Fish Health News You Can Use

Brought to you by the Pacific Region Fish Health Program

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This Issue

Contact Us

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In the Next Issue

- How are treatment decisions are made? To treat or not to treat? What treatment should we use?
- "Normal flora"
- Aeromonas bacteria

Introduction

Fish are dying but the tests for infectious diseases all come up negative. What do we do? Are we stuck? Are we at a dead end? Do we send a nasty email to the testing lab? The unfortunate truth is that fish die for all kinds of reasons that do not involve infections that can be easily identified by standard lab tests. Fish health professionals look at lab tests the same way that detectives look at fingerprints. Sherlock Holmes does not drop a case when fingerprints can't be found, and good fish pathologists don't give up when the lab results come back negative. Lab tests are helpful, but they are just one clue. Sorting out a difficult case requires a thorough investigation that includes 1) lab tests, 2) looking for additional evidence and questioning witnesses, and 3) attempts to recreate the "crime."

In this issue of the Fish Health News, we are going to look at the detective side of fish disease diagnosis. We'll start out by looking at the limitations of lab tests, then discuss collecting and interpreting other evidence, and finish up with bioassays that can be used to reveal the cause of fish losses.

Limitations of lab tests

Bacteriology

PROBLEM#1: Not all bacteria grow in cultures.

Most tests for bacteria are based on culture. A sample of tissue from a sick fish is put on a jello-like plate of nutrient agar. Bacteria then use the nutrients to grow and divide.

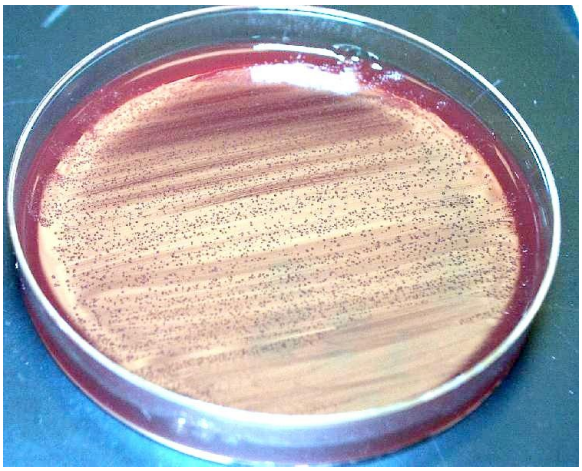


Figure 1: An agar plate with colonies of Aeromonas salmonicida bacteria. This is one of the "atypical" strains that can be very hard to grow.

After a few days in the incubator, colonies of proliferating bacteria are easy to see. The bacteria are then identified through a variety of tests that typically focus on what the bacteria eat and what they secrete [fig -ID test]. That sounds great, but for decades, microbiologists have known that some bacteria are difficult to grow. The basic problem is that a jello-like plates with nutrients from yeast, soybean, or animal extracts are really nothing like the environment inside a fish. We compensate by developing more complicated agar formulations to grow the fussy bacteria, but even then it may require weeks to grow enough bacteria to see. Those are limitations that we have long understood, but modern DNA techniques have also demonstrated that only a very small percentage of all bacteria species will grow on agar plates anyway. This means that a negative bacterial culture test might miss new bacteria that we don't know or understand. It probably isn't a coincidence that the well-known bacterial pathogens of fish all grow well on agar plates!

PROBLEM #2: Just because it grows on the plate doesn't mean that it is the cause of the disease.

Another problem with bacterial culture is that very sick fish, especially Pacific Salmon broodfish, often have malfunctioning immune systems that allow environmental bacteria to become established in the tissues of the fish. This causes two problems, 1) environmental bacteria that invade already-sick fish can be mistaken for the cause of the disease, and 2) fast growing environmental bacteria can overgrow the real disease-causing bacteria so that we don't see them.

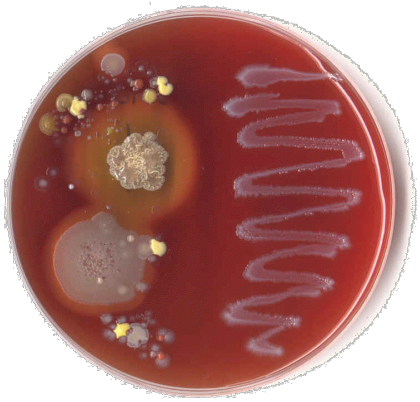


Figure 2: Cultures from two sick fish. The fish on the right grew only a single type of bacteria, an easy diagnosis. The fish on the left is immunocompromised and many types of bacteria grew. The disease bacteria are hidden.

