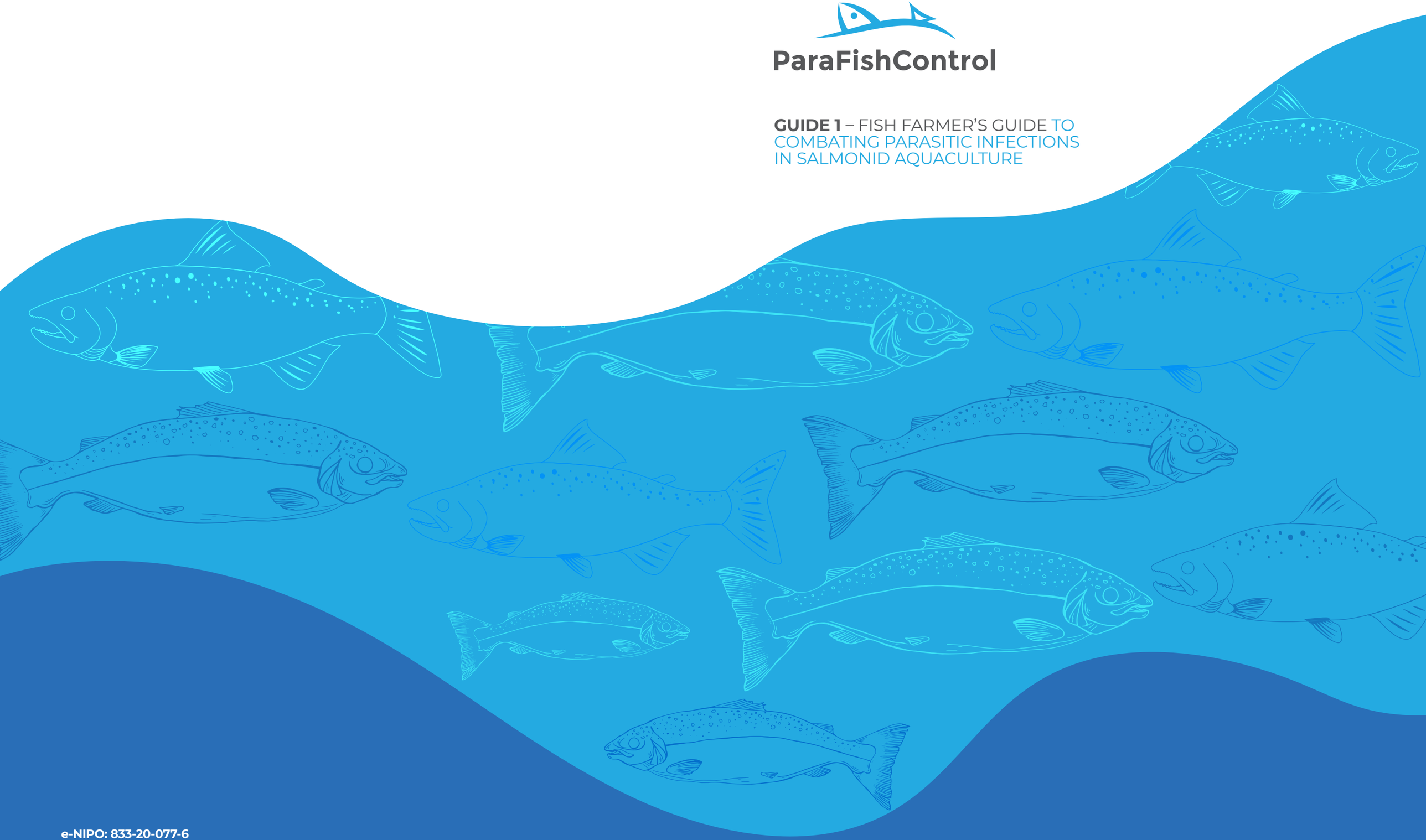
ParaFishControl

GUIDE 1 – FISH FARMER'S GUIDE TO COMBATING PARASITIC INFECTIONS IN SALMONID AQUACULTURE



e-NIPO: 833-20-077-6

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 634429 (ParaFishControl). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

A Series of ParaFishControl Guides to Combating Fish Parasite Infections in Aquaculture. **Guide 1**

ParaFishControl

> “Health is not valued till sickness comes.”
>
> *Thomas Fuller*

Farming of salmonids is expanding on all continents but various parasitic diseases may compromise fish welfare, production and economy if left uncontrolled. This guide provides useful information about five parasites' biological background and how they can be controlled.

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ParaFishControl

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Introduction

Aquaculture production of salmonids involves a wide range of species and rearing techniques across multiple continents. The family Salmonidae is comprised of more than 25 species, but the production in European countries is mainly concerned with Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Smaller production of brown trout (sea trout) (*Salmo trutta*), grayling (*Thymallus thymallus*) and whitefish (*Coregonus spp.*) are primarily directed toward restocking programs in which fry or smolts are released into natural water bodies for restoration purposes.

On a global basis, the annual aquaculture production of Atlantic salmon exceeds 2.3 million tonnes and the annual production of rainbow trout exceeds 750,000 tonnes. In European countries, these two species represent the great majority of salmonid aquaculture production. In the EU they accounted for almost 400,000 tonnes in 2017 (Source: FAO), whereas in Norway they increased to 1.3 million tonnes in 2018 (Source: Statistics Norway). Norway is the largest producer of Atlantic salmon with an output from mariculture systems of more than 1.3 million tonnes in 2019. Scotland, Ireland, Faroe islands, Iceland, Finland and Denmark produce smaller amounts, but the overall output is increasing.

Marine farmed salmonids are anadromous fish species and their physiology is suited both for freshwater and seawater. Atlantic salmon is mainly farmed at sea in mariculture net cages in fjord settings, which are stocked with salmon smolts produced in freshwater farms, over a period of 1-2 years. Rainbow trout has been an important commodity in freshwater aquaculture in Europe for more than 150 years, but it has been shown that mariculture of rainbow trout is a promising commodity in both marine and brackish water areas. Norway, Spain, France, Italy, UK (England and Scotland), Ireland, Germany, Poland, Denmark, Finland, Sweden, Baltic republics, and Turkey are important producers.

Freshwater farming of salmon and trout takes place in different systems. Conventional earth pond systems based on intake of natural surface water (streams, rivers, lakes) are still in use. In addition, organic trout farming relies on this type of rearing facilities. Partial or full recirculation of water allows reuse of water and reduces nutrient output to the environment. This has made this type of salmonid aquaculture a strongly expanding business. Land-based recirculation systems are taking over an increasing part of the production but, in the coming decades, conventional systems with partial recirculation of water are expected to remain the most important technology.

In all systems, disease represents the main threat, particularly that associated with parasitic infections. Thus, fish must be continuously monitored in order to manage and control disease and secure good fish health and welfare.

The Horizon 2020 **ParaFishControl** project has provided a strong basis for future success of European aquaculture by investigating and developing new diagnostic and control systems for parasitic infections.

In this manual, fish farmers can find background information on some of the main parasitic diseases jeopardizing farming of salmonids in European countries. Here the farmer can find valuable information on symptoms, identification of the pathogen, biology of the parasite, the life cycle and recommendations for control. This manual does not provide comprehensive details but may serve as an easily comprehended and necessary support during the daily handling and management of these fish. The guidance provided for individual parasites reflects the current state of knowledge for these pathogens and has been informed, in part, by research conducted through the **ParaFishControl** project.

