Fish Health News You Can Use

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This Issue

Page 1 – Intro to this Issue
Page 2 – Digenes; More than Just a Fluke!
Page 5– Fun Fluke Factoids
Page 8 – Bath Treatment Chemicals
Page 11 - Fun Chemical Factoids
Page 12 - PRFHP Update
Page 12 - Mystery Parasite

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In the Next Issue

- What is a Virus?
- Virus Testing – How does it Work?
- The Great Virus Unknowns
- Salmon Viruses
- PRFHP updates
- Mystery parasite of the day

Intro to This Issue

The Digenes (aka flukes and grubs) are one of the few groups of fish parasites that are big, obvious, and more than a bit off-putting in a fillet. They also have the potential to cause some significant health problems in fish. Despite this down side, the grubs are very fascinating animals with amazing life cycles that utilize many non-fish animals (even people) as hosts. They even come in several colors.

The second major article in this issue is about the chemistry and biology of the chemicals (formalin, peroxide, permanganate, chloramine-T, and copper sulfate) that are commonly used to treat external diseases in fish.

Figure 1: Crystals of copper sulfate and potassium permanganate, two bath treatment chemicals with complex chemistry and regulation.

These chemicals are the brute-force end of fish disease treatments. They are great tools, but it is important to understand their chemistry and the side effects of their use.

Digene Parasites:

More than just a fluke!

Most of the disease organisms that we deal with in fish are too small to see with the naked eye but the group of parasites known as “grubs” are an important exception. Grubs are so obvious in infected fish that they have popular common names like white grubs, yellow grubs, and black grubs. Infected fish do not look very tasty (though they are okay if well-cooked) and, with severe infections, the encysted parasites can impair fish so badly that they die of organ failure or a secondary disease.

Figure 2: Yellow grubs in the pectoral fin of a coppernose bluegill.

Grubs are flatworms (flukes) with complicated life cycles that involve several different animal hosts. The life cycles of yellow, white, and some species of black grubs are all fairly similar. The adult worms attach to the wall of the throat or gut of a fish-eating bird and shed eggs that enter the water when the bird feeds or defecates. Once in the water, the eggs hatch into miracidia, larval forms that invade snails. Inside the snail, the parasites set up shop and go through multiple stages to finally produce a never-ending supply of the next stage in the grub life cycle -- cercaria. Following seasonal cues these cercaria exit through the snail’s skin or gills into the water column. The immature cercaria drift through the water until they encounter a fish host and then invade through their skin or gills. The cercaria attach to the fish, borrow into the fish’s tissue, and develop into immature worms in cysts that are found in skin, muscle, spleen, kidney or other organs (depending on the grub species). These are the “grub” stages that take the fun out of a fish dinner. The encysted worms can live for as long as a year or two. Their life cycle is completed when the infected fish is eaten by a bird. As the fish is digested, the worms exit, attach to the throat or gut of the bird where they mature and begin to shed eggs and start the lifecycle all over again. In short, the lifecycle of the *typical* grub is: bird-snail-fish-bird.

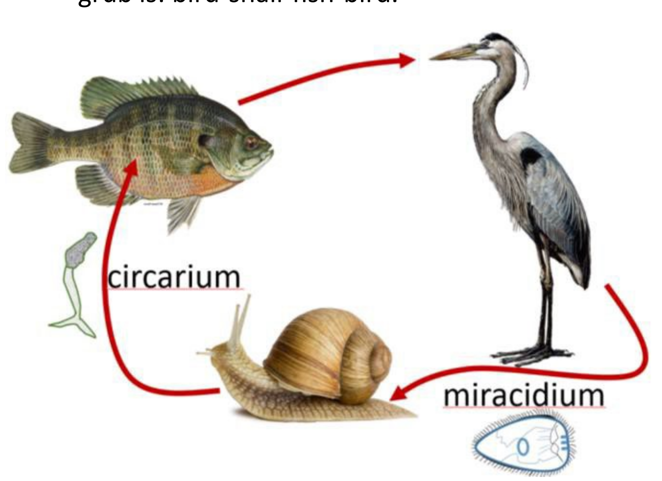

Figure 4: A typical grub life cycle. Circaria and miracidia are free swimming. The fish to bird transfer is accomplished when the bird eats the fish.

