

United States Department of Agriculture

Animal and Plant Health Inspection Service Hazard Identification: *Gyrodactylus salaris*

Veterinary Services

Strategy and Policy

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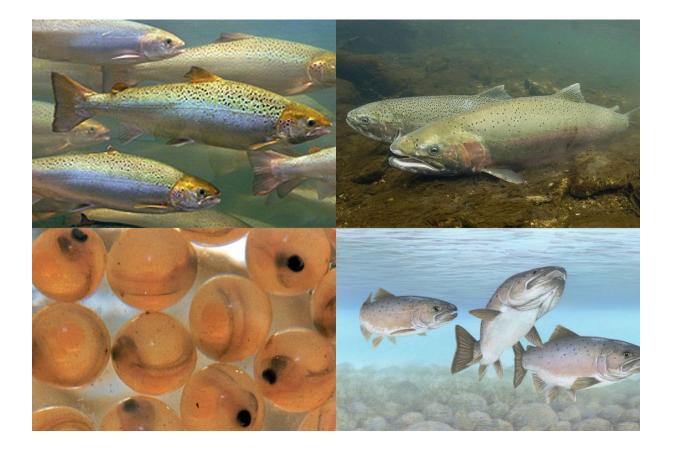


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Introduction

USDA APHIS VS CEAH was asked to generate a Hazard Identification for *Gyrodactylus salaris*. Hazard identification is a process used to identify hazards (biological, chemical, or physical agents in, or the condition of, an animal or animal product) that may result in adverse consequences in susceptible populations.¹ The hazard identification process is used to identify pathogenic agents that may be associated with importation of a commodity (live animals, products of animal origin, genetic material, biological products, or pathological material).¹ The hazard must be relevant to the imported species, and it must be determined if the hazard is a) present in exporting countries; b) present or absent in the importing country; and c) a notifiable disease or subject to control or eradication in the importing country.¹

Subjects within the scope of this document include a description of the hazard (*G. salaris*), identification of susceptible fish species and the geographic distribution of the hazard, and a summary of the epidemiology of the hazard. To conduct this hazard identification, we referenced World Organisation for Animal Health (WOAH) resources, subject matter expert consultation, and available published data and literature relative to *G. salaris* epidemiology. Knowledge and data gaps were present that affected complete evaluation of some tenets of this hazard identification document.

This document follows:

- The WOAH Handbook on Import Risk Analysis for Animals and Animal Products import risk analysis framework, which is accessible via a link in <u>Appendix, Table 1.</u>²
- WOAH criteria for determination of host species susceptibility as described in the WOAH Aquatic Animal Health Code and the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases (<u>Appendix, Table 1</u>).^{3, 4}
 - Fish species described in published literature that do not meet these criteria or in which infection was inferred using diagnostic methods that are not validated according to WOAH protocols are not included in this assessment. Briefly, species susceptibility to a pathogen requires that:
 - the experimental transmission is consistent with natural pathways of infection,
 - the pathogen is adequately identified, and
 - the presence of the pathogen in the host constitutes an infection.
- Standards in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code (<u>Appendix, Table 1</u>)^{5, 4} regarding improvement of animal health and welfare, safe international trade in aquatic animals and their products, and diagnostic approaches to disease diagnosis.
- The understanding that epidemiologically, disease occurs as an interaction occurring in environmental spaces (natural and anthropogenically influenced or derived) where host and pathogen tolerance limits for essential biotic (living) and abiotic (nonliving) environmental factors overlap. 6. 7. 8. 9
- Definitions of animal agriculture biosecurity as:

- a series of management steps and practices that identify, prevent, control, and mitigate introduction and spread of pathogens in an animal population, and spread of pathogens to other susceptible populations,¹⁰ and
- measures based on current epidemiological information and understanding of relevant knowledge and data gaps.^{11, 12, 13, 14}

Subjects that are not within the scope of this document include an assessment of potential entry and exposure pathways and summaries of likelihood, uncertainty, consequences and overall risk. This document is intended for internal USDA APHIS VS use and distribution to external stakeholders.

Key Findings

- Gyrodactylosis, caused by *Gyrodactylus salaris*, is a World Organisation for Animal Health listed notifiable disease.
- Gyrodactylosis is a reportable foreign animal disease in the United States.
 - Confirmatory testing at the National Veterinary Services Laboratory is required following first detections.
 - Molecular testing is required for definitive diagnosis.
- *Gyrodactylus salaris* is a cold-water-adapted parasite living primarily in freshwater rivers and lakes.
- All freshwater fish species are capable of serving as hosts.
 - o Duration, intensity of infestation, and development of disease is species variable.
- All salmonid fish species should be considered potentially susceptible to infestation.
 Atlantic salmon are highly susceptible to infestation and develop high mortality disease.
- Gyrodactylosis is an economically important disease of farmed rainbow trout in Europe.
 - Importation, translocation, and movement of infested farmed rainbow trout are considered primary pathways introduction into rainbow trout aquaculture throughout Europe.
- Control in natural environments requires aggressive mitigation, including eradication of all fish.
- Control in aquaculture requires depopulation, disinfection, and fallowing.
 - Stringent biosecurity measures can decrease the risk of introduction.
 - Re-introduction into fish farms and hatcheries can occur if the water supply to the farm is not secure.
- Susceptibility of wild and farmed salmonids in the United States to *G. salaris* is not described in the literature and is unknown.
- Some countries require testing live salmonid fish, eggs, and gametes for *G. salaris* prior to export from the United States.

Gyrodactylus salaris

Introduction

Gyrodactylus salaris is an environmentally and economically significant pathogen of wild Atlantic salmon and farmed rainbow trout in Europe. The WOAH Manual of Diagnostic Tests for Aquatic Animals (<u>Appendix, Table 1</u>) defines the disease gyrodactylosis as infection with the pathogenic trematode (flatworm) ectoparasite *Gyrodactylus salaris* (salmon fluke; Family Gyrodactylidae, class Monogenea, genus *Gyrodactylus*).^{5, 15}

The genus *Gyrodactylus* is comprised of a large diverse group (approximately 400 species) of viviparous (live bearing) ectoparasites capable of parasitizing fish at high population densities for long periods of time.^{16, 17, 18, 19} In general, *Gyrodactylus* spp. are considered obligate (requires a host to complete the life cycle) and host specific, parasitizing only one host or closely related host species.¹⁹ Most *Gyrodactylus* spp. do not cause disease in host fish; however, clinical signs of disease, morbidity, and mortality are associated with some North American *Gyrodactylus* spp.^{20, 19}

Gyrodactylus salaris was first described in the 1950s as an ectoparasite of Baltic Atlantic salmon which display tolerance to the parasite.^{21, 22} In the 1970s, hatchery reared Baltic salmon were translocated to Norway, resulting in introduction of *G. salaris* into freshwater river systems.²³ By 2002, 45 river systems were infested, leading to catastrophic losses of native Atlantic salmon populations and highly impactful ecological and economic consequences.^{24, 25, 26, 27} In some rivers, over 80 percent of juvenile Atlantic salmon may die due to infestation, and reductions in wild salmon fisheries catches have been estimated at over 40 percent.²⁸ In 2021, detections were reported in 51 rivers, 13 Atlantic salmon hatcheries or farms, and 26 rainbow trout hatcheries or farms.²⁹ Negative impacts on Atlantic salmon populations in rivers on the Swedish west coast and in the Keret river in Russia have been reported as well.²⁹ Detections of pathogenic and non-pathogenic strains have also been detected on Arctic char.²⁹

Gyrodactylus salaris is a reportable disease in the United States and is included on the USDA APHIS NAHRS and NLRAD lists of reportable diseases (<u>Appendix, Table 1</u>).^{1, 30} State and Federal authorities should be contacted upon suspicion or detection of gyrodactylosis (<u>Appendix, Table 1</u>).¹ Gyrodactylosis is a WOAH listed notifiable disease.¹⁵ Requirements for self-declaration of freedom from infection with *G. salaris* and maintenance of free status are described in the WOAH Aquatic Animal Health code (<u>Appendix, Table 1</u>).¹⁵

Susceptible Fish Species

Fish species listed in the WOAH Manual of Diagnostic Tests for Aquatic Animals that meet the WOAH Aquatic Animal Health Code criteria for listing as susceptible to infection with *G. salaris* are found in Table 1. ⁵. ¹⁵ In the United States, migratory and landlocked Atlantic salmon and steelhead trout, and wild and farmed rainbow trout represent the species of greatest economic concern relative to gyrodactylosis.

