Fish Health News You Can Use

Brought to you by the Pacific Region Fish Health Program

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This Issue

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In the Next Stressful Issue

- Stress, what is it?
- Stress, what does it do?
- Stress, is it bad?
- Managing and preventing stress

Introduction

In an earlier edition of Fish Health News, we shared some fascinating virus factoids.

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

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

In this issue we are going beyond these old factoids (and adding some new factoids) to look at how viruses work, and what they mean for salmon in the Pacific NW.

What is a Virus?

Viruses exist somewhere in a gray zone between biology and chemistry. They are either the simplest living things or the most complex chemical reactions. In reality, they function more like small machines made of protein molecules.

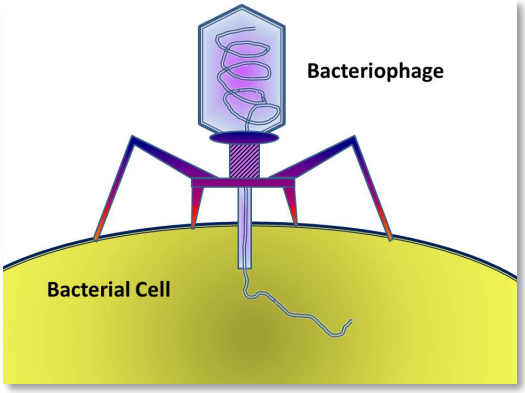

Figure 1: Bacteriophages infect bacteria. They look like lunar landers that settle on the surface of bacteria and inject their DNA.

The fundamental difference between viruses and other living things is that viruses lack many of the systems that we associate with life. On their own, they are unable to feed, reproduce, make or use energy, secrete or absorb anything from their environment, or even move. They exist by infecting living cells, stealing control of cell functions, and then forcing the infected cell to make more viruses.

Within the viruses, there is tremendous diversity is shape, size, genetic components, and life cycles. Let’s look at a few important virus characteristics.

Virus Structure

Genetic material (DNA and RNA): Some viruses have double stranded DNA that, just like in plants and animals, must be translated by cell machinery into RNA that then serves as the template for building proteins.

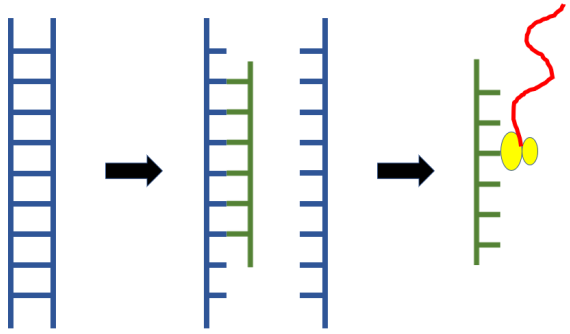

Figure 2: The usual system for making proteins from DNA. Blue DNA molecules separate into mirror images. One side is copied into an RNA molecule (green), that is translated into a protein (red) by a ribosome (yellow).

Other viruses have no DNA and only carry only RNA. To make matters more complicated, there are viruses that have single instead of double stranded DNA or even double stranded RNA (not something that you would find in a normal cell). Of those that are single stranded RNA or DNA, some are ready to be directly translated into proteins and others must first be copied and then translated. That’s just the tip of the iceberg.

Genes: Some viruses are able to infect cells, evade the host immune system, and direct the production of copies of themselves with as few as 5 genes. Rabies, and fish disease viruses like IHNV and VHSV, work with just 6 genes. Other viruses have far more genes. The koi herpes virus has more than 150 genes. The “megaviruses” may have up to 1000 genes.

Structure: A really important characteristic of viruses is the type of outer structure that encloses and protects their genetic material. Viruses have a tough protein shell (a capsid) that is strong enough to protect their genetic material from most things that the virus will encounter in the environment or in their hosts.

Shape: Virus capsids come in many shapes including icosahedrons, bullets, rods, spheres, and even something that looks like a lunar lander (*Figure 1*).

