ONTARIO FISH HEALTH MANUAL

VOLUME I
March 2022

ONTARIO FEDERATION OF ANGLERS AND HUNTERS

Community HATCHERY Program

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Technical support for the development of this manual was provided by the Ministry of Northern Development, Mines, Natural Resources and Forestry and written with funding provided by the Ontario Federation of Anglers and Hunters

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March 2022

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Since 1982 the Ministry of Northern Development, Mines, Natural Resources and Forestry (NDMNRF) has supported community fish hatcheries across Ontario through a variety of programming such as the Community Fisheries and Wildlife Involvement Program (CFWIP) which resulted in the foundation for stocking millions of fish into public waters. Community fish hatcheries contribute to fisheries management objectives, biodiversity conservation goals and increased recreational angling opportunities across Ontario.

In 2013 the NDMNRF moved to a new model of support for community fish hatcheries with the introduction of the Community Hatchery Program (CHP). This program is administrated by the Ontario Federation of Anglers and Hunters (OFAH) and funded by NDMNRF with the goal of providing strategic and targeted support for Ontario's community fish culture and stocking efforts. From 2013 to 2021, over 40 community hatcheries were funded each year by the CHP. Over 1,200 volunteers across the province support community hatcheries annually with more than 60,000 person hours towards raising and stocking over six million fish into public waters.

The new Ontario Fish Health Manual will be a valuable resource for all community hatchery volunteers by providing technical information, best fish health practices, as well as a tool for knowledge transfer among hatchery volunteers. The manual is organized into sections that support all aspects of fish health for hatcheries in Ontario.

We appreciate your continued efforts and contribution towards sustainable fish populations and recreational fishing opportunities. Inquiries regarding the guide or the CHP should be directed to the Coordinator, Community Hatchery Program, Ontario Federation of Anglers and Hunters. We may be contacted at P.O. Box 2800, 4601 Guthrie Drive, Peterborough, Ontario, Canada K9J 8L5 and more information on the CHP may be found by visiting our webpage at www.communityhatcheries.com.

Introduction

This manual was written expressly for the Ontario Federation of Anglers and Hunters' Community Hatchery Program (CHP). Topics discussed remain within the scope of species relevant to the CHP.

This manual is meant as a guide for those caring for fish in community hatcheries and can serve to train new volunteers as well as reference for more seasoned staff in case of a novel event. Please note that this does not replace consultation with a qualified veterinarian in the case of a mortality event.

Fish Health

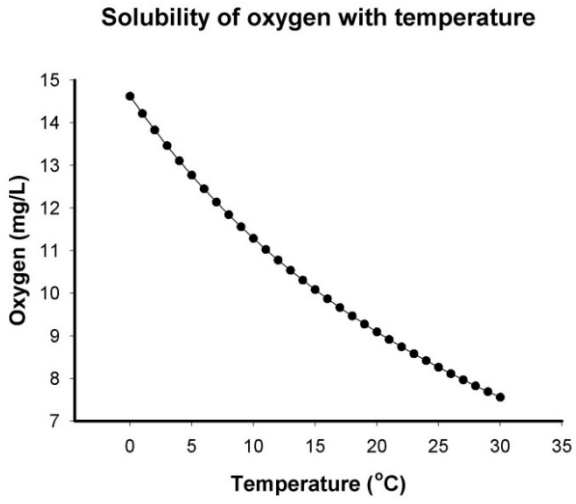
Water quality

Water quality parameters are the single most important factor in fish health. They are often the underlying reason for a disease outbreak. In most species of fish, rapid fluctuations in water quality parameters like pH or temperature can cause significant stress and lead to disease or death. Important to note is that these rapid changes can lead to disease caused by a pathogen that is ubiquitous, that is, a pathogen that is normally present in the environment and generally does not cause disease, however it will cause disease under certain conditions that are not favourable to your fish and/or favourable to the pathogen. This is the case for most fungi, bacteria and parasites in Ontario fish.

Here we will review some of the most important water quality parameters and how they can impact fish health. While there are many other water quality parameters that can influence fish health, here we will focus on those parameters that are most important and those that can be measured either with equipment already on hand or using readily available test kits.

Oxygen

All living animals require oxygen for survival and fish are no exception. The primary site of oxygen extraction for fish species that we grow in Ontario, is in the water through the gills. Dissolved oxygen (DO) is the measurement that we use to reflect how many O_2 molecules are present in solution, that is how much oxygen is in the water; this can be reflected as a percentage or parts per million (ppm). Parts per million can also be expressed as the number of milligrams per litre (mg/L). This is the true measure of O_2 molecules in the water, the percentage, must consider temperature to be converted to the number of mg/L of O_2 in the water. It is therefore best to speak of DO as ppm or mg/L. As temperature rises, the relative number of dissolved oxygen molecules decreases and as the temperature decreases, the relative number of dissolved oxygen molecules increases (see the graph below).



The conversion from % to mg/L is a complex algorithm and more detail than we wish to share here, however there are many good online calculators that you can use to convert % DO to mg/L. For most species in Ontario, especially in farmed conditions, 6 mg/L is the minimum dissolved oxygen requirement. This means that for optimal health conditions, you should remain above this. Remember that energy is required to extract oxygen from the water column, therefore the more fish need to expend energy to breathe or extract oxygen from water, the less energy goes towards growth and fighting off disease. If your current instrumentation measures % saturation, as a general rule, remaining over 80% saturation will maintain DO levels above 6 mg/L (for temperatures up to 30°C).

Temperature

All animals have a range of temperature in which they function optimally however this is even more critical in fish as they are ectotherms, meaning that their core or body temperature is that of their environment. This means that the rate at which their bodily functions operate such as their digestive and immune system, are dependent on the temperature of the water in which they are submerged. The finite range of temperature in which they thrive, often referred to as the preferred optimal temperature (P.O.T) range. This range can vary greatly, even between families like salmonids which are more commonly kept in Ontario. However, in general the optimal temperatures will be between 6 - 20°C with the middle of this range being most ideal. Some species, such as yellow perch, can do very well in the higher ranges up to 24°C whereas Rainbow Trout at this temperature, especially in aquaculture conditions, will be at high risk of succumbing to secondary infections.

Temperature is also important because it directly influences many other water quality parameters or their degree of toxicity; such examples include dissolved gases such as oxygen and carbon dioxide as well as ammonia.

pH

Technically speaking, pH represents to concentration of hydrogen ions (H^+) in a solution with a numerical value generally represented between 0 and 14. In more common terms, pH refers to a solution either being acidic or alkaline in nature. A pH value of 7 indicates a neutral solution, while a value below 7 is said to be acidic and alkaline if the value is above 7. The pH of a solution influences the toxicity of various compounds and as with other water quality parameters, has a tolerated range for each species. Optimal pH for fish grown in Ontario is 6.5 to 8. Salmonids have a much narrower pH tolerance range than other species such as Yellow Perch (3.5 to 9.5) or Walleye (6.0 -9.0).

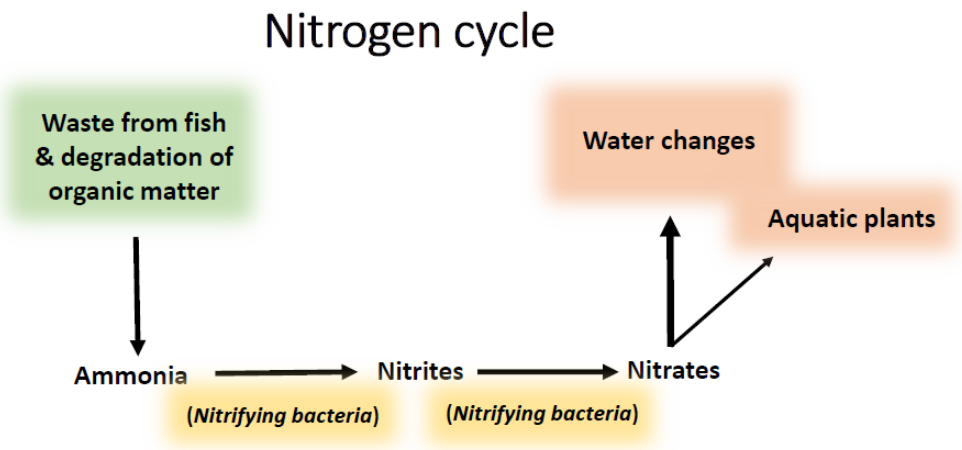
It's important to remember that the pH scale is logarithmic ($pH = [-\log (H^+)]$) which simply means that a change in value of 1 on the pH scale is a 10x change in acidity or hydrogen ion concentration while a change in value of 2 on the pH scale means 100x change in acidity. Consistency is the key with pH; rapid fluctuations are highly stressful and can be lethal to fish. For those with natural

water sources such as ponds, it should be noted that algal blooms can cause the pH to shift dramatically between day and night.

Nitrogenous Waste

The nitrogen cycle is a very important concept to understand when caring for fish, especially in a closed system like land-based recirculating water systems. In flow-through or open water systems, the concern and need to monitor is greatly lessened as fresh water is continually added and not recirculated.

Nitrogenous waste is excreted from fish as ammonia. This compound can be highly toxic to fish. In solution, free ammonia (NH_3) can reversibly convert to its ionized form, ammonium (NH_4^+), which is less toxic to fish. The amount of free ammonia will vary depending on pH and temperature. The majority of test kits will measure total ammonia which is the sum of free ammonia and ammonium. You can use calculators and tables online to determine the amount of free ammonia based on the measurement of total ammonia. In a system ammonia is converted to nitrite and then nitrites to nitrates by various species of nitrifying bacteria. Nitrate is the least toxic of these compounds.