Table 1. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to infestation by *Gyrodactylus salaris*.^{5, 15}

Genus species	Common Name
Salvelinus alpinus	Arctic char
Salmo salar	Atlantic salmon
Salveinus fontinalis	Brook trout
Salmo trutta	Brown trout
Thymallus thymallus	Grayling
Onchorhynchs mykiss	Rainbow trout

Geographic Distribution

The native range extends from the eastern regions of the Baltic Sea to the Karelian isthmus and drainages of Onega and Ladoga lakes in Russia.^{15, 18} The parasite may also occur naturally in low numbers in some Swedish and Finnish rivers that drain into the Baltic Sea.^{31, 32} Introductions into other countries have occurred via import and translocation of infested fish. Search of the WOAH WAHIS database (<u>Appendix, Table 1</u>) for years that data was available (2005–2022) identified reports of *G. salaris* in unidentified farmed and wild fish species in Costa Rica, Finland, Norway, Sweden, and Vietnam.³³ Other countries reported in the literature include Denmark, Estonia, Georgia, Germany, Italy, Latvia, North Macedonia, Poland, Romania, Russia, and Ukraine.³² There have been no reports of this parasite in North America, including the United States.

Public Health

Gyrodactylus salaris is not a zoonotic pathogen. There are no threats to human health.^{34, 35}

Epidemiology

In this section, the epidemiology of *G. salaris* in susceptible host species (e.g., salmonid fish) is summarized. However, all epidemiological factors associated with this parasite are not fully described in available literature or data sources.

Host Characteristics

Gyrodactylus salaris infestation has been most extensively studied in salmonid fish species. Occurrence of clinical disease has only been reported in Atlantic salmon. However, all salmonid species that inhabit freshwater, or migrate to and from the ocean, are considered potentially susceptible to infestation.^{5, 15, 18}

Atlantic Salmon

Gyrodactylus salaris infestation occurs under natural conditions on Atlantic salmon that reside in or return to freshwater environments.^{32, 33} All life stages are susceptible to infection. Prevalence and abundance of the parasite and development of clinical disease are greatest in fry and parr stages and may be related to lack of immunity to the parasite.^{36, 15} Differences in susceptibility to infestation and development of clinical disease among Atlantic salmon populations has been observed.^{25, 37, 26} Baltic Atlantic salmon are described in the literature as both tolerant (the host is unharmed with no direct negative effects on the parasite) and resistant (the host is protected at the expense of the parasite), likely due to co-evolution of the parasite and salmon population.^{25, 37, 26} Atlantic salmon in other European regions (e.g., Norway, Denmark, the United Kingdom) are highly susceptible to infestation and development of clinical disease under natural and experimental conditions.^{25, 37, 26} Review of available peer reviewed literature did not find published research describing the susceptibility of North American wild or farmed Atlantic salmon to *G. salaris*.

Rainbow Trout

Gyrodactylus salaris is widely distributed in wild and farmed rainbow trout in Europe.^{38, 37, 32, 28} In some areas (e.g., Italy), gyrodactylosis is a common and economically significant disease.^{39, 32} In other regions (e.g., Sweden) infestation occurs, but clinical disease is rare.⁴⁰ The cause of this variability is unknown, but may be due to host factors or environmental conditions specific to the different farms or regions in Europe.⁴⁰ Rainbow trout exhibit reservoir host potential in that infestation, while often self-limiting, has been observed to persist for over 90 days.^{25, 40, 32} The susceptibility of wild and farmed rainbow trout populations present in North America has not been described in published literature.

Arctic Char

Arctic char are capable of serving as long-term reservoir hosts.^{25, 41, 42, 43} Asymptomatic infestation under natural conditions is common in some rivers and lakes where this species is present.^{41, 42, 32} Variable susceptibility to infestation has been reported. In some endemic areas, infestation in the absence of other suitable hosts has been observed for approximately 20 years.^{44, 43}

Brook Trout

In Europe (e.g., Romania), *G. salaris* infestation in the absence of clinical disease has been reported in farmed brook trout.⁴⁵ Experimentally, this species develops self-limiting infestation that was almost fully resolved by 70 days (the end point of the experimental study).^{46, 47} Review of published literature did not identify any studies examining the susceptibility of brook trout in North America.

Brown Trout

Gyrodactylus salaris infestations of wild and farmed brown trout in Europe have been reported in the literature.^{45, 32, 28} Susceptibility to infestation is described as limited. In Norway, infestation was noted in rivers during the first year of epizootics when infestation pressure was high and brown trout were observed feeding on dead or moribund Atlantic salmon.⁴⁰ In subsequent years, low to zero levels of infestation were detected.⁴⁰ Experimentally, some population-based variability in susceptibility has been observed.^{48, 40} Some fish were observed to be resistant to infestation while others maintained parasite presence for over 100 days.^{48, 40} The susceptibility of North American wild or farmed brown trout to *G. salaris* is not reported.

Grayling

Grayling are susceptible to *G. salaris* infestation.^{38, 28} Under experimental conditions, infestation was self-limiting up to 35 days (the end date of the study).³⁸ Grayling are also susceptible to infestation with *G. thymalli*, which is similar morphologically to *G. salaris*.³⁸ Molecular assay is required to differentiate between the two parasites.⁴⁹

Other Fish

Review of published literature finds references to detection or experimental infestation of *G. salaris* in other freshwater fish species including North American lake trout, freshwater eels, Adriatic trout, and European flounder. 50, 51, 48, 40, 32 Infestations in these species were self-limiting and did not result in clinical disease.

Environmental Characteristics

Gyrodactylus salaris is a cold-water-adapted parasite that lives in freshwater rivers and lakes. Environmental conditions influencing the presence, growth, and mortality rates of the parasite include salinity and water temperature.^{26, 40, 18} Freshwater is the optimal environment; however, the parasite can survive in brackish water for salinity and temperature dependent intervals of time. Experimentally, adult parasites exposed to water salinity levels ranging from 10–20 ppt survived for 42–240 hours at low water temperatures (1.4 °C/34.5 °F), and for 10–72 hours at water temperatures of 12 °C/53.6 °F.^{52, 26, 18} *Gyrodactylus salaris* is incapable of survival when water salinities approach that of seawater (35 ppt).^{52, 28, 18} Reproduction is water salinity and temperature dependent (5–6 ppt and 2.5–19 °C/36.5–66.2 °F, optimal temperature, 10 °C/50 °F).^{40, 15} Survival rates of detached parasites are also temperature dependent, ranging from 132 hours at 3 °C/37.4 °F to 24 hours at 19 °C/66.2 °F.^{53, 54, 15} Survivability at temperatures above 25 °C/77 °F is not reported.⁵⁵ Survival of the parasite on dead Atlantic salmon is also temperature dependent. Experimentally, survival times of 72, 142, and 365 hours have been observed at water temperatures of 18 °C/64.5 °F, 12 °C/53.5 °F, and 3 °C/37.5 °F, respectively.³³

