



Fish Health News You Can Use

Brought to you by the Pacific Region Fish Health Program

Volume 1, Issue 2

August 2017

Inside This Issue

- Transfer Permits: Requirements and Responsibilities Explained 1
- DI, FI, Turnover, and Water Velocity, A Fish Health Perspective 3
- Who Makes the Fish Health Rules? 5
- Why is Ich so Hard to Treat? 6
- Changing Rules Governing Antibiotic Use in Fish 7
- New Science 8
- PRFHP Updates 9
- Fish Health Factoids 9
- Mystery Parasite of the Day 11

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

<https://www.fws.gov/pacific/Fisheries/fishhealth/>

In The Next Issue

- The 2017 Wild Fish Health Survey - A New Approach
- Amphibian Diseases, Who Cares?
- Which Fish Diseases are Transmitted to the Next Generation within Fish Eggs?
- RAS Systems and Fish Health
- Biosecurity on Fish Hatcheries
- Fish Vaccines
- New Science
- PRFHP Updates
- Mystery Parasite of the Day

Transfer Permits: Requirements and Responsibilities Explained

Transfer permits for interstate and intrastate fish movements are often required by state and tribal wildlife agencies. Because these permits directly address disease risks, the PRFHP is often called upon to assist in the transfer permit process.



A Fish Hauler Getting Ready to Transfer Fish from the Dworshak NFH.

The staff of the PRFHP is very knowledgeable about permits and is happy to assist with any step in the permit process, but the primary responsibility for obtaining transfer permits falls on hatchery and production program managers. The sending and recipient managers must agree on who will obtain the permits. The responsible manager must then start working with state authorities long before the intended shipment to make sure that the requirements for the permit are known and addressed in time for the permit to be in place for the shipment.

The requirements for transfer permits usually include pre-shipment fish health inspections. While PRFHP fish health folks try to anticipate fish transfers and anticipate and complete the required testing ahead of the shipping date, it is vitally important that hatchery managers contact their fish health representative to make sure that everyone is aware of transfer dates and of any additional fish health work that needs to be done. Remember that virus inspections take almost a month to complete!

Successfully obtaining transfer permits is all about early and continuing communication between the receiving hatchery, the shipping hatchery, fish health, and state and tribal regulators. These are the steps in the process:

1. At least 9 weeks before transfer, the sending and receiving managers communicate and decide who will obtain the transfer permits.
2. At least 8 weeks before transfer, determine all states and tribal reservations that fish/eggs will be **transported through or transported to**. Contact each of these states and tribes to determine the requirements, including disease inspections, for the fish transfer.
3. At least 6 weeks before the transfer, contact your fish health specialist to set up the required inspections. The fish health specialist may add inspections required by FWS policy. The six weeks lead time is required to achieve the scheduling, sampling, and incubation needed for virus testing.
4. At least 5 weeks before transfer, submit the required permit applications to the state and tribal authorities. At this time the fish health test data may not yet be complete but this allows the permitting agency time to consider the transfer and to make a provisional decision pending the fish health results.
5. One to two weeks prior to the transfer, the PRFHC will submit the lab testing results to the hatchery of origin, the receiving hatchery, and to the state and tribal authorities.
6. The state or tribal authority issues the transfer permit.
7. The transfer can occur. Remember that the hatchery must have the permit prior to transfer and that a hard copy must be carried in the transport truck.

Additional Considerations

- Remember that new transfers for Washington State will require application for changes to the Washington State future brood document.
- If fish need to be transported on short notice due to an emergency, state and tribal authorities must still be consulted. However, permitting agencies may allow the process to be streamlined with facility history and previous fish health exams used to determine whether a permit will be issued. Contact your fish health specialist for assistance.

Below are the State Contacts for Transfer Permits:

Washington Department of Fish and Wildlife (WDFW)
600 Capital Way North
Olympia, WA 98501-1091
Contact: Todd Kassler (360) 902-2722,
Todd.kassler@dfw.wa.gov
Contact: Joan Thomas (360) 902-2667,
Joan.thomas@dfw.wa.gov
Application for fish transport/ Import permit:
http://wdfw.wa.gov/licensing/fish_transport/transport_app.html

Oregon Department of Fish and Wildlife (ODFW)
4034 Fairview Industrial Drive SE
Salem, OR 97302
Contact: Guy Chilton (503) 947-6249,
Guy.s.chilton@state.or.us

Idaho Department of Fish and Game
Eagle Fish Health Laboratory
1800 Trout Road
Eagle, ID 83616
Contact: Doug Munson (208) 939-2413,
doug.munson@idfg.idaho.gov
Application for live fish transfer/ Import permit:
<https://idfg.idaho.gov/sites/default/files/live-fish-transport-import-permit-application.pdf>

DI, FI, Turnover, and Water Velocity, a Fish Health Perspective

Let's start out by defining some terms.

