Risk assessment of amoebic gill disease

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

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Assessed and approved

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Acknowledgment

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

The Norwegian Food Safety Authority (NFSA) asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) for a risk assessment of amoebic gill disease (AGD). The NFSA has asked for this risk assessment to assess whether the disease should be listed on List 3 of Annex 1 of the Regulation 17 June 2008 no. 819 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals. To prepare scientific background documents necessary to answer the questions, the VKM, Panel on Animal Health and Welfare, established a project group consisting of both VKM members and external experts.

Amoebic gill disease in farmed Atlantic salmon (*Salmo salar*) was originally described in Tasmania, Australia, in the mid-1980s. Later, the disease has continuously caused severe economic losses to the Tasmanian production of Atlantic salmon. Since the mid-1990s, AGD has occurred sporadically in different farmed fish species in the Mediterranean Sea and in the North-East Atlantic. In Norway, AGD was observed for the first time in association with health problems in farmed Atlantic salmon at four sites in the autumn 2006. After 2010, the occurrence of AGD in farmed Atlantic salmon has increased significantly in the North-East Atlantic, first in Ireland and Scotland in 2011-2012 and later northwards on the Orkney Islands, in Shetland, Norway and on the Faroe Islands in 2012-2013.

The disease affects Atlantic salmon throughout the seawater phase, and in particular post-smolts during the first autumn in sea. In Norway, AGD has additionally been observed in farmed rainbow trout (*Oncorhynchus mykiss*), ballan wrasse (*Labrus bergylta*) broodstock and juveniles and in wild caught corkwing wrasse (*Symphodus melops*) used as cleaner fish in farms producing salmonids.

Amoebic gill disease caused by *Paramoeba perurans* represents a serious health risk to farmed Atlantic salmon and rainbow trout along parts of the Norwegian coast. The amoeba can cause high mortality, poor fish welfare and reduced growth if not treated early in the eruption phase. High temperature and high salinity are major risk factors. *Paramoeba perurans* is present in Norwegian waters from Vest-Agder county to Møre og Romsdal county, where also gill disease has been diagnosed. In these areas, infections have been difficult to control in farms with traditional open operation. Sporadic detections have been made northward to Troms county, and given the suitable environmental conditions, the event of establishment of *P. perurans* further north seems likely. Restriction on movement of salmonids and wrasse from affected areas could delay the process, but probably not prevent it.

Much of the existing knowledge on *P. perurans* comes from Tasmania, and the relevance for Norwegian conditions is uncertain. Knowledge on the infection reservoir and spreading dynamics is lacking. The amoeba is sporadically detected in wild fish, but the knowledge is

too scarce to make any assessment of the significance. The amoeba may be transmitted from fish to fish. Based on a theoretical analysis, traditional hygienic measures, such as coordinated treatment and fallowing, could reduce the severity of transmission and infection in enzootic areas. Furthermore, in an outbreak situation, it is reasonable to assume that the local pressure of infection correlates with the number of affected fish.

Sammendrag på norsk

Mattilsynet har bedt Vitenskapskomiteen for mattrygghet (VKM) om en risikovurdering av amøbegjellesykdom (AGD). Mattilsynet har bedt om denne risikovurderingen for å vurdere om sykdommen bør være oppført på liste 3 i vedlegg 1 til forskrift av 17. juni 2008 nr. 819 om omsetning av akvakulturdyr og produkter av akvakulturdyr, forebygging og bekjempelse av smittsomme sykdommer hos akvatiske dyr. For å utarbeide vitenskapelige bakgrunnsdokumenter som er nødvendige for å svare på spørsmålene, har VKM, Faggruppe for dyrehelse og dyrevelferd, etablert en prosjektgruppe bestående av både medlemmer fra VKM og eksterne eksperter.

Amøbegjellesykdom hos oppdrettslaks (*Salmo salar*) ble opprinnelig beskrevet i Tasmania, Australia, på midten av 1980-tallet. Senere har sykdommen kontinuerlig forårsaket alvorlige økonomiske tap i produksjon av atlantisk laks i Tasmania. Siden midten av 1990-tallet, har AGD forekommet sporadisk i ulike oppdrettsarter i Middelhavet og i det nordøstlige Atlanterhavet. I Norge ble AGD observert for første gang i forbindelse med helseproblemer hos oppdrettslaks i fire lokaliteter høsten 2006. Etter 2010 har forekomsten av AGD i oppdrettslaks økt betydelig i Nordøst-Atlanteren, først i Irland og Skottland i 2011-2012, og senere nordover på Orknøyene, i Shetland, Norge og på Færøyene i 2012-2013.

Sykdommen rammer atlantisk laks i hele sjøvannsfasen, men spesielt post-smolt den første høsten i sjøen. I Norge har AGD i tillegg blitt observert i oppdrettet regnbueørret (*Oncorhynchus mykiss*), stamfisk og yngel av berggylt (*Labrus bergylta*) og villfanget grønngylt (*Symphodus melops*) brukt som rensefisk i merder med laksefisk.

Amøbegjellesykdom forårsaket av *Paramoeba perurans* representerer en alvorlig helserisiko for oppdrettet laks og regnbueørret langs deler av norskekysten. Amøben kan gi høy dødelighet, dårlig fiskevelferd og tapt tilvekst dersom den ikke behandles tidlig i utbruddsfasen. Høy temperatur og høy salinitet er viktige risikofaktorer. *Paramoeba perurans* forekommer i norske farvann fra Vest-Agder til Møre og Romsdal, hvor det også har vært påvist gjellesykdom. I disse områdene har det vært vanskelig å kontrollere smitte i farmer med tradisjonell åpen drift. Det er gjort sporadiske påvisninger nordover til Troms, og gitt de rette miljøforhold forhold kan man anta at *P. perurans* vil kunne etablere seg lenger nord. Restriksjoner på flytting av laksefisk og rensefisk fra affiserte områder kan forsinke prosessen, men sannsynligvis ikke forhindre den.

Mye av kunnskapen vi har om *P. perurans* kommer fra Tasmania, og relevansen for norske forhold er usikker. Kunnskap om amøbens smittereservoar og spredningsdynamikk er mangelfull. Det er gjort sporadiske funn av amøben på norsk villfisk, men kunnskapen er for liten til å kunne gjøre noen vurdering av betydningen. Amøben kan overføres fra fisk til fisk. I endemiske områder vil tradisjonelle hygieniske tiltak som samordnet behandling og brakklegging teoretisk kunne redusere skadeomfanget.

Ut fra en teoretisk analyse, vil tradisjonelle tiltak som samordnet behandling og brakklegging kunne redusere omfanget av spredning og infeksjon i enzootiske områder. Videre vil det i en utbruddssituasjon være grunn til å anta at det lokale smittepresset korrelerer med antall affiserte fisk.

Abbreviations

AGD   – amoebic gill disease

CSIRO   – Commonwealth Scientific and Industrial Research Organisation, Australia

FOMAS   – a Norwegian company providing fish health services

ISH   – *in situ* hybridisation

MH   – Marine Harvest, Norway

NFSA   – Norwegian Food Safety Authority

NVI   – Norwegian Veterinary Institute

VKM   – Norwegian Scientific Committee for Food Safety

UiB   – University of Bergen, Norway

VAI   – Vet-Aqua International, Ireland

Glossary

Anadrome fish are fish species that migrate from the sea into fresh water to spawn.

Broodstock is mature fish kept for breeding purposes.

Cleaner fish are fish species removing dead skin and ectoparasites on other fish.

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

Real time PCR is quantitative PCR.

SSU rDNA is the DNA encoding the 18S ribosomal RNA component (rRNA) of the small subunit (SSU) of eukaryotic ribosomes.