Pathogen Characteristics

Most *G. salaris* research has focused on the distribution and pathogenicity of this parasite in salmonid populations present in Europe. More recently, genetic studies using mitochondrial DNA analysis have identified numerous *G. salaris* clades (a group of organisms believed to have evolved from a common ancestor), strains (isolates described by a combination of biological and genetic characteristics), and haplotypes (genotypes identified based on genetic testing). The different mitochondrial clades, strains, and haplotypes correspond to geography (i.e., *G. salaris* from different watersheds are genetically different).^{49, 40} Additionally, these clades, strains, or haplotypes are often linked to host specificity (e.g., haplotypes from salmon are not found on grayling and vice versa).^{25, 53, 56} Some haplotypes have strains that differ in virulence. For example, haplotype F has strains that are either pathogenic or non-pathogenic to rainbow trout.^{49, 53, 40} According to Mo (2020), this indicates that there is no established

correlation between genetic strains or haplotypes and pathogenicity. This author and others also state that there is lack of research to develop markers that can unambiguously identify pathogenic strains, that studies evaluating the pathogenicity or nonpathogenicity of the different strains and haplotypes in genetically diverse salmonid fish stocks is lacking, and that such research is required to fully elucidate which *G. salaris* strains are pathogenic in different salmonid species.^{16, 45, 40} By WOAH's definition in the Manual of Diagnostic Tests for Aquatic Animals, all *G. salaris* strains found on Atlantic salmon are highly pathogenic to that species, while strains recovered from Arctic char in Norway and rainbow trout in Denmark are not pathogenic.^{16, 57, 40, 5} Review of the literature did not identify any research exploring the susceptibility of North American Atlantic salmon or other salmonid fish to any of the identified *G. salaris* clades, haplotypes, or strains.

Genetic testing has resulted in synonomization of *G. salaris* with *G. thymalli*, a gyrodactylid parasite found exclusively on grayling.^{58, 40, 32} According to the WOAH Manual of Diagnostic Tests for Aquatic Animals, *G. thymalli* has never been observed on Atlantic salmon and does not appear to be pathogenic in this species based on experimental trials.^{49, 31, 5} As of 2023, WOAH has not accepted this synonymization^{40, 5}; therefore, it will not be described further in this hazard identification

Gyrodactylus salaris is a social (group living) ectoparasite that lives and feeds on the surface of freshwater fish hosts. Preferential feeding sites are the fins, skin, and gills (less common).^{5, 18} The distribution of the parasites in these preferential sites is affected by the intensity of the infestation.^{48, 5} Unlike many *Gyrodactylus* spp., *G. salaris* has a relatively broad host range. Most host species exhibit some symbiosis or resistance to the parasite and do not develop clinical disease associated with infestation.⁴⁵ Atlantic salmon (except for Baltic Atlantic salmon) are the only salmonid fish that develop clinical disease. According to Paladini et al. (2014), *G. salaris* may transiently attach to any freshwater fish species for a short period of time.⁴⁸ Reproduction may occur on some of these fish, but at levels insufficient for development of persistent infection.⁴⁸ When not attached to a host, *G. salaris* is not parasitic. It can survive for 5–6 days, floating in bottom sediments or in the water column, opportunistically seeking a host.^{48, 18} The parasite has no known predators.

This parasite is hermaphroditic, asexually viviparous (bears live young), and has a direct life cycle involving only one host.^{58, 26, 54, 33} The parasite gives birth to one live young at a time. The young parasite is almost as large as the parent parasite, and already has a developing embryo inside of it.^{26, 40} This reproduction strategy creates a short generation time, rapid population growth, and potential for a single parasite to elicit an epizootic under appropriate environmental and host conditions.^{58, 54} Reproduction is temperature dependent and occurs throughout the year. When temperatures are optimal the gestation is period is 24 hours. This can lead to a very high infection intensity of several thousand parasites on a single fish host.^{40, 54, 18} At the upper end of the optimal temperature range, populations can double in four days. Gestation and reproductive rates decrease in the winter due to direct effects of low temperature on the parasite and indirect host effects (decreased activity and metabolism).^{40, 54, 18}

Gyrodactylus salaris attaches to its host using an attachment organ (the opisthapor) that has two median anchors and 16 marginal hooks.^{26, 54} The mouth is located at the opposite end of the

parasite. When feeding the parasite uses cephalic glands to attach to the host, everts its pharynx through its mouth, and releases a digestive solution containing proteolytic enzymes which dissolve the host's skin.^{18, 59} Mucus and dissolved skin are then ingested. The attachment and feeding sites result in wounds in the host's epidermis that can subsequently lead to osmoregulatory failure, secondary infections, debilitation, and death.^{60, 40, 59}

Transmission

Transmission is horizontally direct via contact with infested live or dead fish, detached parasites in the water column, and parasites present in or attached to bottom sediments and substrates.^{53, 40, 15 Risk of transmission via these routes is greatest in waterbodies that are hydrologically and/or geospatially proximate and where natural movement or migration of infested fish may occur.^{40, 33} Release of effluent water from aquaculture facilities where infested fish are present may also be a source of introduction into proximate areas.⁴⁵}

Long distance translocation of *G. salaris* is thought to occur via anthropogenic activity, because *G. salaris* is not capable of long-distance movement independent of a host.⁶¹ Translocation of infested fish is considered the primary route of introduction throughout natural watershed and aquaculture facilities in Europe.^{51, 26, 45, 32, 33} Rainbow trout are most often associated with the spread of *G. salaris* in Europe, however, any fish that the parasite attaches to can serve as a transport vector.³³ Indirect transmission may also occur via fomites because *G. salaris* can survive for several days on damp materials (e.g., boats, packaging materials, recreational and commercial fishing gear, waders, and other fomites).³³

Clinical Signs and Pathogenicity

In most host species infestation follows a pattern of initial exposure, an increase in the number of parasites present on the host, and a variable interval during which parasites decrease in number until disappearance.⁴⁰ An exception to this pattern is the fulminant infestation leading to clinical disease and mortality observed in Atlantic salmon.⁴⁰ Prevalence of infestation is variably dependent on environmental factors (e.g., water quality, salinity, and temperature, seasonal factors, geographic location), the fish species, the age and health status of individual fish, and fish population factors (e.g., population density).^{26, 31, 33} In susceptible wild and farmed Atlantic salmon prevalence can reach 100 percent.³³ In wild Baltic Atlantic salmon, prevalence is highly variable (0 to 70 percent) based on environmental factors and geographic location.⁶² Prevalence in other susceptible species is similarly variable. In farmed rainbow trout, prevalence rates of less than 10 percent have been reported.⁶²

G. salaris primarily infests the skin, and the dorsal, pectoral, and pelvic fins.^{40, 33, 43} When the parasite burden is high, parasites may also be found on the gills and head, including the eyes and nostrils.⁴⁰ Development of clinical signs in susceptible fish species is associated with the parasite burden and the damage to the host tissues that occurs via the repetitive attachment and feeding of the parasites.²⁶ Some fish species (e.g., Arctic char, farmed rainbow trout) may be infested with low numbers of parasites for years without exhibiting any clinical signs of disease.^{40, 63, 43} In highly susceptible Atlantic salmon, especially those in the part stage, the parasite burden can reach thousands of parasites.^{40, 28} Clinical signs may take several weeks to appear, and can include anorexia, behaviors such as flashing (rubbing against substrates or net

pen surfaces), darting and erratic swimming, erosion of the fins, epithelial hyperplasia, focal areas of redness, irritation of the skin which leads to increased mucous production and gives affected fish a grayish color, lethargy, lesions and ulcerations on the skin, and osmoregulatory impairment.^{45, 40, 33} Moribund fish may lie on the bottom or congregate in areas with low water flow rates.⁴⁰ Development of secondary bacterial, viral, or fungal (e.g., *Saprolegnia* spp.) infections is common.^{40, 54, 33}

