

Fish Health News You Can Use

Brought to you by the Pacific Region Fish Health Program

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Contact Us

Pacific Region Fish Health Program Manager Andrew Goodwin

US Fish and Wildlife Service, Pacific Region

911 N.E. 11th Avenue Portland, OR 97232 Phone: (503) 231-6784

Email: andrew goodwin@fws.gov

Website

www.fws.gov/pacific/Fisheries/fishhealth/

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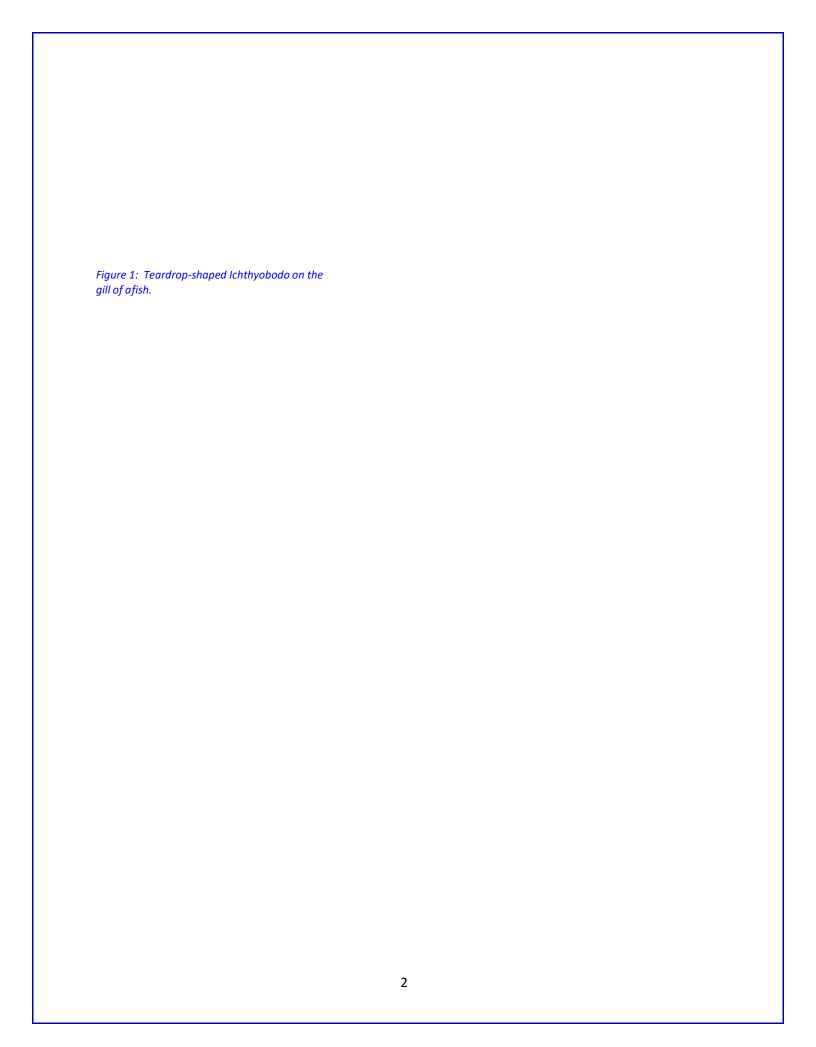
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Flagellated Parasites Inside and Out

Fish that live in surface water are constantly exposed to parasites that can cause disease. Low levels of parasite infestation are normal, and common parasites are unlikely to cause serious problems. Among the common parasites that are quick to take advantage of a weakened host are several single celled protozoans (Ichthyobodo, Cryptobia, and Hexamita) that possess flagella (whip-like extremities) to swim and to attach to fish or substrates. All four of these parasites are common in hatcheries and they are also carried by wild fish at low levels.



Ichthyobodo necator (formerly known as *Costia necatrix*) is a very small parasite that lives on the external surfaces of fish, including the gills.