DI is the Density Index. This is the weight of fish per volume in the culture vessel. Small fish are more metabolically active than large fish so the DI is then corrected by dividing it by the length of the fish. This means that small fish raise the DI more than an identical weight of larger fish. In the Pacific Region we generally use English units so the final number is usually expressed in pounds of fish per cubic foot per inch of fish length. A smaller DI means that the fish are less crowded.

FI is the flow index. This is the weight of the fish per volume of inflowing water. As with the DI, small fish are more metabolically active than large fish so the FI is corrected by dividing it by the length of the fish. This means that small fish raise the FI more than an identical weight of larger fish. In the Pacific Region the final number is usually expressed in pounds of fish per gallon/minute of flow per inch of fish. A smaller FI means that the fish are getting more water.

Turnover time is a measure of how long it takes to completely replace the water in a culture vessel. For example, if it is a 100 gallon container and we are adding 10 gpm of freshwater, the turnover time would be 10 minutes. Of course, the turnover time number does not take mixing into account. In a long raceway with laminar flow, new water flowing in the head end forces old water out of the tail end and, in our example above, a ten minute turnover period would pretty much completely change out the water in the raceway. Things are different in a mixed system like a circular tank. In a perfectly-mixed system, half of the water going down the drain is old water and the other half is fresh water. Thus, that 10 minute turnover would actually only replace about half of the old water in a circular tank (the actual amount depends on the flow pattern in the tank). A short turnover time means that fish wastes like carbon dioxide and ammonia are more quickly removed.

Water velocity is a measure of the speed at which the water is moving through the culture vessel. We usually report it as feet/second. Fast velocities mean that fish waste solids are swept out of the system rather than settling on the raceway floor. In circular tanks, it takes water velocities of between 0.75 and 1 foot/second. One of the advantages of circular tanks is that you can have fast water velocities without huge inputs of water.

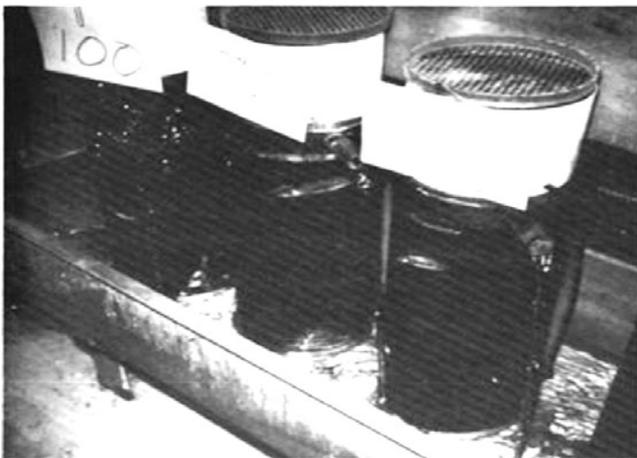
There are many different guidelines for appropriate, DI, FI, turnover, and velocity numbers, and voicing a strong opinion is a good way to start an argument at a hatchery management meeting. The PRFHC staff often sites the conservative guidelines set by Ray Brunson (below, all in English units), but studies done by Joe Banks at Carson, Willard, and Spring Creek NFHs, by Olson at Warm Springs NFH, by Ewing et al. at Willamette Hatchery, and by Clarke et al. at Umatilla Fish Hatchery, together demonstrate that relationships between DI and FI and survival are far more complex than this simple table indicates.

| Fish Species | DI | FI | Turnover | Velocity |
|------------------------------------|-------|-------|-------------|--------------|
| Spring Chinook – low BKD risk | <0.10 | <1.00 | <15 minutes | >0.10 ft/sec |
| Spring Chinook – moderate BKD risk | <0.06 | <0.60 | <15 minutes | >0.10 ft/sec |
| Fall Chinook | <0.15 | <1.00 | <15 minutes | >0.10 ft/sec |
| Coho | <0.20 | <1.00 | <15 minutes | >0.10 ft/sec |
| Steelhead | <0.25 | <1.00 | <15 minutes | >0.10 ft/sec |