Guides are provided for five parasites: the salmon louse, *Lepeophtheirus salmonis*; *Neoparamoeba perurans*, causative agent for amoebic gill disease (AGD); *Ichthyophthirius multifiliis*, causative agent for fish whitespot disease (“Ich”); *Saprolegnia parasitica*, a fungus-like oomycete; and *Tetracapsuloides bryosalmonae*, causative agent of proliferative kidney disease (PKD). Each guide provides a background on the parasite’s biology, an examination of key risks for infection and disease progression and up-to-date guidance for the management and control of the parasite. Because the expression of parasitic disease can be affected by many different factors and is therefore very much site, stock and environment dependent, instigation of any of the suggested control measures should be accomplished with the assistance and guidance of suitable fish health professionals, including veterinary practitioners and farm based health and welfare professionals. These guides also provide details for expert contacts within Europe who may be consulted for further support.

1. Fish farmer's guide to combating *Lepeophtheirus salmonis* infections

Figure 1. *Lepeophtheirus salmonis* (photo: L. A. Hamre, SLRC - Sea Lice Research Centre)

Introduction

The salmon louse (*Lepeophtheirus salmonis*), is a marine copepod ectoparasite of salmonid fish (Figure 1). Susceptible species include Atlantic salmon (*Salmo salar*), Sea trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and chum salmon (*Oncorhynchus keta*). In general, coho salmon (*Oncorhynchus kisutch*) and pink salmon (*Oncorhynchus gorbuscha*) are resistant towards juvenile salmon louse stages.

Biological life cycle

The louse goes through eight life stages, each separated by a moult (Figure 2). After two free-swimming planktonic nauplius stages, the salmon louse copepodid attaches to the skin of the host fish, where the louse feeds on host skin, mucus and blood while it passes through two chalimus stages and two preadult stages before the final moult to the adult stage.

Adult female lice produce eggs continuously, which are deposited in batches (egg-strings). Salmon lice are long-lived and females can produce at least 11 sets of egg strings, each with several hundred eggs. During the copepodid growth phase, it attaches to the fish by appendages, its antennae and maxillipeds. Close to the moult, a frontal filament is extruded, which restricts the chalimus stages to feeding on a small circle of the skin surrounding the point of attachment. Preadults and adults, on the other hand, adhere to the fish by the aid of a suction cup shaped cephalothorax, assisted by staple-like antennae, allowing free movement over the host surface and blood feeding is commonly observed.

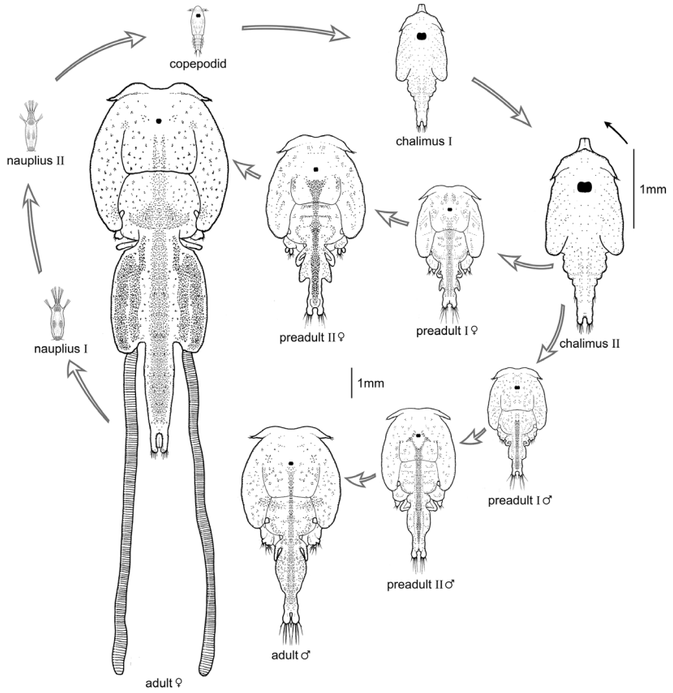


Figure 2. The life cycle of salmon lice. Note the two scale bars, where the first applies to the nauplius, copepodid and chalimus stages, whereas the second applies to the remaining stages. Illustration: "SLRC Lepeophtheirus salmonis life cycle" by Sea Lice Research Centre (licensed under a Creative Commons Attribution-ShareAlike 4.0 International License).

Seasonality

Developmental rate and egg production in salmon lice is positively influenced by higher seawater temperatures. Therefore, the level of salmon lice on fish will often have a cyclic variation with the lowest number of lice in the spring and the highest in the autumn. However, sometimes, the inability to treat in winter months may lead to high numbers. In a natural ecosystem, the absence of salmonid hosts in coastal areas also contributes to low populations of salmon lice during winter.

Age / mean weight susceptibility

Sea lice can infect salmonids in any marine phase. However, small, newly smoltified fish have a much higher mortality rate due to infestation with salmon lice.

Risk predisposing factors

One of the main predisposing factors is related to high stocking density of fish farms and/or high number of farms within an area, with production throughout the year, as the salmon louse reproductive capacity is adapted to highly seasonal availability of hosts.

A) What clinical signs should alarm me?

External signs

Severely infested fish will often display grazing damage at the back of the head and behind the dorsal and adipose fins when viewed from above while swimming. Mobile lice may also be visible in these areas when viewed from above. Lice are observed upon inspection of the external surfaces of the fish, with adult females particularly concentrating in the midline behind dorsal, adipose and anal fins (Figure 3). Copepodids normally attach to fins and skin, but can occasionally be observed in the mouth and gills. The smaller larval stages (copepodids and chalimi) can be difficult to spot and require close inspection and good light. Epidermal erosions, ulceration and haemorrhage can be seen at the attachment site if infected with mobile stages.

Internal lesions

Typically, no internal lesions. With severe infestations, anaemia can be observed.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Visual inspection for salmon lice is typically done every week in Norway (minimum 20 fish). In the spring, only 0.2 adult female lice/fish are allowed to protect the migrating wild salmon leaving rivers and moving into the sea. The rest of the year, 0.5 adult female lice/fish are allowed before any mitigation action has to be taken. Treatment efficacy has to be evaluated afterwards. In Scotland, lice is also counted every week, where 3 adult female lice/fish will lead to increased surveillance of a given farm site. Mitigation action will be taken if the number of lice is not kept below 8 adult female/lice within 4 weeks after exceeding the initial limit.

