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Risk assessment of fish health associated with the use of cleaner fish in aquaculture

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

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to answer the request from the Norwegian Food Safety Authority. The project group consisted of three VKM members from the VKM Panel on Animal Health and Welfare, one external expert, and a project leader from the VKM secretariat. One VKM member from the Panel on Biological Hazards and one from the Panel on Microbial Ecology reviewed and commented upon the manuscript. The Panel on Animal Health and Welfare evaluated and approved the final opinion drafted by the project group.

Authors of the opinion

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Assessed and approved

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

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

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VKM would like to thank Lucy Robertson, VKM Panel on Biological Hazards, and Tor Gjøen, VKM Panel on Microbial Ecology, for reviewing and commenting the manuscript. We are grateful to Lucy Robertson for proofreading the manuscript. VKM would also like to thank the hearing experts Vidar Vold, Lerøy Seafood Group, and Unni Austefjord, Marine Harvest Group, for sharing their views on challenges related to the use of cleaner fish in aquaculture.

Competence of VKM experts

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

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Summary

Background

Cleaner fish are widely used for delousing farmed salmonids. The aquaculture industry is dependent on both wild-caught and farmed cleaner fish to contain louse infestations. At present, mandatory fallowing requires that all fish, including cleaner fish, are removed from the farm after each production cycle of salmon. Due to sustainability concerns, a change in legislation has been proposed, in which reuse of cleaner fish over several production cycles would be allowed.

Terms of reference

In February 2017, the Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food and Environment for an opinion on the risks of infectious disease spread to the next production cycle of salmonids, should cleaner fish from the previous production cycle be kept at the farm during compulsory fallowing. Furthermore, VKM was asked to assess the risk of introducing pancreas disease (PD) by relocating cleaner fish from an area endemic for PD to an area free from this disease.

Working group and evaluation of risk assessment

VKM established a working group comprising three members from the Panel on Animal Health and Welfare and an external expert from the Norwegian Veterinary Institute. One VKM member from the Panel on Biological Hazards and one from the Panel on Microbial Ecology have reviewed and commented upon the manuscript. The Panel on Animal Health and Welfare has evaluated and approved the final report draft submitted by the working group.

Methodology

Since data on the disease status of wild-caught cleaner fish are limited, and so is the availability of relevant diagnostic tools, the working group chose a qualitative approach to assess the risk of transmitting disease from cleaner fish to farmed salmonids. The probability of transmission is a function of time. As the strategies applied for sea louse control by the industry change rapidly, the time perspective for estimation of probability for transmission used in this report is two-three production cycles at sea sites, i.e., 4 years.

Hazard identification and characterization

Cleaner fish have their own pathogens. Some of these pathogens are host species-specific, but others may be promiscuous regarding host species. We focused on those agents that have the potential to be transmitted from cleaner fish to farmed salmonids.

Ten viruses were identified as possible hazards: *Piscine myocarditis virus* (PMCV), *Viral haemorrhagic septicaemia virus* (VHSV), *Infectious salmon anaemia virus* (ISAV), Salmonid alphavirus (SAV), *Infectious pancreas necrosis virus* (IPNV), Nodavirus, *Piscine orthoreovirus* (PRV), Lymphocystis virus, Lumpfish ranavirus, and Lumpfish flavivirus.

Ten bacterial agents were identified as possible hazards: *Aeromonas salmonicida* subsp. *salmonicida*, atypical *Aeromonas salmonicida*, *Vibrio anguillarum* (primarily serotypes O1 and O2a), *Vibrio ordalii*, *Vibrio* spp., *Pasteurella* sp., *Pseudomonas anguilliseptica*, *Piscirickettsia salmonis*, *Moritella viscosa,* and *Tenacibaculum* sp.

Two parasites were identified as possible hazards: *Paramoeba perurans* and *Caligus elongatus*.

Conclusions

The origins and life histories of the cleaner fish are important factors that affect the risk. The disease status of wild-caught cleaner fish is, in general, poorly known. Translocations of such fish may result in the introduction of new pathogens to farmed salmonids. Stocking and screening of wild-caught cleaner fish prior to their introduction to the salmonid net pens could mitigate the risk. The risk of introducing cleaner fish-specific pathogens is considered higher than the risk of introducing salmonid pathogens.

In Norway, amoebic gill disease (AGD) is the only known example of a disease for which there have been evidence-based suspicions of transmission from cleaner fish to farmed Atlantic salmon. Farmed Atlantic salmon are protected against furunculosis and classical vibriosis by vaccination. The risk of cleaner fish transmitting bacterial diseases to salmonids is therefore considered low. However, opportunistic infections caused by *Vibrio* spp., *Tenacibaculum* sp. and *Piscirickettsia salmonis* should not be disregarded. Both wrasses and lumpfish are susceptible to VHSV infection. VHSV is an example of an infection for which the prevalence in wild-caught cleaner fish will influence the probability of introduction, and may represent an example of a high pathogen transmission risk for salmonids. Wrasses are susceptible to PMCV infection and can therefore be considered potential reservoirs for this virus. The risk of cleaner fish transmitting PMCV to salmonids is considered moderate.

Lumpfish are never or very seldom, reused. The probability of transmitting infection and disease to the following production cycle due to retaining cleaner fish for future reuse, therefore applies almost exclusively to wrasses. The probability will generally increase if cleaner fish are replenished from different sources. Potential risk-reduction measures include avoiding reuse of cleaner fish that have been in contact with salmonids experiencing disease outbreaks; periodic complete fallowing, including cleaner fish; quarantine stocking and fish health inspection; and reuse of only a small proportion of cleaner fish.

Currently, it would be important to screen cleaner fish kept during the fallowing period with the intention of reuse for the following pathogens: VHSV, PMCV, and *Paramoeba perurans.* However, such a list may change quickly. Furthermore, disease-causing agents that are important for cleaner fish health, and for which there are no available vaccines, could also have been considered.

SAV is not considered to infect cleaner fish. The use of wild-caught cleaner fish that are moved from areas endemic for PD to areas free of PD has the potential to result in mechanical transmission of SAV. The risk of transmission of SAV to farmed salmonids under these circumstances is considered to be high. The probability and consequence of transmitting SAV varies, depending on environmental, management, and regulatory factors.

Data gaps and uncertainties

The working group recognizes a number of data gaps and uncertainties related to transmission of pathogens to farmed salmonids from cleaner fish. Information on the disease status of wild-caught cleaner fish is scarce. Very little is known about the pathogenic potential of *Vibrio* spp., lumpfish flavivirus, lumpfish ranavirus, or *Piscirickettsia salmonis* in cleaner fish. The lack of basic knowledge of disease development and the absence of specific diagnostic tools for cleaner fish infections are factors limiting a complete and evidence-based evaluation of the risks.

Key words: VKM, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, cleaner fish, wrasse species, lumpfish, Atlantic salmon, rainbow trout, aquaculture, reuse, fallowing, disease transmission, viral diseases, bacterial diseases, parasitic diseases, pancreas disease, infectious salmon anaemia, amoebic gill disease, viral haemorrhagic septicaemia

Sammendrag på norsk

Bakgrunn

Rensefisk brukes til avlusing i lakseoppdrett. Behovet er stort og akvakulturindustrien er avhengig av tilgang til både villfanget og oppdrettet rensefisk for å bekjempe lakselus. Ved hver avsluttet produksjonssyklus av laks skal all fisk, inkludert rensefisk, fjernes fra anlegget. Mattilsynet vurderer å endre regelverket ved å åpne for gjenbruk av rensefisk over flere produksjonssykluser.

Oppdrag

Mattilsynet ba våren 2017 Vitenskapskomiteen for mat og miljø (VKM) om en vurdering av risiko for overføring av smitte og sykdommer til neste produksjonssyklus av laks, dersom rensefisk fra forrige produksjonssyklus oppbevares i anlegget i den obligatoriske brakkleggingsperioden. Videre ble VKM bedt om å vurdere risikoen for at flytting av rensefisk kan føre til at pankreassykdom (PD) spres fra områder der sykdommen er endemisk til områder som er fri for denne sykdommen.

Arbeidsgruppe og evaluering av risikovurdering

VKM nedsatte en arbeidsgruppe bestående av tre medlemmer fra faggruppen for dyrehelse og dyrevelferd og en ekstern ekspert fra Veterinærinstituttet. To VKM-medlemmer fra faggruppene for hygiene og smittestoffer og for mikrobiell økologi har gått gjennom og kommentert manuskriptet. Faggruppen for dyrehelse og dyrevelferd evaluerte og godkjente den endelige rapporten.

Metodikk

Det er begrenset informasjon om sykdomsstatus i villfanget rensefisk, og begrenset mengde relevante diagnostiske verktøy. Arbeidsgruppen brukte derfor en kvalitativ tilnærming for vurdering av risiko for overføring av sykdommer fra rensefisk til oppdrettslaks. Sannsynligheten for smitteoverføring er en funksjon av tid. De strategier som brukes innen industrien for å kontrollere lakselus, kan fort endres. Tidsperspektivet ved estimering av sannsynlighet for smitteoverføring er derfor satt til to-tre produksjonssykluser i sjø, det vil si 4 år, i denne rapporten.

Identifisering og karakterisering av fare

Rensefisk har sine særegne smittsomme agens. Noen av disse er vertsspesifikke, mens andre kan infisere mange forskjellige arter fisk. Vi fokuserte på de agens som potensielt kan overføres fra rensefisk til oppdrettslaks.

Ti virus ble vurdert som farer: *Piscint myokarditt virus* (PMCV), *Viral hemoragisk septikemi virus* (VHSV), *Infektiøs lakseanemi virus* (ILAV), *Salmonid alfavirus* (SAV), *Infeksiøs pankreas* *nekrose virus* (IPNV), *Nodavirus, Piscine orthoreovirus* (PRV), *Lymfycystis virus, Lumpfish ranavirus* og *Lumpfish flavivirus*.

Ti bakterielle agens ble vurdert som farer: *Aeromonas salmonicida* subsp. *salmonicida*, atypisk *Aeromonas salmonicida*, *Vibrio anguillarum* (primert serotypene O1 og O2a), *Vibrio ordalii*, *Vibrio* spp., *Pasteurella* sp., *Pseudomonas anguilliseptica*, *Piscirickettsia salmonis*, *Moritella viscosa,* og *Tenacibaculum* sp.

To parasittarter ble vurdert som farer: *Paramoeba perurans* og *Caligus elongates.*

Konklusjoner

Rensefiskens opprinnelse påvirker risiko. Sykdom- og infeksjonsstatus hos villfanget rensefisk er generelt dårlig kjent. Bruk av villfanget rensefisk kan medføre at oppdrettslaksen eksponeres for nye smittestoffer. Risikoen vil kunne reduseres ved at villfanget rensefisk oppbevares og undersøkes før de introduseres til laksemerdene. Risikoen for å introdusere smittsomme agens som er spesifikke for rensefisk, anses å være høyere enn risikoen for å introdusere smittsomme agens som kan gi sykdom hos laks.

Amøbegjellesykdom (AGD) er det eneste kjente eksempelet der det er berettiget mistanke om overføring av sykdom fra rensefisk til oppdrettslaks. Oppdrettslaks er vaksinert og beskyttet mot blant annet furunkulose og klassisk vibriose. Risikoen for at bakterielle sykdommer skal overføres fra rensefisk til laksefisk anses å være lav. Opportunistiske infeksjoner, som blant annet kan forårsakes av *Vibrio* spp., *Tenacibaculum* spp. og *Piskirickettsia* sp., bør imidlertid ikke utelukkes. Både leppefisk og rognkjeks, som er vanligst å bruke i oppdrett, er mottakelige for VHSV-infeksjon. VHSV er et eksempel på en infeksjon der prevalensen hos villfanget rensefisk vil påvirke sannsynligheten for introduksjon. Det er høy risiko for at VHSV kan overføres til laksefisk. Leppefisk er mottakelig for PMCV og kan derved anses som potensielle reservoarer for dette viruset. Risikoen for at rensefisk skal overføre PMCV til laksefisk anses som moderat.

Rognkjeks blir aldri eller veldig sjelden gjenbrukt. Sannsynligheten for overføring av smitte og sykdom til etterfølgende produksjonssyklus knyttet til oppbevaring av rensefisk for fremtidig bruk, gjelder derfor nesten utelukkende leppefisk. Sannsynligheten for smitteoverføring vil generelt øke hvis rensefisken kommer fra ulike kilder.

Et mulig risikoreduserende tiltak er å unngå gjenbruk av rensefisk som har vært i kontakt med laksefisk som har opplevd sykdomsutbrudd. Det inkluderer periodisk, fullstendig brakklegging inklusive rensefisk, karanteneopphold og fiskehelseinspeksjoner, samt gjenbruk av kun en liten andel av rensefisk.

Følgende smittestoffer vil per i dag være viktig å undersøke hos rensefisk som er påtenkt for gjenbruk under brakkleggingsperioden: VHSV, PMCV og *Paramoeba perurans*. En slik liste kan imidlertid fort bli endret. Der det ikke finnes tilgjengelige vaksiner, bør det derfor også undersøkes for sykdomsfremkallende agens som er viktige for rensefisk.