Smolt is juvenile salmon ready to migrate from fresh water to seawater.

Wrasse is any species of the family Labridae used as cleaner fish for removal of sea lice in fish farms.

Background

Amoebic gill disease (AGD) in farmed Atlantic salmon (*Salmo salar*) was originally described in Tasmania, Australia, in the mid-1980s. Later, the disease has continuously caused severe economic losses to the Tasmanian production of Atlantic salmon. Since the mid-1990s, AGD has occurred sporadically in different farmed fish species in the Mediterranean Sea and in the North-East Atlantic. In Norway, AGD was observed for the first time in association with health problems in farmed Atlantic salmon at four sites in the autumn 2006. After 2010, the occurrence of AGD in farmed Atlantic salmon has increased significantly in the North-East Atlantic, initially in Ireland and Scotland in 2011-2012, and later northwards on the Orkney Islands, in Shetland, Norway and on the Faroe Islands in 2012-2013.

The disease affects Atlantic salmon throughout the seawater phase, but particularly post-smolts the first autumn in sea. In Norway, AGD has additionally been observed in farmed rainbow trout (*Oncorhynchus mykiss*), ballan wrasse (*Labrus bergylta*) broodstock and juveniles and in wild caught corkwing wrasse (*Symphodus melops*) used as cleaner fish in a salmon farm.

Outbreaks of AGD are often long-lasting. The most important factor for disease outbreaks appears to be high salinity. In Tasmania, AGD is usually associated with water temperatures above 12 °C, but in North-East Atlantic outbreaks have been observed at temperatures down to 7 °C. There is limited knowledge about the survival and infectiousness of the causal agent, the amoeba *Paramoeba perurans* (syn. *Neoparamoeba perurans*), at lower temperatures. The most effective known treatment against AGD is fresh water, but hydrogen peroxide is also reported to be useful.

The Norwegian Food Safety Authority (NFSA) has asked for this survey in order to allow assessment on whether the disease should be listed or not. The NFSA intends to use the risk assessment to evaluate whether AGD should be listed on List 3 of Annex 1 of the Regulation 17 June 2008 no. 819 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to undertake a risk assessment of the following:

1. Does AGD in Atlantic salmon and rainbow trout represent a substantial risk for the fish health in Norwegian fish farms?
   1.1. What is known about the distribution of *Paramoeba perurans* in farmed and wild fish in Norway?
   1.2. What is known about the distribution of AGD in farmed and wild fish in Norway?
   1.3. What factors influence the development of AGD?
   1.4. What is known about the spreading dynamics?

2. Is it possible to control the infection and the disease at site level?
   2.1. If yes, what methods/measures will be most relevant for the control of the disease?

3. Is it possible to achieve and maintain areas free from the pathogenic agent? Is it possible to achieve and maintain areas free from the disease?
   3.1. If yes, what measures will be most relevant to achieve and maintain areas free from pathogenic agents and/or free from the disease?

4. Can the disease constitute a threat to wild stocks of fish if not treated and/or kept at a controlled low level?

5. Can the disease have substantial consequences for the Norwegian aquaculture industry?

The NFSA requested that the risk assessment should be completed and submitted by the 1st of November 2014.

Assessment

1 Hazard identification and characterisation

1.1 Literature search

The working group used collections of scientific papers and reports accumulated during their many years of engagement in research on fish health and also specifically on amoebic gill disease (AGD). The literature on AGD is extensive, but limited subsequent to the discovery of the etiological agent *Paramoeba perurans* (syn. *Neoparamoeba* perurans) in 2007. Older literature has been used with care. Due to the broad literature database already present, and the limited amount of publications on AGD after 2007, initial formal conduction of structured searches was not carried out.

1.2 The history of amoebic gill disease

Amoebic gill disease in farmed Atlantic salmon (*Salmo salar*) was originally described in Tasmania, Australia, in the mid-1980s (Munday et al., 1990; 2001). Later, the disease has continuously caused severe economic losses to the Tasmanian production of Atlantic salmon (Nowak, 2012). Since the mid-1990s, AGD has occurred sporadically in different farmed fish species in the Mediterranean Sea and in the North-East Atlantic Ocean (Dyková et al., 1998, 1999; Mitchell and Rodger, 2011). Amoebic gill disease has also been observed in farmed salmonids in USA, Washington State (Kent et al., 1988), Chile (Bustos et al., 2011) and South Africa (Mouton et al., 2014). After 2010, the occurrence of AGD in farmed Atlantic salmon has increased significantly in the North-East Atlantic, initially in Ireland and Scotland in 2011-2012, and later northwards on the Orkney Islands, in Shetland, Norway and on the Faroe Islands in 2012-2013.

In Norway, AGD was observed for the first time in association with health problems in farmed Atlantic salmon at four sites in the autumn of 2006 (Steinum et al., 2008). In three of the farms, the mortality was 10-20 %, and AGD was the only diagnosed disease. In the fourth farm, the mortality was approximately 80 %, and AGD was found in association with several other gill diseases. After 2006, AGD was not detected in Norwegian fish farms for some years. In late autumn 2012, AGD was again diagnosed at five fish farm sites at the South-West coast of Norway. As the disease development was slow and the sea temperature was low, the fish were not treated. The disease disappeared in the following months and the amoebae could not be found after February 2013. Still, surveys for *P. perurans* by *real-time* PCR were done in several Norwegian fish farms, and in September 2013, the amoeba was

detected again at several farm sites. Within a couple of months, the amoeba and AGD were observed in a large number of Norwegian fish farms.

1.3 *Paramoeba perurans* - the etiological agent

The amoeba responsible for AGD has only recently, in 2007, been identified and described as a new species. Hence, for more than 20 years, the etiological agent has been referred to by various other names.

Extensive salmonid aquaculture was established in Tasmania in 1984. Cage rearing of Atlantic salmon in high salinity (>32 ppt) seawater and maintenance of the fish during the warmer months immediately led to problems with amoebic gill disease (Munday, 1985; Munday et al., 1990). The causative amoeba was initially not identified (Munday et al., 2001). Subsequent ultrastructural studies revealed that amoebae from AGD affected salmonids in both Tasmania and Washington State, USA, were similar to *Paramoeba pemaquidensis* (Kent et al., 1988; Roubal et al., 1989). Page (1987) erected the genus *Neoparamoeba* for *P. pemaquidensis* and other paramoebids lacking surface microscales, and later *Neoparamoeba* sp. was adopted as designation of the microscale-lacking causative agent of AGD (Dyková et al., 2000). Elliott et al. (2001) identified the *Neoparamoeba* isolates from AGD affected salmonids in Tasmania, Ireland and Washington State as *N. pemaquidensis* based on small subunit (SSU) rDNA sequence identity. However, Dyková et al. (2005) found that another species, described as *N. branchiphila*, occurred together with *N. pemaquidensis* in the gills of Tasmanian Atlantic salmon with AGD.

Amoebic gill disease can be elicited in naïve Atlantic salmon by exposing them to crude gill extracts or isolated paramoebae from diseased fish (Zilberg et al., 2001; Morrison et al., 2004). However, it was not possible to induce AGD by challenging with paramoebae from cultures with several passages (Kent et al., 1988; Howard et al., 1993; Findlay, 2001; Morrison et al., 2005; Vincent et al., 2007). This controversy was solved by Young et al. (2007), who discovered that a third non-cultivable species of *Neoparamoeba* was present on the gills of Atlantic salmon with AGD. *In situ* hybridisation studies showed that this species, named *N. perurans*, was directly associated with the characteristic gill lesions in AGD affected Atlantic salmon, while *N. pemaquidensis* and *N. branchiphila* were not (Young et al., 2007; 2008b). Eventually, *N. perurans* was cultured *in vitro*, and used to experimentally induce AGD in Atlantic salmon (Crosbie et al., 2012). Feehan et al. (2013) suppressed the genus *Neoparamoeba* and transferred its species, including *N. perurans*, to genus *Paramoeba*. Consequently, the valid name for this causative agent of AGD in seawater reared fish is *Paramoeba perurans* (Young, Crosbie, Adams, Nowak and Morrison, 2007).