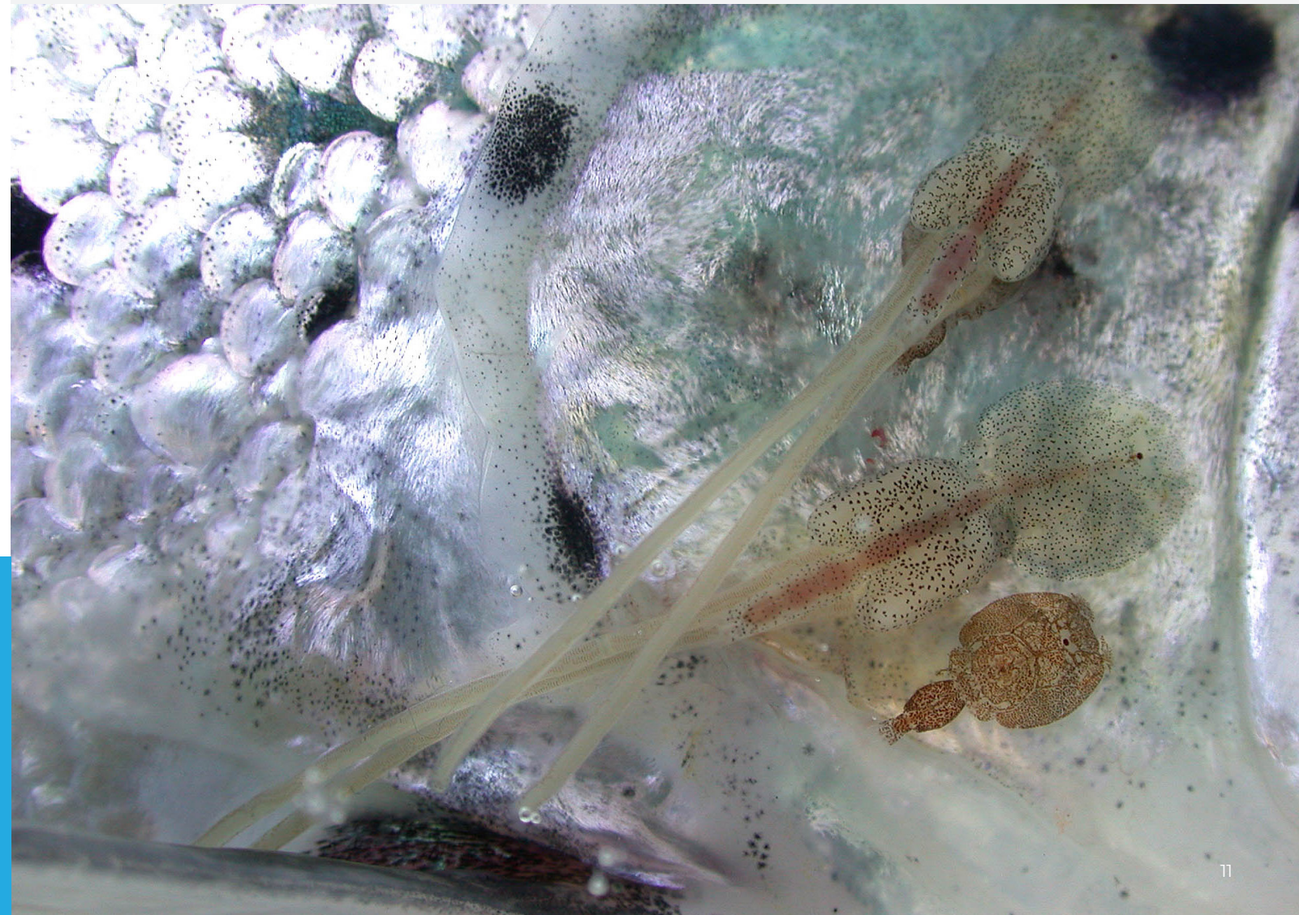
2. Recommendations for the submission of samples to be diagnosed

No recommendation since it is an ectoparasite which is easily diagnosed at farm level.

3. Contact laboratories

- Sea Lice Research Centre, Department of Biological sciences, University of Bergen, Norway.
- Institute of Aquaculture, University of Stirling, Scotland.
- Laboratory of Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark

Figure 3. Salmon lice attached to host (photo: L. A. Hamre, SLRC - Sea Lice Research Centre)



C) Action plan after diagnosis

There is no single magic bullet solution to combating sea lice, and integrated solutions are the best way to manage this parasite (Figure 4).

1. Prevention

Skirts around cages, snorkel cages and deep water feeding.

2. Farm management

Development of coordinated production zones with synchronized fallowing and treatments is important in areas with high density of farms.

3. Treatment

- Medicinal treatment: Anti-parasitic chemotherapeutants are used to treat lice infestations in most countries where salmon aquaculture is practiced. Organophosphates, pyrethroids and hydrogen peroxide are administered through bath treatments, whereas avermectins (emamectin benzoate) and diflubenzuron (not in UK) are administered as additives in medicated feeds.
- Non-medicinal treatment: As widespread resistance towards available chemotherapeutants has spread throughout Atlantic salmon producing countries using veterinary drug treatments, non-medicinal alternatives to control salmon lice have been developed. Some promising techniques, including electromagnetism/ultra-sound and vacuum, are still under evaluation. Freshwater treatment in tarpaulins or well-boats, mechanical delousing with flushing/spraying the lice off the fish and thermal treatments using brief exposure to warm water have all been used successfully. Five main species of cleaner fish (wrasse: *Labrus bergylta, Ctenolabrus rupestris, Centrolabrus exoletus, Symphodus melops;* lumpsuckers: *Cyclopterus lumpus*) are also in use for delousing and are increasingly being cultured explicitly for this purpose.

4. Management of co-infections

Mechanical treatments deployed against salmon lice, as well as crowding activities prior to a range of treatments, can stress the fish and cause external lesions, so that underlying diseases or opportunistic pathogens can lead to disease and death, e.g. viral diseases that initially do not cause clinical signs but after a stressful delousing, lead to mortalities. It has also been shown that fish infected with salmon lice can be more susceptible to other diseases, possibly through the parasite's immunomodulatory activities e.g. infectious salmon anaemia virus (ISAV). Presence of other disorders e.g. complex gill disease / amoebic gill disease may provide a barrier to conducting lice treatments (e.g. hydrogen peroxide treatment) due to compromised gills / reduced respiratory capacity, particularly at higher water temperatures. High water quality, clean nets, prompt treatments and good general health / hygiene practices can minimise impact of other infectious agents.

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Toolbox for integrated pathogen management of sea lice in Atlantic salmon farming