SAV anses ikke å infisere rensefisk. Bruk av rensefisk som flyttes fra områder som er endemisk for PD til områder som er fri for denne sykdommen, kan potensielt resultere i en mekanisk overføring av SAV. Sannsynligheten for overføring av SAV til oppdrettslaks ved flytting av rensefisk anses som moderat, men konsekvensene er potensielt alvorlige, og risikoen vurderes derfor som høy.

Kunnskapshull og usikkerheter

Prosjektgruppen identifiserte en rekke kunnskapshull og usikkerheter knyttet til overføring av smittestoffer fra rensefisk til oppdrettslaks. Informasjon om sykdomsstatus hos villfanget rensefisk er begrenset. Hos rensefisk er veldig lite kjent om patogent potensial for *Vibrio spp., Piscirickettsia salmonis,* flavivirus og ranavirus hos rognkjeks. Mangel på grunnleggende kunnskap om sykdomsutvikling og fravær av spesifikke diagnostiske verktøy for infeksjoner i rensefisk, er faktorer som begrenser en mere fullstendig vurdering av risiko.

Nøkkelord: VKM, Vitenskapskomiteen for mat of miljø, Mattilsynet, rensefisk, leppefisk, rognkjeks, atlantisk laks, regnbueørret, akvakultur, gjenbruk, brakklegging, sykdomsoverføring, virussykdommer, bakterielle sykdommer, parasittsykdommer, pankreas sykdom, infeksiøs laksanemi, amøbegjellesykdom, viral hemoragisk septikemi

Abbreviations and/or glossary

Abbreviations

AGD = Amoebic gill disease

CMS = Cardiomyopathy syndrome

EHNV = Epizootic haematopoietic necrosis virus

i.p. = Intraperitoneal

IPNV = Infectious pancreatic necrosis virus

ISAV = Infectious salmon anaemia virus

LFV = Lumpfish flavivirus

NNV = Nervous necrosis virus

OIE = World Organization for Animal Health

PCR = Polymerase chain reaction

PD = Pancreas disease

PMCV = Piscine myocarditis virus

PRV = Piscine orthoreovirus

RAS = Recirculating aquaculture system

RNA = Ribonucleic acid

RT-qPCR = Quantitative polymerase chain reaction

SAV = Salmonid alphavirus

sp./spp. = species (singular/plural)

SRS = salmon rickettsial syndrome

subsp. = subspecies

VHSV = Viral haemorrhagic septicaemia virus

wpc = Weeks post challenge

UV = Ultraviolet

Glossary

Atlantic salmon = *Salmo salar*

Broodstock = Sexually mature fish used in aquaculture for breeding.

Cleaner fish = In this context fish species that remove ectoparasites from farmed salmonids.

Doorstep species: a species expanding its geographical range, likely to establish in the actual area.

Fallowing = For disease management purposes, an operation in which an aquaculture establishment is emptied of aquatic animals susceptible to a disease of concern or known to be capable of transferring the pathogenic agent, and, where feasible, of the carrying water for a defined period. In Norway, it is mandatory to fallow at the end of a production cycle for a minimum of two months.

Lumpfish = *Cyclopterus lumpus*. Often referred to as lumpsucker.

Mechanical transmission = Transfer of a pathogen from an infected individual to a susceptible individual without any propagation of the pathogen during the transfer.

Primary males: Male fish which are born males (not undergone a sex change).

Rainbow trout *= Oncorhynchus mykiss*

Reuse of cleaner fish = Collection and transfer of cleaner fish from one production site to another or collection of cleaner fish at one site and reuse in the consecutive production cycle after the fallowing period. The common practice of collecting cleaner fish from net pens during salmonid slaughtering, and transferring them to net pens on the same site in the same production cycle, is not considered under the term 'reuse' here.

Salmonid = Fish belonging to the family Salmonidae*.*

Secondary males. Dominating male fish which have changed sex from female to male.

"Sneaker": male which look like female, not being recognised by the dominating male, thus entering the spawning site and fertilizing eggs.

Smolt = Young salmon, in which the physiological changes for adaptation to living in seawater have taken place.

Wrasse = Fish belonging to the family Labriadae. In this risk assessment, wrasse refers to the species used as cleaner fish in Norway: Ballan wrasse (*Labrus bergylta*), corkwing wrasse

(*Symphodus melops*), rock cook (*Centrolabrus exoletus*), goldsinny wrasse (*Ctenolabrus rupestris*) and cuckoo wrasse (*Labrus mixtus*).

Background as provided by the Norwegian Food Safety Authority

The aquaculture industry spends considerable resources on delousing, and use of cleaner fish to feed on sea lice parasitizing salmonids in pens is an extensively applied strategy. However, the use of cleaner fish may also introduce the risk of spread of diseases to Atlantic salmon and rainbow trout.

<u>Fallowing</u>

Paragraph 40, third line in the national regulations for operation of aquaculture, states that "fish farms in sea containing food fish and broodstock fish shall be emptied and fallowed for 2 months minimum". This means that a fish farm must be emptied after each production cycle. The Norwegian Food Safety Authority (NFSA) is considering the possibility of allowing cleaner fish to be kept at the site during the fallowing period. This change would allow cleaner fish from the previous production cycle to remain on the site during the fallowing period, and, furthermore, would allow cleaner fish to be put into pens during this period, provided that this is in accordance with appropriate biosecurity measures.

The purpose of changing the current regulations is to provide the possibility for the reuse of cleaner fish from the same farm in several consecutive production cycles, and thus removing the requirement for mandatory euthanasia or translocation from the farm during the fallowing period. As mandatory fallowing requires the locality to be emptied, current legislation does not allow the cleaner fish to be kept at the farm. As cleaner fish are living animals and a limited resource, it is important that efforts should be made to ensure that cleaner fish are able to live as long as possible, and can be used in multiple production cycles, provided that fish health and welfare is not compromised.

Relevant risk factors for transmission of infection between production cycles include: mortality, time elapsed since previous detection of disease in the farm or nearby area, and the probability that the cleaner fish may act as reservoirs of pathogens. These factors need to be assessed. Such an assessment would be expected to be conducted and documented according to the Norwegian regulation of internal control, in order to fulfil aquaculture legislation. In case of detection of serious disease, NFSA would, nonetheless, have the authority to demand removal and destruction of cleaner fish in an appropriate manner. Should there be suspicion or detection of a listed disease, there would be mandatory removal and destruction of the cleaner fish.

NFSA requests VKM to assess the disease risks to salmonids associated with retaining cleaner fish on a farm during the fallowing period, in the situation that the farm and nearby area have experienced only low mortalities and no contagious diseases during the preceding production cycle. Furthermore, an assessment of the disease risks to salmonids associated with retaining cleaner fish on site during the fallowing period, despite a non-listed disease being present, is also requested.

Mandatory fallowing includes the nets and related equipment at the farm being taken out for washing and disinfection. It is assumed that cleaner fish retained on site during the fallowing period are transferred to an appropriately cleaned and suitable production unit. Stocking of cleaner fish prior to the introduction of food- and broodstock fish would improve the welfare of cleaner fish, and hence improve their survivability. We would like to emphasize that cleaner fish are "aquaculture animals" and therefore require daily care and feeding, according to the national regulations for operation of aquaculture.

NFSA requests an assessment of the risk of infection to the next production cycle of salmonid fish when cleaner fish are allowed to remain on site during the fallowing period.

Relocation of cleaner fish

Aquaculture animals can be put into an aquaculture facility provided that the fish are healthy and do not originate from a facility with increased mortality of unknown cause, as stated in paragraph 11 of the Regulation of trade and disease control of aquatic animals. Relocation of cleaner fish that have been used may thus be permitted, regardless of whether the fish comes from an area endemic for pancreatic disease (PD). This is currently allowed, despite there being an unknown risk of spread of this disease. NFSA has already concluded that relocation of cleaner fish from an area where the presence of infectious salmon anaemia (ISA) is suspected or confirmed is not allowed. Furthermore, NFSA advises that cleaner fish from areas with PD are not relocated. However, based on current regulations, NFSA cannot prevent such relocations.

Under the present regulations, there are no restrictions regarding the capture of cleaner fish from PD-endemic areas.

NFSA requests an assessment of the risk of spreading PD by translocation of cleaner fish from areas endemic for PD to areas free from PD. Both wild-caught cleaner fish and cleaner fish that have already been used in a PD-zone should be evaluated. The assessment will be considered if changes in the regulations are deemed necessary to prevent the spread of PD due to translocations of cleaner fish.

Terms of references as provided by the Norwegian Food Safety Authority

NFSA requests the Norwegian Scientific Committee for Food Safety (VKM) to assess the risks of transmission of infection and disease to salmonids in the following production cycle if cleaner fish are allowed to remain on the site of a salmonid aquaculture facility during fallowing after the previous production cycle. Furthermore, we request VKM to assess the risks of transmission of PD when cleaner fish are relocated from areas endemic for PD to areas considered free for PD.

Cleaner fish refers to the following species: Ballan wrasse (*Labrus bergylta*), corkwing wrasse (*Symphodus melops*), rock cook (*Centrolabrus exoletus*), goldsinny wrasse (*Ctenolabrus rupestris*), cuckoo wrasse (*Labrus mixtus*), and Lumpfish (*Cyclopterus lumpus*).

Salmonid fish refers to Atlantic salmon (*Salmo salar)* and rainbow trout (*Oncorhynchus mykiss*).

Specifically, we request that the following questions are addressed:

1. Which diseases can be transmitted between cleaner fish and salmonids? Specify those diseases to which cleaner fish are susceptible and those diseases for which cleaner fish might only act as vectors.

2. What is the risk of transmission to salmonids associated with transferring cleaner fish after use in a production cycle to another site for storage?

3. What is the risk of transmitting infection and disease to the next production cycle of salmonid fish, when cleaner fish are kept at the farm during fallowing?

4. Which measures can be implemented in order to reduce the risk of transmission of infection to salmonids when cleaner fish are retained at the farm during fallowing?

    a. For which infectious agents should cleaner fish be screened while being held at a farm site during the fallowing period?

5. What is the risk of spread of PD when cleaner fish are relocated from areas endemic for this disease to areas without it?

Assessment

1 Introduction

1.1 Salmon lice in Norwegian salmonid aquaculture

Salmon louse (*Lepeophtheirus salmonis*) causes significant economic losses in the aquaculture of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Various medicinal compounds have been used for the treatment of salmon louse infestation (Roth et al., 1993; Burka et al., 1997; Burridge et al., 2010), and this has resulted in the development of drug resistance (Treasurer et al., 2000; Tully and McFaden 2000; Fallang et al., 2004; Sevatdal et al., 2005; Jones et al., 2008; Lees et al., 2008).

1.2 Cleaner fish used for biological delousing in aquaculture

The aquaculture industry currently needs alternative methods for delousing. Several non-medicinal treatments have been developed or are under development. Cleaner fish (see Box 1) living together with the salmonids in the net pens has been most successful (Costello 1993; 1996; Sayer et al., 1996; Tully et al., 1996; Kvenseth and Kvenseth, 1997; Treasurer, 2002). The use of cleaner fish in Norwegian aquaculture started in the 1980s, when researchers at the Institute of Marine Research in Norway tested the use of wrasses to reduce infestations with salmon lice on farmed Atlantic salmon (Bjordal, 1988; 1990). Results from the first laboratory-scale trials were promising. The trials were scaled up, and the use of wrasses were tested in full-scale production, and gradually adopted by commercial-scale operations (see e.g. Bjordal, 1992; Treasurer, 1994; Deady et al., 1995; Kvenseth, 1996).

In the years immediately following these trials, salmon lice were kept under control by the use of newly introduced anti-parasitic drugs, and interest in the use of wrasses was only moderate. However, during the last decade, the demand for cleaner fish has increased due to the increasing lack of efficacy of drug treatment. This is reflected in the recent steep increase in the number of cleaner fish used in Norwegian aquaculture (Figure 1.2-1).

In 2016, more than 22 million wrasses were captured in Norway (www.fiskeridir.no). In addition, at least one million wild-caught fish were imported from the Swedish west coast, and approximately 1.1 million farmed Ballan wrasse were obtained from two wrasse farming companies (see section 1.5). Around 60 % of Norwegian salmon farms reported active use of cleaner fish during 2016, and this proportion increases to 75 % when only the coastline south of Nordland county is considered (Lusedata.no).