In fish, *P. perurans* is an ectoparasite occurring mainly on the gills, but can also be observed on other surfaces such as skin and fins. Despite being known primarily as a fish parasite, the amoeba is likely to also inhabit yet unknown environmental reservoirs, either free-living or associated with living organisms.

1.4 The host range of *P. perurans*

As outlined above, a greater part of the literature accumulated on AGD since 1985 has dealt with several amoebae. *Paramoeba* spp. infections have been detected in many species of fish with AGD, but the presence of *P. perurans* has not been confirmed in all.

Paramoeba perurans infections have been verified in eight species of fish, representing the families Salmonidae, Osmeridae, Labridae and Scophthalmidae (Table 1.4-1). Hence, the amoeba appears to be unspecific with regard to its fish hosts. In several regions with AGD problems, such as Tasmania, South Africa and Chile, this disease only affects exotic fish species in seawater aquaculture. In the North-East Atlantic, however, AGD affects native farmed fish species. In Norway, *P. perurans* infections have been detected on the gills of at least two cleaner fish species, ballan wrasse (*Labrus bergylta*) and corkwing wrasse (*Symphodus melops*) (Hjeltnes, 2014) (Figure 1.4-1). Two wrasse species, ballan and cuckoo wrasse (*Labrus mixtus)*, have also been found infected in nature, in Hordaland in Western Norway, verified by SSU rDNA sequencing (A. Nylund, UiB, pers. comm.). In 2013, *P. perurans* infections and AGD were observed in farmed ballan wrasse without any association with other fish species (Karlsbakk et al., 2013; Hjeltnes, 2014). In experimental challenge experiments, lumpsucker (*Cyclopterus lumpus*) is susceptible and may develop the gill lesions typical for AGD, but the species is far less susceptible than Atlantic salmon (L. Andersen, ILAB, pers. comm.). The observations of *P. perurans* infections in both labrids and salmonids, suggest that *P. perurans* shows low host specificity also in Norway.
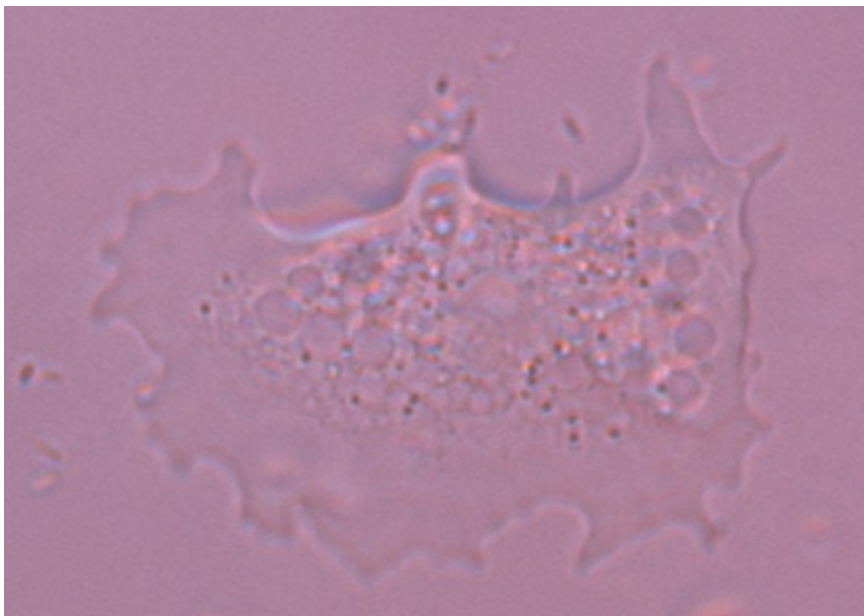
Figure 1.4-1   Attached form of an amoeba, showing hyaline ectoplasm with pseudopodia and granular endoplasm with vacuoles. The amoeba originates from a culture obtained from farmed ballan wrasse and is highly likely *Paramoeba perurans* (photo by E. Karlsbakk).

Atlantic salmon is susceptible to cultured *P. perurans* originating from ballan wrasse, and may develop AGD (Mo et al., 2014). However, in this preliminary challenge experiment, amoebae from ballan wrasse appeared less virulent to Atlantic salmon than those isolated from salmon.

Table 1.4-1 Host species in which *Paramoeba perurans* infections have been verified, either by *in situ* hybridisation (ISH) or by PCR (conventional or *real-time* PCR). All verified *P. perurans* infections represent farmed fish diagnosed with AGD. Only published records are listed. W: Western, NW: North-Western, S: Southern.

Host/region	Agent detection	References
Atlantic salmon (*Salmo salar*)		
Tasmania, Australia	ISH, PCR	Young et al., 2007; 2008b
Washington, USA	ISH, PCR	Young et al., 2008b; Nowak et al., 2010
Chile	ISH, PCR	Bustos et al., 2011; Rozas et al., 2011; 2012
S South Africa	ISH, PCR	Mouton et al., 2014
Galway, Ireland	ISH	Young et al., 2008b
Hebrides, Scotland	ISH	Young et al., 2008b
W Norway	PCR	Steinum et al., 2008; Nylund et al., 2008; 2011
Rainbow trout (*Oncorhynchus mykiss*)		
Tasmania, Australia	ISH	Young et al., 2008b
Los Lagos region, Chile	PCR	Rozas et al., 2012
W Norway	PCR	Hjeltnes, 2014
Chinook salmon (*Oncorhynchus tshawytscha*)		
Queen Charlotte Sound, New Zealand	ISH	Young et al., 2008b
Coho salmon (*Oncorhynchus kisutch*)		
Los Lagos region, Chile	PCR	Rozas et al., 2012
Ayu (*Plecoglossus altivelis*)		
Fukui, Japan	ISH, PCR	Crosbie et al., 2010
Ballan wrasse (*Labrus bergylta*)		
W Norway	PCR	Karlsbakk et al., 2013; Hjeltnes, 2014
Corkwing wrasse (*Symphodus melops*)[1]		
W Norway	PCR	Hjeltnes, 2014
Turbot (*Psetta maxima*)		
NW Spain	ISH	Young et al., 2008b
W South Africa	ISH	Mouton et al., 2014

[1] Wild caught, stocked with salmon as cleaner fish

1.5 Other possible hosts of *P. perurans*

1.5.1 Farmed fish

Paramoeba spp. has been isolated from gills with typical AGD lesions in several fish species other than those listed in Table 1.4-1. Culturing methods applied for amoebae from such lesions may have selected for *P. pemaquidensis* and *P. branchiphila* (Crosbie et al., 2012). Hence, some *in vitro* cultured and clonal amoebal strains obtained and identified by SSU rDNA sequencing belonged to these species (Fiala and Dyková, 2003; Dyková et al., 2005). The discovery of *P. perurans* as an additional non-culturable species from AGD lesions now suggests that this amoeba may have been present also in these cases of AGD. *Paramoeba perurans* may have been competitively outnumbered by other *Paramoeba* spp. in the cultures. The first successful culture of *P. perurans* was recently described by Crosbie et al. (2012), who also demonstrated that the amoebae did not lose their virulence during long term culture of 125 days.

