Fish Health News You Can Use

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Inside This Issue

- Total Dissolved Gas Pressure and "Gas Bubble Trauma", What We Learned at Dworshak 1
- What Different Diagnostic Tests Tell Us About Bacterial Kidney Disease (BKD) 3
- Changes in State Laws That Regulate How Veterinarians Serve Fish Hatcheries 4
- Where We Are Headed with the National Wild Fish Health Survey 4
- New Fish Health Science – Just Published 5
- Fish Health Factoids 5
- Current Status of the Fish Health Reorganization 6
- PRFHP Staffing Update 6
- Name that Parasite 7

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In The Next Issue

- Transfer Permits: Requirements and Responsibilities Explained
- DI, FI, Turnover, and Water Velocity, a Fish Health Perspective
- Who Makes the Fish Health Rules?
- "Why is ICH so Hard to Treat?"
- Changing Rules Governing Antibiotic Use in Fish
- New Science
- PRFHP Updates
- Mystery Parasite of the Day

Total Dissolved Gas Pressure and "Gas Bubble Trauma", What We Learned at Dworshak

Heavy snow pack, plenty of rain, and generator repairs at Dworshak Dam led to high levels of spill this spring. High spill plunges a mixture of air and water deep beneath the dam's tailwaters where the high water pressure causes the gasses to dissolve. The higher the spill rate, the more gasses dissolve. When this water is then pumped into shallow raceways, the water pressure there is much lower and the gasses come back out of solution as bubbles (just like when the lid is removed from a bottle of soda). If fish in raceways are in this gas "supersaturated" water, the excess gasses are taken up by fish through their gills and skin and the extra gas then comes out of solution to form bubbles in the gills, lateral line, fins, and eyes. The bubbles block blood circulation and, if they grow large enough, may rupture blood vessels, damage tissues, and make the fish susceptible to infectious diseases.

Photo: Gas bubbles in gill blood vessels of a Dworshak steelhead juvenile.

continued from page 1

The most common measurement of gas saturation is the "% Total Gas Saturation" or %TDG. This number is calculated by dividing the total pressures of the gasses trying to get out of the water by the total atmospheric gas pressure (the barometric pressure) that is trying to force gasses into the water. For example, if the pressure of the gasses in tailrace water is 792 mB and the atmospheric pressure is 760 mB, then the %TDG = 792/760 = 104.2%. The more that %TDG exceeds 100%, the more likely bubbles are to form. During March of this year, the %TDG in the Dworshak tailrace was often 125% and water in the hatchery ponds was as high as 106%.

This is what we learned about fish health in the hatchery during that period:

- The scientific literature agrees that the threshold where gas supersaturation begins to cause significant harm to juvenile salmon is about 105%. That's a good number.
- At Dwoshak, gas bubbles began to appear in fish gills at %TDGs of about 102%. They were first seen in the gills, but as %TDGs approached 105% we started to see more and larger bubbles, and they appeared in the lateral line and fins.
- At TDGs less than 105%, bubbles were present but we saw no significant changes in fish health or behavior. At 105% and above, most fish quit feeding and other behavioral changes were obvious.
- When %TDG was dropped from 105% down to 101-102%, fish went back on feed but the bubbles already present did not disappear.

Measuring damage to fish caused by gas bubbles was difficult. We used a bubble rating system developed by the Fish Passage Center to record the number of bubbles present, but that alone does not tell us if serious damage is occurring. Cessation of feeding was a good clue, but the only way that we will really know what happened to the fish is through "histology" where we can look for dead or damaged tissues on a cellular level.

So what is Histology?