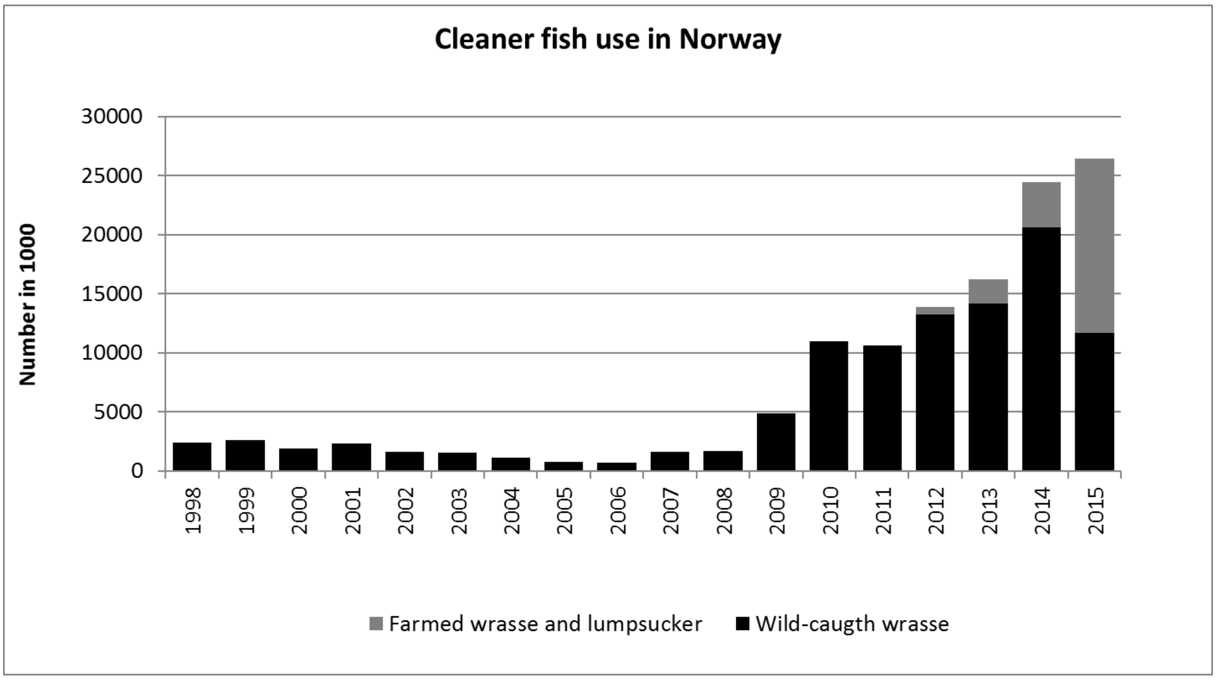

Figure 1.2-1. Number of cleaner fish in Norwegian salmon farms in the period 1998-2015 (source: Norwegian Directorate of Fisheries). Numbers stated in thousand individuals.

Wrasses thrive in temperate waters. The distribution of the different species is temperature dependent (see sections 1.3 and 1.4) and wrasses are active during the summer season. This is also reflected in their effectiveness as cleaner fish. When water temperatures are below 5-7 °C, several European wrasse species enter a state of reduced physiological activity (torpor), presumably rendering them ineffective for delousing purposes. Ballan wrasse and goldsinny wrasse seem to remain more active at lower temperatures than the other species. The use of wrasses as cleaner fish thus decreases towards the north. Wrasses are added to the net pens at a 2-10 % ratio, i.e., 2-10 wrasses per 100 salmon.

During recent years, lumpfish have been introduced as cleaner fish. In the first trials, using wild-caught fish, up to 100 lice could be found in the stomach of a single lumpfish (Willumsen, 2001). After the first documentation of the capacity of lumpfish to act as cleaner fish, several farms started an intensive production (see section 1.5.2). It has been observed that not all lumpfish eat salmon lice, and they are therefore commonly added to the net pens at a higher ratio than wrasses, usually at approximately 15 %. As lumpfish are also active in cold waters, they are suitable for use in northern Norway, where the water temperature is too low for wrasses. Their cleaning efficiency may be reduced during periods with higher temperatures, and when jellyfish and other preferred plankton, representing alternative food sources, are abundant. Only juvenile lumpfish act as cleaner fish, and therefore all lumpfish intended for such use are of farmed origin. They are kept in the net pens until they reach a weight of around 350 grams, and are then removed and killed.

Box 1.

In nature, some fish species have specialized feeding habits, removing and eating ectoparasites that are colonizing the skin, mouth, and gill cavities of larger fish. These so-called "cleaner fish" have been well described from tropical coral reefs, where there may be "cleaning stations" where fish position themselves and signal that they are ready to clean “client” fish. Several tropical wrasse species (in the family Labridae) act as cleaner fish (Baliga & Law 2016).

This fish behaviour has not been well described in temperate waters (Potts, 1973; Hilldén, 1983), but there is anecdotal information indicating that migrating wild Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) stop in shallow water areas that may represent cleaning stations.

Both wrasse and lumpfish (*Cyclopterus lumpus*) are used for sea-lice control in salmon farming.

There are seven wrasse species in North European waters: Ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*), corkwing wrasse (*Symphodus melops*), rock cook (*Centrolabrus exoletus*), cuckoo wrasse (*Labrus mixtus*), scale-rayed wrasse (*Acantholabrus palloni*), and Mediterranean rainbow wrasse (*Coris julis*).

The Mediterranean rainbow wrasse is not indigenous in Scandinavian waters, but considered a “doorstep species”. Scale-rayed wrasse live in deeper waters than the other species, and are not used as cleaner fish.

The different wrasse species have different habitat requirements, and the distribution of species and population densities vary according to latitude, temperature conditions, wind/energy, and bottom vegetation (Skiftesvik et al., 2014a).

1.3 Species used as cleaner fish

Five wrasse species are currently used for sea-lice control in Norway. The dominant species are Ballan wrasse, goldsinny wrasse, and corkwing wrasse.

Ballan wrasse (Figure 1.3-1) is the largest species of wrasse used in Norwegian aquaculture, growing to over 60 cm in length. It is found north to Trøndelag. The Ballan wrasse is abundant in the seabed vegetation, particularly in the lower seaweed zone and kelp forests. It is well adapted to exposed, high energy habitats. It is a long-lived species, that may survive up to 25 years. Ballan wrasse are hermaphroditic, and all individuals are born female. Knowledge is scarce regarding what triggers the development of functional testes in some individuals, but the change is probably related to size/age, and perhaps also the female-male ratio in the population (Dipper et al., 1977; Dipper and Pullin, 1979; Dipper, 1987; Hilldèn, 1984; Leclercq et al., 2014). The Ballan wrasse is relatively robust and may be used as cleaner fish together with large salmon. The fish farmers want small Ballan wrasse that may be held (and grow) together with the salmonids throughout the production cycle. However, juvenile Ballan wrasse constitute a very small proportion of catches (surprisingly), and their market value is therefore higher than for the other species. There was a targeted fishery for juvenile Ballan wrasse on the Norwegian south coast, but the sustainability of this practice was questioned, and it was terminated in 2015. In order to supply the fish farms with young Ballan wrasse, farming has been established (see section 1.5.1).

Figure 1.3-1 Ballan wrasse, *Labrus bergylta* (S. Mortensen, Institute of Marine Research).

Goldsinny wrasse (Figure 1.3-2) is the smallest species of wrasse used in Norwegian aquaculture, with a maximum length of approximately 20 cm and usually up to 14 cm. It may live to 20 years of age. The goldsinny has the widest distribution and is found along most European coastlines, north to Troms county, although its occurrence is sparse in the northernmost part of the distribution range. It inhabits low-energy, shallow water habitats with vegetation and shelter, and is the most commonly captured wrasse in many areas. Around half of the total catch of wrasses are goldsinny. It is the main species imported from Sweden.

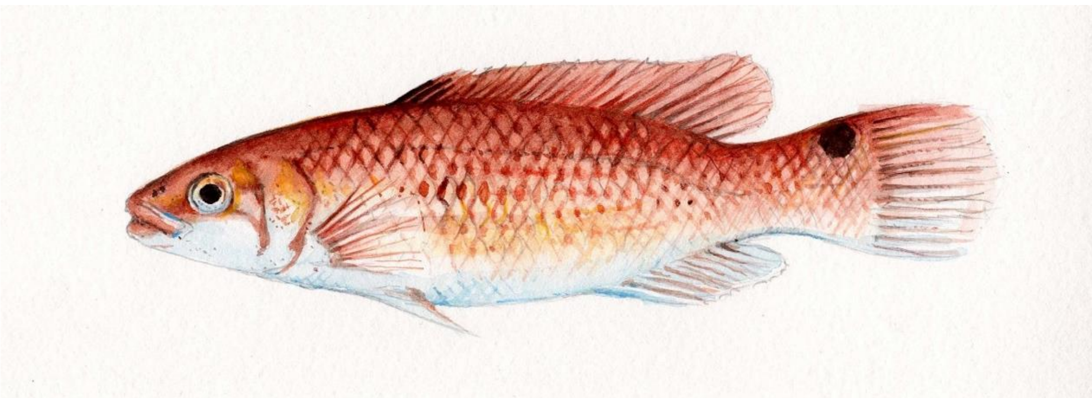

Figure 1.3-2 Goldsinny wrasse, *Ctenolabrus rupestris* (S. Mortensen, Institute of Marine Research).

Corkwing wrasse (Figure 1.3-3) is found north to Mid-Norway (Trøndelag). It is particularly numerous along the west coast. The corkwing prefers shallow water habitats, with dense vegetation where the males establish territories and build nests during the mating season. Some males are morphologically identical to females, and act as "sneakers" during the mating season. This species may live for 10 years, but does not usually survive for so long. On the south coast, they commonly live to three years of age. The corkwing wrasse is not considered very robust, and high mortalities have been recorded during and after transport and after transfer to the net pens. It appears vulnerable to bacterial infections, and high losses have been linked to catch and use during the spawning season (Nilsen et al., 2014; Skiftesvik et al., 2014a).

Figure 1.3-3 Male (above) and female (below) corkwing wrasse, *Symphodus melops*. (S. Mortensen, Institute of Marine Research).

Rock cook (Figure 1.3-4) is typically found in the same habitats as the corkwing wrasse, but has a more southern distribution range. It is usually less numerous. The rock cook is protected in Sweden, and thus not imported to Norwegian fish farming areas. The rock cook is considered a less efficient cleaner fish than Ballan and goldsinny wrasses, and many farmers do not use it.

Figure 1.3-4 Rock cook (male), *Centrolabrus exoletus* (S. Mortensen, Institute of Marine Research).

Cuckoo wrasse (Figure 1.3-5) may be found in a wider range of habitats than corkwing, rock cook, and goldsinny wrasses, in some areas a little deeper, but also often together with these species. It is usually not numerous, and found north to Trøndelag. Cuckoo wrasses are hermaphrodites, and may change sex from female to male at 22 – 25 cm length / 7 years of age. Females and secondary males are bright orange-red. Dominant (primary) males change colour to blue. It is not considered an efficient cleaner fish, and fish farmers report high mortalities after transfer to the net pens. There is only a limited use of this species, mainly on the Norwegian west coast.

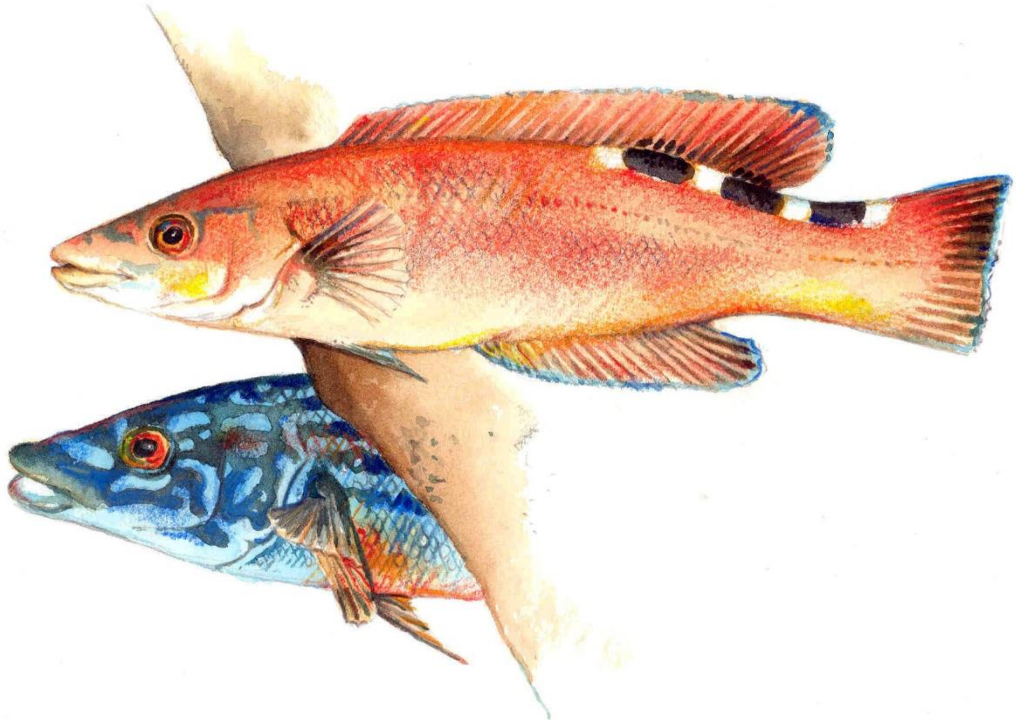

Figure 1.3-5 Cuckoo wrasse, *Labrus mixtus*. Female or secondary male (top) and primary male (bottom, behind kelp) (S. Mortensen, Institute of Marine Research).