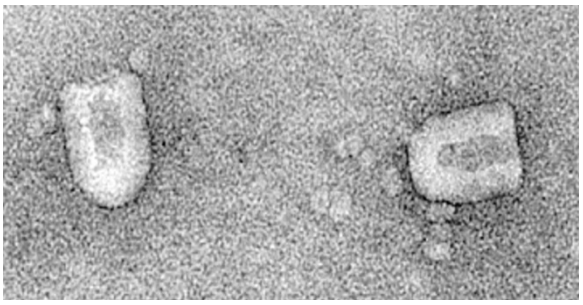

Figure 3: Spring viremia of carp virus under an electron microscope. These bullet-shaped viruses are close relatives of VHSV and IHNV (salmon viruses) and rabies.

Size: The megaviruses are up to 1 µm in diameter (the size of small bacteria). The Porcine circovirus is only 17 nm in diameter (it would take 18 million of them to span a foot).

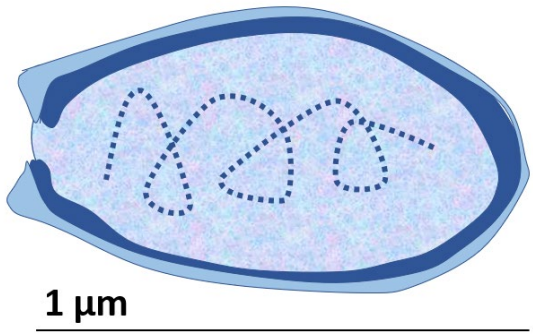

Figure 4: Pandoravirus is a member of the Megaviruses and infects amoeba. It is as big as typical bacteria. Its DNA molecule is coiled and ready to deploy through a pore in its capsid.

Envelopes: Some viruses have the shell capsid enclosed in a membrane (an "envelope") stolen from the cells that they infect. The membrane has many advantages, but it also makes the virus much more sensitive to drying out and to disinfectants. With "enveloped" viruses, exposure to a mild detergent or fat solvent (like alcohol), or just hours or days somewhere dry, is enough to inactivate the virus.

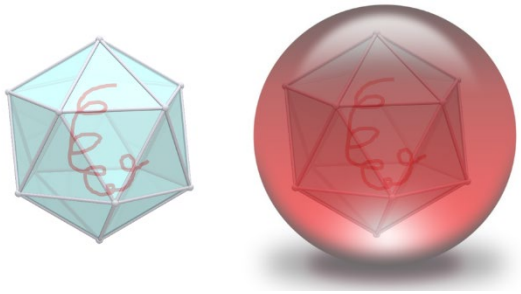

Figure 5: A "naked" virus (left) and a virus with a lipid envelope (right).

Host specificity: Some viruses are so finely tuned that they are able to complete their reproductive cycle only in a single cell type in one host species. Other viruses are able to invade cells, avoid immune defenses, take over cell machinery, and reproduce in hosts so diverse that a single virus species may be able to infect both vertebrates and invertebrates.

Infectious dose: Some viruses are so efficient that it may take as few as a single virus to start a successful infection. Other viruses must infect individual cells simultaneously in large groups in order to overwhelm host defenses.

Virulence and Transmission: Some viruses cause horrible diseases and may quickly kill the host. Other viruses replicate at low levels and are shed by the host without any sign of disease. Viruses that rapidly kill their host are usually very contagious to ensure that a new host is found before the old host dies. Viruses that don't cause obvious damage to their hosts can take their time even if they successfully spread to new hosts only on very rare occasions.

Latency: Some types of viruses infect a host cell and immediately begin the replication process. Other viruses may enter the cell and lie low for

days or even years until conditions are right for a successful infection. They have all kinds of mechanisms for evading detection. Some viruses even nick the host DNA and insert their own DNA right into the host DNA strand. This has the unfortunate side effect of causing cancer in some animals, especially dogs, and in humans (human papilloma virus).

Lysis: Some viruses replicate and accumulate in the host cell until their numbers get so high that the cell explodes and the viruses are released. Other viruses don't kill their host cell and are shed continuously from infected host cells over long periods of time.

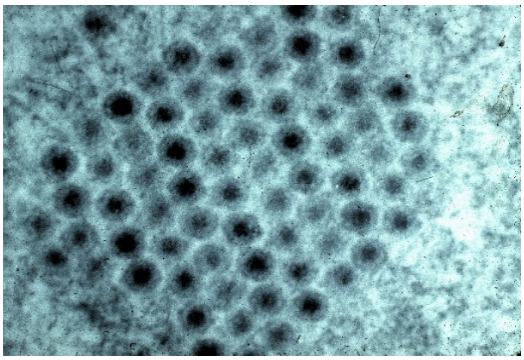

Figure 6: Virus accumulation in an infected fish cell. The are densely packed is a crystalline array and will all be released when the cell ruptures.

