Fish Health News You Can Use

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This Issue

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Salt Use, Parasites, Stress, and Osmoregulation

Maintaining adequate amounts of salt in their blood is a critical, difficult, and energy intensive task for all fish. Like us, fish have almost 9 grams per liter (9 parts per thousand or 9 ppt) of salt in their blood. However, the freshwater environment (especially here in the Pacific Northwest) has almost no salt. This means that fish are constantly loosing salt as it leaks from their skin and gills into the water. The only way to keep up with that salt loss is to either 1) take in new salt in food, or 2) to use energy to pump salts from the environment in through the gills and back into the blood.

Fish gills are an especially critical area for salt management. In the gills of the fish, the only barrier between the blood and the environment is the gill membrane. That membrane is super thin (a few thousandths of a millimeter thick) to efficiently allow oxygen in and carbon dioxide out. It's a marvel of engineering, but it is so thin that salt leaks out through the gill membranes at a prodigious rate. In order to compensate for that salt loss, freshwater fish must actively pump salt back in through their gills. These pumps use large amounts of energy derived from the fish's food. If the fish does not keep up with salt loss by using these gill pumps and eating salty foods (like other animals) then the

salt concentration in the blood drops and muscles and nerves begin to malfunction.

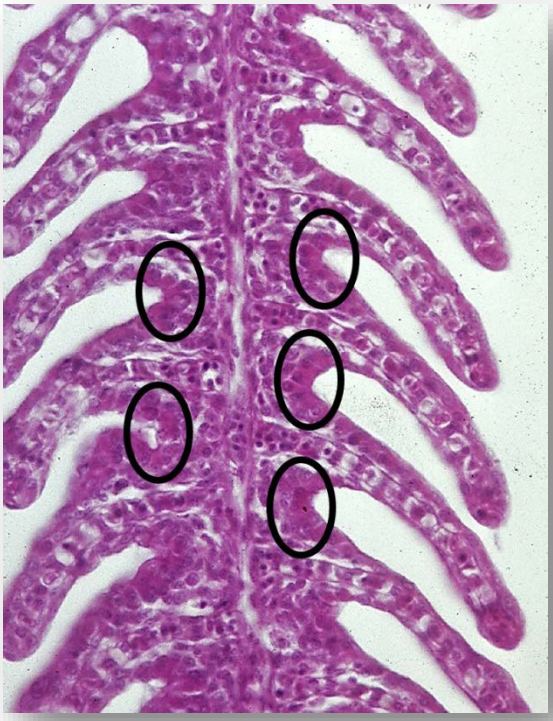


Figure 1: A stained slice of gill tissue as seen through a microscope. The pumps that move salt from the environment into the fish's blood are found in the membranes of chloride cells. The chloride cells (circled) are found in the troughs between the gill lamellae.

Salt management is a constant struggle for fish, but when they are stressed or handled (marking and tagging, transport, cleaning raceways, grading…) things get even more difficult. When fish are stressed and handled, they breathe faster, their hearts beat faster, and they open up all of the blood channels in their gills. This helps the fish to get more oxygen to its brain and muscles but the increased blood flow also greatly increase the rate of salt loss. To make matters even worse, we often fast fish before handling. Fasting prevents some problems, but it also deprives the fish of dietary salt sources and the easy energy that it can use to pump in more salt.

There are many other things that can increase salt balance problems for freshwater fish:

- Low oxygen or high CO_2 levels that make fish breathe faster
- Disease treatments that may both damage the salt pumps in the gills and make gills and skin leakier
- Infections of the skin or gills may cause damage that increases salt loss or damages salt pumps
- High levels of ammonia or carbon dioxide in the water directly slow salt pump function
- If there is a kidney disease, like BKD, additional salt may be lost through the kidneys.

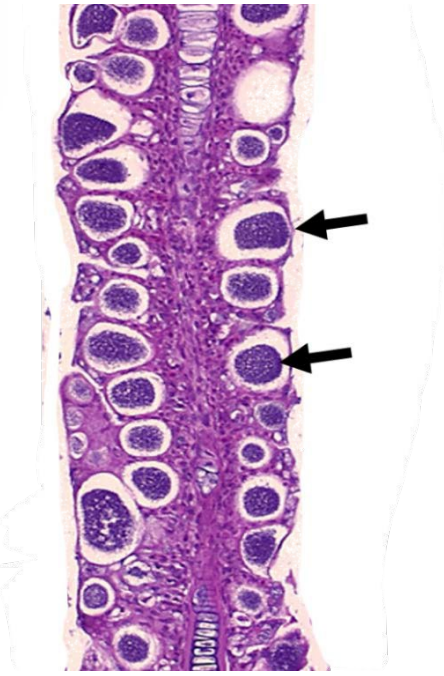


Figure 2: Epitheliocystis (arrows) infection of a gill. In this gill, epitheliocystis (a primitive bacterium) fills the spaces between the gill lamellae. The bacterium and host response effectively block the gill salt pumps.

Salt loss is only half of the story. At the same time freshwater fish are losing salt, water is also leaking in through their skin and gills. This incoming water can dilute the blood and drop the fish's salt concentration to dangerous levels. All of the same factors that cause salt loss (faster breathing, more blood circulating through the gills, gill and skin damage) also increase water uptake. Freshwater fish compensate for water uptake by producing large amounts of very dilute, low-salt urine. Kidney health is extremely important so BKD and other diseases of the kidney can have devastating effects on salt balance.

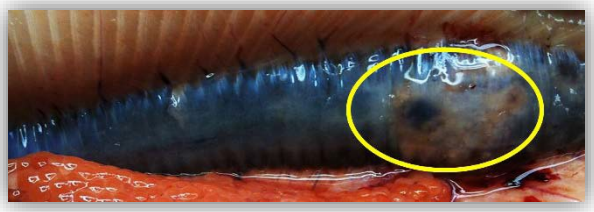


Figure 3: BKD damage (circled) in the kidney of a fall Chinook salmon.

