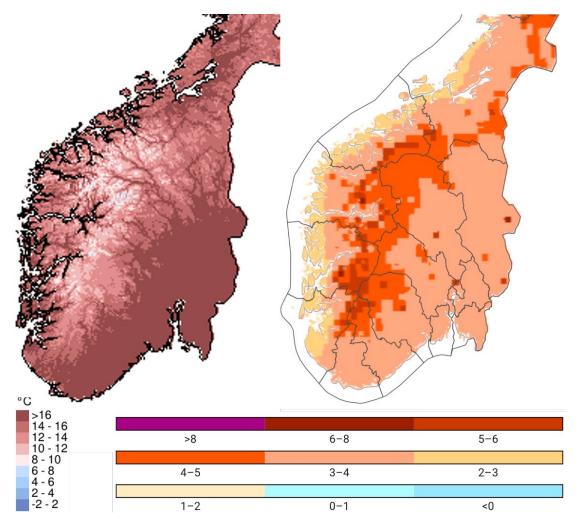


Figure 1.8-1: The average maximum summer temperatures for the climate reference period 1971-2000 for Norway (left) and projected change in maximum summer temperatures (°C) from 1971-2000 to 2071-2100 using the greenhouse gas emission scenario RCP 8.5 (right). The maps were adapted from www.klimaservicesenter.no.

Given the model uncertainties (about +/-1.1 °C for the climate period 2071-2100 for Eastern Norway), and as a precautionary principle, we assume an annual mean temperature of 5.0-6.5 °C for the area near Trysilelva in 2071-2100 and with the maximum temperature during the summer above 20 °C (Figure 1.8-1), which is in accordance with scenario RCP8.5. Using this scenario has been recommended by the Norwegian Biodiversity Information Centre (Sandvik et al. 2015) and in the national policy that deals with future climates.

1.7.2 Effects of climate change on the biology of the watershed

Climate change may impact both water discharge and temperature in watersheds (Hedenskog et al. 2015). Increased air temperatures will impact the seasonal variation in water discharge in Trysilelva/Klarälven, because precipitation, storage as snow and rainwater in the precipitation area, and evaporation throughout the seasons will change (Hedenskog et al. 2015). It is expected that the variation in water discharge during the year will be reduced, periods with high discharge during the winter may increase, the spring flood may be reduced and even disappear towards the end of the century, and the water discharge during spring and autumn may decrease. Water temperatures in the watershed will increase because of increased air temperatures, periods of lower water discharge, and less snowfall (Hedenskog et al. 2015). Climate change has already impacted Trysilelva/Klarälven. The air temperatures during the last 20 years have, in general, been unusually warm (Hedenskog et al. 2015), and the water temperatures have increased accordingly. In the outlet of Femunden, the water temperature for the period 15 June – 30 September increased by 0.05 °C each year in the period 1985-2012. In general, the river water temperatures increase from Femunden and southward. It is likely that the temperatures in downstream river stretches have increased in the same order of magnitude as the outlet of Femunden. In Vänern, the water temperature has increased by 0.036 °C each year during the period 1973-2012, based on measurements taken at 0.5 m, 5 m, and 10 m depths in June, August, and October (Hedenskog et al. 2015).

1.7.3 Relevant potential effects of temperature increase

Following an increase in temperature, at least four phenomena may occur: 1) some species with a preference for temperatures that are warmer than currently found in the area may be able to survive and establish, 2) some species may survive long enough to transmit infectious pathogens, 3) virulence shifts in some pathogens. For examples, water bodies in large areas in southern Norway will experience temperatures of sufficient warmth and for sufficient duration for WSSV to cause crayfish mass mortalities. The virus can be sustained in latent infections, and then cause high mortality rates when the temperature rises above 20 °C. And lastly 4) some species, such as Arctic charr and Atlantic salmon, may experience physiological stress that makes them more susceptible to infectious pathogens.

2 Methodology and Data

The risk of negative impacts on native biodiversity and ecosystems by importing Atlantic salmon eggs or live adults from Klarälven to Norway were assessed. Native biodiversity and ecosystems include the Norwegian part of Trysilelva/Klarälven watershed, but also other watersheds in Norway, because of the connection between Klarälven and Glomma watersheds through canals, and because organisms may be moved accidentally also to other watersheds in Norway via transport of for instance gear, fish or water.

Three methods of importing eggs or live adults were assessed, according to the terms of reference provided by the Norwegian Environment Agency:

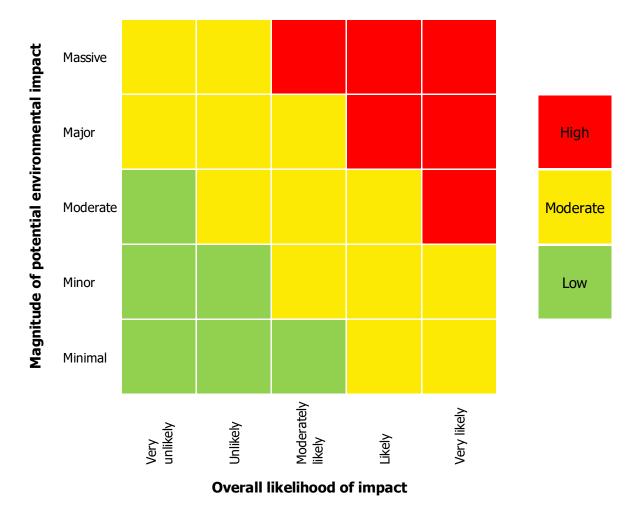
- I. Import of fertilised eggs to a local hatchery in Norway, which are planted in the river in the spring or hatched and released as juveniles or smolts.
- II. Import of fertilised eggs that will be used to establish a long-term broodstock in Norway using the gene bank model, from which eggs can be planted into the river, or transferred to a local hatchery with subsequent release of juveniles or smolts.
- III. Import of adult salmon spawners that are captured in the lower parts of Klarälven in Sweden, transported in tanks and released in the Norwegian parts of the watershed.

The risk assessment was based on a literature review and qualitative assessment of each of the three methods of importing eggs or fish. The risk of impacts on native biodiversity and ecosystems was assessed for relevant infectious agents, including parasites, bacterial pathogens, and viruses, and for other alien organisms. For each of the infectious agents and other alien species (hazards), the magnitude of the potential negative impact to native biodiversity and ecosystems was assessed, and the likelihood that negative consequences would be expected to occur. The likelihood of impact includes a combined assessment of the likelihood; 1) that the infectious agents and alien species will be transferred to Norway with the egg or fish transports, and 2) that the infectious agents and alien species that are not known to be present in Väneren today, assessment of the likelihood commenced by assessing the likelihood of introduction to Vänern during the period 2021-2100. The conclusion of the assessments in terms of low, moderate, or high risk, was based on the product of the magnitude of the potential negative consequences would be expected to occur.

A project group of experts drafted the qualitative assessments. The experts were selected to make sure the group members covered both broad and in-depth expertise on the potential hazards necessary to perform the assessment. VKM assessed potential conflict of interest for each of the members in regard to participation in the project group.

2.1 Methodology for risk assessment

We have used a qualitative risk assessment approach. The overall risk is the product of the magnitude of the consequences of the event and the likelihood that the event will occur, as judged by the project-group experts.



The resulting risks are presented in figures such as that of Figure 2.1-1.

Figure 2.1-1: The conclusion of the risk assessments (low, moderate, or high) is based on the overall likelihood of the negative impact and the magnitude of the potential consequences of that impact on Norwegian biodiversity.

In order to provide clear justification of why a particular rating is given in the risk assessment, the project group 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 2.1-1–2.1-3 below.

Rating	Descriptors
Minimal	No known impact on native biodiversity and ecosystems
Minor	Potential impact on biodiversity and ecosystems, but only occasional deaths of individuals, hence minor effect on biodiversity and ecosystems
Moderate	Impact may cause moderate reduction in viability and adaptability of native populations, hence moderate impacts on biodiversity and ecosystems
Major	Impact may cause severe reductions in native populations, hence major impacts on biodiversity and ecosystems
Massive	Impact may cause severe reductions in native biodiversity (local extinctions), hence massive impacts on biodiversity and ecosystems

Table 2.1-1 Ratings used for the assessment of the magnitude of the negative impact.

Table 2.1-2 Ratings used for the likelihood of impact.

Rating	Descriptors
Very unlikely	Negative consequences would be expected to occur with a likelihood of 0-5%
Unlikely	Negative consequences would be expected to occur with a likelihood of >5-10%
Moderately likely	Negative consequences would be expected to occur with a likelihood of >10-50%
Likely	Negative consequences would be expected to occur with a likelihood of >50-75%
Very likely	Negative consequences would be expected to occur with a likelihood of >75-100%

Table 2.1-3 Ratings used for describing the level of confidence for both impact and likelihood.

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 Information gathering and literature search

We have used ISI Web of Science core collection as primary source of scientific information. In addition, we performed general searches in Google Scholar. The searches included species name (or synonyms or common name) AND specific terms, such as "invasiveness", "invasive", "alien", non-native", "introduced", "disease", "parasite", "pathogen", "virus", "bacteria", "fish migration", "power station", geographic name, such as "Trysil", "Vänern", "Klarälven", "Sweden", and "Glomma".

We conducted a general Google search using the scientific or English species name. These searches sometimes revealed webpages or grey literature with relevant information. Finally, the involved experts used their extensive databases of relevant scientific literature.

The watershed and fish populations are extensively and thoroughly described by Hedenskog (2015). We have used their report extensively, including the references therein and other studies from the watershed regarding descriptions and understanding of the area and the local Atlantic salmon populations and other fish species.

3 Potential hazards to biodiversity in Norway

VKM assessed three proposed methods of import and release of eggs or live fish from landlocked salmon from Klarälven in Sweden to Trysilelva in Norway. The impact of the potential hazards is equal for all the three methods, but the likelihood of them occurring varies among the methods. The three methods are:

- <u>Method I</u>: Import of fertilized eggs that are planted in the river, or hatched in a local hatchery and released as fry or smolts.
- <u>Method II</u>: Establishment of a designated gene bank from which eggs are planted in the river, or hatched in a local hatchery and released as fry or smolts.
- <u>Method III</u>: Import of adult salmon that are captured in Klarälven, transported to Norway and released in the Norwegian parts of the watershed prior to spawning.

Methods I and II are both based on import of salmon eggs, but the two methods imply a significant difference in the number of eggs that will cross the Swedish-Norwegian border, which is relevant for the risk analysis. During a ten-year period when the gene bank is established (method II), 300 eggs from 10 female salmon per year will be transported across the border, which is 3,000 eggs per year and a total of 30,000 eggs during the ten-year period. In addition, new eggs will need to be imported periodically to refresh the gene pool. Method I, however, will require the import of a much higher number of eggs. Based on the same effort (number of female fish) per year, eggs from 10 female salmon, with an average body weight of 3.5 kg and 1450 eggs per kg body mass, will result in 50,750 eggs being transported across the border per year. Over 80 years, this will add up to over 4 million eggs being imported. This is a calculation example, and clearly in the lower range for method I, because 50,750 eggs per year constitutes less than 0.2% of the spawning target on the Norwegian side of the border. In comparison, the gene bank model will be able to produce 1 million eggs per year once established.

In the assessment of the three methods, we assume that current regulations are followed. When applicable, established practices beyond the minimum level implicated in the regulations are also assumed. Specifically, the risk assessment includes the following assumptions:

Method I: We assume that eggs are disinfected as described in section 1.4.1, and that kidney tissue from female broodfish are tested for *R. salmoninarum* by a validated method with high sensitivity; if positive, we assume eggs from these fish are not used. We also assume that target tissues kidney, myocardium and spleen from up to six female fish are pooled and tested on cell cultures (BF-2 and FHM) and that cytopatogen effects are further investigated with appropriate methods (ELISA, qPCR and sequencing). We assume that a potential holding period in a hatchery does not influence the risk.