Take a look at how these virus characteristics affect disease in the real world.

Virus Structure and Disease

Lipid Envelopes: On cruise ships one of the biggest disease problems are infections caused by the noroviruses. Noroviruses have a tough protein capsid and no lipid envelope. This means that detergents and alcohol hand sanitizers are not very effective and that the virus can survive a trip through the digestive system. The best defense is vigorous scrubbing and hand washing to physically remove the viruses from hands and other surfaces. We are lucky that the COVID virus is a fragile virus with a lipid envelope, but this virus seems to make up for its fragility by being extremely contagious so that it can get to a new host before it is inactivated. In fish, the IHNV and VHS viruses are enveloped and easy to kill with disinfectants. The IPN virus is not enveloped and is much harder to kill (fortunately IPNV is not currently a problem in the NW).

Latency: Before vaccines were developed, almost all children were infected by chicken pox. The herpes virus that causes chicken pox has a very clever trick. When the host immune response gets too strong, the virus hides its DNA in nerve cells. Nerve cells are long lived and are not killed by the immune system so they are a safe place for the virus to hang out. Decades later a human carrying the chicken pox virus may experience some life event that impairs their immune system. The virus senses that, leaves the nerve cell nucleus, and slides down the nerve cell axon to skin wired to that nerve. The virus then emerges from the nerve and infects skin producing extremely painful and persistent "shingles" disease. In the fish world, the koi herpesvirus will infect koi or common carp and then remain dormant for months or years until conditions (temperature and stress) are right for it to replicate. There is also evidence that fish that survive KHV disease may shed the virus years later when the fish is stressed.

Figure 7: Gill lesions caused by the koi herpesvirus.

Transmission and virulence: One of the best examples is from human medicine. The Ebola virus rapidly kills its host so in order to persist it must be very efficient at infecting new hosts before its current host dies. The HIV virus take many years to kill its host and it is very inefficient at moving between humans. The HIV infection isn't spread easily, but while Ebola had only days to find a new host, HIV has years to get the job done.

In fish, an IHNV strain in wild populations (where fish are spread out) needs to keep its host alive for long enough for the virus to find another host. A different strain of IHNV that kills quickly may be unsuccessful in the wild where transmission to a new host is difficult. However, when fish are close together on hatcheries and fish farms, it is easy for the IHNV virus to move from fish to fish so producing rapid mortality may not be a problem for the virus. This is another reason why natural resource agencies often respond aggressively to outbreaks of IHN disease in salmonids living on hatcheries.

Another interesting aspect of fish virus transmission is whether the virus is vertically transmitted (from adult to progeny) or horizontally transmitted (fish to fish). The simplest kind of vertical transmission happens when virus is stuck to the outside of eggs from an infected broodfish. The virus just patiently waits on the egg's shell and infects fish when they hatch. The good news is that this kind of transmission is easy to block by bathing the eggs in iodine to inactivate the virus. That is why iodine baths of trout and salmon eggs is required by FWS policy. Much more difficult are viruses that travel inside eggs where they can't be reached by iodine treatments. Fortunately, IHNV and VHSV are on the outside of eggs and iodine is very effective if done carefully and good hatchery biosecurity is in place.

Figure 8: Coho salmon eggs being treated with iodine to kill viruses and bacteria on egg surfaces.

Some viruses cause cancer: You may be familiar with the human papilloma virus and the relationship between long standing infections and cervical cancer, but this is just one of many examples of tumor viruses in fish, reptiles, and mammals.

Figure 9: A Masu salmon in Japan with a jaw tumor caused by the herpesvirus OMV.