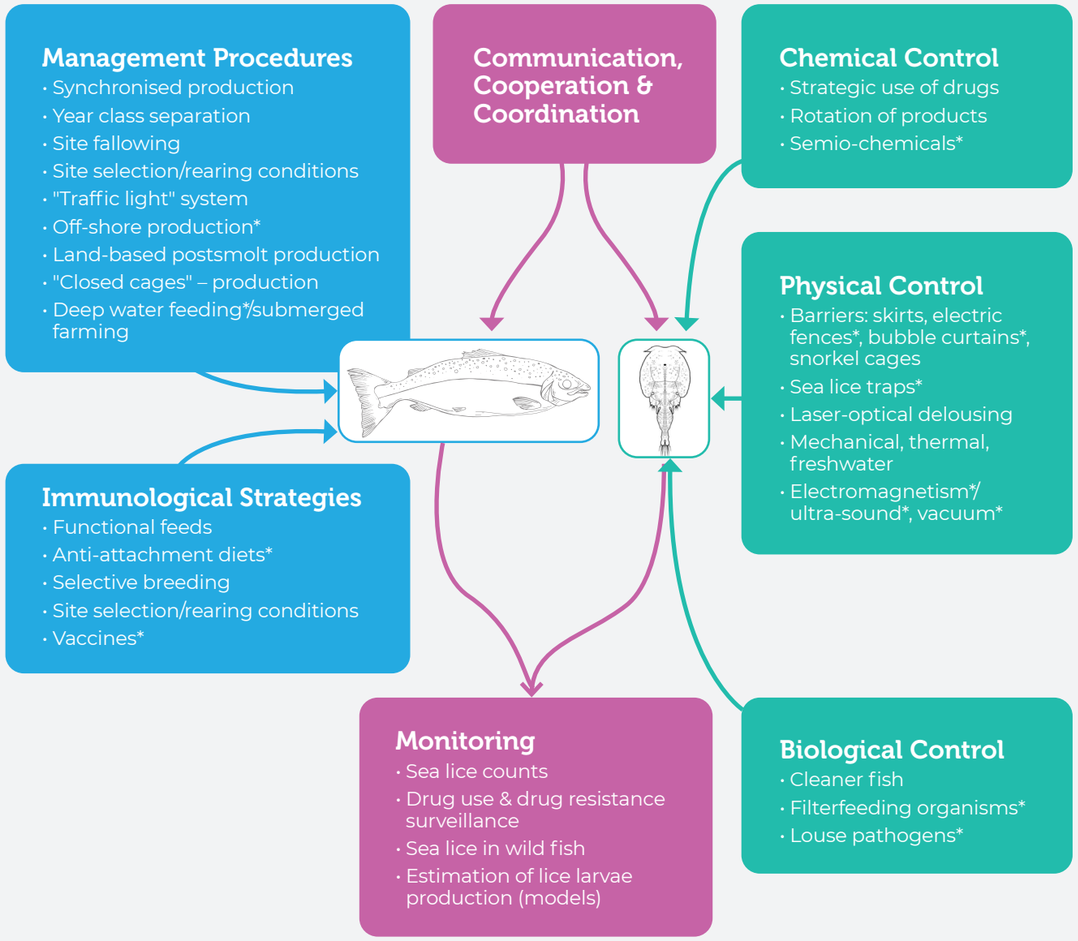

Figure 4. Toolbox for integrated pathogen management of sea lice in Atlantic salmon farming. *Strategies for future development. Drawings: *Salmo salar* from Linnaeus, 1758. commons.wikimedia.org/wiki/File:Salmo_salar_Linnaeus_1758_Fig_123_(Matschie_et_al._1909).svg. Fig. 123; *Lepeophtheirus salmonis* redrawn by Dr. F. E. Montero (UV) from Whelan, K., 2010. A review of the impacts of the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837) on wild Salmonids. Atlantic Salmon Trust, 1–27, Fig. 5.1. Adapted from Sitjà-Bobadilla A, Oidtmann B, 2017.

References: Torrissen, O., *et al.,* (2013). Salmon lice – impact on wild salmonids and salmon aquaculture. *J. Fish Dis.* 36 (3): 171-194.

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Global Salmon Initiative (2019). New approaches to sea lice management currently under development/investigation. Available at: globalsalmoninitiative.org/en/what-is-the-gsi-working-on/biosecurity/non-medicinal-approaches-to-sea-lice-management/

Sitjà-Bobadilla A, Oidtmann B (2017) Integrated Pathogen Management Strategies in Fish Farming, Chapter 5, In: Jeney, G. (Ed.). (2017). Fish Diseases: Prevention and Control Strategies. Academic Press. eBook ISBN: 9780128045855, Paperback ISBN: 9780128045640

2. Fish farmer's guide to combating *Neoparamoeba perurans* infections

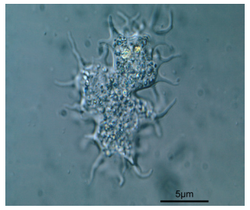

Figure 5. *Neoparamoeba perurans* showing extension of multiple tentacle-like pseudopodia (photo: James Bron, University of Stirling)

Introduction

Amoebic Gill Disease (AGD) is caused by the free-living marine amoeba *Neoparamoeba perurans* (Figure 5), and is responsible for mortalities, welfare impacts and severely reduced production outcomes for a number of cultured fish species. In particular, the parasite affects farmed Atlantic salmon (*Salmo salar*), but it also infects Coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), turbot (*Scophthalmus maximus*) and ayu *(Plecoglossus altivelis)*. A number of cleaner fish species used for biological control of sea lice in Atlantic salmon aquaculture are also affected, including ballan wrasse (*Labrus bergylta)*, corkwing wrasse (*Symphodus melops)* and lumpfish (*Cyclopterus lumpus)*. Infection by *N. perurans* may also contribute to the wider disorder termed Complex Gill Disease (CGD), which is believed to result from the impact of a diverse range of pathogens and adverse environmental conditions.

Free-living *N. perurans* can be common in the marine environment. However, under suitable environmental conditions (including high water temperature and salinity) they may attach to fish gills, where their presence can cause pathology, including visible white raised lesions (Figure 6), usually beginning at the base of gill filaments and scattered across the gill arch. Excessive mucus secretion can also be observed when routine gill examinations are performed on infected fish. Fusion of gill filaments due to an excessive tissue growth response (hyperplasia), which is clearly evident in histology (Figure 7), reduces the gill surface area available for respiration and can therefore cause a range of clinical behavioural signs including respiratory distress, an increased rate of opercular movement and lethargy.

Figure 6. Excised AGD-affected gill from Atlantic salmon showing white lesions (circled) caused by *N. perurans* (photo: Sophie Fridman, University of Stirling)

Biological life cycle

The parasite takes three forms according to conditions. In the water column, the parasite extends pseudopodia in all directions in order to keep it floating, while on a substrate the parasite becomes more two-dimensional spreading pseudopodia around its edges to move and capture food. Finally, under adverse conditions such as during freshwater treatment, the parasite rounds up to give a pseudocyst, which is more resistant to attack.

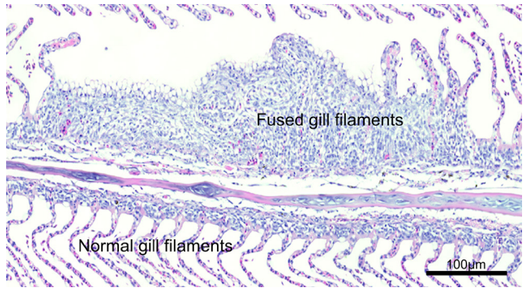

Figure 7. Section of AGD-infected salmon gill showing normal gill filaments (secondary lamellae) and filaments fused together by hyperplasia (photo: Sophie Fridman, University of Stirling)

Seasonality

AGD generally follows a seasonal pattern, beginning at water temperatures above 12°C in the Northern hemisphere, with outbreaks becoming most severe at 15°C or above.

Age / mean weight susceptibility

All marine phase salmonid sizes and ages are susceptible to infection.

Risk predisposing factors

Other risk factors are thought to include high salinity >32%, gill damage resulting from jellyfish swarms or algal blooms, prior history of gill disease, biofouling of nets, fish quality / size, farming area and incidence of AGD on other farms in the vicinity.

A) What clinical signs should alarm me?

External signs

Behavioural signs of infection relate to loss of respiratory capacity caused by the disease. These can include increased opercular movement, respiratory distress and lethargy, with impaired respiration increasingly apparent at high temperatures and during stressful events such as crowding for treatment.

