

INFECTION WITH *GYRODACTYLUS SALARIS*

1. Scope

Infection with *Gyrodactylus salaris* means infection with the pathogenic agent *Gyrodactylus salaris* (*G. salaris*) of the Genus *Gyrodactylus* and Family *Gyrodactylidae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Several strains or clades of *Gyrodactylus salaris* have been identified on the basis of genotyping with the mitochondrial cytochrome oxidase 1 (CO1) marker (Hansen et al., 2003; Hansen et al., 2007b; Meinilä et al., 2002; Meinilä et al., 2004). Although there does not seem to be any correspondence between strains as identified by CO1 and pathogenicity (Hansen et al., 2007a), all strains recovered from Atlantic salmon that have been studied in laboratory experiments, so far, are highly pathogenic to strains of Atlantic salmon. Strains non-pathogenic to salmon have been recovered from non-anadromous Arctic charr (*Salvelinus alpinus*) in Norway (Olstad et al., 2007a; Robertsen et al., 2007) and from rainbow trout (*Oncorhynchus mykiss*) in Denmark (Jørgensen et al., 2007; Lindenstrøm et al., 2003).

2.1.2. Survival outside the host

Survival of detached parasites is temperature dependent, e.g. about 24 hours at 19°C, 54 hours at 13°C, 96 hours at 7°C and 132 hours at 3°C (Olstad et al., 2006). Likewise, survival attached to a dead host is temperature dependent: maximum survival times for *G. salaris* can survive on dead Atlantic salmon are 72, 142 and 365 hours at 18, 12 and 3°C, respectively (Olstad et al., 2006).

2.1.3. Stability of the agent (effective inactivation methods)

Gyrodactylus salaris is known to survive between temperatures of 0°C to 25°C. Tolerance to temperatures above 25°C is unknown. It is not resistant to freezing. *Gyrodactylus salaris* is sensitive to desiccation. *Gyrodactylus salaris* dies after a few days at pH≤5. It is more sensitive to low pH (5.1<pH<6.4) in association with aluminium and zinc than the host Atlantic salmon (Poléo et al., 2004; Soleng et al., 1999) (see also Section 2.4.2).

2.1.4. Life cycle

Gyrodactylus salaris is an obligate parasite with a direct life cycle. Parasites give birth to live offspring, and there are no other life stages.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing a species as susceptible to infection with *G. salaris* according to Chapter 1.5. of the *Aquatic Animal Health Code (Aquatic Code)* include: Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), North American brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *G. salaris* according to Chapter 1.5. of the *Aquatic Code* include: none known.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have identified *G. salaris* on the following organisms, but a long-term active infection has not been demonstrated: [Under study].

2.2.3. Susceptible stages of the host

All stages of the host are susceptible but mortality has only been observed in fry and parr stages.

2.2.4. Species or subpopulation predilection (probability of detection)

Not applicable.

2.2.5. Target organs and infected tissue

Gyrodactylus salaris usually occurs on the fins of infected Atlantic salmon, but the parasite distribution on the host may vary depending on intensity of infection (Jensen & Johnsen, 1992; Mo, 1992). Parasites are also commonly found on the body and less commonly on the gills. On other hosts, the distribution may be different, but in general the parasite is relatively less abundant on the fins and relatively more common on the body compared with Atlantic salmon.

2.2.6. Persistent infection

Not applicable.

2.2.7. Vectors

Not applicable.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Gyrodactylus salaris has spread between rivers and farms mainly by the translocation of live fish. Fish migrating through brackish water can also spread the parasite between rivers (see also Section 2.3.5). Populations located near infected rivers are at great risk of infection if they are located within the same brackish water system. If *G. salaris* introduced into a farm with Atlantic salmon, there is a high probability that all fish in the farm will become infected, depending on the layout of the farm.

2.3.2. Prevalence

Prevalence in susceptible strains of Atlantic salmon reaches close to 100% in parr in rivers (Appleby & Mo, 1997; Johnsen & Jensen, 1991); similarly prevalence in farmed Atlantic salmon (in freshwater) rises to close to 100% within a short time after introduction of the parasite. Prevalence in resistant strains in rivers and farms is unknown. Prevalence in other susceptible species is usually much lower and can be below 10% (e.g. in farmed rainbow trout; Buchmann & Bresciani, 1997).