Morbidity and Mortality

Morbidity and mortality vary among different fish species and populations. In many susceptible species (e.g., Arctic char, Baltic Atlantic salmon, farmed rainbow trout, grayling), morbidity and mortality rates are negligible to low.³³ In highly susceptible Atlantic salmon, high rates of morbidity and mortality (85 percent to100 percent) are frequently observed in fry, parr, and smolt.^{54, 33}

Treatment

There are no vaccines, chemotherapeutic, or immunostimulation therapies available.⁴⁰ Gyrodactylosis can be controlled in aquaculture facilities using commonly used bath treatments containing high salinity salt water, or compounds containing chlorine or iodine, and formaldehyde (see <u>Prevention and Control</u>).^{40, 15} Total eradication of the parasite requires depopulation of the affected fish population, drying the fish rearing structures, and instituting a fallow period.^{24, 29, 28} Chemicals such as rotenone or aluminum sulphate are used to treat natural water bodies (e.g., lakes, rivers) to completely eradicate affected fish populations (see <u>Prevention and Control</u>).^{24, 29} Experimentally, in Europe, selective breeding of Atlantic salmon and rainbow trout has resulted in increased survival rates among offspring.⁴⁰ However, fish are still susceptible to infection.^{40, 15}

Diagnostic Testing

Gyrodactylus salaris cannot be visualized on affected fish without magnification. Observable gross lesions include cutaneous ulcers, epidermal thickening, excess mucus on the skin giving a grayish appearance, frayed fins that may appear eroded, white, and thickened, and sloughing of the skin.^{54, 15} Secondary fungal infections (*Saprolegnia* spp.) may be observed.¹⁵ There are no definitive histopathological signs.⁵⁴

All *Gyrodactylus* spp. are similar morphologically.^{24, 40} Confirmatory diagnosis requires morphological identification of the parasite under magnification in combination with molecular testing (PCR and DNA sequencing of the ribosomal internal transcribed spacer region [ITS]).^{24, 45, 5} WOAH recommended protocols for specimen selection, sample collection, transport and handling, and diagnostic methods are described in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code (<u>Appendix, Table 1</u>).^{5, 15} In the United States, confirmatory testing at the USDA APHIS National Veterinary Services Laboratory (NVSL) is required following first detections. Samples should be collected and submitted under the direction of State and Federal authorities via guidelines provided by NVSL (<u>Appendix, Table 1</u>).⁶⁴ Experimentally, environmental DNA assays (eDNA) have been used for surveillance in natural water bodies.^{65, 66}

Prevention and Control

Control of the spread of *G. salaris* should include application of risk-based approaches for surveillance and control in natural waterbodies and in aquaculture systems, and development of regulations to safeguard susceptible fish populations.^{40, 28} In Europe, legal and illegal translocation and importation of infested fish are identified as the most significant pathways for introduction of *G. salaris* into naïve ecosystems and aquaculture facilities.^{24, 48, 40} The next most significant potential pathway is introduction via equipment used for fishing and recreational water sports (e.g., bait, boats, canoes, kayaks, fishing tackle, nets, paddle boards waders).⁴⁰

Control efforts in some European Union countries (including those that are currently *G. salaris* free) include regulatory standards such as: 45, 67, 32, 28

- controls on transfer, local movement, and international importation of fish,
- surveillance programs for farmed and wild fish populations,
- eradication plans for natural waterbodies,
- eradication plans for hatcheries and fish farms,
- regulations for drying or disinfection of boating, fishing, and diving equipment prior to movement between or within watershed systems,
- guidelines for use of live or dead bait in certain regions,
- requirements for gutting and cleaning of fish or discharging of fish waste in natural waters,
- controls for disposal of water anywhere other than where it was collected, and
- contingency plans that include outbreak control measures, movement restrictions, establishment of buffer zones, treatment plans, eradication measures, and public outreach frameworks.

In the EU, trade of live fish species susceptible to gyrodactylosis is only permitted between countries, zones, or compartments of equivalent health status (or from higher to lower status).^{45, 32} Many countries also have prohibitions on the transport of live fish to rivers containing wild Atlantic salmon unless the source of the fish is known to be *G. salaris* free.⁵⁵ Surveillance plans are applied to natural waterbodies and aquaculture facilities. The intent is to document freedom from *G. salaris* in unaffected areas and aquaculture facilities, to detect and trace spread of the parasite from natural areas or aquaculture facilities where it is present to new sites, and to evaluate post-eradication disease freedom.⁶⁷

There are currently no requirements for routine *G. salaris* surveillance in United States aquaculture facilities. The USFWS does conduct routine surveillance on wild fish populations and maintains a database which catalogs testing data by species, year, and location (<u>Appendix</u>, <u>Table 1</u>). Surveillance protocols are not focused specifically on *G. salaris*; however, they do involve examination for external parasites. Additionally, the USDA and other Federal agencies periodically conduct structured surveillance for research or regulatory purposes that include sampling fish with susceptibility to *G. salaris*. There may be relative State and Tribal regulations with additional local aquatic animal health and import requirements that would detect *G. salaris* or other external parasites (<u>Appendix</u>, <u>Table 1</u>). When required by importing countries, evaluation for *G. salaris* is conducted by APHIS-approved laboratories or accredited

veterinarians, who are obligated to report to the Federal and State animal health officials in their region. As of 2023, USDA APHIS has negotiated health certificates for the export of live salmonid fish, eggs, and gametes with around fifty-eight countries; at least forty-six of these countries include pre-export testing requirements for *G. salaris*.

Gyrodactylus salaris is capable of surviving without a fish host for several days in damp environments but is susceptible to desiccation and temperatures outside of its optimal thermal range.^{40, 63, 33} Boats, equipment, fishing gear, nets and other potential fomites should be completely dried for several days and can be disinfected by placing them in water at temperatures ranging from 20 °C/68 °F for 24 hours, 40 °C/104 °F for 5 minutes, or 50–60 °C/122–140 °F for one minute, freezing at -18 °C/-0.4 °F for 24 hours, or they can be treated with disinfectants efficacious for *G. salaris*.^{53, 55, 40, 54, 33} Recommended disinfectants include 1 Virkon S® (1percent for 15 minutes), iodine-based compounds (e.g., Wescodyne®), and sodium hydroxide-based compounds (e.g.,BiosolveTM Plus).^{55, 23, 54, 33} In some EU member states, equipment must be accompanied by a certificate of disinfection issued by a competent professional in the country of origin.²⁸

There are no drugs that demonstrate efficacy against *G. salaris*. Most of the insecticide or parasiticide treatments tested also exhibit toxicity to the fish hosts.^{40, 59} Chemical bathing of fish in formalin (0.017percent to .025 percent for 30 minutes) or high salinity water (200–250 ppt x 30 minutes) will remove *G. salaris* from infested fish. These treatments do not eradicate the pathogen; therefore, repeated treatments are required to control the parasite in aquaculture facilities.⁴⁰ Experimentally hydrogen peroxide (H₂O₂) treatments have been used to control infestations on host fish.^{68, 40} The WOAH Manual of Diagnostic Tests for Aquatic Animals states that treatment of farmed fish populations with bath treatments reduces the prevalence and abundance of *G. salaris* on affected fish; however, fish may remain infested, and detection of the parasite will become more difficult.⁵ lodine-containing compounds have been used to disinfect eggs that may be surface contaminated via contact with contaminated water.⁵