Despite the similarity of their name, this is not the parasite commonly referred to as "Ich." Ichthyobodo is hardy and can survive in a wide range of temperatures, from about 36 to 86°F (2°-30°C) but in salmonids it tends to cause disease in the lower end of its temperature range. Heavy infestations of this parasite can cause large-scale mortality events, particularly in fry and younger fish.

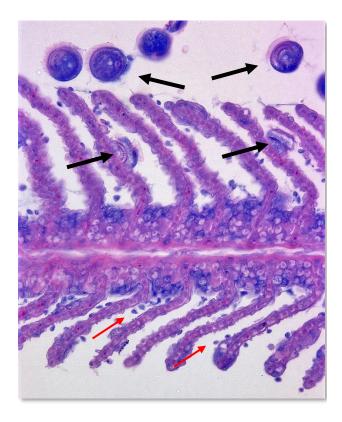


Figure 2: Ichthyobodo on the surface of a fish gill. This is histology (thin slices of gill that have been stained). The small purple dots on the gill lamella are Ichthyobodo (red arrows). The large purple circles at the top and in profile in lamella (black arrows) are trichodina.

The parasite irritates the skin and gills causing flashing, redness of the skin, skin abrasion and sloughing, and some fish may have a bluish cast to their skin due to increased mucus production.



Figure 3: Blue slime disease caused by the heavy Ichthyobodo infection of a rainbow trout.

Because the parasite damages the mucus barrier and embeds into the skin and gills, a heavy infestation can interfere with a fish's ability to maintain correct water and salt balance in the blood. This parasite also makes the host more susceptible to viral and bacterial diseases that find it easier to enter and infect the fish when Icthyobodo has reduced immune function and damaged the skin surface.

Ichthyobodo is often seen in one of two life stages: the mobile "swimming" stage and the attached phase, where the parasite attaches to the fish's skin or gills. The mobile form can be identified in wet mounts of gill and skin scrapes as a very small parasite (approximately the size of a red blood cell) swimming across the slide. The parasite is crescent to oval shaped and flattened with two flagella on the posterior end. The attached form uses its flagella to penetrate and hold on to skin and gill cells. It is more difficult to identify in wet mounts than the freeswimming form but it may be seen "flickering" on the gill surface. The flagella are not visible on the embedded form. Formalin is the most effective treatment for heavy Ichthyobodo infections, but the disease management plan must also include an evaluation of environmental conditions, water quality, and diet to see if there are underlying stressors that are making the fish susceptible.



Figure 4: Icthyobodo causing reddining of the skin in a koi.

Cryptobia are another flagellated parasite that can be found on the body surface, but they can also be found in the gastrointestinal tract, and in the blood of multiple species of fish. They usually only cause clinical disease if there is underlying immune system dysfunction or another disease already present that is weakening the fish. Heavy infestations in the gills can cause respiratory dysfunction by disrupting the cells on the surface of the gills. The signs of Cryptobiosis are general and nonspecific and include lethargy, listlessness, reduced appetite, and poor body condition. Fish that are severely affected by Cryptobia infestation on the gills may show increased breathing rates, gaping, or piping. Diagnosis is based on identifying the protozoa in wet mounts of skin scrapes, gill clips, feces, or in gastrointestinal squash preps. The parasite is about twice the size of Ichthyobodo and is a flattened tear-drop shape with a single flagellum at the anterior and posterior end. As

with Ichthyobodo, formalin is an effective treatment for external infections but significant Cryptobia disease warrants a full evaluation of stressors that might be weakening the fish's defenses.



Figure 5: A Cryptobia parasite from the gill of a salmon. Note the flagella that are characteristic of this group.

Hexamita are flagellated protozoa that, like Cryptobia, can infect fish both internally and externally. Hexamita and Spironucleus are often used as interchangeable terms for these parasites, however these refer to different species of protozoal parasites. Spironucleus are more common in freshwater tropical fish, like cichlids and angelfish, whereas Hexamita are more likely to be encountered in cold and temperate water fish culture. A small population of Hexamita parasites is often

present in the gastrointestinal tract of fish, however, in instances of disease or poor water quality parasite numbers can skyrocket and cause disease.

