GILL DISEASES OF FISH IN ONTARIO FARMS

© Ontario
Ministry of Agriculture, Food and Rural Affairs
Acknowledgements

The Fish Pathology Laboratory receives much of its funding from the Ontario Ministry of Agriculture, Food and Rural Affairs. John Lumsden has a Medical Research Council Fellowship. We thank Gord Cole for reviewing the manuscript.
GILL DISEASES OF FISH
IN ONTARIO FARMS

Hugh W. Ferguson, John S. Lumsden,
Dan D. MacPhee, and Vaughn E. Ostland,
Fish Pathology Laboratory, Pathology Department,
Ontario Veterinary College, University of Guelph
(519) 824-4120 ext. 4640
ACKNOWLEDGEMENTS  
• Inside Front •

INTRODUCTION  
• 1 •

GILL STRUCTURE AND FUNCTION  
• 2 •

THE DISEASE HISTORY  
• 3 •

RESPONSES OF GILLS TO INJURY  
• 4 •

SAMPLE SUBMISSION  
• 5 •

MICROSCOPICAL CHANGES  
• 6 •

CONSEQUENCES OF GILL DISEASE  
• 6 •

SPECIFIC DISEASE PROBLEMS  
• 7 •

CONCLUSIONS AND FUTURE DIRECTIONS  
• 14 •

SELECTED REFERENCES  
• Inside Back •
Gill diseases are among the most important problems affecting farmed fish in Ontario. In two case-surveys of fish brought in for diagnostic help to the Fish Pathology Laboratory of the Ontario Veterinary College, from 1977-1981 and from 1982-1988, diseases which targeted the gills ranked number one amongst all other problems; they represented approximately 51% of all of the cases submitted. This figure applies only to infectious diseases; if we include water quality problems, such as toxic pollutants or metabolic waste products, which can also target the gills, the number would have been even higher.

This obvious susceptibility has stimulated our research program, which over the past few years has been investigating the most common gill diseases of Ontario farmed fish, as well as some of the more fundamental aspects of gill function. The following discussion is a brief overview of some of our findings.
Before we can understand how the gills become diseased, and the consequences of their responses to agents that can damage them, it is necessary to have a good basic understanding of their organization.

The gills have a very large surface area; in many species, including salmonids, it is several times larger than the rest of the body. This fact results in an intimate and extensive contact with the surrounding water. Water is drawn in through the mouth and leaves through the opercula or gill covers. In salmonids, this occurs by means of a two-pump system; the pumps operate in synchrony, one pulling while the other is pushing, to achieve a smooth and continuous flow of water over the gills.

The gills are responsible for absorbing oxygen from the water. Compared to air, water contains relatively little oxygen even under optimum conditions, and the gills must therefore make very efficient use of the little that is available. Water is also very heavy compared to air and a lot of energy is therefore required merely to pump it over the gills.

The gills are composed of four pairs of gill arches; each arch supports rows of finger-like filaments (Figure 1a-c). On both sides of each filament are the plate-shaped lamellae, and it is from here that the oxygen is absorbed into the blood which percolates between their thin walls. The efficiency of oxygen uptake is enhanced by the direction of the blood flow - it travels along a route opposite to that of the water as it moves between the lamellae. A normal fish
under resting conditions uses only a fraction (35-50%) of its gill capacity; in times of increased demand for oxygen, such as during energetic swimming, this reserve capacity can be called upon to enhance uptake.

The cells that cover the lamellae have numerous fingerprint-like ridges on their surface (Figure 2a and b). These microridges are believed to act mainly as anchors for the mucus which flows as a continuous blanket over the gills and which helps to clear debris from the gill surface. The mucus is released by cells found mainly on the filaments and on the arches.

We understand relatively little about the properties of the mucus coat, but we do know that it is critically important to proper gill functioning and health. It represents the actual interface between the gill and the watery environment of the fish, and it is likely that many of the early important events in any disease process occur within this layer. Mucus normally contains a whole range of substances, including antibodies, which conspire to keep the gill surface free of potential disease-causing agents.