Ammonia (NH_3)

Ammonia is the primary waste product of fish created from the breakdown of proteins and is excreted via gills and urine. It is highly toxic to fish as it interferes with oxygen transport across the gills. Ammonia should always be zero in a system. Signs of ammonia toxicity are generally non-specific ranging from neurological symptoms, buoyancy disorders, difficulty orienting in water column, lethargy, loss of appetite and death. The higher the pH and temperature, the more toxic ammonia (NH_3) there is compared to the relatively harmless ammonium (NH_4^+). Ammonium is more prevalent at low pH.

$$H_2O + NH_3 \leftrightarrow NH_4^+ + OH^-$$

Nitrite (NO_2^-)

Nitrite is produced by oxidation of ammonia by nitrogen fixing bacteria and is also very toxic to fish. Ideally, nitrites are not measurable in a system however a low level can be tolerated. An accumulation of nitrites can cause "brown blood disease" or methemoglobinemia in fish. Nitrite competitively binds with hemoglobin leading to signs of hypoxia and fish can asphyxiate despite potentially acceptable DO levels in the water. This will lead to a brown appearance of blood.

Nitrate (NO_3^-)

Nitrates are produced by oxidation of nitrite by nitrogen fixing bacteria. This compound is generally considered safe at low levels however for some species even low levels are not well tolerated and high levels can cause problems similar to ammonia. In a closed system, ammonia is primarily removed via water changes. Aquatic plants can remove some nitrates. High levels can encourage prolific growth of filamentous algae. Acceptable levels vary per species however remaining under 40 mg/L is considered ideal.

Alkalinity

Alkalinity or KH indicates water's buffering capacity or amount of buffers in the water, that is, the ability of a substance to resist a change in pH. Buffering substances or buffers help bind hydrogen ions. Alkalinity is primarily a measurement of the concentration of anions carbonate, hydroxide, phosphate, silicate, and borate in solution.

Three main methods of expressing alkalinity in aquaria:

1. Milligrams of calcium carbonate (mg $CaCO_3$/L)
2. Milliequivalents per litre (meq/L)
3. Degrees of carbonate hardness (dKH)

You may have heard alkalinity referred to as "carbonate hardness" in the aquarium industry. In seawater, KH comes mainly from carbonate and bicarbonate liberated during dissociation from various cations. Hence the term 'carbonate hardness'. This has led to much confusion.

Hardness

People often confuse or use interchangeably alkalinity (KH) and hardness (GH). GH is the abbreviation for *Gesamthaerte*, German for 'total hardness', not general hardness. GH relates directly to molecules in solution primarily those containing calcium, some of which have buffering capacities. Hardness is rarely an issue unless it is extremely low (soft). With very hard water, you can get very mild issues, primarily due to mineral deposits.

Carbon dioxide

Carbon dioxide (CO_2) is a by-product of respiration in fish. If the dissolved CO_2 concentration in water is elevated, this decreases the fish's ability to release its own CO_2 into the water. This will

lead to acidosis, a condition in which the fish's blood is too acidic. Acidosis can negatively impact multiple body systems and can lead to immune system compromise, poor growth, etc. CO_2 should be maintained below 10 mg/L for long-term health. Carbon dioxide is primarily a concern with closed systems that hold high stocking densities of fish and have a poor capacity to aerate. A rapid and simple solution to elevated CO_2 is simply to off-gas or aerate water through a degassing tower or other mechanical equivalent (anything that will vigorously stir up water and allow gases to escape).

Water Quality Parameter	Optimal Range or Value
Oxygen	> 6 mg/L
Temperature	6 - 20°C
pH	6.5 - 8
Ammonia (NH_3^+)	0.00 mg/L
Nitrite (NO_2^-)	0 – 0.5 mg/L
Nitrate (NO_3^-)	0 - 40 mg/L
Alkalinity (KH)	< 80 mg/L
Carbon dioxide (CO_2)	0 - 5 mg/L

Important animal observations

Your daily observations of fish's behaviour can help signal early on that something is awry. In fact, most people who work with fish will first note behavioural changes before any other overt sign of disease like a skin lesion. Some important observations have been listed below with any questions that you should be able to answer about your fish population. It is especially important to ask these questions during a mortality event or when you note that something is "off" about your fish. Specific signs of disease are covered in more detail in the diseases section however this list is simply meant to highlight important observations to note on a regular basis in your fish.

Behavioural Observation	Questions	What could this indicate?
Appetite	Are fish eating? How much are they eating (if not a specific amount, then compared to their usual or what is expected)? Is the feed response good or normal?	A decrease in appetite can indicate several things but are most common in diseases affecting gills, especially in smaller fish. Other diseases in fingerlings or larger fish such as Lactococcosis, can cause a decrease in appetite.
Use of the water column	Are fish resting along the bottom? Are fish schooling? Are fish congregating near inflow points?	In certain diseases, fish can rest on the bottom of tanks, one such example is with whirling disease. Fish that no longer school can be a general sign of disease from lethargy. Fish that congregate near inflow points can have gill disease (bacterial or nodular gill disease) or this could also indicate low dissolved oxygen.
Swimming	Are fish swimming in a normal orientation? If not, how are they swimming: side swimming, head up-tail down or vice-versa, struggling to swim, buoyant at the top of the column? Do they flash (erratically swim side to side) or rub?	Abnormal buoyancy can indicate signs of systemic disease affecting the swim bladder. Flashing or rubbing are most commonly indicators of the presence of ectoparasites.
External lesions	Do you see sores? Are the gills sticking out from the operculae (gill covers) or are the operculae shortened? Are their fins eroded, if so which ones? Does the skin appear different and if so, how (white patches, pinpoint white spots, generally milky appearance)? Are the eyes bulging out or cloudy?	Sores or ulcers can indicate specific diseases, for example bacterial cold water disease. Gills sticking out from the operculae can indicate that they are swollen or clubbed or that the operculae are shortened; this is generally the case for disease targeting gills such as BGD. Eroded fins can indicate disease but can also be suggest high stocking densities. A generalized milky appearance to skin can be an indicator of changes in water quality, such as a drop in pH. Eyes bulging out can indicate systemic bacterial disease such as Lactococcosis.

Diseases

The diseases section was written as a short summary of key points that may aid in the identification of each disease. Detailed scientific knowledge about each pathogen (parasite, fungus, bacteria) will not be covered here. For each pathogen, history, behavioural signs, gross lesions, diagnosis and samples will be listed. History is what you may note in the environment during an outbreak. Behavioural signs would relate specifically to how the fish are behaving when infected. Gross lesions would be what physical and external changes or wounds you might notice in live or dead infected fish. The samples point outlines what type of sample and test is most likely to confirm this diagnosis for you. Treatments are briefly touched on however you should always confirm with your veterinarian prior to initiating any treatment. There are significant risks associated with the use of incorrect doses, treating the wrong disease and sampling after treatment has been initiated can significantly decrease the chances of being able to obtain a diagnosis if your treatment attempt does not work.

Parasites

'Ich' or White Spot Disease (*Ichthyophthirius multifiliis*)

History: This disease occurs in warmer water (15-25°C), but the parasite can survive at <10°C. Other conditions can exacerbate the disease including high stocking densities and any stressor.

Behavioural signs: You may note that fish are flashing, darting or rubbing. They may also display an increased respiratory rate or heavy gilling.

Gross lesions: The classic sign noted are white spots (~1mm) on the skin and gills. In early and late stages of the disease, this may simply look like missing scales. Fish can also have a darker skin colouration overall.

Samples: This disease is relatively easy to diagnose with wet mounts of skin scrapes and gill clip. It is possible to miss the diagnosis with a low burden in which case, skin and gills can be sampled and placed in formalin for histology.

Treatment: Formalin baths remain the front line for treatment of this ectoparasite. There has been some experimentation with the use of natural supplements such as garlic powder and oregano oil in treatment of this disease however none that have been nearly as effective as formalin to date.