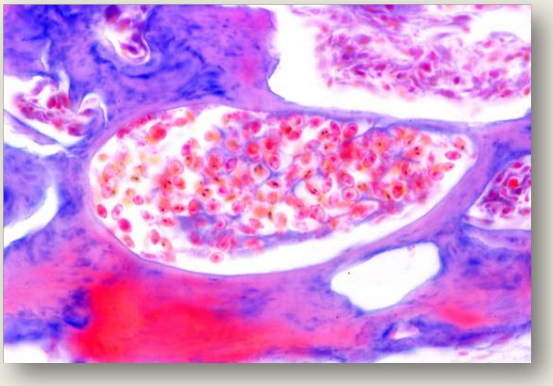

Photo: Histopathology

Histology is a way to look for damage to fish tissues on the cellular level. Bits of the fish are preserved in formalin and then the water in the tissue is replaced by wax. Next we make slices of the tissue that are just two 1/1000ths of a millimeter thick, put them on slides, stain them, and examine them under a microscope. In this picture you can see the spores from whirling disease parasites encysted in the bones of a trout head (orange cells with "eyes" in the center).

Of course, the true measurement of gas supersaturation damage is fish performance in the wild. If downstream migration and SARs for these Chinook and steelhead are good, we can probably assume that no serious harm was done. However, if these same measures are lower than normal the answers are less clear because it can sometimes be difficult to differentiate between the impacts of gas damage and impacts attributable to conditions in the rivers and ocean.

So who needs to worry about gas saturation? Gas supersaturation can happen anywhere that 1) the water source is a dam tailrace, 2) water is pumped from a deep source including both wells and sub-surface lake water, 3) springs, 4) any system where pumps are used (gas bubbles mixed with water dissolve very quickly under pressure in pumped systems), and 5) any system where water is heated (heating increases gas pressure and thus increases %TDG).

continued from page 2

How do you know if you have a gas saturation problem? Any hatchery with an at-risk water source should regularly be measuring TDG with a good and carefully-calibrated saturometer. The fish health staff does look for bubbles in gills when doing inspections and diagnostic work.

What Different Diagnostic Tests Tell Us About Bacterial Kidney Disease (BKD)

BKD is a bacterial infection that is especially severe in spring Chinook salmon. It is caused by a slow-growing bacterium (*Renibacterium salmoninarum* or "Rs" or "Rsal") that often causes progressive tissue damage leading to disability or death. Almost all Chinook salmon in the Pacific NW carry the bacteria at some level.

Photo: A BKD infection in the muscle of a Chinook.

The ELISA Test: This test looks for a protein released by the bacteria into the fish's blood or tissues. The advantages of the ELISA are that 1) the bacterial protein is spread throughout the fish so we can detect it in a kidney sample even when we miss the infected part of the kidney, or even when the infection is limited to the gills or skin, and 2) the higher the ELISA reading, the more intense the infection so we can cull eggs from adult fish with high ELISA levels that will produce Rs laden eggs that may lead to diseased juveniles. The disadvantages of the ELISA are that 1) it is time consuming, expensive, and technically complex, 2) it doesn't directly detect RS bacteria so recovering fish may still have high ELISA values for some time after an infection has subsided (like after antibiotic treatments), and 3) we suspect that there may be some false positives caused by reactions of the test with proteins from sources other than Rs. **Best Use:** The ELISA assay is very effective for culling the highly-infected females that produce young at a high risk for serious BKD losses.

Culture: We can grow Rs bacteria on agar plates in the lab. The advantages are that 1) the test is positive only if live bacteria are present, 2) we can do all kinds of follow-up tests to unambiguously identify the bacteria, and 3) we can do antibiotic sensitivity testing. Unfortunately, the bacteria can be difficult to isolate and may take weeks to grow and identify. We may also miss a localized infection by culturing a part of the fish kidney when the infection is somewhere else in the fish (such as gills or skin). **Best Use**: Confirmation and antibiotic sensitivity testing for juvenile fish from populations with fish dying from BKD.