For a proper understanding of gill diseases, it is important to appreciate that in addition to their role in oxygen uptake, the gills are also responsible for regulating some aspects of salt balance and for the excretion of carbon dioxide and other metabolic wastes such as ammonia. A disease that attacks the gills may interfere with any one or with all of these processes and the variety of responses seen in fish having different diseases is therefore a result of differences in the extent to which each of these normal processes is altered.

The Disease History

There are relatively few ways in which the gills can respond to damage and there are virtually no changes that are specific to one particular type of causative agent such as a fungus or a toxic chemical. As a result, we tend to see a limited array of responses, and it is part of the challenge for the diagnostician to integrate the results of
the tests which are employed (tests such as histopathology and blood chemistry) with the history provided by the farmer, in order to arrive at a correct diagnosis and to be in a position to recommend the correct approach to treating the fish. As a result, a thorough disease history is absolutely critical to the diagnostic process.

Some of the ways that the farmer can help to pinpoint the cause of the damage include a careful direct observation of the fish in the troughs, tanks, or cages, not forgetting their responses to frightening stimuli and to feed. Fish with gill disease are often more lethargic than normal, swimming slowly at the surface of the water, or round the edges of the tanks, although the type of response can vary greatly with the species involved. They may be dark and react poorly or not at all when frightened. They may show little interest in food, or their feeding responses may be undiminished. Some gill diseases can affect the entire population of a tank, leading to explosive and high mortality, while others may cause only low numbers of deaths that grumble along slowly for long periods of time.

Careful observation of the fish can also help to distinguish between the various types of damage: if the fish tend to aggregate at the outflow to the tank, you may be dealing with a water-borne toxin. If, on the other hand, the fish tend to congregate round the inflow to the tank or pond or to gasp near the surface, this can suggest that there is a relatively low oxygen level.

Fish with gill disease often breathe more rapidly than normal and they may appear to cough frequently; this is seen as a rapid, spasmodic clamping together of the opercula. Mucus may be seen streaming from the edges of the opercula, which may themselves be flared outwards, so that when seen from above, the fish have an arrowhead-like appearance (Figure 3). Indeed, fish that have died from gill disease often die with flared opercula.

Each and all of these observations are vital clues to unravelling the cause of the problem.

**Responses of Gills to Injury**

One of the very early indicators of gill damage is the release of copious amounts of mucus. This can happen whether the injury is caused by a water-borne toxin, a parasite or by bacteria. The mucus can be released in such large quantities that the water actually foams, and this foam may be
seen floating on the surface of the troughs, raceways, or ponds. Of course, the skin also contains a large number of mucus-producing cells which react in a similar way to injury, so it is important to distinguish carefully between skin injury and gill injury, although they can often occur together.

Increased quantities of mucus can cause the gills to appear pale and shiny. In other circumstances, the mucus can lead to trapping of organic detritus so that the gills appear brown.

Other changes which can be seen with the naked eye include a thickening or “clubbing” of the tips of the filaments. Some gill diseases cause the gills to bleed, and small spots of hemorrhage are also easily seen. (Figure 4)

A common finding in fish that have had gill disease for a long time is erosion of the edges of the opercula (Figure 5). This is often severe enough to expose the underlying gill tissue. The consequences of this change are poorly understood (and they may even be seen as a consequence of heavy stocking) but they probably include a reduction in the efficiency of pumping water over the gills; this is bad enough in otherwise healthy fish, but in those that have gill disease, it may be serious indeed, impairing their ability to compensate for damaged tissue.

If gill disease is suspected, the need for a close, direct examination of the gill tissue cannot be overemphasized. This can be accomplished by gently scraping the surface of the gills, or by clipping off a few filaments, and placing these on a glass slide. If a drop of tap water (for freshwater fish) is added to this material, and a cover-slip is placed on top, it can be examined through a microscope. With a little practice, it becomes easy to recognize the various parts of the gills, and to identify some common parasites - the fact that they are moving can help to distinguish them from gill tissue!