Lumpfish (Figure 1.3-6) is found along the North Atlantic coastline. It lives most of the time pelagically, feeding on plankton and pelagic invertebrates. Spawning takes place in shallow waters, often in the lower littoral zone. Eggs are laid in clumps and the male guards the eggs for approximately 60 days. The male stays close to the eggs, strongly attached to the substrate with the modified pelvic fins forming a suction disc. Juveniles stay in the vegetation in shallow waters for two years, and are often observed attached to seaweed and other surfaces, as they have no swim bladder. This ability and willingness for attachment to any firm surfaces must be considered when keeping lumpfish in captivity, e.g., in terms of equipment design for stocking and transport. Lumpfish used as cleaner fish are farmed (see section 1.5.2).

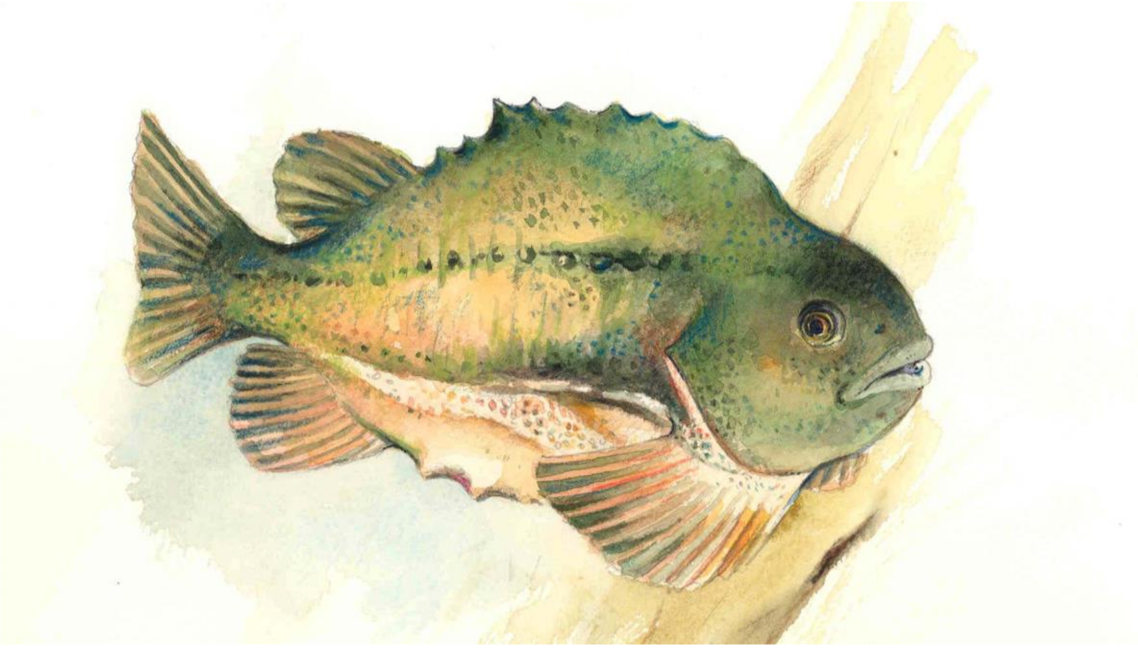

Figure 1.3-6 Lumpfish, *Cyclopterus lumpus* (S. Mortensen, Institute of Marine Research)

1.4 The fishery of wrasses

Wrasses stay in shallow waters during the warm season, usually from May until October. They are particularly active during the spawning season in the summer. Spawning periods vary between species, and depend on water temperature. Norwegian wrasse species that are commonly used as cleaner fish are highly autochthonous, and do not migrate long distances from their natural in-shore residence. It is therefore conceivable that initially conspecific wrasse populations may, as a result of long-term geographical separation, have evolved into distinct subspecies/races that are particularly adapted to their local environment (Sundt and Jørstad, 1993; 1998; Espeland et al., 2010; D'Arcy et al., 2013; Knutsen et al., 2013; Jansson et al., 2017).

Wrasses are caught with fyke nets and pots. When the fishery increased in around 2009 (Figure 1.2-1) the fishermen mainly used eel fyke nets. The eel fishery had ceased, so the fishermen had eel fyke nets available. Suppliers of fishing gear have recently developed both fyke nets and pots specifically designed for capturing wrasse.

The fishery is controversial, mainly due to the increasing fishing effort, bycatch, and a general lack of information on the fishery's effect on the ecosystem. Regulations from the Norwegian Directorate of Fisheries (effective from 2015 and 2016) have therefore been implemented:

- Onset of the fishery is now based on surveys performed by the Institute of Marine Research, in order to ensure that the main spawning periods are concluded before the fishery starts.

- Quotas are enforced, regulating the maximum catch in each fishing region (under evaluation for the 2017 season).
- Allowable catch sizes for each of the wrasse species have been established.
- There is a requirement for selective capture devices, allowing escape of under-sized wrasses and bycatch, and preventing access for sea otters and larger fish.
- Daily handling of the equipment is required.
- Release of by-catch and under-sized wrasses must take place in shallow water/in catch area.

Detailed practical guidelines (in Norwegian) for assumed best practices for capture and temporary storage of wild-caught wrasse are available at the website lusedata.no. A group comprising representatives from various industrial partners in the Norwegian aquaculture industry manages the webpage, and the guidelines have been developed in cooperation with several scientific institutions.

1.4.1 Availability and translocations of wild-caught cleaner fish

The wrasse species indigenous to Norwegian waters have different geographical distributions as well as differing habitat preferences (Skiftesvik et al., 2012a;b; Skiftesvik et al., 2015). In general, catches are mixed, but vary according to fishing area. In some areas on the west coast, corkwing wrasse dominate the catches, whereas goldsinny commonly dominate along the North-west and Trøndelag coasts.

There is a mismatch between the availability of wild-caught wrasse and the demand for cleaner fish in Norwegian aquaculture. In this respect, the Norwegian coast may be divided into three regions:

1. In the south, including the Oslofjord-area and Telemark and Agder counties, there are areas with high densities of wrasses, and intensive fishing. There is, however, almost no salmonid farming and this region has become a net export area for wrasses. There is also a fishery along the Swedish west coast.
2. Along the western Norwegian coast there is an abundance of wrasses and a high number of fish farms. This region is essentially self-supplied with cleaner fish. Many farms use wild wrasses that have neen captured locally.
3. Along mid-Norway and northwards, the wrasse populations become scarcer. This region has intensive production of farmed salmon, and there is a high demand for cleaner fish. It is therefore a net "importer" of wild-caught wrasse from Sweden and the southernmost areas in Norway.

In order to meet the demand, wrasses are fished in the south and transported, primarily by truck, to the fish-farming regions further north. The transport includes approximately one million wrasses annually (not registered at the Norwegian Directorate of Fisheries) from the fishing areas on the Swedish west coast.

1.5 Farming of cleaner fish

The increasing demand for cleaner fish in Norwegian aquaculture in recent years, combined with the ethical, practical, ecological, and biosecurity-related concerns associated with the use of wild-caught wrasse, has given rise to a whole new industry devoted to production of farmed cleaner fish. A shift towards the use of farmed, instead of wild-caught, cleaner fish will facilitate improved infection control (e.g., through vaccination and screening), targeted breeding (e.g., towards domestication and increased lice-eating activity), and less season-dependent delivery. It may also contribute to relieving the exploitation pressure imposed upon wild wrasse populations.

Due to the increase in farms producing lumpfish, the majority of cleaner fish used in Norwegian aquaculture have been of farmed origin since 2015 (Figure 1). Lumpfish represent the dominant species in terms of numbers produced (>90 %). The rest of the farmed cleaner fish are Ballan wrasse.

1.5.1 Ballan wrasse

Only a few Ballan wrasse farmers remain active in Norway today, and in 2015 approximately 1.3 million farmed Ballan wrasses were reported to have been sold in the country (Norwegian Directorate of Fisheries). Through a joint effort by the aquaculture industry and several research partners, a guide for farming of Ballan wrasse has been developed (the LeppeProd-manual), which is publically available online through the Norwegian Seafood Research Fund - FHF fhf.no (project # 900554).

The Ballan wrasse broodstock used today are wild caught, and production of consecutive generations is desirable for obvious reasons. One challenge in this regard, however, lies in the fact that all individuals are born female, and spawn as females at first spawning. Wrasse fish are farmed in marine recirculating aquaculture systems (RAS). The inlet water is deep sea water, filtered and treated with UV. Farmed Ballan wrasse may, depending on required size, be ready for delivery to salmon farms between 9-18 months after hatching (approximately 20-50 g). Vaccination of farmed Ballan wrasse against selected bacterial fish pathogens has been initiated.

1.5.2 Lumpfish

Lumpfish farming activity in Norway has increased radically in recent years, with more than 30 active producers in 2016, and 13.4 million lumpfish reported to have been sold in 2015 (Norwegian Directorate of Fisheries). While official numbers for 2016 are not yet available, the industry reports 17.5 million lumpfish produced, and ambitiously forecasts a production of more than 30 million fish for 2017.

The increased popularity of lumpfish compared with wrasses seems, essentially, to derive from a perception that lumpfish are more robust to transport/handling etc., retain their lice-eating activity at lower water temperatures, and have a considerably shorter production time.

Lumpfish broodstock are wild caught, although efforts are being directed towards achievement of consecutive farmed generations. Vaccination programs for farmed lumpfish have been implemented. However, due to fast growth, they may reach a desirable cleaner fish size (≥7 g) at 5 months age, but current recommendations for achieving adequate protection by vaccination would result in a deliverable fish size of approximately 18-30 g.

1.6 Management of cleaner fish in the net pens

The net pens with salmonid fish represent an environment that is very different from the shallow water zone inhabited by these cleaner fish species naturally. In the wild, wrasses will always stay near the bottom, close to available shelter, and lumpfish juveniles often attach to seaweed and hide in the vegetation. Lack of shelter and areas to rest will presumably result in an increased stress level and reduced welfare for these fish. Most fish farmers try to improve the conditions for the cleaner fish, e.g., by offering shelters made of rows of artificial (plastic) seaweed or different kinds of artificial shelter like stacked tubes or boxes with "windows". Larger lumpfish should have access to firm surfaces for attachment. Areas with artificial shelter in the net pens may function as cleaning stations for the salmon.

It has been shown in experimental trials that wrasses held in clean net pens – without other food than the sea lice – lose weight due to starvation (Skiftesvik et al., 2013). Cleaner fish thus have to be monitored and offered supplemental feed. In general, wrasses are perceived as 'picky eaters' compared with lumpfish, and different feed compositions and administration techniques are required for the different species.

Species-specific practical guidelines, offering advice on best perceived practices for management (covering, e.g., refuges and feeding) and overwintering of cleaner fish in salmon farms, are available at lusedata.no.

1.6.1 Termination or reuse of cleaner fish

Lumpfish are held in the net pens until they reach approximately 350 g and are thereafter removed. Lumpfish are seldom suitable for reuse.

Although it is assumed that very few cleaner fish survive through the course of a full salmon production cycle, cleaner fish stocks are frequently replenished and a certain proportion will remain in the net pens when the salmon are ready for slaughtering. When a net pen is emptied during slaughtering, it is a common practice to collect the cleaner fish and transfer them to another net pen at the same site. In this report, we do not consider this under the definition 'reuse'. According to the hearing experts, collection of surviving cleaner fish and storing them on site or at a separate holding facility during fallowing is not highly demanded. This would be a labor-intensive procedure, involving having to provide care and feeding for a very small fish population. To our knowledge, it is common to transfer the remaining cleaner fish to another site for immediate reuse. At the new site, the cleaner fish will be mixed with salmonids and cleaner fish that are already stocked on the site and also probably supplemented with cleaner fish collected from other sites, farmed cleaner fish, or newly caught wild fish. The practical guidelines available at lusedata.no advise that cleaner fish (wrasses and lumpfish) may be reused at a different site, but also specify that a thorough biosecurity risk assessment must be undertaken in each individual case, prior to relocation. None of the cleaner fish may remain in the net pens during fallowing. Intentional release of live fish (including cleaner fish) from aquaculture facilities is not allowed, and surviving cleaner fish not destined for reuse must be euthanized and destroyed according to applicable legislation.

Reuse of cleaner fish across salmon production cycles represents a compromise between biosecurity and ethical considerations. Work is still ongoing in order to identify practices by which this can be undertaken with minimal risk for transmission of infectious agents.

1.6.2 Losses and welfare issues of cleaner fish in the net pens

High mortalities indicate that the disease situation of the cleaner fish held in salmon net pens is not good. Despite the extensive use of cleaner fish in Norway, the disease situation of cleaner fish has not been thoroughly studied, particularly after release into in the sea pens. There is an almost complete loss of the cleaner fish population in the salmon net pens during a salmon production cycle (reviewed by Mortensen and Karlsbakk, 2012). The high losses result in a need for frequent restocking with new cleaner fish (Skiftesvik et al., 2014b). The high turnover of cleaner fish is incompatible with good animal welfare.

Moderate losses are often not registered or thoroughly investigated, and factors contributing to losses include infectious diseases, wounds, physical damage from handling/transport, sexual maturation, escapes, predation, and (in the case of corkwing wrasse) fish age (e.g., Nilsen et al., 2014).

1.6.2.1 Escapes

Wrasses are small, and even small holes in the nets represent potential escape routes. This applies in particular to goldsinny wrasse (Woll et al., 2013). When fish farmers change from nets with a small mesh size to nets with a larger mesh size, small wrasses often escape. Ballan wrasse and lumpfish grow relatively fast, increasing the likelihood that they may be retained following such procedures.