Method II: We assume that the gene bank is situated in Norway and is dedicated to nonanadromous salmonids. Furthermore, we assume that the same procedures and practices as in other Norwegian gene banks are used, and that any transport of eggs or live fish for release in the watershed uses groundwater. We also assume that imported eggs are disinfected according to procedure described in 1.4.1.1., and that all broodfish (male and female) are subject to a thorough postmortem examination, sampling of suitable target tissue (head kidney) and tested for IPNV, *R. salmoninarum*, and PRV-1. Upon detection of any of these agents, gametes are rejected. We further assume that reestablishment is based on either planting of eggs from the gene bank directly in Trysilelva, or that eggs from the gene bank are moved to a local hatchery and subsequently released as fry or smolts. We assume that a potential holding period in a hatchery does not influence the risk.

Method III: In this risk assessment, we assume that broodfish are captured in the trap at Forshaga and subsequently transported to a holding place near the Höljes dam. We further assume that all broodfish, immediately before transfer to Norway, are held continuously in a tank with at least 25ppt salinity for 14 days and that during this period, new fish have not been added to the tank. We also assume that the broodfish are held in water from the river upstream of the Höljes dam or in groundwater, such that reinfection is avoided.

3.1 Introduction of agents infectious for fish

Many infectious agents included in this risk assessment were unknown until disease caused by the agents emerged in aquaculture. Accordingly, a significant proportion of available knowledge stems from experience and research developed in the aquaculture setting. For Atlantic salmon, knowledge has especially developed in conjunction with marine aquaculture. Although aquaculture is an important industry in Northern Europe, it is a relatively young industry, still expanding in terms of production volume, production forms, areas, and aquaculture species. New knowledge is constantly emerging from the aquaculture setting, and there is a lack of knowledge about the presence of specific infectious agents, their epizootiology, biophysical properties in the ecosystems, and range of susceptible hosts.

This risk assessment aims to assess the impact of introducing infectious agents that are novel to the Trysilelva ecosystem. There is a lack of knowledge of how many and which of these agents have the potential to interact with the fish species that are present in this ecosystem, and how environmental factors will influence this interaction.

A particular challenge for this risk analysis is the potential presence of unknown and/or undescribed infectious agents in Vänern, Trysilelva/Klarälven, and the connecting waterways. A further challenge is the potential for future introductions to Vänern and Klarälven of known or unknown infectious agents through aquaculture, release of imported European eels, putand-take industry, ballast water, and other human activities from present date, and until year 2100.

During the nearly 80-year time frame from 2021 to 2100 that is covered by this risk analysis, it is likely that various factors that are highly relevant to the risk analysis will change. It is

predicted that the climate will continue to change. Increasing temperatures may worsen the impact of infectious agents that are present in the area today and could also facilitate establishment of new infectious agents if these are introduced to the area.

The main routes of introduction of infectious agents are by movement of fish and fish eggs (Peeler et al., 2011). This applies both at the country level, and on the micro level such as a production area, farm, water catchment, river, or pond. It is a stated strategy for the Swedish authorities (Jordbruksverket) to increase aquaculture food production, and also to sustain growth within tourist fishing (put-and-take fishing) (Regeringens prop. 2016/2017:104). Although aquaculture production in Sweden has increased over the last decade, a drop in production during 2017-2018 was partly explained by disease-related losses (Jordbruksverket 2020). The Swedish aquaculture industry is not self-sufficient with eggs and juvenile fish, resulting in an increasing demand for import of fertilized roe and fry. This, in turn, entails risks of introducing infectious agents. The main species farmed in Sweden are rainbow trout and Arctic char, but there is also a development towards aquaculture based on import of new and even exotic fish species, such as tilapia. The ongoing imports of wild-caught European eel also represent a risk concerning a wide range of pathogens relevant to this risk assessment, including IPNV, IHNV, and rhabdoviruses (other than VHSV).

Even if the probability of introducing at least one infectious agent in one import is low, the cumulative probability will increase with increasing number of imports (Figure 3.1-1). If P is the probability of introducing at least one infectious agent through a random import, then (1-P) is the probability of not introducing an infectious agent during that import. Then, the cumulative probability of importing at least one infectious agent during n imports can be calculated by the formula 1- $(1-P)^n$.

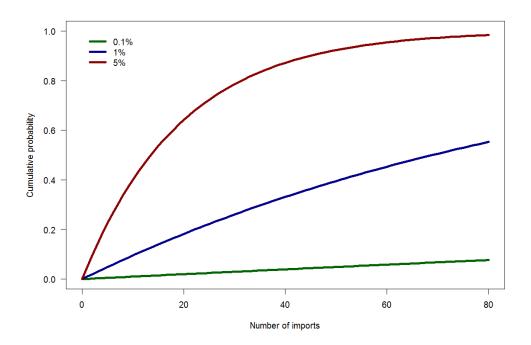


Figure 3.1-1: The cumulative probability of introducing at least one infectious disease agent increases with the number of imports, shown at three different levels of probability (0.1%, 1%, and 5%) (Jarp et al. 1996).

3.1.1 Introduction of viruses

3.1.1.1 HAZARD IDENTIFICATION

Some of the viral agents that are associated with serious diseases in fish in aquaculture or wild fish are described in chapter 1.6.3. The most important ones have been assessed here.

Infectious haematopoietic necrosis virus (IHNV)

Infectious haematopoietic necrosis virus (IHNV) causes infectious haematopoietic necrosis (IHN) and mortality in a range of species, including Atlantic salmon (reviewed by Dixon et al., 2016). It is primarily a disease of juvenile fish.

Viral haemorrhagic septicemia virus (VHSV)

Viral haemorrhagic septicemia virus (VHSV) has caused mass mortalities in several fish species in the Great Lakes in USA and Canada. VHSV has been detected in more than 80 different fish species and has a pronounced ability to adapt to new host species.

Infectious salmon anaemia virus (ISAV)

Infectious salmon anaemia virus (ISAV) is potentially a serious pathogen for farmed Atlantic salmon, but there are currently no indications that ISAV is present in the export or import

areas discussed in this report. It can occur as asymptomatic infections in salmon (HPR0) or brown trout. The ecological impacts on wild fish populations are unknown.

Salmonid alpha virus (SAV)

The salmonid alpha virus (SAV) subtype SAV3 may cause pancreas disease in farmed salmon, but there are currently no indications that SAV is present in the export or import areas discussed in this report. The SAV2 variant may cause sleeping disease in farmed rainbow trout and arctic char. The ecological impacts on wild fish populations are unknown.

Infectious pancreatic necrosis virus (IPNV)

Infectious pancreatic necrosis virus (IPNV) may cause disease in farmed salmonids (mainly at juvenile stages). The virus is found in many wild and farmed fish species, and is capable of vertical transmission. It is resistant to disinfection and drying. IPNV is present in Vänern and has the potential to cause disease in wild fish.

Salmon gill poxvirus (SGPV)

Salmon gill poxvirus (SGPV) may cause disease in farmed salmon, but has not been verified in other relevant salmonids (trout, char, rainbow trout). There is limited information regarding how SGPV may impact wild fish (salmon) at different life stages. There are currently no indications that SGPV is present in the export or import areas discussed in this report.

Piscine orthoreovirus (PRV-1 and PRV-3)

Piscine orthoreovirus (PRV) is associated with disease in Atlantic salmon (PRV-1), rainbow trout (PRV-3), and brown trout (PRV-3). Both PRV-1 and PRV-3 cause disease and mortality in fish at all life stages. Both viruses infect the red blood cells, but also cause pathology in the myocardium, which means that infected fish are susceptible to other stressors.

Rhabdoviruses (other than VHSV and IHNV)

These are emerging diseases in Europe, and knowledge is being acquired following mortalities in different fish species for after entry into aquaculture. Mass mortalities in wild species have also been recorded. They apparently have the ability to adapt to several species.

3.1.1.2 HAZARD CHARACTERIZATION

Introduction of new infectious agents, including virus, to a naïve population can have dramatic consequences. Viral diseases may have a negative impact on wild-fish populations by causing disease outbreaks with mass mortality (die-offs) and subsequent population reductions beyond what can be compensated for by surplus juveniles produced in the system. Infectious diseases may also have more subtle effects, such as reduced fitness and

growth, with secondary impacts on reproduction (fecundity), longevity, and susceptibility to other infectious agents. However, infections that have dramatic consequences in densely populated tanks and cages in an aquaculture facility do not necessarily have the same consequences in wild fish nor on the biodiversity in ecosystems.

Infectious haematopoietic necrosis virus (IHNV)

IHN is mainly a disease in juveniles, in which case the disease may be difficult to observe in wild fish. Infection in other life stages has been observed in aquaculture. Infected fish that survive remain lifelong carriers. The infection can be reactivated during spawning, with consequent shedding of virus in ovarian fluid and milt. Thus, vertical transmission in wild fish is likely. IHNV is expected to have a **major impact** in species that are susceptible and develop disease. Further spread of IHNV after introduction to Trysilelva is also expected due to migration of fish in connected watersheds. Effective measures that limit the spread of infection or reduce the impact of the disease are not available. **Moderate confidence**.

Viral haemorrhagic septicaemia virus (VHSV)

VHS has caused mass mortalities in the North American Great Lakes, affecting more than 28 fish species, and therefore it is expected that introduction and establishment of VHSV in Trysilelva would have a **massive impact** with severe reductions in local biodiversity and serious consequences for ecosystem functions and services. The epizootic in North America is still ongoing and has spread to new catchments. Further spread of VHSV after introduction to Trysilelva by migration of fish in connected watersheds would also be expected. Effective measures that limit the spread of infection or reduce the impact of the disease are not available. **Very high confidence**.

Infectious salmon anaemia virus (ISAV)

For ISAV, it is necessary to distinguish between infections with the non-virulent ISA-HPR0 and the virulent ISA-HPRdel. Whereas, ISA-HPR0 causes a transient infection of epithelial cells of gills and the skin, ISA-HPRdel infects and propagates in endothelial cells, causing serious disease and mortality. The transition from HPR0 to viable HPRdel occurs rarely, and is assumed to occur in a stepwise fashion, with intermediate stages with lower ability to transfer and establish. The host range of these viruses limits their impact, although the susceptibility of pike, perch, European whitefish, Arctic char, and grayling is unknown.

ISA-HPR0 **Minimal impact, medium confidence** (information is lacking regarding the impact on freshwater species)

ISA-HPRdel **Moderate impact**, **low confidence** (information is lacking regarding impact on freshwater species)

Salmonid alphavirus (SAV)

Introduction of SAV to the Trysilelva watershed may have an impact on the native fish populations. **Moderate impact** with **low confidence**

Infectious pancreatic necrosis virus (IPNV)

IPNV is present in the export area and can be transmitted vertically and horizontally among many fish species. Survivors of infection can establish persistent-shedder status. Transfer of this virus to Trysilelva would be expected to have **minor impact** with **medium confidence**.

Salmon gill poxvirus (SGPV)

SGPV is a potential pathogen for salmon and other salmonids in Trysilelva. However, it is not present in the export area. **Minimal impact, low confidence**

Piscine orthoreovirus-1 (PRV-1)

This virus could have an impact on Atlantic salmon in Vänern, while the susceptibility of, and the impact on, other species, such as pike, perch, European whitefish, Arctic char, and grayling, is unknown. Current knowledge suggests the ability for this virus infection to become endemic in populations of Atlantic salmon and certain populations of Pacific salmon. However, it is not certain that the reestablished populations of Vänern salmon will be large enough to sustain an endemic population. It is therefore possible that the infection would die out after introduction. **Minor impact, low confidence.**

Piscine orthoreovirus-3 (PRV-3)

This virus has an impact on brown trout, but the susceptibility and impact on pike, perch, European whitefish, Arctic char, and grayling are unknown. Brown trout is an established species in the Trysilelva ecosystem. As PRV-3 is endemic in sea trout along the coast of Norway, the virus could become endemic in brown trout if introduced to this ecosystem. **Moderate impact, medium confidence.**

Rhabdoviruses other than IHNV and VHSV

Viruses in this group have caused disease and mortality in a wide range of species, including species that are present in Trysilelva. **Moderate impact, very low confidence**

3.1.1.3 LIKELIHOOD

Infectious haematopoietic necrosis virus (IHNV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of IHNV is as follows:

• <u>Method I</u>: **Unlikely**, with **high confidence**.

- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

Viral haemorrhagic septicaemia virus (VHSV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of VHSV is as follows:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

Infectious salmon anaemia virus (ISAV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of ISAV (ISA-HPR0 and ISA-HPRdel) is as follows:

- <u>Method I</u>: **Unlikely**, with **high confidence**.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

Salmonid alpha virus (SAV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of SAV is as follows:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.
- Infectious pancreatic necrosis virus (IPNV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of IPNV is as follows:

- <u>Method I</u>: Very unlikely, with high confidence.
- Method II: Very unlikely, with high confidence.
- <u>Method III</u>: Likely, with medium confidence.
- •
- Salmon gill poxvirus (SGPV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of SGPV is as follows:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Unlikely**, with **medium confidence**.

• *Piscine orthoreovirus-1 (PRV-1)*

Salmon in Vänern are likely susceptible to PRV-1 infections. There is a lack of knowledge regarding the presence of PRV-1 in Vänern. The project group therefore assesses that the likelihood of negative impact on biodiversity in Norway of PRV-1 is as follows:

- <u>Method I</u>: **Unlikely**, with **low confidence**.
- <u>Method II</u>: Very unlikely, with low confidence.
- <u>Method III</u>: Moderately likely, with medium confidence.

Piscine orthoreovirus-3 (PRV-3)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of PRV-3 is as follows:

- <u>Method I</u>: Very unlikely, with low confidence.
- <u>Method II</u>: Very unlikely, with low confidence.
- <u>Method III</u>: **Unlikely**, with **medium confidence**.

Rhabdoviruses (other than IHNV and VHSV)

The project group assesses that the likelihood of negative impact on biodiversity in Norway of other rhabdoviruses is as follows:

- <u>Method I</u>: **Unlikely**, with **very low confidence**.
- <u>Method II</u>: Very unlikely, with very low confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

3.1.1.4 RISK CHARACTERIZATION

Infectious haematopoietic necrosis virus

The project group assesses that the overall risk of negative impact on biodiversity in Norway of IHNV is as follows:

- <u>Method I</u>: **Moderate**, with **medium confidence**.
- <u>Method II</u>: Moderate, with medium confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

Viral haemorrhagic septicaemia virus

The project group assesses that the overall risk of negative impact on biodiversity in Norway of IHNV is as follows:

- <u>Method I</u>: Moderate, with medium confidence.
- <u>Method II</u>: **Moderate**, with **medium confidence**.

• <u>Method III</u>: High, with medium confidence.

Infectious salmon anaemia virus - HPRO

The project group assesses that the overall risk of negative impact on biodiversity in Norway of ISAV-HPR0 is as follows:

- <u>Method I</u>: Low, with medium confidence.
- <u>Method II</u>: Low, with medium confidence.
- <u>Method III</u>: Low, with medium confidence.

Infectious salmon anaemia virus - HPRdel

The project group assesses that the overall risk of negative impact on biodiversity in Norway of ISAV-HPRdel is as follows:

- <u>Method I</u>: **Moderate**, with **medium confidence**.
- <u>Method II</u>: Low, with medium confidence.
- <u>Method III</u>: Moderate, with medium confidence.

Salmonid alpha virus

The project group assesses that the overall risk of negative impact on biodiversity in Norway of SAV is as follows:

- <u>Method I</u>: Low, with medium confidence.
- <u>Method II</u>: Low, with medium confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

Infectious pancreatic necrosis virus

The project group assesses that the overall risk of negative impact on biodiversity in Norway of IPNV is as follows:

- <u>Method I</u>: Low, with medium confidence.
- <u>Method II</u>: Low, with medium confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

Salmon gill poxvirus

The project group assesses that the overall risk of negative impact on biodiversity in Norway of SGPV is as follows:

• <u>Method I</u>: Low, with low confidence.

- <u>Method II</u>: Low, with low confidence.
- <u>Method III</u>: Low, with low confidence.

Piscine orthoreovirus-1

The project group assesses that the overall risk of negative impact on biodiversity in Norway of PRV-1 is as follows:

- <u>Method I</u>: Low, with low confidence.
- <u>Method II</u>: Low, with low confidence.
- <u>Method III</u>: **Moderate**, with **low confidence**.

Piscine orthoreovirus-3

The project group assesses that the overall risk of negative impact on biodiversity in Norway of PRV-3 is as follows:

- <u>Method I</u>: Low, with low confidence.
- <u>Method II</u>: Low, with low confidence.
- <u>Method III</u>: **Moderate**, with **low confidence**.

Rhabdoviruses (other than VHSV, IHNV, and SVCV)

The project group assesses that the overall risk of negative impact on biodiversity in Norway of other Rhabdoviruses is as follows:

- <u>Method I</u>: **Moderate**, with **low confidence**.
- <u>Method II</u>: **Low**, with **low confidence**.
- <u>Method III</u>: **Moderate**, with **low confidence**.

3.1.2 Introduction of bacterial pathogens

3.1.2.1 HAZARD IDENTIFICATION

Renibacterium salmoninarum - aetiological agent of bacterial kidney disease (BKD)

This is a potentially serious pathogen of salmonids, particularly juvenile fish, but also has the potential to induce lifelong subclinical carrier status. There is a proven capability for intracellular vertical transmission, and it is difficult to remove by disinfection. Historic spread via anthropogenic activities has been documented. Likely low-grade endemic amongst wild salmonids in some areas of western Norway. Although previously confirmed in Vänern/Klarälven's drainage basins, it has not been detected here since the mid 90s despite extensive screening of broodfish. The possibility for low-grade endemism amongst wild salmonids in Vänern/Klarälven can however not be ruled out. It is present in some Swedish rivers, with a few annual detections.

Aeromonas salmonicida subspecies *salmonicida* - aetiological agent of furunculosis

This is a potentially serious pathogen of salmonids and also some other fish species. Passive vertical transmission may occur. It is readily removed by disinfection. *A. salmonicida* subsp. *salmonicida* is endemic amongst wild salmonids in one area of mid-Norway, and is also present in Sweden, with a few annual detections, but has not been reported in Vänern/Klarälven.

'Atypical' Aeromonas salmonicida - aetiological agents of 'atypical' furunculosis

This group constitutes a diverse group of bacteria with worldwide distribution, with the wide range of variants often displaying specific host preferences. May cause serious disease in their respective hosts. Several variants have been reported from Sweden, including some connected to Vänern/Klarälven.

Flavobacterium psychrophilum - aetiological agent of flavobacteriosis or rainbow trout fry syndrome

This is a potentially serious pathogen of salmonids and some other fish species. It is common in temperate freshwater environments, and virulent strains have likely been spread anthropogenically. Vertical transmission can probably occur and it may be resistant to some disinfection procedures. Outbreaks are common amongst farmed rainbow trout in Sweden, but it is unknown if virulent strains exist in Vänern/Klarälven.

Flavobacterium columnare - aetiological agent of columnaris disease

This is a potentially serious pathogen of many different freshwater fish species, including salmonids. Clinically, it is essentially irrelevant at water temperatures below 15 °C. Although it has been reported in salmonid fish farming in Sweden, it has never been reported in Norway. Unknown status in Vänern/Klarälven.

Yersinia ruckeri - aetiological agent of yersiniosis or enteric redmouth disease

This is a potentially serious pathogen of farmed salmonids worldwide. A few anthropogenically spread, host-specific strains are apparently responsible for most outbreaks in aquaculture globally, with environmental strains common in freshwater environments. It is considered susceptible to disinfection. The international 'rainbow trout' strain has been detected in Sweden, but not in Norway. It is unknown whether virulent strains exist in Vänern/Klarälven.

3.1.2.2 HAZARD CHARACTERIZATION

The impacts (cf. Table 2.1-1) of introducing the bacteria described above into Femund- and Trysilvassdraget, as outlined below, are assessed with the assumption that these agents (or virulent strains thereof) are not already present here, and that native susceptible fish populations are naïve concerning these bacteria. For neither of the listed agents, this is known with certainty.

Renibacterium salmoninarum

Introduction may potentially entail serious consequences for wild populations of salmonids. BKD outbreaks, particularly amongst juvenile fish, may involve high mortalities. Although the disease essentially only affects salmonid fish species, some uncertainty exists regarding the susceptibility of these species in the wild. Atlantic salmon are considered amongst the most susceptible hosts. As this bacterial species is intracellular, can transmit vertically, and has the potential for inducing lifelong, subclinical carrier status in exposed individuals, it is particularly challenging to eradicate once it has been introduced into a population of susceptible hosts. For these reasons, we consider the **impact** of introduction to be **major**, with **medium confidence**.

Aeromonas salmonicida subspecies salmonicida

Introduction may involve serious consequences for wild salmonid populations. Should the pathogen become established here, furunculosis outbreaks could occur, particularly when water temperatures are high. Although less likely, populations of non-salmonid fish could also be affected. We therefore consider the **impact** of introduction to be **major**, with **medium confidence**.

'Atypical' Aeromonas salmonicida

The potential consequences of introduction will likely depend largely on the host range and virulence of the bacterial strain. Some strains may cause serious disease, with high mortality, in their hosts. Overall, we consider the **impact** of introduction to be **minor**, with **low confidence**.

Flavobacterium psychrophilum

The consequences of introduction would likely be partially dependent on the bacterial strain and its virulence. Environmental strains are common in temperate freshwater environments. Although virulent strains may cause serious disease in salmonid fish, the salmonid species native to Femund- and Trysilvassdraget are not considered among the most susceptible. We consider the **impact** of introduction to be **minor**, with **low confidence**.

Flavobacterium columnare

The consequences of introduction could potentially be significant, and the range of susceptible fish species may be wide. Elsewhere in the world, virulent strains have occasionally caused high mortalities amongst populations of wild fish. However, such outbreaks do require higher water temperatures than those normal for Femund- and Trysilvassdraget today; but these may change over time. We consider the overall **impact** of introduction to be **moderate**, with **low confidence**.

Yersinia ruckeri

The potential consequences of introduction would likely be partially dependent on the bacterial strain. Presumed avirulent/low-virulent strains are prevalent in many freshwater environments. Outbreaks in salmonid aquaculture due to virulent strains sometimes occur

subsequent to a possibly triggering stress component. We consider the **impact** of introduction to be **minimal**, with **medium confidence**.

3.1.2.3 LIKELIHOOD

Renibacterium salmoninarum

The likelihood of introduction and impact will depend largely on the import method used. Although we do not find reports of *R. salmoninarum* being detected in Vänern/Klarälven over recent years, it has been detected in this region previously, and low-grade endemism amongst wild salmonid populations here cannot be ruled out.

Traditional screening methods for this species involve lethal sampling from kidneys, and although non-lethal methods have been developed, these have not been comprehensively tested *in natura*. This particularly regards their ability to detect subclinical carrier fish, which can be a lifelong condition for *R. salmoninarum*. Thus, if subclinical carriers occurred at low prevalence within the source population, certification (by sampling) of individual fish batches as being infection-free would be inexpedient, as this would require a relatively large number of fish to be sacrificed.

Furthermore, *R. salmoninarum* is intracellular and can be transmitted vertically, and therefore superficial disinfection of ova (by, e.g., iodine) is ineffective. Notably also, broodstock screening efforts in Sweden have concentrated exclusively on vertical transmission via ova and disregarded the undetermined possibility of spread via milt. Thus, according to currently applicable regulations and practices (see chapter 1.4), in Sweden only female broodstock sourcing eggs would be screened for *R. salmoninarum*, while this would involve both male and female broodstock in a Norwegian gene bank. Imports by both methods I and II would thus involve some form of screening for *R. salmoninarum*, but this would not apply to method III, according to current practices.