Direct Fluorescent Antibody Tests (dFAT): In this test we take a smear of tissue on a microscope slide, label the bacteria with fluorescent antibodies that stick to the RS, then look for the bright green bacteria under a special microscope. The advantage of the DFAT test is that it is quick and relatively simple, and that it clearly visualizes live bacteria present in the tissues. On the down side, the test is only positive if you get the right piece of fish tissue on the slide and it often isn't good at detecting low levels of bacteria. **Best Use**: Quickly confirming BKD in sick juveniles.

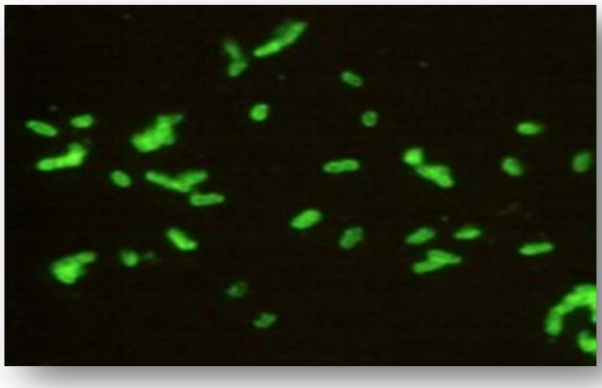

Photo: DFAT showing Rs bacteria in the kidney of a Spring Chinook salmon with BKD.

continued from page 3

PCR Tests: PCR tests look for the DNA of Rs bacteria in fish tissues. This test is very sensitive and specific, and it can detect just a few bacterial cells in a sample. It can also be done on tissues that have been frozen or preserved in alcohol for many years. The downside is that it requires expensive instrumentation and highly-trained technicians. It also uses a very small sample of tissue so the bacteria can be missed when the infection is present, but not at the sample site.
Best Use: Answering the question, is this fish infected by Rs?

Changes in State Laws That Regulate How Veterinarians Serve Fish Hatcheries

The work that veterinarians do on NFHs is governed by many state and federal regulations. Recent changes in FDA rules about drug use are placing the authority to use many fish drugs (specifically antibiotics) only in the hands of veterinarians. State laws are also changing to place more of the responsibility for drug use decisions, and outcomes, on the veterinarian's shoulders. If the veterinarians don't follow the state regulations, they could lose their license to practice.

In addition to FDA changes that require veterinarians to write prescriptions for fish antibiotics, extra-label drug uses, and medicated feeds, the State of Washington has made some very significant changes in requirements for the working relationship between veterinarians and fish hatcheries. In order to support any drug or chemical use on hatcheries, veterinarians must maintain a formal "Veterinary Client Patient Relationship (VCPR)" with the hatchery. This VCPR requires that the veterinarian knows the fish, the hatchery, the people, and the disease problem and is actively involved in the management of the disease. In addition, in the State of Washington, the hatchery must now agree in writing to keep the veterinarian informed about all fish health problems on the hatchery and to follow all treatment recommendations made by the veterinarian.

Our PRFHP veterinarians have now put these written agreements in place with the FWS and Tribal hatcheries that they serve in Washington State. The PRFHP greatly appreciates that the hatcheries and veterinarians were able to put these agreements in place so quickly after the regulations changed. It is a real credit to the team-based approach in R1, and to the trust that exists between our fish health folks and the hatcheries that they serve.

Where We Are Headed with the National Wild Fish Health Survey

The National Wild Fish Health Survey started out as a survey to determine the geographic distribution of the whirling disease parasite. As concern about whirling disease waned, the survey broadened to include efforts to determine the national distribution of all important fish diseases. In more recent years, it has been recognized that resources are not sufficient to achieve this goal, and regions have increasingly focused the funding and effort to solving specific fish health problems that occur in fish living in the wild. Two good examples of successes have been work that the PRFHP has done to support bull trout relocation projects, and a large interagency collaboration to determine the cause of juvenile and adult salmon mortality in the Deschutes system (spoiler alert: the answer turned out to be high levels of the parasite *C. shasta*).

