Amoebic gill disease: a growing threat



Jessica Johnson-Mackinnon^A, *Tina Oldham*^A *and Barbara Nowak*^{A,B} ^AUniversity of Tasmania, Institute of Marine and Antarctic Studies, Locked Bag 1370, Launceston, Tas. 7250, Australia ^BCorresponding author. Tel: + 61 3 6324 3814, Email: B.Nowak@utas.edu.au

The risk of disease outbreaks is predicted to increase due to climate change. For farmed fish an example is amoebic gill disease (AGD). While initially reported only in farmed salmonids in Washington State, USA, and Tasmania, Australia, it has now become an issue for Atlantic salmon farming worldwide and affects a range of other farmed marine fish species. Local high temperature anomalies and a lack of rainfall have been associated with the outbreaks of AGD. This worldwide presence is at least partly due to the cosmopolitan nature of the parasite and its low host-specificity. The disease can be treated using freshwater or hydrogen peroxide baths, but the treatments increase the cost of salmon production. Management of AGD contributes 20% to production costs of Atlantic salmon in Tasmania.

AGD, caused through infection of fish gills by the facultative parasite *Neoparamoeba perurans*, was first documented in sea-caged salmonids in 1985¹. Since its initial observation AGD has become a primary health concern globally for the marine salmonid industry, resulting in mortalities as great as 80% when left untreated². Clinical signs of AGD include respiratory distress, lethargy and inappetence, which are associated with grossly visible gill lesions³. Histologically, gill lesions are characterised by epithelial hyperplasia, interlamellar vesicles with associated amoebae and lamellar fusion⁴ (Figure 1).

Because *N. perurans* was only recently identified⁵ and shown to cause AGD⁶, minimal information is available on its biology and ecology. Amoebae of the genus *Neoparamoeba* (Amoebozoa,

Dactylopodida) are ubiquitous in the marine environment⁷, and *N. perurans* specifically have been detected throughout the water column on and near Atlantic salmon (*Salmo salar* L.) farms^{8,9}. All species from the genus *Neoparamoeba* harbour at least one intracellular endosymbiont known as a *Perkinsela amoebae*-like organism (PLO)¹⁰. The details of the symbiotic relationship between the PLOs and *Neoparamoeba* are unknown; however, the strict phylogenetic congruence of PLOs and their *Neoparamoeba* hosts suggests that PLOs are vertically transmitted from parent to daughter cells during mitotic division¹⁰. Species of *Neoparamoeba* all

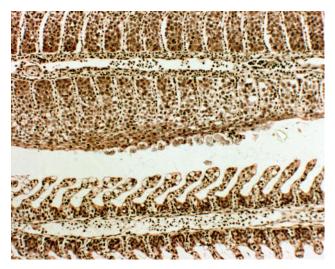


Figure 1. Histological section showing two filaments from gills of infected Atlantic salmon, the top filament is affected and the bottom one is normal. Epithelial hyperplasia and lamellar fusion associated with presence of numerous amoebae can be seen in the top filament.

share the same general ultrastructural characteristics and cannot be differentiated morphologically¹¹.

Despite numerous studies investigating potential reservoirs of *N. perurans*, no significant reservoir outside farmed salmon has been identified⁹. Extensive surveys of the water column^{8,12} and wild fish^{13–15} have detected only minimal evidence of *N. perurans*. Studies of metazoan ectoparasites, for example copepods or isopods, on farmed salmon have detected low frequencies of *N. perurans*^{16,17}, but no evidence of a reservoir population. Additional studies using genus specific identification methods detected *Neoparamoeba* spp. in sediment samples¹⁸ and net biofouling¹⁹, but as yet no species specific testing has been conducted to detect *N. perurans* in these potential reservoirs.

Along with seemingly no reservoir, *N. perurans* is also an opportunistic parasite with no apparent host specificity. The pathogen has been detected not only in the commercially important Atlantic salmon²⁰, but also in a variety of farmed and non-farmed finfish species around the world⁹ (Figure 2). Presently AGD is a major issue for aquaculture in Tasmania, Ireland, Scotland, Norway and the United States with varying levels of impact from 10% to 82% mortality in some cases⁹. Additional outbreaks have been reported in Chile, France, Spain, South Africa, and most recently Canada and the Faroe Islands^{9,21}.

Beyond salmon, *N. perurans* has been found on the gills of an additional 14 finfish species including ayu in Japan²², sea bass in the

Mediterranean²³, and olive flounder in Korea²⁴. There is no traceable pattern from one of these outbreaks to another making it unlikely that it is a specific sub-population that causes the disease or that amoebae are transferred from one outbreak site to another. What is known of its lifecycle suggests an asexual clonal evolutionary pattern. It has been postulated by statistical analysis that the sheer number of individuals in any given microbial species is so large that dispersal would rarely be restricted by contrived geographical barriers²⁵, especially in marine environments⁷.