Three species of fish farmed in the Mediterranean Sea are known to develop AGD. *Paramoeba* sp. was identified from juvenile European sea bass (*Dicentrarchus labrax*) from a farm with mortalities due to AGD (Dyková et al., 2000; Dyková and Novoa, 2001). *Paramoeba* sp. was also isolated from farmed sharpsnout seabream (*Diplodus puntazzo*) with AGD (Dyková and Novoa, 2001). Athanasspoulou et al. (2002) observed lesions typical for AGD in farmed gilthead sea bream (*Sparus aurata*) from Greece. *Paramoeba* sp. was detected in these by a fluorescent antibody raised against *Paramoeba* sp. from Atlantic salmon in Tasmania. The same host species was affected by the disease also in Spain (Dyková pers. comm. in Nowak et al., 2002).

In France, AGD was diagnosed in farmed sea trout (*Salmo trutta*), in addition to Atlantic salmon and rainbow trout. Details from these French AGD outbreaks have not been published (Akhlaghi et al., 1996; Baudin Laurencin in Munday et al., 2001).

1.5.2 Wild fish

In Tasmania, wild fish collected in or in the vicinity of Atlantic salmon farms were screened for *Paramoeba* spp. infections and gill lesions by histology. The purpose was to detect possible natural reservoir hosts. Samples of salmon from the farms were also examined. All farmed salmon was found to be heavily infected and suffering from AGD. None of the 325 wild fish, representing 12 different species, were found infected (Douglas-Helders et al., 2002). Challenge experiments were performed with two species, seahorse (*Hippocampus abdominalis*) and greenback flounder (*Rhombosolea tapirina*), with naïve Atlantic salmon serving as positive controls. Both species were found infected with paramoebae on the gills, but did not develop lesions compatible with AGD pathology. All Atlantic salmon became heavily infected and developed lesions characteristic for AGD. Adams et al. (2008) found a single blue warehou (*Seriolella brama*) collected from Atlantic salmon pens in Tasmania infected with *Paramoeba* sp. The fish had macroscopically unapparent, but histologically

detectable, gill lesions associated with amoebae. Specimens of the common jack mackerel (*Trachurus declivis*) and blue mackerel (*Scomber australicus*), also collected from the pens, were not infected. Foster and Percival (1988) detected *P. pemaquidensis* in the gills of wild couta (*Thyrsites atun*) caught in the vicinity of Atlantic salmon farms in Tasmania (Zilberg and Munday, 2006).

1.6 Environmental sources of *P. perurans*

Several Tasmanian studies searched for environmental reservoirs of the fish parasitic *Paramoeba* spp. prior to the discovery of *P. perurans* (Tan et al., 2002; Crosbie et al., 2003, 2005; Douglas-Helders et al., 2003b; Dyková et al., 2005; 2007). The environmental paramoebae detected by immuno-fluorescent antibody technique and SSU rDNA sequencing in these studies were mostly identified as *P. pemaquidensis*.

Nowak et al. (2010) used a *P. perurans* specific PCR (Young et al., 2008a) when examining sediment, biofouling and environmental invertebrate samples from Puget Sound, USA, and around Vancouver Island, Canada. None of the samples were positive for *P. perurans*. This was not unexpected in the Canadian samples, since there was no evidence of infection with the amoeba in the Atlantic salmon in the examined farms. However, sediment samples (N=6) and invertebrate samples (N=7) collected in the vicinity of an Atlantic salmon farm in the Puget Sound were also negative, and in this farm, all examined salmon (N=20) was infected with *P. perurans*. Bridle et al. (2010) developed a more sensitive *real-time* PCR assay specific for *P. perurans*, and used this for detection of the amoeba in water samples taken at 0.5 and 15 meters of depth in Tasmania. The amoeba was readily detected at or in the vicinity of an Atlantic salmon farm in high salinity seawater, but not in a freshwater affected farm or in an area without fish farms (Bridle et al., 2010). The amounts of amoebae in the water samples were highly variable and did not reveal any pattern. Bridle et al. (2010) also detected large amounts of *P. perurans* in swabs from dead salmon.

In Norway, the inlet seawater from four land-based aquaculture facilities has been examined for the presence of *P. perurans* with *real-time* PCR on DNA from filters. Presence of *P. perurans* in the inlet water was revealed by positive PCR at three of the facilities. These received water from 70 - 90, 150 and 160 meters of depth, which in the case of the latter two was known to be just above the sea bottom. *Paramoeba perurans* was however not detected in the filters from the fourth facility, which obtained water from 50 meters of depth (H. Glosvik, MH, pers. comm.). *Paramoeba perurans* was detected in the inlet water of the northernmost of the examined farms. This farm was situated in Sør-Trøndelag county, which hitherto has not been affected by the disease in farmed fish.

Both in Tasmania, and more recently in South Africa, initiation of Atlantic salmon farming in high salinity seawater immediately led to AGD problems due to *P. perurans* (Munday et al., 2001; Mouton et al., 2014). In South Africa, the affected tank reared locally hatched Atlantic salmon, excluding any possibility for introduction of the amoeba with the fish (Mouton et al., 2014). These observations therefore tend to support suggestions that *P. perurans* is

cosmopolitan in the marine environment (Young et al., 2008b). The widespread occurrence in seawater together with a lack of fish-host specificity may suggest that *P. perurans* is an opportunistic parasite. Its normal habitat and mode of life is so far unknown.

1.7 Transmission among farms

The mechanisms responsible for the transmission of free-living amoebae to fish farms and the extent of transmission between fish farms are unknown. The transmission between fish individuals is horizontal. Amoebic gill disease may be produced experimentally either by cohabitation with infected fish, by exposure to cultured *P. perurans* or by amoebae isolated from the gills of fish affected by AGD (Crosbie et al., 2012; Nowak, 2012). As few as 10 amoebae per litre of seawater may cause AGD in naïve Atlantic salmon (Morrison et al., 2004). In amoebae cultures, floating stages with very long pseudopodia are observed (T.A. Mo, pers. obs.). These are likely to act as transmission stages, and they may be transported for long distances by ocean currents. The survival of these stages in high salinity seawater is unknown, but could probably be of several weeks duration (Douglas-Helders et al., 2003a). In culture flasks, the floating stages sink after retraction of the pseudopodia and establish at the bottom (T.A. Mo, pers. obs.). In the sea, a floating stage may retract its long pseudopodia upon encounter with a fish and establish on the gills.

Since amoebae are readily transferred among cohabitants and occur in seawater samples taken in the vicinity of Atlantic salmon farms with AGD, *P. perurans* appears to be continuously shed from infected fish. This shedding is likely to be proportional to the infection intensities, and may be responsible for farm-to-farm transmission in Norway during the autumn. However, *real-time* screening of Atlantic salmon in Norway suggests that the amoebae disappear from the salmon during winter. If this involves shedding of live amoebae, it may represent input to the yet unknown environmental reservoir.

In addition to fish, *P. perurans* has been detected on salmon lice (*Lepeophtheirus salmonis*) collected from farmed Atlantic salmon in the Puget Sound area of Washington State (Nowak et al., 2010). These lice were obtained from Atlantic salmon infected with *P. perurans*, and may have harboured the amoebae externally (Nowak et al., 2010). Whether lice play a role in transmission of *P. perurans* is unknown.

Escaped Atlantic salmon may disperse rapidly and cover more than an area of 500 square kilometers by a week (Skilbrei et al., 2010). Infected escaped individuals therefore have a high potential for spreading the amoeba.

1.8 Transmission between farmed and wild fish

The reservoir for *P. perurans* outside the fish farms is unknown. It could be wild fish, other biota or free-living amoebae in the sediments. There are very few findings of *P. perurans* in wild fish species, however extensive studies in Norwegian waters are lacking. The amoeba can infect several host species (Table 1.4-1). *Paramoeba perurans* infections and AGD may

be induced experimentally in Atlantic salmon and ballan wrasse in challenge experiments by adding *P. perurans* to the water (Crosbie et al., 2012; Mo et al., 2014). The water around farms with AGD may show elevated densities of the amoeba (Bridle et al., 2010). Therefore there appears to be a potential for amoebal transmission between farmed fish with AGD and wild fish in the vicinity of farms.