The Brunson guidelines are based on Ray's long experience and conservative outlook, but they are not universally accepted. For example, few if any of our hatcheries manage a 15 minute turnover time and Ray's velocity number is only 1/10th of the water speed that is actually required to sweep waste to the end of the raceway. In addition, while it is clear that reducing the number of fish in a raceway reduces losses from infectious disease, it is hard to know whether that beneficial effect results from decreases in DI, FI, or both.

continued from page 3

Questions about DI and FI often arise when water or space availability are impacted by weather, mechanical problems, or unanticipated production needs. At times like that we often end up in fish health discussions about which is the most important, DI or FI. To put it in the most simple terms, can we put more fish in the raceway (increasing the DI) if we compensate by increasing the flow to keep the FI at acceptable levels? Two years ago the PRFHP conducted an extensive review of the science and concluded that the best evidence was that the FI (the amount of freshwater available per fish) had a much stronger influence on survival and returns than did the DI. Most fish don't seem to mind being crowded as long as they have plenty of fresh water to bring in oxygen and flush out waste. There are probably some exceptions related to fish behavior where crowding does lead to behavioral problems (like fin nipping in steelhead), and it is possible that increased fish to fish contact might increase disease transmission, but in general fish seem to tolerate some crowding if the water quality is good. In support of this argument, hatchery managers have pointed out that fish often congregate at the head end of raceways and thus voluntarily live at a DI that is several times higher than the DI that they would experience if they spread out evenly through the raceway.



A picture from a [1983 Progressive Fish Culturist paper](#) by Poston documenting that rainbow trout grew just as well at very high densities (DI of 3.1) as long as there was adequate flow (FI of 1).

FI may be the most important parameter, but there are some complexities to basing culture conditions on FI. In a multiple pass system, do we count third-use water in the same way that we count first-use fresh water? It has higher levels of waste products, usually a lower oxygen level, and it may have higher concentrations of fish diseases organisms, so it does not provide the benefits per gallon that truly fresh water does. Similarly, in circular reuse systems, is the FI calculated based on the flow of fresh water or on the total flow of fresh water and reused water combined? What probably matters to the fish are appropriate carbon dioxide, ammonia, and oxygen levels along with low amounts of particulate matter to prevent gill diseases and a good dilution of fish pathogens in the water. If the flow is sufficient to keep oxygen high, carbon dioxide and ammonia low, particulates and pathogens at acceptable levels, and crowding doesn't lead to problems like fin nipping, the fish will probably do fine.



A circular tank water re-use system at Hagerman NFH. How do we calculate FI in re-use systems?

Let's conclude with some take home messages:

DI and FI are useful guidelines, but they must be used cautiously when water is re-used or incoming water quality is low.

FI is usually more important than DI unless there are behavioral complications.

Disease losses are usually reduced if numbers of fish in the raceway are reduced, but we aren't sure if the benefits result from lower DI, FI, or both.

continued from page 4

If velocity is too low, cleaning effort and gill disease are both increased. If velocity is too high (greater than 2 body lengths per second), fish are stressed.

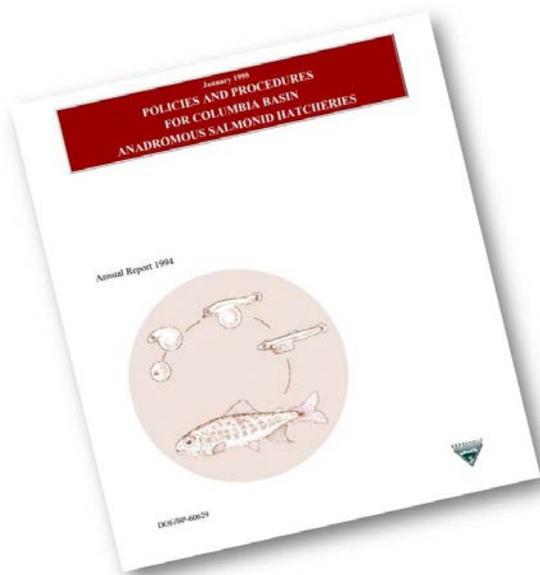
Turnover may be important in Ich management. Maintaining flow while decreasing raceway depth increases the turnover rate and velocity while increasing the DI. There is evidence that the faster turnover may rinse free-swimming Ich out of the raceway and reduce fish exposure to the parasite.

DI, FI, turnover, and velocity are not independent. Changing any one parameter changes the others.

Most importantly, DI and FI vary not just with fish species and health status, but also with water chemistry, fish health, water temperatures, feeding strategies, and other parameters. Using survival and adult returns as the benchmark, each hatchery must work out the best culture density and water flows for its own facilities and programs.

Who Makes the Fish Health Rules?