Physical observations

Visual examination of gills of infected fish shows raised white lesions (Figure 6), usually beginning at the base of gill filaments and scattered across the gill arch, with the proportion of gill affected increasing with increased severity of infection. Unlike similar lesions seen in proliferative gill disease, white patches seen in AGD may be removed by light brushing with finger. At higher severity, gills will show evidence of excessive mucus secretion.

B) How to detect the parasite at farm level

Monitoring plan (what to measure and how often) and trigger level for action

Routine daily monitoring of fish may reveal some of the behavioural signs listed above, and examination of gill arches of euthanised moribund fish or fish under anaesthesia will show clear evidence of lesions indicative of the presence of the disease. To assess severity, gill scoring is normally undertaken according to the protocol of Taylor *et al.* (2016), which scores gills from 0 (clear) to 5 (extensive lesions) by examination of all hemibranchs. Presence of the parasite can be confirmed by light microscopy of on-site gill smears, histology of sampled fish tissue or qPCR of material from gill swabs or biopsies. Specific monitoring for AGD should be undertaken more frequently as temperatures rise above 12°C and treatment instigated at low gill scores (1-2) in order to pre-empt more serious infection. qPCR of gill swabs is the most sensitive detection method and provides an estimate of level of infection for following infections and triggering treatment. It should be noted when sampling that amoebal numbers are not always highest at the site of lesions.

2. Recommendations for the submission of samples to be diagnosed

The target organ for the infection is gills. Gill hemibranchs or smaller samples can be formalin fixed (10% NBF, weight/volume 1:10) for histopathological examination, while gill swab samples or gill biopsies for qPCR confirmation of presence and levels of *N. perurans* on gills may be placed in ethanol/RNAlater (weight/volume 1:10) for PCR or qPCR testing.

3. Contact laboratories

Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK; Institute of Aquaculture, University of Stirling, UK.

C) Action plan

1. Farm management for prevention

Prevention and mitigation of the disease may be improved through adoption of a tailored farm management strategy and development of an appropriate production plan. Factors to be considered are temperature profile of the water over the production cycle, previous history of infection at site, presence in the site vicinity of other farms subject to infection, health / susceptibility of stock (e.g. presence of other diseases such as sea lice) and additional risks such as algal blooms and jellyfish. Close monitoring of fish (particularly during periods of high water temperature) is key to control of AGD, with treatment decisions being made rapidly when low gill scores / qPCR levels indicative of low infection are obtained. Good site hygiene (including maintenance of clean nets) and good health and welfare practices can help reduce risks of severe infections. When clinical outbreaks with increased mortality due to AGD occur, best management practices include reduction of stressors (minimising handling and possibly reducing feeding) and maintenance of high water quality parameters.

2. Treatment

Two approaches are used to treat AGD within the aquaculture industry. One involves the use of a 3-4 hour freshwater or low salinity water bath, although some areas have limited access to freshwater. In areas where high water temperatures are less common, freshwater bathing can be substituted with the use of the oxidant hydrogen peroxide. However, this chemical can cause major safety problems at higher temperatures or when treatment is applied to fish that are already compromised by advanced AGD and hence timings and concentrations must be closely controlled. Freshwater may be replaced by low salinity water (3%) to help protect cleaner fish if present, and soft water is more effective than hard water in killing amoebae.

3. Management of co-infections

During warmer months, AGD often occurs as a co-infection with sea lice *Lepeophtheirus salmonis* and *Caligus elongatus*. Both AGD and sea lice may be controlled using freshwater or hydrogen peroxide baths, although for the latter treatment concentrations are normally higher for sea lice. Critically, reduced respiratory capacity associated with impaired gill function in AGD should be taken into account when subjecting fish to stressful events (which involve handling) such as sea louse treatments.

References: Taylor, R., *et al.,* (2016). Gill Score Guide - Amoebic Gill Disease (AGD) management training document. Obtainable from: researchgate.net/publication/319978353_Gill_Score_Guide_-_Amoebic_Gill_Disease_AGD_management_training_document

3. Fish farmer's guide to combating *Ichthyophthirius multifiliis* infections

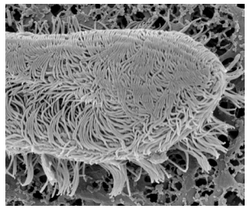

Figure 8. Scanning electron microscope image of *Ichthyophthirius multifiliis* (photo: Ole S. Møller, University of Copenhagen)

Introduction

I. multifiliis is a freshwater parasite able to infect all freshwater fish tested so far. It causes problems both in flow-through systems and in recirculated systems. It causes a disease commonly referred to as *white spot disease* due to the macroscopically visible trophonts in the skin and fins (Fig. 9). It can survive in the temperature range from 1 to 30 °C, but as a thermophilic species it needs temperatures above 15 °C to propagate fast and efficiently.

The main species affected in European aquaculture are rainbow trout, Atlantic salmon, perch, pikeperch, European eel, common carp, and European catfish. Although the infection has been considered one of the worst parasitic diseases in these species due to the frequent use of earth pond systems for culture, it is now even more serious due to the use of recirculation systems in which infective parasitic stages become continuously recirculated. This can cause a major increase in the level of fish exposure to the parasite.

Biological life cycle

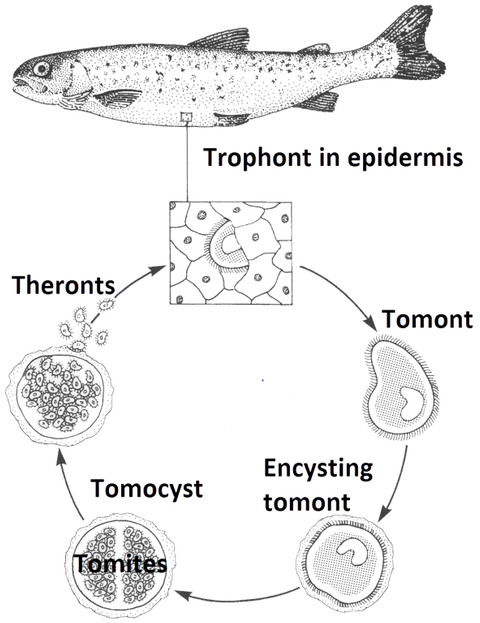

Figure 10. Life cycle of *Ichthyophthirius multifiliis* showing the trophont in the fish skin, the released tomont, the encysted tomocyst and the tomites which are released as theronts. From Buchmann & Bresciani (2001)

I. multifiliis is a protozoan parasite, which means that it is a single-celled parasite. It is covered with numerous hair-like cilia (Figure 8), firmly attached to its external cell membrane and belongs to the taxonomic group termed Ciliophora (translation: organisms carrying cilia). The beating of these cilia allow the parasite to move and swim. The parasite has a characteristic horseshoe shaped nucleus and several micronuclei. The genus comprises only one species and it has its own family, Ichthyophthiriidae, which also includes its marine counterpart *Cryptocaryon irritans* (which is strictly marine and needs salinities near 30 ppt). Many other parasites are relatively specific when they choose their host species, but *I. multifiliis* is not specific in its host choice and can infect all freshwater fish species tested so far. The life cycle of the parasite is direct, which means it can be transmitted from fish to fish. It includes a trophont stage residing in the fish surface (gill epithelia, skin and fin epidermis). This stage is the feeding stage which continuously ingests cellular debris and live host cells in its epidermal location, making the parasite able to grow rapidly over a short time - depending on temperature.