So how does a virus cause cancer? They do it two different ways. In an organ with a virus infection, cells are constantly being killed and replaced. This constant turnover provides many more opportunities for mistakes as DNA is copied. These mistakes (mutations) can sometimes lead to uncontrolled cell division (cancer). The other mechanism is a bit more complex. As we mentioned above, viruses sometimes snip the infected cell's DNA and

insert their own DNA. The virus' DNA contains codes designed to trigger fast copying of the viral DNA. In some cases, the virus DNA sequence instead triggers rapid copying of one of the cell's genes. If it is a gene involved in regulating cell replication this can lead to cancer. In a completely opposite way, when the virus snips the host DNA, it might separate a gene from the DNA that regulates it, another mutation that can lead to cancer. On fish, the OMV virus (one of those 5 gene rabies-like viruses) causes jaw tumors in salmon in Japan.

Some viruses trigger things that just look like cancer: One strategy used by viruses is to trigger replication of the type of cells that they need to copy themselves. This proliferation can result in growths of cells and accompanying inflammation that look like tumors. Human warts and carp pox are excellent examples.

Figure 10: Tumor like warts on the skin of a koi with Carp Pox (actually a herpesvirus).

An even more spectacular example is provided by the lymphocystis virus in fish. Not only does the virus cause cells to multiply, but the viral particles that accumulate in those cells blow the infected cells up to such large sizes that they can be seen by the naked eye. This produces spectacular knobby growths that look cancers. They are not cancer because when the infection is over the growths disappear.

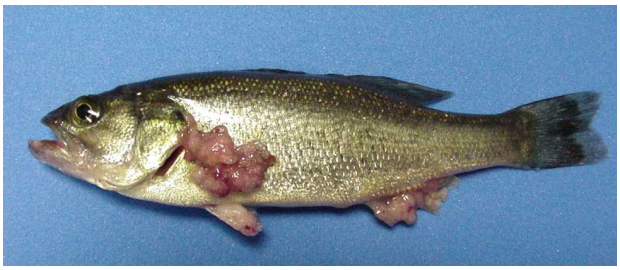

Figure 11: A lymphocystis infection producing tumor-like growths on the fins of a wild largemouth bass.

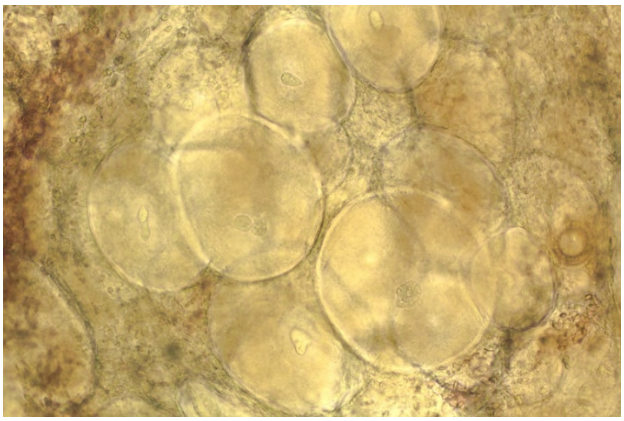

Figure12: A view through the microscope of the bass fin lesion from the previous figure. Cells are swollen to enormous sizes by an accumulation of virus particles.

Virus strains, types, and species

Virus species names are assigned by the International Committee on Virus Taxonomy (ICTV). Higher level taxonomic groups are based primarily on their DNA or RNA type and how the virus replicates. These higher-level groups are broken down into sub-groups that are based on other characteristics including structural differences and DNA or RNA sequence similarities, and then further by host range and associated diseases. Virus species names are currently being revised as free-form binomials, so they will all be two words: the first word is the genus, and the second word is a species-specific term. This will not change the name of the viruses themselves, which is the terms we

all recognize (IPNV, VHSV, etc.), just the species they belong to. For example, the virus infectious hematopoietic necrosis virus (IHNV) is still IHNV, but its taxonomic species is *Novirhabdovirus salmonid*. This is like the difference between "human" and *Homo sapiens*. For many fish viruses the binomial species names trend to simple systems like the *Aquareovirus* genus where the viruses are named *Aquareovirus A* and *Aquareovirus B* and so on. As of today, viral taxonomy includes 4 realms, 9 kingdoms, 16 phyla, 2 subphyla, 36 classes, 55 orders, 8 suborders, 168 families, 103 subfamilies, 1421 genera, 68 subgenera, and 6590 species.