Fish culture and fish movements in the Pacific Northwest must be done according to fish health policies and regulations designed to prevent the introduction or spread of new fish diseases, but who makes the rules that we must follow?



IHOT 1995

- There is FWS policy (713, under revision) that describes required inspections for fish raised on the National Fish Hatcheries.
- There are the Integrated Hatchery Operations Team documents (1995) in which the Service agreed to follow certain fish health rules.
- There are individual state laws that govern the fish health side of fish movements into or within specific states.
- There is the Washington Co-Managers Policy where the service has promised Washington State and the Northwest Indian Fisheries Commission that we will take certain measures to monitor and control diseases during production and transfer.
- There are a few Federal Rules that restrict movements of fish that might carry a few high-priority diseases (viruses like the Great Lakes VHS-IVb virus, ISAV, and SVSV).
- In addition to inspections and testing, our drug use is regulated by the FDA, and the release of water that might contain drugs is regulated by the EPA and by state environmental agencies.
- The work done by our veterinarians is constrained by the FDA and by state licensing boards that set the conditions under which veterinarians work and also set the rules for veterinarian's use of fish drugs.

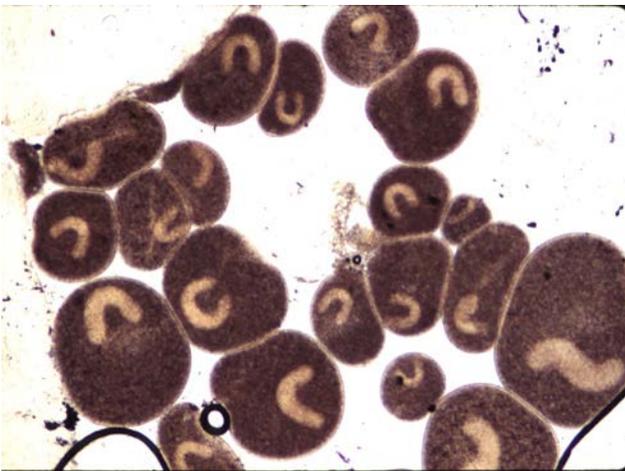
When the PRFHP is planning fish health inspections for our FWS and partner hatcheries, we start out by looking at the hatchery's operations and its production programs and then match those with the relevant policy and regulatory requirements. Sorting that out can be quite a puzzle as the policies and regulations are sometimes vague, or confusing, or they often don't address exactly what it is that we are trying to do. Frequently we must work with hatchery managers to call state regulators to negotiate requirements for specific transfers.

continued from page 5

Once we have worked out what it will take to meet all of the policy and regulatory fish health requirements, we take a step back and ask “What do we need to know about the health status of these fish so that we can protect both our cultured fish and the wild fish that live in the same rivers and streams?” Then we add to that whatever specific testing may have been requested by our hatchery clients or by a funding agency. The final result is a hatchery testing plan designed to meet all of the regulatory, policy, biological, and client needs associated with the hatchery’s production plans. It’s a complex process, especially when emergencies cause unanticipated fish transfers.

“Why is Ich so Hard to Treat?”

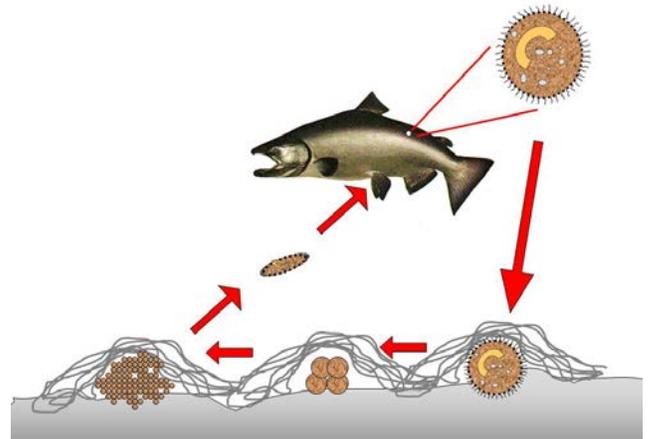
Ich is a very important parasite not just in salmon hatcheries, but in everything from tropical fish to catfish farming. It seems like it should be easy to kill Ich with a formalin bath, but instead it can take weeks to get the parasite under control. To understand why Ich is so hard to treat, we first need to look at the life cycle of Ich.



Mature Ich cells from the skin of a fish. The light colored crescents in the Ich cells are a special protozoan cell structure called a “macronucleus” that houses extra copies of the cell’s DNA.