The guidelines at lusedata.no offer species-specific recommendations with regard to maximum net mesh size in order to reduce cleaner fish escapes. Farmed cleaner fish can more easily be sorted according to size prior to transport, allowing delivery of uniformly sized fish.

1.6.2.2 Predation

Small wrasses held together with large salmon or rainbow trout may be eaten by the salmonids. Predation is most common during the periods when the salmon or trout are starved prior to slaughter. Sufficient access to artificial cleaner fish refuges could possibly reduce this problem.

1.6.2.3 Losses due to handling

Physical damage due to rough transport or handling during stocking may cause acute mortalities or result in ulcerative infections (see 1.6.2.4). Any handling of the salmonids, like chemical delousing or changing of nets, may cause cleaner fish escapes or mortalities. Wrasses have a closed swim bladder and cannot rapidly regulate the gas pressure in the swim bladder. Those wrasses that stay in the dead-fish collector nets at the bottom of the net pen may die due to high pressure in the swim bladder, if the lift is pulled too rapidly to the surface. The same problem may occur if the pen nets are lifted too fast. Sufficient access to artificial refuges will minimize cleaner fish residence in the mortality nets. Cleaner fish may also be injured or killed during the cleaning of nets or other operations.

1.6.2.4 Losses due to infectious disease

Fish that are injured during capture or transport often die within a few weeks after release in the net pens, commonly due to opportunistic infectious disease. Several infectious agents (primarily bacterial) may cause high mortality in cleaner fish, independent of predisposing factors (see chapters 1.7.1 and 2). Stress-induced activation of infection in sub-clinically infected individual fish following capture and salmon farm stocking, is presumably an important contributor to outbreaks of infectious disease in cleaner fish (e.g., Gulla et al., 2016a).

1.7 The disease status of cleaner fish in Norway

Mortalities due to infectious disease represent a large proportion of the cleaner fish losses in the net pens. Different trials of keeping wrasses in holding tanks and pens have shown that a significant number of fish die, and the causes of mortality have been reviewed by Nilsen and colleagues (2014). Furthermore, the Norwegian Veterinary Institute compiles fish disease diagnostic data in the annual Fish Health Report (e.g., Bornø and Gulla, 2016;2017). Data are also contributed by Institute of Marine Research, independent laboratories (Harkestad, 2011; Skiftesvik et al., 2014), and fishermen and fish farmers.

The pattern of mortality varies between cleaner fish species. Amongst wild-caught wrasse, Ballan appear to be the most robust, whereas higher mortality rates occur for rock cook, goldsinny and cuckoo wrasse after release in the net pens. Farmed Ballan wrasse are reported to adapt better to a life in captivity than wild-caught individuals. Lumpfish are perceived as more robust to handling than all the wrasse species, but appear equally, or perhaps even more, susceptible to infectious diseases.

In 2015, the number of sites with atypical furuncolosis diagnosed in cleaner fish, registered at the Norwegian Veterinary Institute, was 83, which dropped to 45 in 2016 (Bornø and Gulla, 2017). This is the most important disease of cleaner fish in Norwegian aquaculture, and affects both wrasses and lumpfish. Most cleaner-fish farmers have introduced vaccination against atypical furunculosis, as well as against a few other bacterial agents/diseases. It remains to be seen whether the apparent drop in atypical furunculosis prevalence is solely a result of vaccination. Nevertheless, vaccination regimes for farmed cleaner fish still require optimization.

1.7.1 Screening and disease diagnostics of cleaner fish

Regulatory guidelines for screening of cleaner fish for particular infectious agents do not exist currently. On-site veterinary health inspections prior to stocking are often conducted in order to identify clinically diseased fish. However, such measures will not enable detection of subclinical carriers. Use of farmed cleaner fish will facilitate improved infection control through vaccination and screening programs. Several cleaner-fish farmers have already implemented such procedures in order to combat some of the most detrimental infectious agents.

Being aquaculture animals, regular health controls of cleaner fish in salmon farms are obligatory, and these are usually carried out simultaneously with salmon health controls. There is a legal requirement for informing the fish health services should an onset of high cleaner fish mortalities be observed. Episodes of increased cleaner fish losses in salmon farms are, however, not always subject to thorough investigation, partly because a large proportion of the dead fish is never recovered (Nilsen et al., 2014).

The need for specific diagnostics for cleaner fish has risen in line with increased usage and production. Since cleaner fish are new species in aquaculture, limited knowledge and fewer diagnostic tools are available. This is especially true for lumpfish, for which much basic knowledge and diagnostic tools are lacking. Experience with normal anatomy and physiology is scarce, and access to cell lines for cultivation of viruses is lacking. With growing knowledge and more frequent use of cleaner fish in aquaculture, new diseases are expected to emerge. Today, the majority of diseases that are investigated are associated with bacterial infections, with *Aeromonas salmonicida* and *Vibrio* spp. dominating. In addition, recently discovered potential pathogens, e.g., *Pasteurella* sp. and lumpfish flavivirus, have been associated with disease outbreaks in lumpfish.

A significant proportion of cleaner fish die in the cages, and how this mortality is recorded and categorized varies. Basic diagnostics are carried out by the fish health services. In situations when there is a need of further disease investigation or there is suspicion of a possible notifiable disease, specialized laboratories are contacted. Until recently, the

Norwegian Veterinary Institute carried out the majority of extended disease diagnosis. Today, several other laboratories also offer this service. Although the obligation to report notifiable infectious diseases also applies for cleaner fish, it may be challenging to obtain an overview of the health status of the cleaner fish population, and acquire information about new, potentially serious diseases.

2 Hazard identification and characterization

2.1 Literature search strategy

Literature searches were made in PubMed, Web of Science, Google, and by searching databases at the Norwegian University of Life Sciences, the Institute of Marine Research, and the Norwegian Veterinary Institute. The date of publications was restricted to from 1995 onwards. The working group also provided information that was, if relevant, included in the assessment, based on their expertise on the topic(s).

Initial searches used the keywords (with different combinations): «cleaner fish», «wrasse», «lumpfish», «salmon», «disease», «virus», «bacteria», «parasites». These often yielded relatively few hits, and searches were therefore repeated using fewer keywords.

2.1.1 Relevance screening

Titles of the articles obtained were screened in relation to terms of reference. Much of the information is found in conference papers, which are not listed on PubMed or Web of Science, and were identified via notifications through various networks or workshops. These were also screened for relevance and used when suitable.

2.1.2 Other sources of information

Hearing experts from the aquaculture industry were invited by VKM in August and September in 2017 to present challenges related to the use of cleaner fish. Experts from Marine Harvest Group and Lerøy Seafood Group presented their views and experiences, and answered questions from the working group.

2.2 Pathogenic agents

Cleaner fish have their own pathogens. Some of these pathogens are host species-specific, but others may be promiscuous regarding host species. Ballan wrasse and lumpfish are farmed at high population densities, potentially promoting emergence of new diseases. Several of the pathogens of farmed salmon, such as ISAV, have emerged under farming conditions from viruses assumed to be non-pathogenic. Therefore, there is a potential for management-related emergence of new diseases in cleaner fish. New pathogens that emerge may be specific for cleaner fish, but potential pathogenicity for salmonids should not be disregarded.

Identification of disease etiology by laboratory diagnostics depends on sampling early in a disease outbreak. Laboratory diagnostics performed on cleaner fish sampled post mortem are easily confounded by saprophytic colonization.

The disease status of wild-caught cleaner fish is, in general, poorly known. The use of cleaner fish caught in areas where PD is endemic in salmon farms could be associated with mechanical transmission of SAV. However, keeping and screening of the fish prior to use would mitigate this risk. The possibility for transmission of salmon pathogens from wrasses caught along the southern coast of Norway or the Swedish coast (i.e., in areas where salmon farming is absent or minimal), is virtually unknown. Translocations of such fish may result in the introduction of new pathogens. The risk of introduction of cleaner-fish specific pathogens through this mode of transmission is considered higher than the risk of introduction of salmonid pathogens.

Smolts that have recently been transported from a smolt-producing facility are generally more susceptible to diseases, due to the effects of stress during transport and sea launching.

All identified hazards are summarized in a table at the end of this chapter (Table 2-1).

2.2.1 Infectious diseases of wrasses

2.2.1.1 Viral infectious agents

Nodavirus – Nervous necrosis virus (NNV)

Nodaviruses are, in general, not host species-specific, but infections are not commonly observed in salmonids. Brain samples from wrasses from the Swedish west coast and the Norwegian coast north to Tysfjord were recently screened for NNV by RT-qPCR (Korsnes et al., 2017). Positive samples were analysed by sequencing and phylogenetic analysis of parts of the RNA2 gene segment. The study showed that NNV is present in wild Ballan, corkwing, and goldsinny wrasse along the coastline of Sweden and Norway. The overall prevalence in the sampled wrasse species ranged between 6.3 and 18 %. The wrasse RNA2 NNV sequences revealed high genetic variation, forming three phylogenetic clusters (Korsnes et al., 2017).

These results are in contrast with the general statement from The Fish Health Report 2015, where it is stated that earlier testing (not specified further) of wild-caught cleaner fish in Norway did not detect nodavirus (Bornø and Gulla, 2016).

***Viral haemorrhagic septicaemia virus* (VHSV)**

VHSV infects a wide range of marine fish species. It has been isolated from more than 80 wild and farmed fish species (OIE, 2017). VHSV is divided into genogroups I-IV (Einer-Jensen et al., 2004). Differences in virulence can be ascribed to a few amino acids and low-virulence strains can mutate into highly virulent strains (Ito et al., 2016; Baillon et al., 2017).

Among salmonids, there are indications that rainbow trout are more susceptible to VHS than Atlantic salmon (OIE, 2017). For rainbow trout, there is genetic variability for susceptibility (Henryon et al., 2002). Consequently, all variants of VHSV are notifiable.

Norway

VHSV is present in marine fish populations in Norwegian coastal waters. In a relatively large survey including many different species of fish, VHSV genotype Ib was detected in Atlantic herring (*Clupea harengus*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), and silvery pout (*Gadiculus argenteus*) (Sandlund et al., 2014). Testing of wild-caught cleaner fish in Norway has not detected VHSV (Bornø and Gulla, 2016).

Scotland

A population of wild-caught wrasses, consisting of Ballan, corkwing, cuckoo, goldsinny, and rock cook, and kept in a land-based holding facility in the Shetland Isles, Scotland, experienced an outbreak of mortality due to infection with VHSV genotype III (Munro et al., 2015). The outbreak was followed up by experimentally determining the susceptibility of goldsinny wrasse to VHSV genotype III by immersion or intraperitoneal (i.p.) challenge. Cumulative moribund fish were 17 % and "more than" 50 % after 14 days post-challenge for immersion and i.p. challenge, respectively. The most pronounced histopathological changes were found in the heart, and thus differ from those described for VHS in salmonids. Virus clearance and heart tissue recovery were noted (Matejusova et al., 2016). The same authors also performed a cohabitation experiment that showed that goldsinny wrasse may shed viable VHSV, and thus can transmit the infection (Matejusova et al., 2016).

***Infectious pancreatic necrosis virus* (IPNV)**

IPNV is, in general, not particularly host species-specific. Bath challenge experiments have shown goldsinny wrasse to be susceptible to IPNV isolated from Atlantic salmon (Gibson et al., 1998). At 2-weeks post challenge (wpc) the infection rate culminated with 30 % of fish infected, and at 4 wpc the virus was no longer detectable. There were no clinical signs, histopathological changes, or mortality (Gibson et al., 1998). Testing of wild-caught cleaner fish in Norway has not detected IPNV (Bornø and Gulla, 2016).

Salmonid alphavirus (SAV)

Salmonid pancreas disease virus, more commonly known as Salmonid alphavirus (SAV), causes pancreas disease (PD). Infection with SAV is the most important viral disease in farmed salmonids in Norway (The Fish Health Report, 2016). Disease due to SAV has only been described in salmonid fish, and thus should be considered to be rather specific in terms of host species. However, there are reports of SAV-positive marine flatfish species being found in the vicinity of SAV-infected salmon farms (McCleary et al., 2014; Snow et al., 2010). The virus is spread horizontally, with shedding through natural excretions/secretions such as faeces and mucus (Graham et al., 2012), and tends to be associated with fat droplets leaking from dead fish (Stene et al., 2016). Although there are variations in the data presented in the literature, it has been found that how long SAV remains infective in seawater is dependent upon temperature and content of organic matter. The half-life of SAV was estimated to be 61 days at 4 °C and no organic matter, and 1-3 days at 20 °C with organic matter present (Graham et al., 2007). The water temperature during the summer will not normally be high enough to cause a significant reduction in the virus load. The transmission potential of water-bodies containing the virus depends on hydrographic conditions and may show great variation, depending on time and geography.

Although neither wrasse nor lumpfish have been shown to be susceptible to SAV, they, along with the water used during transportation, may serve as vectors/vehicle for transmission of the virus. Wild-caught cleaner fish that are exposed to untreated water from SAV-endemic areas during collection, storage, or transportation represent a particularly significant hazard. Increased risk of transmitting SAV has been associated with close proximity to infected salmon populations (Kristoffersen et al., 2009). Epidemiological surveys have shown that uninfected fish inhabiting sites within a 20-km radius of an infected fish farm have a high probability of being infected within one year. The infection pressure in coastal areas varies during the year, but is highest in the summer during which fishing for cleaner fish takes place.

