



VKM Report 2014: 12

Risk assessment of fish health and welfare in freshwater production systems for rainbow trout, brown trout and Arctic char

Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2014: 12
Risk assessment of fish health and welfare in fresh water production systems for rainbow
trout, brown trout and Arctic char

Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee
for Food Safety
06.11.2014

ISBN: 978-82-8259-150-8
Norwegian Scientific Committee for Food Safety (VKM)
P.O. Box 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

Cover photo: Oddmund Grøttan

Suggested citation: VKM (2014) Panel on Animal Health and Welfare; Risk assessment of fish
health and welfare in fresh water production systems for rainbow trout, brown trout and
Arctic char, VKM Report 2014: 12 [89 pp], ISBN:978-82-8259-150-8, Oslo, Norway. Available
online: www.vkm.no

Risk assessment of fish health and welfare in fresh water production systems for rainbow trout, brown trout and Arctic char

Authors preparing the draft opinion

Ulf Erikson (chair), Dag Berge, Eirik Biering, Brit Hjeltnes and Espen Rimstad

(Authors in alphabetical order after chair of the working group)

Assessed and approved

The opinion has been assessed by Panel on Animal Health and Welfare. Members of the panel are: Brit Hjeltnes (chair), Øivind Bergh, Edgar Brun, Knut Egil Bøe, Carlos Goncalo Afonso Rolhas Fernandes das Neves, Jacques Godfroid, Roar Gudding, Kristian Hoel, Cecilie Mejdell, Stein Mortensen and Espen Rimstad

(Panel members in alphabetical order after chair of the panel)

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority. Project leaders from the VKM secretariat have been Ingrid Slaatto Næss and Angelika Agdestein. The members of the working group, Ulf Erikson (previous member of the Panel on Animal Health), Dag Berge (Norwegian Institute for Water Research, NIVA), Eirik Biering (Norwegian Veterinary Institute, NVI), Brit Hjeltnes (Panel on Animal Health and Welfare) and Espen Rimstad (Panel on Animal Health and Welfare) are acknowledged for their valuable work on this opinion.

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.

Table of contents

Summary	7
Sammendrag	10
Glossary	13
Background	15
Terms of reference as provided by the Norwegian Food Safety Authority	16
1. The risks associated with the establishment of cage-based on-growing fish farms in fresh water with rainbow trout, brown trout and Arctic char	16
2. The risks associated with the establishment of land based on-growing farms with rainbow trout, brown trout and Arctic char with intake of water from and effluent of water to a freshwater reservoir.	17
3. The risks associated with the establishment of land based hatcheries with rainbow trout, brown trout and Arctic char with effluent of water to a freshwater reservoir.....	18
Assessment	19
1 Introduction	19
1.1 Literature search.....	19
1.2 Fish farming systems in fresh water.....	19
1.2.1 Farms in lakes or rivers.....	19
1.2.2 Land based single-pass farms.....	19
1.2.3 Land based farms using recirculating aquaculture systems.....	20
1.3 Monitoring of water quality for farmed fish.....	20
1.4 Current water quality guidelines for fish farming in Norway	21
2 Hazard identification and characterisation	22
2.1 Hazards related to water quality parameters	22
2.1.1 Oxygen	23
2.1.2 Carbon dioxide.....	23
2.1.3 Acidity.....	24
2.1.4 Ammonia and ammonium	24
2.1.5 Total organic carbon.....	25
2.1.6 Gas supersaturation.....	25
2.1.7 Total suspended solids.....	26
2.1.8 Alkalinity.....	26
2.1.9 Hardness	27
2.1.10 Metals	27

2.1.10.1	Copper.....	27
2.1.10.2	Aluminium.....	28
2.1.10.3	Iron.....	28
2.1.11	Temperature.....	29
2.2	Hazards related to impact on water quality and environmental status in the recipient	30
2.3	Hazards related to fish stocking density	31
2.4	Hazards related to infectious agents	33
2.4.1	Viral infections	34
2.4.1.1	Infectious pancreatic necrosis virus.....	34
2.4.1.2	Infectious salmon anaemia virus.....	35
2.4.1.3	Salmon pancreas disease virus	35
2.4.1.4	Viral haemorrhagic septicaemia virus.....	36
2.4.1.5	Piscine orthoreovirus.....	36
2.4.1.6	Infectious haematopoietic necrosis virus.....	37
2.4.1.7	Piscine myocarditis virus	37
2.4.2	Bacterial infections	37
2.4.2.1	Flavobacterium psychrophilum.....	37
2.4.2.2	Renibacterium salmoninarum.....	38
2.4.2.3	Aeromonas salmonicida subsp. salmonicida	39
2.4.3	Parasitic infections.....	39
2.4.3.1	Gyrodactylus salaris.....	39
2.4.3.2	Myxobolus cerebralis.....	39
2.4.3.3	Tetracapsuloides bryosalmonae	40
3	Risk characterisation.....	41
	Method.....	41
3.1	Risk of changes in water quality for farmed and wild fish.....	41
3.1.1	Floating cages.....	41
3.1.2	Land based single-pass farms.....	42
3.2	Risk of introducing infection.....	43
4	Uncertainties.....	50
5	Answers to the terms of reference.....	51
ToR 1.	The risks associated with the establishment of cage-based on-growing fish farms in freshwater with rainbow trout, brown trout and Arctic char	51
ToR 2.	The risks associated with the establishment of land based on-growing farms with rainbow trout, brown trout and Arctic char with intake of water from and effluent of water to a freshwater reservoir.	54

ToR 3. The risks associated with the establishment of land based hatcheries with rainbow trout, brown trout and Arctic char with effluent of water to a freshwater reservoir..... 56

6 Conclusions 58

7 Data gaps 60

7.1 Water quality and fish welfare..... 60

7.2 Infections and fish health 60

8 References 61

Appendix I..... 77

Monitoring of recipient impacts..... 77

Appendix II 80

Water quality guidelines of the Norwegian Food Safety Authority 80

Appendix III..... 81

Interrelation of concentration of un-ionised total ammonia nitrogen (TAN = NH₃ + NH₄⁺) with pH and temperature of the water 81

Appendix IV..... 82

Hatcheries and spreading of unwanted organisms other than fish diseases..... 82

Appendix V 84

Animal health requirements for moving live eggs and fish to aquaculture farms in Norway . 84

Summary

The NFSA received in a letter dated 25 November 2011 a request from the Ministry of Fisheries and Coastal Affairs (FKD) to review the legislation in order to improve the opportunities for freshwater aquaculture. Freshwater aquaculture comprises land based production with runoff to freshwater, as well as cage production in freshwater. The request is restricted to rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) for fish production but is in some areas also relevant to hatchery production. The NFSA has hitherto been restrictive in granting permission to fish production in freshwater. Until recently, it was described in the NFSAs guidelines to the regulations on the establishing of aquaculture farms (etableringsforskriften) that permits to cage production in land based freshwater aquaculture should generally not be given. This practice was established in the 1980s and 1990s in connection with sanitation of production sites in the combat against *Gyrodactylus salaris*. However, this is now removed from the guidelines.

Parts of the regulations related to fish health and welfare have primarily been developed for traditional marine aquaculture and rarely been considered in relation to inland freshwater aquaculture. Due to lack of clarity in the rules and guidelines of the legislation, the NFSA needs an assessment from VKM on the risk of infection and the risk of poor animal welfare in cage production in freshwater and land based farming with runoff to freshwater.

To prepare the scientific background report necessary to answer the questions from the NFSA, the VKM Panel on Animal Health and Welfare appointed a working group consisting of three experts from the panel and two national external experts. The working group was chaired by Dr. Ulf Erikson. The Panel on Animal Health and Welfare supports the conclusions from the working group.

The health risk for farmed fish and wild fish with regards to transmission, establishment and development of serious diseases is linked to the source of biological material used for farming. If only local stocks are used, the risk is low. Use of other sources of roe and fish will increase the risk. Use of Norwegian or imported, disinfected roe is associated with low to moderate risk, depending on the source. The risk with use of Norwegian or imported fish is considered to be moderate to high. The same risk profile applies for the use of slaughter pens. If the pens are established to keep fish originating from another water system, new diseases may be introduced and the health risk will increase. Well trained farmers and fish health professionals, such as veterinarians and fish health biologists, will increase the likelihood of rapid detection of an introduced disease and possibilities to take prompt action. Mandatory harvesting, fallowing and disinfection will reduce the chance of an undetected disease to establish itself in the environment and have impact on health of next generation fish stocked on the site as well as on wild fish. The reduction will depend on the nature of the pathogen, the site and the wild fish population.

Based on available literature, various suggestions for recommended fish densities are given for some types of salmonids. However, the available data are not entirely consistent, and factors such as environmental factors, water quality, and fish size are not always taken into consideration. Moreover, the data do not necessarily match the quality requirements requested by the NFSA. Due to lack of data, it is not possible to compare different concepts of large scale fish farming. Nevertheless, it seems tentatively appropriate to recommend fish densities of less than 80 kg/m³ in case of rainbow trout, whereas for Arctic char higher densities can be recommended, possibly within the range of 75-150 kg/m³. Relevant fish density data for brown trout is basically lacking. It must be emphasized that data are limited, particularly for the farming of Arctic char. This is a particular concern when evaluating the total fish welfare at these densities.

Due to lack of specific water quality parameters evaluated under commercial conditions, or in different fish farming systems, it is still recommended to use the current guidance norm for water quality.

Correct monitoring of key water quality parameters is important to ensure that the fish are farmed under good conditions. It is technically possible to monitor dissolved oxygen, pH, carbon dioxide and temperature continuously. Assessment of total ammonium levels requires subsequent analysis of water samples and may be checked sporadically. Total gas pressure should be measured in cases where gas supersaturation is suspected.

If the water intake to land based farms is from a river or lake affected by acid rain, it is particularly important to monitor the pH-value of the water, the conductivity, and the concentration of labile aluminium. The aluminium tends to precipitate on the gills, and analyses of aluminium on the gills will give an early warning of subsequent fish death. If the influx of water is taken from an unpolluted river or lake, it is primarily acidification that can pose a threat to fish health. If the water has low alkalinity, there is a possibility for episodes of acidic waters, which may cause fish death.

Regarding potential differences in fish health and welfare between cage-based aquaculture in fresh water and seawater, some differences are indicated that may affect the fish. Basically, the physical aspects in lakes, like currents, water renewal, depths, water volume, recipient capacity for pollutants, etc., are much more restricted than in the sea. This means that the fish farms have to be smaller in lakes. Impacts from fish farms will appear at an earlier stage than in the sea. This may result in stress symptoms for both farmed and wild fish. For the aquatic environment, the impacts that arise are mostly eutrophication of the free water masses of the lake, and saprobiation of the bottom areas underneath the farms. The marine bottom areas recover faster after the fish farm is fallowed/moved, due to stronger and more frequent currents and water renewal. The lakes have less currents and more stagnant deep waters, which gives much slower recovery. In cases of infection, there is always a great possibility for spreading of infective agents downstream in the watercourse, but also upstream through fish migration. This applies particularly for trout and rainbow trout, whereas to a less degree for char. Some diseases, such as the lethal infection of

salmon by the parasite *Gyrodactylus salaris*, spread in fresh water, but not in seawater. Both rainbow trout and char can be host for this dangerous salmon parasite. Most fish diseases have, however, similar spreading capacity in fresh water and in seawater.

Keywords: Norwegian Scientific Committee for Food Safety, VKM, risk assessment, fish farming, fish health, fish welfare, water quality

Sammendrag

Mattilsynet har i brev datert 25.11.2011 fått en bestilling fra Fiskeri- og kystdepartementet (FKD) om å gå gjennom regelverket med sikte på bedre tilsettelegging for ferskvannsoppdrett. Med ferskvannsoppdrett menes landbasert produksjon som har avløp til ferskvann, eller merdbasert produksjon i ferskvann. Oppdraget er begrenset til oppdrett av regnbueørret (*Oncorhynchus mykiss*), ørret (*Salmo trutta*) og røye (*Salvelinus alpinus*) for matfiskproduksjon, men er på enkelte områder også relevant for settefiskproduksjon. Mattilsynet har til nå vært restriktive med å gi tillatelse til matfiskproduksjon i ferskvann. Inntil nylig stod det i Mattilsynets retningslinje til etableringsforskriften at tillatelser til merdbasert oppdrett i ferskvann som hovedregel ikke skulle gis. Det var en praksis som ble etablert på 80- og 90-tallet i forbindelse med sanering av anlegg og bekjempelse av *Gyrodactylus salaris*. Dette er nå fjernet fra retningslinjen.

Deler av regelverket som regulerer fiskehelse og fiskevelferd, har først og fremst vært utarbeidet med tanke på den tradisjonelle marine havbruksnæringen, og har i liten grad vært vurdert med tanke på ferskvannsoppdrett i innlandet. Uklarheter i regelverket og retningslinjer til regelverket gjør at Mattilsynet har behov for en vurdering fra Vitenskapskomiteen for mattrygghet (VKM) om smitterisiko og risiko for dårlig dyrevelferd ved merdbasert oppdrett i ferskvann og landbasert oppdrett med avrenning til ferskvann.

For å utarbeide en vitenskapelig bakgrunnsrapport som var nødvendig for å svare på spørsmål fra Mattilsynet, etablerte VKMs Faggruppe for dyrehelse og dyrevelferd en prosjektgruppe bestående av tre eksperter fra faggruppen og to nasjonale eksterne eksperter. Prosjektgruppen ble ledet av Dr. Ulf Erikson. Faggruppen for dyrehelse og dyrevelferd støtter konklusjonene fra prosjektgruppen.

Helserisikoen for oppdrettsfisk og villfisk med tanke på smitteoverføring, etablering og utvikling av alvorlig sykdom henger sammen med opprinnelsen til det biologiske materialet som brukes. Ved bruk av lokale stammer er risikoen lav. Bruk av rogn og fisk fra andre steder øker risikoen. Bruk av norsk eller importert desinfisert rogn er forbundet med lav til moderat risiko, avhengig av kilden. Risikoen ved bruk av norsk eller importert fisk anses som moderat til høy. Tilsvarende risikoprofil gjelder for bruk av slaktermerder. Hvis merdene brukes til fisk fra andre vassdrag, kan nye sykdommer introduseres og helserisikoen øke. God kunnskap hos fiskeoppdrettere og fiskehelsepersonell, som veterinærer og fiskehelsebiologer, øker sannsynligheten for at sykdommer oppdages raskt og dermed også for muligheten for å iverksette tiltak på et tidlig tidspunkt. Pålagt utslakting, brakklegging og desinfeksjon vil redusere muligheten for at en uoppdaget sykdom skal kunne etablere seg i miljøet og påvirke neste utsett på lokaliteten og villfiskpopulasjonen. Graden av reduksjon avhenger av egenskaper hos det gitte agens, samt av den enkelte lokalitet og fiskebestand.

Basert på tilgjengelig litteratur, er det gitt ulike forslag til anbefalte fisketettheter for de enkelte typer laksefisk. Imidlertid er ikke tilgjengelige data helt konsistente, og miljøfaktorer,

vannkvalitet, og fiskens størrelse er ikke alltid tatt hensyn til. Videre trenger dataene ikke nødvendigvis å samsvare med de krav og forhold som ligger til grunn for forespørselen fra Mattilsynet. På grunn av manglende data, er det ikke mulig å sammenlikne ulike former for storskala oppdrett. Ut fra tilgjengelige vitenskapelige data og erfaring, anbefales fisketettheter på mindre enn 80 kg/m³ for regnbueørret. For røye kan høyere tettheter anbefales; muligens innenfor intervallet 75-150 kg/m³. Det mangler relevante tetthetsdata for brunørret. Imidlertid må det understrektes at datagrunnlaget er begrenset, særlig det som er relatert til oppdrett av røye. Dette gjelder spesielt for å vurdere den totale fiskevelferden ved disse tetthetsgrensene.

Grunnet mangel på spesifikke vannkvalitetsparametere som vurderes under kommersielle forhold, eller i ulike oppdrettssystemer, er det fortsatt anbefalt å bruke de nåværende retningslinjene som norm for vannkvalitet.

Riktig overvåking av viktige vannkvalitetsparametere er viktig for å sikre at fisken er produsert under gode forhold. Det er teknisk mulig å overvåke oppløst oksygen, pH, karbondioksid og temperatur fortløpende. Vurdering av ammoniumnivået krever påfølgende analyse av vannprøver og kan kontrolleres sporadisk. Det totale gasstrykket bør måles i tilfeller hvor det er mistanke om gassovermetning.

Hvis vanninntaket til landbaserte anlegg er fra en elv eller en innsjø som er påvirket av sur nedbør, er det særlig viktig å overvåke pH-verdien i vannet, konduktivitet, og konsentrasjonen av labilt aluminium. Aluminium har en tendens til å opphopes på gjellene, og analyser av aluminium på gjellene vil gi et tidlig varsel om nær fiskedød. Dersom inntaket av vann er tatt fra en forurenset elv eller innsjø, er det primært forsuring som utgjør en trussel for fiskehelsen. Hvis vannet har lav alkalitet er det en risiko for perioder med surt vann, som igjen kan føre til fiskedød.

Med tanke på mulige forskjeller i fiskehelse og velferd mellom merdbasert akvakultur i ferskvann og sjøvann, er det noen indikasjoner om ulike forhold som kan påvirke fisken. Fysiske forhold i innsjøer som vannmengde, vanddybder, mottakerkapasitet for forurensning, strømninger og fornyelse av vann, er mye mer begrenset enn i sjøen. Dette betyr at anleggsstørrelsen må være mindre i ferskvann. Tegn til forurensning fra oppdrettsanleggene vil vises på et tidligere stadium enn i sjøen. Dette kan resultere i stresssymptomer for både oppdrettsfisk og villfisk. De ulike typer forurensning som oppstår i vannmiljøet er for det meste eutrofiering av de frie vannmassene i innsjøen og saprobiering av de nederste sjiktene under produksjonsanleggene. De marine bunnområdene kommer seg raskere etter at produksjonsanleggene er flyttet/brakklagt på grunn av sterkere strømninger og bedre vannutskiftning. Innsjøene har mindre strøm og mer stillestående dypt vann. Dette fører til mye langsommere gjenvinning av opprinnelig miljøtilstand når merdene flyttes fra et område som har blitt forurenset over tid. Ved infeksjon er det alltid en stor mulighet for spredning av smittsomme sykdommer nedstrøms i vassdraget, men også oppstrøms gjennom fiskemigrasjon. Dette gjelder spesielt for ørret og regnbueørret, men i mindre grad for røye. Enkelte sykdommer, som den dødelige infeksjonen i laks forårsaket av parasitten

Gyrodactylus salaris, spres i ferskvann, men ikke i sjøvann. Både regnbueørret og røye kan være vertskap for denne farlige lakseparasitten. De fleste fisesykdommer har imidlertid lignende spredningskapasitet i ferskvann og sjøvann.

Glossary

Bog is a wetland area with peat substrates.

Buoyancy is the power to float or rise in a fluid.

Cages are fish containing enclosed structures constructed with net and may be floating or fixed to the bottom substrate but still permitting water interchange from below.

Effluent is liquid discharge.

Epilimnion is the top-most layer in a thermally stratified lake.

Eutrophication of water bodies is the process of increase in the rate of supply of organic matter and the resulting algal bloom and oxygen depletion

Fallowing is to leave sites empty of fish at the end of a production period.

Feed conversion efficiency/rate is the total weight of feed divided by the net production (final weight minus starting weight of fish).

Fingerling is a young and small salmon or trout, less than one year of age and about the size of a finger.

Fry is recently hatched fish.

Hatcheries are facilities for breeding, nursing and rearing seed of fish to fry, fingerlings or juvenile stages.

Headrace is a channel that feeds water into a turbine.

Milt is the seminal fluid of fish.

Minnow is small freshwater fish of the carp family.

Saprobiation is the decay of organic material in water bodies.

Opercula (sing. operculum) are the gill covers of the fish.

Pens are fish farming water areas confined by net allowing natural water interchange.

Periphyton is a mixture of algae, cyanobacteria, heterotrophic microbes, and detritus that is attached to submerged surfaces in most aquatic ecosystems.

Recirculating aquaculture systems (RAS) is a system for intensive fish farming where the water in the fish tank flows to a treatment unit before it is returned to the tank.

Quarry is an excavation from which stone is obtained by cutting or blasting.

Schooling is fish swimming in the same direction in a coordinated manner.

Smolt are juvenile anadromous salmonids of one to six years of age, measuring 12–20 cm.

Spawning is the release of eggs or sperm by aquatic animals.

Stripping is to manually induce spawning by stroking the anaesthetised fish.

Tailrace is a channel that carries water away from a turbine.

Background

The Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety (VKM) conceives the commission from the Norwegian Food Safety Authority (NFSA) as follows:

The NFSA received in a letter dated 25 November 2011 a request from the Ministry of Fisheries and Coastal Affairs (FKD) to review the legislation in order to improve the opportunities for freshwater aquaculture. Freshwater aquaculture comprises land based production with runoff to freshwater, as well as cage production in freshwater. The request is restricted to the breeding of rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) for fish production, but is in some areas also relevant to hatchery production.

The NFSA has hitherto been restrictive in granting permission to fish production in freshwater. Until recently, the NFSAs guidelines to the regulations on the establishing of aquaculture farms (etableringsforskriften), stated that permits to cage-based aquaculture production in freshwater should generally not be given. This practice was established in the 1980s and 1990s in connection with sanitation of production sites in the combat against *Gyrodactylus salaris*. However, this is now removed from the guidelines.

Parts of the regulations related to fish health and welfare have primarily been developed for the traditional aquaculture and rarely been considered for inland freshwater aquaculture.

Due to lack of clarity in the rules and guidelines of the legislation, the NFSA has the need for an assessment from VKM on the risk of infection and the risk of poor animal welfare in cage production in freshwater and land based farming with runoff to freshwater.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority requests the following assessments from the Norwegian Scientific Committee for Food Safety:

1. The risks associated with the establishment of cage-based on-growing fish farms in fresh water with rainbow trout, brown trout and Arctic char

1.1. The health risk for farmed fish and wild fish, with particular emphasis on the risk of transmission, establishment and development of serious infectious diseases from farmed fish to wild stocks of fish.

1.2. Which factors may be relevant to reduce the health risk for farmed fish and wild fish, including harvesting and fallowing of the facilities every winter?

1.3. The welfare risk for farmed fish including non-infectious diseases related to the production of farmed fish, with particular emphasis on the risks associated with density, water quality, accumulation of sediments under farms and accumulation of ice on cages. We specifically request the following:

1.3.1. An overview of the available information on the impact different densities may have on fish welfare for the different species in cage-based on-growing farms in fresh water. In this case, fish in on-growing farms means fish with a weight over 250 grams.

1.3.2. An assessment of whether the guidance norm for water quality and measurable parameters for land based hatcheries with salmonids may be suitable as a guidance norm for water quality for the different species in cage-based on-growing farms in fresh water.

1.3.3. An assessment of water quality parameters which are suitable for monitoring in cage-based on-growing farms in fresh water with rainbow trout, brown trout and Arctic char. Such monitoring could be more or less continuous and may be an addition to requirements on density.