For these reasons combined, the **likelihoods** for *R. salmoninarum* having negative impacts are evaluated as follows:

- <u>Method I</u>: **Unlikely**, with **medium confidence**.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: Likely, with low confidence.

Aeromonas salmonicida subspecies salmonicida

The status of Vänern/Klarälven with regard to *A. salmonicida* subsp. *salmonicida* is uncertain, although no detections have been reported here in recent years. Although the primary route of transmission is horizontal, passive vertical transmission via sexual products may be possible, but the species is susceptible to most standard disinfection procedures. Within endemically infected fish populations, infection prevalences are likely to increase during the summer, when most furunculosis outbreaks occur. For *A. salmonicida* subsp. *salmonicida*, the **likelihoods** of negative impacts are thus evaluated as follows:

• <u>Method I</u>: **Unlikely**, with **medium confidence**.

- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

'Atypical' Aeromonas salmonicida

Members of this bacterial group have been confirmed in Vänern/Klarälven, but genotype data are not available. Various genotypes/variants, some with apparent predilections for salmonid fish, have previously been characterized from Sweden. Transmission pathways and disinfection susceptibility are likely to be similar as those for *A. salmonicida* subsp. *salmonicida*, but temperature preferences may differ between the variants. The **likelihoods** for the 'atypical' *A. salmonicida* group having neagive impact are evaluated as follows:

- <u>Method I</u>: **Unlikely**, with **low confidence**.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

Flavobacterium psychrophilum

This species is common in temperate freshwater environments, and probably also Vänern/Klarälven. It may reside intracellularly within egg cells and can likely transmit vertically via sexual products. However, tests for this are not routinely conducted. It is only sporadically found in Atlantic salmon. Whether standard superficial disinfection measures (e.g., iodine) result in full elimination is uncertain. The **likelihoods** for *F. psychrophilum* having negative impact are evaluated as follows:

- <u>Method I</u>: **Unlikely**, with **low confidence**.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: **Moderately likely**, with **low confidence**.

Flavobacterium columnare

As with other members of the *Flavobacterium* genus, this species is likely to be naturally present in many freshwater environments, but is generally considered to thrive at water temperatures above those usually recorded in this area. However, these may increase over time. Survival in water and sediment is prolonged. The **likelihoods** for *F. columnare* having negative impact are evaluated as follows:

- <u>Method I</u>: **Unlikely**, with **low confidence**.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: **Unlikely**, with **low confidence**.

Yersinia ruckeri

Recent studies indicate this species to be common in many freshwater environments, and thus also likely in Vänern/Klarälven. Subclinical carrier status has been documented to occur, but it is unknown how long this may last. The **likelihoods** for *Y. ruckeri* having negative impact are evaluated as follows:

• <u>Method I</u>: **Unlikely**, with **low confidence**.

- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: Moderately likely, with low confidence.

3.1.2.4 RISK CHARACTERIZATION

Based on the impacts and likelihoods assessed in the preceding sections, the overall risks associated with the identified bacterial hazards, per proposed import method, are evaluated below.

Renibacterium salmoninarum

- <u>Method I</u>: **Medium**, with **medium confidence**.
- <u>Method II</u>: **Medium**, with **medium confidence**.
- <u>Method III</u>: **High**, with **low confidence**.

Aeromonas salmonicida subspecies salmonicida

- <u>Method I:</u> Medium, with medium confidence.
- <u>Method II:</u> Medium, with medium confidence.
- <u>Method III:</u> Medium, with low confidence.

'Atypical' Aeromonas salmonicida

- <u>Method I:</u> Low, with low confidence.
- <u>Method II:</u> Low, with medium confidence.
- <u>Method III:</u> Medium, with low confidence.

Flavobacterium psychrophilum

- <u>Method I:</u> Low, with low confidence.
- <u>Method II:</u> Low, with low confidence.
- <u>Method III:</u> Medium, with low confidence.

Flavobacterium columnare

- <u>Method I: Medium</u>, with low confidence.
- <u>Method II:</u> Low, with low confidence.
- <u>Method III:</u> Medium, with low confidence.

Yersinia ruckeri

- <u>Method I:</u> Low, with low confidence.
- <u>Method II:</u> Low, with medium confidence.
- <u>Method III:</u> Low, with low confidence.

3.1.3 Introduction of the parasite Gyrodactylus salaris

3.1.3.1 HAZARD IDENTIFICATION

Gyrodactylus salaris (see chapter 1.6.5.1) is a monogenean that parasitises the external surfaces (skin, fins and gills) of salmonids, mainly Atlantic salmon. This parasite uses one host to complete its lifecycle and is thus only harmful to its fish host. *G. salaris* is an invasive species in Norway that has been introduced several times and later spread to 51 rivers and 39 fish farms with translocation of live fish, with infested escaped farmed fish, or with migrations of wild fish between rivers. In Norwegian rivers, *G. salaris* has caused mass mortalities of Atlantic salmon parr. The yearly mean parasite induced mortality in Atlantic salmon juveniles is estimated to be 86%.

In Klarälven and Vänern, two (or three) haplotypes of *G. salaris* occur on salmon. These haplotypes differ from those found in Norway and their pathogenicity to Norwegian salmon is unknown. However, as Norwegian salmon populations are, in general, highly susceptible to *G. salaris*, it is assumed that the haplotypes in Klarälven and Vänern will cause gyrodactylosis in any Norwegian salmon population.

3.1.3.2 HAZARD CHARACTERIZATION

If *G. salaris* is introduced and established in the Atlantic salmon in Trysilelva, the potential negative consequences for Norwegian Atlantic salmon populations are **massive** with **high confidence.** After establishment on salmon in Trysilelva, and potentially on other salmonids in the associated lakes, *G. salaris* can be spread with migrating salmon or other salmonids to the Atlantic salmon population in Glomma river. Furthermore, *G. salaris* can be spread otherwise to rivers with wet outdoor equipment such as fishing gear and nets, waders, canoes, and small transportable boats. The distance from Trysilelva watercourse to river stretches with anadromous Atlantic salmon in some of the rivers draining to Trondheimsfjorden is short, both in kilometres and in driving time.

3.1.3.3 LIKELIHOOD

The project group assesses that the likelihood of introduction of *G. salaris* and subsequent negative impact on biodiversity in Norway is:

- <u>Method I</u>: **Unlikely**, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: Likely, with medium confidence.

3.1.3.4 RISK CHARACTERIZATION

The Norwegian Authorities assume that a treatment in 25 parts per thousand salinity for 14 days will kill all *G. salaris* on broodfish. However, this has yet to be documented.

Furthermore, it is likely the this treatment procedure will fail at least once in the period until 2100. An introduction of one *G. salaris* specimen is sufficient for establishment of *G. salaris* in a susceptible Atlantic salmon population.

The project group assesses that the likelihood of introduction of *G. salaris* and subsequent negative impact on biodiversity in Norway is:

- <u>Method I</u>: **Moderate**, with **high confidence**.
- <u>Method II</u>: **Moderate**, with **high confidence**.
- <u>Method III</u>: **High**, with **high confidence**.

3.1.4 Introduction of other parasites

3.1.4.1 HAZARD IDENTIFICATION

Five selected parasite species, in addition to *G. salaris*, are described in chapter 1.6.5.2.-6. We are not aware of examinations of salmonids to document their absence or presence in Klarälven/Vänern, nor in the Norwegian part (Trysilelva/Femunden) of the watercourse. However, it is likely that some, if not all, are present in Vänern, especially considering their establishment opportunities after the Göta Canal was built.

The five selected parasite species use fish as either definitive or intermediate host, but none of them can be transmitted vertically from mother broodfish to eggs. Thus, these parasites will not be imported with eggs. On the other hand, bath treatments of broodfish will not kill any of the parasites, because they live inside the fish or they live attached to the gills and are not significantly affected by the bath treatment.

Ichthyophthirius multifiliis (chapter 1.6.5.2)

This parasitic ciliate has caused severe disease outbreaks and mortality in farmed and wild salmonids in many countries and recently in European eel in a river on the Swedish west coast. *I. multifiliis* cause skin damage to the fish host. This parasite uses one host to complete its lifecycle. Ichthyophthiriosis, often termed Ich or white spot disease, occurs mostly in the warm period of the year

Tetracapsuloides bryosalmonae (chapter 1.6.5.3)

This myxozoan parasite causes proliferative kidney disease (PKD) in salmonids and has caused severe disease outbreaks in farmed and wild salmonids in many areas of the northern hemisphere. *T. bryosalmonae* mainly causes damage to the kidneys of the fish host, but other organs can also be severely negatively affected. The frequency and severity of PKD outbreaks are associated with climate change.

Myxobolus cerebralis (chapter 1.6.5.4)

This myxozoan parasite causes whirling disease in salmonids and has caused severe disease outbreaks in farmed and wild fish. *M. cerebralis* causes cartilage damage and skeletal deformities to the fish host, as well as damage to the nervous system that results in the whirling behaviour. This parasite uses two hosts to complete its lifecycle. The definitive host is oligochaetes (*Tubifex*) can also be affected if the parasite is introduced.

Salmincola spp. (chapter 1.6.5.5)

These parasitic crustaceans (copepods) attach to, and feed mainly on, the gills of salmonids. Large parts of the gill lamellae may disappear and tissue responses in the remaining parts may result in reduced oxygen uptake. This can reduce the migratory capacity of its host, and also reduce host survival in warm periods with less dissolved oxygen in the water. These parasites use one host to complete their lifecycle, and most species use only one host species.

Anguillicoloides crassus (chapter 1.6.5.6)

This invasive, Asian parasitic nematode (roundworm) causes inflammation and bleeding in the swim bladder of European eel and has caused disease outbreaks in wild and farmed eel. This parasite uses several hosts at several trophic levels to complete its lifecycle and these hosts can also be affected if the parasite is introduced. This is a food-web transmitted parasite and it is likely dependent on the presence of European eel to be established in an ecosystem.

3.1.4.2 HAZARD CHARACTERIZATION

The impacts (cf. Table 2.1-1) of introducing the selected five parasite species into Femundand Trysilvassdraget, as outlined below, are assessed with the assumption that these parasites are not already present here, and that native susceptible fish populations are naïve with regards to these pathogens.

Ichthyophthirius multifiliis

Ich disease outbreaks occur in most year-classes of salmonids and other freshwater fish species, especially in the warm period of the year. Diseased or weakened fish may have altered behaviours and may be more likely to be eaten by a predator. Outbreaks of Ich may be expected to increase with climate change. We consider the impact of introduction to be **moderate**, with **medium confidence**.

Myxobolus cerebralis

This myxozoan causes whirling disease in salmonid yearlings, especially Atlantic salmon and brown trout juveniles. This parasite affects the nervous system and cause damages to the cartilage and formation of backbones early in the life of salmonids. This will affect their swimming capacity and thus, affected fish are likely more frequently eaten. As this parasite uses two hosts to complete its lifecycle, populations of oligochaetes (*Tubifex* spp.), the

definitive host of *M. cerebralis*, may also be negatively affected. We consider the impact of introduction to be **moderate** with **low confidence**.

Tetracapsuloides bryosalmonae

This myxozoan causes PKD, which is a severe disease in Atlantic salmon juveniles, and even more severe in juvenile brown trout. PKD also affects other salmonids such as grayling and Arctic charr. As this parasite uses two hosts to complete its lifecycle, populations of bryozoans (several species), the definitive host of *T. bryosalmonae*, may also be negatively affected. For these reasons, we consider the impact of introduction to be **major** with **high confidence**.