Virus Culture

Problem #3: Many viruses don't grow in our cell lines

Most virus testing is similar to bacteria testing. Tissues from the fish are placed in flasks onto layers of living fish cells.

If there is a virus, it infects and kills the cells. We can see that cell death through a microscope as "cytopathic effect." Unfortunately, virus culture suffers from the same problems as bacteria culture. We have many kinds of fish cells to use, but many viruses don't grow in any of those cell lines.

Problem #4: Unimportant fast-growing viruses may hide an important slow-growing virus in culture.

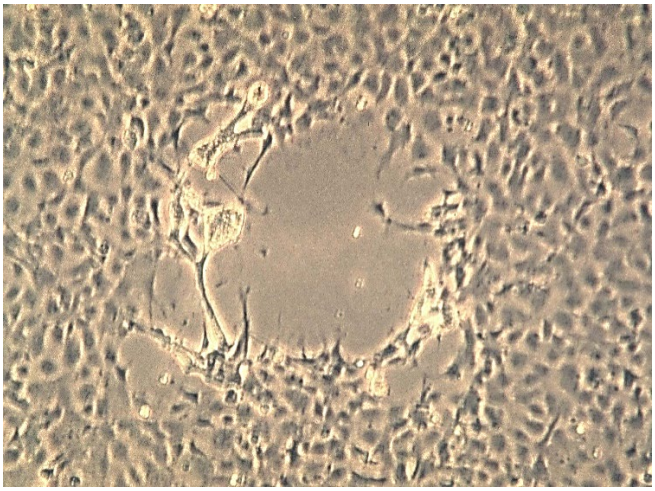
There are other virus culture problems similar to those that we see with bacteria. If we have more than one virus present, a common virus that was not causing disease may outcompete the one that we are looking for.

Problem #5: Viruses masquerading as the problem when they are really the result, not the cause.

Again like the bacteria, high loads of some viruses can look impressive, but their presence is actually the result of a disease, not the cause of it. The best example are the aquareoviruses. They infect healthy fish and idle along at very low levels for long periods of time. When the fish gets stressed by another disease or environmental condition, the virus takes advantage of the compromised immune system and propagates to high numbers in the tissues of the fish. This virus didn't cause the disease, and it may not even be causing problems for the fish, but it is suddenly easy to find, the high levels correlate with disease, and it is easy to mistakenly assume that it is the actual problem.

Problem #6: When we find a new virus, we don't know if it causes disease

Lastly, there are many, many viruses out there that can show up in cell cultures. When it is a new one, we don't know if it is causing a disease, or a harmless incidental finding.



DNA Tests

Problem #7: You only find what you are looking for.

Tests that use PCR methods to find pathogens are super specific and very sensitive. This sounds great, but it also causes problems. The PCR tests are based on specific DNA and RNA

sequences found in target disease organisms. They are so specific that you only find organisms that you are looking for. If you run tests for organisms A, B, C, and D but it is E that is killing the fish, all the tests will be negative.

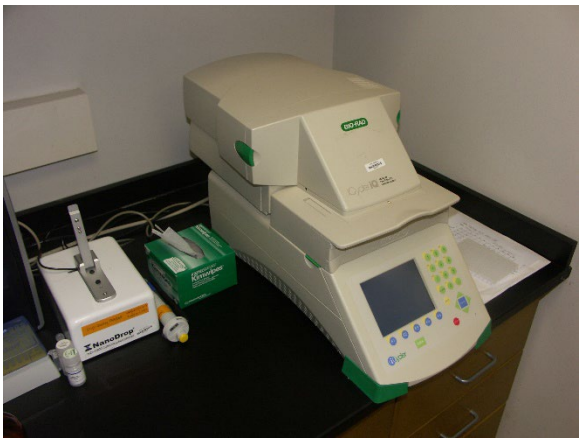


Figure 5: This PCR machine can make billions of copies of pathogen DNA sequences in 96 samples in just over an hour.

Problem #8: PCR tests are so sensitive that they detect even dead bacteria and fragments of old virus infections.

Sensitivity is also a problem. If there are a few DNA sequences in the fish that were left over from a mild viral infection months before, the test will come up positive. Looking at it another way, if the fish had a mild asymptomatic case of disease "A" a month ago, a PCR test for "A" will come up positive even though the fish may be dying of "B".

Antibody based tests

Problem #9: Antibodies against "A" won't help diagnose an infection caused by "B".

There are tests that use antibodies to detect disease organisms. Unfortunately, as with PCR, you only find what you are looking for. In addition, for some diseases fish may develop an antibody response after exposure to the pathogen without actually developing the disease so you may not be able to tell whether the fish was actually sick because of a pathogen or if they just developed an appropriate immune response to the pathogen.