**Sample Submission**

Recognizing more subtle changes requires preserving (“fixing”) the gills in fixatives such as 10% formalin (the concentrated form is 37% formaldehyde and this is known as 100% formalin) followed by special
“processing” of the tissue in a diagnostic laboratory. As a first preference, diagnosticians like sick but live fish, followed by freshly dead ones on ice. Fish that have been dead for more than two hours, or frozen fish, are much less useful. Providing water samples can be especially useful; inflow and outflow samples can help differentiate certain types of problems. In all cases where disease is suspected, we recommend that you contact the diagnostic laboratory and discuss the problem with us. We can then advise you on the types of samples to collect.

Microscopical Changes

Changes commonly seen in these “processed” tissue sections (histological slides) include a loss of the normal covering of the lamellae, due to cell death. Alternatively there may be fusion of the lamellae, swelling or enlargement of the cells and an increase in their number. During a disease process, all of these changes may be encountered, but at different stages throughout its course. Putting an approximate “age” on the changes seen can be an important piece of information, as it may give an indication of the length of time expected for recovery.

Consequences of Gill Disease

Taken together, the consequences of these changes are to alter the even flow of water over the gills, creating turbulence, and thereby reducing the efficiency of gas exchange, as well as altering the other normal gill functions, namely ammonia excretion and salt regulation. As may be imagined, the results of the changes may be to reduce the efficient uptake of oxygen, and to impair the excretion of ammonia and other metabolic bi-products. Other results in freshwater may include a loss of salts, a process that can be thought of as “respiratory diarrhea.” Once again, some or all of these processes may occur together, or at different times throughout a disease process. An appreciation for all of the possible combinations is important when developing a rational approach to treatment.

Approaches to Treatment

These are dealt with under the specific disease problems section. While a few generalities do apply, such as reducing the feed before and after treatment, we tend to treat each case individually, depending on the type of disease, the numbers of fish affected, and the severity of the outbreak. When calculating dosages of chemical and volumes of water, mistakes in arithmetic are easily made! As a rule, therefore, we recommend that any treatment be tested on a small batch of fish before being used on the whole population. We also recommend re-submitting fish after treatment, to see if the problem is persisting.

One very interesting result of our surveys was the finding that successful treatment of gill disease of all kinds depended on a proper diagnosis. Success of treatment without a diagnosis was as low as 21%, even in well-established farms with experienced
management, whereas with a proper diagnosis, success rates rose to almost 89%.

T
he two most common gill disease problems in Ontario fish farms are the infectious ones of bacterial gill disease (BGD) and nodular gill disease.

1. Bacterial gill Disease
This disease affects mainly young fish of all species, but it is not uncommon in growers and even broodstock. As with many bacterial diseases, brook trout seem to be especially susceptible. While it is common in Ontario, it is a disease with a worldwide distribution. It can occur throughout the year, but outbreaks are more common in spring than in late summer and fall. Outbreaks often occur within 48 hours of a period of heavy rainfall, but as with almost all diseases, the precise pattern seen varies greatly from farm to farm.

Specific Disease Problems

6. Filaments showing swelling of the tips; this is called "clubbing", and can often be seen with the naked eye.

7a. Direct examination of fresh gill tissue from brook trout with bacterial gill disease. The bacteria can be seen as fine hair-like organisms on the cell surface.

7b. Scanning electron micrograph of fish with bacterial gill disease. The bacteria can be seen as numerous thread-like structures on the surface of the lamellae. Several disc-shaped red blood cells can also be seen.

Typically the disease progresses rapidly through a population. Affected fish stop feeding and show a reduced fright response. They lie listlessly at the sides and top of the tank, breathing laboriously, and they have flared opercula (Figure 3). In chronically affected fish, the gill filaments may be clubbed (Figure 6).

Mortalities can be extremely high if the fish are not treated. In the microscope, large numbers of bacteria can be seen attached to the surface of the gills (Figure 7a-c); the lamellae may also be fused and distorted.

The cause of the disease in Ontario is a bacterium called Flavobacterium branchiophilum. This is a long thread-like or filamentous organism that is extremely difficult to
grow in the laboratory. When this is achieved on special media, the colonies are a bright yellow colour. The bacterium is covered in fine hair-like structures called pili and these are probably responsible for its ability to attach to the gills (Figure 8). While we do not know precisely how attachment of the bacteria to the gill surface results in disease, nor where they come from, we do know that the organism is extremely widespread, although it is not found on the gills of healthy fish.