1.4. Could the health and welfare of farmed fish and wild fish be set at risk if slaughter pens are established in fresh water?

1.5. To the extent it is possible, we require an assessment of the health risk and welfare risk for farmed fish and wild fish associated with cage-based farming in fresh water compared to

cage-based farming in seawater. If the risk is different, which factors cause the risk to differ between cage-based farming in freshwater and cage-based farming in seawater?

2. The risks associated with the establishment of land based on-growing farms with rainbow trout, brown trout and Arctic char with intake of water from and effluent of water to a freshwater reservoir.

The questions in this section are limited to fish farms with mainly flow-through systems.

2.1. The health risk for farmed fish and wild fish if effluent water is released untreated to a freshwater reservoir, with particular emphasis on the risk of transmission, establishment and development of serious infectious diseases from farmed fish to wild stocks of fish.

2.2. Which factors may be relevant to reduce the health risk for farmed fish and wild fish, including harvesting and fallowing of the facilities every winter and treatment of effluent water? Specifically an assessment of the risk if the effluent water is treated in accordance with a method that satisfies the requirements in § 10 point 1 or § 10 point 5 of the Water Treatment Regulation.

2.3. The welfare risk for farmed fish including non-infectious diseases related to the production of farmed fish, with particular emphasis on the risks associated with density and water quality. We especially request the following:

2.3.1. An overview of the available information on the impact different densities may have on fish welfare for the different species in land based on-growing farms using fresh water. In this case, fish in on-growing farms means fish with a weight over 250 grams.

2.3.2. An assessment of whether the guidance norm for water quality and measurable parameters for land based hatcheries with salmonids may be suitable as a guidance norm for water quality for the different species in land based on-growing farms using fresh water.

2.3.3. An assessment of water quality parameters which are suitable for monitoring in land based on-growing farms using fresh water with rainbow trout, brown trout and Arctic char. Such monitoring can be more or less continuous and may be an addition to requirements on density.

3. The risks associated with the establishment of land based hatcheries with rainbow trout, brown trout and Arctic char with effluent of water to a freshwater reservoir.

The questions in this section are limited to hatcheries with mainly flow-through systems.

3.1. The health risk for wild fish if untreated effluent water is drained to a freshwater reservoir, with particular emphasis on the risk of transmission, establishment and development of serious infectious diseases to wild stocks of fish.

3.2. An assessment of the risk if the effluent water is treated in accordance with a method that satisfies the requirements of § 10 point 1 or § 10 point 5 in the Water Treatment Regulation.

3.3. An assessment of whether the guidance norm for water quality and measurable parameters for land based hatcheries with salmonids may be suitable as a guidance norm for water quality in land based hatcheries with brown trout and Arctic char.

3.4. An assessment of water quality parameters which are suitable for monitoring in land based hatcheries with rainbow trout, brown trout and Arctic char. Such monitoring can be more or less continuous and may be an addition to requirements on density.

The Norwegian Food Safety Authority asks that any knowledge gaps that are identified during the process of the risk assessment are pointed out in the report.

Assessment

1 Introduction

1.1 Literature search

The working group used collections of scientific papers and reports accumulated during their many years of engagement in research on fish health and water quality. Initial formal conduction of structured searches was not carried out. Supplementary searches were however performed continuously in the databases of Science Direct and NCBI PubMed, but due to the broad thematic in the report, it seems unfeasible to define these searches in retrospect.

1.2 Fish farming systems in fresh water

Different operational modes of fish farming in freshwater systems are possible in Norway (Figure 1.2-1). A brief description of the principal differences of the various systems is given below.

1.2.1 Farms in lakes or rivers

Fish are kept in cages located in lakes or rivers, and water exchange and water quality in the cages are therefore governed by the natural conditions in the lake. Seasonal changes in flow and exchange of water, as well as in water quality may occur. An example is the formation of ice during winter. In such cases water is circulated artificially by water agitators to prevent ice formation in the cages. Discharges from aquacultural activity are directly transmitted to the ecosystem.

1.2.2 Land based single-pass farms

In this case, the fish are farmed in tanks by using the flow-through principle. Water is supplied from an external source, such as a lake, river or ground water, and discharged to a recipient water body. Inlet water can be aerated to minimize gas supersaturation or to increase oxygen saturation. It is often filtrated to remove particles and debris before the water enters the fish tanks. Other parameters, such as dissolved substances, pH, temperature and possible presence of microorganisms and parasites are governed by that of the water source. The water can be discharged directly or after water treatment to the recipient lake or river. However, a minimum of water treatment by filtering of the effluent to remove particles is performed in most cases. Large volumes of water passing through such systems make comprehensive water treatment a challenge.

1.2.3 Land based farms using recirculating aquaculture systems

Almost the entire volume of water used in recirculating aquaculture system (RAS) farms is continuously reused after passing a water treatment unit, where several steps to maintain adequate water quality are completed. It is possible to maintain a stable water quality in such systems since seasonal effects can be eliminated. For example, water temperature can be adjusted for optimal growth throughout the year, and water quality can be controlled by using various methods and technology. In RAS, where the degree of recirculation is high, efficient farming can be done without the need for large volumes of water from a freshwater source. Discharge volumes to the recipient are modest, making efficient water treatments feasible.

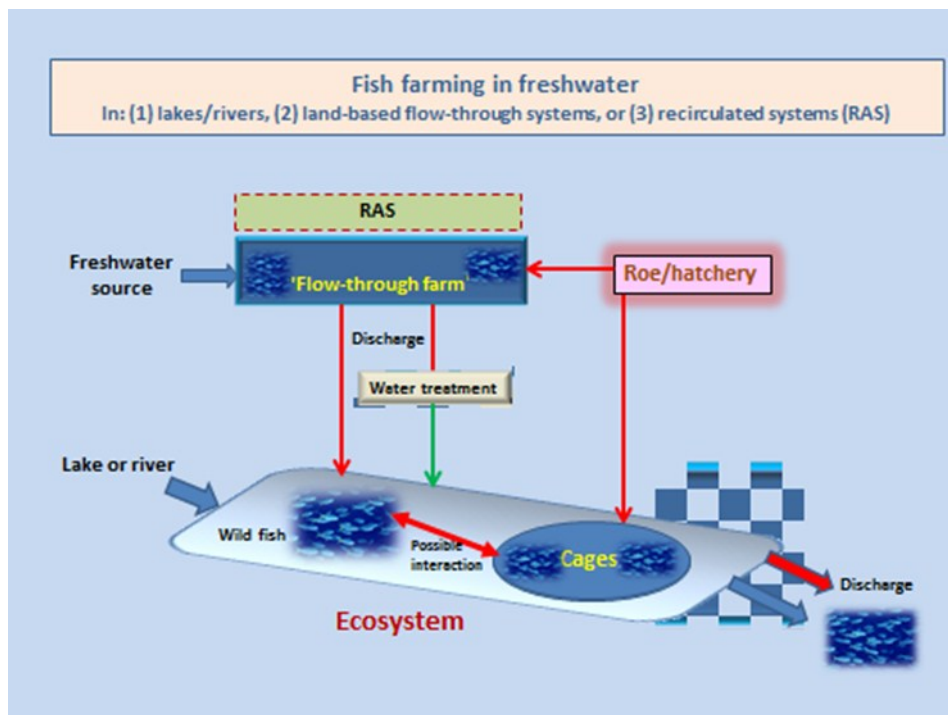


Figure 1.2-1 Different operational modes of fish farming in freshwater systems and their relation to the ecosystem.

1.3 Monitoring of water quality for farmed fish

Correct monitoring of key water quality parameters, such as dissolved oxygen, pH/CO₂, TAN, total gas pressure and temperature, is important to ensure that the fish are farmed under good conditions. Therefore, adequate quality assurance of the relevant analytical methods and sensors must be considered as a prerequisite. In aqueous environments, biofilms will gradually develop on all exposed surfaces including those of various sensors. Munro et al. (1996) reported that biofilm fouling of pH electrodes may impair the function and disturb the precision of pH measurements. In a recent study, Kolarevic et al. (2011) tested the precision of several online pH measurement systems towards manual recordings and the effect of

automated cleaning procedures of the electrodes. The study concludes that automated probe cleaning may be feasible, or alternatively, that pH should be measured with two or more instruments regularly to improve the precision of pH monitoring. These considerations are relevant for other instrumentation as well, but more data are requested. It is important that the fish farms have adequate quality assurance routines to make sure that sensors and equipment are frequently calibrated and checked for correct monitoring of water quality.

Each fish farm needs a tailored monitoring programme of the intake water and the water within cages or pens, to secure good living conditions for the fish. Fresh water varies a lot in quality, and the different water qualities require different monitoring programmes, parameters, sampling frequency, and sensor set-ups.

For monitoring of recipient impacts, consult Appendix I.

1.4 Current water quality guidelines for fish farming in Norway

The Norwegian Food Safety Authority (NFSA) uses internal guidelines for recommended safe levels of major water quality parameters (Appendix II). These values are basically in line with the respective recommended values given in the following chapter. The suggested criteria for good water quality should, however, be used with caution. In many cases the maximum or minimum levels should be considered as guidelines only. The published criteria were derived from experiments carried out under different conditions. Often the focus of these studies was not on the intensive production of fish in cultures. The effect of only one water quality parameter at a time is usually reported in the literature. Although single-factor studies are valuable to improve knowledge about the specific mechanisms affected in the fish, such results can be of limited value to predict the joint impacts of several water quality parameters in a fish farming system.

2 Hazard identification and characterisation

2.1 Hazards related to water quality parameters

Deterioration of water quality is regarded a potential factor that can compromise welfare. Fish are in intimate contact with the environment, which makes them particularly vulnerable to poor water quality and waterborne pollutants (Huntingford et al., 2006; MacIntyre et al., 2008). In a poorly managed fish farm, water quality may gradually deteriorate. In such cases, suboptimal levels of specific water quality parameters will induce a chronic stress response in the fish. Eventually, this can result in altered behaviour, reduced growth, development of diseases, or mortalities. Some studies have been done on the effect of suboptimal water quality and how this might affect fish at various levels of the relevant substances. In most cases, the results are evaluated in terms of 'stress' rather than in terms of 'welfare'. A system for assessment of salmon welfare in aquaculture has recently been developed. Two versions exist; one intended for fish farmers (Stien et al., 2013) and one intended for fish health professionals (Pettersen et al., 2013).

Most research on water quality and fish is usually carried out under controlled conditions where the effect of only one component at a time is studied. Hence, the listed effects of several of the water quality parameters should be used with caution. In practical aquaculture it is nevertheless common to use threshold values of single variables as guidelines for water quality assessments.

For appraisal of water quality in freshwater cages and land based systems, the same assessment will basically apply as was done in connection a previous risk assessment on RAS in salmonid hatcheries, published by VKM in 2012. Recirculating aquaculture systems are not included in the present risk assessment.

Both the previous and the present risk assessment deal with water quality of salmonids in freshwater systems. However, the present risk assessment gives a shorter and more basic description for several of the water quality parameters, compared to the RAS risk assessment, which is referred to for a detailed description. Nevertheless, some additional references, particularly on Arctic char, have been added to the present risk assessment, whereas other issues, such as the effect of ozone in relation to improvement of water quality in RAS, have been left out.

In land based freshwater farming systems, the use of RAS is clearly a technical relevant option. A large part of the farmed North-American Arctic char has been produced within RAS (Summerfelt et al., 2004). In recent years, many large Norwegian commercial smolt producing facilities have been built as RAS. The previous risk assessment on recirculation systems should therefore be consulted for RAS-specific issues.

2.1.1 Oxygen

The dissolved oxygen (DO) level is the single most important parameter in any fish rearing system. Low oxygen, hypoxia, induces respiratory distress leading to a reduction in appetite and ultimately mortality. Symptoms include rapid gill movement, gulping, lethargy and absence of active shoaling behaviour. A DO level of at least 56 % saturation is generally recommended in aquaculture (Timmons et al., 2001). For DO concentrations in mg/L at different water temperatures and salinities, please consult the web page <http://www.ysi.com/media/pdfs/DO-Oxygen-Solubility-Table.pdf>. An oxygen saturation level of 56 % is, however, too low for salmonids. Since growth performance of salmon will improve from 70-75 % to 80-85 % air saturation (Bergheim et al., 2006), it therefore seems reasonable to suggest that at least 85 % saturation should be considered the lower DO limit in practice (Thorarensen and Farrell, 2011).

Oxygen saturation above 100 %, hyperoxia, can be harmful. Such supersaturation can induce emboli in tissues, which leads to gas bubble disease and can cause even greater problems when associated with nitrogen (Noga, 2000). Fish exposed to DO supersaturated water changes behaviour with regards to swimming activity, number of turns, panic reactions, demonstrating signs of pain and discomfort (Espmark et al., 2010). On the other hand, when rainbow trout were reared in flow-through systems at DO saturation levels of 180 % and 94 % for 125 days; no differences were observed in growth and feed conversion, nor was mortality affected. Similar results were observed for cutthroat trout reared in 183 %, 127 % or 97 % oxygen-supersaturated water for 91 days (Edsall and Smith, 1990).

2.1.2 Carbon dioxide

Normally, the concentration of carbon dioxide (CO₂) in water in equilibrium with air is 0.5 –1 mg/L. Metabolically produced carbon dioxide is excreted through the gills and will gradually accumulate in the blood if the CO₂ is not removed from the environment. This results in a decrease in the oxygen carrying capacity (Sanni and Forsberg, 1996). Excessive levels of dissolved carbon dioxide, hypercapnia, can cause stress and several compensatory adaptations in fish (Eddy et al., 1977; Crocker and Cech, 1996; Fivelstad et al., 2003a; 2003b). Hypercapnia might also lead to calcification of kidneys and reduced growth (Fivelstad et al., 1999b). A combination of high carbon dioxide levels, low pH, and high aluminium levels can be a major threat to animal welfare (Fivelstad et al., 2003a; 2003b). Rainbow trout change their normal swimming behavior when carbon dioxide levels exceed 35 –60 mg/L. Equilibrium is lost at about 150 mg/L CO₂, and above 155 mg/L, narcosis is induced after three min at 14 °C (Clingerman et al., 2007). Atlantic salmon become lightly sedated at 70 - 80 mg/L. At 180 - 250 mg/L, narcosis is induced and if the water quality is not improved, the fish will eventually start to die as a result of cessation of respiration (Erikson, 2011). Reduced growth rates for Atlantic salmon has been reported at levels of 20 mg/L CO₂ or less (Fivelstad et al., 1999b; 2003a; Hosfeld et al., 2008), and particularly at ≥ 30 mg/L at the parr and post-smolt stages (Fivelstad et al., 1998, 2007). Higher mortality rates occur at 19 and 32 mg /L CO₂ than at 7 mg /L (Fivelstad et al., 1999a). Furthermore,

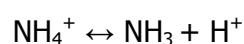
Atlantic salmon are more sensitive to carbon dioxide at low temperatures (Fivelstad et al., 2007). The recommended maximum levels of carbon dioxide, to maintain good welfare and to support maximum growth of salmonids, varies from 10 mg/L CO₂ (Wedemeyer, 1996; Fivelstad et al., 1998) to 20 mg/L (Timmons et al., 2001; Portz et al., 2006).

2.1.3 Acidity

The acidity of the water can be altered by elevated carbon dioxide levels from fish metabolism. This will lead to a drop in water pH which in turn will affect the amount of toxic unionized ammonia (NH₃) in the tank, as described in 2.1.4. Low pH (<4.2 – 5.0) in itself is harmful for salmonids and can be lethal (Randall, 1991). Acidified water causes disturbances in the water and ion metabolism of fish (Audet and Wood, 1988), acid-base regulation (McDonald et al., 1980), transport of oxygen and excretion of carbon dioxide (Randall, 1991), and in the excretion of ammonia (Wright and Wood, 1985). Presence of aluminium in acidified water can increase toxicity for the fish, as described in 2.1.10.2. Moreover, swimming performance of rainbow trout is affected by the acidity of the water (Ye and Randall, 1991), the skin surface is attacked, and the production of mucus is increased (Wendelaar Bonga and Dederen, 1986). Rainbow trout do not acclimatize to acid stress (Audet and Wood, 1988). The recommended levels of acidity are pH > 6 (Randall, 1991), and pH 6.5 – 8.5 (Timmons and Ebeling, 2007).

2.1.4 Ammonia and ammonium

Ammonia (NH₃) is a by-product of the metabolism of mainly amino acids, but also of nucleotides. Important sources of ammonia are urine, solid wastes and excess feed. Accumulation of ammonia in the rearing water will also increase the plasma ammonium ion (NH₄⁺) and ammonia (Wright and Anderson, 2001). In aqueous solutions ammonia is in equilibrium with the ammonium ion:



The total ammonia nitrogen, TAN, of the system is described as:

$$\text{TAN} = \text{NH}_4\text{-N} + \text{NH}_3\text{-N}$$

As shown in the equation, the toxicity of ammonia is dependent of the pH in the system (Suski et al., 2007). Temperature and salinity have only limited effect on TAN toxicity (Randall and Tsui, 2002). The effect of acute toxicity is mainly due to impacts on the central nervous system in vertebrates, and death may follow (Randall and Tsui, 2002). The un-ionized ammonia toxicity is believed to be due to impairment of cerebral energy metabolism resulting in a depletion of high energy compounds in the brain (Smart, 1978) and of glutamate. Additionally, ammonium ions have been suggested to have a depolarizing effect on neurons, by displacement of potassium, eventually leading to cell death (Randall and Tsui, 2002). Chronic exposure to elevated levels of ammonia will increase metabolic rate,

reduce growth rate, disease resistance and fecundity. Major symptoms of ammonia toxicity is lack of foraging, reduced swimming performance, increased gill ventilation, coughing, hyperexcitability, convulsions, coma, gulping, erratic swimming and loss of equilibrium. The pathogenesis is characterized by disruption of enzyme systems and membrane stability, leading to gill damage and histological lesions in various internal organs, as well as osmoregulatory disturbances, ultimately leading to death (Tomasso, 1994; Ip et al., 2001; Thorarensen and Farrell, 2011). To provide good rearing conditions and adequate fish welfare, the safe limits for salmonids in aquaculture range from 0.012 to 0.025 mg /L NH₃ (Westers, 1981; Fivelstad et al., 1995; Wedemeyer 1996, 1997; Timmons et al., 2001). For short time exposure (≤ 4 h), the recommended levels are approximately ten times higher (Wedemeyer, 1996). Appendix III shows the interrelation of the concentration of un-ionised ammonia with the pH and temperature of the water.

Considerably less information is available on the toxicity of the ammonium ion, probably since elevated levels of ammonium ions have generally been considered unharmed (Tabata, 1962), although this view has been questioned (Tomasso, 1994; Linton et al., 1998).

2.1.5 Total organic carbon

Little information is available on the potential effects of total organic carbon (TOC) on salmonid health in fish cultures. Davidson et al. (2009) measured 4.64 and 20.52 mg TOC/L in RAS with high and low exchange rates, respectively. Survival was high (~99 %) in both cases.

2.1.6 Gas supersaturation

The partial pressure of a gas dissolved in water depends on temperature, pressure and the degree of infusion of the gas. If the atmospheric pressure is exceeded by the gas pressure, supersaturation of the water by this gas subsequently follows. Sudden increases in temperature, decreases in pressure, or excessive oxygenation, are all typical causes of gas supersaturation in aquaculture systems. Supersaturation can also occur relatively often in the tailrace water from hydropower stations. The trapped air is exposed to high pressure in the turbine headrace, which gives high concentration of N₂ in the water. The subsequent exposure of the water to the atmospheric pressure of the tailrace easily leads to lethal supersaturation for the fish. Typical external signs of supersaturation are bubbles appearing on the fins, tail, opercula and head. Eventually, the eyes can be driven out from the sockets due to gas behind the eyes, resulting in a condition called pop-eye. Changes in behaviour have also been observed (Weitkamp and Katz, 1980). Ultimately, death can occur as a result of gas emboli, that is, bubbles are blocking the capillaries and preventing normal flow of blood to various tissues, often referred to as gas bubble disease. Embolisms in the heart or other vital organs normally cause death (Wedemeyer, 1996). For example, 50 % of juvenile sockeye salmon (*Oncorhynchus nerka*) exposed to 130 % total gas supersaturation were dead within 37 h (Nebeker et al., 1976). Total gas supersaturation should be < 110 % in

intensive fish cultures (Wedemeyer, 1997). For more details on gas supersaturation and fish, refer to the comprehensive review by Weitkamp and Katz (1980).

2.1.7 Total suspended solids

Suspended solids are defined as particulate matter of organic and inorganic components within the water, with a diameter greater than 1 μm (Chen et al., 1994). However, when water containing clay is filtrated, a 0.45 μm filter will be necessary to effectively produce clear water, and this retention diameter is used to distinguish between particulate and colloidal phase (McNight et al., 1997). Nevertheless, in practical analyses glass fibre filters of mean retention between 0.8 – 1.2 μm are often used to separate particulate matter from the water, as this is sufficient for most purposes. Also, glass fibre filters can be ignited, making it possible to distinguish between organic and inorganic particulate matter.

Suspended solids may originate from human activities, such as tunnel blasting and quarrying near various freshwater systems. In fish farms, typical sources of total suspended solids (TSS) are uneaten fish food, faecal solids, and microfauna. Excessive amounts of feed can cause biofouling, which in turn may affect the welfare of the fish by chronic stress and development of diseases. Accumulation of fine particles (5 – 10 μm) has been associated with lethal effects on rainbow trout (Chapman et al., 1987). Damage to fish gills can occur at TSS levels of 44 mg/L (Magor, 1988). Indirect effects of elevated TSS levels can be increased biological oxygen demand of the culture system, elevated levels of carbon dioxide due to presence of microorganisms associated with the particles, or an increase of fish pathogens. The recommended maximum limit for TSS varies between 15 mg/L (Timmons and Ebeling, 2007), 80 – 100 mg/L (Wedemeyer, 1996), depending on fish species. No damage is seen on natural erosion materials in fisheries when TSS is below 25 mg/L, whereas at 100 mg /L TSS it is considered not possible to have good fish production (Alabaster and Lloyd, 1980). In Asia, rivers have much higher concentrations of particulate matter and still can have good fish productivity, but indigenous fish species are adapted to other levels of particulate matter. Yellow River in China has a mean content of particulate matter of around 10 g/L, whereas in Norwegian lakes and rivers large declines in trout and char productivities have been observed at much lower concentrations (Aass, 1979; 1985).

Tunnel blasting and quarrying activities in specific rock types, such as green stone, soap stone and other soft rocks, have given rise to fish kills due to gill damage (Hessen, 1992; Jacobsen et al., 1987). However, most rocks in Norway are crushed to cubic particle forms, which are far less dangerous than the spine formed crushing products formed by soft rocks.