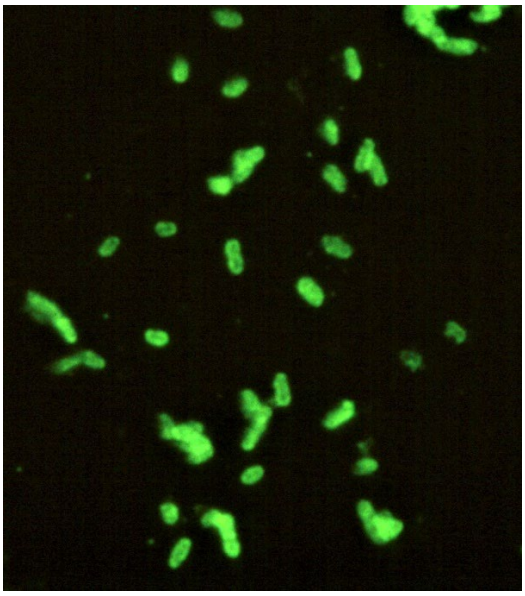


Figure 6: BKD bacteria fluorescing green in an antibody-based test for BKD.

Histology – better news?

Problem #10: While histology can find the result of a disease, it often can't identify the cause.

In earlier issues of the fish health news, we have talked about histology. The fish tissues are preserved in formalin, embedded in wax, sliced much thinner than a hair, placed on glass slides, stained, and examined under a microscope. The beauty of this approach is that the pathologist can look at cells and organs throughout the fish and see exactly what is going wrong. Some diseases cause very recognizable damage that enable a good pathologist to diagnose common diseases like Coldwater or BKD with a high level of confidence. Unfortunately, we are also often faced with a situation where the damage is obvious but can't be associated with a particular disease. "Wow, that's an awesome liver lesion. I wonder what caused it?" If we see these kinds of lesions we still need to determine

if the lesion is associated with the present disease. Fish sometimes have problems that last their entire lives and it can be difficult to determine whether such a lesion, even an impressive one, falls into the “incidental finding” category (ie: we only found it because we were looking) or the “causative” category. The other limitation is that viruses are too small to see. Bacteria can sometimes be seen, but it is hard to tell more than “Hey, there are bacteria present.” So, histology is a great tool and can give us a lot of help with mystery cases, but often it can identify the damage but not reveal the cause.

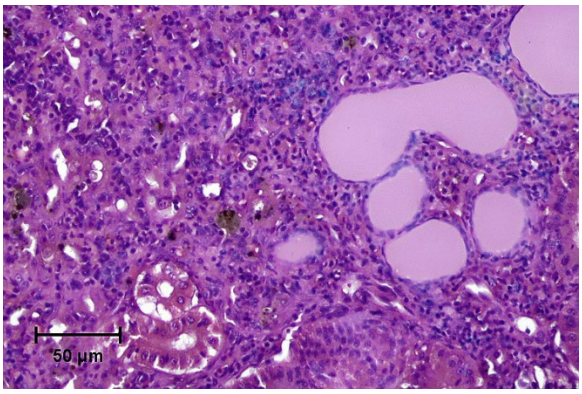


Figure 7: This fish kidney histology reveals large featureless areas filled with water and protein. It isn't normal, but what caused this?

Hey, you haven’t mentioned parasites!

Problem #11: Are these parasites causing this disease or are they the result of some other disease weakening fish immunity?

Many parasites are large enough that they can be easily identified by the naked eye, under a microscope, or through histology. With the exception of a few tricky organisms (like amoeba that are tiny and difficult to distinguish from skin or gill cells), we are confident that between microscopy in the field and histology at the lab, we don’t miss much. That said, one of the pitfalls of histology is that parasites that are on the external surfaces of fish (costia, skin and gill flukes, trichodina, amoeba, and many others) tend to fall off when exposed to the formalin fixatives that are a necessary first step in the histology process.

Figure 8: Some parasites, like these fish "lice" are really easy to diagnose, but others (especially single celled organisms with complex life cycles) can be difficult to find.

And the really big problem…

Problems #12-129: Most of the lab tests that we use are designed to detect infectious diseases, but many fish diseases are not infectious.

Think of the possibilities:

Water quality (particulates, pH, O2, CO2, sodium, chloride, ammonia, nitrite, gas pressure, etc.)

Figure 9: The skin of this channel catfish has eroded around the pores of the lateral line and exposed neuromasts. The cause is unknown, but this occurs most frequently in systems using activated carbon.