Recently we have been able to isolate and culture the bacterium, and we can now produce the disease experimentally in brook trout and rainbow trout of all ages, merely by adding the bacterium to clean tank water. Under these conditions the bacteria attach to the gill surface within one hour and the fish become restless and start to cough. Within 12 hours, the fish are starting to breathe more rapidly, they lose their interest in food, and they become lethargic. They can die within 24 hours, depending on the initial dose of bacteria added to the water, but even with a low dose, the fish will still start to die within four days. If sick fish are added to a tank of healthy ones, the same pattern is repeated, although it can take a little longer to develop. These experiments serve to underline the extremely infectious nature of the disease and the speed with which it can progress through a population. If blood is taken from sick fish for analysis, surprisingly, we can see that fish with BGD are not short of oxygen. They are, however, low in blood sodium and chloride, suggesting that these have been lost through gills damaged by the bacteria.

Other types of non-filamentous bacteria can attach to the gills and also cause disease, although these are uncommon in Ontario.

**Treatment and control of BGD**

In our experience, chloramine-T is the drug of choice for treating fish with BGD. It should be used as a bath treatment at 10 parts per million for one hour. As with all bath treatments,
lowering the water level to half way (and therefore treating for half the normal volume of water) allows for a more rapid lowering of the concentration of the drug in the tank once the water is turned on again. There is usually a very rapid response, and the fish may improve significantly within only a few hours. A second or even a third treatment on alternate days may be needed for severe outbreaks.

In all diseases, the treatment often kills the sickest fish, but these would probably have died anyway. Nevertheless, treatment-associated deaths with chloramine-T are usually lower than with many other treatments, at least in the early stages. The types of changes seen in the gills of chronically affected fish, (having pockets of bacteria protected from the full effect of the drug) may reduce the effectiveness of any bath treatment, and several treatments, or indeed an entirely different approach altogether, may be required.

Alternative chemical treatments include formalin (37% formaldehyde is the concentrated form, and this is equivalent to 100% formalin) at one part formalin in 6,000 to 8,000 parts of water for one hour. This treatment tends to be slightly harsher on the fish than chloramine-T, but our experience suggests that it is more effective for treating mixed infections (i.e. where BGD is complicated by the presence of fungi and/or protozoan parasites). These situations are not uncommon.

We do not recommend using any of the quaternary ammonium compounds (QACs) for treating gill diseases, as we have shown that the gills can be severely damaged even at the recommended dosages; experience has taught us that many more fish than normal died after treatments with these types of chemicals.

Although we have demonstrated that BGD can be reproduced with the bacteria alone, even with the fish in top quality water, we believe that poor water quality can predispose the fish to developing the disease and/or worsen an already existing situation. We know, for instance, that there is a very close association between feeding and disease, and that the condition can be easily modified by withholding food; this is obviously an important area that we are currently investigating. Other recommendations for helping to control the disease that stem from such findings include the following:

1. Increase the water flow wherever possible, even if oxygen levels seem adequate. If this is not possible, consider reducing the water depth so that the turnover time is decreased.

2. Reduce the feed to affected tanks. Many of the sick fish will in any case have lost their appetite. Spread out the daily food ration as much as possible into numerous small amounts, rather than a single large one. Because the disease so commonly follows a heavy rainfall, there may well be a case for reducing feed levels for a few days afterwards.

3. Remove dead and dying fish as soon as possible and try to maintain good hygiene between tanks and troughs by paying strict attention to disinfecting.
nets and other equipment; this is a very infectious disease that is easily spread between tanks on contaminated nets or hands.

2. Nodular Gill Disease
This proved to be the second most common gill disease in both of our surveys. As with BGD, it is primarily a problem of younger fish, although severe disease is not uncommon in older fish. Fish of all species in Ontario are probably susceptible. The disease closely mirrors the situation seen in BGD, and indeed the two conditions would seem to be closely associated.