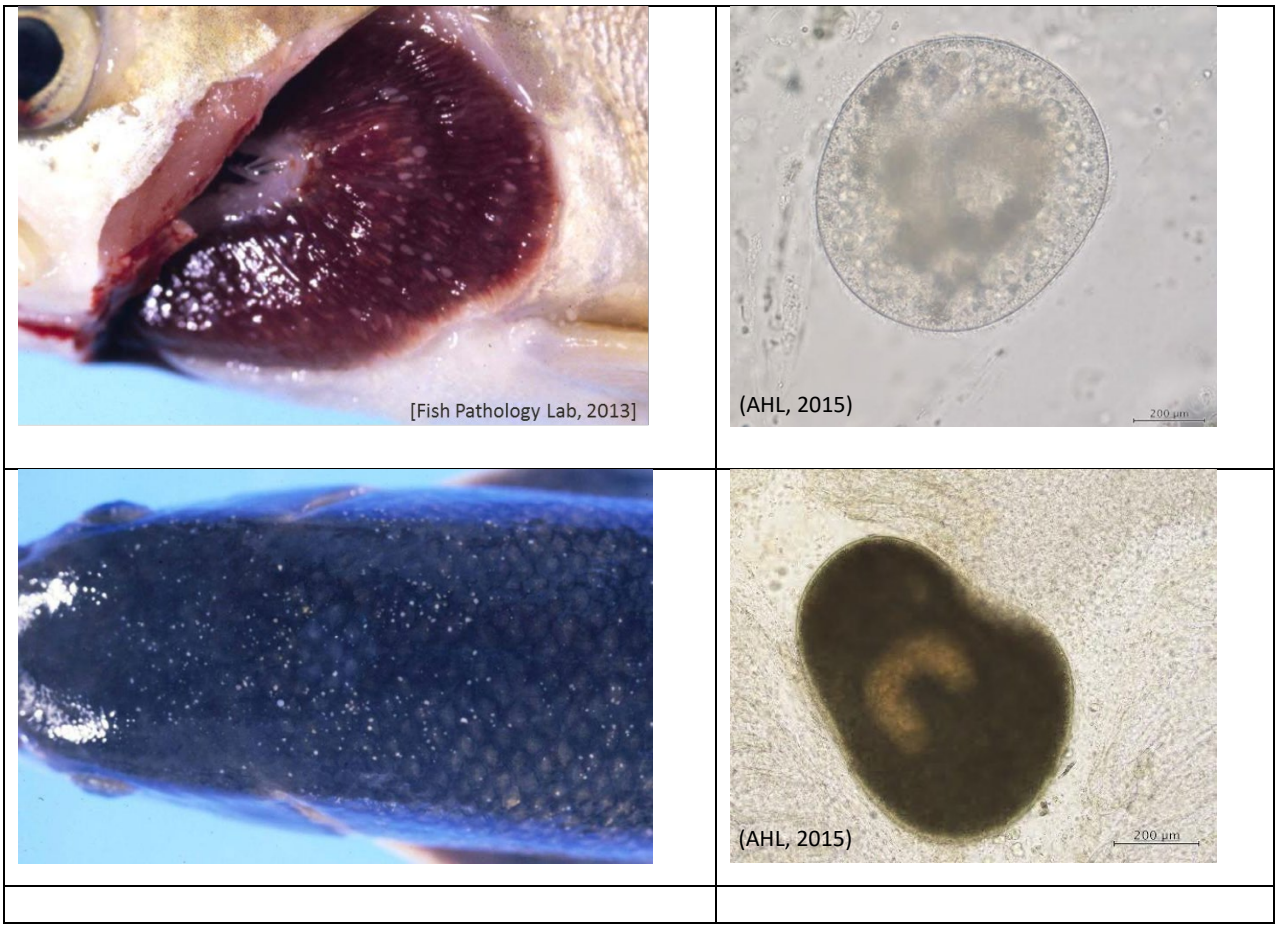


Figure 1: White spots that can be noted on gills (top left) and skin (bottom left) of fish with an Ich infection. Wet mounts of *Ichthyophthirius multifiliis* trophont (top right) and tomite (bottom right).

Trichodina

History: Issues associated with this parasite are generally accompanied by other predisposing factors such as another concurrent disease, poor water quality (high organic waste or total dissolved solids) and poor nutrition. On its own, you will generally only see signs with heavy infestations. Heavy burdens can cause low numbers of chronic mortalities.

Behavioural signs: This parasite can cause fish to be lethargic with a decreased appetite or in some cases, will dart and flash and can also have an increased respiration rate.

Gross lesions: A green sheen to skin and loosened scales as well as poor body condition can develop in some fish with chronic cases.

Samples: Because of its distinctive appearance, the parasite is easily identified on skin scrapes and gill clip wet mounts. Histology of skin and gills (+ other) can also successfully diagnose

Treatment: Formalin or hydrogen peroxide baths are appropriate treatments for these ectoparasites requiring only a single dose. Salt baths can also treat this parasite but generally require more than one dose.

Figure 2: Trichodina under a microscope at an unknown magnification.

Monogenes

History: Monogene outbreaks can originate from spring blooms of monogenes in natural unfiltered water or via the introduction of infected fish in your population. Conditions which can precipitate an adverse event associated with monogenes include high stocking densities, declining water quality, low dissolved oxygen, overcrowding, high ammonia or nitrite and organic pollution. Mortality usually only occurs when parasites are present in high numbers.

Behavioural signs: Signs of monogenes are generic signs of an ectoparasite infection; flashing, darting, rubbing and increased respiration rate.

Gross lesions: Looking at your fish you may be able to note cloudiness to the skin, grey to white irregular to eroded areas on skin (especially behind pectoral fins), eroded fins and small red spots (hemorrhages) on skin.

Samples: Because of the distinctive appearance and larger size of the parasite, you can readily diagnose this with mounts of skin scrapes or gill clips with the parasite. If there is a low burden

that is missed on wet mounts, histology of the skin and gills in formalin can be used in the diagnosis as well.

Treatment: Some types of monogenes can be more resistant to treatment however formalin is best known as first line of treatment. Hydrogen peroxide has also shown good promise. Efficacy of salt baths varies greatly as some species of monogenes can survive in salt water. Having said this, a combination treatment with salt and formalin for example are beneficial. The salt can also support the fish, especially in case of significant damage to the skin and gills by reducing osmoregulatory stress. This is because damaged skin allows fresh water to enter the fish's body, being immersed in a salt solution reduces the rate and extent to which this occurs.

Figure 3: Monogene parasite displaying haptors or hooks on the (bottom of image) which it uses to attach to the fish, feeding with the opposite side (head is a the top of the image).

Gill copepods (*Salmincola* species)

History: This parasite can be found on wild-caught, pond-raised, fish grown in open water cages or fish exposed to a natural unfiltered water source. Fish of all sizes from fingerling to broodstock can be infected. High stocking densities accelerate spread from fish to fish and elevated temperatures accelerate the replication rate of the parasites. This is primarily a problem in late spring to summer.

Behavioural signs: You can note coughing and increased respiration with a heavy infestation. Gill copepods can cause death but only with very heavy infestations.

Gross lesions: Anywhere where the parasite can attach, you may note red spots (hemorrhage), dead tissue or sores. The parasite can easily be observed with the naked eye. While they are primarily found on the gills, they can be also found on the inside of mouth, gill cover and on the skin, generally around the gills but occasionally elsewhere on the body. The main concern with an infestation of gill copepods is the damage to the surface of the gills where the parasite attaches, this can lead leave the fish vulnerable to secondary bacterial infections.

Samples: No special testing or instrumentation is required for a diagnosis as you can simply confirm the presence of the parasite on external examination your fish. Individual parasites can be pulled off gently with forceps or by removing the piece of gill they are attached to avoid damaging the parasite. These can then be placed in a small jar with 70% alcohol (ethanol is best but isopropanol can also be used) which can be used for DNA testing and species confirmation if that is desired.

Treatment: Emamectin benzoate has historically been the go-to treatment for these parasites, and it is effective. There is some concern over the development of resistance of gill copepods in Ontario to this medication therefore it's use is limited to cases where other treatments are not possible and the risk of death or secondary disease in that population of fish are high. In more recent years, hydrogen peroxide has been used and been successful at knocking back the infection substantially. Anecdotally, we have found that the first year a site is infected, the burden can be severe and each subsequent year, the burden is lessened to a point where only mild flare ups occur seasonally that fish can either manage without treatment or by using hydrogen peroxide baths.

Figure 4: Multiple large gill copepods attached to gill filaments.

Ichthyobodo

History: Ichthyobodo outbreaks often occur with a recent drop in temperature or in the presence of other stressors, often an event associated with poor water quality or other gill disease.

Behavioural signs: You may note fish are flashing, darting or rubbing as well as an increased respiratory rate. A decrease in appetite may also be noted with a significant burden. Often, however, there are very subtle or no perceivable changes in behaviour. Ichthyobodo will generally cause low mortalities, but these will be chronic and can worsen over time if the initiating stressor has not been removed.

Gross lesions: Bluish or whitish film over the entire body has been noted in fish with ichthyobodo infestations.

Samples: An experienced observer can diagnose this parasite under the microscope. Wet mount of skin and/or gills will have small round to ovoid parasites adhered to the skin or gills with a thin strand (like a balloon on a string). When alive, the parasite will gently sway from side to side which makes it easier to distinguish from surrounding cells as they will be of a similar size and

shape. If missed under the microscope, samples of skin and gill can be saved in formalin for histology.

Treatment: Formalin baths are efficient at treating ichthyobodo however they must generally be repeated 3 to 4 times to be considered effective. It is very important to correct any issues with water quality prior to or between treatments. If there is another underlying disease, treat concurrently if you can (ex: antibiotic in feed plus formalin baths) or prioritize treating the disease with the most significant impact on your fish first.

Figure 5: Ichthyobodo parasites adhered to gill filaments at 60x magnification.

Chilodonella

History: Poor water quality is often associated with an outbreak. The onset can be quick as the parasite reproduces by binary fission, which is simply by splitting in half and so what started with only a couple organisms can quickly get out of hand. Fish can quickly go from not exhibiting any signs of disease to dying at a rate that increases daily.

Behavioural signs: Fish infected with this parasite can exhibit a gasping behaviour, have a decrease in appetite and can be observed swimming slowly at the top of the water column.

Gross lesions: With a significant burden of the parasite, fish will produce excess mucous (skin and gills), have a dull colouration or white to blue sheen on body and may have a generally tattered appearance to the fins.

Samples: Wet mounts of skin scrapes and gill clips are sufficient for a diagnosis of chilodonella. Histology of skin and gills preserved in formalin can also confirm the presence of this parasite.

Treatment: Formalin baths in combination with salt are the first line of treatment for this parasite.

Figure 6: Multiple oval chilodonella parasites from skin scrapes. Wet mounts at 40x magnification.

Tetrahymena

History: This parasite is present mainly with high organic waste loads. While it can cause problems with fish in cases where there is predisposing stress, especially underlying disease, it is generally not a cause for serious concern.

Behavioural signs: Generally, no changes in behaviour are noted.

Gross lesions: You may be able to appreciate some areas of skin discolouration, occasionally associated with some mild swelling.

Samples: You can easily identify this parasite skin scrape and gill clip wet mounts. Samples of gills and skin in formalin for histology will also allow for identification of an infection.

Treatment: Treatment is generally not necessary with improvement of water quality. Salt baths can be used in most cases requiring treatment. A single application of a formalin bath also works for severe cases.

Whirling Disease (*Myxobolus cerebralis*)

History: Fish with whirling disease are generally wild caught or raised in open water. Fry or fingerling can experience high mortality rates of up to 90%.

Behavioural signs: Characteristic of this disease is swimming in whirling pattern. This may not always be noticed because some fish have been noted to be resting on the bottom until someone bangs on the side of the tank, at which point they stir up and start to swim in a whirling pattern.

Gross lesions: The main lesions that you will note are spinal deformities and a blackened tail. Fish can also have a shortened lower jaw and indentations on the top of the head.

Samples: If whirling disease is suspected based on the observation of the above signs and lesion, fish should be sent whole on ice for testing via molecular methods and special processing of cartilage where spores can be observed.

Treatment: There is no treatment for whirling disease as the parasites and its spore are found in natural water sources where they cannot be eradicated.