2.1.8 Alkalinity

Alkalinity, the total concentration of alkaline substances dissolved in the water, is related to the capacity of water to neutralize hydrogen ions. Thus, water with a certain alkalinity has the potential to stabilize a water system by buffering against large and sudden pH changes. Highly alkaline waters may, however, cause problems for the fish since ammonia excretion

and production can be inhibited (Wilson et al., 1998). Recommended lower and upper limits for alkalinity are >20 mg/L (to provide some buffering capacity), and <100 – 150 mg/L, respectively (Wedemeyer, 1996). Timmons and Ebeling (2007) recommend alkalinities within the range of 50 – 300 mg/L CaCO₃. In water analysis, it is common to use milliequivalents per litre (meq/L = mmol/L) instead of mg/L CaCO₃ as units for alkalinity. An alkalinity of 1 meq/L corresponds to 50 mg/L CaCO₃ (Ruttner, 1963).

2.1.9 Hardness

Hardness is defined as the total concentration, of primarily calcium (Ca²⁺) and magnesium (Mg²⁺), iron, and manganese (Mn) ions present in the water. The concentration is expressed in terms of equivalent mg/L CaCO₃. Thus, hardness is also a measure of the buffering capacity of the water and is therefore important for regulation of pH in aquaculture farms. The total hardness of natural water ranges from <5 to >10 000 mg /L CaCO₃. Water can be classified as soft (0-75 mg /L CaCO₃) up to very hard (> 300 mg/L CaCO₃). Recommended levels range from 20 to 300 mg/L CaCO₃ (Timmons and Ebeling, 2007). Since fish must regulate their blood ion concentrations across the gills, water hardness will affect the amount of energy needed for this purpose, according to the magnitude of the blood-water concentration gradient (Wedemeyer, 1996).

2.1.10 Metals

Metals can be highly toxic to fish (Wedemeyer, 1996). Potential toxicity for fish is governed by the ionic strength and composition prevailing in different lakes and rivers. In nature, fish are more vulnerable to several environmental toxins in water of low ionic strength, such as in the south of Norway, where the water conductivity is normally around 5 – 20 µS/cm. In the more calcium-rich water of the central inland, with conductivity of 50 – 150 µS/cm, the fish can stand higher concentrations, at least for metals, before they start failing to thrive. Only the effects of the most common potential toxic metals found in Norway are mentioned here.

2.1.10.1 Copper

The toxicity of copper is dependent upon alkalinity and hardness of the water, with quite different recommended safety levels, 6 µg/L Cu at alkalinity <100 mg/L and 30 mg/l Cu at alkalinity >100 mg/L (Wedemeyer, 1997; Timmons and Ebeling, 2007). Fish can tolerate higher Cu levels with increasing calcium levels in the water. The toxicity will decrease with increasing pH. For example, in Norwegian copper-impacted mining rivers, like upper Gaula and Ya, fish and other aquatic life died at typical concentrations of 20-25 µg/L Cu. These rivers have relatively high calcium concentrations and ionic strength. In the water of Southern Norway, which is low in calcium, fish die at 5 µg/L Cu. The European Inland Fishery Advisory Commission and Alabaster and Lloyd (1980) give a graded guideline for copper toxicity depending on the calcium concentration in the Water Quality Guidelines for Freshwater Fish. In typical Norwegian water qualities with calcium concentrations below 10 mg/L Ca, copper may be lethal for rainbow trout between 1 – 5 µg/L Cu. Most of the

literature regarding copper toxicity in salmonid fishes is based on experiments with rainbow trout. Toxicity of copper is nevertheless a serious problem in Atlantic salmon smolt production (Åtland et al., 1999). Furthermore, high mortalities due to copper were observed in the start-feeding period, and it seems that salmon could be more sensitive to copper toxicity than rainbow trout. It could be questioned whether the recommended level is too high for Atlantic salmon. Additionally, the concentration of total organic carbon is a key factor to reduce copper toxicity. A suggested toxic mechanism is that copper induces failure in ammonium excretion and sodium uptake. Earlier studies have shown that fish exposed to water contaminated with copper produce high levels of ammonium in the tissues. This waterborne copper toxicity increases with feeding (Hashemi et al., 2008; Kunwar et al., 2009).

2.1.10.2 Aluminium

Aluminium (Al) is toxic to fish has caused water quality problems in Norwegian smolt farms. Even at low concentrations (0.115 – 0.140 mg/L of total Al, 0.010 mg/L of labile Al), the presence of the metal can be toxic in combination with carbon dioxide and reduced pH (Fivelstad et al., 2003b). In turbid rivers like many of the rivers and lakes in Vestfold county, total aluminium content can be as high as 0.3 – 0.5 mg/L without any damage to fish. Most of this aluminium is bound in the minerogenic material making up the turbidity. The reactive aluminium, which can split into labile Al and illabile Al, is normally low in these waters. It is the concentration of reactive aluminium one should be aware of, as the total aluminium is mostly particulate and very little reactive. Recommended maximum levels of labile aluminium are < 0.075 mg/L (Wedemeyer, 1997) and < 0.01 mg/L (Timmons and Ebeling, 2007). The maximum levels depend upon bioavailability and the possibility of aluminium binding to fish gill rather than to humic acid, particles or organic materials. There is a close relationship between aluminium in the water and accumulation on the fish gills of salmon (Kroglund et al., 2001; Teien et al., 2005). In soft water, < 0.010 mg/L labile Al is accepted as a background value. At high concentrations of labile aluminium (0.300 mg/L in freshwater and 0.150 mg/L in seawater (Kroglund and Staurnes, 1999)), the fish die as a consequence of respiratory and osmoregulatory failure (Rosseland and Staurnes, 1994). Physiological changes of welfare interest can be seen at much lower concentrations of labile aluminium (0.100 mg/L in freshwater and 0.040 mg/L in seawater). An often applied recommendation for labile aluminium toxicity for salmon smolts is 0.015 – 0.020 mg/L (Rosseland, 1999).

2.1.10.3 Iron

The toxicity of iron (Fe) may result in a scenario similar to that of aluminium. Ferrous iron (Fe^{2+}) is dissolved from draining bog areas and gradually oxidized to ferric ion (Fe^{3+}) in the rivers or ground waters, where it may precipitate on fish gills in the same way as aluminium. Several hatcheries have experienced this problem due to intake of ground water. It is recommended that the levels of iron (Fe) should be less than 0.15 mg/L (Timmons and Ebeling, 2007).

2.1.11 Temperature

The water temperature in lakes and rivers in Norway varies considerably. The optimal physiological thermal range for brown trout (*Salmo trutta*) is 4-19 °C (Elliott, 1981). The upper critical ranges for brown trout and rainbow trout, depending on life stage and acclimation temperature, are 19 – 30 °C (Elliott, 1981). Exposure of fish to low temperatures blocks reflexes and eventually nerve conduction (Roots and Prosser, 1962; Prosser and Farhi, 1965). This effect (hypothermia) is sometimes exploited as an anaesthetic method for fish. When adult brown trout were abruptly transferred from a water temperature of about 14 °C to a mixture of ice and water (0.2 °C), a cold shock was induced and the fish seemed to be unconscious, did not move or respond to tactile stimuli. However, when the fish were transferred back to the original water temperature after 10 min, they recovered within 10 min, and after 20 min, their body temperature had returned to the one before the treatment (Hyvärinen et al., 2004). If water temperatures drop gradually, for example during autumn and winter, the sensitivity towards low temperature can differ since the fish have time to acclimatize to the gradually changing conditions. The lower lethal temperature for Atlantic salmon is reported to be around -0.7 °C (Saunders, 1986). However, Skuladottir et al. (1990) reported that mortalities started to occur at -1.4 °C in Atlantic salmon with the average weight of 0.4 kg, when the seawater temperature dropped gradually to -1.8 °C. The upper temperature limit for Arctic char (*Salvelinus alpinus*) is about 19 °C (Johnston, 2002). Char have some unusual characteristics in their thermal behaviour as they grow remarkably well at temperatures below 4 °C (Brännäs and Wiklund, 1992) although their high optimum temperature for growth is about 16 °C (Larsson and Berglund, 2005). Nevertheless, in preference tests, the char tended to select temperatures around 11 °C (Larsson and Eriksson, 2009). This finding resulted in the introduction of deep net pens in several Arctic char farms allowing them to access favoured temperatures below the thermocline during warm summer periods (Eriksson et al., 2010). In terms of fish welfare, such farms could constitute an advantage over land based tanks with natural water temperatures. Rearing Arctic char at about 12 °C seems to be a suitable compromise for good on-growth, high feed conversion rate, and reduced hazards related to development of diseases and growth of fungi (Winther et al., 2010). Fletcher et al. (1988) studied lethal freezing temperatures in the presence of ice in Arctic char, brook trout (*Salvelinus fontinalis*), Atlantic salmon, brown trout and rainbow trout with mean fish weights of 104 – 252 g, after two months of acclimatization to seawater at 0 – 1 °C. Arctic char showed the greatest resistance to freezing (-0.99 °C) followed by brook trout (-0.87 °C), brown trout (-0.81 °C), salmon (-0.76 °C) and rainbow trout (-0.75 °C). Notably, the lethal freezing temperatures of all of the salmonids were lower than their plasma freezing temperatures (between -0.79 °C and -0.69 °C) suggesting at least one factor was involved in preventing the fish from freezing. It was hypothesized that the char's epidermis acts as a barrier to prevent propagation of ice. In absence of ice, the authors observed that the Arctic char had survived temperatures of -1.2 °C for at least five days whereas brook trout survived -1.7 °C for three days as long as they did not come into contact with ice. During commercial farming of char in freshwater lakes, the water temperature in the cages has dropped to about 0.6 °C over extended periods of time during the winter. This did not result in excessive mortalities and the low temperatures

were not considered a particular problem for the operation of the cage systems (Oddmund Grøttan, previously employed by Svensk fjällröding AB, pers. comm.).

2.2 Hazards related to impact on water quality and environmental status in the recipient

Other governmental bodies than the NFSA are responsible for laws and regulations concerning environmental issues. It was, however, felt necessary to include these aspects in order to present a complete picture.

In ultra-oligotrophic recipients, defined as waters originally low in nutrients, fish farming can to a certain extent enhance the production of wild fish. This can typically be seen in lakes where the natural fish productivity originally was quenched due to impact of hydropower regulation. The increase in the number of wild fish is to some extent directly caused by release of fodder remains from the farm, but is mainly a consequence of the acceleration of the natural aquatic food web, facilitated by the same nutrient release. However, since an excess of nutrients facilitates eutrophication, it is important to estimate the carrying capacity of the lake for fish farming, recipient capacity, as a dimensioning factor when establishing freshwater production systems. Saprobiation is the build-up of rotting sediments under or downstream of the cages. This constrains the recipient along with other negative impacts, such as spread of unwanted organisms (Appendix IV), fish pathogens and pharmacological agents to the wild. The latter also may raise concerns regarding drinking water safety.

The different types of aquaculture systems differ in their potential of having negative impacts on the recipient. Prevention of environmental pollution from farming in floating net cages represents a major challenge, and is by far the most polluting farming system. In land-based systems, discharge treatment is manageable, even though advanced purification often has economic limitations. Freshwater recipients are much smaller than the marine recipients and are easily overloaded. The recipient capacity is defined by the extent of impact tolerated by a given recipient to still be considered of good ecological status in accordance with the Classification Guidance 02:2013 of the Water Regulation (www.vannportalen.no), which is the Norwegian equivalent of the European Water Framework Directive (WFD). The maximum limit of the strain on the environment that is described by the recipient capacity must be complied with. In water bodies of high ecological status, deterioration of water quality to a degree that leads to the lower class of good ecological status is prohibited.

Assessment models for the recipient capacity for eutrophication impacts are utilized to outline the need for abatement measures in all the 101 water areas in Norway. The same models can be used to assess the recipient capacity for aquaculture. The currently applied methods are described in the State Pollution Control Authority (SFT) Guidance 95:01 (SFT 1997). Calculations of the recipient capacity in accordance with the SFT models also ensure good water quality for wild fish, as they are based on healthy algal growth requirements, which with respect to eutrophication are stricter than for fish.

Effluent treatment is possible to perform in land based aquaculture systems, which allows a greater flexibility for this type of fish farm with regards to increase of production volume, without surpassing the recipient capacity. The required extent of effluent treatment is depending on the recipient capacity for nutrients, oxygen consuming materials, etc., as well as the danger of spreading fish diseases. In freshwater farms using floating cages, however, keeping within the limits of the recipient capacity can be more challenging. In the small lake Espedalvannet in Sogn og Fjordane county, a floating cage plant led to an increase of the algal biomass to levels clearly above the recipient capacity year after year, with an average production of 800,000-1,200,000 smolt per year. It was calculated that the recipient capacity was around 400,000 smolt per year (Berge, 2000). Such a small production was not economically feasible, and the floating plant was closed down. In the large lake Røssvatn in Hattfjelldal, with the same model system, Berge (2010) calculated that it was possible to produce 3,000 tons per year of Arctic char to keep within the recipient capacity of the lake. Taken into account that this is Norway's second largest lake, it gives an indication of the limitation in the potential of floating net cages aquaculture in fresh waters in Norway.

2.3 Hazards related to fish stocking density

At a given flow of water and temperature, the basic factors that govern the amount fish that can be introduced to a system, are related to adequate supply of oxygen and the need to remove metabolic wastes from the fish. Excessive fish densities can cause a stress response, for example as a result of behavioural interactions (Wedemeyer, 1997; Pickering, 1998; Turnbull et al., 2005). Also, inappropriate densities are reported to give reduced growth rate, poor health condition and increased mortality (Wedemeyer, 1997).

Provided good water quality is maintained, North et al. (2006) concluded that it is possible to grow rainbow trout at densities up to 80 kg/m³ without affecting growth, condition factor or mortality. High densities have been shown to reduce growth and feed conversion efficiency in rainbow trout (Ellis et al., 2002; North et al., 2006) although slower growth rate is not considered an indicator of poor welfare in itself (Huntingford et al., 2006). On the other hand, physical and endocrine indicators of poor welfare, such as fin erosion and gill damage, have been reported at high densities (Ellis et al., 2002). When two stocking densities (19-25 kg/m³ and 75-100 kg/m³) were compared with regards to effects on specific growth rate, feed conversion, energetics and welfare of rainbow trout (initial to final weight: 195 to 218 g), it was found that high density reduced specific growth rate and that the fish had poor swimming performance, indicating compromised welfare due to physiological impairment (McKenzie et al., 2012). Partly in contrast to this, Ellis et al. (2002) reviewed available literature on the relationship between stocking density and welfare of rainbow trout, and concluded that no specific limit value should be given. It was suggested to base considerations of acceptable stocking density on water quality parameters as well as effects on fish health and welfare. In recirculation systems, the maximum carrying capacity of rainbow trout has been suggested to be 100 kg/m³ at 12 °C (Roque d'Orbcastel et al., 2009).

Hosfeld et al. (2009) reported a study in which Atlantic salmon parr were reared at different stocking densities, up to a maximum of 86 kg/m³, with continuous adjustment of critical water parameters. The authors underline the importance of maintaining a sufficient food supply at the higher densities, but otherwise, no negative effects were observed. The maximum allowed rearing density for post-smolts in Norway is presently set at 25 kg/m³ (akvakulturdriftsforskriften). Typical fish densities in Norwegian land based farms for post-smolt salmon, as reported in 1995, ranged from 10 to 100 kg/m³ (Forsberg, 1995). Thorarensen and Farrell (2011) concluded after reviewing literature on fish density, ranging from 10 to 125 kg/m³, that it appeared to be no consistent effect on the growth, survival and welfare of Atlantic salmon post-smolts in closed-contained systems up to a fish density of up to 80 kg/m³.

For most fish species, including salmonids, growth rates are higher at low fish densities whereas aggression tends to escalate with increasing densities until a level is reached, at which the frequency of interactions decline, although stress tends to increase and food consumption decreases (Brown et al., 1992). Arctic char seems to be an atypical salmonid in this respect as they show higher growth rates at higher densities (Wallace et al., 1988; Baker and Ayles, 1990). Maximum growth rates are observed at densities of 100-110 kg/m³ provided good water quality can be maintained (Wallace et al., 1988; Jobling et al., 1993; Jørgensen et al., 1993). Notably, the studies of Brown et al. (1992), Jørgensen et al. (1993) and Wallace et al. (1988) were carried out with relatively small fish within the ranges of 85, 52 – 55 and 5 – 35 g, respectively. According to Baker and Ayles (1990), the optimal stocking density for char at 40-200 g is about 40 - 60 kg/m³ when fish health and economic yield is taken into consideration, compared with 10 - 25 kg/m³ for other salmonids. Furthermore, Siikavuopio and Jobling (1995) reported that char kept at 30 kg/m³ had more fin damages and higher weight losses than those kept at 90 and 150 kg/m³. In grow-out cages in Landösjön in Sweden, where char were eventually slaughtered when reaching market size of 0.5 – 1.0 kg, the stocking density is typically around 75 kg/m³ (Oddmund Grøttan, pers. comm.). It has been suggested that increased growth rates at higher densities for char may be behaviourally mediated, such as decrease in agonistic interactions and other changes in social structures (Jobling, 1985; Wallace et al., 1988). This can increase growth rates by reducing stress and energy requirements. The easy adaption to crowding is probably related to their origin as a schooling lake-dwelling species (Brown et al., 1992). The frequency of bites from dominant fish, affecting the welfare of a fish population, is related to stocking density. The proportion of total bites accounted for by top-ranked char and rainbow trout decreased from 87 to 15 % and from 66 to 15 %, respectively, when moving from low to high densities (Alanära and Brännäs, 1996). Particularly relevant for land based farming of Arctic char, it is important to provide for sufficient depth in the tanks. If the water level is kept too low, the fish may develop the so-called 'swim bladder stress syndrome'. The fish will then be unable to control its buoyancy since the swim bladder is filled with excessive amounts of air, resulting in the fish floating at the water surface. To avoid this, the recommended depth should be at least 35 cm (Kolbeinshavn and Wallace, 1985).

Currently, there is little rearing of post-smolts, rainbow trout and char (>150 g) in land based facilities in Norway, but this situation may change rapidly. Based on available literature, various suggestions for recommended fish densities are given for salmonids. However, the data are not entirely consistent and factors such as water quality (not determined in all studies), fish size (literature covers mostly effect of density on smaller fish than the market-sized fish, which is addressed in the present risk assessment), as well as factors related to the farming systems, are either varying, insufficient or even lacking altogether. It is therefore difficult to draw explicit conclusions from the various studies addressing fish density, and further studies in this field are strongly recommended, particularly with larger fish in grow-out cages or tanks. Nevertheless, in case of rainbow trout and Atlantic salmon, it seems appropriate to tentatively recommend fish densities of less than up to 80 kg/m³, whereas for Arctic char higher densities can be recommended possibly within the range of 75-150 kg/m³.

2.4 Hazards related to infectious agents

In this chapter, the working group addresses identification and characterization of infectious hazards related to freshwater fish production. There is limited experience in Norway on cage farming in fresh water. In Chile, cage farming in inland lakes was for many years an integrated part of the salmonid farming strategy, and infections with *Saprolegnia* sp. and *Flavobacterium* sp. were common disease problems. There are also indications that infectious salmon anaemia virus (ISAV) infections in escaped individuals in the lakes infected the fingerlings stocked in the cages.

Most of the viral pathogens that traditionally are found in the seawater phase of Norwegian commercial Atlantic salmon farming can be found in freshwater farming in other geographical areas and under other farming management practices. This can be explained by the negligible influence of the water salinity on the intracellular environment, within which viruses reside. Most fish viruses of anadromous fish are therefore able to establish infections independent of whether the host is in fresh water or seawater.

OIE (World Organisation for Animal Health) publish health standards for international trade in animal and animal products. Fish diseases are listed internationally or nationally to establish standards in the control of fish diseases. In the EU/EEA area, the List 1 and 2 are declared by the European Commission, and the Norwegian national List 3 by the Ministry of Trade, Industries and Fisheries. The infectious agents that are described below are those currently considered to be of most interest for fresh water farming of rainbow trout and Arctic char in Norway. Such a list cannot be exhaustive.

2.4.1 Viral infections

2.4.1.1 Infectious pancreatic necrosis virus

Infectious pancreatic necrosis virus (IPNV) is found in both seawater and freshwater commercial salmon and rainbow trout farms in Norway. It belongs to the genus *Aquabirnavirus*, a group of viruses with a very large host range that includes fish, crustaceans and bivalves in both fresh water and saltwater (Wolf, 1988; Saint-Jean et al. 2003). Infectious pancreatic necrosis is a highly contagious viral disease of young salmonid fish species held under intensive rearing conditions (Wolf et al., 1960) and may cause disease in a variety of salmonid species, including members of the genera *Salmo*, *Salvelinus* and *Oncorhynchus*. The disease characteristically occurs in rainbow trout, brook trout, brown trout, Atlantic salmon, and several Pacific salmon species (*Oncorhynchus* spp.). During the early 1970s, IPN was recognized to cause high mortalities in European and Japanese rainbow and brown trout farms (Ball et al., 1971; Vestergård-Jørgensen and Kehlet, 1971). The virus is not easily inactivated. The infectivity of the virus survives for nearly a year at 4°C and nearly 2 months at 15°C in buffer (Dorson, 1982) and also for long periods in seawater, brackish water and unsterilized fresh water (Moewus-Kobb, 1965; Toranzo and Hetrick, 1982). This stability increases the possibility for transmission of the virus from carrier or clinically ill fish to susceptible fish in the watershed.

The disease is transmitted horizontally via the water route, and vertical transmission has been demonstrated for brook trout and rainbow trout (Ahne and Negele, 1985). Surface disinfection of fertilized roe is not entirely effective in preventing vertical transmission (Bullock et al., 1976). Control methods rely on control policies and of hygiene practices in farming management, through the avoidance of the introduction of IPNV contaminated fertilized roe and the use of a virus free water supply. The virus seems to be difficult to eradicate once introduced into a hatchery.

The IPN virus is not restricted to commercial farming but also found in fish from remote regions that have had no known contacts with commercial farming or restocking of fish. Souter et al. (1986) isolated IPNV from nearly half of 229 anadromous Arctic char prespawners collected from the Mackenzie River delta and rivers in the Yukon Territory where there was no known contact with hatchery-reared fish. The high level of the virus in this location indicates that natural infections may be widespread and that the maintenance of the carrier state in fish is efficient under natural conditions. In Norway some hundred wild broodfish (mainly Atlantic salmon) are screened each year for the presence of IPNV, but positive findings are rare (Hjeltnes, 2014, and earlier issues in the report series).

Infectious pancreatic necrosis has been one of the most common diseases in Norwegian aquaculture during the last decades, and is found in both fresh water and saltwater (Hjeltnes 2014 and earlier issues in the report series). Recently, the number of cases reported to the Norwegian Veterinary Institute has decreased, probably due to effective breeding programs targeting IPN and better hygienic routines. In salmon farming, the course and outcome of

IPNV-infections varies significantly (Roberts and Pearson, 2005). Infectious pancreatic necrosis has been removed from the Norwegian list of nationally notifiable diseases.