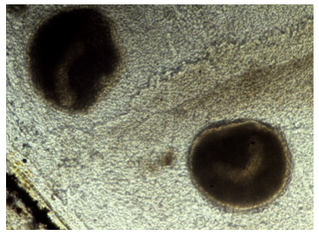

Figure 9. Trophonts of *Ichthyophthirius multifiliis* (diameter 300 µm) in the epidermis of a rainbow trout tail fin (light microscopy with subillumination). The horseshoe-shaped macronucleus is visible. (photo: Kurt Buchman, University

When the trophont has reached a certain size (100-1000 µm), it will break out of the host epidermis and swim freely as a tomont (also covered by cilia). After minutes to hours, the tomont attaches to any surface in the fishpond or fish tank and produces a thick, gelatinous cyst wall. This is termed the *tomocyst* stage. Within the tomocyst, a series of mitotic cell divisions take place and, depending on temperature, up to 1000 resulting daughter cells (tomites) are produced. These escape the tomocyst by penetrating the cyst wall, whereafter they swim in the fish tank water searching for a fish host, which they will penetrate fast and efficiently if it is naïve and non-immunized. The life cycle of *I. multifiliis* is illustrated in Figure 10, showing the trophont, the tomont, the tomocyst, the tomites and the infective theronts.

Seasonality

The life cycle is highly temperature-dependent. In open earth pond systems in Northern Europe, this means that the main disease problems appear from the month of April, when water temperature increases, until October, when temperatures decrease. However, the use of recirculated systems, also at higher latitudes, ensures a rather high mean temperature throughout the year and therefore the parasite is a major concern across all seasons.

Age / mean weight susceptibility

All age classes from the yolk sac larva via the fry to adult fish are susceptible to infection. However, a fish surviving a moderate infection is able to develop immunity against re-infection.

Risk predisposing factors

Water temperatures between 15 and 30 °C increase the risk of spreading the disease, but even 10 °C allows infection. High density of hosts allows efficient transmission and likelihood of the parasite.

A) What clinical signs should alarm me?

External signs

White spots (diameter 0.5 to 1.0 mm) are visible on fins, skin and gills. Infected fish are clearly affected by the presence of the parasites in the fish surface, and may rub their surface against firm objects in the fish pond. Highly infected fish become emaciated, lethargic, anorexic and discoloured (dark).

Internal lesions

The infection induces a strong systemic stress response.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

During high-risk periods, the fish should be monitored daily. All fish tanks must be monitored. Any sign of epidermal spots should alert the personnel. Trigger level for action is observation of one trophont on the fish surface.

2. Recommendations for the submission of samples to be diagnosed

Microscopic evaluation of skin scrapings at farm level is necessary, as the disease can spread rapidly. In case no sufficient equipment and skilled personnel are available, contact laboratories for rapid diagnostic aid.

3. Contact laboratories

- Laboratory of Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark
- Institute of Aquaculture, University of Stirling, Scotland, UK
- Veterinary Medical Institute, Budapest, Hungary
- Bioloxía Celular, Dpto. de Bioloxía Funcional, Santiago de Compostela, Spain
- Università degli Studi di Udine, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Sezione di Patologia Veterinaria, Udine, Italy
- Instituto de Acuicultura Torre de la Sal (IATS), CSIC, Castellón, Spain

C) Action plan after diagnosis

1. Prevention

Daily addition of hydrogen peroxide containing auxiliary products such as peracetic acid or sodium percarbonate will kill infective theronts and thereby decrease infection pressure. Formalin may be applied in case these more environmentally friendly products are unavailable. Recirculated fish farm systems may sustain a high NaCl concentration (10 g/L) over 14 days in order to prevent production of theronts in tomocysts and thereby new infections.

2. Farm management

Mechanical filtration of fish tank water with mesh sizes 40-80 µm will continuously remove a part of the tomonts in the fish tanks and prevent their subsequent attachment and proliferation.

3. Treatment

No medical treatment is available for trophont stages in the fish surface. Novel biological compounds - investigated in the ParaFishControl project - such as microbial surfactants can kill all external life cycle stages.

4. Management of co-infections

The use of hydrogen peroxide containing compounds and formalin reduce the bacterial exposure of skin wounds caused when trophonts leave the fish surface.

References: Al-Jubury, A., *et al.* (2018). Impact of *Pseudomonas* H6 surfactant on al external life cycle stages of the fish parasitic ciliate *Ichthyophthirius multifiliis*. J. Fish Dis. 41: 1147-1152. Doi: 10.1111/jfd.12810
Buchmann, K. (2019). Immune response to *Ichthyophthirius multifiliis* and role of IgT. Parasite Immunology 2019;00:e12675. DOI.org/10.1111/pim.12675 (pp 1-6).
Buchmann, K. & Bresciani, J. (2001). An Introduction to Parasitic Diseases of Freshwater Trout. DSR Publishers. ISBN 87 7432 580 9

4. Fish farmer's guide to combating *Saprolegnia parasitica* infections

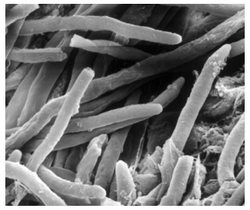

Figure 11. *Saprolegnia* sp. hyphae on Atlantic salmon fry. Scanning electron micrograph (photo: Kurt Buchman, University of Copenhagen)

Introduction

Saprolegnia parasitica is a fungal-like oomycete (order Saprolegniales, family *saprolegniaceae*) infecting a wide range of freshwater fish species (eggs, yolk sac-larvae, fry, juveniles, adults including spawners). Susceptible aquaculture host species include Atlantic salmon, rainbow trout, brown trout and common carp. All types of aquaculture hatchery and production systems may be affected, including ponds, flow-through systems and recirculated systems.

S. parasitica is a fungal-like filamentous coenocytic (cytoplasma with many nuclei) oomycete consisting of aseptate branching mycelia, giving a furry appearance to external lesions (Figure 11).

Biological life cycle

Mycelia produce terminal sporangia releasing biflagellate zoospores which subsequently encyst to cytospores. These will excyst and release secondary zoospores which may give rise to extensive branched mycelia. Sexual reproduction occurs with male and female sexual structures (oogonia and antheridia) on the same mycelium leading to production of oospores. These may germinate and establish new mycelia. Sporangia, oogonia and antheridia are separated from the remaining mycelium with septae (Figure 12).