The bottom line is that for freshwater fish, maintaining adequate salt levels in their blood and ridding themselves of excess water is a constant and energy-intensive battle. All kinds of common things (handling, disease, etc.) make it more difficult to maintain salt and water balance. If salt concentrations get low, fish die.

One way to help fish to deal with salt balance problems is to add salt to the water. Even at relatively salt low levels, salt loss is decreased. More importantly, added salt makes it far easier for the salt pumps in the gills to bring in new salt and it reduces the amount of energy needed to do that pumping. At the same time, it makes things easier for the kidney because the fish call allow some salt to leave through the urine. Low levels of salt in the water helps to keep the fish's muscles and nerves workingefficiently and frees up energy for other important purposes.

So how much salt does it take? Levels as low as 1 gram of salt per liter (one part per thousand) have easily demonstrable benefits. Even a tenth as much (100 ppm) may increase the availability of salt to the fish by 5-10 fold. Most freshwater fish benefit from 1-2 ppt of salt whenever they are handled, hauled, or subjected to acute stresses. Anadromous fish like salmon benefit from levels as high as 5 ppt (0.5%). In some species less robust than Pacific salmon, 2 ppt makes the difference between 100% mortality during transport and nearly 100% survival.

What about salt and disease treatment? Salt is sometimes used external parasites like ich, costia, or flukes.

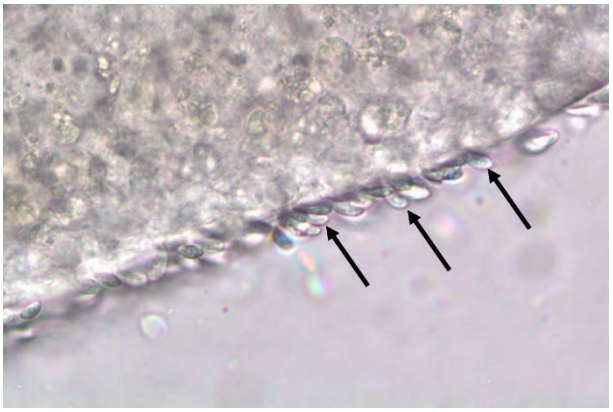


Figure 4: Costia on a fish gill. Salt, at concentrations tolerated by freshwater fish, does not kill costia but salt treatments still provides significant indirect benefits.

Surprisingly, scientific evidence that low levels of salt (1-5 ppt) are effective against parasites is spotty at best. So why do people think that salt is effective? The reason is simple. External parasites can cause a lot of damage to fish skin

and kills. Salt treatments don't kill the parasites, but they help the fish to better survive the damages and subsequent salt loss that the parasites cause. Salt is not directly effective against most parasites or bacteria, but it does keep fish alive. It's just like when you go to the hospital with an infection. That saline IV drip doesn't kill the bacteria, but it keeps you alive long enough for your immune system to do the job.

So when should we use salt?

Fish that are being hauled on trucks should always be in 2-5 ppt of salt. It's cheap, it's easy, and it provides a clear benefit.

Salt treatments should be considered for fish in flow through raceways and ponds whenever they have been subjected to a severe stress. However, at very high turnovers, it may sometimes be impractical to add enough salt to make a difference.

Calculating salt treatments

1 ppt = 1 gram per liter, or 3.8 grams per gallon, or 14 oz (0.84 lbs)/100 gallons

100 ppm = 0.1 ppt = 16 pounds/10,000 gallons

How do you know if the salt concentration is correct? The PRFHP staff are equipped with conductivity meters that provide a quick and easy measurements of salinity.

Conclusion: Salt should always be used when hauling fish. Low concentrations of salt in raceways or ponds during periods of stress may be beneficial.

Fungi

In fish health, most of the things that we call "fungus" are not fungus. With the advent of DNA sequencing, taxonomists realized that the common fish "fungi" don't belong in the Kingdom "Fungi" but instead belong in the "Chromista" Kingdom with the diatoms, the brown and golden algae, and a bunch of weird colorless organisms that you've never hear of (some of which are parasites in fish and humans, and many of which that are so obscure that you can't even find a picture in "Google Image!)" The correct common name for the fish disease "fungi" is "water molds" or more technically "oomycetes".

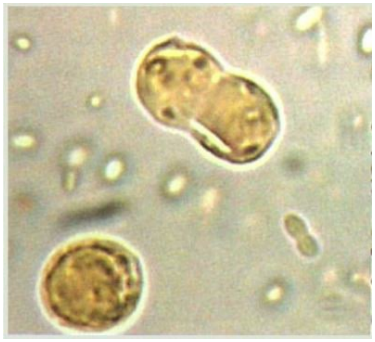


Figure 5: The Chromistan parasite Blastocystis

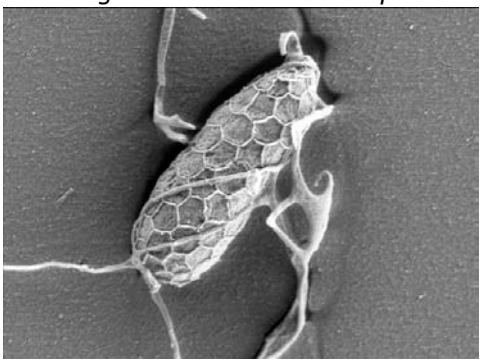


Figure 6: A Chromistan "slime net" organism from the surface of a seaweed.

The water mold that we see most often in salmon is Saprolegnia, the white fuzz often present on the skin of adult salmon. Saprolegnia is ubiquitous in aquatic environments where it feeds on decaying organic matter, but it is also happy to take advantage of a fish that is damaged or immunocompromised. The two ingredients for a saprolegnia outbreaks are 1) a compromised host, and 2) high densities of saprolegnia spores in the water. These conditions occur most commonly in adult salmon that have dedicated their energy reserves to reproduction instead of immunity, that often have skin injuries of all sorts, and that are in water where there is a lot of decaying organic material like plants or dead fish. The condition can be made worse by high temperatures that damage immune function and increase the rate of decay.