Waterborne toxins (algae, industry, accidents, metals, organics, new materials)

Feed problems (vitamins, minerals, toxins)

Broodfish nutrition (thiamine deficiency, energy and fats to the eggs)

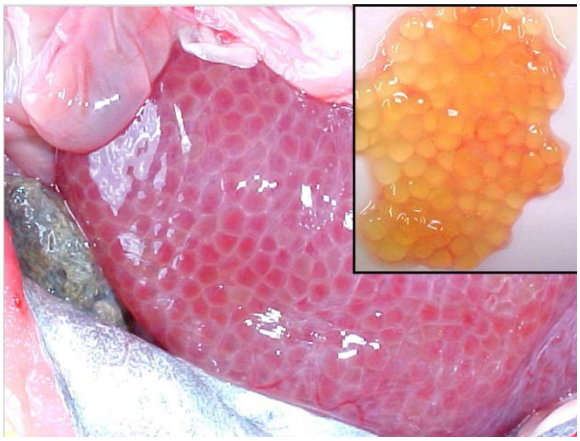


Figure 10: Blue catfish eggs that are purple, instead of orange (inset), because of pigments derived from its diet.

Mechanical injuries (nets, rough surfaces, fin abrasion, jumping, fishing)

Chronic stress (crowding, social interactions, predators, overflowing the stress cup – see Issue #10)

Genetic problems (missing, damaged, or duplicated chromosomes, mutations)

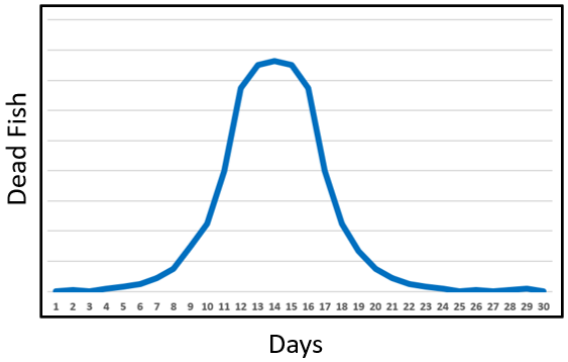
Developmental problems (UV or extreme temps during incubation, chemical exposure, poor egg quality)

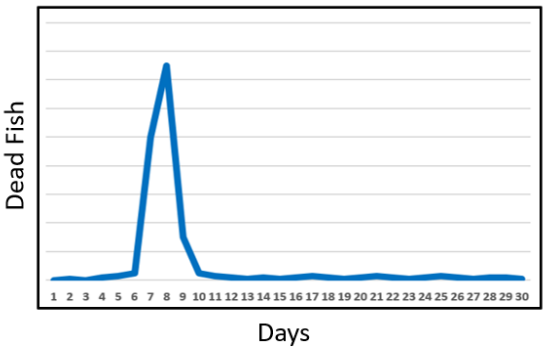
Stray electrical voltage

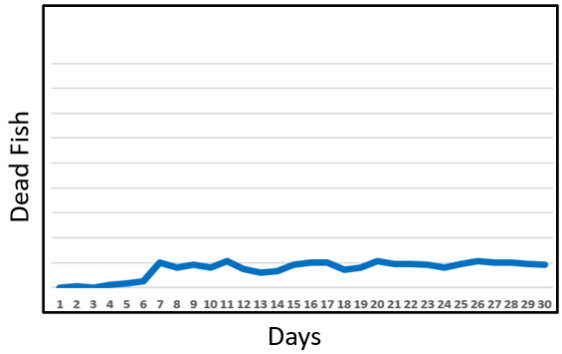
Collecting and Interpreting other Evidence

Lab tests are great, but they often fail to reveal the cause of fish losses. When that happens, we must look for other evidence. The first big question is whether or not it is an infectious disease or an environmental problem. These are some of the ways that we figure that out.

Time course: If we look at the number of dead fish per day over time, the shape of the graph provides strong hints about the cause of death. Infectious diseases typically build up day by day, reach a peak, and then decline producing a classic bell-shaped curve.







Number of culture vessels involved: In a facility with 10 raceways, an outbreak of an infectious disease may eventually work its way through all of the raceways, but it is very unlikely that it will happen the same way, at the same time, in all of the raceways. If it happens in a few raceways and spreads, that might be an infectious disease. If it happens everywhere at once, that's an environmental problem.

Number of species involved: Infectious diseases behave very differently in different species of fish. If there are two species of fish under similar conditions in the same water and both species start dying at the same time, it is probably an environmental problem and not and infectious disease.

Lesions: Infectious diseases often kill specific tissues in characteristic ways. The types of lesions, or lack of them, provides great clues.

Figure 11: The white gill tips of this koi are a hint that the fish has been exposed to a waterborne toxicant. In this case, an accidental formalin overdose.