2.4.1.2 Infectious salmon anaemia virus

Infectious salmon anemia virus (ISAV) has been found to cause disease in seawater in commercial salmon farms in Norway, but was established in fresh and brackish water during the large ISA epidemic in Chile in 2007 – 2010. The disease has only been detected in farmed Atlantic salmon. The virus can replicate in several other fish species, but no disease has been observed under aquaculture conditions. It has been found in feral Atlantic salmon and sea trout (*Salmo trutta*) (Nylund and Jakobsen, 1995). Replication of ISAV occurs in experimentally infected Atlantic salmon, brown trout, rainbow trout, Arctic char (Snow et al., 2001) as well as in several species of Pacific salmon and in some marine fish species (OIE: Manual of Diagnostic Tests for Aquatic Animals, 2014). In experiments with inbred rainbow trout, disease and mortality have been induced (Biacchesi et al., 2007).

Waterborne transmission in fresh water has been demonstrated in cohabitation experiments, indicating that it is important for the spread of ISA, and in seawater by horizontal spreading from infected farms (Thorud and Djupvik, 1988). The virus is stable between pH 5.7 – 9.0, and the virus has a 3-log₁₀ reduction in titre after four months when maintained in sterile seawater at 4 °C (Rimstad and Mjaaland, 2002). In Chile, ISAV was introduced into several freshwater lakes used for production of salmonid smolt and fingerlings in floating cages. This may have been an important vehicle for spread of the virus during the ISA crisis in Chile.

2.4.1.3 Salmon pancreas disease virus

Salmon pancreas disease virus (SPDV), also called salmonid alphavirus (SAV), is only found to cause disease in the seawater phase in commercial salmon and rainbow trout farms in Norway. The virus is also found in freshwater rainbow trout farms in central Europe where the disease is called sleeping disease. Salmonid alphavirus is currently classified into six subtypes, and most of these have specific geographic distributions in Europe. These viruses have been shown to transmit efficiently horizontally via water (McLoughlin et al., 1996). There is no evidence for vertical transmission of SAV in Atlantic salmon (Kongtorp et al., 2010). Screening of marine fish species by *real-time* PCR in areas remote from aquaculture has indicated the presence of SAV in marine species including common dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*) (Snow et al., 2010).

Brown trout is listed as a susceptible species (OIE: Manual of Diagnostic Tests for Aquatic Animals, 2014). The virus is sensitive to high and low pH, high temperature and common virucidal disinfectants (Graham et al., 2007a; 2007b). It can survive for more than two months at low temperatures.

2.4.1.4 Viral haemorrhagic septicaemia virus

Viral haemorrhagic septicaemia virus (VHSV) is common in freshwater rainbow trout farming in Europe, but also a common infection of many marine fish species. The virus is able to infect a multitude of fish species, and is occasionally found in rainbow trout seawater farms in Norway. In Finland, VHSV has repeatedly caused outbreaks in freshwater farms. Transmission of VHSV occurs primarily horizontally through contaminated water by direct excretion of virus from infected fish. In salmonids, VHSV primarily causes disease of farmed rainbow trout. Viral haemorrhagic septicaemia virus has been isolated from a broad range of freshwater and marine species (Skall et al., 2005), and has in the last decade caused mass mortalities in wild fish in the Great Lakes region of North America, involving at least 31 freshwater fish species (Kim and Faisal, 2011). In Denmark, it has recently been demonstrated that it was possible to eradicate VHSV from river systems after a previous introduction many years ago by fallowing and sanitizing infected farms (Olsen et al., 2013).

In the late sixties and seventies there was a number of outbreaks of VHS in rainbow trout in fresh water in Norway. Most likely this could be attributed to import of infected or contaminated roe from Denmark. However, there is no evidence that VHS was established in wild fish populations.

Disinfectants such as UV, chlorine and hypochlorite or iodophores are very efficient to inactivate rhabdoviruses.

2.4.1.5 Piscine orthoreovirus

Piscine orthoreovirus (PRV) is the cause of the disease heart and skeletal muscle inflammation (HSMI) which traditionally has been observed in seawater in commercial salmon farms in Norway (Palacios et al., 2010, Finstad et al., 2012). However, the virus is also commonly found in salmon in the freshwater phase, and the virus may possibly be related to other disease conditions than HSMI in fish as well. In Norwegian aquaculture, HSMI is one of the most common diagnoses, with approximately 150 confirmed cases each year since 2007 (Hjeltnes, 2014).

Piscine orthoreovirus can be considered as ubiquitous in marine farming of Atlantic salmon in Norway and has also been found in farmed Atlantic salmon from Chile and Canada (Kibenge et al., 2013). The virus is present in the freshwater stage, but at a lower prevalence than in seawater farms (Løvoll et al., 2012). In Norway, PRV is commonly found in returning wild Atlantic salmon spawners (Garseth et al., 2013c), and phylogenetic analysis substantiates transfer of virus between farmed and wild salmon (Garseth et al., 2013b). Piscine orthoreovirus has been found in sea trout (*Salmo trutta* L.), the sea going form of brown trout, but so far not in Arctic char (Garseth et al., 2013c; Biering et al., 2013). In addition the marine species great silver smelt (*Argentina silus*), capelin (*Mallotus villosus*), Atlantic horse mackerel (*Trachurus trachurus*) and Atlantic herring (*Clupea harengus*) have tested PRV-positive by PCR (Wiik-Nielsen et al., 2011). Infection routes of PRV have not been

thoroughly studied, but the possibility of vertical transmission cannot be excluded (Wiik-Nielsen et al., 2012).

The effect of commonly used disinfectants on the infectivity of PRV is unknown, partly due to lack of an efficient *in vitro* cultivation system. However, avian and mammalian orthoreoviruses are remarkably stable and withstand extremes of ionic conditions, temperatures up to 55 °C, pH values between 2 and 9, lipid solvents and detergents.

2.4.1.6 Infectious haematopoietic necrosis virus

Infectious haematopoietic necrosis virus (IHNV) belongs to the genus *Novirhabdovirus* of the family *Rhabdoviridae*. The virus comprises two genogroups that are geographically related (Kurath et al., 2003). Isolates from farmed rainbow trout in Europe appear to have originated from North America (Enzmann et al., 2005).

The disease affects wild and farmed rainbow trout, many species of Pacific salmon and Atlantic salmon. Brown trout and Arctic char have been found to be infected in the wild or shown to be susceptible by a natural route of infection (OIE: Manual of Diagnostic Tests for Aquatic Animals, 2014.). In Norway, IHN has never been detected.

2.4.1.7 Piscine myocarditis virus

Piscine myocarditis virus (PMCV) is involved in cardiomyopathy syndrome (CMS), which is a disease of large fish in seawater in commercial salmon farms in Norway (Løvoll et al., 2010). The virus has been detected in returning broodfish of wild Atlantic salmon in 2 out of 797 and one out of 453 tested fish (Garseth et al., 2012; Biering et al., 2013). There is no available information regarding the susceptibility of rainbow trout or Arctic char.

2.4.2 Bacterial infections

2.4.2.1 *Flavobacterium psychrophilum*

Flavobacterium psychrophilum is one of the most important bacterial fish pathogens worldwide and is well-known from freshwater aquaculture in Europe (Dalsgaard et al., 1990; Wiklund et al., 1994; Dalsgaard and Madsen, 2000). Susceptible species probably includes all salmonids in addition to several non-salmonids including eel (*Anguilla anguilla*), carp (*Cyprinus carpio*), and tench (*Tinca tinca*) (Nematollahi et al., 2003). As the infection is of great importance in aquaculture globally and in farming of several important salmonid species, there is still a major focus on occurrence, spread and control of *F. psychrophilum*.

The bacterium is the causative agent of bacterial coldwater disease (BCWD) and rainbow trout fry syndrome (RTFS) and has since the mid-1980s caused high mortality in farmed rainbow trout in Europe. In Norway, *F. psychrophilum* has been isolated from Atlantic salmon and brown trout with fin rot and ulcers without significant associated mortality in hatcheries

(Brit Hjeltnes, pers. comm.). However, in 2007 – 2008, systemic infections with *F. psychrophilum* were described (Nilsen et al., 2011a; 2011b). This, together with epidemiological data, suggests that a new virulent clone has been released in Norway (Nilsen et al., 2014). So far, this possible new clone seems to be restricted to certain areas in Western Norway. The bacterium has the potential to establish in freshwater and brackish water systems. No impact has so far been reported on wild stock, but such virulent variants represent a significant treat to farming of rainbow trout.

In addition to its presence in Norway, the bacterium occurs in every country and species from which it is relevant to import roe (Nematollahi et al., 2003). Vertical transmission occurs, and broodfish is a possible source of infection and transmission of *F. psychrophilum* both vertically and horizontally (VKM, 2011).

2.4.2.2 *Renibacterium salmoninarum*

Bacterial kidney disease (BKD) is a serious disease in salmonids caused by the intracellular bacterium *Renibacterium salmoninarum*. The disease occurs only in salmonids, and examples of susceptible species are salmon and brown trout (*Salmo* spp.), Pacific salmon and rainbow trout (*Oncorhynchus* spp.), Arctic char (*Salvelinus* spp.) and grayling (*Thymallus thymallus*) (Kettler et al., 1986; Bullock and Herman, 1998). Bacterial kidney disease is widespread worldwide, with the possible exception of Australia. In Norway, there are occasional discoveries in both wild and farmed fish.

Renibacterium salmoninarum is transmitted directly from fish to fish, both vertically and horizontally. The bacterium is an obligate parasite that cannot survive long outside the host (Evelyn, 1993). There is no indication of vectors or reservoir in other species. The bacterium can be detected in mussels, but infectivity does not seem to be sustained over time (Paclibare et al., 1994). Viability in sediments or faecal material for up to 21 days has been reported (Austin and Rayment, 1985). Horizontal transmission occurs by direct contact between infected individuals and healthy fish, as well as by consumption of infected tissue (Mitchum and Sherman, 1981). Faecal - oral transmission can also occur (Balfry et al., 1996).

Vertical transmission of *R. salmoninarum* by intra-ovum transmission to offspring seems to be the most important naturally occurring infection route (Evelyn et al., 1984; 1986). Ovarian fluid is an important source of infection for eggs, but bacteria can also enter the egg during maturation in the ovary (Evelyn et al., 1984, 1986, 1993).

Bacterial kidney disease was first diagnosed in Norway in 1980, and the disease spread to both wild and commercial fish populations (Tore Håstein, pers. comm.). The problem was resolved by mandatory testing of all wild caught broodfish, increased disease surveillance of commercial broodfish and culling of infected populations. In Norwegian farmed salmon, BKD is now a sporadic diagnosis, and *R. salmoninarum* is seldom detected in wild caught fish.

2.4.2.3 *Aeromonas salmonicida* subsp. *salmonicida*

Aeromonas salmonicida subsp. *salmonicida* is a serious fish pathogenic bacterium causing furunculosis in both freshwater as well as marine aquaculture. The bacterium is mainly a pathogen of salmonids and widespread in Europe. In Norway, outbreaks of the disease in fresh water were reported in the sixties after import of Danish rainbow trout. Furunculosis was reintroduced to Norway in 1985 after import of infected Atlantic salmon smolts from Scotland (Egidius, 1987). *Aeromonas salmonicida* subsp. *salmonicida* soon spread around the Norwegian coast to all the main marine fish farming areas in Norway. For several years, furunculosis was one of the most serious fish diseases in marine aquaculture and in hatcheries adding seawater to improve the water quality. The disease spread to several river systems (Johnsen and Jensen, 1994) where it may appear in wild salmon in the summer, especially at high temperature and low water flow (Johansen et al., 2009). However, *A. salmonicida* is not common in inland waters in Norway. The bacterium is transmitted horizontally from fish to fish through the water.

2.4.3 Parasitic infections

2.4.3.1 *Gyrodactylus salaris*

Gyrodactylus salaris is an important pathogenic ectoparasite of Atlantic salmon. Atlantic stocks of salmon are unable to mount an effective immune response, and infections thus lead to serious disease and usually death (Bakke et al., 1990). *Gyrodactylus salaris* is endemic in northern Russia, northern Finland and western Sweden, and it has been introduced into Norway, Denmark (Nielsen and Buchmann, 2001) and Germany (Bakke and Harris, 1998). The parasite has a direct lifecycle. It reproduces and survives permanently only on Atlantic salmon and rainbow trout, but it can live for many days on other salmonid and non-salmonid hosts (Peeler et al., 2004). *Gyrodactylus salaris* will not survive full seawater salinity (Soleng and Bakke, 1997). After introduction into Norway with infected fish in the early 1970s (Mo, 1994), *G. salaris* spread to 48 rivers and has been the cause of a lengthy and costly eradication campaign. Still 14 rivers remain infected, whereas 14 others are in the process of being declared free after treatment. The parasite is killed by the disinfectants commonly used to treat fish eggs (T.A Mo, pers. comm.; Peeler et al., 2004). Introduction or spread of *G. salaris* is only likely via import or movement of fish. The consequences of an introduction into salmon-running water systems are likely to be severe.

2.4.3.2 *Myxobolus cerebralis*

The myxosporean parasite *Myxobolus cerebralis* causes whirling disease in salmonids. It has two hosts; in addition to salmonid fish it needs an oligochaete worm of the genus *Tubifex* to complete its life cycle (Hedrick and El-Matbouli, 2002). The parasite was first described from Europe and has been introduced into North America with severe consequences for wild salmonids (El-Matbouli et al., 1992). Rainbow trout is considered to be the most susceptible host for whirling disease. *Myxobolus cerebralis* is believed to occur in salmonids in many

Norwegian rivers, but to date it has not represented any disease problem for farmed fish (VKM, 2005). The parasite may be introduced by movement or importation of fish, given the presence of an oligochaete host.

2.4.3.3 *Tetracapsuloides bryosalmonae*

Proliferative kidney disease (PKD) caused by the myxozoan *Tetracapsuloides bryosalmonae*, is a widespread condition of both farmed and wild salmonids in Europe and North America. In Norway, it was identified as the cause of mortality of Atlantic salmon in the Åbjør watercourse and in the river Jølstra from 2002 – 2004 (Sterud et al., 2007). Ensuing investigations identified the parasite in 15 of 18 Norwegian rivers examined (Forseth et al., 2007). *Tetracapsuloides bryosalmonae* is also widespread in Denmark in rainbow trout, brown trout and salmon (Skovgaard and Buchmann, 2012), and Arctic char is also considered susceptible (Morris et al., 2000). Spread of the parasite appears not to be linked to movement of fish, but rather to environmental factors like increasing temperature and availability of its primary host, a freshwater bryozoan (Okamura et al., 2011). It has been speculated that fish are a dead-end host for *T. bryosalmonae*, but at least brown trout and brook trout are permissive hosts (Morris and Adams, 2006; Grabner and El-Matbouli, 2008).

3 Risk characterisation

Method

The present report is a qualitative risk assessment. The working group builds the risk characterization on the identified hazards in the previous chapter and estimates the probability of these events to occur as well as the magnitude of their consequences.

Low probabilities define events that are unlikely to occur, whereas high probabilities are used for events that could be expected to occur. If there is less than an even chance of the event occurring, the probability is classified as moderate.

Limited consequences associated with the given event are mild or insignificant for fish health and welfare, and the disease is easy to control. Serious consequences for fish health and welfare are for instance high mortality or high morbidity with significant pathological changes, affecting a high number of fish during a longer time span. Moderate consequences are less profound for fish health and welfare.

Risk is defined as probability multiplied by consequence, and in a qualitative risk assessment each hazard is assigned a low, moderate or high risk.

3.1 Risk of changes in water quality for farmed and wild fish

Risks associated with changes in water quality are shown in Figure 3.1.-1.

3.1.1 Floating cages

When fish are farmed in cages located in lakes or rivers, water exchange and water quality in the cages are governed by changes of the natural conditions in the lake as well as by potential accidental pollution of the ecosystem. Since farms are located at sites with good water exchange, sufficient supply of oxygen and effective removal of carbon dioxide and total ammonia nitrogen would not, under normal conditions, constitute a problem in terms maintaining good water quality. The alkalinity and hardness of the water source (lake or river) will determine how well the water can compensate with potential changes in environmental pH.

Potential changes in water quality due to pollution could involve changes in pH, and the levels of total organic carbon, gas supersaturation, total suspended solids and metals (copper, aluminium and iron). Such changes may cause considerable deterioration of water quality that could seriously compromise fish welfare and could ultimately cause mortalities.

Summer water temperatures in Norwegian lakes and rivers would not normally exceed tolerance levels of the fish. During winter, water circulation in cages will keep water temperatures above the freezing point.

To promote good fish welfare it is also necessary to consider the relationship between fish stocking density and the ability of the system to maintain appropriate water quality. Seasonal changes in flow and exchange of water, as well as in water quality, may occur. Formation of ice will occur in most lakes during winter. However, practical experience has shown that circulation of water artificially by water agitators will effectively prevent ice formation in the cages.

Discharges from aquacultural activity are directly transmitted to the ecosystem without treatment posing a potential risk to wild fish welfare. Relocating or re-locating the cages can improve the recipient conditions at the farm site.

3.1.2 Land based single-pass farms

In this case, the fish are farmed by using the flow-through principle. Water is supplied to the tanks from an external source, such as a lake, river or ground water, and then discharged to a recipient water body.

The inlet water can be aerated to minimize possible gas supersaturation (ground water) or to increase oxygen saturation levels. Also, the inlet water is often filtered to remove particles and debris before it enters the fish tanks. Otherwise, the risks related to changes in water chemistry will largely be similar to the floating cage system.

The water can be discharged directly, or after water treatment, to a recipient lake or river. Usually, the water treatment includes filtering of the effluent to remove particles. Otherwise, the large volumes of water passing flow-through farms make comprehensive water treatment a challenge. Thus, the impact on the ecosystem, including the risk of affecting wild fish welfare, may not in practice be very different from floating cage fish farms.

		Probability		
		Low (A)	Moderate (B)	High (C)
Consequence	Serious (c)	cA Low O ₂ levels in cages, flow-through systems and for wild fish Increased NH ₃ levels	cB Insufficient removal of particles	cC
	Moderate (b)	bA High CO ₂ levels in cages and for wild fish Gas supersaturation High levels of suspended solids	bB High CO ₂ levels in flow-through systems Lack of personnel knowledge on how to operate farms safely	bC
	Limited (a)	aA pH out of range Increased NH ₄ ⁺ levels	aB	aC

Figure 3.1-1 A summary of the risk assessment related to water quality factors, based on available literature data for salmonids. Green (aA, aB, bA): Low risk. Yellow (aC, bB, cA): Moderate risk. Red (bC, cB, cC): High risk. See text for limitations on validity and data gaps.

3.2 Risk of introducing infection

The annual fish health report gives a summary of disease outbreaks in the Norwegian aquaculture industry (Hjeltnes, 2014), and indicates the prevalence of disease-causing agents in farmed salmonids in Norway. However, most diseases in farmed salmonids are found in seawater, and the report is therefore not completely relevant to assess the risk of transmission of infectious agents in freshwater cage farming. Examples of differences between current seawater farming and possible future freshwater farming in open water are fish species farmed (salmon versus rainbow trout or Arctic char), reservoir of infectious agents in wild fauna, spread of infectious agents and risk of establishing reservoirs that may be threats to wild fauna as well as other fish farms. Nevertheless, to prevent spread of infectious agents and keep the infection pressure low, biosecurity measures similar to those used in seawater farming can be used in fresh water farming as well. In general, such

measures are based upon the field experience from aquaculture in combat of infectious diseases and knowledge of properties of agents and their epidemiology.

Serious outbreaks of disease are relatively rare among wild fish (Johansen et al., 2011). This is due to relative low population density compared to farming, good water flow and effective removal of diseased individuals, all of which lead to low infection pressure and low opportunity for epidemics. However, diseases may spread from farmed fish to wild populations. Following the reintroduction of furunculosis to Norway with smolts from Scotland in 1985 (Egidius, 1987), the disease quickly spread to both farmed and wild salmon populations and became a major disease problem (Johnsen and Jensen, 1994). The introduction of effective vaccines and other biosecurity measures, such as obligatory disinfection of roe, reduced the number of outbreaks in farmed salmon. Furunculosis is now a rare condition in both farmed and wild salmon, except regular episodes among wild fish in the river Namsen, usually coinciding with low water level and high temperature (Johansen et al., 2009). Due to hygienic measures and an effective vaccination program, furunculosis is well controlled in marine Norwegian aquaculture. With proper disinfection, the probability of roe transmission is very low.

When fish is taken from its natural habitat and stocked in pens in water or tanks on land, this will increase the likelihood of pathogen transmission, pathogen propagation and disease outbreaks. This holds true for all kinds of aquaculture operations, even though stocking densities usually are much lower for the less intensively run stock enhancement hatcheries compared those of the commercial operations. Accordingly, regular health controls conducted by authorized personnel such as veterinarians or fish health biologists, are mandatory for all aquaculture facilities in freshwater as well as in seawater, pursuant to the Regulation on the Operation of Aquaculture Establishments (FOR-2008-06-17-822). Use of only local fish stock, will not absolve an aquaculture facility from these obligations.

The spread of BKD in both wild caught and commercial Norwegian broodfish populations since the first occurrence in Norway in 1980, illustrates the risk of propagation of pathogens in aquaculture (Tore Håstein, pers. comm.). The problem of BKD was resolved by mandatory testing of all wild caught broodfish and increased disease surveillance of commercial broodfish populations. However, the finding of seven BKD-positive broodfish in the river Vosso in 2012 was a reminder of the presence of BKD in wild salmon populations (Eirik Biering, pers. comm.). It will be difficult to prevent introductions of BKD to new freshwater areas with increased use of roe from non-indigenous fish, but the risk is considered to be moderate as the biosecurity measures already in place seem to be sufficient to control the disease.

Viruses may also be propagated in aquaculture conditions. In 2010, a new virus called piscine orthoreovirus (PRV) was identified and linked to the disease heart and skeletal muscle inflammation (HSMB) (Palacios et al., 2010). Kidney samples from wild caught salmonid broodfish were screened for PRV (Garseth et al. 2013c), and it was found that fish released from stock enhancement hatcheries had a higher rate of infection than wild salmon.

Furthermore, the highest rate of PRV positives was found in escaped farmed salmon (Garseth et al., 2013a). It was also shown that there is extensive exchange of PRV between wild and farmed salmon populations (Garseth et al., 2013b), illustrating the possible risk posed by multiplication of pathogens within aquaculture. The high prevalence of PRV in both farmed and wild fish indicate that the virus is highly contagious, but the consequence of an introduction into a naïve population is uncertain. Thus, it cannot be excluded that an introduction may affect the survival of wild populations. Also, the introduction of IPNV from farms into wild populations is possible, due to a large host range and the possibility of vertical transmission of the virus. The consequence of such an introduction is difficult to predict.

Vertical transmission means the transmission of a pathogen from a parent aquatic animal to its progeny via its sexual product (The Aquatic Animal Health Code, OIE, 2008). The aspect of vertical transmission is highly relevant when it comes to evaluating the risk of introducing infection by using genetic material, such as roe or fry. The probability of spread of an infectious agent by vertical transmission depends on parameters such as pathogen load in the gonads of parent fish, intracellular or extracellular transmission and efficacy of disinfection. In general, there is a lack of detailed knowledge regarding these variables. The probability of spreading of infectious agents through vertical transmission is therefore evaluated by use of epidemiological and phylogenetic information.