Taxonomy in viruses is more complex than in animals and plants. Traditional definitions of a species as "a group of organisms that interbreed and produce fertile offspring" don't work in viruses where reproduction is achieved by each virus making copies of itself. In addition, the self-replication process in viruses is somewhat messy so mutations are common. Most of these mutations are just back-ground noise within a species. However, sometimes a mutation will give rise to a lineage of viruses that is more successful and thus becomes a significant sub-group that has a behavior that sets it apart from other viruses within the species. Important differences may include everything from suitable hosts, severity of disease, transmission, and interaction with host immune systems. As these lineages of viruses evolve and diverge more and more, there comes a time when they are different enough to reclassify into two species. Unfortunately, there are no good rules for determining when new names should be assigned, so that is the task of study groups in the ICTV.

To deal with that naming problem and recognize the diversity among virus lineages, we often break virus species down into strains, types, clades, genotypes, or other sub-groups that are useful for understanding how the virus behaves. The members of these smaller groups within a virus species can be identified by clustering of their DNA or RNA sequences in phylogenetic trees, but the subgrouping is often correlated with noticeable differences in geographic range, host range, or virulence. The pathogen IHNV in salmon (species *Novirhabdovirus salmonid*) is an excellent example. IHNV has been divided into "upper, middle, and lower" subgroups referred to as genogroups U, M, and L, that differ in their dominant geographic region, and further by variations in host range and disease severity.

So, are these different sub-groups of viruses within a species correctly named as clades, genogroups, strains, or types? The definitions of these terms overlap and there is little consistency in their usage. Basically, subgroups within each species usually follow the terminology used by whoever first publishes a description of the subgroups. This is why we say VHSV exists as 4 genotypes (I-IV), but IHNV exists as 5 genogroups (U, M, L, E J). As long as you follow the accepted terminology within the literature on a species it will make sense!

(Many thanks to Dr. Gael Kurath at the Western Fisheries Research Center in Seattle for editing a draft of this section, to Bill Gale who asked for an explanation of this confusing topic, and to any Fish Health News readers that stuck with us through an explanation of virus taxonomy!)

Figure 13: A common carp with red spots and bulging eyes from an infection by "Genogroup 1" of the Spring Viremia of Carp Virus.

Viruses of Salmon in the NW

IHNV

The virus that impacts fish health the most in our Region if Infectious Hematopoietic Necrosis Virus (IHNV). It is a bullet-shaped RNA virus with an envelope and only 6 genes. It is a relative of rabies and of VHSV (coming up next). In the Pacific NW it has evolved into several genogroups including the upper, middle, and lower genogroups that are named based on their predominant north/south geographic regions. Within these three genogroups are many different strains that all emerge, decline, and kill fish depending on the evolutionary pressures in the system. The steelhead, rainbow trout, and sockeye are the most sensitive to IHN disease. Problems in Chinook are uncommon in the Columbia, but they do occur in California where the "lower" IHNV strains are found. New strains are emerging that seem to pose more or a threat to Chinook in the Columbia. Infections have also occurred in pink and Chum salmon, cutthroat trout, and brook and brown trout. Coho don't seem to be harmed by IHNV.

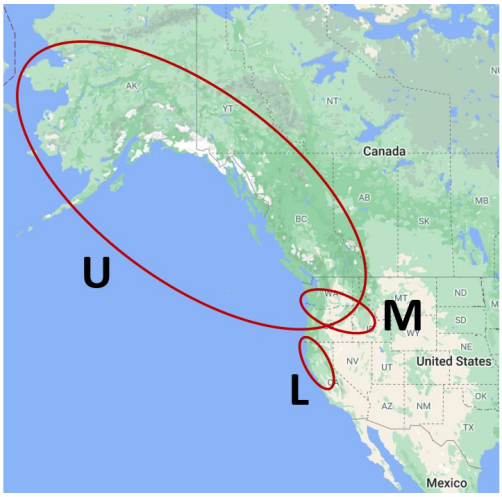

Figure 14: The geographic ranges of the U, M, and L genogroups of IHNV.

IHN disease occurs mostly in small fish, in freshwater, and at temps below 15 C (60 F). It is transmitted through eggs, but only on egg surfaces so iodine treatments are very effective in preventing transmission from young fish to adults. When conditions are right (including specific strains of IHNV) mortality from IHNV in hatchery settings can be catastrophic.