Figure 7: Brown Trout with blackened tail and bent spine.

Bacteria

Bacterial Cold Water Disease (*Flavobacterium psychrophilum*)

History: Historically bacterial cold water disease (BCWD) has been a disease of lower water temperatures (<10°C) however over the past decade, it has occurred in warmer water, up to 15°C or more. BCWD is most common in the spring and can often be associated with recent fish movement (shipping, grading, tank transfer). The disease can be either acute with a sudden onset of moderate to high mortalities or chronic with lower numbers of mortalities extending over a long period of time.

Behavioural signs: In the acute presentation of the disease, there are often no clinical signs. With the chronic presentation fish can have a decreased flight responsef, be sluggish.

Gross lesions: With the chronic presentation skin sores and fin erosion with yellow skin colouration around the wound are most common. Fish can have swollen bellies, appear to have darkened skin and have a large spleen on necropsy.

Samples: A definitive diagnosis can only be made through the laboratory. Sending whole fresh fish on ice for bacterial cs and histology can confirm a diagnosis of BCWD. You can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section.

Treatment: Systemic antibiotics are the treatment of choice for BCWD.

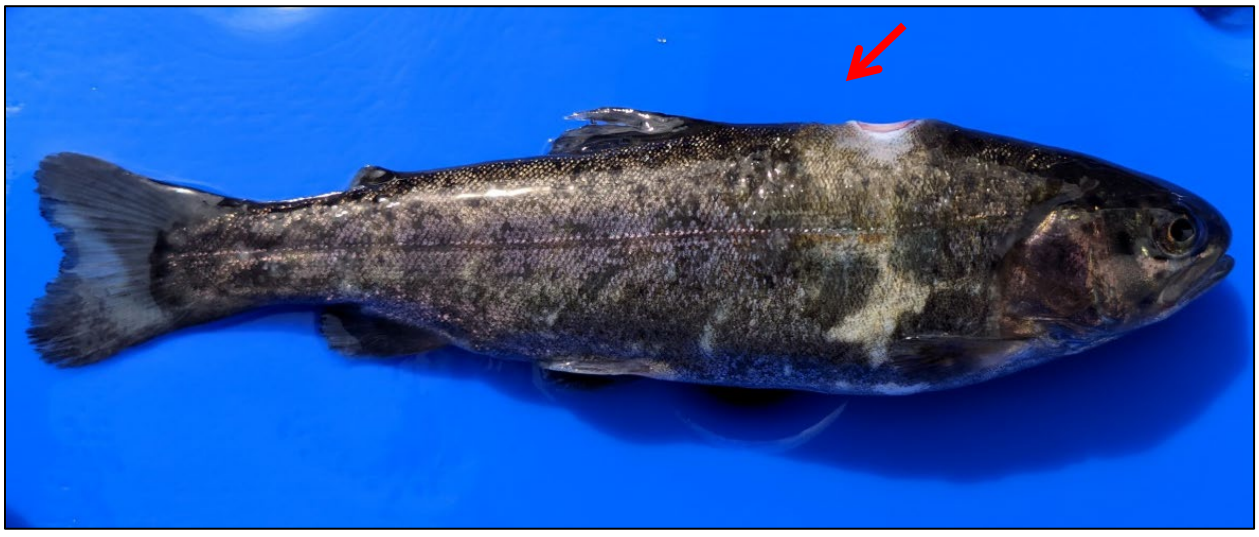


Figure 8: Rainbow Trout fingerling with skin ulcer (red arrow) along its dorsum and darkened skin colouration.

Bacterial Gill Disease (*Flavobacterium branchiophilum*)

History: Bacterial gill disease (BGD) is primarily a disease of hatcheries because conditions such as overcrowding, low dissolved oxygen, high ammonia and high turbidity (recent precipitation with natural water sources) will precipitate and outbreak. In this disease, mortalities often increase rapidly.

Behavioural signs: Fish will generally show some clinical signs despite the rapid onset, these may include flared opercula, fish are slow or sluggish, coughing, increased respiratory rate, piping, swimming along surface or riding high in the water column and loss of appetite.

Gross lesions: With BGD you may note excess mucous trailing from the gills and exposed gills due to a shortened operculum. When you look at the gills, they can appear pale with clubbed gill filaments (see below).

Samples: With an experienced examiner, the long strand or filament like bacteria can be noted on wet mounts of gill clips. Sending whole fresh fish on ice for bacterial cultures and histology can confirm a diagnosis of BGD. You can send a combination of fresh fish on ice and some gills in formalin.

Treatment: Bath treatments with either chloramine-T or hydrogen peroxide have been effective in controlling mortalities associated with BGD.

Figure 9: Rainbow Trout fingerling with eroded operculum and gill clubbing.

Columnaris Disease (*Flavobacterium psychrophilum*)

History: Columnaris disease manifests in warmer waters (>14°C), occurring in the late spring to early fall. Outbreaks can be associated with a recent stressor. During warmer weather (>20°C) mortalities can increase suddenly and continue to increase until fish are treated.

Behavioural signs: Fish with columnaris disease can have an increased respiratory rate, appear sluggish or simply be found dead with no signs leading to the death.

Gross lesions: We see two different presentations of the disease in Ontario; 1) Where lesions are restricted to the gills. You can see dead gill tissue with yellow mucous like material along the edges (see image below) and 2) Systemic disease where bacteria have gone to the whole body and caused lesions like skin sores that have yellow edges.

Samples: With an experienced examiner, the long strand or filament like bacteria can be noted on wet mounts of gill clips forming characteristic stacks. Sending whole fresh fish on ice for bacterial cultures and histology can confirm a diagnosis of columnaris disease. You can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section.

Treatment: Systemic antibiotics are the treatment of choice for columnaris disease.

Figure 10: Gills of a Rainbow Trout with columnaris disease. There are multiple areas of dead gill tissue containing yellow pigment. The yellow pigment is from the bacteria; *Flavobacterium* in latin meaning 'yellow bacterium'. Photo credit: Gabriela Alarcon, 2020.

Furunculosis (*Aeromonas salmonicida* subspecies *salmonicida*)

History: Underlying stressor, increase in temperature. Acute to chronic morbidity / mortality.

Behavioural signs: In acute cases, often no signs, high mortality. In chronic cases, low-grade mortality, lethargy, decreased appetite. In acute cases, often no signs. In chronic cases; raised reddened skin lesions which can rupture, lethargy, decreased appetite, skin darkening, hemorrhage at base of ventral fins, vent and internal organs, large spleen, enlarged belly.

Gross lesions: In acute cases, often no lesions. In chronic cases; raised redened skin lesions which can rupture, skin darkening, hemorrhage at base of ventral fins, vent and internal organs, large spleen, distended coelom. Skin ulcers and furuncles

Samples: A definitive diagnosis can only be made through the laboratory. Sending whole fresh fish on ice for bacterial cultures and histology can confirm a diagnosis of furunculosis. You can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section.

Treatment: Systemic antibiotics can be used to treat furunculosis however these have been found to be ineffective in some cases. For furunculosis, prevention is ideal; either through pathogen free sources of fish or vaccination.

Bacterial Kidney Disease (*Renibacterium salmoninarum*)

History: Bacterial kidney disease (BKD) tends to manifest in the spring, during a rise in temperature. Generally the disease is introduced via an infected fish or eggs.

Behavioural signs: Similar to other bacterial diseases with acute to chronic stages, in severe acute cases there are often no signs with fish simply being found dead. In a more mild and chronic case, fish can appear slow or sluggish and display a loss of balance.

Gross lesions: Infected fish can exhibit dark skin colouration, bulging eyes, pale gills, swollen bellies, redness around the vent (anus) or base of fins and skin blisters or sores. Internally, they can have swollen kidney with white-grey nodules (these can also be found in other organs like the spleen), and there may also be holes in the muscle, usually under the area where you note sores on the skin.

Samples: A definitive diagnosis can only be made through the laboratory. Sending whole fresh fish on ice for molecular testing, bacterial cultures and histology can confirm a diagnosis of BKD. You can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section.

Treatment: Systemic antibiotics have historically not been successful at treating this disease. BKD is very difficult to control as there may be asymptomatic carriers of the disease. Prevention is therefore of utmost importance with this disease. Acquiring a pathogen-free source of fish (testing batches of eggs and fish before entry to your facility) is likely the simplest strategy. Vaccination has even proved to be of variable efficacy.

Figure 11: Exposed kidneys of a Rainbow Trout with BKD displaying white nodules or plaques characteristic of the disease.

Enteric Red Mouth Disease (*Yersinia ruckeri*)

History: As is the case with many bacterial diseases, an outbreak of enteric red mouth disease (ERM) is usually accompanied by an underlying stressor, often another disease. Acute to chronic diseases associated with mortalities can also present with this disease.

Behavioural signs: In acute cases, there are often no signs. In chronic cases, fish are unable to find food as they become blind. Fish can also have abnormal balance and be slow or sluggish.

Gross lesions: Fish with ERM can have darkened skin, swollen bellies filled with fluid, bulging eyes, and can also show redness around and inside the mouth and jaw and gill tips.

Samples: A definitive diagnosis can only be made through the laboratory as many of these lesions overlap with other bacterial diseases. Sending whole fresh fish on ice for bacterial cultures and histology can confirm a diagnosis of ERM. You can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section.

Treatment: Systemic antibiotics are the treatment of choice for enteric red mouth disease. As a preventative measure, one vaccine is approved for use against ERM in Canada.

Figure 12: Red areas along the inside of the mouth characteristic of ERM disease.

Lactococcosis (*Lactococcus garvieae*)

History: Lactococcosis caused by the bacterium *Lactococcus garvieae* is an emerging pathogen in Ontario. It was first identified in the province in the summer of 2020 in rainbow trout. Typically mortality events from lactococcosis occur in temperatures over 16°C however infections may occur at lower temperatures. This disease can also present as acute or chronic. As is often the case, in the acute presentation, fish die before there are any behavioural signs or gross lesions. Those described below are found in more chronic cases.