Saprolegnia infections in adult salmon returning to hatcheries are difficult to manage if fish arrive at the hatchery with skin injuries already fuzzy with water mold infections. The best management is to avoid further skin damage, to provide the lowest fish densities and highest practical water flows, and to provide the cleanest and coolest water available. When that isn't enough, we resort to antifungal treatments with formalin or hydrogen peroxide. These treatments kill water molds that are exposed to the water, but have little effect on those already working their way into the tissues of the fish. In fish with weakened immunity, treatments slow the spread and growth of water molds, but they cannot cure the disease.

Saprolegnia infections in young fish are a different story. Young fish should have very active immune systems and intact skin. If water mold infections are present it is a sign that something else is very wrong. Management of the outbreak should be focused on addressing the husbandry or environmental problems that have allowed the water mold to get the upper hand.

Figure 7: Saprolegnia infection on an adult salmon (photo by USGS)

We do see other watermold infections in fish. In fry, especially those that have been fed at a too-early age, we see swim bladder infections by *Phoma herbarum* that may also invade other tissues or the gut. There is no treatment and diseased fish will die. Fortunately, it usually effects only a small part of the population and serious outbreaks can usually be prevented by good husbandry.

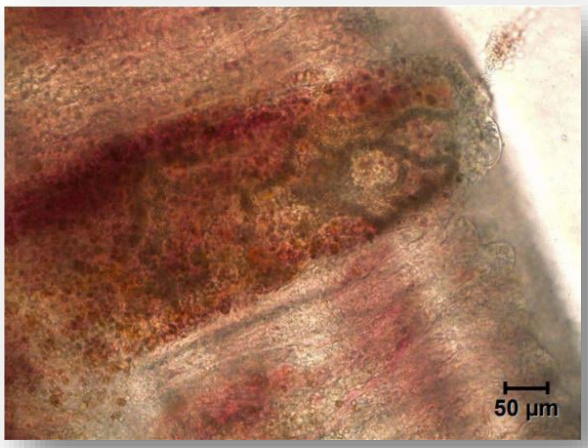


Figure 8: Branchiomyces, a watermold, growing within a gill lamella in a bass.

The most important thing to remember about the watermolds is that they are ubiquitous in

the environment and that the same water molds that we find on fish are just as happy infecting a plant or decomposing organic matter on the river bottom. These are not dedicated disease organisms with specialized mechanisms to overcome the immune systems of fish. They are decomposers of organic material that only afflict fish when the fish's immune system has been severely compromised. The formula for a fungus disease is a compromised fish along with high levels of watermold spores in the environment. Prevention means keeping fish healthy and reducing decaying organic materials in the water supply. Treatments of formalin or hydrogen peroxide can prevent new infections and slow existing infections of the skin and gills, but chemical baths cannot kill water molds that have already invaded tissues beneath the surface of the fish.

Interesting fungus facts

The water molds produce motile spores that can easily be mistaken for protozoan gill parasites

Figure 9: Saprolegnia. The gray structures at the tips of the filaments are filled with motile zoospores.

The flagella on watermold spores are of two types – tinsel and whiplash. The tinsel flagellum takes the lead.

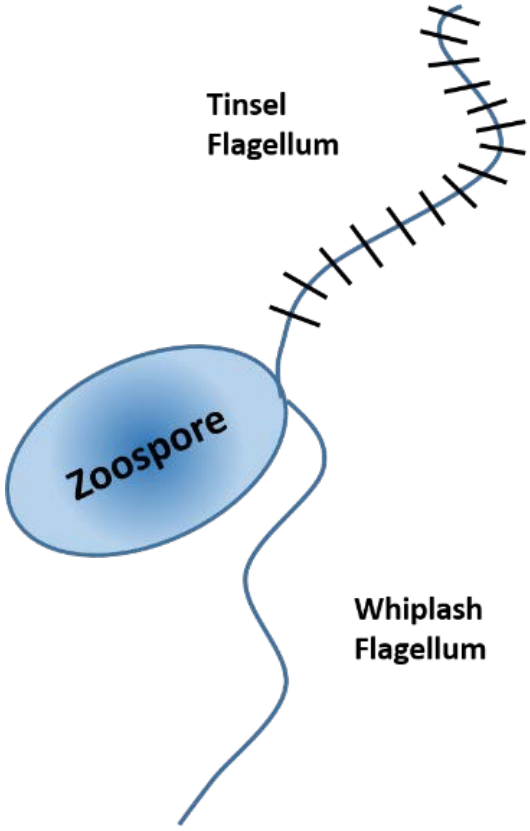


Figure 10: Tinsel and whiplash flagella on a zoospore.

Some fungal infections in fish grow very slowly and induce a vigorous inflammatory response. The resultant lesions are easily mistaken for cancer.

The Irish Potato Famine of the 1840s that ravaged Ireland and other parts of Europe was caused by a watermold.

Kelp are close relatives of the water molds that infect fish.

Figure 11: Kelp, a close relative of saprolegnia and a home for slime net organisms

PRFHP Update

Following the opening of a contract with the Washington Animal Disease Diagnostic Laboratory (WADDL), the reorganization of the PRFHP has begun in earnest. Testing from the FWS laboratory in Olympia will be switched to WADDL on August 1, 2018. We plan to be out of our current building in Olympia by early September. Veterinarian Sonia Mumford of the Olympia office has taken a new position as the manager of the Eaton National Fish Hatchery in North Carolina. Chris Patterson and Sharon Lutz will move in across the parking lot to share office space with the PSOPFWCO.