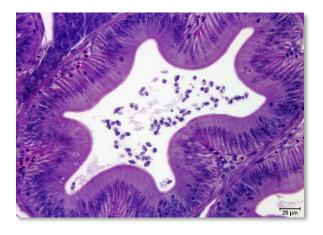


Figure 6: Hexamita (purple spots in the picture center) in a histological section of the digestive system of a salmon. This section (slice) is across one of the digestive caeca.

Affected fish lose weight and often have poor body condition, skin irritation, pop-eye, distended bellies, and lethargy. Hexamita parasites can sometimes be seen on skin scrape and gill clip, but are most readily detected on fecal exams or intestinal squash preps. The parasite is identified by their egg-shaped, tapered body and eight flagella (six anteriorly and two posteriorly). Noting the number of parasites on a wet mount is important because treatment and prognosis of Hexamitosis is dependent upon the burden of protozoa present. It is not uncommon to diagnose Hexamitosis as part of a mixed infection along with bacterial, viral, or other parasite pathogens. Heavy infestations are treated by top-coating the feed with Epsom salts; the Epsom salts act as a cathartic to flush the parasites out of the GI tract.

Trypanosomes are yet another flagellated protozoan parasite, but they differ from the previous parasites in that they can be found living in the fish's blood. These parasites can

infect a wide variety of fish species, especially cold water species, and are transmitted by leeches. The leech is a necessary component to the trypanosome lifecycle because the parasite develops in the leech's gut where it becomes infective to fish. A leech can transmit the parasite when it attaches to the fish and takes a blood meal. When trypanosomes infect a fish host they multiply within the blood stream and can infect any organ system. Heavy trypanosome infection usually manifests as anemia, organ dysfunction, and death. Trypanosomes are identified on blood smears, gill clips, and organ impression smears of infected fish by visualizing the elongated, eelshaped parasite with a single flagellum. The key to controlling infestation is to control the leech population in the fish-rearing environment.

Trypanoplasma are parasites similar to the trypanosomes in that they are spread from one fish to another by leeches and they infect the fish host's bloodstream. Unlike trypanosomes, trypanoplasma parasites will migrate out of the blood and into the host's organs so that, eventually, the parasite can no longer be detected in the bloodstream. Trypanoplasms can be seen on blood smears in the early infection stage and in organ smears in later infection stages; the appearance of the parasite is very similar to Cryptobia, so much so that some scientists group Cryptobia and Trypanoplasma into the same genus. The most common species of trypanoplasm to infect salmonids, *Trypanoplasma salmositica*, causes anemia, exophthalmia (pop eye), belly distention, and enlargement of the spleen. As with trypanosomes, the only effective treatment and control for Trypanoplasma is to eliminate leeches.

Several species of trypanosomes and Trypanoplasma have been identified in wild fish in the Pacific Northwest, particularly during the fish's ocean phase. Many of the leeches that have been studied as vectors of blood parasites are marine leeches and their survival in freshwater is limited. While there are freshwater fish leeches in the Pacific Northwest, they are significantly less diverse than their saltwater counterparts. The ability of freshwater leeches to sustain and transmit blood parasites has not been thoroughly studied. At present, there is a low risk of these parasites naturally infecting hatcheries but it is important to keep in mind that returning adults may be hosting one or more species of blood-borne parasites.

Fun Flagellated Factoids

Hexamita are thought to be involved in the development of head and lateral line erosion, or "hole-in-the-head disease," in fresh and saltwater tropical fish like cichlids and tangs. The parasites migrate into the sensory pores along the lateral line and face and cause inflammation and tissue injury, resulting in the characteristic erosions or "holes" that the disease is named after.



Figure 7: A cichlid with "hole-in-the-head" disease. Photo by Tony Griffitts at http://www.aquaworldaquarium.com/

There are species of Hexamita that inhabit the GI tracts of birds and mammals (humans, too!). Like in fish, adult animals with normal immune systems are usually able to keep the parasite in

check but overpopulation of the parasite or host immune suppression can result in disease.

Hexamita are closely related to Giardia sp., which can cause GI illness is mammals.