Farming of rainbow trout, brown trout or Arctic char in fresh water depends on supply of roe and/or fry. This material may originate from local populations, from other domestic sources or be imported. These options of origin entail different risk scenarios with regards to infectious agents.

Local production of roe and fry from indigenous populations excludes the possibility of introducing new infectious agents to the water system. However, there is a possibility of in-farm amplification of enzootic pathogens, and this may increase the infectious pressure on wild species. It is likely that this can be reversed by removal of the farmed population. The Norwegian environmental authorities allow only indigenous fish to be used for stock enhancement activities. For commercial fresh water farming the NFSA sets no such limitations in the current fish health legislation. However, the County Governor only allows use of indigenous stocks for farming of Arctic char.

Even if disinfection procedures are performed according to regulations, there is still a possibility for vertical transmission and introduction of IPNV, *Renibacterium salmoninarum* and *Flavobacterium psychrophilum* when roe from non-indigenous fish are used. Furthermore, when new diseases emerge, the possibility of vertical transmission is unknown. In Norway, there is mandatory screening of wild caught parent fish for *R. salmoninarum* but there is no mandatory screening for IPNV and *F. psychrophilum*. However, all deaths or behavioural deviations that occur during the nine months prior to stripping or other types of reproduction which are not obviously caused by technical faults or related to illness, are to be investigated by autopsy and other potentially relevant methods.

The general requirements for domestic trade of live salmonids are that the fish must be free of notifiable diseases, be clinically healthy and not originate from a population with unexplained high mortality. Thus, there is a possibility for non-notifiable diseases to be spread by movement of live fish. Notifiable diseases may also be spread if diagnostic procedures fail to detect the agent or if regulations are violated. Importation of roe requires a general health certificate, and disinfection according to official manuals. Importation of fish requires a general health certificate and disease status of the exporting country equal to or better than in Norway for listed diseases. As with national trade, there is a possibility for non-notifiable diseases to be spread by movement of roe and live fish. This possibility for introducing new emerging diseases may be somewhat higher with importation due to the overview and transparency of the fish disease situation and data in Norway.

Most of the rainbow trout roe used in Norway is produced in Norway. In recent years, a small part has been imported from Denmark (5 – 10 %). All Arctic char roe is produced in Norway. There are no imports of brown trout roe as only native stocks are used.

Evaluation of the probability for vertical transmission of fish viral and bacterial pathogens has been performed earlier by VKM (2011), and excerpts for particulate pathogens are given in Table 3.2-1. Information on the knowledge on vertical transmission of the remaining pathogens presented in Chapter 2 of the present risk assessment is given in Table 3.2-2.

The working group has specifically considered risks and consequences of exposure to the infectious agents addressed in 2.3. In the assessment it is assumed that specific regulations in Norway, such as disinfection of roe, disinfection of waste water from slaughter houses and processing plants, containment of dead fish in farms etc. are complied. Risks associated with infections and diseases are shown in Figure 3.2-1.

Animal health requirements for moving live eggs to aquaculture farms in Norway are outlined in Appendix V.

		Probability		
		Low (A)	Moderate (B)	High (C)
Consequence	Serious (c)	cA Viral haemorrhagic septicaemia virus (VHSV) Infectious haematopoietic necrosis virus (IHNV) <i>G. salaris</i>	cB	cC New emerging diseases???
	Moderate (b)	bA Infectious salmon anaemia virus (ISAV)? Salmonid alphavirus (SAV/SPDV)? <i>A. salmonicida</i> subsp. <i>salmonicida</i> (furunculosis)	bB <i>R. salmoninarum</i> (Bacterial kidney disease, BKD) ¹	bC <i>F. psychrophilum</i> (bacterial coldwater disease / rainbow trout fry syndrome (RTFS)) ¹
	Limited (a)	aA <i>T. bryosalmonae</i> (Proliferative kidney disease, PKD)?	aB <i>Myxobolus cerebralis</i> (Whirling disease)	aC Infectious pancreatic necrosis virus (IPNV) ¹ Piscine orthoreovirus (PRV)??

Figure 3.2-1 A summary of the risk assessment for infectious agents of importance for fresh water farming of rainbow trout, brown trout and Arctic char, when using non-indigenous roe. It is further assumed that the specific regulations in Norway are complied, including disinfection of roe. Green (aA, aB, bA): Low risk. Yellow (aC, bB, cA): Moderate risk. Red (bC, cB, cC): High risk. The number of question marks is correlated to the degree of uncertainty. Piscine myocarditis virus (PMCV) is not included in the risk chart, due to lack of data on distribution, transmission and reservoir. The low risk of *Tetracapsuloides bryosalmonae* is explained by its widespread occurrence in Norwegian rivers, which makes the consequence of an introduction limited. Additionally, disinfected roe is not a probable source of this agent. ¹Vertical transmission is known to occur.

Table 3.2-1 Probability of vertical transmission of viral and bacterial fish pathogens as previously evaluated by VKM (2011)

Infectious agent	Probability of vertical transmission
Infectious salmon anemia virus (ISAV)	Vertical transmission of ISA virus cannot be excluded, but there is no indication of any impact of this on the disease ISA in Norwegian fish farming.
Salmon pancreas disease virus (SPDV) / Salmonid alphavirus (SAV)	Epidemiological and experimental investigations have not found indications of vertical transmission of SPDV in farmed Atlantic salmon (Kongtorp et al., 2010). There is no evidence that SAV has been transferred by roe exports. Based on this, the risk of vertical transmission of SAV is estimated to be negligible.
Infectious pancreatic necrosis virus (IPNV)	It has been shown that vertical transmission of IPNV occurs in several salmonid species. There is only indirect evidence for vertical transmission of IPNV in Atlantic salmon, but the estimates are that this occurs. Current disinfection procedures are not fully adequate to prevent this.
Piscine orthoreovirus (PRV)	Due to lack of an adequate <i>in vitro</i> cultivation system there is no available information regarding efficacy of disinfectants. In a relatively small study on the prevalence of PRV in Atlantic salmon broodfish and progeny, RNA specific for PRV was not found in fertilized roe. This indicates that vertical transmission is not a major route of transmission for PRV. However, the study was small and there are many findings of PRV in the fresh water phase. Wild caught broodfish are often infected (Garseth et al., 2013c), and PRV is also found on a regular basis in ovary fluid and milt from commercial broodfish (Vidar Aspehaug, PatoGen Analyse AS, pers. comm.). The question about vertical transmission of PRV has thus not been settled.
<i>Flavobacterium psychrophilum</i>	Vertical transmission occurs, and broodfish is a possible source of infection. As current disinfection procedures of roe do not adequately prevent transmission introduction of <i>F. psychrophilum</i> into water systems through freshwater farming cannot be excluded.
<i>Renibacterium salmoninarum</i> (BKD)	Vertical transmission is known to occur.

Table 3.2-2 Knowledge on vertical transmission of fish pathogens not listed in Table 3.2-1

Infectious agent	Knowledge on vertical transmission
Viral haemorrhagic septicaemia virus (VHSV)	Vertical transmission is not known to occur.
Infectious haematopoietic necrosis virus (IHNV)	Vertical transmission is not known to occur.
Piscine myocarditis virus (PMCV)	No data available

Infectious agent	Knowledge on vertical transmission
<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> (furunculosis)	Vertical transmission is not known to occur.
<i>Gyrodactylus salaris</i>	Vertical transmission is not known to occur.
<i>Myxobolus cerebralis</i>	Vertical transmission is not known to occur.
<i>Tetracapsuloides bryosalmonae</i> (proliferative kidney disease/PKD)	No data available

4 Uncertainties

New emerging diseases represent significant uncertainties in the present risk assessment. Also, the lack of initial formal conduction of structured searches might have contributed to some degree of general uncertainty. Further considerations and details on relevant uncertainties are given in Chapter 5, Data gaps.

5 Answers to the terms of reference

The terms of reference (ToR) to the risk assessment requested by the NFSA are answered by VKM as follows:

ToR 1. The risks associated with the establishment of cage-based on-growing fish farms in freshwater with rainbow trout, brown trout and Arctic char

ToR 1.1. The health risk for farmed fish and wild fish, with particular emphasis on the risk of transmission, establishment and development of serious infectious diseases from farmed fish to wild stocks of fish. The risk will vary depending of the origin of the farmed fish. If only fish of local stock are used, the risk will be limited to amplification of pathogens already present in the system and their reintroduction into the environment and also transmission to other aquaculture facilities operating in the same water system. These other aquaculture facilities may be hatcheries and smolt production sites for the marine salmon industry. It is likely that consequences for the environment can be reversed by fallowing and harvesting with return to the initial conditions. This will also be the case for other aquaculture facilities in the water system. Thus, if only local stocks are used, the risk is considered to be low, also for wild stocks of fish.

Several serious fish diseases and pathogens have been introduced into Norway with import of fish. Examples here are *G. salaris* and furunculosis. These examples clearly show that import poses a risk. In Norway, there are several examples (*G. salaris*, *Flavobacterium*) indicating the risk of introducing new diseases to new areas by movement of fish. Generally, this risk is reduced by the use of fish from the same geographical area. If Norwegian or imported disinfected, fertilised roe is used, the risk is limited to known and hitherto unknown diseases with vertical transmission. Known pathogens with proved or probable vertical transmission include IPNV, *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*. These are already present in Norway but they may be spread to new water systems or new subtypes with different virulence may be introduced. The risk is considered to be low to moderate for both wild and farmed fish.

If non-indigenous Norwegian or imported live fish are used, the range of possible pathogens expands dramatically together with the probability of introducing infected material. In this case the risk is considered to be moderate to high for transmission, establishment and development of serious infectious diseases, either known or hitherto unknown, from the introduced material to Norwegian wild fish or Norwegian farmed fish.

ToR 1.2. Which factors may be relevant to reduce the health risk for farmed fish and wild fish, including harvesting and fallowing of the facilities every winter? Use of local stocks will reduce the risk of introducing diseases into a new

geographic area. Use of other sources of roe and fingerlings will increase the risk. Training of fish farmers for ensuring rapid recognition of signs that are suspicious of a disease situation and mandatory fish health inspection by qualified veterinarians or fish health biologist will make it possible to detect an early introduction of a disease. An early detection increases the possibilities for successful disease eradication.

Mandatory harvesting, fallowing and disinfection will reduce the chance of an undetected disease to establish itself in the environment and have impact on health of next generation fish stocked on the site as well as on wild fish. The reduction will depend on the nature of the pathogen, the site and the wild fish population.

ToR 1.3. The welfare risk for farmed fish including non-infectious diseases related to the production of farmed fish, with particular emphasis on the risks associated with density, water quality, accumulation of sediments under farms and accumulation of ice on cages. We specifically request the following:

ToR 1.3.1. An overview of the available information on the impact different densities may have on fish welfare for the different species in cage-based on-growing farms in freshwater. In this case, fish in on-growing farms means fish with a weight over 250 grams. Based on available literature, various suggestions for recommended fish densities are given for some salmonids. However, the data are not entirely consistent and factors such as water quality (not determined in all studies), fish size (literature covers mostly effect of density on smaller fish than the market-sized fish addressed in the present risk assessment), as well as factors related to the farming systems are either varying, insufficient or even lacking altogether. It is therefore difficult to draw explicit conclusions from the various studies addressing fish density, and further studies in this field are strongly recommended, particularly with larger fish in grow-out cages or tanks. Nevertheless, in case of rainbow trout and Atlantic salmon, it seems appropriate to tentatively recommend fish densities of less than 80 kg/m³. Based on the fact that Arctic char prefer high fish densities, available literature on relatively small char indicate that fish densities up to at least 150 kg/m³ can be used. Anecdotal information from an Arctic char fish farm stated that large fish could be reared at 75 kg/m³ or higher without encountering special problems.

Operational experience from Arctic char farms has shown that accumulation of ice on cages can be avoided by providing for adequate water circulation by water agitators in the vicinity of the cages.

ToR 1.3.2. An assessment of whether the guidance norm for water quality and measurable parameters for land based hatcheries with salmonids may be suitable as a guidance norm for water quality for the different species in cage-based on-growing farms in fresh water. Due to the lack of specific water quality data for different types of salmonids in aquaculture, it is

recommended to use the guidance norm for land based hatcheries (pH, dissolved oxygen, carbon dioxide, TAN, nitrite, total organic carbon and aluminium).

ToR 1.3.3. An assessment of water quality parameters which are suitable for monitoring in cage-based on-growing farms in fresh water with rainbow trout, brown trout and Arctic char. Such monitoring could be more or less continuous and may be an addition to requirements on density.

Correct monitoring of key water quality parameters (dissolved oxygen, pH/CO₂, TAN, total gas pressure and temperature) is important to ensure that the fish are farmed under good conditions. It is technically possible to monitor dissolved oxygen, pH, carbon dioxide and temperature continuously. Assessment of total ammonium levels (TAN) requires later analysis of water samples and may be checked sporadically. Total gas pressure should be measured in cases where gas supersaturation is suspected.

ToR 1.4. Could the health and welfare of farmed fish and wild fish be set at risk if slaughter pens are established in fresh water? If the pens are established to keep fish originating from an aquaculture facility in direct contact (flow-through or pens in water) with the same water system, the health risk will not differ from the health risk posed by the aquaculture facility. If the pens are established to keep fish originating from another water system, new diseases may be introduced and the health risk will increase. The welfare risk will be dependent upon local conditions, like water quality and ice conditions, but will generally not differ from floating cages.

ToR 1.5. To the extent it is possible, we require an assessment of the health risk and welfare risk for farmed fish and wild fish associated with cage-based farming in freshwater compared to cage-based farming in sea water. If the risk is different, which factors cause the risk to differ between cage-based farming in freshwater and cage-based farming in seawater? The physical aspects in lakes, like currents, water renewal, depth, water volume, recipient capacity for pollutants, etc., are much more restricted than in the sea. This means that the fish farms, as well as the different cages, have to be smaller. Signs of pollution from the fish farms will appear at an earlier stage than in the sea. This may result in stress symptoms both farmed and wild fish. For the aquatic environment, the pollution types that arise are mostly eutrophication of the free water masses of the lake, and saprobiation of the bottom areas underneath the farms.

The marine bottom areas recover faster after the farm is moved, due to frequent currents and water renewal. The lakes have less currents and more stagnant deep waters, which gives much slower recovery when the cage farm is moved from an area that has been polluted over time.

With respect to risk of spreading fish diseases, there is always a great risk for spreading of infective diseases downstream in the watercourse, but also upstream through fish migration. This applies particularly for trout and rainbow trout, whereas to a less degree for char. Some diseases, such as the lethal infection of salmon by the parasite *Gyrodactylus salaris*, spread

in fresh water, but not in seawater. Both the rainbow trout and the char can be host for this dangerous salmon parasite. Most fish diseases have, however, similar spreading capacity in fresh water and in seawater.

About 75 % of all Norwegian freshwaters are regulated for hydropower purposes, which often includes transfer of water between watercourses. This should be given particular attention for fish farming in regulated water bodies.

ToR 2. The risks associated with the establishment of land based on-growing farms with rainbow trout, brown trout and Arctic char with intake of water from and effluent of water to a freshwater reservoir.

The questions in this section are limited to fish farms with mainly flow-through systems.

ToR 2.1. The health risk for farmed fish and wild fish if effluent water is released untreated to a freshwater reservoir, with particular emphasis on the risk of transmission, establishment and development of serious infectious diseases from farmed fish to wild stocks of fish. The fish in a land based facility with untreated water intake from and untreated water outlet to the same freshwater reservoir will be in full contact with this reservoir. There will be no hygienic barriers to prevent or limit contagion between the facility and the reservoir. Thus, the risks will be similar to ToR 1.1. with pens in the water reservoir itself. If the fish is taken from local stock, the risk is considered to be low. If disinfected fertilized roe is imported, the risk is considered to be moderate. If other life stages are imported, the risk is considered to be high.

ToR 2.2. Which factors may be relevant to reduce the health risk for farmed fish and wild fish, including harvesting and fallowing of the facilities every winter and treatment of effluent water? Specifically an assessment of the risk if the effluent water is treated in accordance with a method that satisfies the requirements in § 10 point 1 or § 10 point 5 of the Water Treatment Regulation. Factors mentioned under ToR 1.2 will be relevant. In addition, a decrease in the use of effluent water will reduce the chance of introducing new pathogens. Recirculating aquaculture systems will release less water compared to a flow through system. Treatment of the effluent water in accordance with the Water Treatment Regulation will reduce the chance of introducing new pathogens to negligible if the method that satisfies the requirements in § 10 point 4 is used. Use of a method that satisfies the requirement in § 10 point 1 is not approved for use in laboratory facilities working with list 1 disease, exotic pathogens or un-characterised pathogens. Use of a method that satisfies the requirement in § 10 point 5 will not cause significant reduction in the health risk for farmed or wild fish. The method is simple and only filtering by 40 µm is required. This step will only restrain larger pathogens like *Gyrodactylus salaris*.

ToR 2.3. The welfare risk for farmed fish including non-infectious diseases related to the production of farmed fish, with particular emphasis on the risks associated with density and water quality. We especially request the following:

ToR 2.3.1. An overview of the available information on the impact different densities may have on fish welfare for the different species in land based on-growing farms using freshwater. In this case, fish in on-growing farms means fish with a weight over 250 grams. Due to lack of data differentiating between various fish farming systems, only generalized data for salmonids can be obtained, as stated in answer to ToR 1.3.1. Hence, based on available literature, various suggestions for recommended fish densities are given for salmonids. However, the data are not entirely consistent and factors such as water quality (not determined in all studies), fish size (literature covers mostly effect of density on smaller fish than the market-sized fish addressed in the present risk assessment), as well as factors related to the farming systems, are either varying, insufficient or even lacking altogether. It is therefore difficult to draw explicit conclusions from the various studies addressing fish density, and further studies in this field are strongly recommended, particularly with larger fish in grow-out cages or tanks. Nevertheless, in case of rainbow trout and Atlantic salmon, it seems appropriate to tentatively recommend fish densities of less than 80 kg/m³, whereas for Arctic char higher densities can be recommended possibly within the range of 75-150 kg/m³.

ToR 2.3.2. An assessment of whether the guidance norm for water quality and measurable parameters for land based hatcheries with salmonids may be suitable as a guidance norm for water quality for the different species in land based on-growing farms using freshwater. As with ToR 1.3.2, due to the lack of specific water quality data for different types of salmonids in aquaculture, it is recommended to use the guidance norm for land based hatcheries (pH, dissolved oxygen, carbon dioxide, TAN, nitrite, total organic carbon and aluminium).

ToR 2.3.3. An assessment of water quality parameters which are suitable for monitoring in land based on-growing farms using freshwater with rainbow trout, brown trout and Arctic char. Such monitoring can be more or less continuous and may be an addition to requirements on density. As with ToR 1.3.2, correct monitoring of key water quality parameters (dissolved oxygen, pH/CO₂, TAN, total gas pressure and temperature) is important to ensure that the fish are farmed under good conditions. It is technically possible to monitor dissolved oxygen, pH, carbon dioxide and temperature continuously. Assessment of total ammonium levels (TAN) requires later analysis of water samples and may be checked sporadically. Total gas pressure should be measured in cases where gas supersaturation is suspected.

If the water intake is from a river impacted by acidification, it is important monitor the pH of the water, conductivity, and the concentration of labile aluminium. The aluminium tend to precipitate on the gill surface, and analyses of aluminium on the gills will give an early warning of coming fish kill (see values in the Classification Guidance to the Water Regulation (www.vannportalen.no)).

ToR 3. The risks associated with the establishment of land based hatcheries with rainbow trout, brown trout and Arctic char with effluent of water to a freshwater reservoir.

The questions in this section are limited to hatcheries with mainly flow-through systems.

ToR 3.1. The health risk for wild fish if untreated effluent water is drained to a fresh water reservoir, with particular emphasis on the risk of transmission, establishment and development of serious infectious diseases to wild stocks of fish. The risk will vary depending of the origin of the farmed fish. If only fish of local stock are used, the risk will be limited to amplification of pathogens already present in the system and their reintroduction into the environment and also transmission to other aquaculture facilities operating in the same water system. These other aquaculture facilities may be hatcheries and smolt production sites for the marine salmon industry. It is likely that consequences for the environment can be reversed by fallowing and harvesting with return to the initial conditions. This will also be the case for other aquaculture facilities in the water system. Thus, if only local stocks are used, the risk is considered to be low.

If disinfected, fertilized roe is imported, the risk is limited to known and hitherto unknown diseases with vertical transmission. Known pathogens with proved or probable vertical transmission include IPNV, *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*. These are already present in Norway but they may be spread to new water systems or new subtypes with different virulence may be introduced. The risk is considered to be moderate for both wild and farmed fish.

ToR 3.2. An assessment of the risk if the effluent water is treated in accordance with a method that satisfies the requirements of § 10 point 1 or § 10 point 5 in the Water Treatment Regulation. Factors mentioned under the answer to ToR 2.2 are all relevant.

ToR 3.3. An assessment of whether the guidance norm for water quality and measurable parameters for land based hatcheries with salmonids may be suitable as a guidance norm for water quality in land based hatcheries with brown trout and Arctic char. As with ToR 1.3.2 and ToR 2.3.2, due to the lack of specific water quality data for different types of salmonids in aquaculture, it is recommended to use the guidance

norm for land based hatcheries (pH, dissolved oxygen, carbon dioxide, TAN, nitrite, total organic carbon and aluminium).

ToR 3.4. An assessment of water quality parameters which are suitable for monitoring in land based hatcheries with rainbow trout, brown trout and Arctic char. Such monitoring can be more or less continuous and may be an addition to requirements on density. Correct monitoring of key water quality parameters (dissolved oxygen, pH/CO₂, TAN, total gas pressure and temperature) is important to ensure that the fish are farmed under good conditions. It is technically possible to monitor dissolved oxygen, pH, carbon dioxide and temperature continuously. Assessment of total ammonium levels (TAN) requires later analysis of water samples and may be checked sporadically. Total gas pressure should be measured in cases where gas supersaturation is suspected.

It is labile aluminium that is toxic to fish. This compound is however complicated to analyse. The content of reactive aluminium, pH, and conductivity will indicate when toxic levels are approached. The aluminium tends to precipitate on the gill surface, and analyses of aluminium on the gills will give an early warning of coming fish kills. The problem with toxic aluminium is mainly confined to areas susceptible to acidification. If the intake water is taken from an unpolluted river or lake, it is only acidification that can be dangerous for the fish health. If the water has low alkalinity, it is at risk for having episodes of acidic waters, which may cause fish kills. Here the pH of the intake water must be monitored, as well as concentrations of labile aluminium. Aluminium on fish gills could also be monitored if the fish farms are fed by intake water susceptible for acidification.