Fish with nodular gill disease, however, do not stop feeding and they maintain their fright response. Instead of large numbers of sick and dying fish, as seen in BGD, nodular gill disease produces lower mortalities that occur over a longer period of time. And there is usually a poor response to the normal treatments for BGD, namely chloramine-T. The fish keep on dying, a few at a time, but constantly. When the fish are examined, grey-white nodules can be seen attached to the gill surface (Figure 9). This reaction can be so pronounced that an entire gill arch can be obliterated (Figure 10). Some farmers have interpreted these nodules as “mucus that won’t detach”.

The cause of the disease is a protozoan parasite, an ameba called Cochlidiopodium (Figure 11). The close association between BGD and nodular gill disease is probably due to the fact that the bacteria on the surface of the gills attract the parasite and these then start to eat the bacteria. In the process, enzymes are probably released and these damage the gills, which then respond by fusing together and proliferating. The “nodules” seen with the naked eye
therefore represent this damaged and proliferated gill tissue. Treating the fish with chloramine-T removes the bacteria, but not the parasites. We do not yet know why fish with this disease die, but we suspect that it is not from salt loss (as with BGD). Instead, we feel that it is probably due to oxygen deficiency; the ever-encroaching fusion and proliferation of the gill tissue forces the fish to utilize its tissue reserves and when these finally run out, it dies.

In our experience, treatment is difficult but can be successful using formalin at high doses and for several consecutive days. As with all gill diseases, but especially so for nodular gill disease, the regime that we recommend varies according to how sick the fish are. Nevertheless, levels of at least one part formalin in 6,000 parts of water for one hour are needed for there to be any significant effect on this parasite. And this usually needs to be repeated for three consecutive days. This relatively high dose rate may have to be reduced to one in 8,000 for young fish. Formalin removes oxygen from the water and is therefore more dangerous to use at higher water temperatures. Regardless of the temperature, mortality due to the treatment itself can be reduced if the water is aerated during the treatment. Because the fish probably die due to oxygen deficiency, we recommend that oxygen levels be optimized, or increased, if at all possible. Reducing the feeding level is also recommended. As a general rule, fish should not be fed all, 24 hours before, and 24 hours after any treatment.

3. Columnaris Disease (also known as “saddlepatch” or “saddleback”) This is quite a common problem, especially in cages and on bait-fish farms, but mostly it occurs in summer when water temperatures get close to or exceed 18°C. It is often regarded therefore as a warmwater disease. It can affect all species and all ages of fish, although as with most diseases, mortality is highest in smaller fish. It does not restrict itself to the gills, the skin and the mouth being other sites commonly affected, but it is possible that certain strains of the bacteria do prefer to attack the gills.

The cause is another long filamentous bacterium called *Cytophaga* (formerly *Flexibacter*) *columnaris*. The bacteria can aggregate on the skin and gills to form cottonwool-like “columns”, clumps or haystacks, which can sometimes become large enough to be seen with the naked eye.

On the skin, the typical change is a light-coloured saddle-patch round the dorsal fin, which may be extensive enough to form a pale band round the
mid-section of the fish. The mouth is commonly affected in bait fish (Figure 12) and the lips may be almost entirely destroyed. If present in large numbers, the bacteria can turn the skin round the mouth a yellow colour. In the gills, the bacteria are especially destructive, often causing massive damage and bleeding, while other areas in the gills can be pale. The increased production of mucus that accompanies the infection tends to trap uneaten food and other debris; this can turn the gills a patchy brown colour (Figure 13). When this is combined with bleeding, which with time can also turn brown, the gills can be particularly striking.

4. Fungal Disease
These gill infections are most common in young fry. The mats of Saprolegnia-like fungi grow extensively within the gill cavity and they can even march down the esophagus into the stomach or up into the swimbladder. The fungi are very destructive and affected fish usually die, although it is uncommon for large numbers to be affected at any one time. In our experience, the disease is often associated with poor feeding methods, such as giving the wrong size of crumble or pellet. If these are too big, they can lodge in the esophagus and act as a “beachhead” for the fungi, which can then grow out to attack the other tissues. Similarly, if the feeding intervals are too long (sometimes over a weekend), the fish may be tempted to eat a pellet they would otherwise leave alone.