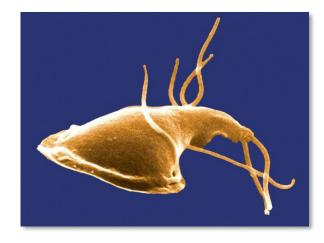


Figure 8: An electron micrograph of a Giardia parasite (CDC)

The scientific name for Ichthyobodo necator combines Greek and Latin roots and translates to fish-killing Bodo. When it was first discovered in 1884 the parasite was named Bodo necator (Latin for "killer" or "slayer"). In 1928 Ichthyo (Greek for "fish") was added to distinguish it from other flagellates in the Bodonida order.

Cryptobia on the surface of fish may feed on bacteria and behave as "ectocommensals".

For many years, the AFS Fish Health Section logo had a picture of an Ichthyobodo that had four flagella instead of two. It was later recognized that this happens only just prior to cell division.

Intestinal Hexamita infections pose a difficult chicken or the egg conundrum. Are thin fish that are off-feed more susceptible to Hexamita, or does Hexamita cause fish to be thin and go off feed? It may work both ways.

Steatitis in Fish

Steatitis is an inflammatory disease of fat tissue commonly associated with nutritional deficiencies. This condition has been seen in many different animals including mammals and reptiles. It was first recorded in fish in 1979 in rainbow trout and was linked to a batch of pelleted diet that had become rancid. When feed becomes rancid, the fish oil in the feed undergoes chemical processes (oxidation) that result in the formation of harmful compounds. When ingested, these compounds cause widespread damage to the fish on a cellular level. Salmon feeds contain a high level of fish oil that oxidizes easily making these feeds more prone to spoilage. Feed spoilage is caused by improper storage (high temperatures, direct sunlight), age, or problems during manufacturing (using old oil or fishmeal, low levels of antioxidants like vitamin E).

Another contributor to the development of steatitis is exposure of fish to sunlight. The UV in the sunlight causes further chemical changes in oxidized fats stored under the skin of fish eating feeds that are spoiled.

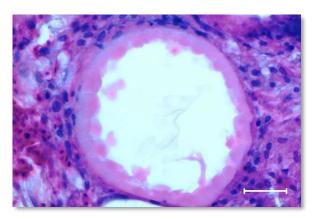


Figure 9: A rim of oxidized fat (smooth pink) lining the inner wall of a fat cell in the skin of a fish. The purple dots surrounding the cell are white blood cells that have arrived as part of an inflammation response.

Most severe cases of steatitis seem to result from a combination of feed problems and

sunlight so the first noticeable sign of problems is discoloration or damage to skin on the top of the fish where UV exposure is the greatest.

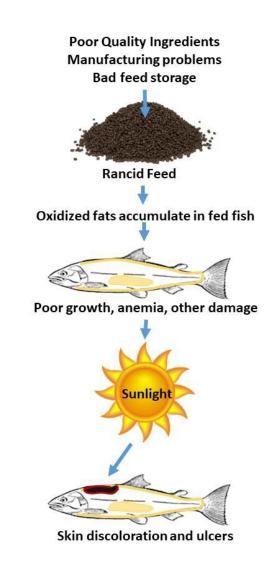


Figure 10: The likely mechanism for most cases of steatitis in fish.

Steatitis is not always associated with typical skin lesions. In trout, affected fish appear emaciated, have darkened coloration, and exhibit scale loss, pale gills, and necrotic fins. Affected catfish have fin loss, skin ulcers, and deposits of yellow lipid-containing substance in the fatty tissue.



Figure 11: A catfish with oxidized (yellow) fat accumulating at the base of the dorsal fin.



Figure 12: A catfish where inflammation around oxidized fats has led to ulceration.



Figure 13: Inflammation and ulcers have weakened fin structures to the point where the fin has peeled off.



Figure 14: Classic steatitis-induced ulceration of the dorsal side of a catfish head.

Steelhead with steatitis may not have any external signs of the disease. Rainbow trout with steatitis had pale livers and grey discolored swimbladders. Affected fish may also be anemic. With these non-specific internal and external signs, steatitis must be diagnosed through histology (microscopic examination of very-thin stained slices of tissue) where the damaged fats and associated problems are clear. Feed should always be collected and submitted for analysis. This can be difficult if the feed thought to be related to the disease has been fed out.