2.3.3. Geographical distribution

Gyrodactylus salaris is restricted in its distribution to Europe. It has been recovered from farmed Atlantic salmon or farmed rainbow trout in several (mainly northern) European countries. In the wild, the parasite has been found on wild salmonids, mainly Atlantic salmon parr, in rivers in Russia, Sweden and Norway. Infection with *G. salaris* is more common in farmed rainbow trout than previously thought, and is likely to be present in more countries than those currently known. In 2006, infection with *G. salaris* was reported from fish farms in Italy (Paladini et al., 2009) and, in 2007, from fish farms in Poland (Rokicka et al., 2007) and Macedonia (Zietara et al., 2007). In 2009, *G. salaris* was identified by the OIE Reference Laboratory, from fish farms in Romania. The United Kingdom and Ireland have been demonstrated to be free of the parasite.

2.3.4. Mortality and morbidity

Mortality in farmed Atlantic salmon fry and parr can be 100% if not treated. Mortality in wild Atlantic salmon fry and parr in Norwegian rivers can be as high as 98%, with an average of about 85%. Mortality in other susceptible host species is usually low or not observed.

2.3.5. Environmental factors

Although *G. salaris* mainly lives in fresh water, it reproduces normally at salinities up to 5–6 ppt. Survival at higher salinities is temperature dependent. For example at 1.4°C, *G. salaris* may survive for 240 hours, 78 hours and 42 hours at 10 ppt, 15 ppt and 20 ppt salinity, respectively, while at 12°C it may survive for 72 hours, 24 hours and 12 hours at the same three salinities, respectively (Soleng & Bakke, 1997).

2.4. Control and prevention

2.4.1. Vaccination

Vaccines are not available.

2.4.2. Chemotherapy

Gyrodactylus salaris is sensitive to changes in the chemical composition of the water. It is sensitive to the most commonly used chemicals for bath treatment of farmed salmon parr and salmon eggs (e.g. high salinity salt water, formaldehyde and compounds containing chlorine and iodine). Furthermore, *G. salaris* is sensitive to acidic solutions (pH 5.0–6.0) of aluminium sulphate ($[Al_2(SO_4)_3]$) (Soleng et al., 1999). As aluminium sulphate is less toxic to fish than to *G. salaris* in moderately acidified waters, this chemical has been used in attempts to eradicate the parasite from river systems in Norway.

2.4.3. Immunostimulation

Immunostimulation is not available.

2.4.4. Resistance breeding

In laboratory experiments, selected breeding has resulted in increased survival among the offspring (Salte et al., 2010). However, selected breeding has not been applied to wild salmon stocks, mainly because the stock will remain infected and thus the parasite may spread to more rivers.

2.4.5. Restocking with resistant species

Restocking with resistant strains of Atlantic salmon (e.g. Baltic Neva strain) in affected rivers is not compatible with existing strain management of Atlantic salmon.

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Eggs that are transferred from infected farms should be disinfected (iodine-containing compounds have been used).

2.4.8. General husbandry practices

The general recommended husbandry practices for avoiding the spread of infective agents between units in freshwater fish farms apply to *G. salaris*. Equipment (e.g. fish nets) used in one unit should not be used in another without adequate disinfection.

3. Sampling

3.1. Selection of individual specimens

In cases where sampling is performed and infection is not suspected, a random sample with an adequate number of fish should be taken from, for example, a river. In farms, if fish show clinical signs of infection (as described in Section 4.1.1), these fish should be selected.

3.2. Preservation of samples for submission

Fish should be killed immediately and should not be allowed to dry out before preservation. Whole fish should be preserved in 80–100% EtOH in bottles large enough to provide excess space and preservative. The concentration of EtOH after preservation should not be below 70%. As a rule of thumb this concentration is obtained if the proportion of fish to EtOH does not exceed 1:9. If the concentration is lower, the mucous and epidermis may disintegrate and *Gyrodactylus* specimens, even if they are preserved, may drop off. Bottles should have an opening wide enough to avoid the possibility of scraping off *Gyrodactylus* specimens when fish are put into the bottle or when taken out for examination. Bottles should be stored in a horizontal position until the tissue is fixed/preserved to prevent the fish curling. This facilitates examination of the fish as they can easily be turned with a pair of forceps under the microscope. When preservation of the fish is complete, the bottles can be stored in a vertical position.

As *G. salaris* is common on fins of Atlantic salmon, fins cut off from the body and stored in EtOH as described above can also be submitted. This is especially suitable for larger fish and under field conditions where, for example, transport is limited.