Recently, AGD, caused by *P. perurans*, was found in farmed ballan wrasse in Norway (Karlsbakk et al., 2013; Hjeltnes, 2014) and in corkwing wrasse stocked as cleaner fish with infected Atlantic salmon (Hjeltnes, 2014). Wrasse is extensively used as cleaner fish, to combat salmon lice infestations in Atlantic salmon aquaculture. Both farmed and wild-caught wrasses are used. Infected wrasse may represent a source of *P. perurans* when introduced into the Atlantic salmon farms, and the possible role of wrasse as a vector for the amoeba thus needs to be further studied (Karlsbakk et al., 2013). Conversely, since these wrasse species have been observed to be susceptible, they may possibly also become infected in the wild as a consequence of elevated infection pressures posed by AGD outbreaks in farmed salmonids. However, at present, there is no evidence supporting such an impact.

1.9 Disease development

Amoebic gill disease can affect farmed salmonids throughout the seawater phase, but the fish are most susceptible during their first autumn at sea. Larger fish tend to be more robust than smaller fish. It could be assumed that older fish have developed more effective responses against infective organisms including amoebae, but other factors may also be involved. In 2013, Norwegian fish health services reported differences in AGD development between one year old smolt released in the spring (S1) and off-season smolt released in the autumn (S0), revealing delay in S0 fish (S. Nygaard, FOMAS, pers. comm.). An AGD outbreak can last for several months. The disease period and severity is likely dependent on the interaction between various abiotic and biotic factors.

The first clinical signs of AGD are increased gill mucus in pale areas on the gills that can be observed with the naked eye. However, such changes may also be due to other causes. In fish suffering from AGD, but also from other gill problems, the swimming activity is reduced and the gill ventilation increased. Reduced feeding is a typical observation during AGD development. Studies from Tasmania show that there is a significant hypertension in AGD-affected Atlantic salmon, with lowered cardiac output and cardiac stroke volume, as well as elevated systemic vascular resistance (Leef et al., 2007). The cause for the vasoconstriction and hypertension associated with AGD is unknown, but these pathophysiological effects may also contribute to the elevated mortality seen in conjunction with stress induced by a hydrogen peroxide treatment.

1.10 Diagnostics of AGD

In the field, multifocal patches of white to grey swollen tissue and increased mucus may be an indication of AGD. A gill score system, based on the extent of macroscopically visible gill

changes, has been developed (Taylor et al., 2009). The average gill score is an aid in the consideration of treatment. The simplest way to check for amoebae is examination of a fresh smear from a gill patch in a light microscope, whereas a light microscopic examination of gill tissue in histological sections is considered to be the «gold standard» for an AGD diagnosis. In AGD, hyperplasia, hypertrophy and lamellar fusion are typical, and usually associated with increased mucus cell activity. Large numbers of amoebae may be seen at the surface of the irritated gill tissue, but one can expect these to be washed away to some extent during processing of histological sections (Nygaard et al., 2014).

When AGD is diagnosed, the amoeba involved should be identified. Diagnostic laboratories mainly use molecular methods for detection and identification of *P. perurans*. At present, mainly *real-time* PCR is used for the detection of *P. perurans* (Fringuelli et al., 2012). So far, no method exists for the discrimination between isolates of *P. perurans*, but work is in progress in several laboratories. As *real-time* PCR methods are very sensitive and often used in surveillance for infective organisms in fish, this method is commonly used in Norwegian fish farms to document the presence or absence of *P. perurans*. A single *P. perurans* cell contains an estimate of 2880 rRNA gene copies (Bridle et al., 2010), while the published *real-time* PCR assays are able to detect less than 14 rDNA copies (Bridle et al., 2010; Fringuelli et al., 2012). An even higher sensitivity is likely when the analyses are based on RNA rather than DNA.

Host species in which *P. perurans* infections have been verified, either by *in situ* hybridisation or by PCR are presented in Table 1.4-1.

1.11 Implications of surveillance and monitoring

Methodological sampling and analysis facilitate surveillance, aiming to uncover *P. perurans* infection in farmed salmon in an area, or to demonstrate freedom from the infection. Surveillance may allow detection of a prospective spread of *P. perurans* and subsequently AGD into new areas. In an enzootic area, monitoring to assess changes in the level or distribution of *P. perurans* may possibly be used to estimate the risk of outbreaks of AGD.

To define freedom from AGD within a specified population, sufficient evidence of its absence in the fish is needed. However, lack of detection does not prove the absence of AGD, but demonstrates confidence that a hypothetical presence of the disease would be at a lower level than that specified by the design of the surveillance. This is the current situation in northern parts of Norway.

Similarly, freedom from infection involves providing sufficient evidence to demonstrate that *P. perurans* is not present in a specified population. With respect to the northern parts of Norway, which are currently free from AGD, *real-time* PCR detections of *P. perurans* suggest that there is no general freedom of infection. However, the area is large, and further sampling is necessary, preferentially when the population is at risk, in particular from September to December.

1.12 Prevention, control and treatment

Specific measures to prevent AGD are not known, but a good environment and healthy, robust fish would in general reduce disease problems. Cleaning of the nets might be of importance, since *Paramoeba* spp. can be detected in fouling organisms that attach themselves to cages and mooring equipment (H. Rodger, VAI, pers. comm.). From Tasmania, it is known that different strains and breeding lines of Atlantic salmon have different susceptibility or resistance to *P. perurans* (Taylor et al., 2007; Kube et al., 2012). Breeding for increased resistance is also relevant for Norwegian farmed Atlantic salmon, should AGD become a permanent problem.

In association with control of AGD and treatment against *P. perurans*, early detection of the parasite is of importance. The longer the disease has progressed, the more difficult it is to implement effective treatments. Also, the treatment stress may aggravate mortality. Therefore, it is important that fish gills are examined regularly by gill scoring, looking for raised pale patches. This can be done simultaneously with lice counts. In the case of suspicious clinical signs, gill smears should be examined for the presence of amoebae by direct microscopy at the farm. This can be done without killing the fish. Alternatively, gill swabs or gill samples can be fixed and sent to a laboratory for PCR and/or histological examination.

Freshwater is the most efficient treatment against *P. perurans* and AGD, and has been the treatment of choice in Tasmania. The fish are treated with freshwater for two to three hours. Alternatively, hydrogen peroxide can be used. However, this is less effective and causes a higher risk for fish mortality compared to fresh water. In 2013, mainly hydrogen peroxide was used for treatment against AGD in Norwegian fish farms, due to logistic and regulatory issues related to freshwater treatments. However, some successful treatments were carried out with fresh water in well boats. In the future, if the logistic problems are solved, Norwegian fish farms will likely increase the capacity for freshwater treatments, both because it is preferred and because freshwater is cheap, with plentiful supply along the Norwegian coast.

2 Answers to the terms of reference

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

ToR 1. Does AGD in Atlantic salmon and rainbow trout represent a substantial risk for the fish health in Norwegian fish farms?

The numerous AGD outbreaks along the Norwegian coast in 2013 (and recently in 2014 – see answer to ToR 1.2.), may indicate that *P. perurans* has increased in abundance and distribution. Possibly, the number of outbreaks will increase in the coming years. The geographical distribution and severity of the outbreaks are likely to depend on the seawater temperature and salinity. Other factors, such as biomass and distance between fish farms, may also be of importance. Fish affected by AGD usually have respiratory and circulatory disorders and may also loose appetite. Therefore, fish surviving AGD often show reduced growth and may suffer from emaciation.