Beginning in 2017, Region 1 is organizing meetings between the PRFHP, NFHs, and FWCOs to identify new studies that will be as successful as our Deschutes and bull trout work. We are emphasizing wild fish health questions that directly affect the FAC programs and mission.

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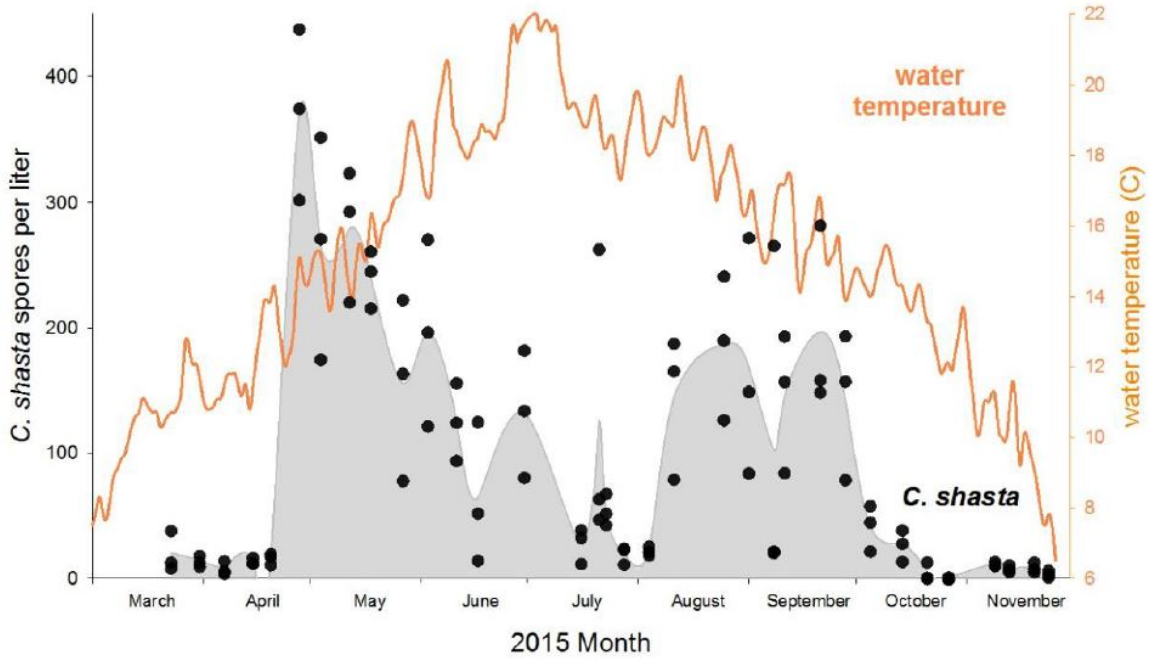

Figure: A graph from the Deschutes C. shasta study showing C. shasta spore numbers in water. Remarkably, numbers higher than 10 spores per liter are considered a threat to fish health.

New Fish Health Science – Just Published

For inspections where the goal is to determine if a fish population carries the IHN virus, a new study shows that non-lethal fin clips are as good as or better than traditional lethal kidney and spleen samples. *Journal of Aquatic Animal Health (2017) 29:67-73*

A new strain of the VHS fish virus (VHSV-IVb) emerged in the Great Lakes a few years ago and caused huge fish kills in several species of fish. New work shows that this VHSV strain persists in aquatic systems by infecting amphipods. It isn't known if amphipods can also serve as a host for our West Coast VHS virus (VHSV-IVa). *Journal of Aquatic Animal Health (2017) 29:31-42.*

Photo: An amphipod.

A new study by NOAA scientists has shown that the major declines that occurred in the early 1990s in Alaska's Pacific herring populations were most likely due to factors unrelated to the Exon Valdese oil spill. PLOS One 12(3): e0172898.