Anguillicoloides crassus

If established, this parasite will infect many invertebrate and vertebrate species at several trophic levels and may affect these host species in different ways and severity. We consider the impact of introduction to be **moderate** with **low confidence**.

Salmincola spp.

These parasitic copepods are only occasionally lethal to their fish host. However, by feeding on the gills, the respiratory capacity of the fish may be reduced and make them more vulnerable to predation and other negative effects when water temperature increases and dissolved oxygen in reduced. We consider the impact of introduction to be **minor** with **moderate confidence**.

3.1.4.3 LIKELIHOOD

The project group assesses that the likelihood of negative impact on biodiversity in Norway of the five selected parasite species are as follows:

Ichthyophthirius multifiliis:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: **Moderately likely**, with **medium confidence**.

Myxobolus cerebralis:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: Likely, with medium confidence.

Tetracapsuloides bryosalmonae:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with medium confidence.

• <u>Method III</u>: Very likely, with medium confidence.

Anguillicoloides crassus:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Unlikely**, with **medium confidence**.

Salmincola spp.:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Moderately likely**, with **medium confidence**.

3.1.4.4 RISK CHARACTERIZATION

The project group assesses that the overall risk of negative import on biodiversity in Norway of the five selected parasite species are:

Ichthyophthirius multifiliis

- <u>Method I</u>: Low, with high confidence.
- <u>Method II</u>: Low, with high confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

Myxobolus cerebralis

- <u>Method I</u>: Low, with high confidence.
- <u>Method II</u>: Low, with high confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

Tetracapsuloides bryosalmonae

- <u>Method I</u>: Low, with high confidence.
- <u>Method II</u>: Low, with high confidence.
- <u>Method III</u>: **High**, with **high confidence**.

Anguillicoloides crassus

- <u>Method I</u>: Low, with high confidence.
- <u>Method II</u>: Low, with high confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

Salmincola spp.

- <u>Method I</u>: Low, with high confidence.
- <u>Method II</u>: Low, with high confidence.
- <u>Method III</u>: **Low**, with **medium confidence**.

3.2 Introduction of other organisms

Several alien and potentially invasive organisms can occur in the Swedish part of the watershed (see chapter 1.2.7). Those that have any potential of being transferred through any of the three methods have been assessed separately below. The project group assesses that it is very unlikely that methods I-III for re-stocking of salmon will lead to the introduction of Chinese mitten crab, round goby, and WSSV to Norway. These species have not undergone a full risk assessment.

The Chinese mitten crab has small larval stages that easily can be transported with water into Norway, however, it is unlikely to establish. Should these larvae reached Trysilelva, they need to reach maturity and migrate to estuarine habitats to spawn, which is very unlikely to happen. The nearest estuary is in Fredrikstad, at the outlet of Glomma, about 300 kilometers from Trysilelva through a complex system of rivers, timber flumes and lakes.

The round goby is currently not present in the Vänern area. The eggs of the round goby are deposited on stones, shells, and aquatic plants. This suggests that eggs will not be introduced by water. The likelihood of introduction to Norway will increase if the round goby establishes in Vänern.

WSSV can cause mass mortality in native populations of noble crayfish at temperatures above 20 °C. Many freshwater habitats in Norway presently have periods of sufficient duration when the water temperatures are above 20 °C. However, the disease has not been detected in nature in Europe to date. The likelihood of introduction to Norway will increase if WSSV establishes in the Vänern area.

3.2.1 Introduction of Aphanomyces astaci

3.2.1.1 HAZARD IDENTIFICATION

Crayfish plague is caused by infection with the oomycete (a fungus-like eukaryote) *A. astaci*. Crayfish plague causes up to 100% mortality in noble crayfish. *A. astaci* is native to North America, and North American crayfish are healthy carriers of *A. astaci*. As a result of widespread introductions of the North American signal crayfish (*P. leniusculus*), 98% of all crayfish populations in Sweden were eradicated between 1900 and 2020. The crayfish plague has also eradicated several populations of noble crayfish in Norway. Signal crayfish is established in Vänern. *A. astaci* can spread with its crayfish host, and by water even without its host.

3.2.1.2 HAZARD CHARACTERIZATION

A. astaci causes crayfish plague, and thereby eradication of red-listed noble crayfish populations. The magnitude of impact in Norway caused by *A. astaci* is limited to the noble crayfish, and to regions where this species is present. The noble crayfish is not present in

Trysilelva. However, the timber flumes connect Trysilelva to Glomma, where there are noble crayfish populations. These populations will be at risk. The project group assesses that the overall risk of negative impact on biodiversity in Norway, following introduction of *A. astaci* is **massive** with **high confidence**.

3.2.1.3 LIKELIHOOD

A. astaci can be spread by: 1) a crustacean host, 2) water transport of spores through the timber flumes, 3) biological vectors such as imported salmon (*A. astaci* survive gut passage), mammals, and birds (in moist fur or feathers), or 4) mechanical transport of spores by fishing gear, boats etc. The likelihood of entry of *A. astaci* to Norway depends on the likelihood of import of crustacean carriers with pathogens, fish with pathogens in the gut, or water with pathogens. The likelihood is closely linked to the source and amount of water imported from Sweden. Pathogens need to spread from Trysilelva and to areas with native crayfish, such as Glomma, in order to have an impact. The likelihood of spread depends on several factors, such as the number of spores transmitted, the presence of susceptible crayfish at the site of release, the conditions to which the spores/cysts are exposed during transfer, and the number of years with restocking of the salmon population. *A. astaci* remains viable for five days, and possibly longer, in crayfish kept in water at 21 °C after dying of crayfish plague. The project group assesses that the likelihood of negative impact on biodiversity in Norway of *A. astaci* is as follows:

- <u>Method I</u>: Very unlikely, with medium confidence.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: Moderately likely, with medium confidence.

3.2.1.4 RISK CHARACTERIZATION

There is a high risk associated with *A. astaci* because it can cause mass mortality of the noble crayfish. *A. astaci* can be introduced from Sweden with crustaceans (crayfish and Chinese mitten crab), in fish guts, water, mammals, birds, and equipment used by humans. The project group assesses that the overall risk of negative impact on biodiversity in Norway of *A. astaci* is as follows:

- <u>Method I</u>: Low, with medium to high confidence.
- Method II: Low, with medium to high confidence.
- <u>Method III</u>: **Moderate**, with **medium** to **high confidence**.

3.2.2 Introduction of Canadian pondweed (Elodea canadensis)

3.2.2.1 HAZARD IDENTIFICATION

E. canadensis is an invasive macrophyte that has spread throughout Europe. In Norway, it is present in more than 100 sites. It is widely distributed in Sweden, including in the Lake Vänern area.

3.2.2.2 HAZARD CHARACTERIZATION

E. canadensis forms dense populations that reduce water movement, cut off light, produce anoxic conditions, and trap sediments. These populations may reduce the quality of water and habitats, and these factors can affect the entire ecosystem, including the composition of native plants and crayfish populations. The project group assesses that the overall risk of negative impact on biodiversity in Norway, following introduction of *E. canadensis* is **major** with **high confidence.**

3.2.2.3 LIKELIHOOD

The spread of *E. canadensis* it facilitated by a broad ecological tolerance and asexual reproduction, including vigorous re-growth of small fragments. These fragments can also spread by human activities, such as boating, angling, and equipment used to transfer fish. The likelihood of entry into Norway depends on the likelihood of import of plant fragments, which is a function of the amount of water and number of years of transfer. Dispersal of overwintering dormant apices and fragments by water and by waterfowl can result in rapid spread from Trysilelva. The project group assesses that the likelihood of negative impact on biodiversity in Norway of *E. canadensis* is as follows:

- <u>Method I</u>: Very unlikely, with medium confidence.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: **Unlikely**, with **medium confidence**.

3.2.2.4 RISK CHARACTERIZATION

E. canadensis can negatively affect entire ecosystems and potentially spread by release of plant fragments. This implies that the plant can be imported in water or equipment used by humans. The project group assesses that the overall risk of negative impact on biodiversity in Norway of *E. canadensis* is as follows:

- <u>Method I</u>: **Moderate** (bordering to low), with **medium** to **high confidence**.
- <u>Method II</u>: **Moderate** (bordering to low), with **medium** to **high confidence**.
- <u>Method III</u>: **Moderate**, with **medium** to **high confidence**.

3.2.3 Introduction of zebra mussel (Dreissena polymorpha)

3.2.3.1 HAZARD IDENTIFICATION

D. polymorpha is among the most aggressive invasive species in freshwaters worldwide. It is found in lakes in south eastern Sweden, but not in Vänern or other parts of western Sweden.

3.2.3.2 HAZARD CHARACTERIZATION

D. polymorpha can have negative impacts on the native ecology, especially since it is an ecosystem engineer that alters the environment of invasion. It can also outcompete native freshwater pearl mussels. The project group assesses that the overall risk of negative impact on biodiversity in Norway of *D. polymorpha* is **major** with **high confidence**.

3.2.3.3 LIKELIHOOD

D. polymorpha is currently found in lakes in south eastern Sweden and not in Vänern or other parts of western Sweden. Each female can produce 30,000 to 1,000,000 eggs in one year, suggesting a potential for spread to Western Sweden by humans. The project group assesses that the likelihood of negative impact on biodiversity in Norway of *D. polymorpha* is as follows:

- <u>Method I</u>: Very unlikely, with high confidence.
- <u>Method II</u>: Very unlikely, with high confidence.
- <u>Method III</u>: **Unlikely**, with **medium confidence**.

3.2.3.4 RISK CHARACTERIZATION

D. polymorpha can negatively affect entire ecosystems. Relevant for salmon stocking, one female produces up to 1,000,000 microscopic eggs annually that can spread via water or on equipment. This is not likely to occur from Vänern at present as *D. polymorpha* is absent from western Sweden. The risk for negative impacts in Trysilelva would be considerably higher if the species first spread to the Mälern area. The project group assesses that the overall risk of negative impact on biodiversity in Norway of *D. polymorpha* is as follows:

- <u>Method I</u>: **Moderate** (bordering to low), with **high confidence**.
- <u>Method II</u>: **Moderate** (bordering to low), with **high confidence**.
- <u>Method III</u>: Moderate, with medium to high confidence.

3.2.4 Introduction of signal crayfish (Pacifastacus leniusculus)

3.2.4.1 HAZARD IDENTIFICATION

The signal crayfish is a North American species that was introduced to Europe in the 1960s. The signal crayfish is now considered invasive across Europe and there are about 5,000 sites with signal crayfish in Sweden (Bohman 2021), including the Vänern watershed.

Signal crayfish, even at moderate densities, may have negative effects on biological communities, including stream invertebrates, freshwater pearl mussel, noble crayfish, salamander (*Lissitriton vulgaris* and *Triturus cristatus*), and salmonid fish (Velle et al. 2021). It may also dig burrows, which can reach high densities (14 per m²) and cause collapse of riverbanks.

3.2.4.2 HAZARD CHARACTERIZATION

Alterations to local ecosystems through digging, predation and competition will presumably affect a number of species. However, the most negative effects will be primarily in those areas where red-listed species are present, such as noble crayfish, salamanders, and freshwater pearl mussels. The project group therefore assesses that the overall consequences on local ecosystems to be moderate to major with medium confidence.

Signal crayfish can be spread by intentional and illegal introductions for use as a food source. It can also be spread by accidental release through fish movements and transport on equipment, such as fishing nets and boats. Once at a new site, it can migrate up and down rivers, and over land. Their colonization rate is relatively slow (about 1 km per year) (Stanton, 2004).