In the Gorge, Spencer Meinzer now works for the Willard NFH but also does their fish health work. Ken Lujan is the assistant manager of our Spring Creek NFH but continues to do the Wild Fish Health Survey in the summer months and take care of Spring Creek's fish health during the rest of the year. Susan Gutenberger still works for the PRFHP out of the Carson NFH. Mary Peters retired this spring. David Thompson is still working out of the FWS lab in Willard, but will move to space in the Spring

Veterinarian Trista Becker and new Animal Health Tech Taylor Scott-Moelder work out of office space on Leavenworth NFH. They have an awesome new "building in a building" that provides great modern office space without changing the historic hatchery building in which they reside.

In Idaho, veterinarian Guppy Blair has moved on to a new position at the Aquatic Animal Drug Approval Program in Bozeman MT. Term employee Tim Bundy heads to R2 in August. Corie Samson and Laura Sprague are busily taking care of the Idaho hatcheries and will continue to run that laboratory until late 2019 or early 2020 when the testing will be switched to WADDL. Corie and Laura will continue to occupy office space at Dworshak.

We are in the process of seeking approval to hire two temp employees to help get us through the fall 2018 season and are seeking waivers to hire new veterinarians.

The goal of the PRFHP reorganization is to have veterinarians and other fish health professionals dispersed throughout the region to locations proximate to concentrations of FWS and partner hatcheries, and to have all of our testing done under full internationally-recognized accreditation. We now have PRFHP staff working out of 8 duty stations and testing is switching over to the internationally-recognized and fully-accredited WADDL laboratory. As we re-do reimbursable agreements and budgets, it is clear that even during this transitional time the costs for running the reorganized PRFHP are going to be significantly less.

Dissolved Gasses and Gas Bubble Disease

Author's note: The challenges that we've faced at Dworshak NFH in the last two years have made us all work harder to understand the relationship between dissolved gas pressure and gas bubble disease. The essay below is one that I wrote to help me to better understand what we were seeing. Kyle Hanson was a great help and let me try out some ideas on him. He even did a big edit for me. Thanks Kyle! Hopefully this will help some of you like it helped me. We'll start out by defining some terms.

Glossary

Total Dissolved Gas Pressure (TDGP): Is the total pressure exerted by a mixture of gasses dissolved in a liquid. In an open vessel at equilibrium, the TDGP is equal to the atmospheric pressure (barometric pressure). In other words, gas from the air is entering the water at the same rate that gas is leaving the water and entering the air. In aquaculture, TDGP is often expressed as a percentage of normal atmospheric pressure.

Bubble forming conditions: A situation where the TDGP pushing gasses out of a fluid exceeds the gas pressure pushing gasses into the fluid. In an open vessel this means that the TDGP in the water would exceed the barometric pressure so gas would be leaving the water faster than it was entering the water. Under these conditions, gas in the water also enters microbubbles (normally present in water or blood) faster than gas is leaving the bubbles and bubble growth results.

Gas Bubble Disease (GBD): The TDGP in fish blood is normally in equilibrium with the TDGP in the water around the fish. If bubble forming conditions are present in a fish's environment (a TDGP higher than the barometric pressure), then bubble forming conditions also exist in fish blood. This causes microbubbles in the blood to expand into larger bubbles that block blood vessels. Additional gas may leave the blood and overinflate the swim bladder. GBD can exist in chronic and acute forms depending on the number and size of bubbles present within the fish. It can be reversed if the fish experience conditions where bubbles shrink (TDGP is less in the environment than in the fish's blood).

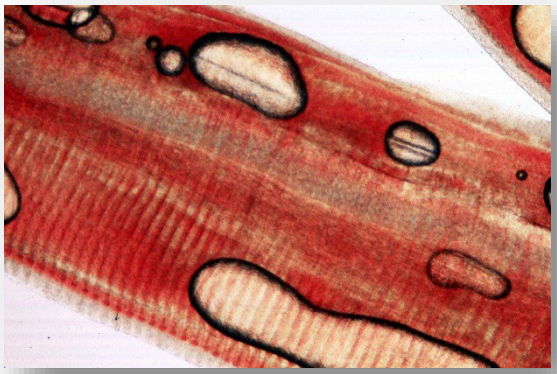


Figure 12: Gas bubbles within the blood vessels of a fish gill filament.

Gas Partial Pressures: Just like the barometric pressure is the sum of the gas pressures contributed by nitrogen oxygen, argon, carbon dioxide, and water vapor, the TDGP is the sum of all the pressures exerted by gasses dissolved in water. In water exposed to air, the dissolved gasses would be a mixture of the same gasses present in the air and the pressure of each gas in the water would equal the pressure of each gas in the air.

Gas saturation and **total gas pressure**: When the pressure exerted by a gas in a fluid is in equilibrium with the pressure of a gas in the

atmosphere above the fluid, we call that "100% saturation". If gasses have been dissolved in water under higher-than-barometric pressure, then the TDGP in the water will be greater than that of normal atmospheric pressure. If we take the TDGP in the water under these conditions and divide it by the barometric pressure we end up with a saturation above 100%. We would often call this "supersaturation", a condition that is often associated with bubble forming conditions and GBD.

Gas saturation and **partial gas pressures**: It is also possible to calculate % saturation for individual gasses in a mixture of gasses. In an open vessel in air, the pressure of the oxygen going into the water is the same as the pressure of the oxygen going out of the vessel. The same is true for nitrogen and the other gasses and the total pressure of all the gasses in the water equals the total pressure of all the gasses in the air above the water. Under these conditions, if you divide (for example) the oxygen pressure in the water by the oxygen pressure in the air, the oxygen saturation would be 100%. If you change the composition of the gasses in the atmosphere, the composition of gasses in the water follows suit. This can result in major changes to the concentrations of the gasses in the water, but as long as it is an open vessel (not a pressurized system) the TDGP in the water and in the fish remains at 100% and bubble forming conditions do not occur. Note though that if you take the oxygen pressure in water aerated with pure oxygen and divide it by the normal atmospheric oxygen pressure, the calculated oxygen saturation will be far higher than 100% even though the TDGP remains at 100%.