The infectivity of SAV decreases after shedding depending on virus load, time, and temperature. Since cleaner fish are not susceptible species, there will be no replication and the infectivity load will be very low.

SAV has been detected in cleaner fish by RT-qPCR when the wrasses have shared sea-cages with salmon during an outbreak of PD (Bornø and Gulla, 2016; 2017). No mortalities or signs of PD were observed when wrasse were experimentally infected with SAV (Gibson and Sommerville, 1996). Homogenates of tissue from experimentally challenged wrasse were injected into salmon, but there was no evidence for transfer of SAV.

Infectious salmon anaemia virus (ISAV)

Goldsinny wrasse have been experimentally challenged with ISAV; there were no mortalities in wrasse injected with ISAV nor in wrasse cohabiting with ISAV-infected salmon (Kvenseth, 1998; Treasurer, 2012). ISAV has been detected by RT-qPCR when wrasses have shared sea-cages with salmon during an outbreak of ISA (Bornø and Gulla, 2017). No clinical diseases were observed in the cleaner fish in either the PD or ISA outbreaks in salmon, and cross-contamination during sampling could not be excluded.

Piscine orthoreovirus (PRV)

PRV is ubiquitous in the marine phase of Atlantic salmon farming. At least three different genogroups of PRV have been found in salmonids, i.e., in Atlantic salmon, Coho salmon, and rainbow trout. The genogroup PRV-1 causes heart and skeletal muscle inflammation in Atlantic salmon (Wessel et al., 2017), and most detection procedures, i.e., RT-qPCR, detect this virus variant. PRV has been found in a few samples of marine fish by PCR (Wiik-Nielsen et al., 2012), and also in gill and kidney samples from wrasses kept in net pens holding infected salmon, according to a student report (Persson and Røsæg, 2013). However, both findings had high Ct-values, at around the cut-off of the detection method used, and, as with the ISAV and SAV detections, possible cross-contamination during sampling should be considered.

Piscine myocarditis virus (PMCV)

Ballan and corkwing wrasse have been found to be susceptible to PMCV. Scholz and colleagues (2017) reported that when Ballan and corkwing wrasse cohabited with a farmed salmon population experiencing cardiomyopathy syndrome (CMS), PMCV were detected in the wrasses at a low viral load. Non-specific heart lesions were present in PCR-positive, but not PCR-negative, wrasse. However, elevated mortality in wrasse was not observed and, based on the findings described, no wrasse mortality was attributed to CMS (Scholz et al., 2017).

CMS is not a notifiable disease in Norway. It is a common disease, mostly appearing in the late production stages, and about 100 outbreaks are registered annually. The disease occurs all along the coast.

Lymphocystis disease virus

Lymphocystis virus has been detected in wrasses living in warm waters (Bluestreak cleaner wrasse, *Labroides dimidatus*). The virus has been found in more than 140 different fish species (Essbauer and Ahne, 2001). Lymphocystis virus belongs to the family *Iridoviridae.*

2.2.1.2 Bacterial infectious agents

Vibrio anguillarum

Vibrio anguillarum, primarily serotypes O1 and O2, causes classical vibriosis in several fish species including Atlantic salmon, but farmed salmon in Norway today are vaccinated against this disease. Both serotypes O1 and O2a (subtype of O2) are occasionally recovered from deceased wrasse used as cleaner fish in Norway (Bornø and Gulla, 2016), and have been verified as being pathogenic towards Ballan wrasse (Biering et al., 2016). *V. anguillarum* occurs ubiquitously in marine environments (Sørensen and Larsen, 1986).

Vibrio spp.

Wrasse are also susceptible to infections with various other members of the *Vibrio* genus, in particular *Vibrio splendidus* and *Vibrio tapetis* (Jensen et al., 2003; Bergh and Samuelsen, 2007; Harkestad, 2011; Colquhoun et al., 2012; Nilsen et al., 2014). Infection trials have, however, provided conflicting results, and recent genetic studies indicate that these bacteria may represent opportunistic pathogens (Gulla et al., 2015; 2017). These relatively small fish seem to undergo particularly rapid decomposition, which may complicate diagnostic work due to colonization by saprophytic bacteria. This includes *V. splendidus*-related strains, which represent a highly diverse group of bacteria that dominate in marine bacterioplanktons (Thompson et al. 2005).

Aeromonas salmonicida

Atypical strains of *Aeromonas salmonicida*, pathogenic to a wide range of freshwater and marine fish species, are often recovered from diseased wrasse (all species) used as cleaner fish in Norway. Disease manifestations in wrasses often include ulcerous and/or granulomatous conditions. Two particular genotypes (A-layer types) dominate among atypical *A. salmonicida* isolates recovered from wrasses in Norway (Gulla et al., 2015). One study reported approximately 4 % prevalence, by qPCR, of this bacterium in apparently healthy wild-caught Norwegian wrasses, prior to stocking in salmon farms, whereas 68 % of the dead wrasses tested from salmon farms were infected (Gulla et al., 2016a). Stress may induce disease and mortality in wrasse (Samuelsen et al., 2002; 2003). Stress-mediated activation of subclinical *A. salmonicida* infections in wrasses used as cleaner fish, with subsequent shedding and disease outbreaks, presumably occurs. Atypical *A. salmonicida* has sporadically been associated with disease in farmed Atlantic salmon in Norway, but most commonly the strains involved are genetically different from those infecting cleaner fish (Gulla et al., 2015).

Typical *A. salmonicida* (*A. salmonicida* subsp. *salmonicida*), which causes the notifiable disease furunculosis in salmonids, has not been reported in Norwegian wrasse in recent years, although experimental studies have shown that goldsinny wrasses are susceptible to this subspecies (Collins et al., 1991; Treasurer and Laidler, 1994; Hjeltnes et al., 1995; Bricknell et al., 1996; Gravningen et al., 1996). Historically, furunculosis has only been reported in wrasses held in co-culture with infected salmon (Treasurer and Cox, 1991). Farmed salmon in Norway are vaccinated against furunculosis.

Tenacibaculum spp.

Tenacibaculum spp. infections are associated with non-systemic ulcerative conditions in many fish species, including salmon. Members of the *Tenacibaculum* genus are often recovered from eroded fins and ulcers in wrasses (Bornø and Gulla, 2016). A recent study found only a very limited degree of association between host fish species and *Tenacibaculum* genotype when examining isolates from various farmed marine fish species in Norway (Olsen et al., 2017). The natural abundance of *Tenacibaculum* spp. in marine environments must, however, be considered, and prior damage to the skin barrier is likely to be strongly predisposing for such conditions.

Moritella viscosa

Moritella viscosa, frequently associated with winter ulcers in salmon, has occasionally been isolated from wrasses used as cleaner fish in Norway. *M. viscosa* causes winter ulcer disease in Atlantic salmon, a disease that is associated with low water temperatures, when the number of cleaner fish in salmon farms (and wrasse activity) is usually low. There are also some indications that the genetic subtypes infecting Atlantic salmon differ from those infecting other marine fish species (including wrasse) (Grove et al., 2010; Johansen et al., 2016), although more isolates should be examined for confirmation.

2.2.1.3 Parasites in wrasse

More than a hundred different species of parasites have been documented from the five wrasse species used as cleaner fish in Norway (E. Karlsbakk pers comm). Most of the parasites seem to be host-specific, but there is currently no complete overview of their host specificity or geographical distribution. Most reports and publications describe parasites infecting Ballan wrasse (Karlsbakk et al., 2001; Askeland, 2002; Askeland et al., 2002; Karlsbakk et al., 2011) and goldsinny wrasse (Karlsbakk et al., 1996; Solberg, 1999). Reviews may be found in Costello (1996) and Treasurer (1997; 2012).

The amoeba *Paramoeba perurans* has been of particular interest due to its capability of infecting several different fish species, and of causing amoebic gill disease (AGD) in salmon as well as wrasse (Karlsbakk et al., 2013).

2.2.2 Infectious diseases of lumpfish

2.2.2.1 Viral infectious agents

***Viral haemorrhagic septicaemia virus* (VHSV)**

VHSVgenotype IV was detected in lumpfish in Iceland in 2015 (Cuenca et al., 2017; OIE, 2015b). The infected fish had been caught for use as broodfish in a lumpfish farm. There was no disease outbreak. Screening has so far not revealed VHSV in lumpfish in Norway.

Lumpfish flavivirus (LFV)

In 2016, a commercial laboratory in Norway reported detection of a previously undescribed virus, allegedly belonging to the Flavoviridae family, in farmed Norwegian lumpfish with liver necrosis (PHARMAQ analytiq). The virus has been tentatively designated Lumpfish flavivirus (LFV). As no peer-reviewed publication or genetic information has been made available to date, it has not been possible to verify this information. Results from RT-qPCR screening reportedly revealed a widespread distribution of the virus amongst farmed lumpfish in Norway. Several cleaner fish producers have acknowledged that they are testing the fish for the presence of this virus (ilaks.no).

Lumpfish ranavirus

Ranavirus is a genus in the family *Iridoviridae*. A ranavirus has been isolated from lumpfish at multiple locations in the north Atlantic area. Initially isolated in the Faroe Islands in 2014, the virus was subsequently found in lumpfish from Iceland in 2015, and in Scotland and Ireland in 2016 (Stagg et al., 2017). The virus causes a cytopathic effect in many cell lines. Partial sequences of eight isolates showed high similarity, and comparison with other ranaviruses showed high homology with ranaviruses from cod (*Gadus morhua*) and turbot (*Psetta maxima*) isolated in Denmark in 1979 and 1999. Phylogenetic analysis suggests that this ranavirus is related to *Epizootic haematopoietic necrosis virus* (EHNV) (Stagg et al., 2017). EHNV is an iridovirus that is widespread in Australia, and is known to affect farmed rainbow trout, causing epizootic haematopoietic necrosis that is notifiable to OIE.

Nervous necrosis virus (NNV)

Nodaviruses are, in general, not host species-specific. They are commonly found in marine fish species, but infections are not commonly observed in salmonids. NNV has, however, not been reported in lumpfish juveniles.

2.2.2.2 Bacterial infectious agents

Vibrio anguillarum

Classical vibriosis caused by *Vibrio anguillarum* (usually serotype O1) is regularly reported in lumpfish used as cleaner fish in Norway (Bornø and Gulla, 2016). The pathogenic potential of *V. anguillarum* towards lumpfish has been verified (Rønneseth et al., 2014).

Vibrio ordalii

Vibrio ordalii, a very close relative of *V. anguillarum*, is sporadically associated with disease in lumpfish used as cleaner fish in Norway (Bornø and Gulla, 2016). *V. ordalii* has caused disease outbreaks in farmed salmon, e.g., in Chile (Colquhoun et al., 2004), but phylogenetic investigations have revealed genetic differences between Pacific- and North Atlantic strains (Steinum et al., 2016).

***Vibrio* spp.**

V. splendidus-related strains are also commonly recovered from deceased lumpfish but, as for the wrasses, their pathogenic potential remains uncertain.

Aeromonas salmonicida

The number of sites with atypical *A. salmonicida* infection diagnosed at the Norwegian Veterinary Institute in farmed lumpfish showed a tenfold increase from 2014 to 2015, from 5 to 51 sites (Bornø and Gulla, 2017). As for the wrasses, however, the number of such cases decreased significantly in 2016, although still remaining one of the most important lumpfish diseases (Bornø and Gulla, 2017). One *A. salmonicida* genotype, i.e. A-layer type, which also affects wrasse, strongly dominates the lumpfish cases in Norway (Bornø and Gulla, 2016).

In 2015 and 2016, *A. salmonicida* subsp. *salmonicida* was repeatedly detected in diseased farmed lumpfish used as cleaner fish in one fjord in Trøndelag (Bornø and Gulla, 2017). No detection from farmed (vaccinated) salmon in the area has been reported, but there are strong indications that the strain involved is endemic to the wild local salmon population (Bornø and Gulla, 2016).

Pasteurella sp.

Pasteurellosis caused by a *Pasteurella* sp. has caused high lumpfish mortalities since its first reported detection in 2012 (Alarcon et al., 2015a). Notably, the aetiological agent is not to be confused with *Photobacterium damselae* subsp*. piscicida* which, despite not belonging to the *Pasteurella* genus, also causes a bacterial disease termed 'pasteurellosis' in farmed marine fish in other parts of the world. The *Pasteurella* sp. involved in lumpfish disease in Norway is genetically closely related to, yet distinct from, *P. skyensis*, which has caused disease outbreaks in farmed salmon in Scotland (Birkbeck et al., 2002). Furthermore, it is even more closely related to the unnamed bacterial species that has, on rare occasions, caused the disease 'Varracalbmi' in farmed Norwegian salmon (Valheim et al., 2000). Isolates from Norwegian lumpfish and salmon appear to belong to distinct serotypes (Gulla et al., unpublished data). The number of sites with pasteurellosis in lumpfish diagnosed at the Norwegian Veterinary Institute has remained steadily high since 2013 (between 8 and 16 sites), but showed a twofold increase from 2015 to 2016, from 14 to 28 sites (Bornø and Gulla, 2107).