The IHN virus is very widespread in the NW and it is commonly detected in adults returning to spawn. It is managed by disinfecting eggs and the holding susceptible fish in virus free water until they are old enough to tolerate IHNV infections. Vaccines are used available in other countries and are widely used in net pen salmon culture. IHNV is heavily regulated in both interstate and international commerce.

The most interesting IHNV situation in our Region is at the Dworshak National Fish Hatchery, Dworshak raises steelhead. They carefully disinfect eggs and then start out the production cycle using water from above Dworshak Dam where there is no fish passage and the system appears to be IHNV free. Later, when the fish are older and more IHN disease resistant, and when infected steelhead adults are no longer common above the hatcheries Clearwater River intake, they are moved to the IHNV positive river water with very few problems. Experience has shown that any premature exposure of steelhead to the Clearwater River water leads to major IHN disease losses.

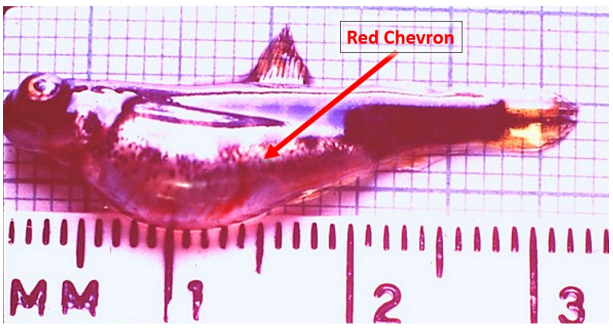

Figure 15: A rainbow trout parr with IHN disease. The red chevron shape is a hemorrhaged group of muscle cells in a single "flake" (a myomere) of trout muscle. This is one of those "Classic disease signs" that is rarely seen.

VHSV

The VHS virus is a close relative IHNV. Like IHNV, the VHS virus exists in several genotypes with many different strains in each genotype. One VHS group causes huge losses in trout culture in Europe. Another strain emerged in the Great Lakes where for several years it caused major fish kills, especially in freshwater drum, muskies, and exotic gobies. Other strains are found in a broad range of marine fish. Fortunately, the strain that is found here in the Pacific NW has not been seen to produce disease in trout and Pacific salmon. It is a rare finding in healthy-looking adult salmon returning from the ocean, but major epidemics do occur in wild populations of Pacific herring.

Figure 16: Wild sardines that succumbed to VHS disease. Port Hardy, BC, Canada. Picture by Garth Traxler.

Because of the impact of some strains of VHSV, it is a highly regulated virus. Here in the Pacific NW, our strain is widely distributed in wild fish populations, but there is great concern about what would happen if exotic VHSV strains were imported into this region.

Figure 17: Dead wild gizzard shad, VHSV IVB, Dunkirk Harbor, NY in 2007 (Lake Erie). *Photo by Andrew Noyes.*

IPNV

The IPNV is an icosahedral-shaped RNA virus without a lipid envelope. As with IHNV and VHSV, the IPN virus exists in many groups and strains. Naturally occurring infections have been detected in dozens of fish species and even in many invertebrates. The strains that cause disease in salmonids are different than those that cause disease in other animals or that are isolated from apparently healthy fish in other species.

The IPN strains that we worry about cause problems in rainbow, brook, and brown trout, and in Atlantic salmon. The disease in trout occurs when the fish are very young, usually around first feeding, and it is not uncommon for total mortality to reach more than 90%.

In the US, the virus is primarily a problem in the Northeast but outbreaks occur as far south and west as Arkansas. The virus is occasionally detected in wild fish in the Northwest but it has been many years since this virus has been a problem on our Region's fish hatcheries. Virologists cannot explain why this virus is no longer a problem on our NW hatcheries. The virus is still here, the host is still here, but fish are not dying.

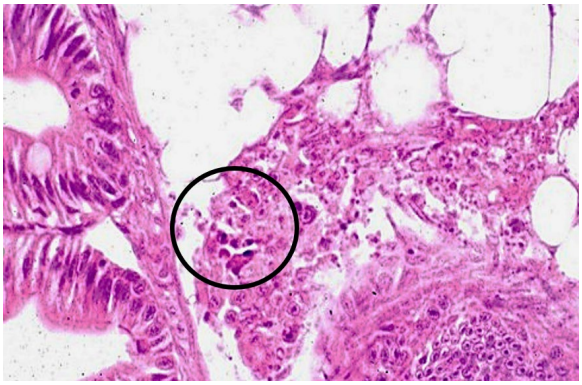

Figure18: Histology from a rainbow trout with IPN. The tissue on the left is intestine. The black circle highlights a group of dying pancreas cells.