Affected fish can be difficult to treat. Control of the disease, therefore, is centred round the idea of preventing its spread by focusing on the need to correct the feeding frequency and to ensure that the food itself is the correct size, aiming for the smallest fish in the tank rather than the biggest, which is sometimes the natural tendency. It is important to regularly remove uneaten food and dead fish from the tank bottom, as these act as reservoirs for the infection; so too do automatic feeders that are hanging too close to the surface of the water. These need to be cleaned regularly to prevent a
build-up of fungus round the edges where the food gets wet.

Fungal infections also quite commonly accompany BGD, in which case the age range follows that of BGD. These mixed infections can be difficult to treat, but we usually aim to target the bacteria first.

5. Parasitic Infections
A variety of different protozoa can target the gills. The most common ones include whitespot (Ichthyophthirius multifiliis), costia (Ichthyobodo necator), Chilodonella, and Trichodina (Figure 14). The first two are considered quite serious, while we tend to view the latter two more as indicators of poor water quality. All may be found as mixed infections along with BGD. Another commonly encountered parasite is the small fluke Gyrodactylus (Figure 15).

Infected fish may show marked irritation and usually the entire population is involved, although some fish only to a degree. While white-spot can be large enough to be seen with the naked eye (individual parasites can be up to 1 mm in diameter) and the parasites are often present on the skin as well as the gills, a simple scraping examined directly under the microscope can quickly inform the farmer that there is a parasite problem. The shape and size vary from one species of protozoan to the next, but it is the movement that is so helpful in differentiating them from gill tissue.

The response of the gills to infection in the early stages of all of these parasites is increased production of mucus. This is often seen streaming from the opercula, and may cause the water to foam. Later responses may include loss of gill tissue and blood salts, followed in chronic cases by thickening and impaired overall functioning.

Treatment of parasitic gill disease is usually successful using formalin at one part in 6,000 to one part in 8,000 for one hour (the precise level varies according to a
number of complicating factors), although this may have to be repeated
two or three times on alternate days. In the case of Trichodina and
Chilodonella, attention should also be
paid to improving water quality,
particularly reducing the amount of
organic debris.

The treatment of white-spot is an
exception to these general rules. Unlike
the other parasites mentioned, which are
located on the outside of the tissue, this
protozoan burrows into the gills. Unless
you actually kill the gill tissue, the only
way to treat it is to wait until it emerges
from the fish in order to encyst at the
bottom of the tank or pond.

Accordingly, the treatment strategy is
aimed at breaking the lifecycle by
killing the infective stages before they
can find the fish again. Once again, this
can be achieved using formalin, but it is
used in a sequence which varies
according to the water temperature, as
the timing of the lifecycle varies with
the water temperature. Vigorously
cleaning the sides and bottom of the
tanks to dislodge the developing
infective stages is also an important part
of the control strategy. Even with these
approaches, white-spot remains one of
the most difficult infections to treat.
Eventually the fish develop an
immunity, so the rationale is usually to
limit the losses until such time as this
can occur.

Conclusions and Future
Directions

Although this article has
concentrated on the things that
can go wrong, it is very important to
emphasize that Ontario’s farmed fish
are some of the healthiest that can be
found. Having said that, gill diseases
remain the number one disease problem
reducing the profitability of the
industry. They can affect fry and
fingerlings, as well as growers, and even
broodstock are not exempt. Even
though farmers can recognize many of
the most common diseases themselves,
and it is very important that they should
frequently inspect their fish, we have
found it is critically important for
successful treatment, that they get an
accurate diagnosis from a laboratory.
Similarly, follow-up submissions of fish
can greatly help to establish the
effectiveness of any treatment approach.

Our research work is presently centred
on discovering if there are many
different strains of the bacterium
responsible for BGD in Ontario farms,
or whether they are basically all the
same. We are also investigating
whether all species and strains of fish
are equally susceptible and if the fish
are capable of mounting an effective
immune response. These approaches
are aimed at trying to select more
resistant fish, and at the possibility of
vaccination. Similarly we are looking at
just how and why different feeding
regimes can lead to higher mortality.
As with so many diseases of farmed
fish, close attention to good
management is of paramount
importance.