3.3. Pooling of samples

Samples can be pooled, although each fish is subsequently examined and analysed separately. Fins of fish from a farm or a river can be pooled and are also examined and analysed separately, but in this instance each fin cannot be related to a certain fish host.

3.4. Best organs or tissues

Fish can be examined as whole specimens either live under anaesthesia (for example, with MS222), freshly killed, or preserved. In addition, fresh or preserved fins can be examined. The same examination method (see Section 4.3.1) is used in all cases. Examination of live, anaesthetised fish is very time-consuming and not recommended.

Instead of examining the whole fish, the fins can be examined (by the method described in Section 4.3.1). When Norwegian salmon parr are infected, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nostrils, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and seems to vary among salmon strains.

3.5. Samples/tissues that are not suitable

Dead fish, stored on ice, are not acceptable for *Gyrodactylus* examination, even if the fish are kept separately in plastic bags, etc. The parasites quickly die if not covered in water, and as these parasites do not have an exoskeleton, dead parasites disintegrate quickly. If such dead fish are rinsed in water, *Gyrodactylus* specimens may be found in the sediment. However, if specimens are not found in the sediment, it cannot be concluded that the fish were uninfected. Formaldehyde-fixed *Gyrodactylus* specimens are difficult to identify morphologically and are unsuitable for DNA analysis.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Usually there are no clinical signs in fish with one or up to a few tens of parasite specimens.

In the early disease phase, increased flashing (fish scratch their skin on the substrate) is typical. Later, fish may become greyish because of increased mucous production and the fins may be eroded. Diseased fish are lethargic and are usually found in slower-moving water.

4.1.2. Behavioural changes

Flashing is common among moderate to heavily infected farmed fish as they scratch their skin on the bottom or wall of a tank or pond. Heavily infected fish may have reduced activity and stay in low current areas.

4.2. Clinical methods

4.2.1. Gross pathology

Heavily infected fish may become greyish as a result of increased mucification, and at a later stage the dorsal and pectoral fins may become whitish as a result of increased thickness (mainly hypertrophy) of the epidermis.

Heavily infected fish may have eroded fins, especially dorsal, tail and pectoral fins, because of parasite feeding.

Secondary fungal infections (*Saprolegnia* spp.) are commonly observed in fish with infection with *G. salaris*.

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Not applicable.

4.2.4. Wet mounts

Scrapings (wet mounts) from skin or fins can be used to detect *Gyrodactylus* specimens on infected fish. In these cases, with high intensity infestation, hundreds or thousands of *Gyrodactylus* specimens are present all over the body and fins. Preparations of wet mounts are usually not suitable for identification of *Gyrodactylus* to the species level and other preparations for morphological or DNA analysis must be made (see below). If the number of *Gyrodactylus* specimens is low, the chances of detecting the parasites by scrapings are limited.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

Not applicable.

4.2.7. Electron microscopy/cytopathology

Not applicable.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

Detection of *Gyrodactylus* and identification of *G. salaris* is a two-step process. Firstly, parasite specimens are observed using optical equipment and secondly, parasites are identified, usually on an individual basis using other equipment and methods.

Optical equipment must be used to detect *Gyrodactylus*. In the case of a suspected outbreak of infection with *G. salaris* where only light microscopy is available, wet mounts can be used to detect *Gyrodactylus* specimens. However, it is strongly advised not to use this method in a surveillance programme as the presumed specificity and sensitivity is low (value not known) and, therefore, the number of fish examined is high.

Fish can be examined as live whole specimens (under anaesthesia), freshly killed or preserved. The same examination method (see below) is used in all cases. Examination of live, anaesthetised fish is very time-consuming and not recommended. *Gyrodactylus* specimens fixed in formaldehyde are also very difficult to identify and are not suitable for DNA analysis. Instead of examining the whole fish, the fins can be examined (by the method described below). When pair of susceptible Atlantic salmon strains are infected, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nares, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and the distributions also seem to vary among salmon strains.

Live anaesthetised fish, freshly cut fins or EtOH-preserved fish or fins should be examined under a binocular dissecting microscope with good illumination. The fish should be placed in a box and completely covered in fresh water. Preserved fish can also be examined in EtOH. Living parasites are more easily detected by their movements, thus disturbing light refraction on the skin of the fish should be avoided. Live *Gyrodactylus* are colourless while EtOH-preserved *Gyrodactylus* specimens are usually only slightly opaque. If the dissecting microscope is illuminated from above, the bottom of the microscope stage should be black. This will increase the contrast and the parasites will be detected more easily. The whole surface of the fish, including gills and mouth cavity, must be examined. It is best to use two forceps for this process. The fins of relatively small fish, usually less than 10 cm, can also be studied using illumination through the bottom of the microscope stage. This way, *Gyrodactylus* specimens on the fins can usually be easily observed.