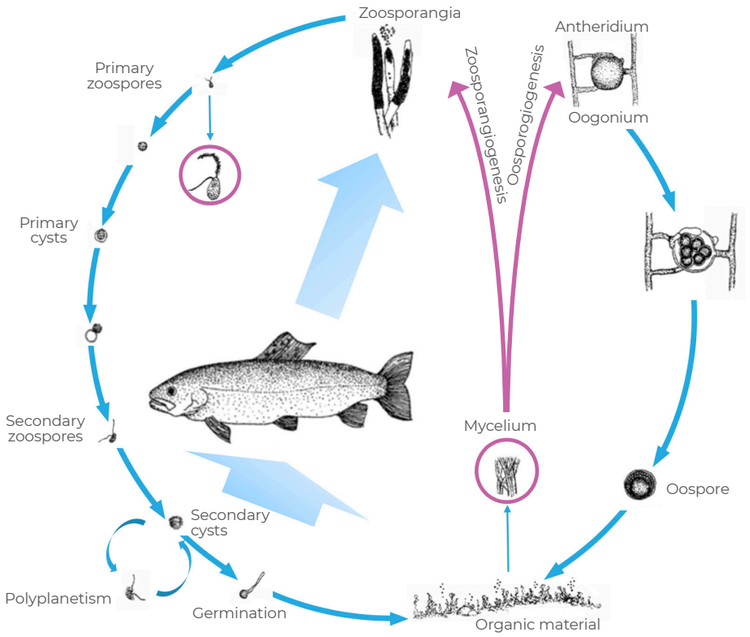

Figure 12. Involvement of fish in the life cycle of *Saprolegnia* spp.: the secondary zoospores are the most important dispersive phase and can encyst on a suitable submersed substrate or on a fish host, giving rise to mycelium made of hyphae that will produce zoosporangia. Secondary zoospores can repeat cycles of encystment and release (termed *polyplanetism*) if a new appropriate substrate is not found. Fish infection and development of lesions are influenced by several environmental, intrinsic and/or management factors.

Seasonality

This oomycete can grow between 5 °C and 37 °C in the laboratory. The infection occurs throughout the year, but lower temperatures weaken the host immune system. creating a predisposition to infection in colder months.

Age/mean weight susceptibility

All stages of freshwater fish species (eggs, yolk sac-larvae, fry, juveniles, adults including spawners) are susceptible to infection. Heavy infections are seen at all stages but the larvae and fry are more delicate with vulnerable epithelia.

Risk predisposing factors

Mechanical injuries of fish surfaces are highly predisposing as *S. parasitica* spores readily germinate in wounds. Low temperature and stressful stimuli are predisposing factors due to the associated lowering of host immunity. Stripping of eggs and milt from spawners cause skin injuries and stresses fish, such that these fish may often develop the disease within days to weeks. Similarly, stress associated with routine vaccination of salmon pre-smolts may predispose fish to saprolegniasis.

A) What clinical signs should alarm me?

External signs: Hyphal overgrowth of incubating eggs in hatching trays. Woollen coats and tufts on skin, fins and gills of fish (Figure 13).

Internal lesions: Hyphae may penetrate internal organs including the gastrointestinal tract and elicit inflammatory reactions.

Figure 13. *Saprolegnia* infected brown trout (photo: Javier Dieguez, Real Jardín Botánico, CSIC)

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Fish should be surveyed on a daily basis and any sign of the disease should trigger action including instigation of preventive measures and water cleaning.

2. Recommendations for the submission of samples to be diagnosed

Saprolegnia infections are easily detected based on the macroscopically visible tuft of hyphae but specific diagnosis may be performed by diagnostic laboratories. Tissue sampled from infected fish (containing mycelium) should be conserved in 96% ethanol and submitted to a diagnostic laboratory for molecular identification.

3. Contact laboratories

- Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK
- Institute of Aquaculture, University of Stirling, Scotland, UK
- University of Aberdeen, International Centre for Aquaculture Research and Development (ICARD), UK
- University of Bologna, Fish Pathology Laboratory of DIMEVET-UNIBO, Italy
- Laboratory of Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark
- Real Jardín Botanico, CSIC, Madrid, Spain

C) Action plan after diagnosis

1. Prevention

Keep the temperature at the optimum for the cultured species, helping to secure optimal immune status. Avoid stressing conditions, survey and secure high water quality. No commercial vaccines are available.

2. Farm management

Ozonisation of fish tank water, regular treatment with hydrogen peroxide containing compounds and continuous water filtration using fine meshed screens.

3. Treatment

Ozonisation of fish tank water, treatment with hydrogen peroxide containing compounds. Eggs in hatching trays can successfully be treated by addition of hydrogen peroxide, formalin, sodium chloride, copper compounds or iodophores. Use of the effective malachite green is banned and various anti-fungal drugs must be used with caution.

4. Management of co-infections

Co-infections should be diagnosed and specific treatments should be instigated.

References: Alderman, D. J. (2008). Fungal diseases of fish. In: Eiras, J. C., Segner, H., Wahli, T., Kapoor, B. G. (eds.). Fish diseases. Vol. 1. 279-349. Bruno, DW & Wood, BP (1999). *Saprolegnia* and other oomycetes. In: PTK Woo and DW Bruno (eds): Fish diseases and disorders, Vol. 3. CABI Publishing, Oxon, UK.,

5. Fish farmer's guide to combating *Tetracapsuloides bryosalmonae* infections

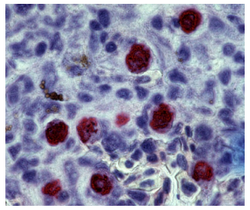

Figure 14. Histology-microscope photo of *Tetracapsuloides bryosalmonae* in a rainbow trout kidney, stained red by a lectin-Fast Red technique and haematoxylin (photo: Kurt Buchmann, University of Copenhagen)

Introduction

Proliferative Kidney Disease (PKD) is a potentially fatal disease of freshwater fish caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*.

The natural hosts of the parasite are brown or brook trout, but rainbow trout (*Oncorhynchus mykiss*) is a blind host, meaning that the overt phase of *T. bryosalmonae* can infect the fish but the infection will not lead to production or release of infective spores. However, in this fish species the parasite causes the most severe disease outbreaks. Apart from rainbow trout, worldwide the clinical disease has also been reported in Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*). It has also been reported that grayling (*Thymallus thymallus*), Arctic charr (*Salvelinus alpinus*) and pike (*Esox lucius*) are susceptible to the disease, while brook trout does not exhibit clinical signs.

Biological life cycle

The life cycle of the parasite includes two separate vertebrate and invertebrate hosts. In the natural environment, such as in European rivers, malacospores (Figure 14) of the parasite in the overt phase (with four polar capsules) are released from bryozoans (such as *Fredericella sultana*) into the environment. The waterborne spores may infect fish such as brown trout (*Salmo trutta*) or brook trout (*Salvelinus fontinalis*) and, once in the fish, the parasite replicates in the kidney tissue. Covert spores (with two polar capsules) are released from the infected fish through urine into the environment and, while free in the water, the parasite again infects a bryozoan host, in which the parasite matures from covert to overt.