Ich are single-celled protozoa with simple life cycles that use only fish as their hosts. In the fish-associated stage of their life cycle, the Ich burrow under the skin of the fish where they feed on fish tissues.

When they have grown to sufficient size (single cells that are so big they can be easily seen with the naked eye), they dig their way back out of the fish and drift through the water until they contact and stick on a hard surface. Once stuck, they coat themselves in a protein “cocoon” and begin to divide. The process continues until the one enormous Ich cell has divided into a thousand or more small Ich cells. These are released into the water where they seek out a new fish host so that they can burrow under the skin, grow, and start the life cycle all over again.

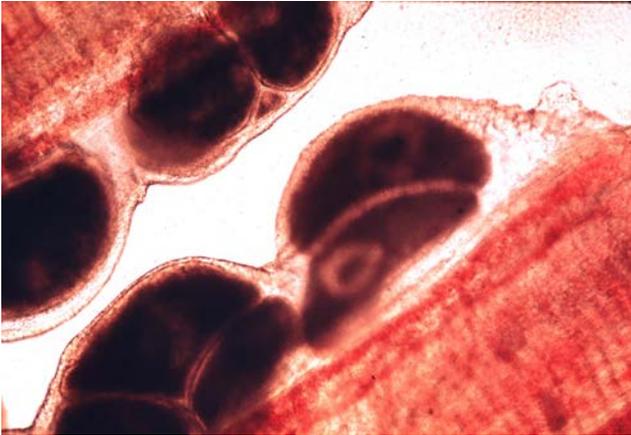


The life cycle of Ich showing a developing Ich cell under the salmon’s skin and the free living stages that are vulnerable to formalin treatments.

So what makes the Ich so hard to kill? The secret to Ich’s success is that they are not on the surface of the fish, but instead are underneath the transparent top layer of the skin or the cells covering the gills. We can’t effectively treat them with formalin while they are in the fish because any treatment potent enough to get to the Ich would also kill the critical outer cell layers of the fish. That cell layer includes cells needed for water and salt regulation, mucus production, immunity, and a host of other functions. Since we can’t get at the Ich under the fish’s skin, the treatment has to be in the water at an effective dose during the brief periods when the parasite is coming and going from its fish host.

continued from page 6

If there is any kind of prolonged lapse in the treatment, Ich will make it through the free-living stage and once again be safely under the skin of the fish to start the infection cycle all over again.



Ich in a fish gill. You can see that the Ich are actually underneath the layers of fish cells that cover the surface of the gills.

The length of the Ich life cycle also has a big effect on treatment strategies. At high temperatures the Ich life cycle may only take a day or two so treatments need to be close together. At cooler temperatures the life cycle might stretch out into many days or even weeks and treatments may be several days apart. The good news about cooler temperatures is that the slower life cycle means that they don't kill fish as quickly, but the dark side is that treatments may have to continue for weeks to catch all of the parasites during their vulnerable stage. If there is any lapse in treatment, some of the Ich will survive the free-living state and successfully start the infection cycle over again.

The reality is that it is almost impossible to kill each and every Ich as it cycles. The way that Ich treatments succeed is by keeping the Ich numbers at an acceptable level until the fish are able to mount an immune response that protects them from the parasite. For Pacific salmon, a strong immune response requires cool temperatures, good nutrition, clean water, and a peaceful environment.

Changing Rules Governing Antibiotic Use in Fish

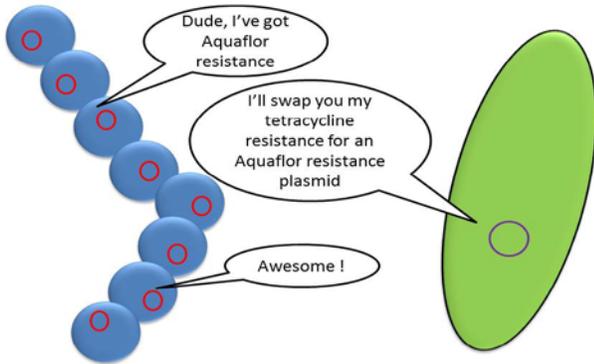
Public health agencies are very concerned about the development of antibiotic resistance in bacteria. Antibiotic resistant bacterial infections kill tens of thousands of Americans every year and we are now seeing bacteria that can resist everything in our antibiotic arsenal. New antibiotics take years and millions of dollars to develop, but the financial rewards for pharmaceutical companies to develop new antibiotics are trivial compared to what they might earn on a successful drug that treats a common chronic disease like high blood pressure or psoriasis. With antibiotic resistance spreading rapidly and very few new antibiotics in the pipeline, it is imperative (for both fish and people) that we don't do anything to make the problem worse.