Behavioural signs: Infected fish will be slow or sluggish, swimming almost in an aimless manor and they will have a decrease or loss of appetite. In cages or larger tanks, mortalities may not be noted as they sink to the bottom therefore it is very important to sweep the bottom of tanks for mortalities, chronic decrease of appetite, poor growth.

Gross lesions: Signs noted are those typical of a hemorrhagic septicemia (bacteria that spread to the whole body and cause bleeding). The most common include dark colouration, bulging eyes, redness around the mouth and eyes, and swollen bellies with fluid inside, slow or sluggish swimming, and abnormal balance. In acute cases, there are few signs with a sudden increase in mortality rate and fish will typically sink, not float to the surface.

Diagnosis: A definitive diagnosis can only be made through the laboratory as many of these lesions overlap with those of other bacterial diseases. Sending whole fresh fish on ice for bacterial cultures,

molecular testing and histology can confirm a diagnosis of lactococcosis. You can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section.

Treatment: Systemic antibiotics can work in some cases however the bacteria seem to have variable resistance against some antibiotics and only appear to have an effect as it likely treats concurrent bacterial diseases like columnaris disease and ERM. Preventative measures such as vaccines are advisable for long-term management of this disease.

Figure 13: Rainbow Trout with Lactococcosis displaying bulging eyes.

Epitheliocystis (*Burkholderiales* species)

History: Epitheliocystis tends to be problematic during times of high precipitation, often occurring during the spring. Mortalities tend to be chronic and low-grade (lower numbers).

Behavioural signs: There are often no clinical signs however, when observable they are mainly restricted to an increased respiration rate and slow or sluggish swimming.

Gross lesions: Pinpoint white or raised spots may be noted on gills (rarely on skin) although these are generally not visible with the naked eye. Gills can appear pale overall and clubbing of gill filaments may also be noted.

Samples: An experienced examiner may note the pinpoint spots on gill clip wet mounts. Formalin-fixed gills for histology and fresh gills for molecular testing can confirm the presence of this pathogen.

Treatment: There are no known treatments for epitheliocystis. Because the bacteria can live inside of cells, this makes the infection very difficult to treat. There is an approved vaccines for a different family of bacteria that cause epitheliocystis in salmonids in Canada however none have been developed for the bacteria that was identified as the causative agent of epitheliocystis in Ontario fish to date.

Fungi

Water Mould or Fungal Infection (*Saprolegnia* species)

History: Fish with fungal infections tend to be significantly immunocompromised in some way; most commonly from a temperature change, recent transport, and skin sore caused by a concurrent bacterial infection.

Behavioural signs: There are generally no clinical signs directly associated with the fungal infection.

Gross lesions: You can readily observe cotton-like growths extending from skin (and/or gills) often with missing skin or a sore underneath.

Samples: Wet mounts of skin scrapes are generally sufficient to identify the fungus as long broad strands that are branching; these are called fungal hyphae. You can confirm the diagnosis or other underlying disease with formalin-fixed tissues for histology.

Treatment: Salt baths are generally sufficient at treating fish with fungal lesions however in more extensive cases where the sores aren't too deep, formalin can be a good choice. Hydrogen peroxide and formalin can also be used on eggs with water mould.

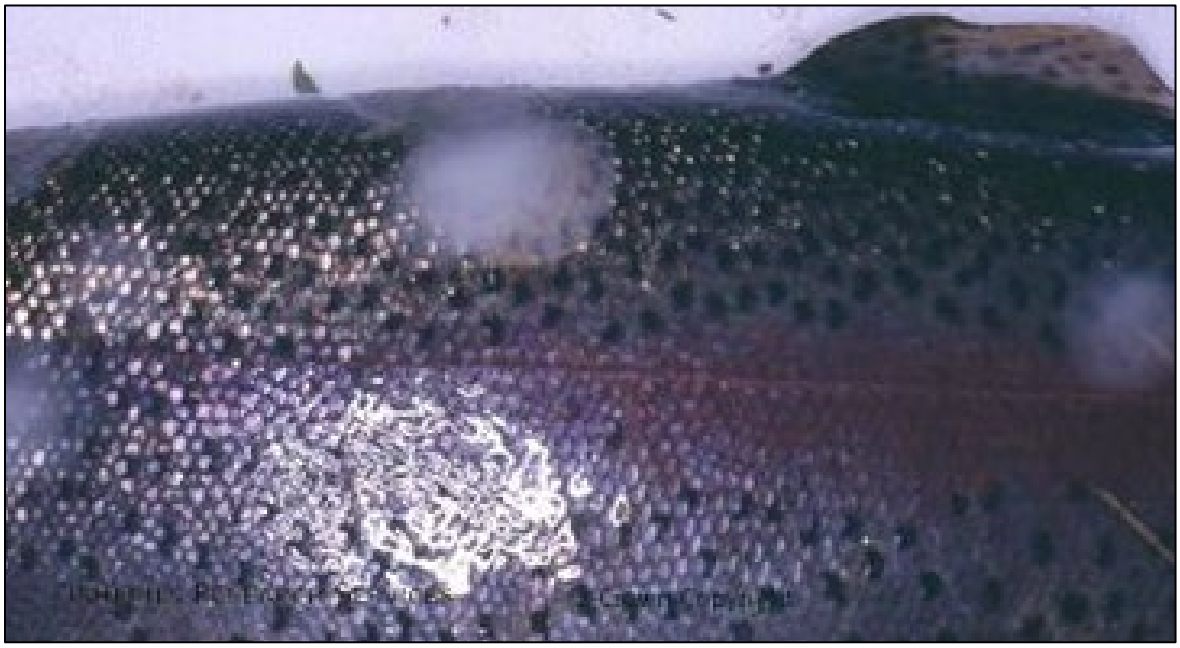


Figure 14: Rainbow Trout with white cotton-like skin growth - characteristic of a fungal infection.

Viruses

Viral Hemorrhagic Septicemia Virus (rhabdovirus)

History: Viral hemorrhagic septicemia (VHS) is typically found in wild fish and can seasonally cause very high mortality rates.

Behavioural signs: Fish with VSH may be noted gasping at the surface or swimming with a corkscrew motion.

Gross lesions: Most commonly observed are hemorrhages (pinpoint red spots) on the skin, eyes, vent, base of pectoral fins and internal organs. Also noted in some cases are bulging eyes, pale gills, a generally dark skin colouration and swollen belly with fluid inside.

Samples: For the diagnosis of viral diseases, it is best to send whole fish on ice for molecular testing and histology. Provided that you have enough fish to sample, you can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section) for histology.

Treatment: There are no treatments for VHS and no approved vaccine in Canada.

Figure 15: VHS hemorrhages (pinpoint red spots) on the skin, eyes, vent, base of pectoral fins and internal organs.

Infectious Pancreatic Necrosis (birnavirus)

History: Infectious Pancreatic necrosis (IPN) will typically occur in fish grown in open water. Rainbow trout, brook trout, lake trout, brown trout, Atlantic salmon and arctic charr are all susceptible to the disease. Fry and fingerling are the most susceptible as fish will gain immunity over 3-4 months of age. Survivors of an outbreak can remain carriers and shed virus intermittently for long periods of time.

Behavioural signs: A population infected with IPN may experience a sudden increase in daily mortality rate which may be the only sign of disease; 10 to 90% cumulative mortality can be expected with this disease. The most common behavioural signs of IPN are; decreased appetite, swimming in a spiral or corkscrew motion, or laying still on the bottom of the pond.

Gross lesions: Fish with IPN can exhibit darkened skin colouration, long thin trailing fecal casts, swollen bellies (fluid-filled), pale gills, bulging eye, hemorrhage or redness along the belly and base of fins, empty stomach and intestines filled with clear to milky mucous.

Samples: For the diagnosis of viral diseases, it is best to send whole fish on ice for molecular testing and histology. Provided that you have enough fish to sample, you can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section) for histology.

Treatment: There is no treatment available for IPN. Multiple vaccines are approved in Canada for IPN.

Infectious Salmon Anemia (orthomyxovirus)

History: While Infectious Salmon Anemia (ISA) is most common in fish grown in seawater, it has also been identified in freshwater. Species which can be infected are Atlantic salmon, brown trout and rainbow trout. There are many strains of ISA virus, many of which do not cause death or even disease.

Behavioural signs: In cases where ISA does cause disease, you can observe a loss of appetite, abnormal swimming patterns (slow swimming, swimming slowly at the surface), and fish gasping at the surface.

Gross lesions: ISA can also lead to a darkened skin colouration, long thin trailing fecal casts, swollen bellies filled with liquid, pale gills, bulging eyes, hemorrhage (redness) area along the belly and base of fins, empty stomach and intestines filled with clear to milky mucous. Internally, you may note a dark and swollen kidney, liver and spleen, pinpoint hemorrhages (red spots) in the fat between organs, hemorrhages along the pyloric cecae, intestines and liver, a pale heart and fluid in the sac around the heart.

Samples: For the diagnosis of viral diseases, it is best to send whole fish on ice for molecular testing and histology. Provided that you have enough fish to sample, you can send a combination

of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section) for histology.

Treatment: There is no treatment for ISA however there are approved vaccine in Canada against the ISA virus.

Spring Viremia of Carp (rhabdovirus)

History: Spring Viremia of Carp (SVC) occurs seasonally in the spring as the name suggests. Species susceptible are mostly common carp-like species but northern pike and fathead minnow have been found to be experimentally susceptible. The disease can present as acute or chronic.

Behavioural signs: There are often no changes in behaviour for acute cases but in chronic cases, fish have been noted lying on the bottom of tank, congregating in corners or sides of tanks or ponds and appearing generally sluggish.

Gross lesions: You may be able to note trailing mucous casts from the anus in some cases, darkened skin colour, bulging eyes, pale gills with or without areas of bleeding, hemorrhages (red spots) in the skin, at the base of fins, eyes, around the anus (vent), in internal body fat, intestines, and stomach, and swollen belly (with fluid inside) and anus (vent).