Piscirickettsia salmonis

In 2017, *Piscirickettsia salmonis* was reported for the first time in diseased farmed lumpfish in Ireland (Marcos-Lopez et al., 2017), but has never been reported from Norwegian lumpfish. *P. salmonis* has represented a significant problem to salmon farming in Chile, where it causes the severe disease Salmon Rickettsial Syndrome (SRS). *P. salmonis* has sporadically been recovered from farmed salmon in Europe, including Norway, but European strains appear less virulent than those in Chile (Olsen et al., 1997; Reid et al., 2004; Rozas-Serri et al., 2017). Genetic investigations indicate that the strain isolated from Irish lumpfish is closely related to isolates previously found from Atlantic salmon in Ireland (Marcos-Lopez et al., 2017).

Pseudomonas anguilliseptica

Pseudomonas anguilliseptica infections have occurred in diseased juvenile lumpfish with fin rot, skin ulcerations, haemorrhages, and ascites in the abdominal cavity (Hellberg et al., 2012; Poppe et al., 2012). The number of sites where this infection has been diagnosed in lumpfish at the Norwegian Veterinary Institute doubled in 2016, from 4 to 8 (Bornø and Gulla, 2017).

***Tenacibaculum* spp.**

As for the wrasses, various *Tenacibaculum* spp. are regularly recovered from skin ulcers from both wild and farmed lumpfish (Bornø and Gulla, 2017; Nilsen et al., 2014). *T. maritimum*, the *Tenacibaculum* species most commonly associated with disease in marine fish globally, was recently detected in cultured juvenile lumpfish with skin lesions in Norway (Småge et al., 2016). Although there are reports that associate this infection with disease in Atlantic salmon (Ostland et al., 1999), *T. maritimum* is primarily considered to represent a threat for juvenile fish (Toranzo et al., 2005). This makes transmission from cleaner fish to farmed salmonids less relevant.

Moritella viscosa

M. viscosa is sporadically recovered from lumpfish in Norway, but the few isolates characterized belong to the same genetic group as for the wrasses, which are distinct from isolates causing winter ulcer in Atlantic salmon (Johansen et al., 2016). It should nevertheless be mentioned that, compared with the wrasses, lumpfish can tolerate lower water temperatures, which is when winter ulcer disease primarily occurs. It may be relevant to be aware of this if lumpfish are used as cleaner fish during the colder season.

2.2.2.3 Parasites in lumpfish

Paramoeba perurans

As for wrasse, AGD, caused by *Paramoeba perurans*, represents a problem in the use of lumpfish as cleaner fish in Norway. This amoeba has been detected both in lumpfish farms and after stocking in salmon farms. There was an increase (from 2 to 8) in the number of sites with AGD detected in lumpfish at the Norwegian Veterinary Institute from 2015 to 2016 (Bornø and Gulla, 2017).

***Gyrodactylus* sp. and *Trichodina* spp.**

The monogenean ectoparasite *Gyrodactylus* sp. has been detected on farmed lumpfish with skin lesions. Wild lumpfish are often infected with *Gyrodactylus cyclopteri*, which primarily infects the gills. In addition, the peritrichous protozoan ciliates *Trichodina cyclopteri* and *T. galyae*-infections are common on the gills of wild-caught lumpfish, and may occur at high densities. They will probably not infect salmon, which host other species of *Trichodina* (Karlsbakk et al., 2014). Ciliates are often observed in skin lesions, together with *Tenacibaculum* spp. and other bacteria (Hellberg et al., 2012), but it is unlikely that they represent the primary cause of the lesions.

Nucleospora cyclopteri

Nucleosporosis, caused by the microsporidian *Nucleospora cyclopteri,* is a hazard to lumpfish farming (Karlsbakk et al., 2014; Alarcon et al., 2016b). This parasite is probably species-specific and only occurs in lumpfish. *N. cyclopteri* is found along the entire Norwegian coast. It develops in the nuclei of some of the white blood cells. Infected cells proliferate, and infected fish often exhibit swollen kidneys. Infections in farmed lumpfish are associated with mortality. It is likely that the infections influence the immune status of the fish and may thus have an effect on immunization (vaccination) and survival after bacterial infections.

Caligus elongatus

Wild lumpfish are important hosts for the sea louse *Caligus elongatus*, and lumpfish held in net pens are frequently infected. *C. elongatus* often moves between fish and may infect many different fish species. At high densities it harms the host, causing skin damage and lesions (Pike and Wadsworth, 1999). If fish are treated with orally administrated pharmaceuticals, the lice may leave the fish and attach to non-treated fish. There are two genetically different variants of *C. elongatus*. These may represent distinct species, one of them particularly affecting lumpfish (Øines et al., 2006; 2007). More research is needed in order to reveal these features. Some problems with *C. elongatus* in lumpfish have been observed in Norway in 2016 (Bornø and Gulla, 2017).

Table 2-1. List of infections in cleaner fish relevant to salmon

Agent	Wrasses*	Lumpfish*	Geographic distribution**
Aeromonas salmonicida* subsp. *salmonicida	+	+	Endemic in some areas
Atypical *Aeromonas salmonicida*	+	+	Ubiquitous
***Vibrio anguillarum* (primarily serotypes O1 and O2a)**	+	+	Ubiquitous
Vibrio ordalii	+	+	Occurs; unknown distribution
***Pasteurella* sp. (close aff. w/ *P. skyensis*)**	-	+	Common
Pseudomonas anguilliseptica	-	+	Occurs; unknown distribution
Piscirickettsia salmonis	-	+	Occurs; unknown distribution
Moritella viscosa	+	+	Ubiquitous
***Tenacibaculum* spp.**	+	+	Ubiquitous
***Vibrio* spp.**	+	+	Ubiquitous
Piscine myocarditis virus (PMCV)	+	-	Common
Viral hemorrhagic septicemia virus (VHSV)	+	+	Occasionally detected in wild fish
Infectious salmon anemia virus (ISAV)	-	-	Sporadic
Salmonid alphavirus (SAV)	-	-	Common south of Nordland county. Sporadic further north.
Infectious pancreas necrosis virus (IPNV)	+	-	Ubiquitous
Nodavirus	+	-/?	Common
Piscine orthoreovirus (PRV)* **	+/?	-	Ubiquitous
Lymphocystis virus	-	-	Ubiquitous?
Lumpfish ranavirus	-	+	Not tested in Norway? Assumed ubiquitous?
Lumpfish flavivirus (LFV)	-	+	Unknown
Paramoeba perurans	+	+	Common from Agder to southern part of Nordland county
Nucleospora cyclopteri	-	+	Ubiquitous
Caligus elongatus	+	+	Common

* As of November 2017: Reported to infect these/this species = "+"; lack of reported infection = "–".
** Relative to marine areas of farming of Atlantic salmon in Norway.
*** One student study reported PRV in wrasse, high Ct, not confirmed by others. Set as +/?

3 Risk characterization

3.1 Methodology

The present report is a qualitative risk assessment. The risk of transmitting pathogenic agents from cleaner fish (wrasse or lumpfish) to salmonids is defined as the probability of a hazard (identified in the previous chapter) to occur, multiplied by the consequences of such an event, as judged by the group of experts.

The working group has chosen a three-grade scale to assess probabilities and consequences. The probability of transmission is a function of time, and is also a function of the volume and management of cleaner fish used for salmon louse control in pens. As the salmon louse control strategies applied by the industry change relatively often, the time perspective for estimation of probability for transmission in this report is two-three production cycles at sea sites, i.e., 4 years. The definition of terms used for probability are:

- Low probability: <5 % probability of transmission occurring.
- Moderate probability: 5-30 % probability of transmission occurring.
- High probability: >30 % probability of transmission occurring.

The definitions of the terms used for categorizing consequence are:

- Limited consequence: Lack of effect or mild consequences for fish health, and the disease is easy to control. No changes in the distribution of the infection.
- Moderate consequence: Moderate consequences for fish health, such as limited number of mortalities or morbidity with limited pathological changes, affecting a moderate amount of fish. Limited changes in the epidemiological distribution of the infection.
- Serious consequence: Severe consequences for fish health, such as high mortality or high morbidity with significant pathological changes, affecting many fish. Significant changes in the epidemiological distribution of the infection.

Risk is defined as probability multiplied by consequence. In this risk assessment, each hazard represents either a low risk (green), a moderate risk (yellow), or a high risk (red).

In general, the disease status of wild-caught cleaner fish is poorly known. Quarantine stocking and screening of the fish prior to use could improve this situation. The possibility for transmission of salmonid pathogens via wrasses caught along the southern coast of Norway or the Swedish coast (i.e., in areas where there is little or no salmon farming), is virtually unknown. Translocations of such fish may result in the introduction of novel pathogens. The risk for introduction of cleaner fish-specific pathogens to new areas through this mode of transmission is beyond the remit of this assessment, but is considered to be higher than the risk for introduction of salmonid pathogens.

Stocking of different cleaner fish species together at holding sites may enable transmission of potential pathogens between different fish species. Such practices may increase the probability for emergence of more virulent pathogens of cleaner fish. Virulent pathogens are normally shed in high quantities, which will increase the extent of exposure, and thus the risk for adaptation, to salmonid hosts.

The use of cleaner fish caught in areas where PD is endemic in salmon farms could result in the mechanical transmission of SAV. Wild cleaner fish may be caught near SAV-infected sites or in the vicinity to holding net pens close to slaughter facilities.

Selection favouring more virulent agents that are specific for cleaner fish can, hypothetically, be forecast if continuous use of cleaner fish is practiced, i.e. if they are reused during fallowing of a salmonid production site. Possible transmission and adaption to salmonids for specific pathogens of cleaner fish can only be speculated.

Consequences of transmission	Probability of transmission: Low	Probability of transmission: Moderate	Probability of transmission: High
Serious	**ISAV**	**SAV****	**VHSV**
Moderate	***P. salmonis**** ***A. salmonicida*** **subsp. *salmonicida#***	**PMCV***	***P. perurans***
Limited	**Nodavirus** **PRV*** **Lymphocystis virus** ***M. viscosa**** ***Pasteurella* sp.** ***N. cyclopteri**** ***V. ordalii*** ***P. anguilliseptica*** **Atypical *A. salmonicida**** ***V. anguillarum#***	**IPNV** ***C. elongatus**** ***Tenacibaculum* spp.*** ***Vibrio* spp.**	

Figure 3. Estimated probability of transmission from cleaner fish to farmed, vaccinated salmonids in Norway and the consequences of infection in salmonids after transmission.

A hashtag# denotes agents for which vaccination is protective. Vaccination against classical furunculosis is protective in farmed salmon. However, the distribution of the agent, A. samonicida supsp. salmonicida is unknown.

An asterisk* denotes uncertainties**.** This is mainly due to limited information for the agents listed in cleaner fish in Norway. It is also due to that detection of some agents (PRV, ISAV, SAV) by PCR may represent contamination somewhere in the sampling process. See chapter 4 regarding uncertainties in general.

Two asterisks** reflects that the probability of transmission of SAV is estimated as moderate when using wild-caught cleaner fish from a SAV-endemic area, but low when using farmed cleaner fish. The consequence of detection of SAV in salmonids in a SAV-endemic zone is moderate, but outside the endemic zone is serious. SAV could therefore be placed at several places in the table, but is put at moderate probability of transmission and serious consequence.

4 Uncertainties

- The extent to which the decrease in numbers of atypical furunculosis outbreaks among cleaner fish, reported from 2015 to 2016, can be attributed to vaccination of the cleaner fish is unknown.
- Vaccination against classical furunculosis is protective in farmed salmon. However, the distribution of the agent, A. samonicida supsp. salmonicida is unknown. The regular appearance of this disease in some wild salmon stocks during particular warm summers indicate the presence of a wild fish reservoir for this pathogen.
- Detection of SAV, ISAV and PRV by PCR from wrasse co-stocked with salmon is assumed to represent contamination somewhere in the sampling process. A study in which homogenate of tissue, taken from experimentally challenged wrasse, was injected into salmon, showed no evidence for transfer of SAV to the salmon (Gibson and Sommerville, 1996).
- PCR-screening can normally not be used when it comes to detecting unknown agents or agents not targeted by the primers and protocol used.
- Unknown viral agents in the process of adapting to a salmonid host will probably be very hard to detect at an early stage. Deep-sequencing could facilitate such detection initially and will presumably become more accessible as diagnostic tools develop in the future.
- Too few fish are tested to detect infections with low prevalence.
- The susceptibility of lumpfish to Nodavirus is uncertain.
- The transmission and adaptation of pathogens specific for cleaner fish to salmonids, and potential consequences, can only be speculated.
- Due to low amounts of virus, the methods used for detection of SAV contamination in wild cleaner fish, including water used for transport, may not be sufficiently sensitive.

5 Risk-reduction measures

The following measures may reduce the risk of transmitting pathogens between cleaner fish and salmonids:

Stocking of cleaner fish

- Minimizing the mixing of cleaner fish from different sources and of different species.
- Implementing mandatory surveillance of clinical disease in the cleaner fish.
- Avoiding the use of wild-caught cleaner fish, where the disease status is poorly known.
- Using farmed cleaner fish only (lumpfish and Ballan wrasse). The involvement of fewer species would also presumably reduce the risk for pathogen transmission.
- Filtering and treating of water used to transport cleaner fish from SAV-endemic areas.