In summer 2019, steatitis was diagnosed in juvenile spring and fall Chinook and Coho salmon at several national fish hatcheries in the Columbia Gorge, on the Olympic Peninsula, and in Central Washington. Given the wide distribution of affected fish, rancid feed from a central supplier is likely. Fish affected by

steatitis experienced inflammation of skin, fat, and muscle on the top surface of the fish just before the dorsal fin. The lesions initially appeared as a linear pale patch but progressively darken. Skin and muscle may become ulcerated and eventually turn white following death of tissue. Secondary bacterial and fungal infections are also often observed in the lesions.

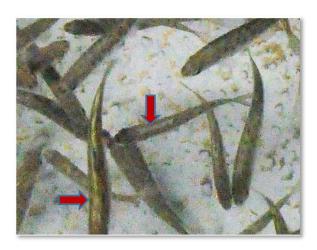


Figure 15: The first step in the development of steatitis, a linear pale patch on top of fish before dorsal fin.



Figure 16: The second step in the development of steatitis, darkening of the linear dorsal patch.



Figure 17: The final step in steatitis. Ulceration of the affected skin and infection by oomycetes (saprolegnia "fungus")

Steatitis is best managed by prevention. Feed should be sored in a cool and dry place. Order feed in smaller batches more frequently to ensure quality of feed. Our Region's FWS hatcheries should be diligent in submitting feed samples to the Abernathy Fish Technology Center for analysis (call Ann Gannam at Abernathy FYC for help). Provide shading on outdoor ponding units to block sunlight and reduce the severity of steatitis. Contact your fish health staff right away if you suspect steatitis or any other disease problem.

Carbon Dioxide and Fish

Fish take up oxygen and then secrete carbon dioxide (CO₂) out through their gills. In normal situations, oxygen gets to dangerously low levels long before CO₂ levels in the water are high enough to harm the fish. However, in cases where supplemental oxygen is added (especially pure oxygen) or where ground water is used, it is possible for CO₂ levels to get high enough to cause behavioral changes, slow growth, and even death.

When pure oxygen is added to the water, the additional oxygen is converted into more CO₂ than would be possible from air alone. The CO₂ gas is very soluble in water so simple aeration with oxygen (oxygen diffusers etc.) is not enough to strip the gas. Removal of CO₂ requires large degassing towers or other more sophisticated degassing technology.

Looking at the math: Every 1 ppm of oxygen used by the fish produces about 1.4 ppm of CO₂. Thus, in a flow through raceway where oxygen levels drop from (for example) 12 ppm at the head to 7 ppm at the tail, the 5 ppm of oxygen used by the fish produces 5 X 1.4 = 7 ppm of CO₂. That much CO₂ is not a big deal. If water is serially used and pics up some addition oxygen splashing from an upper bank of raceways to a lower bank, more oxygen is added so more CO₂ can be made, but some of the CO₂ is stripped from the water by the splashing so CO₂ is still not a big concern. However, if you inject pure oxygen through diffusers or other methods, a raceway (or RAS system) might use a total of 20 ppm of oxygen while the water is passing through. This 20 ppm of oxygen use would result in 28 ppm of CO₂. That is enough to cause serious problems.

To keep CO₂ levels under control, degassing towers are a required part of re-use aquaculture systems (RAS). As the water is re-

used through the system, CO_2 accumulates and would lead to fish losses without a stripping tower. Wells and springs can also have high levels of dissolved CO_2 and may require degassing of the water before it is used in fish culture.



Figure 18: Hatchery Manager Craig Eaton in front of the gas equilibration tower (tan) under construction as part of the RAS system at the former Hagerman NFH.

So how much CO₂ is too much? Levels of CO₂ less than 5 ppm are ideal and up to 10 ppm is usually okay. As CO₂ concentrations approach 20 ppm, there are very significant changes in fish behavior (including unusual schooling, lethargy, unusual positioning in raceways and tanks), growth and feeding are affected, and the risk of death or disease is greatly increased. Above 20 ppm long term exposure is often lethal. Short term exposure, like on fish haulers where pure oxygen is used, is usually

okay as long as levels do not get too high for extended periods.