Samples: For the diagnosis of viral diseases, it is best to send whole fish on ice for molecular testing and histology. Provided that you have enough fish to sample, you can send a combination of fresh fish on ice and some in formalin (see list of standard tissues to sample in the 'Sampling and Diagnostics' section) for histology.

Treatment: There is no treatment or approved vaccine for SVC in Canada.

Walleye Dermal Sarcoma (retrovirus)

History: Walleye dermal sarcoma only affects walleye and sauger and will occur seasonally. While unsightly, this disease is chronic and self-limiting which means that it resolves over time without intervention. It also does not directly cause mortality.

Behavioural signs: Generally, there are no behavioural changes noted with this disease.

Gross lesions: Various-sized, white to pink, pinpoint to mulberry-size masses, especially on skin but also in the mouth and on gills, rarely internally.

Samples: You can send either the whole fish with mass(es) or just the mass(es) on ice for molecular testing and histology. Provided that you have enough fish with masses to sample, you can send a combination of fresh fish on ice and some or a mass in formalin for histology.

Treatment: None (isolate affected, prophylactic antibiotics if ulcerated).

Other

Nodular Gill Disease

History: A mortality event associate with nodular gill disease is usually accompanied by a change or worsening of water quality and/or increase in temperature. The pathogen responsible in this case is an amoeba. Mortality rates will increase rapidly over a period of days.

Behavioural signs: Fish with NGD are often slugging, swim along the surface, have flared opercula and an increased respiration rate.

Gross lesions: Lesions are restricted to the gills and will look very similar to bacterial gill disease with pale gills, clubbing of gill filaments and mucous trailing from the operculae.

Samples: While the amoeba responsible for NGD can be examined on wet mounts of gill clips, they can be very challenging to find even for an experienced examiner. Saving gills in formalin for confirmation via histology is advisable.

Treatment: Formalin and peroxide are good choices for treatment of nodular gill disease.

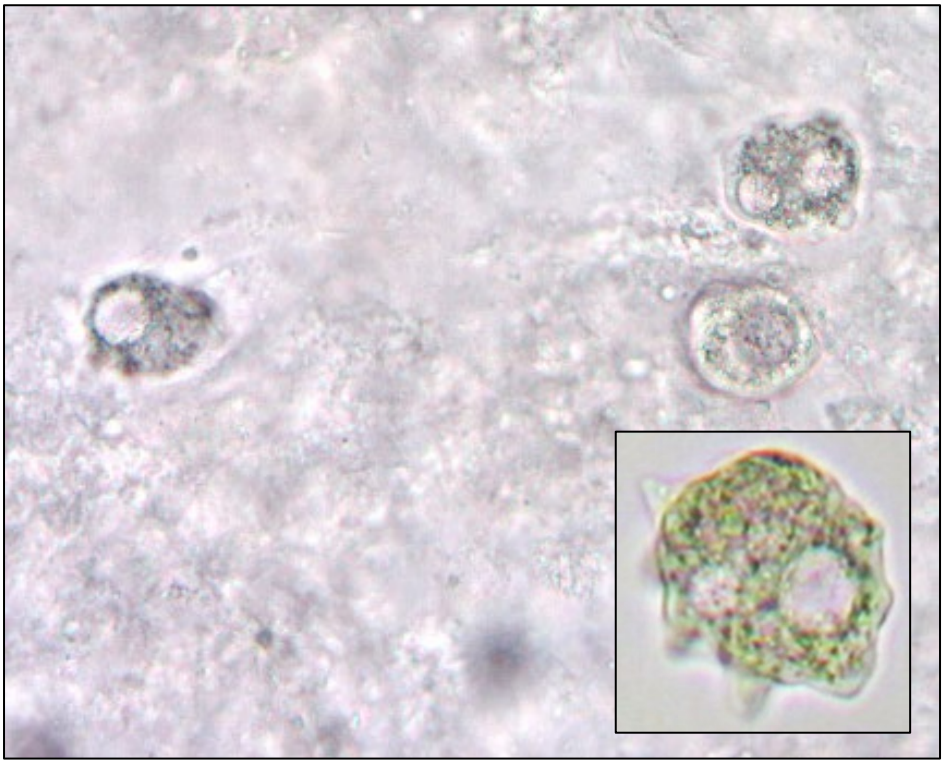


Figure 16: Three amoeba can be seen in the upper half of the image. Characteristic vacuoles and pseudopoda (limb-like projections) can be noted in the magnified image on the bottom right. This image was taken of a gill clip at 40x magnification.

Diatoms

History: Diatoms are a type of algae with a cell wall containing silica. They can be examined from water samples under the microscope and be found in a myriad of intricate patterns. While they are not a pathogen, they can cause problems with fish when there is a bloom. A combination of an increase in lighting (intensity and duration) and high organic waste load can lead to a diatom bloom. In a natural water source (pond, etc.), where there is a thermocline and at the time where there is turnover, is often when a diatom bloom occurs.

Behavioural signs: There are often no changes in behaviour however in severe cases, fish may have an increased respiratory rate and coughing.

Gross lesions: Large numbers of diatoms caught in gill tissue can lead to pale gills, clubbed gill filaments, and increased mucous on the skin and gills.

Samples: Wet mounts of skin scrapes or gill clips will flag the presence of diatoms. Examining a sample of water to see relative numbers can also be done. Formalin-fixed gills for histology will also identify diatoms.

Treatment: A single treatment with hydrogen peroxide can be used. Subsequent removal of the slick of algae that will accumulate at the surface is recommended as this will affect water quality as the diatoms decompose.

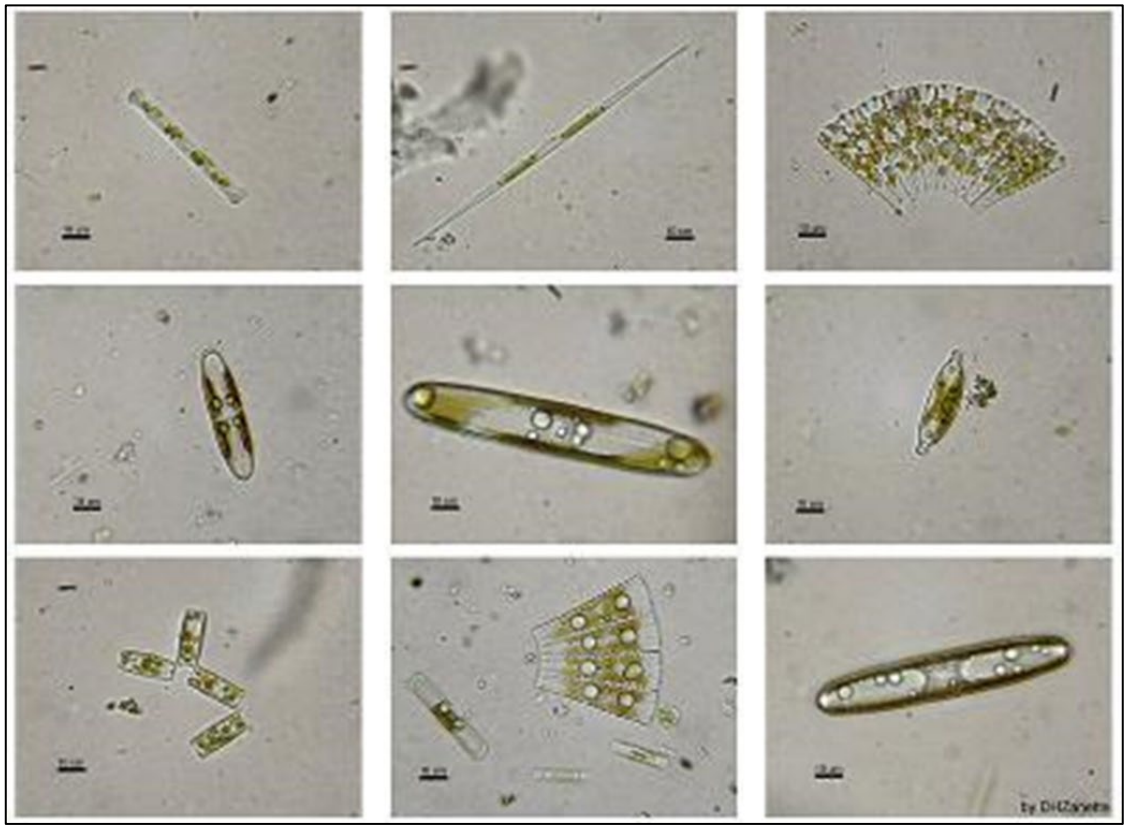


Figure 17: Diatoms under a microscope, unknown magnification.

Biosecurity

What is biosecurity and why is it important for your fish's health?

What is Biosecurity?

Biosecurity is the process of taking precautions to minimize the risk of introduction and spread of infectious organisms into or between populations.

Why is Biosecurity so important?

Since very few effective treatments are available for most aquatic animal diseases, effective biosecurity is the key to preventing these diseases.

How are infectious organisms transmitted?

- Between aquatic organisms in the environment.
- Equipment used to transfer animals from one holding unit or site to another.
- Directly through water by animals releasing infectious agent or sick animals dying.
- Sources of aquatic animal infections:
    - Contaminated feed
    - Contaminated equipment
    - Untreated wastewater
    - Fish-bearing source waters
    - Pests (birds, raccoons, etc.)

What can you do to improve biosecurity at your facility?

How to reduce the risk of disease

- ☐ Strict sanitary measures for personnel and visitors:
    - Provide disinfected or disposable protective clothing
- ☐ Routinely disinfect equipment and water with recommended disinfectants.
- ☐ Restrict vehicle, boat and equipment contact.
- ☐ Maintain a visitor log.
- ☐ Plan flow of personnel movement through the facility.
- ☐ Require disinfection procedures between holding units and/or buildings:
    - Hand sanitizer
    - Foot baths
- ☐ Contain and/or treat effluent and organic waste and prohibit from re-entering your tank or raceway system.
- ☐ Open water facilities, dispose of organic waste on land at a site that has measures to prohibit escape of breakdown products into surrounding waters.
- ☐ Use pest management protocols to keep out birds, vermin and/or predators.
- ☐ Use signage to inform visitors and personnel that there are biosecurity requirements in place such as controlled access, footbaths, video surveillance, etc.