4.3.1.1. Microscopic methods

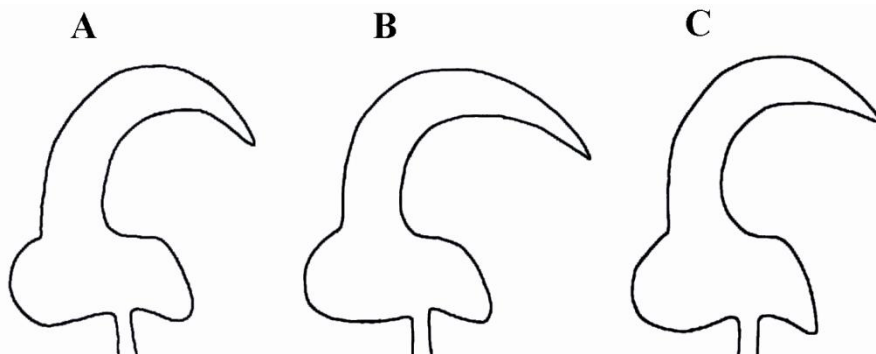
Identification of *Gyrodactylus* species is based on morphology and morphometry of marginal hooks anchors (hamuli) and bars in the opisthaptor (the attachment organ). Good preparation of specimens is a prerequisite for species identification.

Digestion of the soft tissue, leaving the hard parts only, is recommended when high-resolution morphometrics is required for reliable morphometric diagnosis. The soft tissue can be digested in a solution (approx. 1 µl) of 75 mM Tris, 10 mM EDTA (ethylenediamine tetra-acetic acid), 5% SDS (sodium dodecyl sulphate) and 100 mg ml⁻¹ proteinase K, pH 8.0. After adding the digestion solution, the reaction should be inspected in the microscope until completion and then ended by adding a stop solution (1:1 glycerol and 10% neutral buffered formalin). The procedure for digestion is described in detail in Harris et al., 1999. Identification of *G. salaris* should be in accordance with references: Cunningham et al., 2001; Malmberg, 1957; Malmberg, 1970; McHugh et al., 2000; Olstad et al., 2007b; Shinn et al., 2004.

The size of the opisthaptor hard parts in *Gyrodactylus* varies extensively with, for example, temperature, whereas shape is more stable (Mo, 1991a; Mo, 1991b; Mo, 1991c). The capability of linear measurements to capture morphology might therefore not always be sufficient for reliable diagnosis (Olstad et al., 2007b).

Gyrodactylus salaris is morphologically similar to *G. teuchis* from brown trout, Atlantic salmon, and rainbow trout, and to *G. thymalli* from grayling (Figure 1). The species can be differentiated by trained morphologists on the basis of the shape of the marginal hook sickle. *Gyrodactylus teuchis* has a longer and more constantly curved sickle blade, while *G. thymalli* has a small angle on the shaft of the sickle (Cunningham et al., 2001; McHugh et al., 2000; Shinn et al., 2004).

Fig. 1. Marginal hooks of (A) *Gyrodactylus salaris*, (B) *G. teuchis* and (C) *G. thymalli*. Drawings are modified after Cunningham et al., 2001.



4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable.

4.3.1.1.3. Fixed sections

Not applicable.

4.3.1.2. Agent isolation and identification4.3.1.2.1. *Cell culture/artificial media*

Not applicable.

4.3.1.2.2. *Antibody-based antigen detection methods*

Not applicable.

4.3.1.2.3. *Molecular techniques*4.3.1.2.3.1. *Preparation of samples*

Template DNA should be prepared from live/fresh or EtOH-preserved specimens using a suitable DNA preparation protocol. DNA extraction kits may be used according to the manufacturers' instructions.

4.3.1.2.3.2. *Analysis of the ribosomal RNA gene internal transcribed spacer region*4.3.1.2.3.2.1. *Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS)*

For amplification of a 1300 base pair product of the ITS-region, primers, such as 5'-TTT-CCG-TAG-GTG-AAC-CT-3' and 5'-TCC-TCC-GCT-TAG-TGA-TA-3', may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 30 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes (Cunningham, 1997). If partially degraded material is analysed, the ITS1 and ITS2 spacers can be amplified in two separate reactions using primer sets and PCR conditions described in Matejusová et al., 2001.