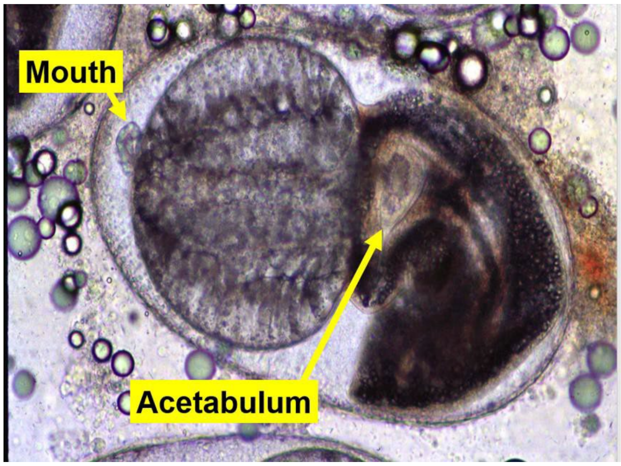

Figure 5: An immature white grub from the spleen of a fathead minnow. When the minnow is eaten by a bird, the oval capsule ruptures releasing the parasite into the bird's gut. The "acetabulum" is a suction cup that the grub uses to attach to the gut lining.

cats, foxes, raccoons, mink, and even humans (one of the many reasons why public health laws usually require that raw fish be frozen and thawed before serving!). The life cycle is typically dog-snail-fish-dog.

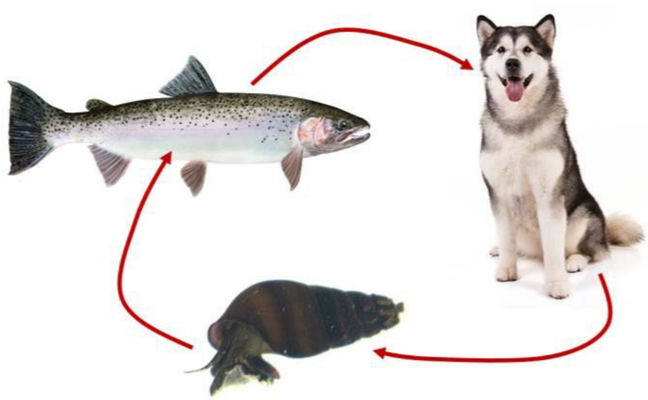

Figure 7: The typical life cycle of Nanophyetus. There is another amazing wrinkle to this story (see the Fun Factoids).

<u>Nanophyetus</u>

Nanophyetus salmincola is found in the internal organs of fish, especially salmonids.

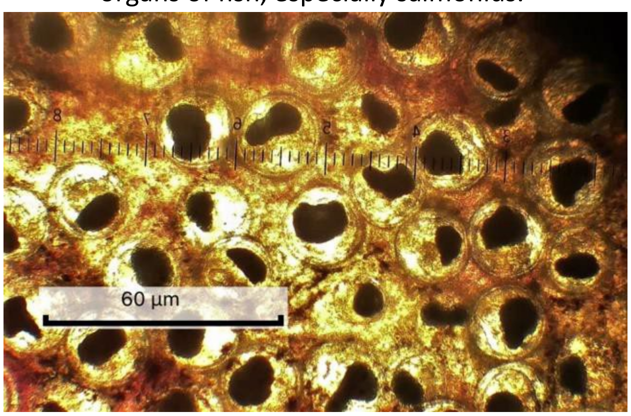

Figure 6. Nanophyetus in the kidney of a salmon.