The cosmopolitan nature of *N. perurans* and lack of host specificity make discerning trends and risk factors for AGD challenging. A recent meta-analysis which considered all reports of AGD to date suggests locally high temperature anomalies, rather than absolute temperature, are related to disease outbreak⁹. Salinity also plays an important role in AGD. *N. perurans* is a marine amoeba with minimal tolerance for low salinity. Freshwater bathing for 2–4 hours is the most commonly utilised commercial treatment for AGD²⁶, and though many reports do not include information on rainfall, the few which have report lower than average rainfall preceding outbreaks^{3,27–29}. Given the predicted increase in ocean temperatures and altered rainfall patterns associated with climate change, there is concern that AGD associated costs will continue to increase for the salmonid industry moving forward³⁰.

Although research into AGD has come a long way in the past 30 years there are still many knowledge gaps in key areas from basic biology



Figure 2. Map showing reported confirmed (PCR and/or ISH) cases of amoebic gill disease (AGD) in farmed Atlantic salmon.

to industry research. For instance, little is known about the parasite *N. perurans*. The mechanisms behind the successful transfer of the PLO from mother to daughter cell, and benefits of the symbiosis, are not yet known. In addition there is little information on how the amoebae cause disease and whether there is potential for vaccines or drug targets. On a more practical side, extensive and thorough testing of sediment, biofouling and other potential reservoirs would also be beneficial for predicting outbreaks and controlling this globally increasing threat.

References

- Kent, M. et al. (1988) Paramoeba pemaquidensis (Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon Oncorbynchus kisutch reared in sea water. Dis. Aquat. Organ. 5, 163–169. doi:10.3354/dao005163
- Steinum, T. *et al.* (2008) First cases of amoebic gill disease (AGD) in Norwegian seawater farmed Atlantic salmon, *Salmo salar* L., and phylogeny of the causative amoeba using 18S cDNA sequences. *J. Fisb Dis.* **31**, 205–214. doi:10.1111/j.1365-2761.2007.00893.x
- Munday, B. et al. (1990) Paramoebic gill infection and associated pathology of Atlantic salmon, Salmo salar and rainbow trout, Salmo gairdneri in Tasmania, in Pathology in marine science. Proceedings of the Third International Colloquium on Pathology in Marine Aquaculture, 2–6 October 1988, Gloucester Point, Virginia, USA. pp. 215–222.
- 4. Nowak, B.F. (2012) Fish parasites, pathobiology and protection. (Woo, P.T.K. and Buchmann, K. eds), pp. 1–18, CAB International.
- Young, N.D. et al. (2007) Neoparamoeba perurans n. sp., an agent of amoebic gill disease of Atlantic salmon (Salmo salar). Int. J. Parasitol. 37, 1469–1481. doi:10.1016/j.ijpara.2007.04.018
- Crosbie, P.B.B. et al. (2012) In vitro cultured Neoparamoeba perurans causes amoebic gill disease in Atlantic salmon and fulfils Koch's postulates. Int. J. Parasitol. 42, 511–515. doi:10.1016/j.ijpara.2012.04.002
- Page, F.C. (1987) The classification of 'naked' amoebae (Phylum Rhizopoda). Arch. Protistenkd. 133, 199–217. doi:10.1016/S0003-9365(87)80053-2
- Wright, D. *et al.* (2015) Depth distribution of the amoebic gill disease agent, *Neoparamoeba perurans*, in salmon sea cages. *Aquacult. Environ. Interact.* 7, 67–74. doi:10.3354/aei00137
- Oldham, T. *et al.* (2016) Incidence and distribution of amoebic gill disease (AGD)—an epidemiological review. *Aquaculture* 457, 35–42. doi:10.1016/ j.aquaculture.2016.02.013
- Young, N.D. *et al.* (2014) Support for the coevolution of *Neoparamoeba* and their endosymbionts, *Perkinsela* amoebae-like organisms. *Eur. J. Protistol.* 50, 509–523. doi:10.1016/j.ejop.2014.07.004
- Dyková, I. *et al.* (2005) *Neoparamoeba branchipbila* n. sp., and related species of the genus *Neoparamoeba* Page, 1987: morphological and molecular characterization of selected strains. *J. Fish Dis.* 28, 49–64. doi:10.1111/j.1365-2761.2004. 00600.x
- Bridle, A. *et al.* (2010) Rapid detection and quantification of *Neoparamoeba* perurans in the marine environment. *Aquaculture* **309**, 56–61. doi:10.1016/ j.aquaculture.2010.09.018
- Stagg, H.E.B. et al. (2015) Detection of Paramoeba perurans in Scottish marine wild fish populations. Bull. Eur. Assoc. Fish Pathol. 35, 217–226.
- Kent, M. *et al.* (1998) Survey of salmonid pathogens in ocean-caught fishes in British Columbia, Canada. *J. Aquat. Anim. Health* **10**, 211–219. doi:10.1577/1548-8667(1998)010<0211:SOSPIO>2.0.CO;2
- Douglas-Helders, G. *et al.* (2002) Wild fish are not a significant reservoir for *Neoparamoeba pemaquidensis* (Page, 1987). *J. Fisb Dis.* **25**, 569–574. doi:10.1046/j.1365-2761.2002.00394.x