The project group assesses that the overall risk of negative impact on biodiversity in Norway, following introduction of *P. leniusculus* is **moderate** with **high confidence.**

3.2.4.3 LIKELIHOOD

Signal crayfish lay round 200–400 eggs that are carried under the female's tail from fall and until they hatch the following spring. The eggs hatch into juveniles that pass through three stages until they are released in early summer as small crayfish. Thus, signal crayfish lack a free-living planktonic life stage, which has implications for the likelihood of introduction into Norway. Specimens, especially small stages, could nevertheless be translocated on equipment used by humans during the re-stocking of salmon. The project group assesses that the likelihood of negative impact on biodiversity in Norway of *P. leniusculus* is as follows:

- <u>Method I</u>: Very unlikely, with medium confidence.
- <u>Method II</u>: Very unlikely, with medium confidence.
- <u>Method III</u>: **Unlikely**, with **low confidence**.

3.2.4.4 RISK CHARACTERIZATION

Signal crayfish can have negative effects on biological communities and single species. It can also dig burrows and cause collapse of riverbanks. Relevant for introduction through salmon re-stocking, the signal crayfish adult carries the eggs until hatching, and it lacks free-living planktonic life stages. Introduction into Norway would therefore be by translocation of small specimens or adults. The project group assesses that the overall risk of negative impact on biodiversity in Norway of *P. leniusculus* is as follows:

- <u>Method I</u>: Low, with medium to high confidence.
- <u>Method II</u>: Low, with medium to high confidence.
- <u>Method III</u>: **Moderate**, with **medium confidence**.

4 Uncertainties

Several uncertainties are associated with this assessment. Considerable uncertainty concerns the potential presence of unknown and undescribed infectious agents in Vänern, Klarälven, and the connecting waterways. Another uncertainty is the potential for future introductions of infectious agents to Vänern and Klarälven through aquaculture, release of imported European eels, put-and-take fisheries, ballast water, and other human activities, from now until year 2100. There are also uncertainties related to the risk of human error and potential consequences that may occur during treatments and sorting of fish during transport.

Much of the knowledge on infectious agents affecting Atlantic salmon comes from disease outbreaks in marine aquaculture. Most infectious agents included in this risk assessment were unknown until diseases caused by these agents emerged in aquaculture. Thus, much of the available knowledge is derived from experiences aquaculture and how much of this can be extrapolated to populations in natural environments is also uncertain. Monitoring of wild Atlantic salmon populations occurs relatively rarely, and even less frequently for landlocked Atlantic salmon populations. In general, there is lack of information on infectious agents from freshwater systems.

During the nearly 80-year time period from 2021 to 2100 that is covered by this risk analysis, it is likely that there will be alterations in several factors that are highly relevant to this risk assessment. We have performed the risk assessment using the knowledge that is available today. However, the climate is predicted to continue to change, and rising temperatures may increase the negative impacts of infectious agents.

Several abiotic and biotic factors determine a species' habitat selection, natural distribution, and ability to spread and colonize new areas. These factors are poorly known for many species. Also, there are large uncertainties related to the environmental impact of alien species, because the impacts of most alien species are poorly understood (Jeschke et al. 2014). The short-term (e.g., caused by predation or harmful diseases) and long-term (e.g., caused by competition or parasitism) effects that two organisms living together in a community have on each other strongly influence evolution. The effects depend on the species involved, on the evolutionary context, and on the concurrent environmental conditions. As a result, ecological interactions are often difficult to define and measure (Harrison and Cornell 2008, Ricklefs 2008). This suggests that it may be difficult to foresee ecological interactions between species that normally do not share habitats, such as interactions between alien and native species. Also, it may take generations before effects become visible.

5 Conclusions with answers to the terms of reference

5.1 Risk of introduction of alien species and infectious agents through import of fertilized eggs (method I)

For method I, import of fertilized eggs to a hatchery that are later planted in the river or released as fry or smolts, the likelihood of impact on native biodiversity and ecosystems from introducing of alien species and diseases is unlikely or very unlikely for the assessed organisms and viruses (figure 6.1-1). However, for *G. salaris, A. astaci,* and VHSV, the negative impacts on native biodiversity and ecosystems are expected to be massive, should they be introduced to the Norwegian parts of the watershed. For *A. salmonicida* subsp. *salmonicida, R. salmoninarum,* IHNV, *T. bryosalmonae, D. polymorpha,* and *E. canadensis,* the impacts are expected to be major, should they be introduced.

The overall risk of negative impacts on native biodiversity and ecosystems associated with the assessed organisms and viruses is moderate or low, with *G. salaris* posing the greatest risk. The overall risk is larger than the risks associated with method II (gene bank model), but smaller than the risks associated with method III (transfer of adult salmon, see below). There are additional risks connected to the potential presence of currrently undescribed infectious agents or agents with unknown presence in Vänern or Klarälven, which we have not been able to specifically assess. For the 80-year period until year 2100 that this risk assessment is covering, there are also additional risks connected to future introductions of infectious agents or alien organisms not described here to Vänern and Klarälven through aquaculture, release of imported European eels, the put-and-take industry, ballast water, fouling organisms on boats and other human activities.

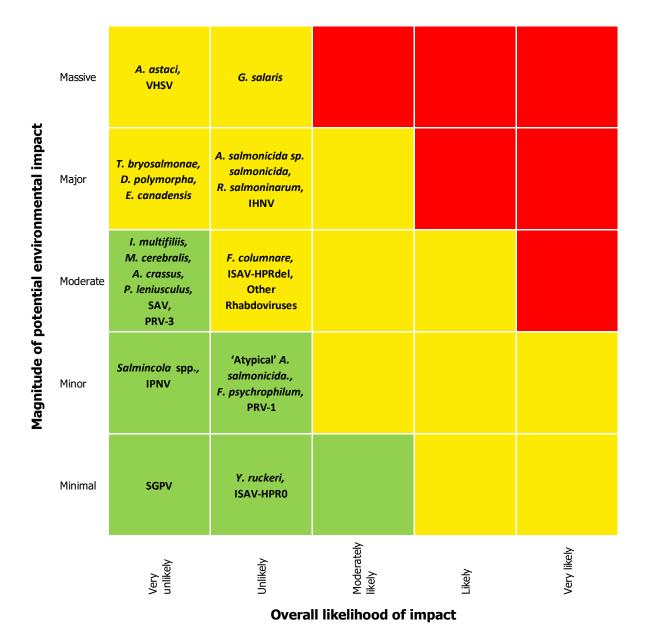


Figure 5.1-1: Risk associated with introduction of parasites, virus, bacteria and other organisms with reestablishment of salmon using method I, which is import of fertilized eggs to a hatchery that are later planted in the river or released as fry or smolts.

5.2 Risk of introduction of alien species and infectious agents when using the gene bank model (method II)

For method II, which is establishment of a designated gene bank from which eggs are planted in the river or hatched in a local hatchery and released as fry or smolts, the likelihood of impact of introducing of alien species and diseases is very unlikely for all the assessed organisms and viruses (figure 6.2-1). However, for *G. salaris, A. astaci,* and VHSV, the negative impacts on native biodiversity and ecosystems are expected to be massive, should they be introduced to the Norwegian parts of the watershed. For *A. salmonicida*

subsp. *salmonicida, R. salmoninarum,* IHNV, *T. bryosalmonae, D. polymorpha,* and *E. canadensis,* the impacts are expected to be major, should they be introduced.

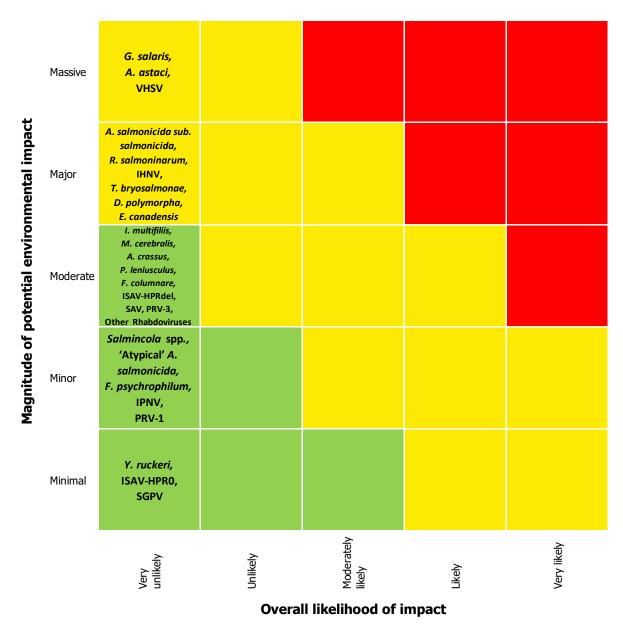


Figure 5.2-1: Risk associated with introduction of parasites, virus, bacteria and other organisms with reestablishment of salmon using method II, which is establishment of a designated gene bank from which eggs are planted in the river, or hatched in a local hatchery and released as fry or smolts.

The overall risk of negative impacts on native biodiversity and ecosystems associated with the assessed organisms and viruses is moderate or low, with *G. salaris*, *A. astaci*, and VHSV posing the greatest risk. The overall risk is smaller than the risks associated with the two other methods of releasing eggs and fish included in the assessment. There are additional

risks connected to the potential presence of currently undescribed infectious agents or agents with an unknown presence in Vänern or Klarälven, which we have not been able to specifically assess. For the 80-year period until year 2100 that this risk assessment covers, there are also additional risks connected to future introductions of infectious agents or alien organisms not described here to Vänern and Klarälven through aquaculture, release of imported European eels, the put-and-take industry, ballast water, fouling organisms on boats and other human activities.

5.3 Risk of introduction of alien species and infectious agents by transfer and release of adult salmon (method III)

For method III, which is import of adult salmon that are captured in Klarälven, then transported and released in the Norwegian parts of the watershed prior to spawning, the likelihood of negative impacts on native biodiversity and ecosystems of introducing of alien species and diseases is very likely for *T. bryosalmonae*, and likely for *G. salaris*, *R. salmoninarum*, *M. cerebralis*, and IPNV (figure 6.3-1). The likelihood of impact of several other organisms and viruses is moderately likely, including *A. astaci*, VHSV, *A. salmonicida* subsp. *salmonicida*, and IHNV, whereas for some organisms and viruses the likelihood of impact is unlikely (figure 6.3-1).

For *G. salaris, A. astaci,* and VHSV, the environmental impact on Norweigan biodiversity is regarded as massive, should they be introduced to the Norwegian parts of the watershed. For *A. salmonicida* subsp. *salmonicida, R. salmoninarum,* IHNV, *T. bryosalmonae, D. polymorpha*, and *E. canadensis*, the impact is regarded as major, should they be introduced (figure 6.3-1).

The overall risk of negative impacts on native biodiversity and ecosystems is high for *G. salaris, A. astaci*, VHSV, *R. salmoninarum*, and *T. bryosalmonae* (figure 6.3-1). For most of the other organisms and viruses assessed, the overall risk is moderate, with *A. salmonicida* subsp. *salmonicida*, IHNV, and *M. cerebralis* posing the highest risk within the moderate group. The overall risk is regarded as low for only three of the assessed organisms and viruses. The overall risk is much higher than the risk associated with the two other methods included in the assessment.

There are additional risks connected to the potential presence of currently undescribed infectious agents or agents with an unknown presence in Vänern or Klarälven, which we have not been able to specifically assess. For the 80-year period until year 2100 that this risk assessment covers, there are also additional risks connected to future introductions of infectious agents or alien organisms not described here to Vänern and Klarälven through aquaculture, release of imported European eel, put and take industry, ballast water, fouling organisms on boats and other human activities.