Several of our hatcheries have surface water supplies that are inhabited by pleated juga snails and the hatchery fish, like the wild fish in that water supply, are infected by *Nanophyetus*. In general, fish can tolerate large numbers of maturing *Nanophyetus* in gill and other tissues, but heavier parasite burdens undoubtedly make the fish more susceptible to secondary health problems and may directly impair organ function enough that migration and saltwater adaptation or smolting may be compromised. There is also increasing evidence that declines in steelhead populations where adults breed in streams emptying into the southern portions of Puget Sound may in part be due to intense infections of *Nanophyetus*. There are concerns that climate change may allow the juga snails to move north with increasing water temperatures, spreading the range of *Nanophyetus* parasites.

Sanguinicola

There are more than 70 different grubs in the *Sanguinicola* group, but they all have life cycles that involve just two animal hosts instead of three. Like other grub species, *Sanguinicola* infect snails as miracidia stage larvae. Within the snail host the eggs develop into cercaria and migrate from the snail into the water column. The cercaria find a fish host and burrow into their tissues, where the parasites attach to the fish's blood vessel walls and mature into adults . Mature *Sanguinicola* shed eggs into the fish's bloodstream. The eggs travel throughout the fish's blood vessels until they reach the gills where the eggs invade the blood vessel wall and develop into the miracidium stage. Following seasonal cues, the miracidia migrate out the water column through the gills and go in search of a new snail. The *Sanguinicola* life cycle is: fish-snail-fish.

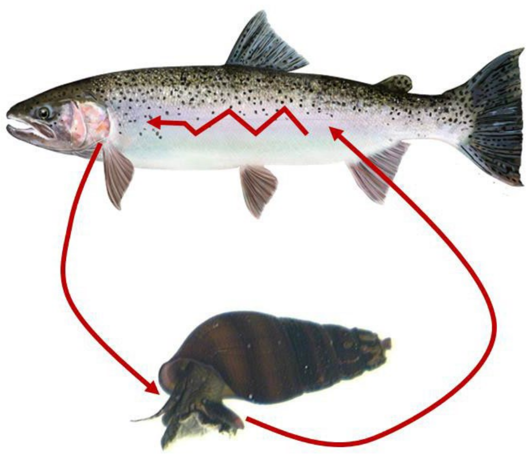

Figure 9: The life cycle of Sanquinicola. This parasite uses the fish for two stages in its life cycle so it doesn't need a third animal like a bird or mammal.

sometimes present in high numbers that clearly impact the health of wild and cultured fish.

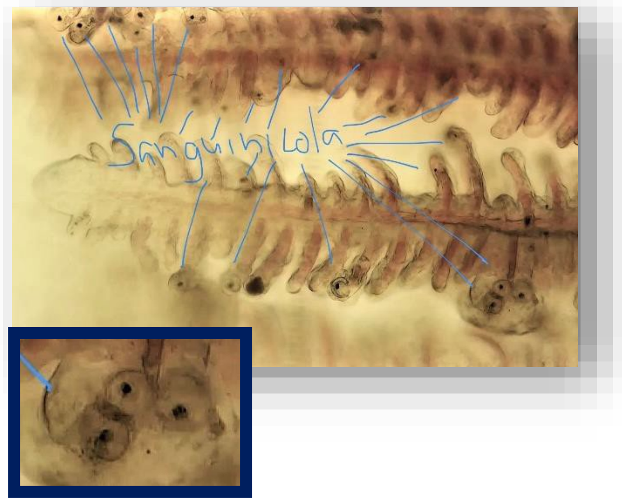

Figure 8: Sanguinicola in the gills of a juvenile salmon. The parasites are identified by blue lines and are recognizable as circles with a black dot (see enlarged inset). These parasites are at the stage where they burrow out through the gill membranes

Treatment and control

Nanophyetus and *Sanguinicola* grubs are very difficult to control. There are no drugs specifically labelled to treat for grubs in fish. In laboratory experiments looking at severe grub infections, killing large numbers of grubs within a fish will often kill the fish as it is unable to deal with the mass of dead parasites and the secondary inflammatory response. The best method for controlling these parasites is prevention but that isn't always easy. Obviously, the best protection for hatcheries is a protected well or spring water supply, but this isn't a possibility for some facilities. Physical filtration systems, like sand filters, could very likely significantly reduce the parasite burden but there isn't much science to support this. Chemical treatments for the incoming water to kill immature grubs before they entered fish might be effective but the treatment would have to be administered 24/7 during the times when the grub miracidia were seeking out fish hosts. Thus, a chemical prevention option isn't practical for a variety of reason both legal and biological.