Low Head Oxygenator (LHO): An aeration device for aquaculture. In its simplest form, oxygen-depleted water exits a raceway and flows onto the top of the LHO. It then passes through a perforated plate and falls through a pure oxygen atmosphere and into the raceway below. The gas pressure in the LHO is equal to the atmospheric pressure, but because the oxygen pressure in the gas phase is now higher than the oxygen pressure in the water, oxygen moves into the water. Likewise, because the nitrogen gas pressure in the LHO is near zero, nitrogen moves out of the water into the gas. The net result is that nitrogen in the water is replaced by oxygen. Oxygen concentrations in water leaving the LHO may be quite high, but the TDGP in the water remains the same so LHOs do not cause bubble forming conditions. LHOs are desirable because they have no moving parts, no electrical requirement, and require only a small drop in head to efficiently transfer pure oxygen into water.

Figure 13: In this fascinating case, a vigorous spring algae bloom added so much oxygen to the water in an earthen pond, so quickly, that the TDGP was well over 100% and the skin of the channel catfish filled with bubbles.

Gas Equilibrium Basics

Gasses in the air normally exist in equilibrium with gasses dissolved in water. Oxygen and nitrogen from the air are always entering the water while oxygen and nitrogen in the water are escaping into the air (passive diffusion). If we start out with gas-free water and expose it

to the air, initially the odds of an oxygen or nitrogen molecule going from air to water are much higher than the probability of those gas molecules going from the water to the air. The gasses therefore start to accumulate in the water. If we let that go on for a while, we get to a point where the probability of an oxygen or nitrogen molecule leaving the water is the same as the probability of those molecules entering the water. With air, this equilibrium happens when there is about 10mg/l oxygen and 15 mg/l nitrogen in the water.

Another way to look at it is to express things as gas pressure instead of probability. There is a gas pressure in the air, but also a gas pressure in the water. Atmospheric pressure is pushing gas into the water. As the amount of gas in the water increases, the gas pressure in the water pushes gas back out. At equilibrium (well aerated water), the gas pressure into the water is the same as the pressure back out and there is no net gain or loss of gas in the water. The total pressure exerted by the nitrogen and oxygen (and smidge of argon, and the water vapor) is equal to the barometric pressure in the atmosphere at the water surface.

Barometric pressure is very relevant to our discussion of GBD. If the barometric pressure drops suddenly, the pressure in the atmosphere will temporarily be less than the gas pressure in the water. When that happens, gas leaves the water faster than gas is coming in. This can happen at the water surface, but it also happens in a micro-bubble present in the water. Gasses start going into the bubble faster than they are going out so the bubbles grow (gas bubble forming condition). We exploit this principle in our vacuum degassers. By dropping the air pressure above the water, the pressure difference between the water and the atmosphere is increased so gasses leave the water more quickly (increased gas bubble forming conditions) and the TDGP in the water drops. When that water leaves the degasser and is back in a normal atmospheric pressure, the now-lowered TDGP in the water is much closer to the gas pressure in the atmosphere so bubble forming conditions may be absent or very greatly reduced.

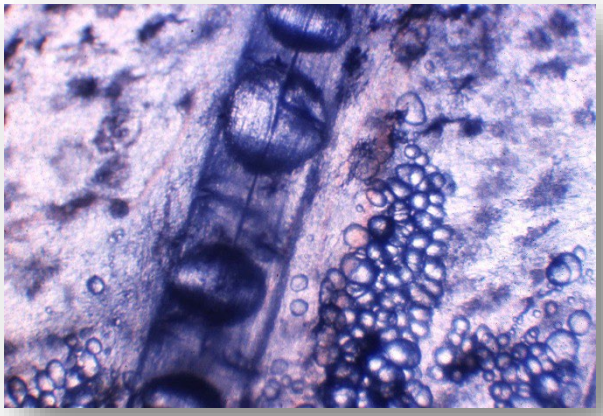


Figure 14: Gas bubbles in the fin of a fish exposed to high TDGPs. The vertical column is a fin ray. It has bubbles too.

How things work with pure gasses instead of mixtures of gasses – intro to LHOs

If you put an airstone in a bucket of water and pump pure oxygen through it, the chances of a nitrogen molecule going from the gas to the water is about zero so the nitrogen molecules that are leaving the water are not replaced by other nitrogen molecules. In addition, as the nitrogen molecules leave, there is now more room in the water for oxygen molecules. Now looking at it as pressure: With the nitrogen gone, this means that the oxygen molecules can spread out and the oxygen pressure drops (it is

like putting the same amount of gas into a bigger tank). This lower pressure allows more oxygen to enter the water until the gas pressure (now almost all nitrogen) equals the atmospheric pressure.

This business of using pure gasses is directly applicable to an LHO. In the LHO the water is exposed to a pure oxygen atmosphere. In a pure oxygen atmosphere, nitrogen molecules leaving the water are not replaced so nitrogen levels in the water drop. The exit of the nitrogen drops the total gas pressure in the water leaving room for more oxygen to enter. The pure oxygen atmosphere in the LHO makes it more probable that oxygen molecule swill enter the water than leave so the oxygen concentration in the water goes up. Thus, the nitrogen gas pressure in the water drops and the oxygen flows into the water until the TDGP in the water is equal to the barometric pressure. At typical temperatures and barometric pressures, the oxygen concentration can get to about 40 mg/l. The nitrogen is gone, oxygen has replaced it, and the result is almost 4 times more oxygen per liter of water than you would see in water exposed to air even though the TDGP has stayed about the same.