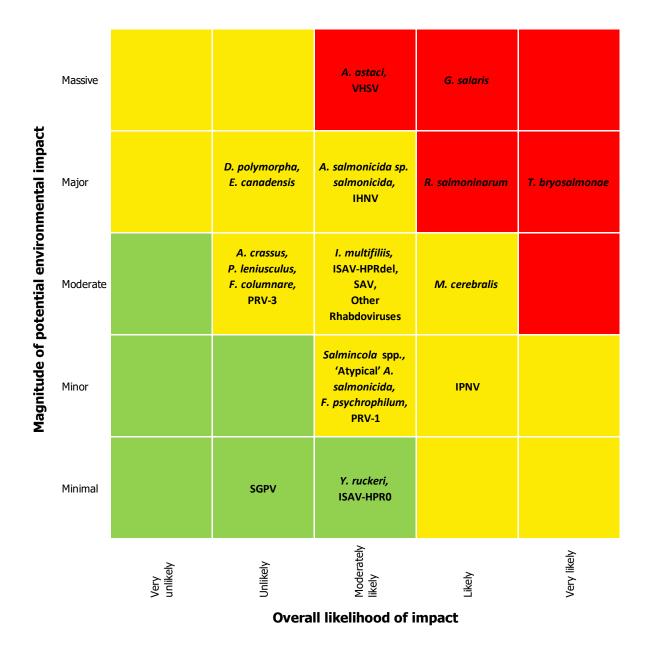


Figure 5.3-1: Risk associated with introduction of parasites, virus, bacteria and other organisms with reestablishment of salmon using method III, which is transfer and release of adult salmon.

5.4 Ethical and other aspects not included in the assessment

VKM was not requested to assess animal welfare and ethical aspects related to release of Atlantic salmon on river stretches in Norway or egg planting that will result in production of juvenile salmon on these stretches. Ethical concerns are related to injuries and mortalities incurred when fish pass the power stations during downstream migration (1.2.9.2). There are also welfare issues related to handling and transport of broodfish involved in the procedures assessed in this report. These aspects may be particularly challenging if procedures need to be developed to handle and transport large numbers of fish. The methods of introducing Atlantic salmon to the Norwegian parts of the watershed involve planting eggs or releasing salmon in the Norwegian parts of the watershed for a one-way downstream migration to the Swedish parts. The panel notes that these methods will, in isolation, be insufficient for reestablishment of salmon in the Norwegian part of the watershed. In order to reestablish a viable salmon population in this area, fish from this population must be able to return to their spawning areas subsequent to downstream migration. Thus, bi-directional movement of fish from and into the population is necessary. The risks to biodiversity connected to opening a two-way movement of fish that is needed to reestablish salmon in the Norwegian parts of the watershed would, necessarily, increase compared with the risks associated with the specific import and release methods, and does not comprise a full risk assessment of complete reestablishment of salmon in the Norwegian parts of the watershed.

6 Risk-reducing measures

Relevant risk reducing measures are those that hinder or reduce the translocation of infective or invasive agents from Sweden to Norway.

Regarding method I, the biosafety level in general, could be improved beyond that afforded by government regulations, by extending health monitoring and test routines, for the broodfish during the captive period, the broodfish following autopsy, the eggs during hatchery-quarantine, the fry before release, and the broodfish that show signs of clinical disease or die during the holding period before stripping. At present, head kidney samples from female broodfish are teste for *R. salmoninarum*. Expanding the test programme to include male fish and also other known vertically transmissible infectious agents, including IPNV, will also contribute towards reducing the risk.

Regarding method II, in addition to the risk-reducing measures described for Method I above, it will be possible to increase the biosafety level within the gene-bank facility. The number of pathogens within the specific-pathogen free (SPF)-specification, and the level of health monitoring of the gene-bank broodfish prior to maturity and stripping, could be continuously adjusted. The technical and technological levels of the installations are regularly improved, and the same is true regarding the level of experience among the staff and managers. The technology for managing water quality is developing rapidly, and it is now possible to adjust most water quality parameters according to changing requirements. The barrier levels and the number of barriers will also be of importance for the biosecurity level within the gene-bank facility. eDNA-based surveillance for specific pathogens could provide relevant information (also from an 80-year perspective), and could also be used as a biobank resource for future monitoring should currently unrecognised pathogens or invasive species become apparent.

Regarding method III, the broodfish could be held in a prolonged quarantine (30 to 60 days). The quarantine period should be accompanied by thorough surveillance for signs of clinical disease and specific tests for infectious agents.

Lethal sampling of a representative sample of broodfish may provide relevant and important information, but will result in a loss of valuable broodfish. A less invasive design of the surveillance program will therefore be to examine all broodfish that die or show signs of disease during the quarantine period. It is also possible to conduct surveillance for infectious agents based on eDNA or biobarcoding by filtering effluent water from each cage.

During this quarantine period, fish with different health statuses will be placed together in tanks enabling exchange of infectious agents. The potential to increase the prevalence and intensity of infectious agents during this period is therefore significant, not least because of the potentially stressful and immunosuppressive exposure to saline water, handling by humans, and cohabitation with other fish. Infectious agents that are capable of inducing prolonged subclinical carrier status (e.g., *R. salmoninarum*) may go undetected through such

quarantine periods, or, at worst, become triggered via stress and spread to other specimens within the holding tank.

Keeping the broodfish at low densities and in several tanks will reduce the likelihood and impact of such transmission.

Prior to transfer of broodfish from Sweden to Norway, the fish can be examined for the presence of infectious agents externally (some parasites) or by non-lethal test procedures (typically blood samples and swabs). However, as infectious agents in wild fish are commonly at low prevalence and have organ-specific locations, the lack of detection of such agents cannot guarantee absence, even when the number of examined fish is large (false-negative results).

The level of biosecurity, fish welfare, and fish health depends on the technical facilities regarding fish tanks, sluices, and lockers. The levels of competence and staffing will in general have a significant effect on the safety level.

Spill water or any other material, such as sediments and equipment used by humans, can be sterilized to kill infectious agents, and other organisms. This should be done using Virkon S, chlorine, saltwater, ethanol, complete desiccation, or boiling. Note that some pathogens, including *A. astaci*, will not be eliminated by ultraviolet sterilizers, and some pathogens may survive contact with saltwater, drying, or freezing.

The risk of further spread from Trysilelva to Glomma can be mitigated by closing waterflow through the timber flumes.

7 Data gaps

There is a general lack of knowledge on infectious agents from freshwater systems, and, more specifically, there is substantial lack of knowledge of which infectious agents and invasive organisms are present in Vänern and Klarälven, including currently undescribed organisms and viruses. For Atlantic salmon, there is lack of knowledge related to wild populations, and for landlocked salmon in particular, because most monitoring and research have been related to marine aquaculture. There is a lack of knowledge about the impact of specific infectious agents alone and in interaction with other host organisms and environmental factors.

Several abiotic and biotic factors determine a species' habitat selection, natural distribution, and ability to spread and colonize new areas. These factors are not well known for many species. In general, the impacts of most alien species are poorly understood.

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9 Appendix I

9.1 Listing of infectious diseases in aquatic animals

Norway has been a member of the World Organisation for Animal Health (OIE) since it was founded in 1924, and was fully harmonized with the EU with regard to animal health requirements for aquaculture animals and products from January 2003. Accordingly, agreements with the three international organizations OIE, EU (EEA), and the World Trade Organization (WTO) have implications for how Norway handle and formulate regulations in the area of infectious diseases in animals.

The primary objective of the European Union (EU/EEA) and WTO is to facilitate international trade. The animal health aspect in these agreements is that animal-health considerations should not unduly act as a barrier to trade, and that measures should be taken to prevent the spread of animal diseases through trade in animals and animal products.

When the OIE was founded, the activity was rooted solely in scientific considerations. The three main objectives were to: (1) promote research on pathology and prevention of animal diseases in areas where international cooperation was desired, (2) gather information on the occurrence of animal diseases and make the information available to the authorities of each Member State, and, finally, (3) establish international agreements and measures on how to prevent spread of animal diseases and assist countries in fulfilling these agreements. The objectives were later expanded to include facilitation of international trade in animals and animal products.

Fish diseases are listed both internationally and nationally to establish standards in the control of fish diseases. The OIE publishes health standards for international trade in animal and animal products. In the EU/EEA area, Lists 1 and 2 were originally declared by the European Commission (Council Directive 91/67/EEC, Annex A) and are included in Part II of Annex IV of the Council Directive 2006/88/EU (Fiskehelsedirektivet). The Norwegian national List 3 has been adopted by the Ministry of Trade, Industry and Fisheries in the Annex I in Regulation 17 June 2008 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (FOR-2008-06-17-819, omsetnings- og sykdomsforskriften, Norwegian Ministry of Trade, Industry and Fisheries).

Both the OIE and EU list diseases, and one disease may have different status in these two organizations, because they operate at different geographical levels.

9.1.1 Disease list of the World Organisation for Animal Health (OIE)

9.1.1.1 Criteria for listing diseases

According to the OIE Aquatic Animal Health Code, the objective of listing diseases is to support Member Countries by providing information needed to take appropriate action to prevent the transboundary spread of important diseases of aquatic animals. This is achieved by transparent, timely, and consistent notification.

Each listed disease has a corresponding chapter in the Aquatic Animal Health Code that assists Member Countries in the harmonization of disease detection, prevention, and control, and provides standards for safe international trade in aquatic animals and aquatic animal products.

9.1.1.2 Criteria for listing of a disease in the OIE

The criteria for inclusion of a disease in the OIE list are as follows:

- International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors, or fomites) is likely, *and*
- At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions in the Aquatic Animal Health Code, *and*
- A precise case definition is available and a reliable means of detection and diagnosis exists, *and*
 - Natural transmission to humans has been proven, and human infection is associated with severe consequences, *or*
 - The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g., production losses, morbidity, or mortality at a zone or country level, *or*
 - The disease has in aquatic animals been shown to, or scientific evidence indicates that it would, affect the health of wild animals resulting in significant consequences e.g., morbidity or mortality at a population level, reduced productivity, or ecological impacts.

9.1.1.3 The disease list in OIE

Article 1.3.1. The following diseases of fish are listed by the OIE:

Infection with Aphanomyce sinvadans (epizootic ulcerative syndrome)

Infection with epizootic haematopoietic necrosis virus

Infection with Gyrodactylus salaris

Infection with HPR-deleted or HPR0 infectious salmon anaemia virus

Infection with infectious haematopoietic necrosis virus

Infection with koi herpesvirus

Infection with red sea bream iridovirus

Infection with salmonid alphavirus

Infection with spring viraemia of carp virus

Infection with viral haemorrhagic septicaemia virus.

Article 1.3.2. The following diseases of molluscs are listed by the OIE:

Infection with abalone herpesvirus

Infection with Bonamia ostreae

Infection with Bonamia exitiosa

Infection with Marteilia refringens

Infection with Perkinsus marinus

Infection with Perkinsus olseni

Infection with Xenohaliotis californiensis.

Article 1.3.3. The following diseases of crustaceans are listed by the OIE:

Acute hepatopancreatic necrosis disease

Infection with Aphanomyces astaci (crayfish plague)

Infection with Hepatobacter penaei (necrotising hepatopancreatitis)

Infection with infectious hypodermal and haematopoietic necrosis virus

Infection with infectious myonecrosis virus

Infection with Macrobrachium rosenbergii nodavirus (white tail disease)

Infection with Taura syndrome virus

Infection with white spot syndrome virus

Infection with yellow head virus genotype 1.

Article 1.3.4. The following diseases of amphibians are listed by the OIE:

Infection with Batrachochytrium dendrobatidis

Infection with Batrachochytrium salamandrivorans

Infection with Ranavirus species.

9.1.1.4 Relevant regulations in the European Community (EC)

The Fish Health Directive (Council Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals), lays down

(a) the animal health requirements to be applied for the placing on the market, the importation and the transit of aquaculture animals and products thereof;

(b) minimum preventive measures aimed at increasing the awareness and preparedness of the competent authorities, aquaculture production business operators, and others related to this industry, for diseases in aquaculture animals;

(c) minimum control measures to be applied in the event of a suspicion of, or an outbreak of, certain diseases in aquatic animals.

Member States remain free to take more stringent measures in the field covered by Chapter II (requirements regarding aquaculture production businesses and authorised processing establishments), Article 13 (regarding disease prevention requirements in relation to transport) and Chapter V (regarding notification and minimum measures for control of diseases of aquatic animals) provided that such measures do not affect trade with other Member States.