Fish and grub parasites have been evolving together for millennia and have reached the point where a stable relationship has developed.

Figure 10: Nixon and Brezhnev, classic Détente. This picture is always relevant when talking about the relationships between parasites and their hosts.

Even impressive numbers of grubs cause surprisingly few problems for the fish and, in turn, the fish don't mount a big immune response in an attempt to kill the parasites. This is why the fish health folks don't usually worry much about grubs. With that in mind, it is still important to recognize that heavy infections of hatchery fish are serious and may result in diminished growth, secondary infectious diseases, slow migration, and difficulty adapting to sea and that these problems may be increased with the higher temperatures associated with climate change. In the wild, the problems that *Nanophyetus* seems to be causing for steelhead in Puget Sound may be increasing because of climate change and other human impacts that are changing the geographic range and density of snail hosts faster than steelhead populations can adapt.

Ever heard of swimmer's itch? Sometimes swimmers visiting warm weedy ponds end up with hundreds of itchy bumps on their skin. Those are caused by digenes (grubs) that have been shed by snails and are searching for their next host. Humans are not that host, but the parasites still burrow into the skin and cause itchy reactions as your immune system kills the parasites and cleans up the mess.

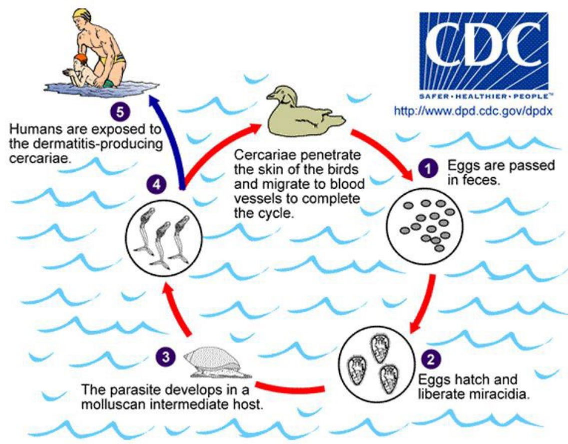

Figure 11: The CDC's diagram of the swimmer's itch life cycle. Note that it normally involves only birds and snails.

One of the favorite tricks of parasites that have complex multi-animal life cycles is to change the behavior of their hosts in ways that make it easier for the parasite to move on to its next home. Spectacular examples are worms that move into the antennae of land snails and make them pulse and flash bright colors, and parasites that make ants climb up grass blades to be eaten by grazers. The worms in the snail antennae look like tasty caterpillars that birds (the next host) like and they even make the snails hang out away from cover in bright light. Still pictures don't do them justice. Check out some of the links on YouTube. [Zombie Snails.]

In the following figure are grubs encysted around the brain of a minnow. In heavy infections the grubs compress the brain causing erratic behavior that makes the infected minnow more likely to be eaten by a bird and thus helps the parasites complete their life cycles. Zombie minnows!

Figure 12: Grubs (in the open ovals toward the top left) compressing the brain (the big pink structure) of a fathead minnow.

The picture (below) shows grubs in the dissected eye of a fish. Not only is the eye a safe and cozy place to develop, but growth here interferes with the fish's vision making it more likely to be eaten by the predator that is needed for the next stage of the parasite's development. Zombie bullhead catfish.

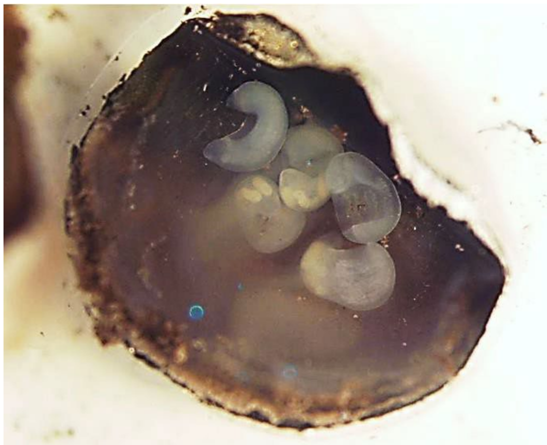

Figure 14: Grubs inside a fish eye.