6 Conclusions

The health risk for both farmed and wild fish related to introduction of serious diseases is linked to the source of biological material used for farming. If only local stocks are used, the risk is low. Use of other sources of roe and fingerlings will increase the risk. Use of Norwegian or imported, disinfected roe is associated with low to moderate risk, depending on the source. The risk with use of Norwegian or imported fish is considered to be moderate to high. The same risk profile applies for the use of slaughter pens. If the pens are established to keep fish originating from another water system, new diseases may be introduced and the health risk will increase. Well trained farmers and fish health professionals (veterinarians and fish health biologists) will increase the likelihood of rapid detection of an introduced disease and possibilities to take prompt action. Mandatory harvesting, fallowing and disinfection will reduce the chance of an undetected disease to establish itself in the environment and have impact on health of next generation fish stocked on the site as well as on wild fish. The reduction will depend on the nature of the pathogen, the site and the wild fish population.

Based on available literature, various suggestions for recommended fish densities are given for some types of salmonids. However, the available data are not entirely consistent, and aspects such as environmental factors, water quality, and fish size are not always taken into consideration. Furthermore, due to lack of data, it is not possible to evaluate or compare different concepts for large scale fish farming (on-growth in cages and flow-through systems or in land based hatcheries). Nevertheless, it seems appropriate to tentatively recommend fish densities of less than up to 80 kg/m³ in case of rainbow trout, whereas for Arctic char higher densities can be recommended, possibly within the range of 75-150 kg/m³. Relevant fish density data for brown trout is basically lacking.

Due to lack of specific water quality data evaluated under commercial conditions, or in different fish farming systems (cages, flow-through, or land based hatcheries), it is still recommended to use the current guidance norm for water quality as issued by the NFSA.

Correct monitoring of key water quality parameters (dissolved oxygen, pH/CO₂, TAN, total gas pressure and temperature) is important to ensure that the fish are farmed under good conditions. It is technically possible to monitor dissolved oxygen, pH, carbon dioxide and temperature continuously. Assessment of total ammonium levels (TAN) requires subsequent analysis of water samples and may be checked sporadically. Total gas pressure should be measured in cases where gas supersaturation is suspected.

If the water intake to land based farms is from a river or lake impacted by acidification, it is particularly important monitor the pH of the water, conductivity, and the concentration of labile aluminium. The aluminium tends to precipitate on the gills, and analyses of aluminium on the gills will give an early warning of subsequent fish kills. If the intake water is taken from an unpolluted river or lake, it is only acidification that can be dangerous for the fish

health. If the water has low alkalinity, it is a risk for having episodes of acidic waters, which may cause fish kills.

Regarding potential differences in fish health and welfare between cage-based farming in freshwater and seawater, some differences are indicated that may affect the fish. Basically, the physical aspects in lakes, like currents, water renewal, depth, water volume and recipient capacity for pollutants, are much more restricted than in the sea. Signs of pollution from the fish farms will appear at an earlier stage than in the sea. This may result in stress symptoms for both farmed and wild fish. For the aquatic environment, the pollution types that arise are mostly eutrophication of the free water masses of the lake, and saprobiation of the bottom areas underneath the farms. The marine bottom areas recover faster after the farm is moved, due to frequent currents and water renewal. The lakes have less currents and more stagnant deep waters, which gives much slower recovery when the cage farm is moved from an area that has been polluted over time. Due to limited recipient capacity of Norwegian lakes, the use of floating net cages in freshwater is considered suitable for farms with modest biomass production only. With respect to risk of spreading fish diseases, there is always a great risk for spreading of infective diseases downstream in the watercourse, but also upstream through fish migration. This applies particularly for trout and rainbow trout, whereas to a less degree for char. Some diseases, such as the lethal infection of salmon by the parasite *Gyrodactylus salaris*, spread in fresh water, but not in seawater. Both rainbow trout and char can be host for this dangerous salmon parasite. Most fish diseases have, however, similar spreading capacity in freshwater and in seawater.

7 Data gaps

7.1 Water quality and fish welfare

Review of literature revealed that there is basically a general lack of scientific data on fish welfare obtained under the environmental conditions typically found in different fish farming systems (multi-factor studies). Fish welfare deals with more than mere survival and not being sick. It includes the ability to fulfill behavioural needs, to avoid distress and perhaps even to experience some positive emotions.

Studies on long-term effect of presence of organic compounds and toxicity of metals on fish health and behavior are lacking, as are studies on the relationship between water quality, composition of microbial communities, and fish health under commercial fish farming conditions. Also, data related to tolerance levels to different water quality parameters and appropriate fish farming conditions for brown trout are very scarce, and there is a need for establishing maximal and optimum stocking densities for adult char in grow-out cages and tanks.

Studies on various water quality parameters, and their combinations, at different levels that are either preferred or actively avoided by fish, are to a large extent lacking. It is possible that if the fish will use the opportunity to choose between areas within the cage environment having different environmental parameters such as temperatures, light intensities, flow velocities, salinities, dissolved oxygen levels and water depths, fish welfare may be improved. A stable and uniform water environment might accordingly reduce the active stress coping strategies of the fish, and lack of environmental stimuli may in itself be a source of mental stress. More studies on these aspects would be important for a better understanding of the concept of fish welfare. In turn, this may enable aquaculturists to design and run fish farms in a way where fish welfare is highlighted.

7.2 Infections and fish health

Infection routes for most relevant pathogens are incompletely understood.

For PMCV, there are several additional aspects with large data gaps, and at present, there is no available information regarding the susceptibility of rainbow trout or Arctic char to this virus.

8 References

- Aass P. (1979) Tilslamming i Hallingdalselva 1966-67. Fisket i Ustedalsfjord og Strandafjord [Turbiditation of the Hallingdal River 1966-67. The fishery in the Ustedalsfjord and Strandafjord]. In T. Gunnerød & P. Melquist (eds.): Vassdragsregulerings biologiske virkninger i magasiner og lakseelver (pp. 93-115). Symposium 1978, NVE - DVF. 294 pp.
- Aass P. (1985) Langvarige fiskeribiologiske forskningsprogrammer i ferskvann. [Long term fishery biological research projects in freshwater]. Fauna 39: 10-17.
- Ahne W., Negele R.D. (1985) Studies on the transmission of infectious pancreatic necrosis virus via eyed roe and sexual products of salmonid fish. In A. Ellis (ed.), Fish and Shellfish Pathology (pp. 262-270). Academic Press, London, UK.
- Alabaster J. S., Lloyd R. (1980) Water Quality Criteria for Freshwater Fish. FAO 1980, Butterworth, London, UK. ISBN 0 408 10673 5, 297 pp.
- Alanära A., Brännäs E. (1996) Dominance in demand-feeding behaviour in Arctic charr and rainbow trout: the effect of stocking density. J. Fish Biol. 48:242-254.
- Audet C., Wood C.M. (1988) Do rainbow trout (*Salmo gairdneri*) acclimate to low pH? Can J Fish Aquat Sci 45:1399-1405.
- Austin B., Rayment J. N. (1985) Epizootiology of *Renibacterium salmoninarum*, the causal agent of bacterial kidney disease in salmonid fish. J Fish Dis 8(6):505-509.
- Baker R.F., Ayles G.B. (1990) The effects of varying density and loading level on the growth of Arctic charr (*Salvelinus alpinus* L.) and rainbow trout (*Oncorhynchus mykiss*). World Aquaculture 21:58-61.
- Balfry S. K., Albright L. J., Evelyn T. P. T. (1996) Horizontal transfer of *Renibacterium salmoninarum* among farmed salmonids via the fecal-oral route. Dis Aquat Org 25(1/2):63-69.
- Bergheim A., Gausen M., Næss A., Hølland P.M., Krogedal P., Crampton V. (2006) A newly developed oxygen injection system for cage farms. Aquacult. Eng. 34:40-46.
- Bakke T. A., Jansen P. A., Hansen L. P. (1990) Differences in the host resistance of Atlantic salmon, *Salmo salar* L., stocks to the monogenean *Gyrodactylus salaris* Malmberg, 1957. J Fish Biol 37(4):577-587.
- Bakke T. A., Harris P. D. (1998) Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. Can J Fish Aquat Sci 55(S1):247-266.

- Ball H.J., Munro A.L.S., Ellis A., Elson K.G.R., Hodgkiss W., McFarlane I.S. (1971) Infectious pancreatic necrosis in rainbow trout in Scotland. *Nature*, 234:417–418.
- Berge D. (2000) Vurdering av miljøtilstanden i Espelandsvatnet i Hyllestad kommune – Sogn og Fjordane [Assessment of the environmental conditions in Lake Espedalsvatnet in Hyllestad municipality – Sogn og Fjordane]. NIVA Report LNR 4181, ISBN 82-577-3798-4.
- Berge D. 2010. Vassdragsreguleringer og biologisk produksjon i innsjøer – Kan bærekraftig fiskeoppdrett være et avbøtende tiltak? [Water course regulations and biological production in lakes – Could sustainable fish farming be a remediation measure?] NIVA Report LNR 6066, ISBN 978-82-577-5801-1.
- Biacchesi S., Le Berre M., Le Guillou S., Benmansour A., Bremont M., Quillet E., Boudinot P. (2007) Fish genotype significantly influences susceptibility of juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), to waterborne infection with infectious salmon anaemia virus. *J Fish Dis* 30:631-636.
- Biering E., Madhun A. S., Isachsen C. H., Omdal L. M., Einen A. C. B., Garseth Å. H., Karlsbakk E. (2013) Annual report on health monitoring of wild anadromous salmonids in Norway.
<http://www.vetinst.no/nor/Publikasjoner/Rapportserie/Rapportserie-2013/Annual-report-on-health-monitoring-of-wild-anadromous-salmonids-in-Norway>.
- Boyd C.E. (1979) Water quality in warm water fish ponds. Auburn University, Alabama.
- Brown G.E., Brown J.A., Srivastava R.K. (1992) The effect of stocking density on the behavior of Arctic charr (*Salvelinus alpinus* L). *J. Fish Biol.* 41:955-963.
- Brännäs E., Wiklund B.S. (1992) Low temperature growth potential of Arctic charr and rainbow trout. *Nordic J Freshwater Res* 67:77-81.
- Bullock G.L., Rucker R.R., Amend D., Wolf K., Stuckey H.M. (1976) Infectious pancreatic necrosis: transmission with iodine-treated and nontreated eggs of brook trout (*Salvelinus fontinalis*). *J Fish Res Board Can*, 33:1197–1198.
- Bullock G.L., Herman R.L. (1988) Bacterial Kidney Disease of salmonid fishes caused by *Renibacterium salmoninarum*. Fish Disease Leaflet 78, US Fish and Wildlife Service, Washington, DC.10 pp.
- Chapman P.E. Popham J.D., Griffin J., Michaelson J. (1987) Differentiation of physical from chemical toxicity in solid waste fish bioassay. *Water, Air, and Soil Pollution* 33:295-308.
- Chen S., Stechey D., Malone R.F. (1994) Suspended solids control in recirculating aquaculture systems. In M.B. Timmons & T.M. Losordo (eds.), *Aquaculture Water*

Reuse Systems: Engineering Design and Management (pp. 61-100). Elsevier, Oxford, UK.

Classification Guidance to the Water Regulation 02:2013 (2013) Klassifisering av miljøkvalitet i vann. Økologisk og kjemisk klassifiseringssystem for kystvann, grunnvann, innsjøer og elver. <https://www.vannportalen.no>

Clingerman J., Bebak J., Mazik P.M., Summerfelt S.T. (2007) Use of avoidance response by rainbow trout for fish self-transfer between tanks. *Aquacult. Eng.* 37:234-251.

Crocker C. E., Cech J.J. (1996) The effects of hypercapnia on the growth of juvenile white sturgeon, *Acipenser transmontanus*. *Aquaculture* 147:293-299.

Daily J.B., Economon P. (1983) Selection of water supplies. In F.P. Mayer, J.W. Warren & T.G. Carey (eds.), *A guide to integrated fish health management in the Great Lake basins* (pp. 37-48). Special Publication No. 83-2, Great Lakes Fishery Commission, Ann Arbor, Michigan.

Dalsgaard I., Hørlyck V. (1990) Koldtvandssyge eller vintersår hos ørreder [Cold water diseases or winter ulcers in trout]. *Ferskvandsfiskeribladet* 88:118-120.

Dalsgaard I., Madsen L. (2000) Bacterial pathogens in rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at Danish freshwater farms. *J Fish Dis* 23:199-209.

Davidson J., Good C., Welsh C., Brazil B., Summerfelt S. (2009) Heavy metal and waste metabolite accumulation and their potential effect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. *Aquacult Eng* 41:136-145.

Dorson M. (1982) Infectious pancreatic necrosis of salmonids: present status of knowledge concerning the viruses and the possibilities of controlling the disease. *B Fr Piscic* 285:195-209.

Eddy F. B., Lomholt J.P., Weber R.E., Johansen K. (1977) Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO₂ tension. *J Exp Biol* 67:37-47.

Edsall D.A., Smith C.E. (1990) Performance of rainbow trout and Snake River cutthroat trout reared in oxygen-supersaturated water. *Aquaculture* 90:251-259.

Egidius E. (1987) Import of furunculosis to Norway with Atlantic salmon smolts from Scotland. *ICES CM Documents* 1987/F:8.

El-Matbouli M., Fischer-Scherl T., Hoffmann R. W. (1992) Present knowledge on the life cycle, taxonomy, pathology, and therapy of some *Myxosporea* spp. important for freshwater fish. *Annu Rev Fish Dis* 2:367-402.

- Elliott J.M. (1981) Some aspects of thermal stress on freshwater teleosts. In A.D. Pickering (ed.), *Stress and Fish* (pp. 209-245). Academic Press, Toronto, Canada.
- Ellis T., North B., Scott A.P., Bromage N.R., Porter M., Gadd D. (2002) The relationship between stocking density and welfare in farmed rainbow trout. *J Fish Biol* 61:493-531.
- Enzmann P.J., Kurath G., Fichtner D., Bergmann S.M. (2005) Infectious hematopoietic necrosis virus: Monophyletic origin of European IHNV isolates from North-American genogroup M. *Dis Aquat Org* 66:187-195.
- Erikson U. (2011). Assessment of different stunning methods and recovery of farmed Atlantic salmon: Isoeugenol, nitrogen, and three levels of carbon dioxide. *Animal Welfare* 20:365-375.
- Eriksson L.O., Alanära A., Nilsson J., Brännäs E. (2010) The Arctic charr story: development of subarctic freshwater fish farming in Sweden. *Hydrobiologia* 650:265-274.
- Espmark Å.M., Hjelde K., Bæverfjord G. (2010) Development of gas bubble disease in juvenile Atlantic salmon exposed to water supersaturated with oxygen. *Aquaculture* 306:198-204.
- Evelyn T.P.T., Ketcheson J. E., Prospero-Porta L. (1984) Further evidence for the presence of *Renibacterium salmoninarum* in salmonid eggs and for the failure of povidone-iodine to reduce the intra-ovum infection rate in water-hardened eggs. *J Fish Dis* 7(3):173-182.
- Evelyn T.P.T., Prospero-Porta L., & Ketcheson J. E. (1986) Experimental intra-ovum infection of salmonid eggs with *Renibacterium salmoninarum* and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin. *Dis Aquat Org* 1(3):197-202.
- Evelyn T.P.T. (1993) Bacterial kidney disease – BKD. In V. Inglis, R.J. Roberts & N.R. Bromage (eds.), *Bacterial diseases of fish* (pp. 177-195). Blackwell Scientific Publications, Oxford, UK.
- Finstad Ø. W., Falk K., Løvoll M., Evensen Ø., Rimstad E. (2012) Immunohistochemical detection of piscine reovirus (PRV) in hearts of Atlantic salmon coincide with the course of heart and skeletal muscle inflammation (HSMI). *Vet res* 43(1):1-11.
- Fivelstad S., Schwartz J., Stromsnes H., Olsen A. B. 1995. Sublethal Effects and safe levels of ammonia in seawater for Atlantic salmon postsmolts (*Salmo Salar* L). *Aquacult Eng* 14:271-280.

- Fivelstad S., Haavik H., Løvik G., Olsen A.B. (1998) Sublethal effects and safe levels of carbon dioxide for Atlantic salmon postsmolts (*Salmo salar* L). *Aquaculture* 160:305-316.
- Fivelstad S., Bergheim S., Kløften H., Haugen R., Lohne T., Olsen A. (1999a) Water flow requirements in the intensive production of Atlantic salmon (*Salmo salar* L): Growth and oxygen consumption. *Aquacult Eng* 20:1-15.
- Fivelstad S., Olsen A.B., Kløften H., Ski H., Stefansson S. (1999b) Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L) smolts at constant pH in bicarbonate rich freshwater. *Aquaculture* 178:171-187.
- Fivelstad S., Olsen A.B., Åsgård T., Baeverfjord G., Rasmussen T., Vindheim T., Stefansson S. (2003a) Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmo salar* L): ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. *Aquaculture* 215:301-319.
- Fivelstad S., Waagbø R., Zeitz S.F., Hosfeld A.C.D., Olsen A.B., Stefansson S. (2003b) A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: physiology and growth. *Aquaculture* 215:339-357.
- Fivelstad S., Waagbø R., Stefansson S., Olsen A.B. (2007) Impacts of elevated water carbon dioxide partial pressure at two temperatures on Atlantic salmon (*Salmo salar* L) parr growth and haematology. *Aquaculture* 269:241-249.
- Fletcher G.L., Kao M.H., Dempson J.B. (1988) Lethal freezing temperatures of Arctic char and other salmonids in presence of ice. *Aquaculture* 71:369-378.
- FOR-1997-02-20-192 (1997) The Water Treatment Regulation, Norwegian Ministry of Trade, Industry and Fisheries (vannbehandlingsforskriften. Forskrift om desinfeksjon av inntaksvann til og avløpsvann fra akvakulturrelatert virksomhet. Nærings- og fiskeridepartementet). <http://lovdata.no/dokument/SF/forskrift/1997-02-20-192>
- FOR-2006-12-15-1446 (2006) The Water Regulation, Norwegian Ministry of Climate and Environment (vannforskriften. Forskrift om rammer for vannforvaltningen., Klima og miljødepartementet). <http://lovdata.no/dokument/SF/forskrift/2006-12-15-1446>
- FOR-2008-06-17-823 (2008) The Regulation on the Establishing of Aquaculture Farms, Norwegian Ministry of Trade, Industry and Fisheries (etableringsforskriften. Forskrift om etablering og utvidelse av akvakulturanlegg, zoobutikker, m.m., Nærings- og fiskeridepartementet). <http://lovdata.no/dokument/SF/forskrift/2008-06-17-823>
- FOR-2008-06-17-822 (2008) The Regulation on the Operation of Aquaculture Establishments, Norwegian Ministry of Trade, Industry and Fisheries

(akvakulturdriftsforskriften. Forskrift om drift av akvakulturanlegg. Nærings- og fiskeridepartementet). <http://lovdata.no/dokument/SF/forskrift/2008-06-17-822>

Forsberg O.I. (1995) Empirical investigations on growth of post-smolt Atlantic salmon in land based farms. Evidence of a photoperiodic influence. *Aquaculture* 133:235-248.

Forseth T., Jørgensen A., Moe T.A. (2007) Pilotkartlegging av PKD i norske laksevassdrag. [Pilot mapping of PKD in Norwegian salmonid waters]. NINA Report 259, 12 pp.

Garseth Å. H., Biering E., Tengs T. (2012) Piscine myocarditis virus (PMCV) in wild Atlantic salmon *Salmo salar*. *Dis Aquat Org* 102:157-161.

Garseth Å. H., Biering E., Aunsmo A. (2013a) Associations between piscine reovirus infection and life history traits in wild-caught Atlantic salmon *Salmo salar* L. in Norway. *Prev vet med* 112(1):138-146.

Garseth Å. H., Ekrem T., Biering E. (2013b) Phylogenetic Evidence of Long Distance Dispersal and Transmission of Piscine Reovirus (PRV) between Farmed and Wild Atlantic Salmon. *PloS one* 8(12):e82202.

Garseth Å.H., Fritsvold C., Opheim M., Skjerve E., Biering E. (2013c) Piscine reovirus (PRV) in wild Atlantic salmon, *Salmo salar* L., and sea-trout, *Salmo trutta* L., in Norway. 2013. *J Fish Dis.* 36(5):483-93. doi: 10.1111/j.1365-2761.2012.01450.x. Epub 2012 Nov 20.

Grabner D. S., El-Matbouli M. (2008) Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) to *Fredericella sultana* (Bryozoa: Phylactolaemata) by various fish species. *Dis Aquat Org* 79(2):133.

Graham D.A., Cherry K., Wilson C.J., Rowley H.M. (2007a) Susceptibility of salmonid alphavirus to a range of chemical disinfectants. *J Fish Dis.* 30(5):269-77.

Graham D.A., Staples C., Wilson C.J., Jewhurst H., Cherry K., Gordon A., Rowley H.M. (2007b) Biophysical properties of salmonid alphaviruses: influence of temperature and pH on virus survival. *J Fish Dis.* 30(9):533-43.

Hashemi S., Blust R., De Boeck G. (2008) The effect of starving and feeding on copper toxicity and uptake in Cu acclimated and non-acclimated carp. *Aquatic Toxicol.* 86:142-147.

Hedrick R. P., El-Matbouli M., Bartholomew J., & Wilson J. (2002) Review: Recent advances with taxonomy, life cycle, and development of *Myxobolus cerebralis* in the fish and oligochaete hosts. In: J.L. Bartholomew & J.C. Wilson (eds.), *Whirling Disease: Reveiw and current topics* (pp. 45-54). Symposium 29. American Fisheries Society, Bethesda, Maryland, USA.

- Hessen D.O. (1992) Uorganiske partikler i vann, effekter på fisk og dyreplankton. [Inorganic particles in water, effects on fish and zooplankton]. NIVA Report O-89179/Lnr.2787. ISBN 82-577-2172-7, 42 pp.
- Hjeltnes B. (ed.) (2014) Fiskehelse rapporten 2013. Oslo: Report, Norwegian Veterinary Institute, Oslo, Norway, 42 pp.
<http://www.vetinst.no/nor/Publikasjoner/Fiskehelse rapporten/Fiskehelse rapporten-2013>
- Hosfeld C.D., Engevik A., Mollan T., Lunde T.M., Waagbø R., Olsen A.B., Breck O., Stefansson S., Fivelstad S. 2008. Long-term separate and combined effects of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L) smolts. *Aquaculture* 280:146-153.
- Hosfeld C.D., Hammer J. Handeland S.O., Fivelstad S., Stefansson S.O. (2009) Effects of fish density on growth and smoltification in intensive production of Atlantic salmon (*Salmo salar* L.). *Aquaculture* 294:236-241.
- Huntingford F.A., Adams C., Braithwaite A., Kadri S., Pottinger T.G., Sandøe P., Turnbull J.F. (2006) Review paper: Current issues in fish welfare. *J. Fish. Biol.* 68:332-372.
- Hyvärinen P., Heinimaa S., Rita H. (2004) Effects of abrupt cold shock on stress responses and recovery in brown trout exhausted by swimming. *J. Fish Biol.* 64:1015-1026.
- Ip Y. K., Chew S. F., Randall D. J. (2001) Ammonia toxicity, tolerance, and excretion. In P.A. Wright & P.M. Anderson (eds.), *Fish Physiology*, Vol. 20. Nitrogen excretion (pp. 109-148). Academic Press, San Diego, California.
- Jacobsen P., Aanes K.J., Grande M., Kristiansen H., Andersen S. (1987) Vurdering av årsaker til fiskedød hos G.P. Jægtvik A/S, Langstein. [Assessment of causes of fish death at G.P. Jægtvik Ltd, Langstein]. NIVA Report O-87114.
- Jobling M. (1985) Physiological and social constraints on growth of fish with special reference to Arctic charr, *Salvelinus alpinus* L. *Aquaculture* 44:83-90.
- Jobling M., Jørgensen E.H., Arnesen A.M., Ringø E. (1993) Review: Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. *Aquaculture Int.* 1:20-46.
- Johansen R., Kongstorp R.T., Bornø G., Skjelstad H.R., Olsen A.B., Flesjø K., Colquhoun D., Ørpetveit I., Hansen H., Garset Å.H., Hjeltnes B. (2009) The Health Situation in Norwegian aquaculture 2008. Norwegian National Veterinary Institute, Oslo. 20 pp.
- Johansen L. H., Jensen I., Mikkelsen H., Bjørn P. A., Jansen P. A., Bergh Ø. (2011) Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. *Aquaculture*, 315(3):167-186.