The cause of antibiotic resistance is antibiotic use. Most antibiotics are derived from organisms like fungi that produce antibiotics to enable them to out-compete bacteria in their quest for food. This means that the war between the bacteria and fungi has been going on for millennia and that somewhere out there is probably a bacterial strain that has evolved antibiotic resistance as a defense against the fungi's antibiotic chemical weapons. When we take the fungi's weapon (penicillin for example) and use it against strep throat bacteria it is initially highly effective because these strep bacteria have probably never seen the fungus or its antibiotic. The antibiotic works great against the strep until the bacteria are able to develop an effective defense. This is how resistance often develops:

- Somewhere in nature is a bacterial species that has been fighting the fungi's chemical weapon (the penicillin) for millions of years and carries a gene for antibiotic resistance.
- Some day that bacterium and the strep throat bacteria cross paths and trade some DNA (bacteria carry these useful genes in little loops of DNA that they trade back and forth between bacterial species).

continued from page 7

- A lucky strep throat bacterium picks up that penicillin resistance gene.
- When that lucky strep bacterium is exposed to penicillin, it survives and proliferates while all of the other sensitive bacteria die.
- Pretty soon all of the strep bacteria present are penicillin resistant and penicillin is no longer effective for strep throat.



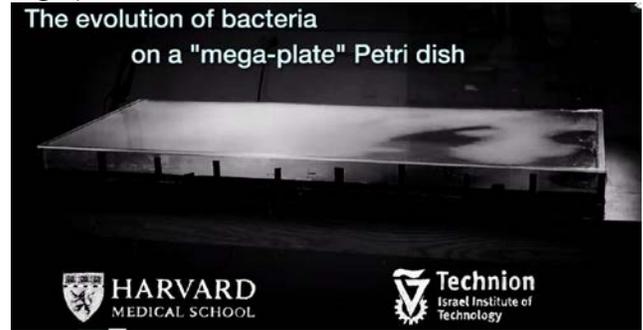
Two bacteria exchanging loops of DNA (plasmids) that carry genes for antibiotic resistance.

The speed at which resistance occurs and is spread is directly related to how often and how widely the antibiotic is used. If we treat fish chronically with an antibiotic that is important in human health, the bacteria on the fish will probably develop resistance and may find an opportunity to hand off that resistance to a bacterium that causes an important disease in humans. Thus, antibiotic use in fish, and the development of resistance, put not only fish health at risk, but also has the potential to increase the likelihood of antibiotic resistant infections in humans. To reduce the likelihood of this happening, the FDA has been making big changes to regulations affecting drug use in fish and in other animals.

- There is no longer any over the counter antibiotic use in fish. All antibiotic use must be prescribed and overseen by a licensed veterinarian.
- Veterinarians are under a lot of pressure from the FDA to be very conservative about antibiotic use and to not use them preventatively, over long periods, frequently, or when there is any other option to protect the health of the fish.

- The training and continuing education that veterinarians receive emphasizes that the indiscriminant use of antibiotics is unethical.

One of the most fascinating demonstrations of the development of antibiotic resistance is shown in a video. In this example, scientists made a giant bacterial culture "mega-plate" (Jello-like nutrient agar).



This is a still photo from the video. It shows the "mega-plate" used to investigate the evolution of antibiotic resistance.

That plate has no antibiotic at the right and left ends, but increasing antibiotic concentrations toward the center. The scientists inoculate the ends of the plate with antibiotic sensitive bacteria. As the bacteria grow and spread, mutations occur that make the bacteria increasingly resistant to the antibiotic until there are eventually strains that can grow at the highest antibiotic concentrations on the plate. In this case the resistance results not from trading genes, but from DNA mutations that change the operation of resistance mechanisms that the bacteria already have on board. Check out the amazing video! ([Click here](#))

The bottom line for fish culture is that we cannot sustain production programs that depend on the routine use of antibiotics. We must think of antibiotics only as an emergency tool of last resort.