Resurgent white pelican populations were visiting catfish ponds and infecting snails with a grub, *Bulbophorus confusus* (aptly named because of the difficulty in definitive identification) with a pelican-snail-fish-pelican life cycle. Deaths in juvenile fish were initially attributed to viral infections because overwhelming numbers of grubs killed the fish before the grubs developed enough to be detected. The industry now combats the disease by monitoring and controlling populations of the snail host. Harassing the migratory white pelicans is illegal and would be ineffective because one late night pelican visitation is enough to infect the snails in the pond.

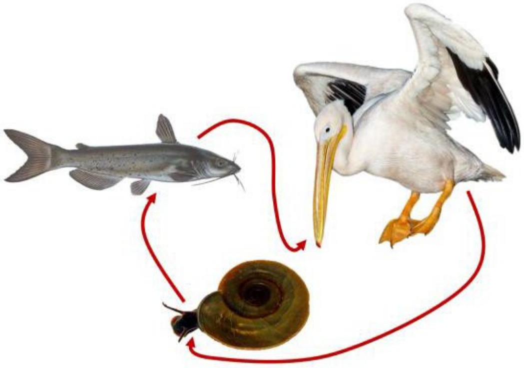

Figure 13: The Bulbophorus life cycle in catfish.

Figure 15: Bulbophorus parasites under the skin of a channel catfish.

In a paper by Drew Mitchell, he documents 2316 white grubs in a single 2 inch fathead minnow. Below is a fathead minnow (the Rosy Red variety) with a heavy white grub infection.

Figure 16: White grubs (white spots) in the liver and spleen of a fathead minnow.

If swimmers itch isn't bad enough, there are grubs that actually target human hosts. Below is the life cycle of the Chinese Liver Fluke. There are an estimated 15 million people infected with this parasite in Southeast Asia. Mature grubs in humans may shed 4000 eggs per day for up to 30 years. They are a major cause of liver disease and even liver cancer in regions where these parasites are common.

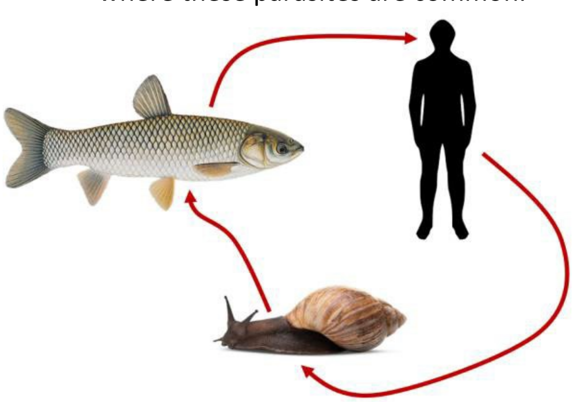

Figure 17: The life cycle of Chinese liver flukes.

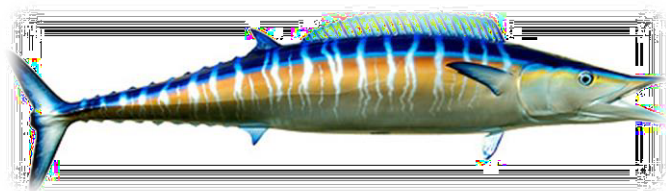

Figure 18: Hirudinella from a wahoo

The endangered fountain darter lives in the warm spring-fed San Marcos River in Texas. An exotic tropical snail became established in the spring and was then infected by a grub that relies on this exotic snail as a host. This completed a heron-snail-darter-heron life cycle that has devastated the darter population. The parasite grows in fish gills where triggers an inflammatory reaction that deforms the gill cartilage and greatly impairs gill function. Fish that can't breathe hang out near the surface and are easy prey for the herons. Zombie darters!