- Johnsen B. O., Jensen A. J. (1994) The spread of furunculosis in salmonids in Norwegian rivers. *J. Fish Biol* 45(1):47-55.
- Johnston W.G. (2002) Arctic Charr Aquaculture. Fishing News Books, Blackwell Publishing, Malden, MA, USA.
- Jørgensen E.H., Christiansen J.B., Jobling M. (1993) Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). *Aquaculture* 110:191-204.
- Kettler S., Pfeil-Putzien C., Hoffmann, R. (1986) Infection of grayling (*Thymallus thymallus*) with the agent of bacterial kidney disease (BKD). *Bull Eur Assoc Fish Pathol* 6:69-71
- Kibenge, M.J., Iwamoto T., Wang Y., Morton A., Godoy M.G., Kibenge F.S. (2013) Whole-genome analysis of piscine reovirus (PRV) shows PRV represents a new genus in family Reoviridae and its genome segment S1 sequences group it into two separate sub-genotypes. *Virology* 453(1):230.
- Kim R., Faisal M. (2011) Emergence and resurgence of the viral hemorrhagic septicemia virus (Novirhabdovirus, Rhabdoviridae, Mononegavirales). *J Adv Res* 2:9–23.
- Kolarević J., Cirić M., Zühlke A., Terjesen B.F. (2011) On-line pH measurements in recirculating aquaculture systems (RAS). Abstract, EAS, Rhodes, October 2011, 570-571.
- Kolbeinshavn A., Wallace J. (1985) Observations on swim bladder stress syndrome in Arctic charr (*Salvelinus alpinus*), induced by inadequate water depth. *Aquaculture* 46:259-261.
- Kongtorp R.T., Stene A., Andreassen P.A., Aspehaug V., Graham D.A., Lyngstad T.M., Olsen A.B., Olsen R.S., Sandberg M., Santi N., Wallace C., Breck O. (2010) Lack of evidence for vertical transmission of SAV 3 using gametes of Atlantic salmon, *Salmo salar* L., exposed by natural and experimental routes. *J Fish Dis* 33:879–88.
- Kroglund F., Staurnes M. (1999) Water quality requirements of smolting Atlantic salmon (*Salmo salar*) in limed acid rivers. *Can. J. Fish. Aquatic Sci.* 56:2078-2086.
- Kroglund F., Teien H.C., Rosseland B.O., Salbu B., Lucassen E.C.H.E. (2001) Water quality dependent recovery from aluminium stress in Atlantic salmon smolts. *Water, Air & Soil Pollution* 130:911-916.
- Kunwar P.S., Tudorache C., Eyckmans M., Blust R., De Boeck G. (2009) Influence of food ration, copper exposure and exercise on the energy metabolism of common carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.* 149:113-119.

- Kurath G., Garver K.A., Troyer R.M., Emmenegger E.J., Einer-Jensen K., Anderson E.D. (2003) Phylogeography of infectious haematopoietic necrosis virus in North America. *J Gen Virol.* 84(Pt 4): 803-814.
- Larsson S. (2005) Thermal preference of Arctic charr, *Salvelinus alpinus* and brown trout, *Salmo trutta* – implications for their niche segregation. *Environ. Biol. Fishes* 73:89-76.
- Larsson S., Berglund I. (2005) The effect of temperature on the energetic growth efficiency of four Swedish Arctic charr populations (*Salvelinus alpinus* L.). *J. Thermal Biol.* 30:29-36.
- Larsson S., Eriksson, L.O. (2009) Thermal performance and preference of Arctic charr – how to farm a cold-adapted fish in warm waters. Abstract 9th charr symposium, 15-18 June, Scotland.
- Linton T.K., Morgan I.J., Walsh P.J., Wood C.M. (1998) Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism. *Can. J. Fish. Aquatic Sci.* 55:576-586.
- Løvoll M., Wiik-Nielsen J., Grove S., Wiik-Nielsen C.R., Kristoffersen A.B., Faller R., Poppe T., Jung J., Pedamallu C.S., Nederbragt A.J., Meyerson M., Rimstad E., Tengs T. (2010) A novel totivirus and piscine reovirus (PRV) in Atlantic salmon (*Salmo salar*) with cardiomyopathy syndrome (CMS). *Virology*, 7:309.
- Løvoll M., Alarcón M., Bang Jensen B., Taksdal T., Kristoffersen A. B., Tengs T. (2012) Quantification of piscine reovirus (PRV) at different stages of Atlantic salmon *Salmo salar* production. *Dis Aquat Org* 99:7-12
- MacIntyre C.M., Ellis T., North B.P., Turnbull J.F. (2008) The influences of water quality on the welfare of farmed rainbow trout: A review. In E. Branson (ed.), *Fish Welfare* (pp. 150-178). Blackwell Scientific Publications, London, U.K.
- Magor B.G. (1988) Gill histopathology of juvenile *Oncorhynchus kisutch* exposed to suspended wood debris. *Can. J. Zool.* 66:2164-2169.
- McDonald D.G., Hobe H., Wood C.M. (1980) The influence of calcium on the physiological responses of rainbow trout, *Salmo gairdneri*, to low environmental pH. *J. Exp. Biol.* 88:109-131.
- McKenzie D.J., Höglund E., Dupont-Prinet A., Larsen B.K., Skov P.V., Pedersen P.B., Jokumsen A. (2012) Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout. *Aquaculture* 338-341:216-222.

- McKnight D. M., Harnish R., Wershaw R.L., Baron J.S., and Schiff S. (1997) Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park., *Biogeochem.* 36:99-124.
- McLoughlin M.F., Nelson R.T., Rowley, H.M., Cox D.I., Grant A.N. (1996) Experimental pancreas disease in Atlantic salmon *Salmo salar* post-smolts induced by salmon pancreas disease virus (SPDV). *Dis Aquat Org* 26:117–24.
- Mitchum D. L., & Sherman L. E. (1981) Transmission of bacterial kidney disease from wild to stocked hatchery trout. *Can J Fish Aquat Sci* 38(5):547-551.
- Mo T. A. (1994) Status of *Gyrodactylus salaris* problems and research in Norway. In A.W. Pike & J.W. Lewis (eds.), *Parasitic Diseases of Fish* (pp. 43-56). Samara Publishing Ltd., Otley, Yorkshire, UK.
- Moewus-Kobb, L. 1965. Studies with IPN virus in marine hosts. *Annals of the New York Academy of Sciences*, 165:328–342.
- Morris D. J., Adams A., Feist S. W., McGeorge J., Richards R. H. (2000) Immunohistochemical and PCR studies of wild fish for *Tetracapsula bryosalmonae* (PKX), the causative organism of proliferative kidney disease. *J Fish Dis*, 23(2):129-135.
- Morris D. J., Adams A. (2006) Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), the causative organism of salmonid proliferative kidney disease, to the freshwater bryozoan *Fredericella sultana*. *Parasitology-Cambridge* 133:701-709.
- Munro W., Thomas C., Simpson I., Shaw J., Dodgson J. (1996) Deterioration of pH electrode response due to biofilm formation on the glass membrane. *Sensors and Actuators B* 37:187-194.
- Nebeker A.V., Bouck G.R., Stevens D.G. (1976) Carbon dioxide and oxygen-nitrogen ratios as factors affecting salmon survival in air-supersaturated water. *Trans Am Fish Soc* 3:425-429.
- Nematollahi A., Decostere A., Pasmans F., & Haesebrouck F. (2003) *Flavobacterium psychrophilum* infections in salmonid fish. *J Fish Dis*, 26(10):563-574.
- Nielsen C. V., Buchmann K. (2001) Occurrence of *Gyrodactylus* parasites in Danish fish farms. *Bull Eur Ass Fish Pathol* 21(1):19-25.
- Nilsen H., Olsen A.B., Vaagnes Ø., Helleberg H., Bottolfsen K., Skjelstad H., Colquhoun D.J. (2011a) Systemic *Flavobacterium psychrophilum* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum), farmed in fresh and brackish water in Norway. *J Fish Dis* 34:403–408.

- Nilsen H., Johansen R., Colquhoun D.J., Kaada I., Bottolfsen K., Vågnes Ø., Olsen A.B. (2011b) *Flavobacterium psychrophilum* associated with septicemia and necrotic myositis in Atlantic salmon *Salmo salar*: A case report. *Dis Aquat Org* 97:37–46.
- Nilsen H., Sundell K., Duchaud E., Nicolas P., Dalsgaard I., Madsen L., Aspán A., Jansson E., Colquhoun D.J., Wiklund T. (2014) Multilocus Sequence Typing Identifies Epidemic Clones of *Flavobacterium psychrophilum* in Nordic Countries. *Appl Environ Microbiol* 2014, 80(9):2728-36. DOI: 10.1128/AEM.04233-13. Published Ahead of Print 21 February 2014.
- Noga E.J. (2000) *Fish Disease: Diagnosis and Treatment*. Iowa State University press, Ames.
- North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J., Bromage, N.R. 2006. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 255:466-479.
- Nylund A., Jakobsen P. (1995) Sea-trout as a carrier of infectious salmon anemia virus. *J Fish Biol* 47:174-176.
- OIE Manual of Diagnostic Test for Aquatic Animals (2014)
<http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/manual-of-diagnostic-tests-for-aquatic-animals/>
- Okamura B., Hartikainen H., Schmidt-Posthaus H., Wahli T. (2011) Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biol* 56(4):735-753.
- Olsen N.J., Skall H.F., Jensen B.B., Henriksen N.H., Møllergård S., Korsholm H.H. (2013) Eradication of viral haemorrhagic septicaemia in Danish aquaculture. In DAFINET Workshop and PhD Course: Book of Abstracts.
- Paclibare J. O., Evelyn T. P. T., Albright L. J., & Prosperi-Porta L. (1994) Clearing of the kidney disease bacterium *Renibacterium salmoninarum* from seawater by the blue mussel *Mytilus edulis*, and the status of the mussel as a reservoir of the bacterium. *Dis aquat org* 18(2):29-133.
- Palacios G., Løvoll M., Tengs T., Hornig M., Hutchison S., Hui, Kongtorp R.T., Savji N., Bussetti A.V., Solovyov A., Kristoffersen A.B., Celone C., Street C., Trifonov V., Hirschberg D.L., Rabadan R., Egholm M., Rimstad E., Lipkin, W. I. (2010) Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. *PLoS One* 5(7):e11487.
- Peeler E. J., Gardiner R., Thrush M. A. (2004) Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. *Prev Vet Med* 64:175-189.

- Petterson J.M., Bracke M.B.M., Midtlyng P.J., Folkedal O., Stien L.H., Steffenak H., Kristiansen T.S. (2013) Salmon welfare index model 2.0: An extended model for overall welfare assessment of caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish health professionals. *Rev. Aquaculture* 5:1-18.
- Pickering A.D. (1998) Stress responses in fishes. In K.D. Black, A.D. Pickering (eds.), *Biology of Farmed Fish* (pp. 222-255). Sheffield Academic Press, Sheffield, UK.
- Piper R.G., McElwain I.B., Orme L.E., McCraren J.P., Fowler L.G., Leonard J.R. (1982) *Fish hatchery management*. U.S. Fish & Wildlife Service, Washington DC, USA.
- Portz D.E., Woodley C.M., Cech J.J. (2006) Stress-associated impacts of short-term holding on fishes. *Rev. Fish. Biol. Fish.* 16:125-170.
- Prosser C.L., Farhi E. (1965) Effects of temperature on conditioned reflexes and on nerve conduction in fish. *Z. vergl. Physiol.* 50:91-101.
- Randall D.J. (1991) The impact of variations in water pH on fish. In D.E. Brune & J.R. Tomasso JR (eds.), *Aquaculture and Water Quality* (pp. 90-104). World Aquaculture Society, Baton Rouge, Los Angeles.
- Randall D.J, Tsui T.K.N. (2002) Ammonia toxicity in fish. *Mar. Pollut. Bull.* 45:17-23.
- Rimstad E., Mjaaland S., (2002) Infectious salmon anaemia virus - An orthomyxovirus causing an emerging infection in Atlantic salmon. *APMIS* 110:273-282.
- Roberts R. J., Pearson M. D. 2005. Infectious pancreatic necrosis in Atlantic salmon, *Salmo salar* L. *J Fish Dis* 28(7):383-390.
- Roots B.I., Prosser C.L. (1962) Temperature acclimation and the nervous system in fish. *J. Exp. Biol.* 39:617-629.
- Roque d'Orbcastel E., Person-Le Ruyet J., Le Bayon N., Blancheton J-P. (2009) Comparative growth and welfare in rainbow trout reared in recirculating and flow through systems. *Aquacult. Eng.* 40:79-86.
- Rosseland B.O. (1999) Vannkvalitetens betydning for fiskehelsen [The significance of water quality to fish health]. In E. Poppe (ed.), *Fiskehelse og fiskesykdommer* (pp. 240-252), Universitetsforlaget, Oslo, Norway.
- Rosseland B.O., Staurnes M. (1994) Physiological mechanisms for toxic effects and resistance to acidic water: An ecophysiological and ecotoxicological approach. In C.E.W. Steinberg & R.F. Wright (eds.), *Acidification of Freshwater Ecosystems: Implications for the Future* (pp. 227-246). John Wiley & Sons Ltd, Chichester, UK.

- Saint-Jean S. R., Borrego J. J., & Perez-Prieto S. I. (2003) Infectious pancreatic necrosis virus: Biology, pathogenesis, and diagnostic methods. *Adv Virus Res* 62:113-165.
- Ruttner F. (1963) Dissolved substances and their transformations. In *Fundamentals of limnology* (pp. 56-61). University of Toronto Press, Toronto and Buffalo, Canada.
- Sanni S., Forsberg O.I. (1996) Modelling pH and carbon dioxide in single-pass seawater aquaculture systems. *Aquacult. Eng.* 15:91-110.
- Saunders R.L. (1986) The thermal biology of Atlantic salmon: Influence of temperature on salmon culture with particular reference to constraints imposed by low temperature. *Rep. Inst. Freshw. Res. Drottningholm* 63:77-90.
- Siikavuopio S.I., Jobling M. (1995) The effects of stocking density on survival and growth of wild-caught Arctic charr. *Nordic J. Freshw. Res.* 71:419-423.
- Skall H.F., Olesen N.J., Mellergaard S. (2005) Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming: A review. *J Fish Dis* 28:509–29.
- Skovgaard A., Buchmann K. (2012) *Tetracapsuloides bryosalmonae* and PKD in juvenile wild salmonids in Denmark. *Dis Aquat Org* 101(1):33-42.
- Skuladottir G.V., Schiöth H.B., Gudmundsdottir E., Richards B., Gardasson F., Jonsson L. (1990) Fatty acid composition of muscle, heart and liver lipids in Atlantic salmon, *Salmo salar*, at extremely low environmental temperature. *Aquaculture* 84:71-80.
- Smart G.R. (1978) Investigation of the toxic mechanisms of ammonia to fish – gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. *J. Fish Biol.* 12:93-104.
- Snow M., Raynard R. S., Inglis J., Bruno D. W. (2001) Investigation into the potential for seawater rainbow trout (*Oncorhynchus mykiss*) to act as vectors of infectious salmon anaemia virus (ISAV). *Bull Eur Ass Fish Pathol* 21:252-262.
- Soleng A., Bakke T. A. (1997) Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): Laboratory studies. *Can J Fish Aquat Sci* 54(8):1837-1864.
- Souter B.W., Dwilow A.G., Knight K., Yamamoto T. (1986) Infectious Pancreatic Necrosis Virus in Adult Arctic Char, *Salvelinus alpinus* (L.), in Rivers in the Mackenzie Delta Region and Yukon Territory. *Can Tech Rep Fish Aquat Sci No. 1441*, Fish Res Board Can, Winnipeg, Manitoba. 11 pp.
- State Pollution Control Authority (SFT) (1997) Environmental objectives for Norwegian water resources: Relation between pollution and effects (Miljøsmål for vannforekomstene – sammenhenger mellom utslipp og virkning, Statensforurensningstilsyn (SFT) 1997,

- Veiledning 95:01, SFT rapport TA-1138/1995) Guidance 95:01, ISBN: 82-7655-257-9, 50 pp.
- Steinberg C.E.W., Wright R.W. (eds.) (1994) Acidification of Freshwater Ecosystems: Implications for the Future. John Wiley & Sons, Chichester, UK.
- Sterud E., Forseth, T., Ugedal O., Poppe T. T., Jorgensen A., Bruheim T., Mo T. A. (2007) Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* (Myxozoa). *Dis Aquat Org* 77:191-8.
- Stien L.H., Bracke M.B.M., Folkedal O., Nilsson J., Oppedal F., Torgersen T., Kittilsen S., Midtlyng P.J., Vindas M.A., Øverli Ø., Kristiansen T.S. (2013) Salmon Welfare Index Model (SWIM 1.0): A semantic model for overall welfare assessment of caged Atlantic salmon: Review of the selected welfare indicators and model presentation. *Rev. Aquaculture* 5:33-57.
- Summerfelt S.T., Wilton G., Roberts D., Rimmer T., Fonkalsrud K. (2004) Developments in recirculating systems for Arctic char culture in North America. *Aquacultural Eng.* 30:31-71.
- Suski C.D., Kieffer J.D., Killen S.S., Tufts B.L. (2007) Sub-lethal ammonia toxicity in largemouth bass. *Comp Biochem Physiol A* 146:381-389.
- Tabata K. (1962) Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbon dioxide. *Bull Tokai Reg Fish Res Lab* 34:67-74.
- Teien H.C., Salbu B., Soerli-Heier L., Kroglund F., Rosseland B.O. (2005) Fish mortality during seasalt episodes-cathment liming as countermeasure. *J. Environ. Monit.* 7:989-998.
- Thorarensen H., Farrell A.P. (2011) The biological requirements for post-smolt Atlantic salmon in closed-contained systems. *Aquaculture* 312:1-14.
- Thorud K E., Djupvik H.O. (1988) Infectious anaemia in Atlantic salmon (*Salmo salar* L.). *Bull Eur Assoc Fish Pathol* 8:109-111.
- Timmons M.B., Ebeling J.M., Wheaton F.W., Summerfelt S.T., Vinci B.J. (2001) *Recirculation Aquaculture Systems*. NRAC Publications, No. 01-002. Cayuga Aqua Ventures, Ithaca, New York.
- Timmons M.B., Ebeling J.M. (2007) *Recirculating Aquaculture*. NRAC Publications, No. 01-007. Cayuga Aqua Ventures, Ithaca.
- Tomasso J.R. (1994) Toxicity of nitrogenous wastes to aquaculture animals. *Rev. Fish. Sci.* 2:291-314.

- Toranzo A.E., Hetrick F.M. (1982) Comparative stability of two salmonid viruses and poliovirus in fresh, estuarine and marine waters. *J. Fish Dis.* 5:223–231.
- Turnbull J., Bell A., Adams C., Bron J., Huntingford F. (2005) Stocking density and welfare of cage-farmed Atlantic salmon: Application of multivariate analysis. *Aquaculture* 243:121-132.
- Vestergård-Jørgensen P.E., Kehlet N.P. (1971) Infectious pancreatic necrosis (IPN) viruses in Danish rainbow trout. *Nordisk Veterinærmedisin*, 23:568–575.
- VKM (2005) Vurdering av faren for spredning av fiskesjukdommer ved bruk av dødfiskensilasje uten varmebehandling og/eller uensilerte biprodukt i pelsdyrfôr [Risk assessment of the spread of fish diseases when using fish silage without heat treatment and/or non-ensilaged biproducts in fur animal feed]. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety, 12 pp.
- VKM (2011) Risikovurdering - stamfiskovervåking og vertikal smitteoverføring [Risk assessment of broodfish surveillance and vertical transmission of infections]. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety ISBN: 978-82-8082-384-7 44 pp.
- VKM (2012) Risk Assessment of recirculation systems in salmonid hatcheries. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety. ISBN: 978-82-8259-048-8 110 pp.
- Wallace J.C., Kolbeinshavn A.G., Reinsnes T.G. (1988) The effects of stocking density on early growth in Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* 73:101-110.
- Wedemeyer G.A. (1996) *Physiology of fish in intensive culture systems*. Chapman and Hall, New York.
- Wedemeyer G.A. (1997) Effect of rearing conditions on the health and physiological quality of fish in intensive culture. In: G.K. Iwama, A.D. Pickering, J.P. Sumpter & Schreck C.B. (eds.), *Fish Stress and Health in Aquaculture* (pp. 35-71). Soc Exp Biol Semin, Ser. 62. Cambridge University Press, Cambridge, UK.
- Weitkamp D.E., Katz M. (1980) A review of dissolved gas supersaturation literature. *Trans. Am. Fish. Soc.* 109:659-702.
- Wendelaar Bonga S.E., Dederen L.H.T. (1986) Effects of acidified water on fish. *Endeavour, New Series* 10:198-202.
- Westers H. (1981) *Fish Culture Manual for the State of Michigan*. Michigan Department of Natural Resources, Lansing, Michigan.