Annex IV in the Fish Health Directive (Council Directive 2006/88/EC) concerns listing of diseases and include criteria for listing diseases (Part I) and the actual listed diseases (Part II).

Criteria for listing diseases

List I Exotic diseases

Exotic diseases meet criteria in point 1 and either point 2 or 3.

1. The disease is exotic to the EEC/EEA, i.e., the disease is not established in EEC aquaculture, and the pathogen is not known to be present in EEC waters.

2. It has potential for significant economic impact if introduced into the EEC, either by production losses in EEC aquaculture or by restricting the potential for trade in aquaculture animals and products thereof.

3. It has potential for detrimental environmental impact if introduced into the EEC, to wild aquatic animal populations of species, which are an asset worth protecting by EEC law or international provisions.

List II Non-exotic diseases

Non-exotic meets the criteria in points 1, 4, 5, 6, 7, and 2 or 3.

1. Several Member States, or regions in several Member States, are free of the specific disease.

2. It has potential for significant economic impact if introduced into a Member State free of the disease, either by production losses, and annual costs associated with the disease and its control exceeding 5% of the value of the production of the susceptible aquaculture animal species production in the region, or by restricting the possibilities for international trade in aquaculture animals and products thereof.

3. The disease has shown, where it occurs, to have a detrimental environmental impact if introduced into a Member State free of the disease, to wild aquatic animal populations of species that is an asset worth protecting under EEC law or international provisions.

4. The disease is difficult to control and contain at farm or mollusk farming area level without stringent control measures and trade restrictions.

5. The disease may be controlled at Member State level, experience having shown that zones or compartments free of the disease may be established and maintained, and that this maintenance is cost-beneficial.

6. During placing on the market of aquaculture animals, there is a risk that the disease will establish itself in a previously uninfected area.

7. Reliable and simple tests for infected aquatic animals are available. The tests must be specific and sensitive and the testing method harmonised at EEC level.

9.1.1.5 Listed diseases EC/EU

Fish Health Directive (Council Directive 2006/88/EC of 24 October 2006)

Exotic diseases	Susceptible species
Epizootic haematopoietic necrosis	Rainbow trout (<i>Oncorhynchus mykiss</i>); redfin perch (<i>Perca fluviatilis</i>)

Non-exotic diseases	Susceptible species
Spring viraemia of carp (SVC)	Bighead carp (Aristichthys nobilis), goldfish (Carassius auratus), crucian carp (C. carassius), grass carp (Ctenopharyngodon idellus), common carp and koi carp (Cyprinus carpio), silver carp (Hypophthalmichthys molitrix), sheatfish (Silurus glanis) and tench (Tinca tinca)
Viral haemorrhagic septicaemia (VHS)	Herring (<i>Clupea</i> spp.), whitefish (<i>Coregonus</i> spp.), pike (<i>Esox lucius</i>), haddock (<i>Melanogrammus aeglefinus</i>), Pacific cod (<i>G. macrocephalus</i>), Atlantic cod (<i>G. morhua</i>), Pacific salmon (<i>Oncorhynchus</i> spp.) rainbow trout (<i>O. mykiss</i>), rockling (<i>Onos mustelus</i>), brown trout (<i>Salmo trutta</i>), turbot (<i>Scophthalmus maximus</i>), sprat (<i>Sprattus sprattus</i>) and grayling (<i>Thymallus thymallus</i>)
Infectious haematopoietic necrosis (IHN)	Chum salmon (<i>Oncorhynchus keta</i>), coho salmon (<i>O. kisutch</i>), Masou salmon (O. <i>masou</i>), rainbow or steelhead trout (<i>O. mykiss</i>), sockeye salmon (<i>O. nerka</i>), pink salmon (<i>O. gorbuscha</i>) chinook salmon (<i>O. tshawytscha</i>), and Atlantic salmon (<i>Salmo salar</i>)
Koi herpes virus (KHV) disease	Common carp and koi carp (Cyprinus carpio).
Infectious salmon anaemia (ISA)	Rainbow trout (<i>Oncorhynchus mykiss</i>), Atlantic salmon (<i>Salmo salar</i>), and brown and sea trout (<i>S. trutta</i>).

9.1.1.6 Listing of diseases in Norwegian regulations

In Regulation 17 June 2008 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (FOR-2008-06-17-819, omsetnings- og sykdomsforskriften, Norwegian Ministry of Trade, Industry and Fisheries) exotic diseases are on List I and non-exotic diseases are on List II. In addition, diseases that are of importance at the national level are on the national list i.e., List III.

9.1.1.7 Criteria for listing diseases on the national list (List III)

The following criteria are used in the assessment of whether a fish disease should be listed on the national list. These criteria were prepared by the Norwegian Food Safety Authority in 2008 in connection with the implementation of the Fish Health Directive:

a) The disease is not listed in the Fish Health Directive (EU).

b) The disease can represent a significant risk to the animal health situation in aquaculture facilities.

c) It is difficult to eradicate the disease and keep it under control at site level.

d) Disease-free areas can be achieved and maintained where this is important for controlling the disease.

e) The disease is clearly defined on the basis of infectious agent and / or pathological findings.

f) The disease can be a threat to wild populations of aquatic animals if it is not controlled and / or kept at a controlled low level.

Based on current criteria, diseases on List 3 must either meet criteria a, b, c, d and e, or a, e and f

	Disease
Fish	Bacterial kidney disease (BKD, <i>Renibacterium salmoninarum</i>)
	Infestation with Gyrodactylus salaris
	Viral nervous necrosis (VNN)/Viral encephalo- og retinopaty (VER) Nodavirus
	Furunculosis (<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i>)
	Pancreas disease (PD, Norwegian salmon alpha-virus)

Listed diseases – National list III in Norway

	Disease	
	Systemic infection with <i>Flavobacterium psychrophilum</i> in rainbow trout (<i>Oncorhynchus mykiss</i>)	
Crustaceans	Crayfish plague (<i>Aphanomyces astaci</i>)	

9.1.2 Relevant regulations regarding import of aquatic animals to Norway

Commission Regulation (EC) No 1251/2008 of 12 December 2008 implements the Fish Health Directive (Council Directive 2006/88/EC) as regards conditions and certification requirements for the placing on the market and the import into the Community of aquaculture animals and products thereof and laying down a list of vector species. In Norway, Commission Regulation (EC) No 1251/2008 was implemented by the Regulations on additional requirements for the transport, sale and import of aquaculture animals and products thereof).

ESA (EFTA Surveillance Authority) has granted large parts of Norwegian land area, including the Norwegian part of the Trysilelva/Klarälven watercourse, disease-free status for *Gyrodactylus salaris* (Ref. Case No. 61388, Event No. 474528, Dec. No. 298/08 / COL). This entails the right to set additional requirements for protection against *G. salaris* when importing fish into this zone. The aim is to protect Atlantic salmon stocks, and susceptible wild fish species in freshwater and freshwater farming (rainbow trout) against infection. The requirements with regards to *G. salaris* are laid down by Commission Regulation (EC) No 1251/2008 (Annex II Part A (Model health certificate)).

Eyed eggs can be imported from Sweden to a *Gyrodactylus*-free zone in Norway, if they have been disinfected with a method that has been proven effective against *G. salaris*. In Norway, the method of choice is a 100 mg/l iodophor solution, with a contact time of at least 10 minutes and at pH between 6.0 and 8.0.

Imported fish must immediately before transfer have been held continuously in a tank at 25 ppt salinity for 14 days. During this period, new fish cannot be added to the tank.

9.1.3 Implementation of the new Animal Health Law in EU

Regulation (EU) 2016/429 of the European parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') is the new animal health regulation that will be implemented in the EU Member States during 2021. The aim of this regulation is to implement the new Animal Health Strategy of the European Union, called 'Prevention is better than cure',

including the 'One Health' principle, stronger emphasis on preventive measures, disease surveillance, disease control, and research. It is also an objective to consolidate the legal framework, converge with international standards, and provide a single, simplified and flexible regulatory framework for animal health.

Commission delegated regulation (EU) 2020/689 of 17 December 2019 regulates movements of aquatic animals within the EU.

9.1.3.1 Disease prevention and control rules to be applied to different categories of listed diseases

The animal health law and Commission delegated regulation (EU) 2020/689 provide a legal framework for the classifications of animal diseases and set some boundaries on movement of live animals and animal products (eggs) across borders. In article 2 of 2020/689, definitions of the various disease categories are listed:

Category A disease: means a listed disease that does not normally occur in the EU and for which immediate eradication measures must be taken as soon as it is detected, as referred to in point (a) of Article 9(1) of Regulation (EU) 2016/429

Category B disease: means a listed disease that must be controlled in all Member States with the goal of eradicating it throughout the EU, as referred to in point (b) of Article 9(1) of Regulation (EU) 2016/429

Category C disease: means a listed disease that is of relevance to some Member States and for which measures are needed to prevent it from spreading to parts of the EU that are officially disease-free or that have eradication programmes for the listed disease concerned, as referred to in point (c) of Article 9(1) of Regulation (EU) 2016/429

Category E disease: means a listed disease for which there is a need for surveillance within the EU, as referred to in point (e) of Article 9(1) of Regulation (EU) 2016/429

Category	Action	Actions that also apply
A	Immediate eradication	(B, D and E also apply)
В	Compulsory eradication	(D and E also apply)

Category	Action	Actions that also apply
c	Optional eradication	(D and E also apply)
D	Measures concerning movements	E also apply
E	Surveillance	

9.1.3.2 Listing of diseases in fish the new Animal Health Law relative to the Fish Health Directive

	Fish Health Directive	New Animal Health Law Category	Susceptible species
Epizootic haematopoietic necrosis	Exotic disease	A+D+E	Rainbow trout (<i>Oncorhynchus mykiss</i>) and European perch (<i>Perca fluviatilis</i>)
Epizootic ulcerative syndrome	Not listed	A+D+E	Genera: Catla, Channa, Labeo, Mastacembelus, Mugil, Puntius and Trichogaster.
Spring viraemia of carp (SVC)	Non-exotic disease	C+D+E	Bighead carp (Aristichthys nobilis), goldfish (Carassius auratus), crucian carp (C. carassius), grass carp (Ctenopharyngodon idellus), common carp and koi carp (Cyprinus carpio), silver carp (Hypophthalmichthys molitrix), European catfish (Silurus glanis) and tench (Tinca tinca)

	Fish Health Directive	New Animal Health Law Category	Susceptible species
Viral haemorrhagic septicaemia (VHS)	Non-exotic disease	C+D+E	Herring (Clupea spp.), whitefish (Coregonus spp.), pike (Esox lucius), haddock (Melanogrammus aeglefinus), Pacific cod (Gadus macrocephalus), Atlantic cod (G. morhua), Pacific salmon (Oncorhynchus spp.), rainbow trout (O. mykiss), rockling (Onos mustelus), brown trout (Salmo trutta), turbot (Scophthalmus maximus), sprat (Sprattus sprattus) and grayling (Thymallus thymallus)
Infectious haematopoietic necrosis (IHN)	Non-exotic disease	C+D+E	Chum salmon (<i>Oncorhynchus keta</i>), coho salmon (<i>O. kisutch</i>), Masou salmon (<i>O. masou</i>), rainbow or steelhead trout (<i>O. mykiss</i>), sockeye salmon (<i>O. nerka</i>), pink salmon (<i>O. gorbuscha</i>) chinook salmon (<i>O. tshawytscha</i>), and Atlantic salmon (<i>Salmo salar</i>)
Koi herpes virus (KHV) disease	Non-exotic disease	E	Common carp and koi carp (<i>Cyprinus carpio</i>).
Infectious salmon anaemia (ISA)	Non-exotic disease	C+D+E	Rainbow trout (<i>Oncorhynchus mykiss</i>), Atlantic salmon (<i>Salmo salar</i>), and brown and sea trout (<i>S. trutta</i>).