New Science

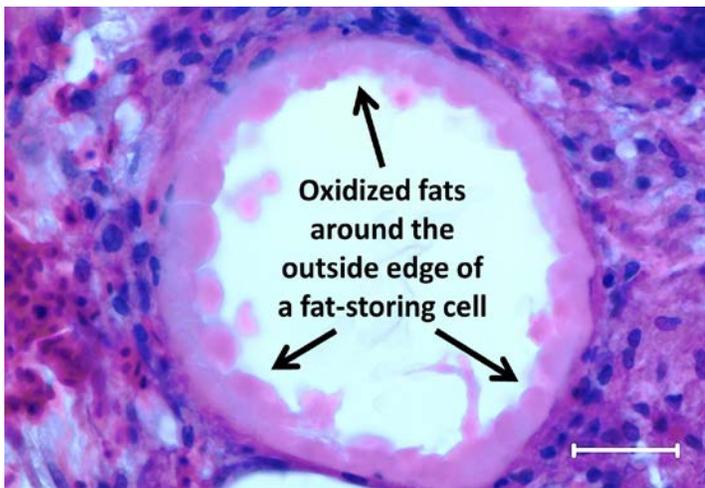
Steatitis (inflammation in fat) that we see in some steelhead populations is causes slower growth and skin lesions.

continued from page 8

A new study (led by our own AFTC!) has now shown that this disease is associated with a combination of high dietary fish oil with UV light exposure. On-line in *Aquaculture*, June 9, 2017.



Steatitis in steelhead. The inflammation is causing darkening of the skin around the dorsal fin.



Histology (a thin slice of fish tissue stained and examined under a microscope) showing a ring of oxidized fat within a fat storing cell in a fish.

Adult Spring Chinook mortality in the Willamette River between 2011 and 2015 is most closely related to minor physical injuries like descaling and scrapes. The authors hypothesize that the minor injuries lead to secondary infections that reduce survival to the spawning grounds. *North American Journal of Fisheries Management*, Volume 37, 2017 - Issue 2

PRFHP Updates

The PRFHP has developed a detailed plan for National Wild Fish Health Survey activities in 2017. This plan emphasizes the study of fish health problems effecting fish living in the wild and strives to provide information of importance to our Region's fisheries managers. Details will be presented at the Project Leader meeting this September.

The original plan for reorganization of the PRFHP called for centralizing our lab testing function in a new accredited laboratory in Olympia while leaving our veterinarians and other fish health professionals at duty stations near the hatcheries that they serve. With new administration guidelines that emphasize hiring restrictions, layering, consolidation, and fiscal restraint, the Pacific Region is now planning to contract the laboratory testing portion of its work to an experienced and accredited third party testing provider. This solution will provide accurate, timely, and fully accredited lab results to support the primary mission of the PRFHP – the prevention, management, and treatment of diseases on Federal and partner hatcheries. This only affects the testing function of the program. Hatcheries will continue to be served by the same great team of veterinarians and fish health professionals. Contact Andy Goodwin, PRFHP Manager, at andrew_goodwin@fws.gov or 503-231-6784 with any questions.

Fish Health Factoids

In the last issue of Fish Health News, we presented numbers that showed that the earth is populated by an amazing number of viruses. Now let's consider bacteria.

continued from page 9

Typical bacteria are about 1 micron (1/1000 of a mm) in size, but they vary from virus-sized mycoplasmas to a free-living marine bacterium large enough to be seen with the naked eye (*Thiomargarita namibiensis*).



The giant bacterium *Thiomargarita namibiensis* featured on a postage stamp from Namibia.

There are enough viruses on earth to make a chain 200 million light years long, but there are enough bacteria to stretch 10 billion light years – more than 50 times as far, and the chain would be about 50 times wider.

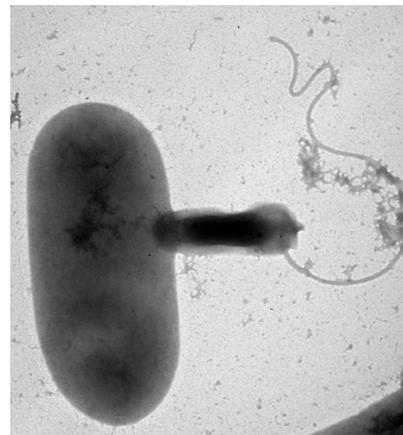
Many bacteria can complete their life cycle (growth and division) in as little as 10 minutes. This means that a single bacterial cell can turn into a billion bacteria in as little as 5 hours.

There are usually several thousand bacteria in a teaspoon of pond water and up to a million in a teaspoon of sediment.

There are about 100 recognized bacterial diseases of fish and several thousand named bacterial species overall, however, a recent DNA study produced solid evidence of at least 1,000,000 bacterial species living in seawater alone.

In human beings, there are 10 times more bacterial cells than human cells, and the navel and appendix may be critical refuges to maintain populations of the bacteria that support our lives by protecting our skin, aiding digestions, and producing critical vitamins.