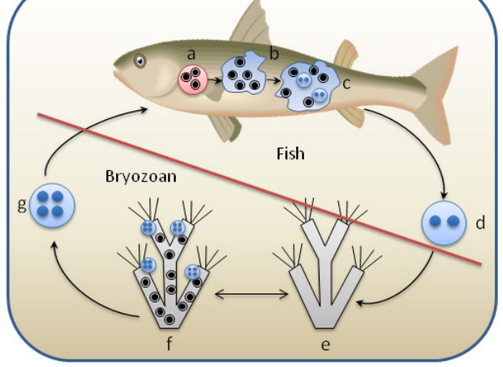

Figure 15. Schematic illustration of the life cycle of the malacosporean *Tetracapsuloides bryosalmonae*, alternating between fish and bryozoans hosts. The extra-sporogonic and pre-sporogonic stages (a-b) develop in the blood and fish tissues and sporogonic stages in the kidney (c) produce fish malacospores with two polar capsules which are released to the water (d) and infect bryozoans causing covert infections (e) of single cell stages. In overt infections (f) sacs are developed and finally malacospores with four polar capsules are produced (g), which are infective for fish. Note that cyclying can occur between covert and overt infections. Drawing by A. Sitjà-Bobadilla

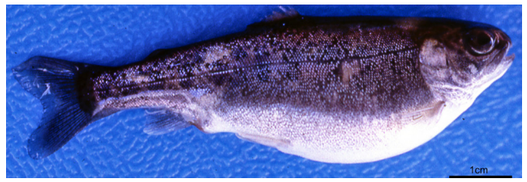

Figure 16. Rainbow trout suffering clinical PKD signs (Photo: Dr J. A. Castillo, University of Zaragoza)

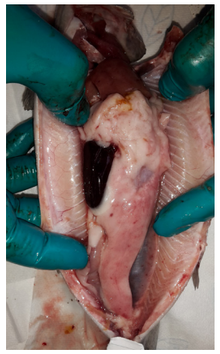

Figure 17. Clinically affected rainbow trout during a PKD outbreak showing enlarged spleen (photo: Niccolò Vendramin, Technical University of Denmark)

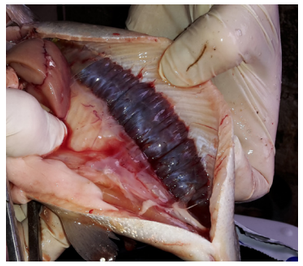

Figure 18. Clinically affected rainbow trout during a PKD outbreak showing enlarged kidney (photo: Niccolò Vendramin, Technical University of Denmark)

Seasonality

The disease generally follows a seasonal pattern, occurring at water temperatures above 15 °C in the summer and becoming less prevalent during the cold season.

Age / mean weight susceptibility

Rainbow trout are particularly susceptible to the disease and the juveniles suffer from more severe mortalities during outbreaks.

Risk predisposing factors

The main risk factor for infection is related to presence of infective stages in the water source. Aquaculture facilities, sourcing water from rivers holding wild populations of brown trout and bryozoans and where PKD outbreaks have been reported are to be considered high risk. Notably, rainbow trout that survive a PKD outbreak acquire immunity against the disease in the following season.

A) What clinical signs should alarm me?

External signs: Rainbow trout suffering clinical PKD may be darker coloured, listless, have protruding eyes, show pale, anaemic gills and abdominal swelling (Figure 16).

Internal lesions: Affected fish show enlarged kidney and spleen (Figures 17 and 18).

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Routine daily monitoring of fish may reveal some of the external signs listed above and euthanasia and dissection of moribund fish will show clear clinical internal signs. Presence of the parasite can be confirmed by histology / immunohistochemistry of sampled fish tissue and experimental work has also shown the possibility of detecting it in water by the use of e-DNA methods.

2. Recommendations for the submission of samples to be diagnosed

The target organ for the infection is kidney. Whole fish, moribund or freshly dead can be shipped to the laboratory wrapped in a plastic bag inside a styrofoam-polystyrene box with cooling elements or ice. Alternatively, kidney tissue samples from moribund fish can be collected and stored in:

- Ethanol/RNA later (weight/volume 1:10) for PCR testing
- Formalin fixed (weight/volume 1:10) for histopathological examination and immunohistochemistry

3. Contact laboratories

- DTU Veterinary Institute, Technical University of Denmark, Division for Fish Diseases, Frederiksberg, Denmark.
- Laboratory of Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark
- Institute of Aquaculture, University of Stirling, Scotland, UK
- University of Bologna, Fish Pathology Laboratory of DIMEVET-UNIBO, Italy
- University of Aberdeen, Scottish Fish Immunology Research Centre (SFIRC), UK

C) Action plan after diagnosis

1. Prevention

Prevention and control of the disease may be achieved through adoption of a tailored farm management strategy and development of an appropriate production plan. The first requirement is to define whether a farm is at high risk or low risk of infection. Factors to be considered are:

- Water source (untreated river water high risk vs ground water low risk)
- Temperature profile of the water over the year
- Presence of natural hosts in the water body supplying the farm (brown trout/brook trout and bryozoans)
- Epidemiological situation of other farms in the same catchment area
  - Do other fish farms source water from the same river?
  - Have these farms experienced PKD outbreaks?

Once the epidemiological situation is clarified, and a farm is considered to be at high risk of suffering PKD outbreaks, the main features of the disease can be used to mitigate the impact and consequences of PKD outbreak.

For a rainbow trout farm producing portion size fish, it is possible to take advantage of PKD seasonality. Therefore, the stocking period in the farm has to be properly planned. The aim is to try to expose larger fish (more resistant than small juveniles) to the end of the shedding period from the bryozoans (typically late summer) and avoid clinical outbreak of disease. In this way it is possible that the production cycle is finalized before the high-risk period of production of parasites in spring.

When a clinical outbreak with increased mortality due to PKD occurs, best management practices include reduction of stressors (including reducing feeding), maintenance of high water quality parameters (oxygen availability, removal of dead fish).

It has to be mentioned that vaccines prototypes are under development and might contribute to improve disease prevention or outcome in the future.

2. Treatment

There is at present no licensed drug for use against PKD. It is known that fumagillin, an antibiotic produced by *Aspergillus fumigatus*, when used in feed may prevent development of early infections.

3. Management of co-infections

Co-infections may occur due to immunosuppression induced by the parasite. These may be treated by licensed antimicrobials. The risk of obtaining co-infections may be lowered by vaccination.

References: Feist, S. W., *et al.*, (2001). Induction of proliferative kidney disease (PKD) in rainbow trout (*Oncorhynchus mykiss*) via the bryozoan *Fredericella sultana*, infected with *Tetracapsula bryosalmonae*. *Dis. Aquat. Org.* 45: 61-68.
Sterud, E., *et al.*, (2007). Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* (Myxozoa). *Diseases of aquatic organisms*. 77. 191-8. 10.3354/dao01846
Buchmann, K. & Bresciani, J. (2001). An introduction to parasitic diseases of freshwater trout. DSR Publishers

Other ParaFishControl Resources

1. Integrated Pest Management Strategies for Sea Lice: bit.ly/3ezeYsg
2. Integrated Pest Management Strategies for *Neoparamoeba perurans*: bit.ly/2XWqbx0
3. Integrated Pest Management Strategies for *Saprolegnia*: bit.ly/2VKDs9c

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 634429 **(ParaFishControl)**. This output reflects only the authors' view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

We would like to thank Dr Linda Andersen from the Industrial and Aquatic Laboratory (University of Bergen) for her thorough review of Figure 4, Toolbox for integrated pathogen management of sea lice in Atlantic salmon farming. We also thank the authors of the photos that illustrate this manual and the European Association of Fish Pathologists for their help with distribution of the manual to key aquaculture stakeholders.

How to cite the guide

Buchmann K., Bron J., Dalvin S., Øvergård A. C., Vendramin N. (2020). Fish farmer's guide to combating parasite infections in salmonid aquaculture. A series of ParaFishControl guides to combating fish parasite infections in aquaculture. Guide 1. Edited by Sitjà-Bobadilla, A. & Bello-Gómez, E. e-NIPO: 833-20-077-6, 2020, 22 pp.

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