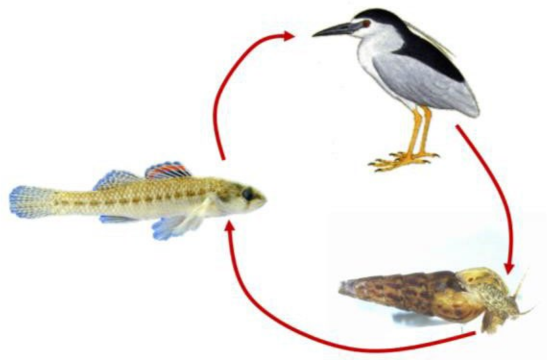

Figure 19: Fountain darter parasite life cycle with an exotic tropical snail species.

Figure 20. A gill arch from a severely infected fountain darter. The dark ovals are immature grubs. Gill cartilages are deformed and bent. Blood is pooled in the damaged circulatory system.

The Chemistry of Disease Treatment Baths

antibiotics that we use are finely tuned to target specific biochemical pathways that are present in bacteria but not in vertebrate animals, thus they're able to kill bacteria with little impact on the fish's biology. Bath treatment chemicals, on the other hand, are very different and react with a wide spectrum of organic molecules present on the surface of both disease organisms and fish cells. They are significantly less targeted than antibiotics and, in most cases, bath treatment chemicals kill fish cells just as easily as they kill the cells of bacteria, aquatic fungi, and parasites. Antibiotics are precision tools while bath treatments are blunt instruments.

The lack of specificity of bath chemicals is both a blessing and a curse. For example, formalin kills ich, aquatic fungi, and even some very small multi-cellular organisms like gill flukes, but it also kills fish cells, like skin and gill cells. Chloramine-T kills bacteria and gill flukes, but also fish cells. Permanganate and peroxide kill bacteria, fungi, and single-celled parasites, and fish cells. Copper kills fungi and many parasites, and fish cells. These chemicals always kill some fish cells so we only use them when the damage caused by the disease is worse than the damage that we expect to be caused by our treatments. This involves a careful evaluation of the fish's overall health and their ability to withstand chemical treatment along with speculating about the course that the current infection would take if left untreated. Knowing which chemical to use for a parasite outbreak is fairly simple but knowing *when* to use that chemical is far more complicated.

At this point you are undoubtedly wondering how we can use these chemicals if they are as toxic to fish as they are to parasites and bacteria. The answer is that these chemicals are very reactive on surfaces but are slow to penetrate tissues. A brief bath treatment is sufficient for the chemicals to penetrate through small parasites, aquatic fungi, bacteria, and the top layer or two of fish skin cells. In these treatments, the disease organisms and the superficial fish cells are both killed by the treatment. The damage that is a disaster for tiny parasites like ich is only minor surface damage for a salmon. As long as the treatment is correct in time and concentration, the benefits to the fish will outweigh the damage that the treatment causes to the fish. The goal is for the damage to the fish to be sufficiently superficial that it is healed by a normal fish immune system in a matter of hours or days post-treatment.

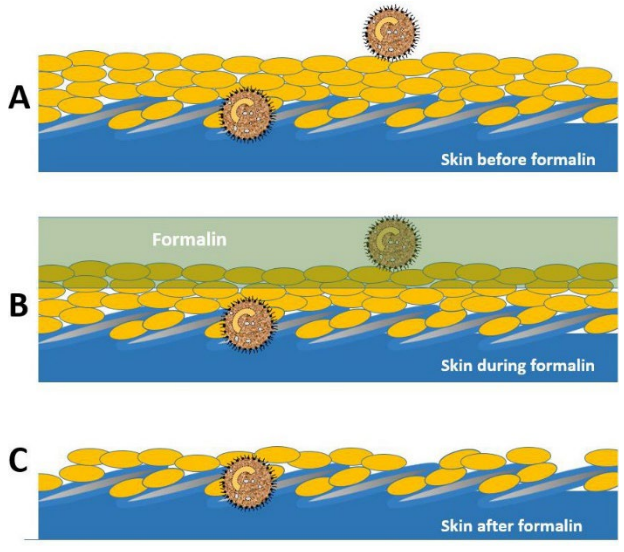

Figure 21: Fish skin before (A), during (B), and after (C) formalin treatment. In "A" the top layer of skin is many cells deep (yellow) and ich are present on and beneath the cell layer. The formalin treatment (green in "B") kills exposed ich and surface cells.