- Wiik-Nielsen C. R., Løvoll M., Sandlund N., Faller R., Wiik-Nielsen J., Bang Jensen B. (2011) First detection of piscine reovirus (PRV) in marine fish species. *Dis Aquat Organ.* 97(3):255.
- Wiik-Nielsen C.R., Ski P.M., Aunsmo A., Løvoll M. (2012) Prevalence of viral RNA from piscine reovirus and piscine myocarditis virus in Atlantic salmon, *Salmo salar* L., broodfish and progeny. *J Fish Dis.* 35(2):169-71.
- Wiklund T., Kaas K., Lönnström L., Dalsgaard I. (1994) Isolation of *Cytophaga psychrophila* (*Flexibacter psychrophilus*) from wild and farmed rainbow trout (*Oncorhynchus mykiss*) in Finland. *Bull Eur Ass Fish Pathol* 14:44–46.
- Wilson J.M., Iwata K., Iwama G.K., Randall D.J. (1998) Inhibition of ammonia excretion and production in rainbow trout during severe alkaline exposure. *Comp Biochem Physiol B* 121:99-109.
- Winther U., Prestvik Ø., Ulgenes Y., Heide M., Nilssen F., Haug L., Uhlig C., Jensen H., Siikavuopio S., Sæther B-S. (2010) En mulighetsstudie for økt vekst innen innenlandsoppdrett [A study of opportunities for increased growth in inland fish farming]. SINTEF Report SFH80 A106041, 123 pp.
- Wolf K., Snieszko S.F., Dunbar C.E., Pyle E. (1960) Virus nature of infectious pancreatic necrosis in trout. *Proceedings of the Society for Experimental Medicine and Biology*, 104:105–108.
- Wolf K. (1988) *Fish viruses and fish viral diseases*. Comstock Publishing Associates, Cornell University Press, Itacha, NY.
- Wright P.A., Wood C.M. (1985) An analysis of branchial ammonia excretion in the freshwater rainbow trout: Effects of environmental pH change and sodium uptake blockade. *J Exp. Biol.* 114:329-353.
- Wright P. A. and Fyhn H.J. (2001) Nitrogen excretion. In: P.A. Wright & P.M. Anderson (eds.), *Fish Physiology*, Vol. 20 (pp.149-186). Academic Press, San Diego, California.
- Ye X., Randall D.J. (1991) The effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiol Biochem* 9:15-21.
- Øye A.K., Rimstad E. (2001) Inactivation of infectious salmon anaemia virus, viral haemorrhagic septicaemia virus and infectious pancreatic necrosis virus in wastewater UVC irradiation. *Dis Aquat Organ* 48:1-5.
- Åtland Å., Kristensen T., Skryseth L.M., Lange G., Fjellheim A. (2009) Giftighet av kobber - et undervurdert problem i settefiskanlegg både Norge og Chile. [Copper toxicity – an underestimated problem in Norwegian and Chilean hatcheries]. *Norsk fiskeoppdrett* No. 7/2009.

Appendix I

Monitoring of recipient impacts

Monitoring of impacts from floating net cage aquaculture in lakes

The present appendix is based on the general recommendations for water monitoring found online at <https://vannportalen.no/hovedEnkel.aspx?m=63831>.

The main challenges for the recipient environment exposed to floating cage aquaculture are confined to the increase of algal growth in the lake, nuisance periphyton growth in the vicinity of the farm, as well as build-up of organic sediments underneath cages.

Water samples should be collected in central parts of the lake. Sampling frequencies and analysis parameters are shown in Table 1.4-1.

Table AI-1 Suggested monitoring of eutrophication impact of fish farming in lakes. In small lakes at one station in the central part of the lake, in large lakes it may be necessary with more than one station.

Time	Sample type	Parameter
May	Mixed epilimnion	Secchi depth, pH, Cond., Tot-P, Tot-N, chlorophyll-a, TOC
June	Mixed epilimnion	Secchi depth, pH, Cond., Tot-P, Tot-N, chlorophyll-a, TOC
July	Mixed epilimnion	Secchi depth, pH, Cond., Tot-P, Tot-N, chlorophyll-a, TOC Phytoplankton species and biomass analysis
August	Mixed epilimnion	Secchi depth, pH, Cond., Tot-P, Tot-N, chlorophyll-a, TOC Phytoplankton species and biomass analysis
	Vertical series ¹	Oxygen, Tot-P, PO ₄ -P
September	Mixed epilimnion	Secchi depth, pH, Cond., Tot-P, Tot-N, chlorophyll-a, TOC
October	Mixed epilimnion	Secchi depth, pH, Cond., Tot-P, Tot-N, chlorophyll-a, TOC

Epilimnion = surface water, Secchi depth = turbidity, Cond.= conductivity, PO₄-P = phosphate, Tot-P = total phosphorous, Tot-N = total nitrogen, TOC = total organic carbon

¹sampling five depths from surface to bottom

Additionally, in August, a total of five periphyton samples are taken from submerged surfaces: One close to the fish farm, one on the stones along the shoreline just inside the fish farm, at two stations 200 m upstream and downstream, as well as at a reference station far away from the farm. These are analysed for species composition, and the ecological quality ratio (EQR) is calculated according to the Classification Guidance 02:2013 of the Water Regulation (vannforskriften). The Water Regulation is a framework governed by the Norwegian Ministry of Climate and Environment.

Furthermore, sediment samples are taken under the cages, at a station 100 m away from the farm both upstream and downstream and at representative reference station far away from the fish farm. Samples are taken with a plexiglass core tube, and the thickness of the sediments is measured. Photos are taken in the field with a scale placed next to the corer. The upper five cm of the sediments are analysed for water content, organic and inorganic dry matter, total phosphorous, total nitrogen, sulphide, total organic carbon, as well as bottom animals. The bottom animals must be sampled in November, when the insect larvae are large enough to allow identification on species level, the sediment cores can be taken once during the year. The samples are analysed according to the procedures of the Classification Guidance of the Water Regulation.

The described monitoring program will provide data necessary for evaluation of the impact on both the local level, as well as on the level of the whole lake ecosystem. If the results of the analysis confirm a good ecological status, this also implies good living conditions for wild fish.

Monitoring of impacts from land based fish farms with discharge to lakes

Land based fish farms already exert a minimum of effluent treatment by using settling tanks that allow removal of fodder remains and fish faeces. Build-up of sediments therefore rarely represents a problem for the recipient. However, eutrophication can be a potential problem due to discharge of nutrients, in particular phosphorous, through the effluent.

Monitoring is conducted at a station centrally placed in the lake. Sampling frequencies and parameters are as described in Table 1.4-1. The effluent itself should also be analysed for the same parameters.

Monitoring of impacts from land based fish farms with discharge to rivers

If the effluent is to a river, samples should be collected from upstream of the discharge point, and in immediate vicinity downstream of the discharge point, as well as 100-200 m further downstream. The effluent itself should also be sampled.

Chemical samples are taken once a month from May to October for analysis of turbidity, pH, conductivity, total phosphorous, phosphate, total organic carbon, total nitrogen, ammonia, ammonium, biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

In August, samples of the attached growth of periphyton and any heterotrophic growth on the river bed substrate are collected. In November, bottom animals should be sampled at the same stations. The biological samples should be analysed and interpreted as described in the Classification Guidance to the Water Regulation. If the ecological status of the lake or river is rated as good by the classification system, satisfying living conditions can be considered secured for wild fish.

Appendix II

Water quality guidelines of the Norwegian Food Safety Authority

Table AII-1 Water quality guidelines used within the Norwegian Food Safety Authority.

Water quality parameter	Limits
pH (inlet)	6.2 – 7.8
Dissolved oxygen	Maximum 100 % saturation (tank) and >80 % saturation (outlet)
Carbon dioxide	<15 mg/L (salmon) <10 mg/L (rainbow trout)
TAN (NH ₄ ⁺ + NH ₃)	<2 mg/L
Nitrite	<0.1 mg/L (freshwater)
Total organic carbon (TOC)	<10 mg/L
Aluminium	<5 µg/L (labile) and maximum 15 µg/g gill (gills)

Appendix III

Interrelation of concentration of total ammonia nitrogen (TAN = NH₃ + NH₄⁺) with pH and temperature of the water

Table AIII-1 Concentrations of total ammonia nitrogen (TAN = NH₃ + NH₄⁺) that lead to concentrations of toxic un-ionized ammonia (NH₃) above 0.025 mg/L. Such values are long-term harmful concentrations for salmonid fish, depending on pH and temperatures in the ambient water (Alabaster and Lloyd, 1980). The table shows TAN-values in mg/L.* How to read the table: For example, at a fish farm where the water has a pH of 6.5 and the temperature is 10 °C, the concentration of TAN must be 42.4 mg/L in order to give an ammonia concentration above 0.025 mg NH₃/L.

Temp. °C	pH-value					
	6.5	7.0	7.5	8.0	8.5	9.0
5	63.3	20.0	6.3	2.0	0.66	0.23
10	42.4	13.4	4.3	1.4	0.45	0.16
15	28.9	9.2	2.9	0.94	0.31	0.12
20	20.0	6.3	2.0	0.66	0.22	0.088
25	13.9	4.4	1.4	0.46	0.16	0.069
30	9.8	3.1	1.0	0.34	0.12	0.056

* Note that the current practice in Norwegian laboratories for water analysis is to present TAN as mg N/L.

Appendix IV

Hatcheries and spreading of unwanted organisms other than fish diseases

Many fish farms have their own hatcheries. Other farms will buy fingerlings/or fry. There is a long tradition and experiences with hatcheries of trout in Norway with the purpose of enhancing fish population in natural lakes and rivers. Even though such plants are not part of our study, it is relevant to learn from the experience from these fish enhancement hatcheries. These hatcheries provide stocking aid to watercourses, which are compensations for different human imposed damages to the livelihood of trout, such as hydropower regulations, acid rain, eutrophication and other pollution of habitats and spawning areas, streams, etc. The hydropower regulation is the largest single stressor on inland fish populations, therefore their branch associations (such as GLB, EBL) have built and operated some big hatcheries (for instance Reinsvoll settefisk AS and Rendalen Settefisk AS). Via stockings from the Reinsvoll hatchery, the Tunhovd trout has been stocked into more than 1000 lakes all over Southern Norway. It has shown indications that the Tunhovd trout is less resistant towards acid rain than the local local trout strains from the Southern Norway (Sørlandet), and there are also indications that the unwanted minnow is spread by stockings from the Reinsvoll hatchery. Therefore Environmental Directorate has required use of locally based fish strains for stockings in the wild. As a result Reinsvoll Hatchery AS is recently closed down. In addition to these former large hatcheries there are a large number of smaller hatcheries engaged in fish enhancement programmes, which are using local fish strains only. These are normally run by local fish associations, with support from municipalities, water works, industries, hydropower companies, etc. Stocking from these locally based hatcheries do also spread different organisms to lakes where they were not present before, though in more restricted scale than from the former big hatcheries.

The OFA hatchery in Sørkedalen is a flow-through system which takes water from River Sørkedalselva, and release into the same river after some treatment. In earlier days they did not have any treatment of the intake water. River Sørkedalselva contains the river pearl mussel (*Margaritifera margaritifera*). This mussel has glochidia larvae which are released into the water and attach to the fish gills, where it has a parasitic stage which often last more than half a year. On the gills the larvae goes through the metamorphoses to an adult mussel. The deployment of fish fry and fingerlings into the wild, is in the period where the larvae are on the gills. In this way OFA was spreading pearl mussels in many rivers in Nordmarka without knowing it. In periods of massive mussel spawning, the number of glochidie larvae on the gills gave increased mortality in the hatchery, as well. Now there is sand filtering of the intake water, so the problem does not exist anymore. The pearl mussel is, however, a wanted species, which has been eradicated from many rivers from pearl fishing and acidification. The duck mussel (*Anodonta piscinalis*) is spread in the same way, having the glochidie larvae on the fins of the fish.

It should be noted that the spread of organisms does not only arise from the fish itself, or the inlet water or outlet water, but often has come from the water filled into the oxygenated plastic bags used to transport the fish. This was earlier, and still often is, filled directly with water from the container in which the fingerlings are living, which is flow-through water from a nearby river. In this way minnows have followed as blind passengers in older stockings where intake water was taken from streams having minnows, and with no treatment of the intake water. The water plague ('vasspest', *Elodea Canadensis*) can also easily be spread in this manner.

There is a growing concern about the danger of spreading unwanted organisms from fish hatcheries and fish stocking programs. The inland aquaculture will face this concern more strictly than the marine aquaculture.

Appendix V

Animal health requirements for moving live eggs and fish to aquaculture farms in Norway

This annex gives a general account of the most important fish health requirements applying to the movement of live eggs and fish of trout, rainbow trout and Arctic char to aquaculture farms in Norway. Section 1 provides an account of the general rules that apply regardless of the place of origin of the animals. Section 2, 3 and 4 provides a general account of the rules which apply respectively, for moving within Norway, moving from EEA countries to Norway, and moving from third countries (countries outside the EEA) to Norway.

1. General fish health requirements applying to the movement of live eggs and fish of trout, rainbow trout and Arctic char to aquaculture farms in Norway – regardless of place of origin

In principle, the same animal health requirements apply to the placing on the market of aquaculture animals within Norway, from other countries within the EU / EEA to Norway, and imports from outside the EU / EEA (third countries). Approval from The Norwegian Food Safety Authority (NFSA) is not required when live eggs or fish are placed on the market within Norway, nor when they originate from other countries within the EU / EEA or from third countries. The regulations set the health requirements that must be met in order for the placing on the market or import to be legal. Below is a summary of these requirements. It is emphasized that other regulations safeguarding considerations other than aquatic animal health issues, managed by The Norwegian Directorate of Fisheries and The Norwegian Environment Agency, also regulates the movement and introduction of live eggs and fish into Norway.

The basic health requirements that apply to all transfers of live eggs and fish to an aquaculture farm are:

- The animals must be clinically healthy. They have to be clinically healthy in relation to all contagious diseases, not only the listed diseases.
- The animals shall not come from a farm where there is any unresolved increased mortality.

Health requirements that apply in addition to this depend on the listing of diseases, species defined as susceptible to the listed diseases, and health status at the place of destination in relation to the listed diseases. A basic principle is that live eggs and fish may be transferred to aquaculture farms with equal or lower health status, but cannot be transferred from farms or areas with a low status to farms with a better health status.

There are three different lists of diseases in Regulation 17 June 2008 no. 819 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals:

List 1 – Exotic diseases:

List 1 diseases are considered as exotic within EU / EEA. The whole of EU / EEA area is considered free from these diseases. List 1 in the Norwegian legislation is fully harmonized with the list of exotic diseases in 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. The Directive also lays down minimum control measures in the case of confirmation of exotic diseases in aquaculture animals.

Fish diseases on List 1 for which trout, rainbow trout and/or Arctic char is defined as susceptible species, and Norwegian status:

Fish disease	Defined as susceptible species in the legislation	Norwegian status
EHN	Rainbow trout – yes Trout and Arctic char are not defined as susceptible species.	Disease-free as the rest of EU / EEA

List 2 – Non-exotic diseases:

List 2 diseases are present within EU / EEA, but some areas are free from these diseases. List 2 in the Norwegian legislation is fully harmonized with the list of non-exotic diseases in Council Directive 2006/88/EC. The Directive also lays down minimum control measures in the case of confirmation of non-exotic diseases. However, the measures applied depend on the ambition and health status in each country.

Fish diseases on List 2 for which trout, rainbow trout and/or Arctic char is defined as susceptible species, and Norwegian status:

Fish disease	Defined as susceptible species in the legislation	Norwegian status
VHS	Trout and rainbow trout - yes Arctic char is not defined as a susceptible species.	Disease-free, except for buffer area along the border to Russia.
IHN	Rainbow trout – yes Trout and Arctic char are not defined as susceptible species.	Disease-free, except for buffer area along the border to Russia.
ISA	Trout and rainbow trout - yes Arctic char is not defined as a susceptible species.	Some areas with brood stock and roe production are disease-free. The major part of Norwegian territory has an undetermined status (category III).

List 3 – National diseases

According to Council Directive 2006/88/EC each Member State may also implement certain national measures to prevent and control diseases not listed in the Directive. National measures that affect trade between Member States are subject to approval. Norwegian national measures that effect trade must to be approved by EFTA Surveillance Authority. Norway has approved national measures for *Gyrodactylus salaris*, but not for any other List 3 diseases.

The major part of Norwegian territory has disease-free status for *G. salaris*. The exception is infected rivers, including their water catchment area, and a buffer zone (Enningdalsvassdraget) along the border to Sweden. Trout, rainbow trout and Arctic char are defined in the legislation as species susceptible to *G. salaris*.

Other fish diseases on the Norwegian List 3 are BKD, VNN / VER, Furunculosis, PD, Francisellosis, Sea lice and Systemic infection with *Flavobacterium psychrophilum* in rainbow trout. Control measures against these diseases are implemented in Norway, but we do not have approved national measures that effect trade with other countries. Therefore, there are no requirements on disease-free status of these diseases when live eggs and fish are introduced to Norway from other countries.

2. Fish health requirements applying to domestic movement of live eggs and fish of trout, rainbow trout and Arctic char to aquaculture farms in Norway

Most of Norway is within the same defined area and health status with respect to VHS, IHN, ISA and *G. salaris*. Therefore, it is usually only the basic and general health requirements that apply when live eggs and fish are moved within Norway:

- The animals must be clinically healthy. They have to be clinically healthy in relation to all contagious diseases, not only the listed diseases.
- The animals shall not come from a farm where there is any unresolved increased mortality.
- Aquaculture animals shall not come from a farm where restrictions are imposed due to suspected or confirmed presence of a listed disease (List 1, 2 and 3 except sea lice), but the NFSA may exempt from this requirement.

In addition, fish has to be examined for PD prior to transport out of the areas covered by PD-zone regulations.

Health certificate issued by NFSA is not required when live eggs and fish are moved within a defined area with equal health status as regards listed diseases. Since that is the case for most parts of Norway, health certificate is not required in most cases of domestic movement

of live eggs and fish. However, health certificate issued by the NFSA is required for consignments of live eggs and fish intended to brood stock farms with disease-free status for ISA, and for consignments of disinfected eggs leaving areas infected with *G. salaris*.

Notification in TRACES is not required for domestic movement of live eggs and fish. The traceability of biological materials is taken care of by the obligation to record all movements of aquaculture animals into and out of a farm.

3. Fish health requirements applying to the introduction of live eggs and fish of trout, rainbow trout and Arctic char from other countries within EU / EEA to aquaculture farms in Norway

Consignments of live eggs and fish from other countries within EU / EEA intended for aquaculture farms in Norway must be accompanied by an animal health certificate. The competent authority in the country of origin must certify on the following general requirements:

- The animals are clinically healthy. They have to be clinically healthy in relation to all contagious diseases, not only the listed diseases.
- The animals do not come from a farm where there is any unresolved increased mortality.
- Aquaculture animals do not come from a farm where restrictions are imposed due to suspected or confirmed presence of a listed disease (list 1 and 2).

Which specific animal health status requirements apply in addition to this depends on the specie in the consignment. If the consignment consist of live eggs or fish of **rainbow trout**, the competent authority must in addition certify that it comply with the following:

- Animals in the consignment must originate from a Member State, zone or compartment declared free of VHS and IHN.
- If animals in the consignment are live fish of rainbow trout, they must also either originate from a Member State, or part thereof, which comply with requirements for freedom from *G. salaris* laid down in the OIE standard, or have been held in water with salinity of at least 25 parts per thousand for a continuous period of at least 14 days immediately prior to the placing on the market.
- If animals in the consignment are live eggs from rainbow trout, they must also have been disinfected by a method demonstrated to be effective against *G. salaris*.

If the consignment consist of live eggs or fish of **trout**, the competent authority must in addition certify that it comply with the following:

- Animals in the consignment must originate from a Member State, zone or compartment declared free of VHS. Trout is not defined as susceptible species to IHN. Disease-free status of IHN is therefore not required.
- The same requirements on *G. salaris* apply for trout as for rainbow trout.

If the consignment consists of live eggs or fish of **Arctic char**, the competent authority must in addition certify that it complies with the same requirements on *G. salaris* as for rainbow trout.

Disease-free status for ISA is not required unless the consignment is intended to an area with disease-free status for ISA in Norway. Trout, rainbow trout and Arctic char are defined as species susceptible to ISA, but disease-free status is not required for consignments intended to areas with unresolved status (category III).

Countries within the EU / EEA area with disease-free status for VHS are Denmark (continental area), Ireland, Cyprus (continental area), Finland (except Åland), Sweden, United Kingdom and Iceland. These areas also have disease-free status for IHN. In other countries there are also smaller areas with disease-free status for both VHS and IHN. Disinfected eggs of trout and rainbow trout from these areas may comply with the requirements in the fish health regulations for legal introduction to aquaculture farms in Norway. Arctic char is not defined as species susceptible to VHS and IHN. Disinfected eggs of Arctic char originating from the whole EU / EEA area may therefore comply with the requirements in the fish health regulations for legal introduction to aquaculture farms in Norway.

Countries within the EU / EEA area with disease-free status for *G. salaris* are Ireland, northern part of Finland (the water catchment areas of the Tenojoki and Näämäjoki), United Kingdom and Iceland. These areas also have disease-free status for VHS and IHN. Live fish of trout, rainbow trout and Arctic char originating from freshwater in these areas may comply with the requirements in the fish health regulations for legal introduction to aquaculture farms in Norway.

Freedom from the relevant diseases must be declared in accordance with Council Directive 2006/88/EC. There is an obligation to notify the relevant diseases to the competent authority, and reports of suspicion of infection of the relevant diseases must be immediately investigated by the competent authority. Additional requirement on risk-based animal health surveillance applies to all aquaculture farms in EU / EEA. However, the requirements in Norway are more stringent than in other countries. This applies to both the required frequency of inspections performed by a veterinarian or fish health biologist, and the investigations that have to be carried out during inspections.

When aquaculture animals are introduced from other countries in EU / EEA, the traceability is taken care of by the obligation to record all movements of aquaculture animals into and out of a farm, animal health certificates and notification in TRACES.

4. Fish health requirements applying to import of live eggs and fish of trout, rainbow trout and Arctic char from third countries to aquaculture farms in Norway

The fish health requirements that apply to the introduction of live eggs and fish of trout, rainbow trout and Arctic char from other countries within EU / EEA to Norway also apply to import from third countries. Additional requirements for imports from third countries are that the consignments must originate from a country or region that has been approved and listed in the fish health regulations. The competent authority in the country of origin must in addition also certify that consignments of species susceptible to EHN originate from areas declared free from EHN. Rainbow trout is defined as susceptible to EHN, but not trout and Arctic char.

The following requirements on status for specific diseases apply:

- Disinfected eggs of rainbow trout must originate from an area declared free from EHN, VHS and IHN.
- Disinfected eggs of trout must originate from an area declared free from VHS.
- Declaration on freedom from specific diseases is not required for disinfected eggs of Arctic char.
- Live fish of rainbow trout must originate from an area declared free from EHN, VHS and IHN, and must comply with requirements for freedom from *G. salaris*.
- Live fish of trout must originate from an area declared free from VHS and must comply with requirements for freedom from *G. salaris*.
- Live fish of Arctic char must comply with requirements for freedom from *G. salaris*.

Freedom from the relevant diseases must be declared in accordance with Council Directive 2006/88/EC or the relevant OIE Standard by the competent authority of the country of origin. There must be an obligation to notify the relevant diseases to the competent authority, and reports of suspicion of infection of the relevant diseases must be immediately investigated by the competent authority. There are no additional requirements on risk-based animal health surveillance.

When aquaculture animals are imported from other third countries, the traceability is taken care of by the obligation to record all movements of aquaculture animals into and out of a farm and animal health certificates. Documents and consignments have to be controlled at Border Inspection Posts.