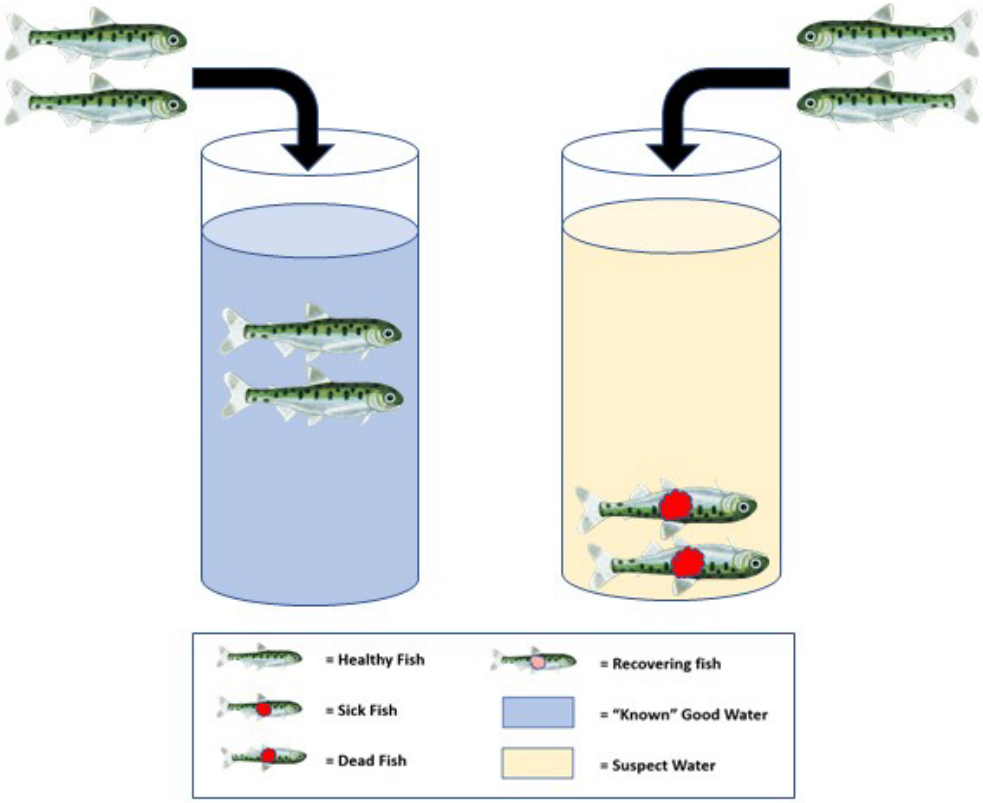
History of recent stressful events: Feed changes, recent handling, weather, water source, water quality, recent treatments, predators. All of these can cause health problems, and may also predispose fish to infectious diseases. Knowing the history is really important when we are trying to determine if a bacterial or parasite infection is the cause of the disease, or the result of it.

Figure 12: These hybrid striped bass were put on a new feed. Intestinal bacteria reacted by producing gas that made the fish so buoyant that their backs were out of the water and some were upside down.

Recreating the Crime

There are some other very powerful approaches to determining if fish losses are from an environmental problem, or from an infectious disease. These fall into the broad category of "Bioassays." If the bioassays establish that the cause of the problem is an infectious disease, there are some additional clever approaches that can discern between bacteria, viruses, and parasites even when nothing is detected by standard lab tests. In the section below are cartoon versions of some of the most useful bioassay types.