The damage to the fish is important in building a treatment plan. Even a correct treatment has the potential to kill enough skin and fin cells that it will 1) require the fish to use energy and material reserves to produce new cells, 2) make it more difficult for the fish to manage water and salt balance, and 3) weaken the fish's barriers to infection by other diseases. These effects are especially noticeable in fish that are the most seriously compromised by disease and unable to respond adequately to the chemical damage. It is not uncommon for bath treatments to kill a small percentage of the sickest and most compromised fish in the group.

Looking at the chemistry, it is not surprising that bath treatments kill some fish cells. Hydrogen peroxide, potassium permanganate, and Chloramine-T are all strong oxidants. They oxidize a wide variety of the organic molecules that are on the surfaces of cells and disrupt cell function enough that the cells die. They don't penetrate very deeply because they are so reactive that they are used up quickly as they seep into tissues. As mentioned before, this relatively short duration of action enables these chemicals to blitz through small single-celled parasites but generally prevents them from going through multiple layers of fish cells.

Hydrogen peroxide is a liquid oxidizer. One of its most desirable characteristics is that it breaks down into water and oxygen fairly rapidly so effluent concerns are minimized. In its concentrated form, it is plenty strong enough to eat holes in clothing, damage equipment, and damage skin and eyes of handlers without appropriate PPE. On unprotected human skin hydrogen peroxide penetrates through the dead outer layer of skin cells before it runs into enzymes that quickly break it down into water and oxygen. In human skin the oxygen production causes a prickly sensation and small bubbles to form rapidly under the skin.

Potassium permanganate is a solid oxidizer. One of the most interesting things about its chemistry is that it produces a purple solution that turns brown as the oxidation potential is used up. It also turns skin and clothing brown and eats holes in cloth. Since it is a crystal, it is very important to dissolve it in treatment water completely so that the crystals don't lodge in fish gills. In a deep-purple permanganate solution, it can be difficult to see if there is still undissolved chemical. Because of its vigorous oxidation activity on organic substrate any algae, food waste, or fish waste in ponds will rapidly deactivate potassium permanganate and interfere with its ability to address pathogens.

Figure 22: Potassium permanganate in water. It is purple when it is still an active oxidizer and brown when it is used up.

Chloramine-T is a powder-based oxidizer that uses some of the same kinds of oxidation reactions that occur with bleach and other chlorine compounds. Its activity is somewhat sensitive to water chemistry so that must be taken into account when planning a Chloramine-T based treatment plan.

Unlike the chemicals above, formalin and copper sulfate are not strong oxidants and they work through different chemistry. Formalin reacts with organic molecules to form strong chemical cross links, especially within and between proteins. These links disable those proteins so that they are no longer able to fulfill their functions. Another difference is that formalin is a gas (formaldehyde) that is dissolved in water. Because it is a gas, it is happy to diffuse back out of the formalin and into your lungs where it starts cross linking proteins in your cells. This is *not good* and chronic exposure to formalin can lead to serious health effects in humans, like lung disease and certain kinds of cancers. As with all of these chemicals properly used and maintained PPE are key!

Copper sulfate chemistry is another chemical that is not directly an oxidizer. Copper ions diffuse into cells and bind to enzymes and proteins that protect the cell from oxidizing molecules that are a normal part of cell chemistry. By disrupting the normal chemistry of the cell, copper sulfate causes cells to become leaky and ultimately die. More than any other treatment compound, copper sulfate is extremely sensitive to water chemistry. In alkaline water with lots of carbonates and bicarbonates, copper very quickly makes copper carbonates that settle out of solution. In acidic water low on carbonates and bicarbonates, the copper stays in its active form for a very long time. It is imperative that copper only be used when the water chemistry is well known so that treatments can be adjusted to be effective but not lethal to fish. In inland waters of the Pacific Northwest, copper is usually too toxic to use safely.