Because there are no efficacious treatments for *G. salaris*, eradication of infested hosts is the recommended control measure. In Norway, rotenone and acidified aluminum sulphate have been used to eradicate *G. salaris* from infested river systems (via killing all the fish present), followed by restocking with eggs and fry from *G. salaris*-free hatcheries.^{24, 55, 40, 67, 54} Experimentally, low doses of sodium hypochlorite (200 μ g Cl/L) have been used in some areas.⁴⁰ Other control methods include use of physical or electric barriers to stop the movement of fish from infested to uninfested rivers.^{24, 69, 55, 67} As of 2021, these efforts resulted in eradication of *G. salaris* from 39 of 44 infested rivers.²⁹ In fish hatcheries and farms, eradication of all affected fish hosts is followed by disinfection of the farm, and a period of fallowing followed by restocking with eggs, fry, and fish from *G. salaris* from all affected hatcheries and fish farms by 2021.²⁹

Recommended aquaculture facility biosecurity measures include: 24, 51, 55, 45, 40, 15

• siting farms rearing susceptible fish in areas where *G. salaris* is not present in wild fish populations,

- use of wells or springs for water sources,
- treatment of influent water prior to use,
- sourcing live fish, eggs, and gametes from sources known to be G. salaris free,
- use of preventative surveillance, use of disinfectants and other cleaning methods known to be efficacious for the parasite,
- use of designated PPE,
- use of site designated equipment,
- maintenance of fish health (avoid overstocking, maintain good water quality and temperature), and
- treatment of effluent water prior to release.

Many countries utilize import/export regulations and recommendations in effort to limit or control the risk of *G. salaris* introduction. A summary of WOAH import/export guidelines specific to *G. salaris*, U.S. regulations, and other regulatory information related to aquaculture in the United States is summarized in the <u>Appendix, WOAH Pathogen Specific Import Export</u> <u>Recommendations</u>.

Summary

Gyrodactylosis is an economically important WOAH-listed parasitic disease affecting salmonid fish. Atlantic salmon are highly susceptible to this parasite and develop clinical signs of disease with subsequent high morbidity and mortality. Other salmonids (e.g., Arctic char, grayling, rainbow trout) are susceptible to infestation, but rarely develop clinical disease. Importation and translocation of sub-clinically infested rainbow trout has been associated with translocation of *G. salaris* throughout Europe.

In EU states where *G. salaris* is present in wild salmonid populations, control is difficult once introduction has occurred. Eradication measures require application of chemicals to natural water systems that result in the death of all wild fish present, with likely ecosystem and local economic consequences. Control in fish farms and hatcheries requires eradication of all fish hosts, followed by disinfection and fallowing, with economic consequences to the producer, and the local economy. Presence of *G. salaris* has resulted in trade regulations relative to the movement and importation of susceptible fish.

The susceptibility of wild, stocked, and farmed salmonid spp. in the United States is not known. However, it may be assumed that farmed, stocked, and wild Atlantic salmon in the Atlantic Northeast, and farmed, stocked, and wild rainbow trout populations might exhibit similar patterns of susceptibility described in European fish. It may also be expected that control and eradication of this parasite in wild and farmed fish populations would be similarly complex, expensive, and impactful.

In the United States, there are no Federal, State, local, or Tribal import regulations specific to *G. salaris*, although the USFWS does require general disinfection of salmonid eggs prior to import. The USFWS does perform routine disease surveillance in wild fish stocks that includes sampling for external parasites. State and Tribal entities may also have local requirements for external parasite sampling. USDA and other Federal agencies may periodically conduct structured surveillance that includes external parasite sampling for research or regulatory

purposes. Best practices to prevent introduction of *G. salaris* into the United States should include development of policies and contingency plans to ensure that imported all live fish, eggs, and gametes are imported form *G. salaris*-free sources and that importation complies with the guidelines described in the WOAH Aquatic Animal Health Code. Because the parasite can potentially live on any freshwater fish species, regulatory measures would ideally control movement of all fish species from areas where *G. salaris* is present. *G. salaris* is a WOAH-listed reportable pathogen. Therefore, detection of gyrodactylosis in cultured fish stocks in the United States would likely result in significant trade impacts.

Appendix

Tables

Table 1. Links to manuals, websites, and other resources relevant to *Gyrodactylus salaris*, aquaculture and aquatic animal diseases.

Resource	Link
Guide to State and Tribal aquaculture regulations	USDA APHIS Interactive Maps
	USDA APHIS Diagnostic Testing at the NVSL
National Veterinary Service Laboratory	USDA APHIS General NVSL Information
National Animal Health Laboratories	USDA APHIS Laboratory Information and
National Animal Health Laboratories	Services
	USDA APHIS Laboratories
USDA APHIS Comprehensive Aquaculture Health	USDA APHIS Comprehensive Aquaculture
Program Standards (CAHPS)	Health Program Standards
USDA APHIS National Animal Health Reporting	USDA APHIS National Animal Health
System (NAHRS)	Reporting System (NAHRS)
USDA APHIS National Aquaculture Health Plan &	USDA APHIS National Aquaculture Health
Standards (NAHP&S): 2021–2023	Plan & Standards (NAHP&S): 2021–2023
USDA APHIS National List of Reportable Animal	USDA APHIS National List of Reportable
Diseases (NLRAD)	Animal Diseases
USDA APHIS Veterinary Services and State	Federal and State Animal Health (usaha.org)
authorities	USDA APHIS Contact Veterinary Services
2017 OIE Report of the Meeting of the OIE ad hoc	a-ahg-susceptibility-of-fish-september-2019.pdf
Group on Susceptibility of Fish Species to Infection	(woah.org)
with OIE Listed Diseases	
World Organisation for Animal Health (WOAH)	Aquatic Code Online Access - WOAH - World
Aquatic Animal Health Code	Organisation for Animal Health
World Organisation for Animal Health (WOAH)	Manual Online Access - WOAH - World
Manual of Diagnostic Test for Aquatic Animals	Organisation for Animal Health
World Organisation for Animal Health (WOAH)	World Animal Health Information System
World Animal Health Information System (WAHIS)	WAHIS - WOAH - World Organisation for
database	Animal Health
World Trade Organization, Sanitary and	WTO WTO Agreements Series: Sanitary and
Phytosanitary Measures	Phytosanitary Measures
The United Nations Code of Conduct for	International Agricultural Law and Organizations
Responsible Fisheries based upon UNCLOS and	Aquaculture Overview - National Agricultural
other international laws.	Law Center (nationalaglawcenter.org)
FAO Aquaculture Regulatory Frameworks	AQUA-CULTURE REGULATORY
· · · ·	FRAMEWORKS (fao.org)
United States Fish and Wildlife National Fish Health	National Wild Fish Health Survey Mapper U.S.
Survey Mapper	Fish & Wildlife Service (fws.gov)
	Steps for Importing Salmonids into the United
	States of America U.S. Fish & Wildlife Service
	<u>(fws.gov)</u>
United States Fish and Wildlife Importation	Information for Importers & Exporters U.S. Fish
Guidelines	& Wildlife Service (fws.gov)
	CFR-2016-title50-vol1.pdf (govinfo.gov)
	Help Center Articles - Do I Need a Permit?
	(servicenowservices.com)

USDA APHIS Import permit information	USDA APHIS Fish, Fertilized Eggs, and Gametes
USDA APHIS International Regulations (IREGS) website	USDA APHIS Animal and Animal Product Export Information) Import/Export Requirements for Aquaculture Products (fdacs.gov)

Table 2. Countries that require testing for *Gyrodactylus salaris* for which APHIS has a negotiated export health certificate that can be used to ship live salmonid fish or eggs.