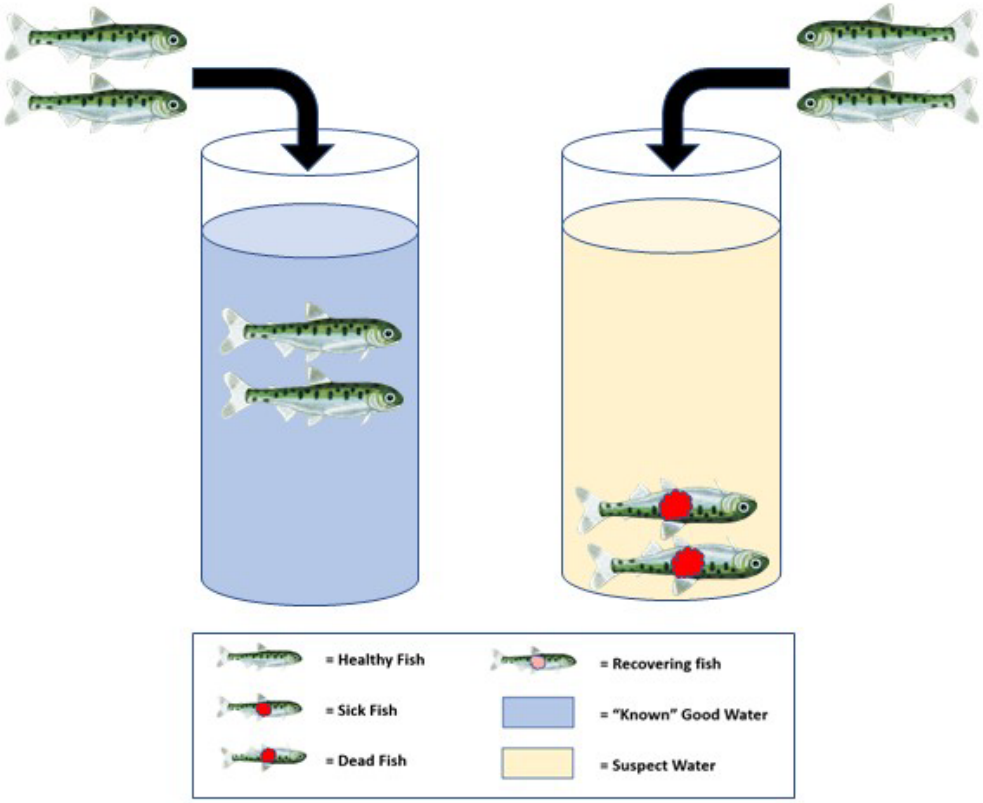
Look at each cartoon on the following pages and try to figure out what the results are telling you. Answers are on the page that follows each puzzle.



Setup: Two tanks. One with water that is "known" to be good and one that is associated with the fish health problem. Healthy fish are placed in each tank. Tanks can be static (aerated) for short term experiments or flow-through for longer term.

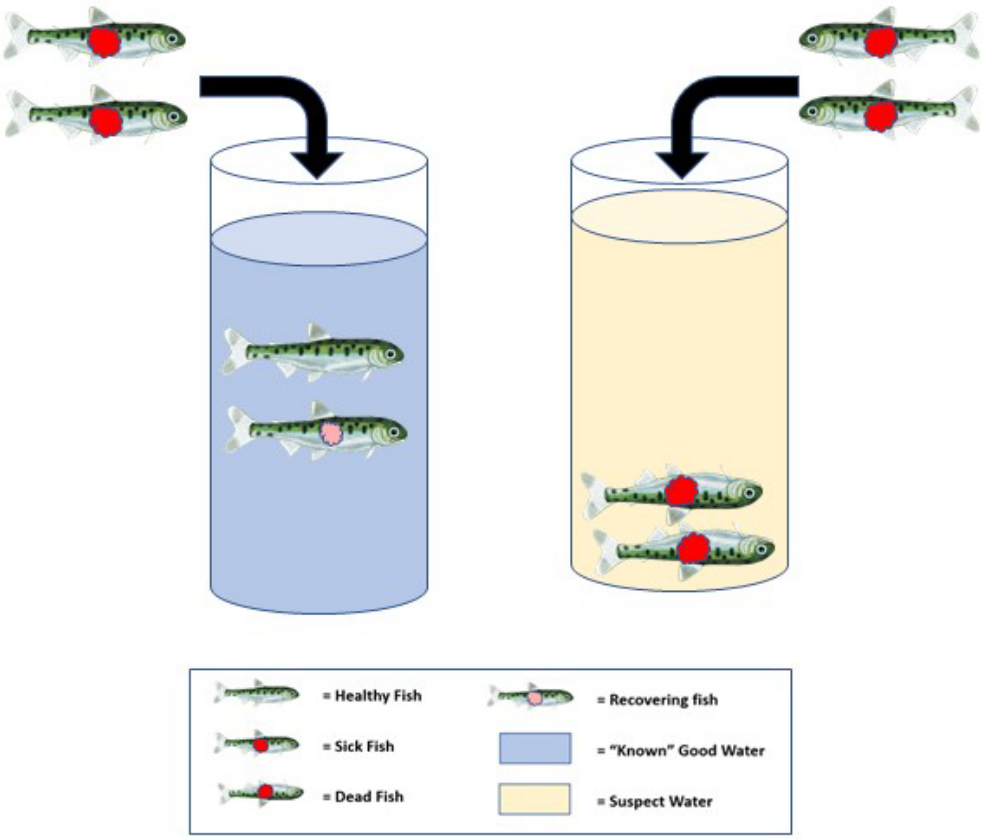
Result: The fish in the "known" good water stay healthy while the fish in the suspect water get sick and die.