There are bacteria that are predators that prey on other bacteria, but we haven't found any way to put them to work treating bacterial infections.



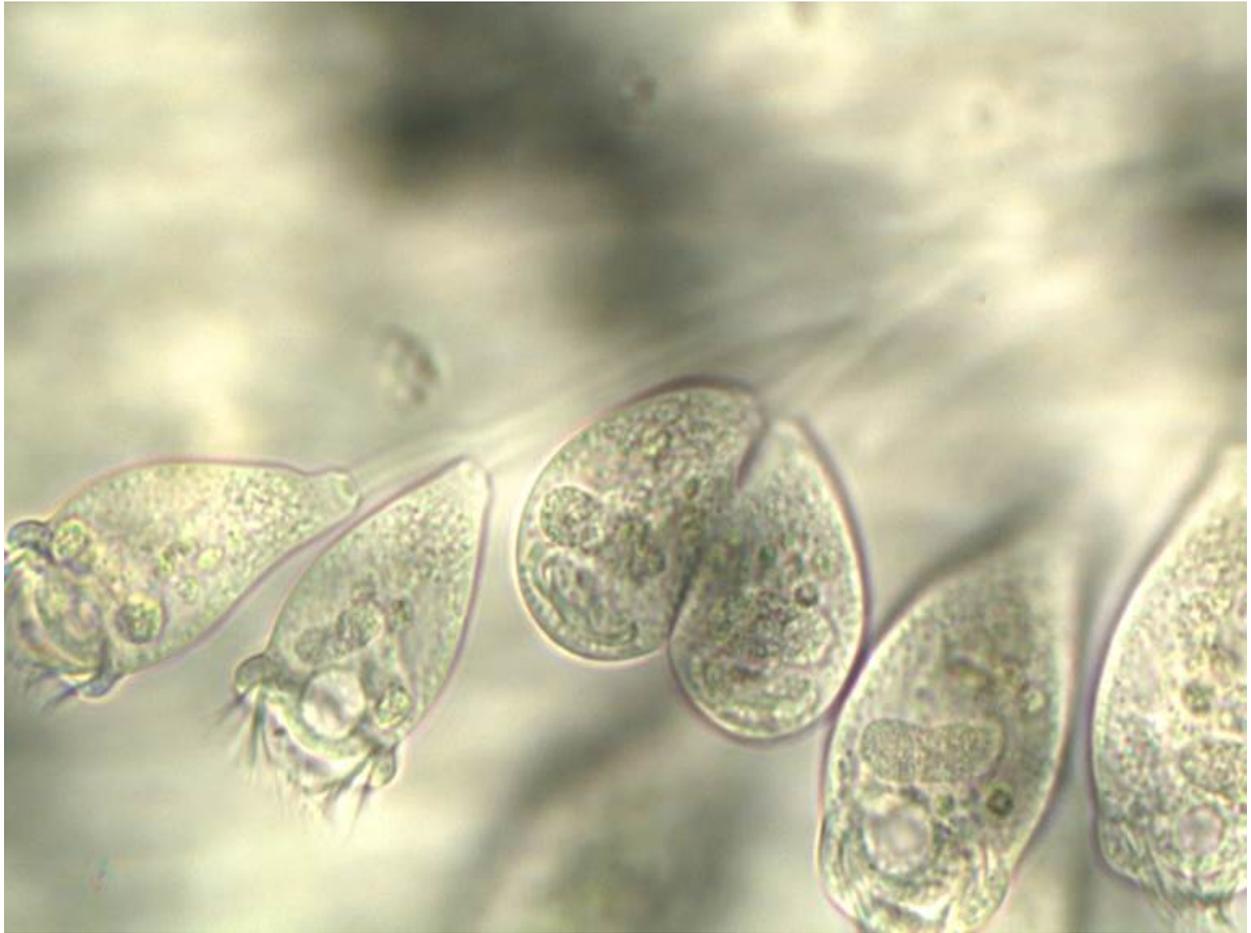
A Transmission Electron Microscopy (TEM) image of a *Bdellovibrio* bacteriovorus bacterial cell attacking a *Shewanella* bacterial cell. Image credit: Mark O. Martin, University of Puget Sound.

In our fish health labs, we have traditionally identified bacteria by what they eat and what they excrete. These tests take days or weeks to perform. New methods use DNA sequencing or mass spectrometry to identify bacterial species in just a few hours.



A "MALDI TOF" machine at the Washington State University Animal Disease Diagnostic Laboratory (WADDL), that can identify bacteria in hours instead of in days or weeks.

Mystery Parasite of the Day



[Answer](#)