Cleaner fish remaining at the farm during fallowing

- As cleaner fish may act as mechanical vectors, prohibiting reuse of cleaner fish that have been in contact with salmon populations that have experienced disease outbreaks.
- Periodic complete fallowing, including remaining cleaner fish, in order to compromise between risks associated with transmission of pathogens and reuse. This approach could be particularly effective for minimizing the risks for host adaption and emergence of novel cleaner fish and salmon pathogens, and should ideally be practiced in a coordinated manner within epidemiologically managed zones (Murray, 2017).
- Allowing reuse of only a limited number of the cleaner fish remaining at start of fallowing.
- Screening for known, specific pathogenic agents and health checks with routine diagnostics to attempt to identify unknown agents that cannot be detected by PCR-screening.
- A quarantine stocking time (based on degree-days) for cleaner fish destined for reuse could facilitate detection of contaminated specimens that have not yet reached the clinical stage of a pathogenic infection, depending on the particular agent(s) of concern.
- Frequent health inspections on sites practicing reuse of cleaner fish.
- Using a quarantine procedure for cleaner fish that are destined for reuse to assist in detection of covert infections.

6 Conclusions (with answers to the terms of reference)

6.1 Which diseases can be transmitted between cleaner fish and salmonids? Specify those diseases to which cleaner fish are susceptible and those diseases for which cleaner fish might only act as vectors.

Diseases that were assessed as having moderate or high probability of transmission from cleaner fish to unvaccinated salmonids in Norway are listed below. In addition to those infectious diseases that depend upon biological vectors for transmission, mechanical vectors can be used to transmit most infectious diseases. In Norway, amoebic gill disease (AGD) is the only known example of a disease where there has been strongly supported, evidence-based suspicion of transmission from cleaner fish to farmed Atlantic salmon. Thus, apart from AGD, such transmission has not been confirmed for any of the diseases listed below. Most reports in the literature focus on detection of specific agents and do not investigate the potential for inter-species transmission.

Bacterial diseases

Furunculosis, caused by *Aeromonas salmonicida* subsp. *salmonicida*: Farmed salmon are protected by vaccination.

Classical vibrosis, caused by *Vibrio anguillarum* (serotypes O1 and O2a): Farmed Atlantic salmon are protected by vaccination.

Tenacibaculosis, caused by *Tenacibaculum* spp.: Atlantic salmon, lumpfish, and wrasse are susceptible to infection with members of the *Tenacibaculum* genus. Various *Tenacibaculum* spp. are ubiquitous in marine environments in Norway, but fish-to-fish transmission cannot be ruled out.

Opportunistic disease, caused by *Vibrio* spp.: *V. splendidus* and other species in the 'Splendidus-clade' occur as dominant members of marine bacterioplanktons. Direct fish-to-fish transmission may not be the main route of infection, but cannot be ruled out.

Piscirickettiosis, caused by *Piscirickettsia salmonis*: Although sporadically detected in Norwegian salmon, European strains appear to be less virulent than strains in Chile.

Viral diseases

Viral haemorrhagic septicaemia, caused by viral haemorrhagic septicaemia virus: Rainbow trout are considered more susceptible than Atlantic salmon, (OIE, 2017). Consequently, the use of cleaner fish with unknown infection status may represent a higher risk in farming of rainbow trout than Atlantic salmon.

Infectious pancreatic necrosis, caused by infectious pancreatic necrosis virus: IPNV is not host species-specific.

Cardiomyopathy syndrome, caused by Piscine myocarditis virus: Ballan and corkwing wrasse are susceptible to PMCV infection. The potential for transmission from cleaner fish to Atlantic salmon is unknown, but cannot be ruled out.

Parasitic diseases

AGD, caused by the amoebae *Paramoeba perurans*: This parasite can infect several fish species including Atlantic salmon, lumpfish and wrasse species.

Agents for which cleaner fish may act as a mechanical vector:

- Salmonid alphavirus
- *Infectious salmon anaemia virus*
- *Piscine orthoreovirus*

6.2 What is the risk of infection transmission to salmonids associated with transferring cleaner fish after use in a production cycle to another site for storage?

As this question is closely related to the next question, we found it more expedient to merge the answers.

6.3 What is the risk of transmitting infection and disease to the next production cycle of salmonid fish, when cleaner fish remain at the farm during fallowing?

Lumpfish are used as cleaner fish until they reach approximately 350 g weight. Thereafter, they are usually euthanized and discarded. Lumpfish are never or very seldom reused. Consequently, for reuse of cleaner fish, only those infectious diseases that could be transmitted by wrasses are considered. The risk level depends on the origin and life history of the wrasses. Reuse of surviving wrasse from areas with disease outbreaks in the salmon farms will be associated with a higher risk of transmission of diseases.

Few cleaner fish survive through a full salmon production cycle, and cleaner fish stocks are frequently replenished during the salmon production cycle. The disease status of wild-caught cleaner fish is, in general, poorly known, resulting in uncertainty in the assessment. Cleaner fish that are transferred to a different location after use in a production cycle are usually used directly and not stored before use at the new location. Thus ToR 6.2 refers to an issue that is not in regular practice in the industry, nor is it widely demanded.

If cleaner fish remain at the site during the fallowing period, they should be kept in cleaned nets or tanks. It is assumed that cleaner fish from the different nets will be grouped together. If cleaner fish remain at a production site, then fallowing of the site will be incomplete. Cleaner fish that remain in the same site during the fallowing period will not introduce agents that are not already present.

When cleaner fish remain at a production site, or are transferred to another location for storage (unusual), then they must be taken care of during the fallowing period. Keeping cleaner fish originating from various productions sites together at a holding facility will increase the risk of exchange of infectious diseases between the cleaner fish, and thus increase the risk for further transmission to salmonids in a subsequent production cycle.

The salmonid diseases for which the pathogen might propagate in the cleaner fish during the fallowing period, provided the presence of infected specimens, are *Viral haemorrhagic septicaemia virus, Infectious pancreatic necrosis virus, Piscine myocarditis virus*, *Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio anguillarum,* and *Paramoeba perurans.*

Both wrasses and lumpfish are susceptible to VHSV infection. A high proportion of wild-caught cleaner fish will increase the probability of introducing VHSV in a holding facility. If VHSV is introduced into a holding facility and the cleaner fish are then used, the risk of transmission to Atlantic salmon or rainbow trout is considered **high**.

Regarding IPNV, currently most farmed Atlantic salmon are selected for IPN-resistance through genetic selection, and soon this is likely to become common for farmed rainbow trout as well. The risk of transmission of IPNV to the next production cycle of salmonids is considered **low.**

One report found that wrasses are susceptible to PMCV infection. Wrasses should therefore be considered as potential reservoirs for PMCV. If wrasses that are to be reused originate from an area where CMS outbreaks have recently occurred, they pose a risk to salmonids for the development of CMS. There is, however, currently no report of transmission from wrasse to salmonids. The risk of transmitting PMCV from wrasses to salmonids is difficult to estimate, and is set to be **moderate** with considerable associated uncertainty.

In general, the development of bacterial diseases in wrasse is considered to be affected by stress associated with sub-optimal living conditions. Environmental and management factors will thus influence the development of diseases of the wrasses in the holding facilities. Salmonids are protected by vaccines against *Aeromonas salmonicida* subsp. *salmonicida* and *Vibrio anguillarum*,. The risk of transmitting bacterial diseases to the next production cycle of salmonids is therefore considered **low**.

Paramoeba perurans, causing AGD, may infect wrasse, and may potentially be transmitted to the next generation of salmonids. The probability of transmitting AGD to the next production cycle of salmonids by wrasse carrying *Paramoeba perurans*, is considered **high**. *Paramoeba perurans* is detected throughout the year in the endemic areas.

The possibility of covert infection of cleaner fish with salmonid pathogens such as SAV or ISAV during the fallowing period is considered unlikely. However, this should be tested and more scientifically reliable data than currently available should be obtained.

6.4. Which measures can be implemented in order to reduce the risk of infection when cleaner fish are retained at the farm during fallowing?

Risk reduction might be associated with the following measures:

- Avoiding reuse of cleaner fish that have been in contact with salmonids experiencing disease outbreak or notifiable disease, regardless of their susceptibility to the agent in question. "Contact" in this regard includes coming from an area where the disease occurs, not necessarily from the same production site.
- Quarantine stocking and fish health inspection of wild-caught fish prior to use, to improve knowledge on their disease. Screening for selected pathogens would add further information.
- Periodic complete fallowing (i.e., including the cleaner fish) may reduce the risk of transmission of pathogens.
- Reusing only a small proportion of the cleaner fish.
- Quarantining cleaner fish to be reused until their disease status has been established.
- More frequent health inspections at production sites where reuse of cleaner fish is practiced.

a. For which infectious agents should cleaner fish be screened while being held at a farm site during the fallowing period?

Screening should be implemented for *Viral haemorrhagic septicaemia virus, Piscine myocarditis virus,* and *Paramoeba perurans.* However, such a list may change and should be re-evaluated periodically. Disease-causing agents important for cleaner fish health, for which there are no available vaccines, could also be considered but was beyond the scope of this mandate.

While several bacterial pathogens occurring in Norwegian waters may infect both cleaner fish species and salmonids, we consider that reuse of cleaner fish would not significantly increase the risk that these agents pose against farmed salmonids in Norway today. This is either due to salmonids being effectively vaccinated (e.g., *V. anguillarum*), or because direct fish-to-fish transmission is considered to play only a minor role in the total epidemiological situation (e.g. *Tenacibaculum* spp.). However, the biology and diseases of cleaner fish are relatively unexplored. Relevant agents with relevance for salmonids in remaining cleaner fish destined for reuse are listed here:

  - *Paramoeba perurans*
  - *Viral haemorrhagic septicaemia virus* - VHSV
  - *Piscine myocarditis virus* - PMCV

- *Infectious pancreatic necrosis virus* - IPNV
- *Salmonid alphavirus - SAV*
- *Infectious salmon anemia virus* – ISAV

Pathogens considered to be of particular relevance for the health of the cleaner fish themselves are listed here:

- *Paramoeba perurans* (in affected areas)
- Atypical *Aeromonas salmonicida*
- *Vibrio anguillarum*
- *Pasteurella* sp. (lumpfish only)
- *Viral haemorrhagic septicemia virus* - VHSV
- Lumpfish flavivirus (uncertain relevance; lumpfish only)
- Lumpfish ranavirus (uncertain relevance)
- *Nucleospora cyclopteri* (uncertain relevance; lumpfish only)

6.5 What is the risk of spread of PD when cleaner fish are relocated from areas endemic for this disease to areas without it?

SAV is not considered to infect cleaner fish. The probability and consequences of transmission of SAV when relocating cleaner fish between areas will depend on several environmental, management, and regulatory factors.

Currently, the farming of cleaner fish depends on wild-caught broodstock fish. If the broodstock fish are caught in SAV-endemic areas, then the possibility for SAV contamination of the wild broodstock is estimated to be **moderate**. With appropriate treatment of inlet water in cleaner fish farms in PD-endemic areas, it is possible to reduce the possibility of contamination with SAV through inlet water to **low.**

The risk of transmitting infection when farmed cleaner fish to be used for the first time (not reuse) are moved from areas endemic for PD to areas without PD, is considered **low**. This is dependent on water treatment and prevention of exposure during transportation. As for wild-caught broodfish, the probability of catching wild cleaner fish exposed to SAV in SAV-endemic areas is **moderate**. For wild-caught cleaner fish being moved from areas endemic for PD to areas free of PD, the probability of transmission of SAV to farmed salmonids is considered to be **moderate**.

The probability of detecting SAV contamination of cleaner fish, whether being relocated or used for the first time, is very low. The amount of virus will be very low, and there are neither clinical symptoms nor pathological changes.

The infective dose of SAV is unknown. The probability that cleaner fish and contaminated water may result in infection of farmed salmonids when introduced to an uninfected population is considered **moderate**.

The consequences of infecting a farmed salmonid population outside the current SAV-endemic area will be **serious**, but depends on the location of the farm, particularly the proximity to and probability of infecting neighbouring farms.

7 Data gaps

- Since cleaner fish are new species in aquaculture, their diseases, including diagnostic and prevention, are not well described. This is especially true for lumpfish, for which most basic knowledge and diagnostic tools are lacking.
- Knowledge of their normal anatomy and physiology is scarce and access to cell lines for cultivation of viruses is lacking.
- Lymphocystis virus has been found in Bluestreak cleaner wrasse, *Labroides dimidatus*, but not in wrasses used as cleaner fish in farming of salmonids.
- Information regarding the pathogenic potential of *Vibrio* spp. in wrasse and lumpfish is lacking.
- Publications or genetic information on lumpfish flavivirus are currently lacking.
- Information on lumpfish ranavirus is scarce.
- Information on *Piscirickettsia salmonis* in cleaner fish is scarce.
- It is unknown whether PMCV can be transmitted from cleaner fish to farmed salmonids.

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