Fish Health Factoids

Viruses are the most common critters on earth. There are several million in a teaspoon of fresh water and 10 nonillion (10,000,000,000,000,000,000,000,000,000,000) viruses on earth. A typical freshwater system produces about 4 million new virus particles in every gallon of water every day. You can put 5 billion viruses on a pinhead (one layer deep) and it would take 100 sextillion (100,000,000,000,000,000,000,000) viruses to fill an 8 ounce cup. Even though viruses are ridiculously small, if you took all of the viruses on earth and lined them up end to end, they would make a chain long enough to go from earth all the way to the Andromeda Galaxy – and back again – 50 times (200 million light years).

continued from page 5

Photo: The Andromeda Galaxy.

The good news for fish and humans is that most viruses are "bacteriophages" that prey on bacteria. There are more than 250 known human viruses and about 150 in fish, but new technology is making it clear that these are just the tip of the iceberg.

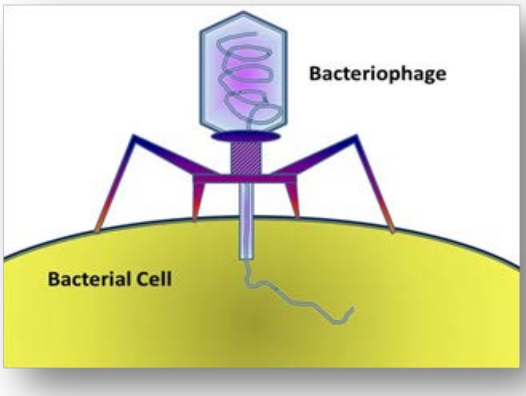

Figure: A bacteriophage (virus) injecting its DNA to infect a bacterial cell.

Current Status of the Fish Health Reorganization

The Pacific Region has for decades run three Fish Health Centers that each did both on-hatchery disease management, and fish disease testing in the laboratory. That made sense for a long time, but changes in regulations, increases in technical complexity, and the availability of reliable overnight shipping have changed how we need to do business. The Fish Health Center reorganization is reshaping fish health as the "Pacific Region Fish Health Program" or PRFHP. The "Fish Health Center" name is now obsolete in the Pacific Region (except for the signage). Instead there is a single PRFHP with staff at duty stations across the region (currently Lacey, Leavenworth, Carson, and Willard, WA and Orofino, ID) that will soon be sending samples by overnight express to a single testing laboratory. The fish health staff is now in one org code and we are well along the way toward centralizing administrative functions that include everything from supervision to budgets.

PRFHP Staffing Update

The fish health reorganization plan calls for teams of veterinarians and vet techs to work together to support a regional group of hatcheries and to ship their samples overnight to a laboratory for testing. As a first step we hired veterinarian Trista Becker last year and located her at Leavenworth NFH to be responsible for the Complex and for the Chief Joseph Hatchery. Then in early January 2017, we hired an experienced vet tech (and former Peace Corps volunteer), Sarah Anderson, and located her at Leavenworth to assist Trista. We are really pleased with how this new arrangement is working and greatly appreciate the willingness of the Complex to find room for Trista and Sarah to work.

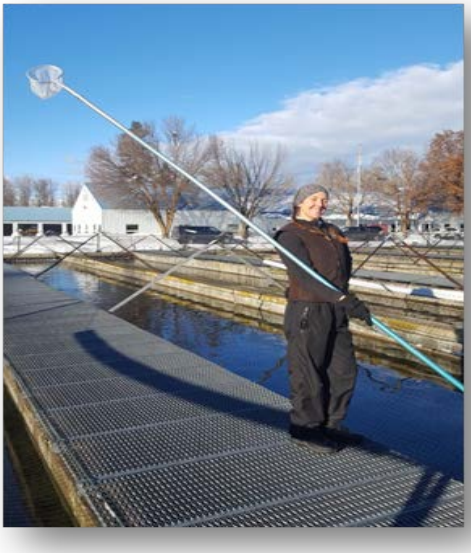

Photo Credit USFWS: Sarah Anderson checking fish on a beautiful day.

continued from page 6

Name that Parasite

This parasite is quite common on fish. It is a single celled protozoan with a simple life cycle that involves only cell division. They are easy to recognize under a microscope where they look like donuts with teeth.