Amoebic gill disease represents a substantial risk for the fish health in Norwegian seawater fish farms.

ToR 1.1. What is known about the distribution of *P. perurans* in farmed and wild fish in Norway?

In 2013, many fish farming companies conducted surveys to monitor the occurrence of *P. perurans*. The surveys were extensive in areas considered to be at high risk, and *real-time* PCR analyses were performed by private laboratories, which is why the results from the surveys have not been made public.

Data was obtained from Patogen Analyse (Appendix) and the Norwegian Veterinary Institute (NVI). In summary, positive samples were found from Vest-Agder in the south to Møre og Romsdal in the north (Hjeltnes, 2014). The bulk of positive samples came from Rogaland and Hordaland. In addition, *P. perurans* has been detected by *real-time* PCR in influx water to a land based marine fish farm in Sør-Trøndelag county, and in the gills of farmed Atlantic salmon from Nordland and Troms (Gjevre et al., 2014).

There are no published records of *P. perurans* in wild fish. However, Are Nylund (UiB, pers. comm.) found infections in two wrasse species, ballan wrasse and cuckoo wrasse, caught in the vicinity of Bergen, which is situated in Hordaland county in Western Norway. The samples were screened by *real-time* PCR for *Paramoeba* spp., and some selected samples were verified to contain *P. perurans* by SSU rDNA sequencing.

ToR 1.2. What is known about the distribution of AGD in farmed and wild fish in Norway?

In 2013, the NVI diagnosed AGD at 58 separate fish farm localities (Hjeltnes, 2014). It is likely that AGD additionally was diagnosed by other institutions, fish health services, as well as by fish farmers themselves. However, a summary of these observations is not publicly available. Among the AGD outbreaks diagnosed at the NVI, the disease was diagnosed in Atlantic salmon at 53 sites, in rainbow trout at two sites, in ballan wrasse at two sites and in corkwing wrasse at one site. In sites producing Atlantic salmon, about 70 % were located in Hordaland county, about 20 % in Rogaland county and the remaining in the counties of Møre og Romsdal, Sogn og Fjordane and Vest-Agder. In about 90 % of the AGD diagnoses, the samples were received in October and November, and the remaining in September and December. In approximately 40 % of the cases, other gill diseases occurred in association with AGD. Even if AGD was the only disease in more than half of the gill disease cases diagnosed, it shows that gill diseases in farmed Atlantic salmon often have a complex causation. Amoebic gill disease has not been observed in wild fish in Norway.

Until early October this year, the NVI has diagnosed AGD in more than 20 farms. The first case was diagnosed by the end of August, which is about 14 days earlier than in 2013. The number of AGD diagnosis at NVI in September 2014 was about three times higher than in September 2013. By early October 2014, AGD has not been diagnosed by NVI in Vest-Agder and Rogaland, but the first case has been diagnosed in Sør-Trøndelag (T.A. Mo, pers.comm.).

ToR 1.3. What factors influence the development of AGD?

Tasmanian studies have shown that the two main risk factors for development of AGD are high seawater salinity (>32 ppt) and high seawater temperature (> 12 °C) (Douglas-Helders et al., 2001, Munday et al., 2001, Nowak, 2012). The disease progress is particularly difficult to control at temperatures above 16 °C (Akhlaghi et al., 1996; Munday et al., 2001). However, in the North-Atlantic fish farmers have experienced AGD outbreaks at lower temperatures (Steinum et al., 2008). In one case AGD was diagnosed at 7 °C in December. However, in most cases clinical disease developed within the two months after temperature maxima in autumn. In Western Norway, the average sea temperature between August and September often exceeds 14°C at one to 10 meters of depth, as reported by the Institute of Marine Research in Norway. The AGD outbreaks in four Norwegian fish farms in 2006 occurred after a period of unusual high seawater temperatures, 3-4°C above the average. The increased seawater temperatures came after a warm period with low rainfall. This likely resulted in increased seawater salinities in coastal areas where freshwater drainage and impact usually is high.

The four AGD outbreaks in 2006 occurred simultaneously in localities that are geographically distant. Thus, the amoeba was either enzootic, or had already at that time a wide distribution along the Norwegian coast. In September 2013, _P. perurans_ occurred in many

more sites and in a wider geographical area compared to 2006. High seawater salinity and high temperature may also have contributed to the increased AGD frequency in 2013. In addition, other factors may be of importance, valid for the spread and outbreak of infectious fish diseases in general. Such factors could be number of fish farms in an area, fish density and biomass in cages, several year classes at one site, daily operation and cleaning, movement of equipment and movement of fish. The depth below the cages could also be of importance.

ToR 1.4.   What is known about the spreading dynamics?

It has been shown that *P. perurans* may be transmitted from infected to naïve Atlantic salmon. The amoebae are shed from infected Atlantic salmon to the surrounding water, and have been detected in water samples collected close to the farm (Bridle et al., 2010).The shedding is considered directly related to outbreaks of AGD. The severity of an outbreak will therefore influence the amount of *P. perurans* in the water. *Paramoeba perurans* likely shows prolonged survival in seawater, at least 14 days (H. Rodger, VAI, pers. comm.), and thus has the potential of being spread far with currents.

The spreading and survival of *P. perurans* in the marine environment may be a function of several factors like temperature, salinity, susceptible hosts or suitable substrates. Several studies have been performed in order to find the reservoirs of the non-parasitic stages. *Paramoeba perurans* may survive and propagate on dead fish in the cages (Bridle et al., 2010). Other *Paramoeba* spp. are known to survive in sediments (Crosbie et al., 2003) and biofouling (Tan et al., 2002) in the farming environment. Nowak et al. (2010) analysed sediment and invertebrate samples collected in the vicinity of a farm with *P. perurans* infected Atlantic salmon in Washington, without detecting the amoeba. Hence, there is lack of data on survival of *P. perurans* on and in the vicinity of aquaculture facilities affected by AGD.

ToR 2.   Is it possible to control the infection and the disease at site level?

It is not considered possible to control *P. perurans* infections at a site level given open cages. In closed systems, the infections may be hindered through inlet water treatments. Considerations are presented in the answer to ToR 3. Experiences from Scotland and Norway have demonstrated that it is possible to control the disease at an infected site if early treatment with fresh water or hydrogen peroxide is applied. Re-infections are common and repeated treatments are often necessary.

Hygienic measures have proven to be very effective in control of many fish diseases. For *P. perurans* and AGD, there is a lack of information regarding zoosanitary control, and theoretical assumptions are thus included in the following:

The amoeba has been detected by PCR in a part of the Norwegian coast with important fish farming areas. In this area, infected farmed salmonids, and also possibly different species of cleaner fish, may act as a *P. perurans* reservoir, particularly during autumn. Movement of such fish represents a substantial possibility of introducing the amoeba into areas where the amoeba has not been previously found, given environmental conditions that favor the parasite. *Paramoeba perurans* seems to disappear from this farmed fish reservoir during the winter, but re-establish the following summer and/or fall. Furthermore, the amoeba is frequently detected at several sites at the same time within a region with no obvious scattering pattern. This strongly indicates the presence of an unidentified important reservoir. Whether *P. perurans* contaminated premises like nets and other equipment and sediments underneath or near infected sites contribute to this reservoir is not demonstrated, but has been suggested. The role of wild fish is unknown, and not possible to control. Synchronised fallowing and treatment within a region have proven to be highly effective at eradicating epidemics when transmission rate is low (Werkman et al., 2011). However, in the presence of a substantial reservoir other than infected farmed fish, such measures will not prevent re-infection of sites. Nevertheless, experience with salmon lice has demonstrated that coordinated measures can lower the impact of the farm associated reservoir.