- Nowak, B.F. et al. (2010) Do salmon lice, *Lepeophtheirus salmonis*, have a role in the epidemiology of amoebic gill disease caused by *Neoparamoeba perurans*² *J. Fisb Dis.* 33, 683–687. doi:10.1111/j.1365-2761.2010.01158.x
- Gonzalez-Poblete, L. (2015) Ectoparasites and associated pathogens affecting farmed salmon during marine grow-out in Chile and Tasmania. PhD Thesis, University of Tasmania.
- Crosbie, P.B.B. et al. (2005) Distribution of Neoparamoeba sp. in sediments around marine finfish farming sites in Tasmania. Dis. Aquat. Organ. 67, 61–66. doi:10.3354/dao067061
- Tan, C.K. *et al.* (2002) Biofouling as a reservoir of *Neoparamoeba pemaquidensis* (Page, 1970), the causative agent of amoebic gill disease in Atlantic salmon. *Aquaculture* **210**, 49–58. doi:10.1016/S0044-8486(01)00858-4
- 20. Munday, B.L. (1986) Diseases of Salmonids, in *Proceedings of the Workshop on Diseases of Australian Fish and Shellfish*.
- Ruane, N. and Jones, S. (2013) Amoebic gill disease (AGD) of farmed Atlantic salmon (*Salmo salar L.*). *ICES Identification leaflets for diseases and parasites of fish and shellfish*.
- Crosbie, P.B.B. et al. (2010) Amoebic gill disease in hatchery-reared ayu, Plecoglossus altivelis (Temminck & Schlegel), in Japan is caused by Neoparamoeba perurans. J. Fisb Dis. 33, 455–458. doi:10.1111/j.1365-2761.2009.01137.x
- Dyková, I. et al. (2001) Comments on diagnosis of amoebic gill disease (AGD) in turbot, Scophthalmus maximus. Bull. Eur. Assoc. Fisb Pathol. 21, 40–44.
- Kim, H.J. et al. (2005) Neoparamoeba sp. infection on gills of olive flounder, Par-alichthys olivaceus in Korea. J. Fish Pathol. 18, 125–131.
- Finlay, B.J. (2002) Global dispersal of free-living microbial eukaryote species. Science 296, 1061–1063. doi:10.1126/science.1070710
- Powell, M.D. et al. (2015) Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: Considerations of water chemistry and fish welfare in Norway. Aquaculture 448, 18–28. doi:10.1016/j.aquaculture.2015.05.027
- Bustos, P.A. *et al.* (2011) Amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*) farmed in Chile. *Aquaculture* **310**, 281–288. doi:10.1016/j.aquaculture. 2010.11.001
- Rozas, M. *et al.* (2012) Epidemiology of amoebic gill disease (AGD) in Chilean salmon industry between 2007 and 2010. *Bull. Eur. Assoc. Fisb Pathol.* 32, 181–188.
- Rodger, H.D. and McArdle, J.F. (1996) An outbreak of amoebic gill disease in Ireland. Vet. Rec. 139, 348–349. doi:10.1136/vr.139.14.348
- Battaglene, S.C. *et al.* (2008) Scoping study into adaptation of the Tasmanian Salmonid Aquaculture Industry to potential impacts of climate change. National Agriculture and Climate Change Action Plan: Implementation Programme report, p. 83. http://www.imas.utas.edu.au/right-column-content/publications-search? queries_publication_type_query=Research+Reports

Biographies

Jessica Johnson-Mackinnon is a Canadian PhD student in the Aquatic Animal Health group at the University of Tasmania. Her primary interest is investing pathogens associated with industry using molecular techniques.

Tina Oldham is a PhD student in the Aquatic Animal Health group at the University of Tasmania. Her primary interest is in development of sustainable, resilient aquaculture.

Barbara Nowak is a Professor at Institute of Marine and Antarctic Studies at University of Tasmania. Her research focuses on aquatic animal health. She has been working on amoebic gill disease for the past 25 years.