One of the biggest challenges with CO_2 is accurately measuring the concentration in raceways and wells. In water, CO_2 interacts with carbonates and bicarbonates (alkalinity) and hydrogen ions (pH) in ways that make accurate measurement complex. The most reliable methods involve measuring the total alkalinity, pH, and temperature and them multiplying the total alkalinity by a factor off a table to calculate the concentration of CO_2 present.

Table 2. Factors for calculating carbon dioxide concentrations in water with known pH, temperature and alkalinity

рН	Temperatures (°C)						
	5	10	15	20	25	30	35
6.0	2.915	2.539	2.315	2,112	1.970	1.882	1.839
6.2	1.839	1.602	1.460	1.333	1.244	1.187	1.160
6.4	1.160	1.010	0.921	0.841	0.784	0.749	0.732
6.6	0.732	0.637	0.582	0.531	0.495	0.473	0.462
6.8	0.462	0.402	0.367	0.335	0.313	0.298	0.291
7.0	0.291	0.254	0.232	0.211	0.197	0.188	0.184
7.2	0.184	0.160	0.146	0.133	0.124	0.119	0.116
7.4	0.116	0.101	0.092	0.084	0.078	0.075	0.073
7.6	0.073	0.064	0.058	0.053	0.050	0.047	0.046
7.8	0.046	0.040	0.037	0.034	0.031	0.030	0.030
8.0	0.029	0.025	0.023	0.021	0.020	0.019	0.018
8.2	0.018	0.016	0.015	0.013	0.012	0.012	0.011
8.4	0.012	0.010	0.009	0.008	0.008	0.008	0.007

Tucker (1984).

"Factors should be multiplied by total alkalinity (mg/L) to get carbon dioxide (mg/L).

For practical purposes, CO₂concentrations are negligible above pH = 8.4.

Unfortunately, most hatcheries in the Pacific Northwest do not have the test kits needed to measure CO₂ accurately and samples shipped or held for any length of time undergo chemical changes and de-gassing so measurements made from them are inaccurate.

As RAS systems become more common, measuring CO2 accurately is becoming increasingly important. In addition, well and spring water can be a problem and CO2 levels can change over time, especially in shallow wells. If you have any concerns about CO2, contact your PRFHP person for assistance.

PRFHP Update

We have hired two new veterinarians. Dr. Katie Royer (author of the Steatitis article in this issue) works with David Thompson out of the Little White Salmon NFH and is responsible for veterinary oversight for the Gorge Complex as well as for the Prosser and Klickitat Tribal Hatcheries. Dr. Christine Parker-Graham (author of the Flagellates article in this issue) is based out of the Puget Sound Olympic Peninsula Complex offices in Lacey, WA and is responsible for the Peninsula hatcheries and for the Abernathy FTC. These two veterinarians join Dr. Trista Becker, based at Leavenworth NFH, to provide veterinary coverage for our entire Region. They work hand in hand with our other full time PRFHP fish health staff (Tim Bundy, Susan Gutenberger, Corie Samson, Laura Sprague, David Thompson) and our hatcheryassociated fish health partners (Ken Lujan, Spencer Meinzer), to provide complete fish health services. The new veterinarians are very fish oriented with a great training, broad experience, and interesting new perspectives on fish health.

The Washington State Animal Disease Diagnostic Laboratory (WADDL) in Pullman, WA is now doing all of the advanced lab tests (virology, bacterial culture and identification, histopathology, supporting the work of the Pacific Region Fish Health Program. The testing that is being done at WADDL is under the highest level of veterinary diagnostic laboratory accreditation. It allows the FWS to take advantage of the economies of scale at WADDL that enable them to have certified clinical specialists and more sophisticated instrumentation while still conducting tests at a lower cost. The WADDL partnership allows our PRFHP staff to spend more time on disease prevention, management, and treatment and less time on repetitive laboratory tests.

Mystery Parasite of the Day



For the answer, click <u>HERE</u>.