They scoot around on gills and skin. They don't often kill fish, but they may develop populations dense enough to cause flashing and reduce feeding. While we call them by one common name, there are actually many species (answer at the end of Fish Health News).

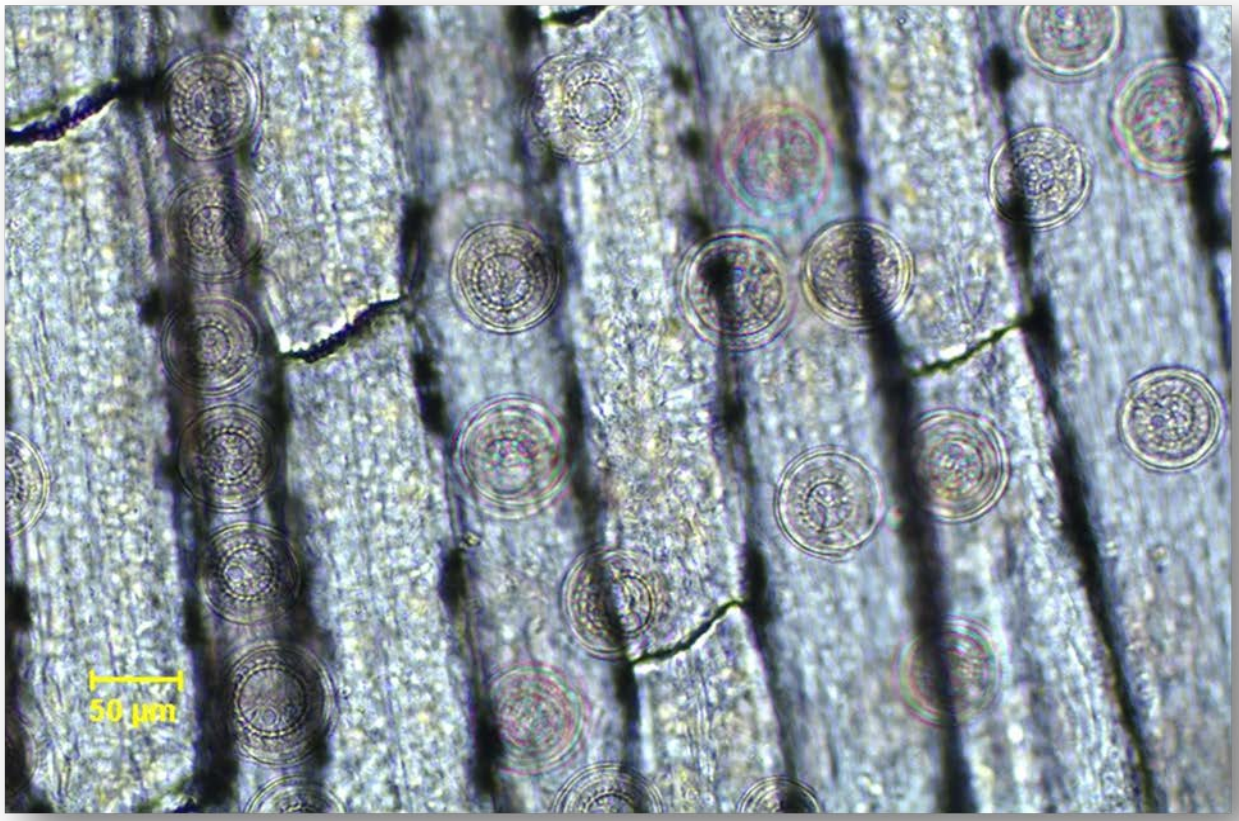

Photo: Name that Parasite.

Click here for the identity of the Mystery Parasite.