Gyrodactylus salaris Freedom Testing Required
No
Yes
No
Yes
Yes
No
No
No

Czech Republic	No
Denmark	No
Estonia	No
Finland	No
France	No
Georgia	Yes
Germanys	No
Greece	No
Hungary	No
Ireland, Republic of	No
Isle of Man	No
Isle of Man Israel	No
Israel	Yes
Israel Italy	Yes
Israel Italy Kazakhstan	Yes No No
Israel Italy Kazakhstan Kyrgyzstan	Yes No No No
Israel Italy Kazakhstan Kyrgyzstan Latvia	Yes No No No No
Israel Italy Kazakhstan Kyrgyzstan Latvia Lithuania	Yes No No No No No No No
Israel Italy Kazakhstan Kyrgyzstan Latvia Lithuania Luxembourg	Yes No No No No No No No No No

Mexico	No
Могоссо	No
Netherlands	No
New Zealand	No
North Macedonia	No
Norway	No
Peru	Yes
Poland	No
Portugal	No
Romania	No
Russian Federation	No
Russian Federation Serbia	No
Serbia	No
Serbia Singapore	No
Serbia Singapore Slovakia	No No No
Serbia Singapore Slovakia Slovenia	No No No Yes
Serbia Singapore Slovakia Slovenia South Africa	No No No Yes
Serbia Singapore Slovakia Slovenia South Africa Spain	No No No Yes No
Serbia Singapore Slovakia Slovenia South Africa Spain Sweden	No No No Yes No No

Turkey	No
Turks and Caicos Islands	No
Ukraine	No
United Arab Emirates	Yes
United Kingdom/ Great Britain	No

Table 3. Countries in which presence of *Gyrodactylus salaris* has been reported (wild and or farmed fish species) from 2010 through 2022. The World Organisation for Animal Health (WOAH) World Animal Health Information System (WAHIS) database should be consulted for information regarding current country status.^{33, 15}

Country	Gyrodactylus salaris
Australia	-
Austria	-
Belgium	-
Canada	-
Chile	-
China	-
Costa Rica	Yes
Croatia	-
Czech Republic	-
Denmark	Yes
Estonia	Yes
Faroe Islands	-
Finland	Yes

France-GeorgiaYesGermanyYesIceland-Iran-Iran-Ireland-ItalyYesJapan-LatviaYesNetherlands-North MacedoniaYesPolandYesRomaniaYesRussiaYesSlovakia-Slovakia-	
GermanyYesIceland-Iran-Iran-Ireland-ItalyYesJapan-LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesSlovakia-Slovenia-	
Iceland-Iran-Iran-Ireland-ItalyYesJapan-LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
Iran-Ireland-ItalyYesJapan-LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
Ireland-ItalyYesJapan-LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
ItalyYesJapan-LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
Japan-LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
LatviaYesNetherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
Netherlands-North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
North MacedoniaYesNorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
NorwayYesPolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
PolandYesRomaniaYesRussiaYesSlovakia-Slovenia-	
RomaniaYesRussiaYesSlovakia-Slovenia-	
RussiaYesSlovakia-Slovenia-	
Slovakia – Slovenia –	
Slovenia –	
South Korea –	
Spain –	
Sweden Yes	
Switzerland -	
Turkey –	
Ukraine Yes	
United Kingdom (England, – Scotland)	

United Kingdom (Scotland)	-
United States	-
Vietnam	Yes

WOAH Import/Export Recommendations for G. salaris

WOAH import/export guidelines specific *G. salaris* are found in WOAH Aquatic Animal Health Code.¹⁵ Briefly,

- 1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with *G. salaris*.
 - a. The importing country's Competent Authority should require that the consignment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country. The international aquatic animal health certificate should state that, based on the procedures described in the WOAH Aquatic Animal Health Code, the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with *G. salaris*.
- 2. Importing aquatic animals or aquatic animal products from a country, zone, or compartment NOT declared free from infection with *G. salaris*.
 - a. The importing country's Competent Authority should assess the risk as described in the WOAH Aquatic Animal Health Code, and consider the following risk mitigation measures: a) direct delivery and lifelong holding of the imported aquatic animals in a quarantine facility; and treatment of all transport water, equipment, effluent and waste materials sufficient to inactivate *G. salaris*; or b) immediately prior to movement require that the aquatic animals are held in water with a minimum salinity of 25 parts per thousand (ppt), and have no contact with other susceptible aquatic animal species.
- 3. Importing eggs for aquaculture from a country, zone, or compartment not declared free from infection with *G. salaris*.
 - Eggs should be disinfected using a method demonstrated to be effective against *G. salaris* and post-disinfection should not come into contact with anything that may affect their health status.
- 4. Importing aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from *G. salaris*.
 - a. The importing country's Competent Authority should a) require deliver of the consignment directly to authorized quarantine facilities where the animals will be held; b) all water (including ice), equipment, containers and packaging materials used in transport are treated to ensure inactivation of *G. salaris* and disposed of

in a biosecure manner as described in the WOAH Aquatic Animal Health Code, Chapters 4.4, 4.8, and 5.5; c) all effluent and waste materials from the quarantine facilities are disposed of in a biosecure manner or treated to ensure inactivation of *G. salaris*; and d) all carcasses are disposed of as described in the WOAH Aquatic Animal Health Code, Chapter 4.8.

References

1. WOAH. (2022). "World Organisation for Animal Health Terrestrial Animal Health Code. World Organisation for Animal Health." from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=sommaire.htm

2. Bruckner, G., S. MacDiarmid, N. Murray, F. Berthe, C. Muller-Graf, K. Sugiura, C. Zepeda, S. Kahn, and G. Mylrea (2010). "Handbook on import risk analysis for animals and animal products." Office International des Epizooties, Paris.

3. WOAH. (2017). "OIE Report of the Meeting of the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases. World Organisation for Animal Health." from https://www.woah.org/app/uploads/2021/10/a-ahg-susceptibility-of-fish-april-2017.pdf.

4. WOAH (2022). Aquatic Animal Health Code, Chapter 10.4, Infection with Infectious Salmon Anemia Virus, World Organisation for Animal Health.

5. WOAH. (2022). "OIE Manual of Diagnostic Tests for Aquatic Animals 2022. World Organisation for Animal Health." from <u>https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/</u>

6. Hedrick, R. (1998). "Relationships of the host, pathogen, and environment: implications for diseases of cultured and wild fish populations." Journal of Aquatic Animal Health 10(2): 107-111.

7. Jiménez-Valverde, A., A. T. Peterson, J. Soberón, J. Overton, P. Aragón, and J. M. Lobo (2011). "Use of niche models in invasive species risk assessments." Biological invasions 13(12): 2785-2797.

8. Engering, A., L. Hogerwerf and J. Slingenbergh (2013). "Pathogen–host–environment interplay and disease emergence." Emerging microbes & infections 2(1): 1-7.

9. James, T. Y., L. F. Toledo, D. Rödder, D. da Silva Leite, A. M. Belasen, C. M. Betancourt-Román, T. S. Jenkinson, C. Soto-Azat, C. Lambertini and A. V. Longo (2015). "Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research." Ecology and Evolution 5(18): 4079-4097.

10. Hovingh. (2017). "Biosecurity Fundamentals." from <u>https://extension.psu.edu/biosecurity-fundamentals#:~:text=What%20is%20biosecurity%3F,to%20other%20herds%20or%20flocks</u>

11. Oidtmann, B. C., E. J. Peeler, M. A. Thrush, A. R. Cameron, R. A. Reese, F. M. Pearce, P. Dunn, T. M. Lyngstad, S. Tavornpanich and E. Brun (2014). "Expert consultation on risk factors for introduction of infectious pathogens into fish farms." Preventive Veterinary Medicine 115(3-4): 238-254.