What does it tell us?



What does it tell us?

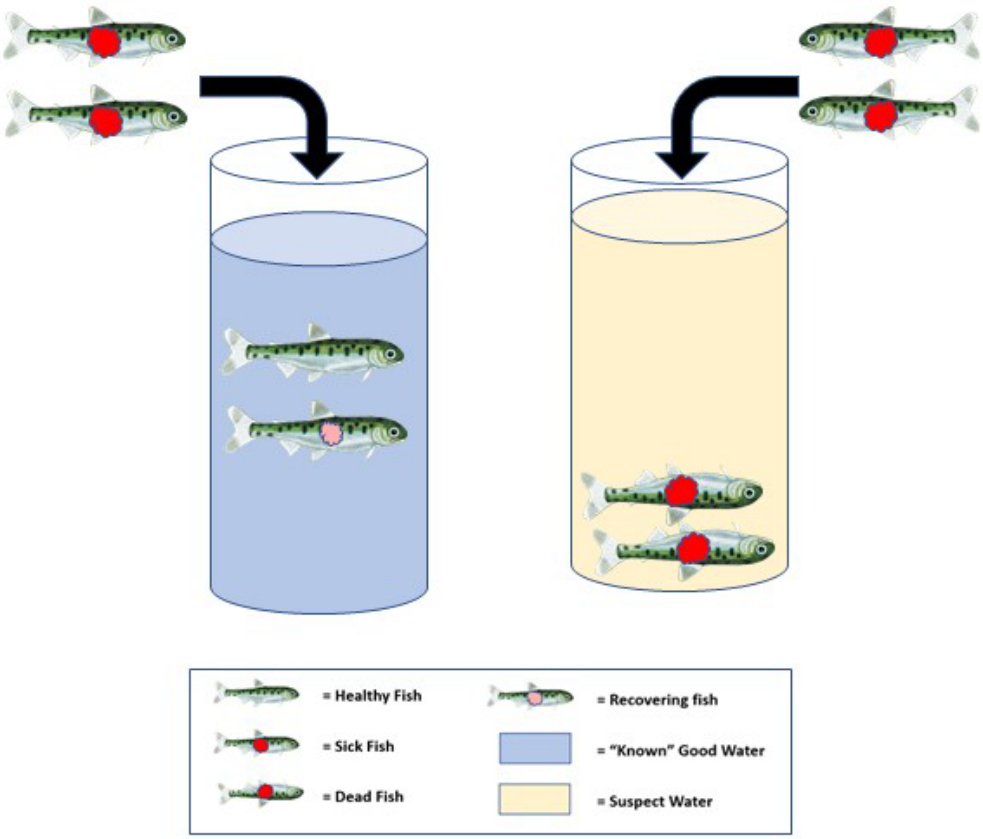
This is evidence that the cause of the fish health problem is poor water quality, including toxins of any sort. Ideally, the disease that is produced in the bioassay is identical to what was seen in during the disease outbreak. Otherwise, the the disease may be the result of something different happening.



Setup: Two tanks. One with water that is "known" to be good and one that is associated with the fish health problem. Diseased fish are placed in each tank. Tanks can be static (aerated) for short term experiments or flow-through for longer term.

Result: The sick fish in the "known" good water stay healthy while the fish in the suspect water get sicker and die.

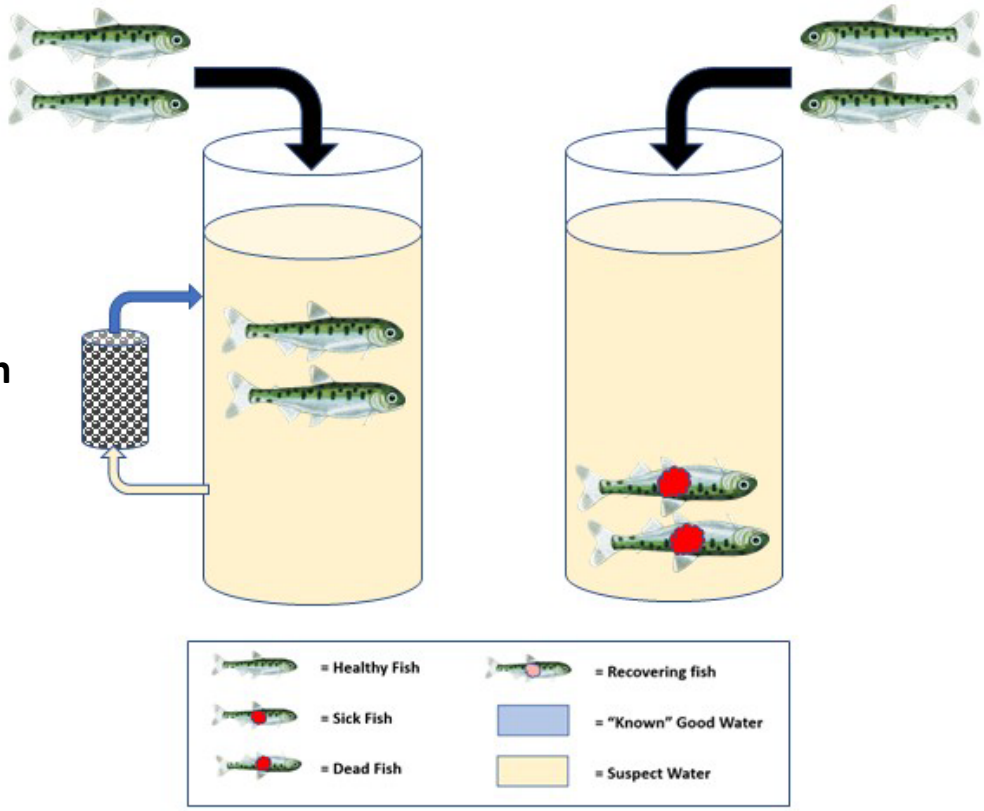
What does it tell us?