4.3.1.2.3.2.2. *ITS sequencing and sequence analysis*

Amplified ITS fragments prepared as in Section 4.3.1.2.3.2.1 above should be sequenced and the sequences subjected to a BLAST search in GenBank/EMBL to establish identity with known sequences. In addition to the PCR primers, at least two internal primers should be used such as; 5'-ATT-TGC-GTT-CGA-GAG-ACC-G and 5'-TGG-TGG-ATC-ACT-CGG-CTC-A (Zietara & Lumme, 2003). Several sequences of other species infecting salmonids, e.g. *G. derjavini*, *G. derjavinooides*, *G. truttae*, *G. teuchis* and *G. thymalli* are available in GenBank/EMBL. *Gyrodactylus salaris* and *G. thymalli* cannot be distinguished by this method, but sequences of ITS distinguishes *G. salaris* and *G. thymalli* from all other known species.

4.3.1.2.3.3. *Analysis of the mitochondrial cytochrome oxidase I gene*4.3.1.2.3.3.1. *PCR amplification of the mitochondrial cytochrome oxidase 1 (CO1) gene*

For amplification of the CO1-gene, the primers 5'-TAA-TCG-GCG-GGT-TCG-GTA-A-3' and 5'-GAA-CCA-TGT-ATC-GTG-TAG-CA-3' (Meinilä et al., 2002) may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes. Additional primer sets for amplification of CO1 can be found in references: Meinilä et al., 2002; Meinilä et al., 2004.

4.3.1.2.3.3.2. *CO1 sequencing and sequence analysis*

Amplified CO1 fragments prepared as described above should be sequenced and compared with other sequences using a BLAST search in GenBank/EMBL. In addition to the PCR primers, at least two internal primers can be used, such as 5'-CCA-AAG-AAC-CAA-AAT-AAG-TGT-TG-3', and 5'-TGT-CYC-TAC-CAG-TGC-TAG-CCG-CTG-G-3' (Hansen et al., 2003).

If the obtained sequence does not have a 100% match in GenBank/EMBL, a phylogenetic analysis should be performed to establish the relationship to other available sequences. Different clades of *G. salaris* and *G. thymalli* can be distinguished with this method.

NOTE: CO1 sequences cannot unambiguously differentiate between *G. salaris* and *G. thymalli* but can be used to assign specimens to a clade. Clades of *G. salaris* and *G. thymalli* generally correspond well to host preferences and/or the geographical distribution of the parasites, with a few exceptions.

Note that some researchers have chosen to submit all their sequences from both Atlantic salmon and grayling as *G. salaris*, causing confusion when comparing sequences (both ITS and CO1) with those in

GenBank/EMBL in a BLAST search. Host identity of sequences in GenBank/EMBL should thus always be checked.

Where the sequence is not assigned to one of the recognised clades of *G. salaris* or *G. thymalli*, advice should be sought from the OIE Reference Laboratory.

4.3.1.2.4. Agent purification

Not applicable.

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

Not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with *Gyrodactylus salaris*

Diagnostic/detection methods to declare freedom are the same as those mentioned in for Section 4.3.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Observation of *Gyrodactylus* specimen(s) on Atlantic salmon or rainbow trout (or other susceptible hosts) either in skin scrapings examined in a light microscope or on fins or skin examined under a stereo-microscope.

7.2. Definition of confirmed case

Infection with *G. salaris* shall be confirmed if the following criteria are met:

- i) Morphology consistent with *G. salaris*;
Or
- ii) Molecular identification of *Gyrodactylus* specimen(s) to *G. salaris* (or *G. thymalli*) by sequencing of ITS followed by sequencing and phylogenetic analysis of CO1 to assign the sequence to the nearest known relative is preferred.

8. References

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NB: There is an OIE Reference Laboratory for infection with *Gyrodactylus salaris*
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
<http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/>).
Please contact the OIE Reference Laboratories for any further information on infection with *G. salaris*.

NB: FIRST ADOPTED IN 1997 AS GYRODACTYLOSIS OF ATLANTIC SALMON (GYRODACTYLUS F);
MOST RECENT UPDATES ADOPTED IN 2018.