TDGP vs % Saturation

One of the challenges associated with pure gasses and LHOs is that we need to separate our consideration of TDGP and % saturation. For example: If you put an air diffuser into a bucket of water and run pure oxygen through it, the oxygen will go up to about 40 ppm or 4 times what it would using plain air. We would call that "400% saturation" because there is 4x more oxygen in the water than under typical atmospheric conditions, but the reality is that the total gas pressure in the water is still exactly equal to the barometric pressure. We've just altered the mixture of gases and changed out almost all of the dissolved nitrogen in the water for oxygen. Since the TDGP in the water is in equilibrium with the atmospheric pressure, then the TDGP is still just 100% and there is zero risk of GBD because gas bubble forming conditions do not exist. Confusion comes from the convention of expressing percent saturation relative to air instead of relative to pure oxygen. If an LHO drives oxygen levels up to "400% saturation" there is no risk of GBD because the TDG is still 100%. Interestingly though, those high oxygen levels do promote oxidation reactions that can damage the tissues of fish.

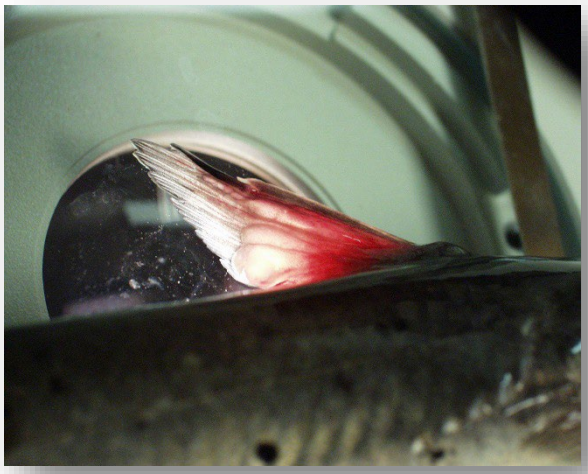


*Figure 15: This fish has been exposed to high TDGP. Gas bubbles formed and damaged the tissue. When conditions changed, the gas went away but the damaged area filled with tissue fluids producing these odd "Jelly Fin" lesions***.**

Dworshak, an Example

When water is spilled over the dam, it entrains small bubbles of air that are carried deep into the river. The weight of the water pushes on the bubbles shrinking them and increasing the gas pressure within them. This drives the gas from the bubbles into the water. When that

water is brought back up to the surface, that extra water pressure isn't there and the gas starts to leave the water and re-enter the microbubbles that remain. The same thing happens when you take the lid off a bottle of coke. Opening the bottle drops the pressure above the Coke so the gas starts to re-enter the microbubbles and makes them grow. *An interesting side note*: When you shake a coke before opening, it fizzes much more. This isn't because you have increased the pressure in the unopened coke by shaking. It is because the shaking made a bevy of microbubbles that were then there to grow quickly when you opened the cap, dropped the pressure, and caused the dissolved CO_2 to flood into the microbubbles. Without the microbubbles, all of the gas exchange has to happen on the soda's surface – a much slower and less messy process.

Figure 16: Adding certain mint candies to a bottle of soda causes very impressive bubble formation. The reason is that the crystal structure on the surface of the candy carries millions of microbubbles. When the candy goes into the freshly-opened soda, the very high TDGP of carbon dioxide causes gas to surge into those microbubbles and expand them producing big quantities of foam.

At Dworshak, when we pump river water with the extra gas into the hatchery, the extra gas is still in the process of leaving the water and we have prime bubble forming conditions. Some of the extra dissolved gas gets into the fish through its gills and skin. As the spontaneous degassing process continues, the gas moves into microbubbles in the fish's blood or into the swim bladder in the same way that the gas is moving out of the surrounding water. This causes bubbles to block blood vessels and swim bladders to be overinflated. As long as the fish stays in this bubble-forming environment, the bubbles within the fish will continue to grow. If the fish is moved to water that is at 100% TDGP, the bubbles will quit growing, but they will not go away. If the fish is moved to water that has a lower gas pressure than is present in the bubbles (for example – water that has been through a vacuum degasser) gas will move out of the bubbles and the bubbles will shrink.

When fish with bubbles are released from a hatchery, bubbles may gradually disappear as the fish swims through areas of lower gas pressure, but the most likely mechanism for bubble resolution is the physics of deeper water. If the fish can dive down a few meters, the increased water pressure will shrink the bubbles. This provides some immediate relief. Shrinking the bubbles also increases TDGP in the bubbles so gas leaves the bubbles and enters the blood stream, ultimately exiting the fish through gills as dissolved gasses. This means that when the fish then returns to the surface, the re-expanded bubbles are much smaller or may be gone altogether.

Solutions at Dworshak

We pump in spilled water under bubble growth conditions (higher pressure pushing gasses out of the water than the pressure pushing gasses

in). If we put it right onto fish, the dissolved gasses enter the fish and bubble formations continues in the fish's blood vessels. To prevent that, we run the water through vacuum degassers before putting it on fish. The degassers drastically drop the air pressure above the water. This speeds bubble formation so it removes the extra gas from the water before it gets to the fish. When the water leaves the degassers it is now much closer to being in gas equilibrium with the air so it is much less likely to cause severe bubbles in the fish.

To provide an extra layer of protection at Dworshak, we also run some of the water through LHOs. Within the LHO, the water drops through a pure oxygen atmosphere. Several interesting things happen.

1. Breaking the water up into fine streams and dropping them through air helps extra gas to escape and reduces the bubble forming tendency of the water (the TDGP)
2. Nitrogen is stripped from the water
3. Oxygen enters the water

The water that leaves the LHO and enters the raceway is likely to still have a TDGP above 100%, but the TDGP will now be more from oxygen and less from nitrogen (the degree of change depends on the inlet TDGP, oxygen flow to water flow ratios in the LHO, and the design of the LHO (drop and hole size)). The TDGP may still be over 100% so bubble forming conditions still exist. If this water is introduced into a standard raceway (high water flows, low fish density), GBD will still result because fish respiration is not sufficient to drop the oxygen-associated TDGP in the head end of the raceway.