ToR 2.1   If yes, what methods/measures will be most relevant for the control of the disease?

Not applicable, due to the answer to ToR 2.

ToR 3.   Is it possible to achieve and maintain areas free from the pathogenic agent? Is it possible to achieve and maintain areas free from the disease?

The occurrence of *P. perurans* infections and AGD in Norway in 2006, 2012, 2013 and 2014 covered a large part of Western Norway. Almost all cases of PCR detection on salmonids or AGD outbreaks have been restricted to the autumn months. Evidence from *real-time* PCR screening of Atlantic salmon from several farms in Rogaland and Hordaland in 2013 suggests that the amoeba was not present during spring and summer. It appeared on the salmon in September, occurred at high intensities during autumn, followed by a decrease in intensity, resulting in *P. perurans* negative samples in the period from January to March. Hence, the available evidence suggests that the farmed Atlantic salmon is colonised by amoebae during autumn, more or less simultaneously over large geographic areas.

This implies that:

- The amoeba occurs throughout the coast of Western Norway
- In other seasons than the autumn, the amoeba does not infect salmonids and cause AGD
- *Paramoeba perurans* may effectively be spread during autumn
- Infections disappear during winter

Also, the infectious agent is probably able to survive at least 14 days in seawater, as addressed in the answer to ToR 1.4.

These considerations are most in agreement with the hypothesis that the amoebae originate from a widespread environmental reservoir, currently present in Western Norway. Therefore, the possibility to control the infection at site level in open pens or cages, or in larger areas, is considered unlikely in this region. However, in closed seawater facilities, filtering and treatment of the inlet water by ultra violet radiation, ozone or other methods, may be developed in order to avoid infection of the fish.

By early October 2014, no observations of AGD have been reported in Northern Norway. However, there are unpublished observations of slight infections in Atlantic salmon in Nordland and Troms, based on *real-time* PCR analysis ("The gill health project", NVI; Gjevre et al., 2014). It is not known if *P. perurans* is present in the environment or in wild fish in Northern Norway. The amoeba has been detected in deepwater from Trøndelag and Western Norway. The water temperature is an important environmental factor that impacts development of AGD. In Tasmania, ADG has usually been associated with water temperatures above 12 °C. In Norway, outbreaks have been observed in autumn at declining temperatures starting at 11-14 and ending at 7-11 °C (Steinum et al., 2008). In these cases, a period with even higher temperature preceded the outbreaks. The water temperature at time of outbreak cannot alone explain freedom of AGD in northern part of Norway. However, it is likely that a period with elevated temperature is necessary for *P. perurans* to be able to colonise and proliferate on salmon. The temperatures in Northern Norway may be too low for significant infections to develop.

In Western Norway, many farmed salmon populations represent major reservoirs of the parasitic stage of *P. perurans* during autumn. The amoeba has also been found on cleaner fish. Restrictions of fish movements from AGD enzootic areas to AGD free areas may therefore help to reduce the spread of amoebae. However, due to the possible impact of wild fish and environmental factors and unknown time of survival of *P. perurans* in the environment, biosecurity measures including restriction on movements of fish cannot guarantee to keep areas free from AGD.

ToR 3.1.   If yes, what measures will be most relevant to achieve and maintain areas free from pathogenic agents and/or free from disease?

Not applicable, due to the answer to ToR 3.

ToR 4.   Can the disease constitute a threat to wild stocks of fish if not treated and/or kept at a controlled low level?

In Tasmania, Chile and South Africa, AGD affects farmed exotic salmonid species in high salinity seawater. Extensive screening of wild fish in Tasmania, both around and trapped inside Atlantic salmon pens with AGD, has not revealed major lesions in these native fish species. In Norway, the disease affects some farmed species that normally live in shallow waters, such as Atlantic salmon and wrasse. Ballan wrasse kept in tanks receiving deepwater from 120-160 meters of depth have occasionally become infected and developed AGD. It is possible that these fish have been exposed to amoebae that are not normally common in their shallow water habitats. Cleaner fish in pens with salmon suffering from AGD also develop the disease. Hence, in Norway, it appears possible that the elevated infection pressure constituted by AGD outbreaks in salmon farms may affect wild fish in the environment, for instance wrasse, in high salinity seawater. However, there is at present no evidence for such an interaction. Further research is necessary to clarify the potential host range of *P. perurans*, and its virulence to different fish species.

Atlantic salmon smolts migrating out of the Norwegian fjords in spring appear not to be at risk, since farmed salmon seems not to be affected during this season. Smolts (N=35) collected at the outlet of the Hardanger fjord, an area where AGD has occurred in the fall, were indeed negative for *P. perurans* in May (Taranger et al., 2014).

Anadromous brown trout (sea trout) in Southern Norway usually descend to sea as smolts in spring when they are two to three years old (Jonsson and L'Abée-Lund, 1993). Both juveniles and adults return to fresh water in summer and autumn in order to overwinter, and adults also to spawn (Jonsson, 1985). A proportion of the sea trout may stay in the sea also during winter, usually in brackish waters (Klemetsen et al., 2003). Since AGD is known from sea trout farmed in France (Akhlaghi et al., 1996; Baudin-Laurencin in Munday et al., 2001), this species is likely susceptible to *P. perurans* infections. However, sea trout feeding in the Norwegian fjords during summer and returning to freshwater in autumn is unlikely to be affected by the amoeba, both since farmed salmon is uninfected during summer and because of the lower salinity in the surface water in the fjords. Sea trout staying in the sea during autumn may be at risk of becoming infected, although they usually will inhabit low salinity estuarine waters at this time of year. However, sea trout from coastal regions where AGD occurs in farmed salmon may be exposed to the amoebae to greater extent. Infections in sea trout are not known, but appear not to have been studied.

Treatment of *P. perurans* infections will also contribute to a reduced infection pressure, both among farms and for wild fish.

ToR 5. Can AGD in fish have substantial consequences for the Norwegian aquaculture industry?

Losses generated by AGD can include direct mortality, loss in growth, increased prevalence of poor conditioned fish, possible increased susceptibility to other diseases, mortality during sea lice bath treatment, and premature harvests. There is no available total estimate of the losses caused by AGD in affected countries. However, some isolated numbers have been communicated. In Tasmania, for instance, where AGD for several years has been recognised as the main disease problem in fish, losses can reach 10 % week (R. Taylor, CSIRO, pers. comm.) if not treated. In 2012, the largest fish health service company in UK, FishVet Group, recognised AGD as the main fish health challenge in Scotland (B. Laxdal, FishVet Group, pers.comm.), and the same year, one company in Scotland estimated their total cost due to AGD to be around 45 million NOK (B. Hjeltnes, pers.comm.). Concurrently, another Scottish producer experienced an AGD related loss of 4,661 tonnes Atlantic salmon in farms in Shetland (B. Hjeltnes, pers. comm.).

The Norwegian aquaculture industry has relatively limited experience with AGD. In 2013 the amoeba was detected at in at least 58 sites, but only a limited number had clinical AGD. Losses were reported in the counties of Hordaland and Rogaland. Treatment with hydrogen peroxide was carried out on several sites, and on some sites it was necessary to perform three successive treatments during the autumn.

If left untreated, it is evident from experiences in Tasmania and Scotland that AGD has the potential to cause major losses. However, VKM has not been able to estimate the potential total losses for the Norwegian farmers.

3 Uncertainties

The conclusions are largely based on short time series of observation of the parasite in Norway, which reflects a relatively high degree of uncertainty. Also, a general lack of available data leads to uncertainties connected to the conclusions in total. Further considerations and details on the uncertainties are given in Chapter 5, Data gaps.