How do I keep my fish healthy?

- Choose a safe water source for land-based facilities such as well water or spring water. Where such water sources are not available, use a disinfection and/or filtration system(s).
- Stock only with certified disease-free eggs and/or fish.
- Schedule routine disease monitoring with a veterinarian and implement a fish health management plan.
- Remove mortalities and moribund animals routinely. When disease is suspected, contact your veterinarian.
- Use caution prior to moving fish between holding units or farms. Fish showing signs of disease should not be sold or transferred to other facilities.
- Minimize handling wherever possible to reduce stress that can predispose fish to infectious diseases.

Standard Operational Measures

- Are there dip nets for each tank?
- How are nets disinfected?
- Is there potential for splashing water from one tank to another?
- Are there foot baths entering each room?
- Are staff members or visitors restricted from entering, required to change clothing/footwear if they were recently at another facility with fish?
- Are there hand wash stations or anti-bacterial solution available?
- Is there a separate area for necropsies or processing?
- How is the necropsy equipment disinfected?
- Do you have Standard Operating Procedures (S.O.Ps) in case of a reportable disease outbreak?

Prophylaxis vs Quarantine

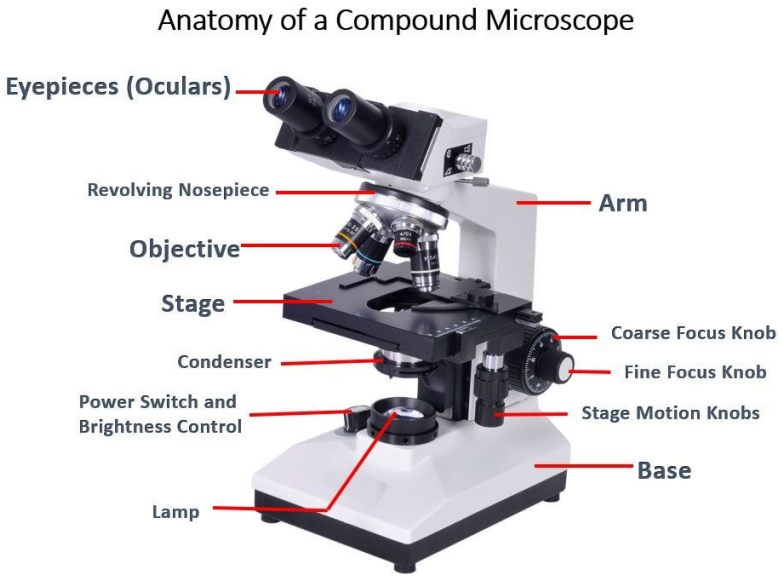
- **Prophylaxis**: along with biosecurity measures, can also include surface treatments prior to transport or in quarantine as well as vaccination.
- **Quarantine**: can simply be a set period of isolation to monitor for disease outbreak. If no abnormalities are noted, animals can be introduced to the remainder of the population. Quarantine can also include prophylactic treatments despite no signs of disease or treatment can be based on specific clinical signs and diagnostic results once disease signs are noted.

Sampling and Diagnostics

How to use a microscope

A microscope can be useful even for novice users to serve as preliminary diagnostics and to aid in the description of an adverse event in your fish population to your veterinarian. A microscope is most useful in the identification of ectoparasites but in a more experience operator can also serve to identify bacterial pathogens, amoeba, and diatoms. In an expert user, you can identify gas bubbles within vessels, various cells of whose arrangement and relative numbers can suggest a type of inflammatory response or disease process.

Before using a microscope, you must know its constituent and their function.



Place your slide containing your sample with cover slip securely on the stage. Always start scanning the sample on the lowest objective or magnification, usually 4x on most microscopes. Scan your slide starting with the top left working your way down to the bottom right corner like you are reading the page of a book. Make notes of the magnification and under which objective you are scanning. For size descriptions use the percent of the field of vision covered by the subject. The size can be calculated using magnification (see table below). Continue to scan your sample at each magnification.

The total magnification of a microscope is the combined magnification of the eye piece and the objective. For example, a microscope with a 10x eye piece magnification and 40x objective, has a total magnification of 400x.

Field Diameter (For a microscope with a 10x eye piece)

4x	10x	20x	40x	60x
5 mm	2 mm	1 mm	500 µm	333 µm

How and what do you collect for testing

When performing your necropsies for the purpose of disease investigation or sending samples to the veterinarian for diagnostics (testing), always try to sample a **fresh dead** (within minutes of death) or fresh euthanized **sick fish**. The reason for this is that:

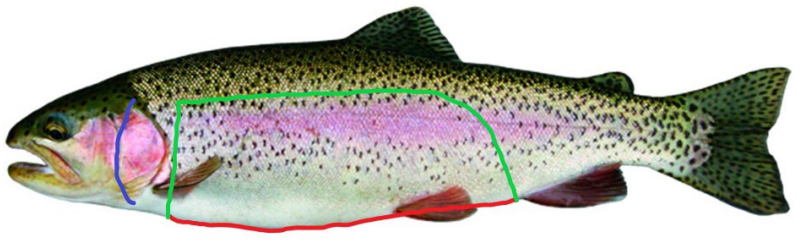
1. Fish that potentially died overnight will be highly decomposed and can also have bacterial overgrowth and parasite infestation which does not reflect the cause of death.
2. If you euthanize a healthy fish (no lesions, normal behaviour in affected tank) for necropsy, it is possible that it is unaffected which also will not reflect the cause of death in your population of fish.

Depending on the size of your population, 5-10 fresh dead fish per affected tank (smaller fish require a larger number for testing). Ideally these are fish that appear sick or have lesions, if there are no observable clinical signs in your sick fish, choose fish at random.

In most cases, you will simply submit whole fish to the laboratory for testing. You may however be asked to save some fish in formalin if you have some on hand. This is not the 36.9% formaldehyde used for surface treatments; the formalin used for sampling is 10% neutral buffered formalin, formulated for tissue preservation and examination via light microscopy or histopathology.

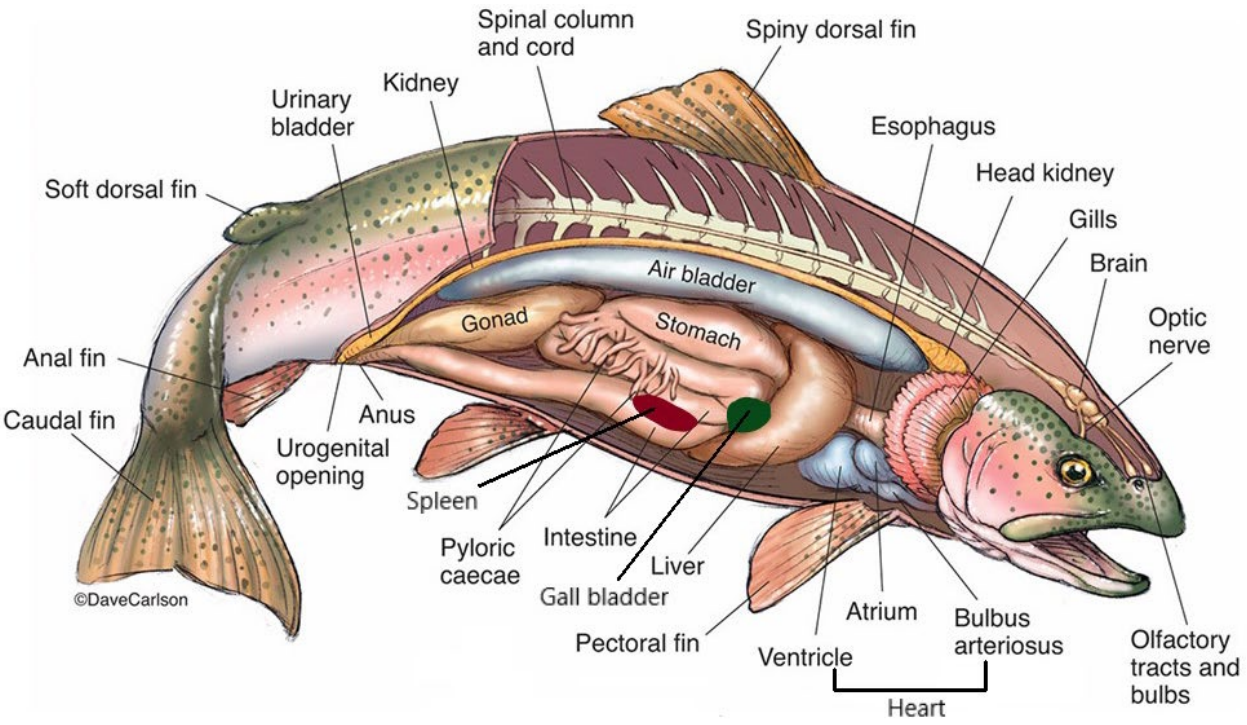
If you are saving tissues in 10% neutral buffered formalin for histology, follow one of the following:

- **Fish < 10 g**: may be placed whole in formalin after the cuts described below (blue, green and red lines) have been made.



- **Fish > 10 g**: the following tissues should be included (see diagram below for reference):
  - ☐ Gills
  - ☐ Skin with muscle (with lesions if there are any)
  - ☐ Heart
  - ☐ Liver
  - ☐ Kidney (anterior and posterior)
  - ☐ Spleen
  - ☐ Stomach
  - ☐ Intestine
  - ☐ Pyloric caeca (finger-like projections that surround the stomach)
  - ☐ Swim bladder (air bladder)
  - ☐ Gonad (ovary or testes)
  - ☐ Eye
  - ☐ Brain (optional, sample in cases where neurological behaviour is observed)

NOTE: There should always be a 9:1 ratio of formalin to tissue for appropriate fixation.