Other Viruses

There are other viruses that are sometimes detected in salmon in our Region, but they have no clear association with disease. These include a strain of the Piscine Reovirus (PRV) and several others. The PRV story is interesting in that other stains of PRV present in Europe are associated with serious diseases in farmed Atlantic salmon, but our NW strain does not appear to cause disease in Pacific salmon. The best evidence is that our NW strain is native to the NW and that it long ago reached a truce with Pacific salmon species

One of the largest challenges facing fish virology right now is that new testing methods are detecting dozens of fish viruses that have no association with disease. When do we worry? Before addressing that, let's take a look at the new technology that finds these new viruses.

Virus Testing Methods

The classic method: We have lines of fish cells that have been adapted to grow in thin layers in plastic flasks. The cells are kept in a liquid growth medium. When we need more cells, we remove them from the flask, split them among several flasks, and wait a few days for them to proliferate and refill the flasks. This can be done time after time for decades so cell lines are well characterized and shared among many labs.

Figure 19: Plastic flasks with thin layers of cells for traditional virus testing.

To use cell lines to detect viruses is quite simple. Tissues are sampled from fish, homogenized, centrifuged, and then the liquid that may contain viruses is added to a flask with a fish cell line. We check the cells daily under a microscope and, if a virus is present, after a few days or weeks, we can see the infected cells die in characteristic ways. Cells may change from flat to round, fuse together into blobs (syncytia), or develop foamy cytoplasm.

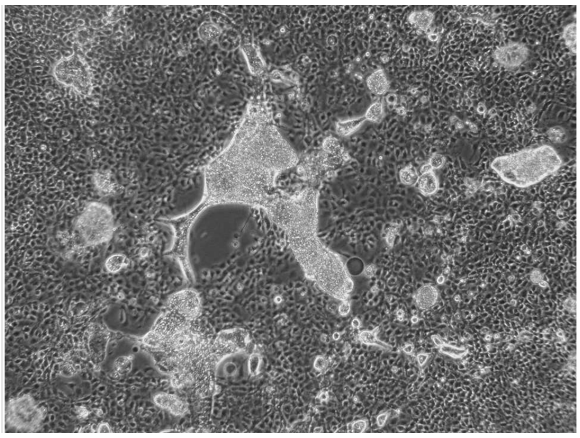

Figure 20: A layer of fathead minnow cells (small black smudges) infected by golden shiner virus (GSV). The GSV has caused hundreds of fish cells to fuse into giant cells (the big bright blobs) with hundreds of nuclei.

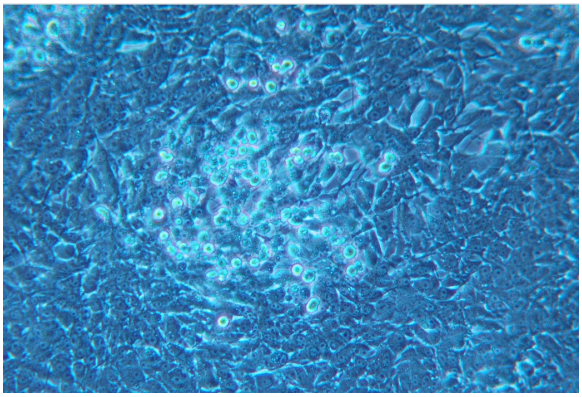

Figure21: Seen at a slightly higher magnification than the previous figure, this picture shows spring viremia of carp virus (SVCV) causing a patch of infected cells to become round and refractile instead of flat.

There are advantages and disadvantages to cell culture.

On the plus side:

- It is low relatively low tech
- You don't have to know what virus you are looking for
- It produces more virus that can be used in other tests, tested in fish, or shared with other labs.

On the minus side:

- While some viruses grow in just a few days, others may take several weeks
- Many viruses don't grow in cell cultures at all
- Cell culture tells you that a virus is present, but not which virus
- Fish test samples need to be fresh so that the viruses are still alive when the testing starts

The other method for looking for viruses is PCR. In PCR we extract the DNA and/or RNA from fish tissues and do some fancy chemistry that produces copies of target virus DNA or RNA. Every time the instrument subjects the sample to a special temperature cycle, enzymes in the test sample copy the viral nucleic acids.