LHOs, Circular RAS, and Opportunities at Dworshak

There is another very interesting aspect of LHO use to resolve high TDGP problems that is applicable to circular RAS systems. Brian Vince ran some calculations for me that show that if water going into an LHO has a TGDP of 120% composed mainly of nitrogen (a worst case scenario), the water coming out of the LHO would have a TDGP still at 115%, but with nitrogen pressure at just 100% and oxygen pressure at 180% saturation relative to air. If this very high TDGP/very high oxygen saturation water was introduced into a standard raceway, respiration by fish would not drop the TDG levels fast enough so fish at the head end of the raceway would likely get GBD and direct damage from oxidation. However, things are very different in a circular tank. In circular tanks, the water is evenly mixed throughout the tank. Fresh, high-TDGP replacement water can be added to the oxygen-depleted water in the tank at a rate that keeps TDGP and oxygen saturation near 100% and, because of the speedy and uniform mixing in these circular systems, fish would not be exposed to high TDGP or oxygen saturation. No GBD or oxidative damage would occur and incoming replacement water would carry enough oxygen to support the fish even at a low replacement/high recycle rate. In a water situation like that which we face at Dworshak, circular RAS incoming water could be treated solely by LHOs without using the current vacuum degassers. While this would require quite a bit of oxygen, we would also be able to utilize all of the oxygen that is currently stripped by the vacuum degassers, save on the electrical costs for the vacuum pumps, and perhaps reduce pumping head.

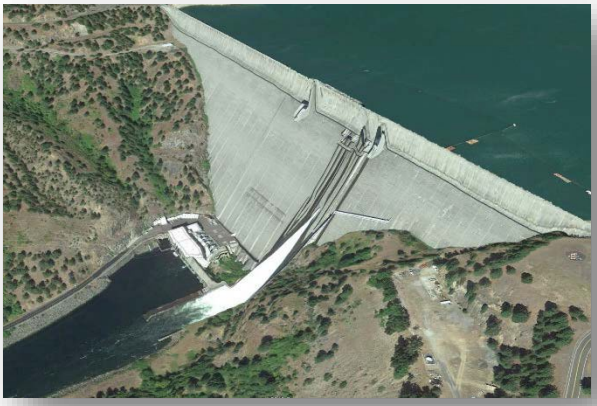


Figure 16: Water leaving the Dworshak Dam through the spillway. Water falls more than 700 feet and forces gas bubbles deep underwater. Picture from Google Earth.

Three Final Points

1. *Nitrogen super-saturation alone cannot cause GBD, it's the TDGP that really matters*

Bubble forming conditions require that the TDGP within the fluid exceed the total gas pressure above the fluid. Only under these conditions will bubbles grow. In a situation where (for example) nitrogen saturation is at 105% and oxygen saturation is at 90%, the TDGP is still less than 100%. Under these conditions the water (when exposed to air) would lose nitrogen and gain oxygen, but bubble growth cannot occur because no overall pressure differential exists to expand the bubbles. The consensus is that bubbles first appear when TDGPs are 101-103% and that significant health effects of chronic exposure begin at 105% independent of the gas composition.

2. *Both nitrogen and oxygen can cause GBD.*

The composition of gasses in bubbles are proportional to the composition of the gasses in the water around the bubble. If the water is supersaturated with oxygen and TDGP is above 100%, then bubbles will form that have a higher oxygen:nitrogen ratio than air. There are many examples of GBD caused by excessive TDGP driven entirely by oxygen. In a case of mixed gas supersaturation (like oxygen and nitrogen below dams) the bubbles are composed of a mixture of oxygen and nitrogen. Either or both gasses are equally effective at forming bubbles and it is the physical presence of bubbles in fish that causes GBD damage.

That said, there are several scientific papers that look at fish mortality following GBD triggered by high TDGP composed of oxygen and nitrogen gas in different ratios.

- Nebeker et al 1976 stated that TDGP was much more important than the oxygen/nitrogen ratio and that no bubbles formed when the TDGP was below 100% (this also supports point 1 above). They did find that at TDGPs of 120-129%, a higher oxygen/nitrogen ratio decreased mortality, but paradoxically noted that bubbles were much more extensive and severe at higher oxygen/nitrogen ratios.
- Rucker 1973 showed that at 119% TDGP, mortality is higher when the nitrogen saturation is over 110%
- Nebeker 1978 showed again that at TDGPs above 130% a higher oxygen/nitrogen ratio decreased mortality

Based on these papers, it is clear that at very high TDGPs, mortality is more severe as more of the TDGP is contributed by nitrogen. It should be noted though that these papers saw this effect at oxygen/nitrogen ratios very different from those that would be expected to naturally occur as a result of gas entrainment below a dam, and at TDGPs far higher than that which