12. Sitjà-Bobadilla, A. and B. Oidtmann (2017). Integrated pathogen management strategies in fish farming. <u>Fish diseases</u>, Elsevier: 119-144.

13. Oidtmann, B., P. Dixon, K. Way, C. Joiner and A. E. Bayley (2018). "Risk of waterborne virus spread–review of survival of relevant fish and crustacean viruses in the aquatic environment and implications for control measures." <u>Reviews in aquaculture</u> **10**(3): 641-669.

14. Romero, J. F., I. A. Gardner, L. Hammell, D. Groman, D. Whelan, N. O'Brien, L. J. Hawkins, H. Burnley and K. Thakur (2022). "Descriptive epidemiology of variants of infectious salmon

anaemia virus in four Atlantic salmon farms in Newfoundland and Labrador, Canada." <u>Journal of Fish Diseases</u> **45**(6): 919-930.

15. WOAH. (2022). "Aquatic Animal Health Code. World Organisation for Animal Health ", from <u>https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/</u>

16. Jørgensen, T. R., T. B. Larsen, L. G. Jørgensen, J. Bresciani, P. W. Kania and K. Buchmann (2007). "Characterisation of a low pathogenic form of Gyrodactylus salaris from rainbow trout." Diseases of Aquatic Organisms 73(3): 235-244.

17. Leis, E., T. K. Chi, and J. Lumme (2021). "Global phylogeography of salmonid ectoparasites of the genus Gyrodactylus, with an emphasis on the origin of the circumpolar Gyrodactylus salmonis (Platyhelminthes: Monogenea)." Comparative Parasitology 88(1): 130-143.

18. ADW. (2023). "Gyrodactylus salaris. University of Michigan Museum of Zoology Animal Diversity Web." from <u>https://animaldiversity.org/accounts/Gyrodactylus salaris/</u>.

19. Tuttle-Lau, M. T., E. M. Leis, A. R. Cupp, L. L. Peterman, J. L. Hebert, R. A. Erickson, S. M. Schleis, and M. P. Gaikowski (2023). "Efficacy of hydrogen peroxide to reduce Gyrodactylus species infestation density on four fish species." Journal of Aquatic Animal Health.

20. Garcia, R. L., A. G. Hansen, M. M. Chan, and G. E. Sanders (2014). "Gyrodactylid ectoparasites in a population of rainbow trout (Oncorhynchus mykiss)." Journal of the American Association for Laboratory Animal Science 53(1): 92-97.

21. Harris, P. D., L. Bachmann, and T. A. Bakke (2011). "The parasites and pathogens of the Atlantic salmon: lessons from Gyrodactylus salaris." Atlantic salmon ecology. Chichester (United Kingdom): Wiley-Blackwell: 221-252.

22. Lumme, J., P. Anttila, P. Rintamäki, P. Koski and A. Romakkaniemi (2016). "Genetic gradient of a host–parasite pair along a river persisted ten years against physical mobility: Baltic Salmo salar vs. Gyrodactylus salaris." Infection, Genetics and Evolution 45: 33-39.

23. NFSA. (2020). "Norwegian Food Safety Authority. The Status of Gyrodactylus salaris in Norwegian watercourses at 1st of June 2020. ." from

https://www.mattilsynet.no/language/english/fish_and_aquaculture/recreationalfishing/how_to_st op the spread of gyrodactylus salaris.10035/binary/How%20to%20stop%20the%20spread% 20of%20Gyrodactylus%20salaris

24. Johnsen, B. O., and A. J. Jenser (1991). "The gyrodactylus story in Norway." Aquaculture 98(1-3): 289-302.

25. Bakke, T. A., P. D. Harris, and J. Cable (2002). "Host specificity dynamics: observations on gyrodactylid monogeneans." International journal for parasitology 32(3): 281-308.

26. Bakke, T. A., J. Cable, and P. Harris (2007). "The biology of gyrodactylid monogeneans: the "Russian-doll killers"." Advances in parasitology 64: 161-460.

27. Hendrichsen, D. K., R. Kristoffersen, K. Ø. Gjelland, R. Knudsen, S. Kusterle, A. H. Rikardsen, E. H. Henriksen, A. Smalås and K. Olstad (2015). "Transmission dynamics of the monogenean G yrodactylus salaris under seminatural conditions." Journal of Fish Diseases 38(6): 541-550.

28. MarineScotland. (2022). "Gyrodactylus salaris Topic Sheet Number 32." from https://www.gov.scot/binaries/content/documents/govscot/publications/factsheet/2019/11/marine

-scotland-topic-sheets-aquaculture/documents/gyrodactylus-salaris-updated-october-2016/gyrodactylus-salaris-updated-october-2016/govscot%3Adocument/gyrodactylus-salaris.pdf

29. Hansen, H., S. Mohammad, H. I. Welde and M. M. Amundsen (2022). "The post-treatment surveillance programme for Gyrodactylus salaris in Norway 2021." Veterinærinstituttets rapportserie.

30. USDA-APHIS. (2022). "National Animal Health Reporting System (NAHRS). United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) ", from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/sa disease reporting/ct usda aphis animal health.

31. Anttila, P., A. Romakkaniemi, J. Kuusela and P. Koski (2008). "Epidemiology of Gyrodactylus salaris (Monogenea) in the River Tornionjoki, a Baltic wild salmon river." Journal of Fish Diseases 31(5): 373-382.

32. Paladini, G., A. P. Shinn, N. G. Taylor, J. E. Bron and H. Hansen (2021). "Geographical distribution of Gyrodactylus salaris Malmberg, 1957 (Monogenea, Gyrodactylidae)." Parasites & Vectors 14(1): 1-20.

33. WOAH. (2022). "World Animal Health Information System (WAHIS) database. World Organisation for Animal Health." from <u>https://wahis.woah.org/#/home</u>

34. Spickler (2007). Epizootic Hematopoietic Necrosis. Center for Food Security and Public Health, Iowa State University.

35. CABI. (2022). "Epizootic Haematopoietic Necrosis. CABI International Digital Library." from https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.83967#sec-5.

36. Vodica (2014). "Impact of Parasitic Disease of Gyrodactylosis on several indicators of growth and welfare in rainbow trout (Onchorynchus mykiss Walbaum 1792) Aged 0+." Proceedings, 4th International Conference of Ecosystems (ICE2014).

37. Dalgaard, M., T. Larsen, S. Jørndrup and K. Buchmann (2004). "Differing resistance of Atlantic salmon strains and rainbow trout to Gyrodactylus salaris infection." Journal of Aquatic Animal Health 16(3): 109-115.

38. Soleng, A. and T. A. Bakke (2001). "The susceptibility of grayling (Thymallus thymallus) to experimental infections with the monogenean Gyrodactylus salaris." International Journal for Parasitology 31(8): 793-797.

39. Paladini, G., A. Gustinelli, M. L. Fioravanti, H. Hansen and A. P. Shinn (2009). "The first report of Gyrodactylus salaris Malmberg, 1957 (Platyhelminthes, Monogenea) on Italian cultured stocks of rainbow trout (Oncorhynchus mykiss Walbaum)." Veterinary Parasitology 165(3-4): 290-297.

40. Mo, T. A. (2020). Gyrodactylosis (Gyrodactylus salaris). Climate change and infectious fish diseases, CABI Wallingford UK: 404-422.

41. Robertsen, G., H. Hansen, L. Bachmann, and T. Bakke (2007). "Arctic charr (Salvelinus alpinus) is a suitable host for Gyrodactylus salaris (Monogenea, Gyrodactylidae) in Norway." Parasitology 134(2): 257-267.