External examination

Quickly but thoroughly examine the outside of the fish including skin, gills, eyes, and inside the mouth. Note any changes in colour from normal or lesions (ulcers, blood, mats of fungus, lumps, etc.). Note all your observations in the submission form and/or in your communications with the veterinarian.

Please note that if you are performing a necropsy on an animal that you wish to save tissues for in formalin, sample ~3 gill arches and immediately place them in **10% neutral buffered formalin** due to fast rate of decomposition of these tissues.

Wet mounts

Gills: gills are the fastest to autolyze (decompose) and so they should be examined first as they often involved in diseases in Ontario aquaculture. Use a small sharp pair of scissors to clip 2-3mm of the gill filaments tips, place on a glass slide, cover with 1-2 drops of water and cover with a cover slip. You have too much sample if you cannot press your cover slip down to create a seal with the water. Examine immediately under the microscope.

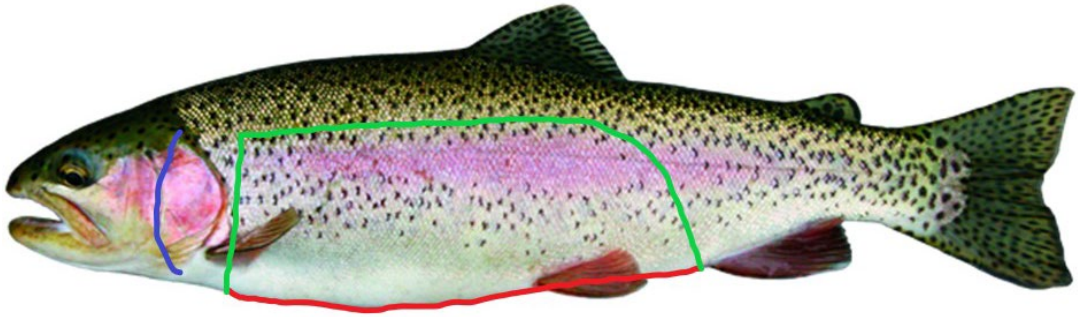
Skin: scrape skin using a scalpel blade or glass slide in the direction with the scales hard enough to remove some scales but not to accumulate too much mucous on a slide. Place 1-2 drops of water over your sample and cover with a single cover slip. You have too much sample if you cannot

press your cover slip down to create a seal with the water. Examine immediately under the microscope.

Fecal: this portion is optional but useful when suspecting gastrointestinal parasites. Massage the ventral abdomen of the fish in an anterior to posterior direction (front to back) to extract a very small amount of feces. Place 1-2 drops of water over your sample and cover with a single cover slip. Examine immediately under the microscope.

Internal examination

Open the coelom creating two cuts; 1) cut along the midline from the vent up to the area between the opercula (**red line**) and then 2) cut from the front of the vent towards the back up to the swim bladder/kidney and around towards the front as far as the coelom extends and then ventrally to meet the end of the first cut (**green line**). As with the external examination, note any changes in colour, size or texture of organs or any lesions that you observe. You can also remove the opercula on both sides to properly examine and sample gills (**blue line**).



Sample submission

Always perform your own necropsy on affected fish when you first notice a problem at your facility. Keep accurate and detailed records of your findings (describe lesions, what you find on wet mounts, behavioural signs, mortality rate, etc.). These findings are very important as part of the history when submitting a case for testing (bacteriology, histology, virology, etc.).

Submit a case to your veterinarian if the problem persists or submit fish immediately in case of a sudden high mortality rate.

How to package samples

Packaging

1) Whole fish are to be placed in sealed waterproof bags* (Ziplock bags or Whirlpack bags are appropriate). Double bagged is ideal in case of leaks.
2) Either place fish individually in bags or separate them by groups (for example, by different tanks or raceways).
3) Label each bag with the tank, raceway or group identifier, date and time of collection.
4) Place fish in bags without water but do not pat the fish dry as this will compromise the outer layer of the fish.
5) Immediately place the fish in a cooler on ice. Place a large amount of ice in the cooler and avoid opening the cooler after placing fish inside to keep the samples cool. Shaved or crushed ice is ideal as it will not crush your fish samples. If you choose to use ice packs or large blocks of ice, place your fish in a solid sealable container to avoid crushing the samples.

In a case where you are unable to ship or drop off fish immediately, freeze fish in labelled bags as described above until you can send fish for testing. This is not ideal for some types of tests but is better than allowing fish to decompose at room or refrigerator temperature, especially if the delay before shipment will be multiple days.

Paperwork

Always fill out a case submission form, submit a copy with the samples, submit an electronic copy to your veterinarian and Community Hatchery Program Coordinator via email *prior* to shipping as this will notify them of an incoming case. To obtain a submission form, either contact the Community Hatchery Program Coordinator or go directly to the Guelph Animal Health Laboratory website (https://www.uoguelph.ca/ahl/), under the "Submissions" tab, select "Submission Forms and Special Projects". This will bring you directly to the page where you can select the PDF form "Fish Submission Form". You can fill, save or print this form as needed.

The URL to access the form directly is:
https://www.uoguelph.ca/ahl/system/files/AHL_Fish_Submission_Form%20_%2020.pdf

Where to submit samples

Shipping

Send all samples via same-day or overnight courier with attention to "AHL Fish Lab (Mycoplasma)" or

Drop-off (in person)

Drop off your cooler at the "Specimen Reception" desk at the back of the Pathobiology and AHL Building. The building is on the University of Guelph campus at the corner of Gordon Street and McGilvray Street and is a large glass building. Please go to the back and drop off specimens at the door beneath the blue and white 'Specimen Reception' sign.

Remember to include a printed copy of the completed submission form with your samples.

Shipping and drop-off address:

Animal Health Laboratory - FISH LAB (Mycoplasma)
Laboratory Services Division
University of Guelph
Building 89, 419 Gordon St.
NW Corner Gordon/McGilvray
Guelph, ON N1G 2W1

Glossary of Terms

Acute: having a sudden onset.

Antibiotic: a compound that has the ability to kill or inhibit microorganisms such as bacteria or fungi.

Asymptomatic: not showing signs of disease or illness.

Bacterium: a microscopic single-celled organism without nucleus. The pleural of bacterium is bacteria.

Bacterial culture: a test that grows bacteria to identify which types are present in a sample, for examples, a swab from a diseased fish's kidneys.

Bath treatment: a type of treatment where medication is put in a water bath (cooler, isolated tank, etc.) and fish are placed in the medicated solution for a set period of time as part of a treatment regime.

Behavioural signs: behaviours (swimming pattern and speed, appetite, congregating at outflows, etc.) deviating from the expected or normal behaviour of a fish which could indicate something specific like a particular disease.

Broodstock: a group of mature fish used for breeding purposes.

Carrier: a fish that can transmit a disease causing agent such as a bacterium, virus or parasite.

Certified disease-free: fish grown under conditions and undergoing specific testing which minimize the risk of infection and spread disease. In Canada, these guidelines and labels are federally mandated.

Chloramine-T: an organic compound that is an antibacterial and disinfectant.

Chronic: continuing for a long time.

Clinical signs: a change either in behaviour or appearance of a fish that indicates disease.

Clubbed gills: severely thickened gill filaments to a point where they look like small clubs.

Coelom: refers to the body cavity of the fish.

Darting: swimming rapidly and erratically.

Density (stocking): refers to the amount of fish per volume of water, primarily expressed as the number of grams of fish per L.

Diagnosis: the identification of the nature of a disease.

Dose: a quantity of a drug recommended to be given at a specific time.

Ectoparasite: a parasite that remains on the outer surface of the fish.

Ectotherms: an animal that is dependent on the environment as the source of its body heat. In fish, the temperature of the water dictates body temperature.

Erosion: loss of the top layer of a fish's skin, a very superficial wound.

Fingerling: juvenile stage trout; called so because they are approximately the size of a human finger.

Flashing: a type of erratic swimming pattern where the fish turns rapidly side to side, showing its sides to the top of the water.

Flight response: a fish's response to potential harm by swimming away from the perceived harm.

Fry: a hatched trout that is less than one inch in length and has learned to search for food and begin eating.

Fungus: a fungus is a group of eukaryotic organisms that includes yeasts, molds, and mushrooms.

Furuncles: another word for a "boil" or skin wound generally caused by a bacterial infection.

Gill filament: the red, fleshy part of the gills, responsible for oxygen and nutrient exchange into the blood.

Gilling: term used for raising and lowering of gill covers representing respiration or breathing.

Hemorrhage: an escape of blood from a broken blood vessel.

Histology: branch of biology concerned with the composition and structure of plant and animal tissues in relation to their specialized functions.

Immunocompromised: having an impaired or compromised immune system.

Lesions: any damage or abnormal change in the tissue of an organism, usually caused by disease or trauma.

Lethargy: a lack of energy, activity and responsiveness.

Morbidity: the condition of suffering from a disease or medical condition.

Mortality rate: a measure of the number of deaths in a population over a defined period of time.

Necropsy: autopsy.

Operculum: gill cover.

Parasite: an organism that lives in or on an organism of another species (its host) and benefits by deriving nutrients at the other's expense.

Pathogen: a microorganism that can cause disease for example; bacteria, parasites, fungi and viruses.

Prophylactic: intended to prevent disease.

Standard operating procedures (S.O.P.): a set of step-by-step instructions compiled by an organization to help workers carry out routine operations.

Secondary infection: an infection that occurs during or after another infection.

Systemic disease: a disease that affects or has spread to the whole body.

Turbidity: the measure of relative clarity of a liquid.

Vent: the cloaca or anus equivalent in fish.

Ventral: refers to the bottom half of the fish.

Virology: the scientific study of viruses.

Virus: a submicroscopic infectious agent the replicates only inside of living cells.