Figure 23: Copper sulfate in solution (left) and as a copper carbonate precipitate in water with high alkalinity (right).

The impact of chemical bath treatments on fish skin and gills varies depending on fish species, fish size, fish density, water temperature, water quality, the presence of other organic material, and the flow pattern and turnover of the water in the culture vessel. In all new situations it is very advisable to do a "bucket bioassay" before proceeding, which can be a good predictor of how a treatment will interact with fish in individual circumstances. It is also important to consider your effluent permitting and to work with your responsible veterinarian to make sure that the proposed treatment is within the limits of the complex regulatory structure that governs their use.

Chemistry factoids

White blood cells in your immune system engulf bacteria and kill them with hydrogen peroxide. For this reason, many bacteria have evolved enzymes that efficiently break hydrogen peroxide down into water and oxygen; thus it takes a high concentration of peroxide to overwhelm these bacterial defenses.

When formalin gets too cold in storage, it polymerizes into an insoluble white compound (paraformaldehyde). It is commonly said that this polymer is very harmful to fish, but the reality is that it is relatively inert and certainly less toxic to fish than formaldehyde itself. The biggest problem with paraformaldehyde is that it removes the formaldehyde from solution and makes it less effective as a disease treatment. Commercial formaldehyde solutions (formalin) often contain methanol to inhibit paraformaldehyde formation.

Formaldehyde is a gas. A saturated solution of formaldehyde gas in water is about 37% formaldehyde by weight. The saturated solution is known as formalin. Thus, a 100% formalin solution contains 37% formaldehyde by weight. This causes no end of confusion. The chemical that you buy is 100% formalin and treatment recommendations are given in ppm formalin.

Automated formalin dosing systems should never be trusted. Treatment concentrations should be checked often using a test kit. The PRFHP staff has these test kits and are happy to help with dosing recommendations and adjustments.

Through an odd bit of chemistry, overdoses of potassium permanganate can be neutralized by hydrogen peroxide. When hydrogen peroxide is added, the purple color of the permanganate disappears, indicating that the product has been neutralized. It takes one molecule of hydrogen peroxide per molecule or permanganate (about 1 gram of peroxide per 3 grams of potassium permanganate).

Formalin does such a good job of cross-linking proteins that it is used to preserve and stabilize fish tissues that are later embedded in paraffin and sliced in 0.002 mm thick slices for histology. These very same properties make it the go-to tool at your local mortuary.

A close, but more reactive, relative of formaldehyde is glutaraldehyde. Trainers in the NFL have sometimes use it to harden and stabilize skin damaged by artificial turf burns.

Figure 24: Astro turf

PRFHP Update

After many long months, there is some movement in our effort to hire a new veterinarian to work out of the Leavenworth Complex. Our goal is to get an announcement out, with the "Recent Grads" option, in time to catch the 2021 graduating class of new fish veterinarians. In the meantime, Dr. Katie Royer (FWS Veterinarian in the Gorge) continues to provide veterinary oversight for the Leavenworth Complex and Yakama Nation hatcheries. Dr. Christine Parker-Graham (FWS veterinarian out of Lacey, WA) continues to provide veterinary support for the Washington Peninsula hatcheries, the Dworshak Complex, the Nez Perce Tribe, and two federal hatcheries in California.

In other news, Dr. Susan Gutenberger (fish pathologist in the Gorge) began phased retirement in November 2020. By using the "phased" option, Susan is providing FWS with an opportunity to make sure that her years of knowledge are passed on in a smooth transition as David Thompson and Katie Royer take over her duties at Carson and Eagle Creek National Fish Hatcheries.

The entire PRFHP staff is still working hard to maintain fish health services while dealing with the complexities of the coronavirus pandemic. We have had to make some tough decisions to help protect crews during fall 2020 spawning operations, but all of the critical spawning samples have been collected and tested.

Mystery Parasite of the Day (super tough!!)

For the answer, click Here.