42. Robertsen, G., K. Olstad, L. Plaisance, L. Bachmann and T. A. Bakke (2008). "Gyrodactylus salaris (Monogenea, Gyrodactylidae) infections on resident Arctic charr (Salvelinus alpinus) in southern Norway." Environmental Biology of Fishes 83: 99-105.

43. Mo, T. A., H. Hansen, and S. Hytterød (2023). "Occurrence and seasonality of Gyrodactylus salaris and G. salmonis (Monogenea) on Arctic char (Salvelinus alpinus (L.)) in the Fustvatnet lake, Northern Norway." Journal of Fish Diseases 46(4): 395-403.

44. Hytterød, S., P. Adolfsen, S. Aune and H. Hansen (2011). "Gyrodactylus salaris funnet på røye (Salvelinus alpinus) i Fustvatnet (Nordland); patogen for laks (Salmo salar)." Veterinærinstituttets rapportserie 11(2011): 14.

45. Hansen, H., C.-D. Cojocaru, and T. A. Mo (2016). "Infections with Gyrodactylus spp. (Monogenea) in Romanian fish farms: Gyrodactylus salaris Malmberg, 1957 extends its range." Parasites & vectors 9(1): 1-10.

46. Bakke, T., P. Harris, and P. Jansen (1992). "The susceptibility of Salvelinus fontinalis (Mitchill) to Gyrodactylus salaris Malmberg (Platyhelminthes; Monogenea) under experimental conditions." Journal of Fish Biology 41(3): 499-507.

47. Sterud and Bakke (1998). "The influence of Gyrodactylus salaris Malmberg 1957 (Monogenea) on the epidermis of Atlantic salmon, Salmo salar L., and brook trout, Salvelinus fontinalis (Mitchill): experimental studies." Journal of Fish Diseases 21(4): 257-263.

48. Paladini, G., H. Hansen, C. F. Williams, N. G. Taylor, O. L. Rubio-Mejía, S. J. Denholm, S. Hytterød, J. E. Bron and A. P. Shinn (2014). "Reservoir hosts for Gyrodactylus salaris may play a more significant role in epidemics than previously thought." Parasites & vectors 7(1): 1-13.

49. Hansen, H., L. Bachmann, and T. A. Bakke (2003). "Mitochondrial DNA variation of Gyrodactylus spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden." International Journal for Parasitology 33(13): 1471-1478.

50. Bakke, T., P. Jansen, and L. Hansen (1991). "Experimental transmission of Gyrodactylus salaris Malmberg, 1957 (Platyhelminthes, Monogenea) from the Atlantic salmon (Salmo salar) to the European eel (Anguilla anguilla)." Canadian Journal of Zoology 69(3): 733-737.

51. Peeler, E., R. Gardiner, and M. Thrush (2004). "Qualitative risk assessment of routes of transmission of the exotic fish parasite Gyrodactylus salaris between river catchments in England and Wales." Preventive veterinary medicine 64(2-4): 175-189.

52. Hopkins, C. C. (2002). Introduced marine organisms in Norwegian waters, including Svalbard. Invasive aquatic species of Europe. Distribution, impacts and management, Springer: 240-252.

53. Olstad, K., G. Robertsen, L. Bachmann and T. Bakke (2007). "Variation in host preference within Gyrodactylus salaris (Monogenea): an experimental approach." Parasitology 134(4): 589-597.

54. Chong, R. S.-M. (2022). Infection with Gyrodactylus salaris. Aquaculture Pathophysiology, Elsevier: 513-515.

55. Koski, P., P. Anttila and J. Kuusela (2015). "Killing of Gyrodactylus salaris by heat and chemical disinfection." Acta Veterinaria Scandinavica 58: 1-6.

56. Jørgensen, T. R., M. K. Raida, P. W. Kania and K. Buchmann (2009). "Response of rainbow trout (Oncorhynchus mykiss) in skin and fin tissue during infection with a variant of Gyrodactylus salaris (Monogenea: Gyrodactylidae)." Folia Parasitologica 56(4): 251.

57. Ramírez, R., T. A. Bakke, and P. D. Harris (2015). "Population regulation in Gyrodactylus salaris–Atlantic salmon (Salmo salar L.) interactions: testing the paradigm." Parasites & Vectors 8: 1-14.

58. NEA (2006). "The Synonymisation of Gyrodactylus thymall and gyrodactylus salaris: Implications for NASCO. North-East Atlantic Commission. NASCO."

59. Aspatwar, A., A. Bonardi, H. Aisala, K. Zueva, C. R. Primmer, J. Lumme, S. Parkkila and C. T. Supuran (2023). "Sulphonamide inhibition studies of the β -carbonic anhydrase GsaCA β present in the salmon platyhelminth parasite Gyrodactylus salaris." Journal of Enzyme Inhibition and Medicinal Chemistry 38(1): 2167988

60. Petterson, E., M. Stormoen, Ø. Evensen, A. B. Mikalsen and Ø. Haugland (2013). "Natural infection of Atlantic salmon (Salmo salar L.) with salmonid alphavirus 3 generates numerous viral deletion mutants." Journal of General Virology 94(9): 1945-1954.

61. Reyda, F. B., S. M. Wells, A. V. Ermolenko, M. S. Ziętara and J. I. Lumme (2020). "Global parasite trafficking: Asian Gyrodactylus (Monogenea) arrived to the USA via invasive fish Misgurnus anguillicaudatus as a threat to amphibians." Biological Invasions 22: 391-402.

62. Kuusela, J., R. Holopainen, M. Meinilä, P. Anttila, P. Koski, M. S. Ziętara, A. Veselov, C. R. Primmer and J. Lumme (2009). Clonal structure of salmon parasite Gyrodactylus salaris on a coevolutionary gradient on Fennoscandian salmon (Salmo salar). Annales Zoologici Fennici, BioOne

63. Alarcón, M., T. Moldal, M. Dverdal Jansen, M. Aamelfot, H. Sindre, T. M. Lyngstad and K. Falk (2021). "Infectious salmon anemia virus detected by RT-qPCR in Norwegian farmed rainbow trout, Oncorhynchus mykiss (Walbaum, 1792)." Journal of Fish Diseases 44(4): 479-481.

64. USDA-APHIS. (2022). "General NVSL Information. United States Department of Agriculture (USDA) Animal and Plant Health Inspection Program (APHIS) ", from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/SA_About_NVSL/CT_About_nvsl.

65. Rusch, J. C., H. Hansen, D. A. Strand, T. Markussen, S. Hytterød and T. Vrålstad (2018). "Catching the fish with the worm: a case study on eDNA detection of the monogenean parasite Gyrodactylus salaris and two of its hosts, Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss)." Parasites & vectors 11(1): 1-12

66. Fossøy, F., H. Brandsegg, R. Sivertsgård, O. Pettersen, B. K. Sandercock, Ø. Solem, K. Hindar and T. A. Mo (2020). "Monitoring presence and abundance of two gyrodactylid ectoparasites and their salmonid hosts using environmental DNA." Environmental DNA 2(1): 53-62.

67. NEA (2021). "Report of the Meeting of the Working Group on Gyrodactylus salaris in the North-East Atlantic Commission Area. North-East Atlantic Commission. NASCO."

68. Thrush, M. A., T. Hill, and N. G. Taylor (2019). "Development of a non-lethal hydrogen peroxide treatment for surveillance of Gyrodactylus salaris on trout farms and its application to testing wild salmon populations." Transboundary and emerging diseases 66(5): 2107-2119.

69. Mo, T. (1994). "Status of Gyrodactylus salaris problems and research in Norway." Parasitic diseases of fish 2: 43-56