Cycles	DNA Copies
1	2
5	32
10	2,048
15	65,536
20	524,288
25	67,108,864
30	2,147,483,648

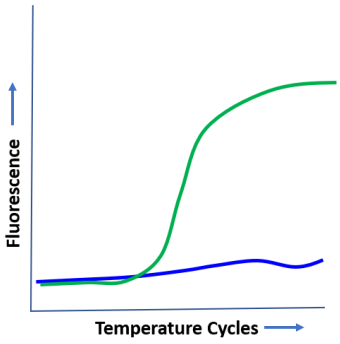

Figure 22: The table above shows how the number of DNA copies doubles with every PCR temperature cycle. The graph on the right shows how fluorescence increases as the cycles happen. The green line is a positive sample, the blue line is negative.

These copies are easy to detect with fluorescent dies. If the copies are made, we know that the virus is present. Again, there are advantages and disadvantages to this test method.

On the plus side:

- PCR is fast. It can be done in one day.
- PCR works on viruses that won't grow in cell cultures
- PCR provides information on the identity of the virus

On the minus side:

- For PCR, you must know what virus you are looking for
- PCR is more technically complex and prone to contamination
- PCR does not provide viable virus that can be propagated for other uses.
- PCR demonstrates that virus DNA or RNA is present but does not prove that there is intact virus capable of causing disease.

Cell culture has for many decades been the gold standard for fish health testing. PCR tests are used primarily to identify viruses that have been detected in culture. In human and other veterinary medicine, cell culture has mostly been replaced by PCR. We can see that trend happening in fish medicine, but concerns over the "you only find what you are looking for" nature of PCR, the limitations of not being able to make more live virus for further testing, and disagreements over the interpretation of test results have slowed the adoption of PCR in fish health.

New Virus Challenges

New technology based on DNA sequencing has made it possible to sort through the DNA and RNA of a fish in search of sequences that are associated with viruses. This approach

inevitably reveals all kinds of sequences related to known and unknown viruses. The challenge is that these sequences in no way prove that intact viruses are, or have ever been, present. The technology also does not differentiate between a dangerous fish virus, virus-like genes that may be part of the fish, and the many viruses that are found in healthy-looking animals in low numbers but have never associated with a disease. It is a powerful tool, but one that must be used and interpreted with great caution.

Summing it all up

It is very important to recognize that there are very dangerous trout and salmon viruses that occur in other geographic regions but not in the Pacific Northwest. This is in part due to differences in host species, climate, water chemistry, and other environmental conditions, but it is very important to be very conservative about movements of potentially infected fish between watersheds.

Most major disasters with fish viruses have occurred when we move a virus to a new region, or when we move fish to a new region where there is a virus that the fish have not seen before. The biggest fish kills, especially in the wild, occur when a fish population and a virus meet for the first time. Examples:

- The VHSV IVb virus moved from the North Atlantic to the Great Lakes and caused major fish kills, especially in drum, shad, gobies, and muskellunge.

- A herpesvirus introduced to Australia caused fish kills in 1995 and 1998/99 that went clear around the coast and killed billions of fish. The kill spread along the coast at about 15 miles per day and collapsed entire food chains.

Viruses are constantly evolving and new strains with new characteristics emerge all the time. The naming system for those strains is messy.

Biosecurity methods for viruses differ based on physical characteristics of the virus that include its protein shell (capsid) and whether or not it has a lipid envelope.

Other than vaccines, viral diseases are prevented or controlled by management techniques and not by any drug or chemical use. The most important tools that we have are iodine treatments of eggs, the use of virus-free spring or well water for the fish's early life stages, and constant surveillance.

Figure 23: Christine Parker-Graham of the PRFHP checking on the health of fish on a Service hatchery.

Virus testing methods are at an awkward in-between stage where older methods that have many advantages but are slow (21 days), are being replaced by faster new methods (PCR) that miss viruses that we don't know to look for.

Thanks to careful management, viruses are not ***currently*** a major source of Pacific salmon losses on Service hatcheries in the NW. However,

It is really important that we don't let down our guard!