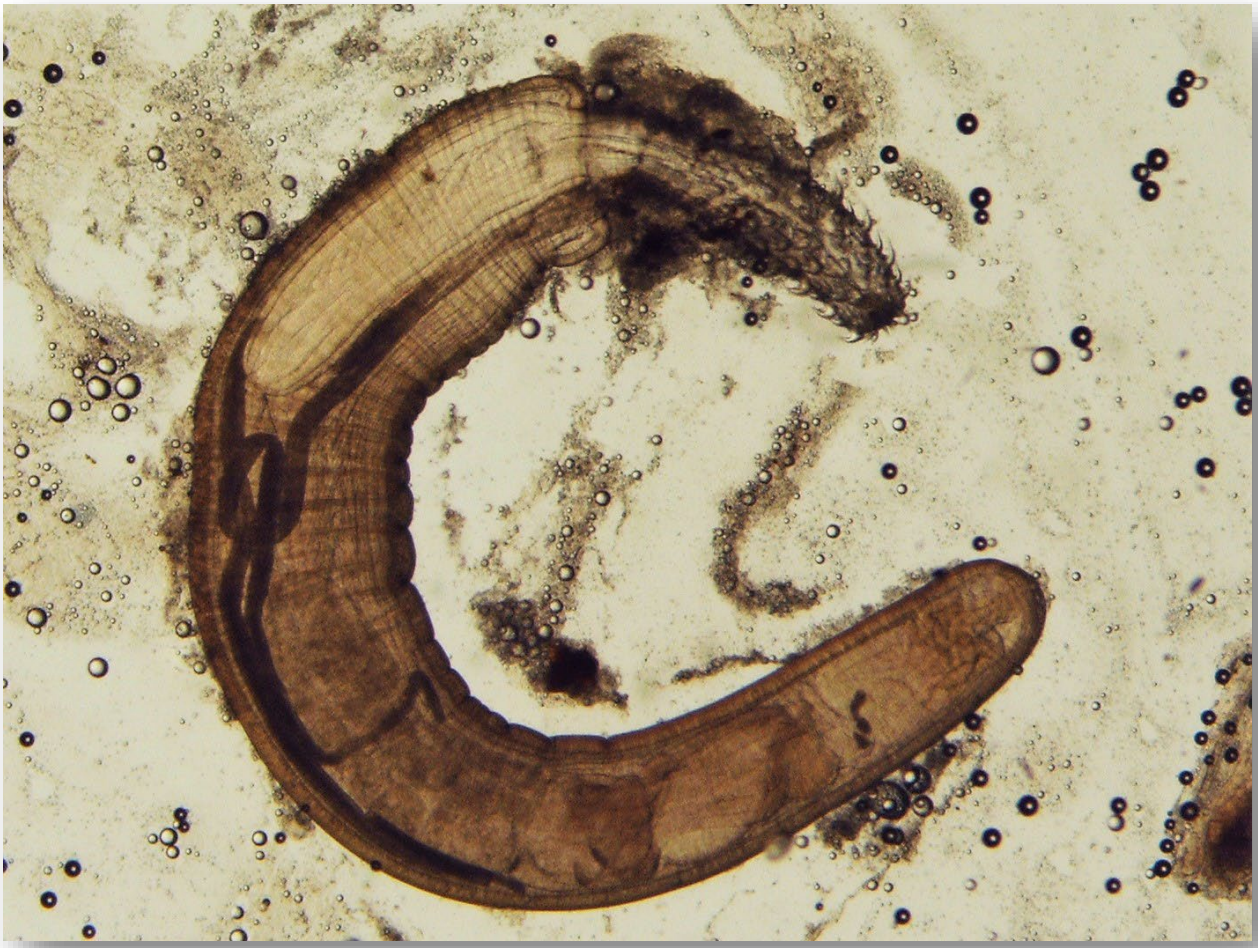
our hatchery fish experience within the hatchery. In some treatments the oxygen/nitrogen ratio was so low that fish were stressed by low DO. So, the oxygen/nitrogen ratio does have an effect at very high TDGPs, but it is not usually significant under normal conditions (dams and hatcheries) so TDGP regulations ignore it. An explanation of the gas ratio effect seen in research studies may be that at very high TDGP associated with very high oxygen/nitrogen ratios, sufficient oxygen may still diffuse into tissues that are downstream of a blood vessel blocked by a bubble. This would explain why this oxygen/nitrogen ratio effect is seen only under these high TDGP and high oxygen ration conditions.

3. *TDGPs over 100%, caused by oxygen alone, are not safe*

Water aerated using pure oxygen may have very high oxygen concentrations, but the overall TDGP will not increase above 100% in an open container. To increase the DO from the normal maximum of about 10 mg/l in air all the way up to 20 mg/l is easy to do with pure oxygen and, in this example, it results in an oxygen saturation of 200% (compared to air). Fish are pretty happy at that 20 mg/l and while they may eventually develop some chronic oxidative damage to their gills, no GBD will occur because in an open un-pressurized container, the TDGP remains at 100%. The important point here though is that the "200% saturation" is calculated relative to air and not relative to the atmosphere that was above the water (pure oxygen) and that the TDGP is still at 100%. Looking at the extreme, with pure oxygen aeration at normal atmospheric pressure (with an airstone or LHO) you can drive the DO up to almost 40 mg/l and the nitrogen to almost zero. Calculated according to air this would give you an "oxygen saturation" of almost 400%, but the all-important **TDGP remains at 100%** and no GBD will occur. The oxygen replaces the nitrogen and the TDGP does not increase. As it is normally calculated in aquaculture, it is possible to have a 200% oxygen saturation along with a TDGP of a nice safe 100%. However, if you take water and increase the oxygen saturation to 200% without a concomitant drop in nitrogen pressure (only possible in a pressurized system), then the TDGP would be well over 100% and GBD would result.

This can also be looked at in terms of partial pressures. In water where the TDGP is over 115% as the result of adding pure oxygen under pressure (in a U-tube for example), the partial pressure of oxygen in the water might be at 120% and the nitrogen partial pressure at 100%. When the water leaves the pressurized system, the oxygen gas partial pressure in the water will exceed the oxygen gas partial pressure in the air. This will cause oxygen molecules to move into microbubbles more quickly than oxygen molecules are leaving and bubble expansion will occur. As the bubbles fill with oxygen, the partial pressure of nitrogen within the bubble will drop and there will also be a net movement of nitrogen from the water into the gas bubbles. Oxygen levels in the water will decline as the partial pressure differential drives oxygen into the air and into bubbles, but the nitrogen leaving in the bubbles will be replaced by nitrogen diffusing into the water from the atmosphere. The net effect is that oxygen levels in the water drop and nitrogen levels remains the same. Bubble growth will continue until the TDGP in the water equals the atmospheric pressure above it.

Mystery Parasite of the Day



Answer