4 Conclusions

Amoebic gill disease represents a substantial risk for the fish health in Norwegian seawater fish farms. So far, the disease has been observed in 2006, 2012, 2013 and 2014. *Paramoeba perurans* has been detected in farmed fish from Vest-Agder to Møre og Romsdal in the period September to February, but the distribution of *P. perurans* in wild fish in Norwegian waters is unknown.

It is presumed that *P. perurans* has an unknown environmental reservoir that plays a key role in the spread of the amoeba, and it is therefore not considered possible to control the infection at site level in open cages. Development of amoebic gill disease, however, can be controlled at site level, given early detection and treatment, but it is not considered possible to achieve and maintain areas free from the pathogenic agent. Hygienic measures may have a positive impact on the control.

If the environmental conditions permit, the amoeba can affect large parts of the Norwegian fish farming area. Known parameters that will have impact on this are salinity and temperature. However, actual threshold levels in Norwegian waters are not known. *Paramoeba perurans* is transmitted from fish to fish through the water, but there is a lack of information regarding the spreading dynamics. Restrictions on movement on marine fish such as cleaner fish or salmonids transferred to sea, may possibly delay an introduction of the amoeba to new areas.

The available knowledge is not sufficient to allow assessment on whether this disease represents a threat to wild stocks.

If left untreated, AGD could cause substantial losses in Norwegian fish farming areas. However, the currently inadequate amount of available data makes it unfeasible to estimate the total potential for future losses for the whole industry.

These conclusions are largely based on short time series of observation of the parasite in Norway, which reflects a relatively high degree of uncertainty.

5 Data gaps

The available information on the ecology of *P. perurans* in nature outside the fish farm cages is very limited. There is evidence suggesting the presence of an environmental reservoir, where the amoebae reside spring and summer. The reservoir could be constituted by biota, such as wild fish and invertebrates, or by water and sediments. The application of molecular methods on environmental samples is the simplest way to seek natural reservoirs. It is important to obtain more information on the prevalence and potential impact of *P. perurans* in wild fish. These may represent natural hosts for the amoeba, but may also suffer due to an elevated infection pressure near AGD outbreaks.

The apparent common occurrence of *P. perurans* in deepwater should be examined by analysis of further water and sediment samples covering all depths. If elevated densities of *P. perurans* indeed occur in deepwater, it is likely that the breakdown of stratification and vertical mixing of the water in autumn may be of importance. Such perturbation could explain the apparent sudden appearance of *P. perurans* infections in September over large geographic areas.

Opportunistic protists infecting fish, such as scuticociliates, often live as scavengers in nature. It is possible that *P. perurans* and other *Paramoeba* spp. act as scavengers, since these amoebae may occur in large numbers on dead fish in pens (Douglas-Helders et al., 2000; Dyková et al., 2007; Bridle et al., 2010). This hypothesis can readily be tested, in order to identify relevant risk factors.

Further information is needed on the impact of temperature and salinity on the infectivity and proliferation rate of *P. perurans* in Norway. Data from elsewhere may not be valid for the North-East Atlantic. A combination of *in vitro* studies and experimental challenge experiments may provide such data.

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Appendix
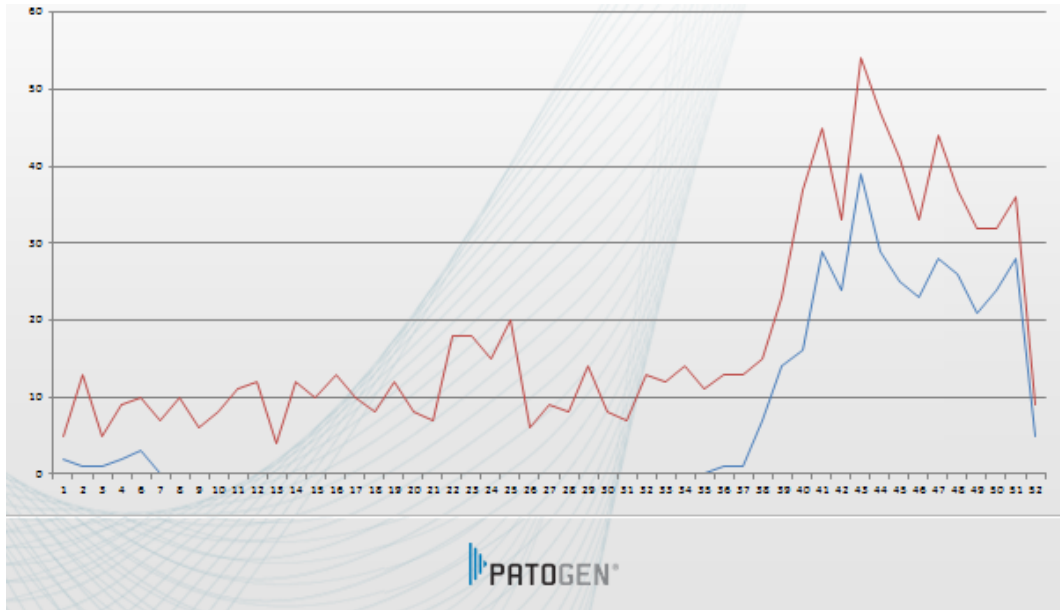
Figure A-1   Number of samples submitted (red) and the amount of samples positive at PCR analysis for *Paramoeba perurans* (blue) per week in 2013 (Source: PatoGen Analyse AS, Norway, reprinted with permission).
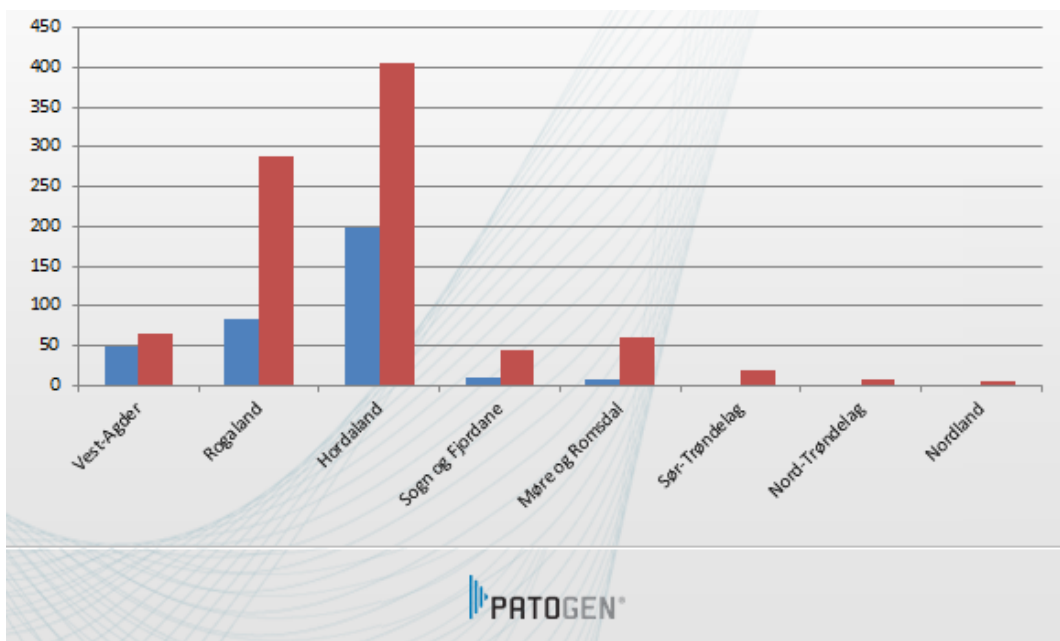
Figure A-2   Number of submitted samples (red) and the amount of samples positive at PCR analysis for *Paramoeba perurans* (blue) per county in 2013 (Source: PatoGen Analyse AS, Norway, reprinted with permission).