What does it tell us?

This is more evidence that the cause of the fish health problem is poor water quality, including toxins of any sort. What does it mean if the fish die in both tanks? It might mean that it is an infectious disease, but it might also mean that it really was bad water but that the fish were so sick that they couldn't recover. What does it mean if the fish get better in both tanks? This result is not very helpful, but it may mean that some other factor, not included in this bioassay design, might be the problem.

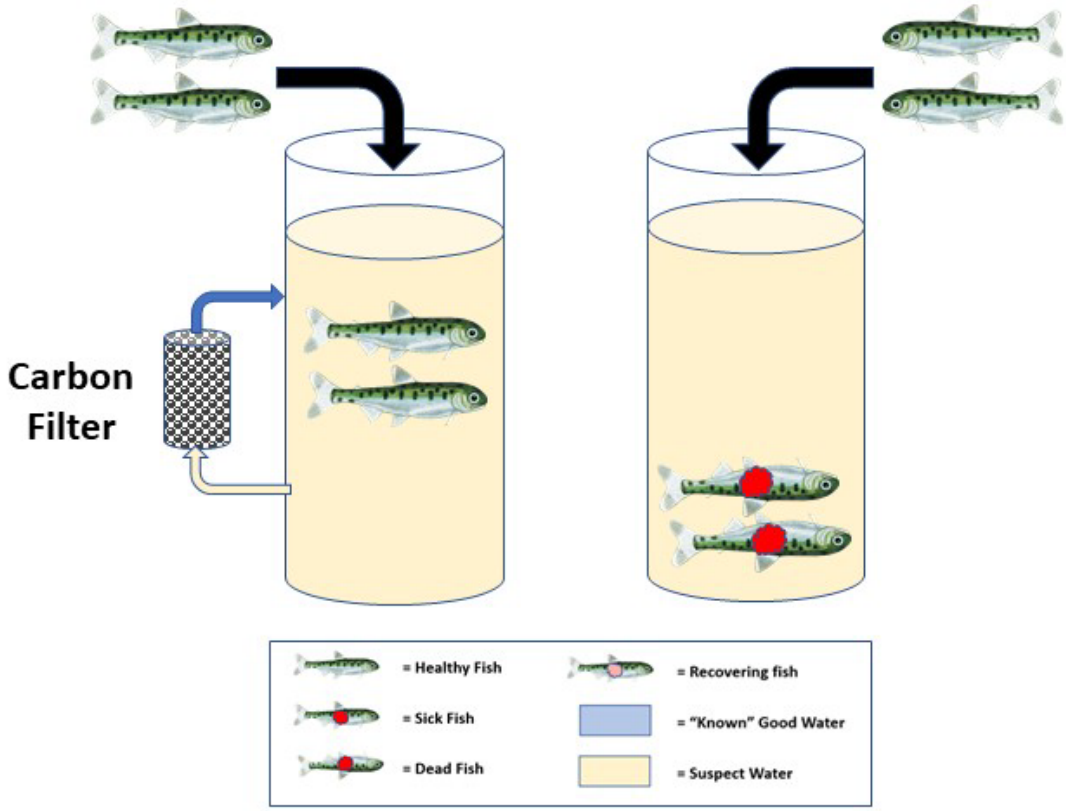
Carbon Filter



Setup: Two tanks, both filled with water that is associated with the fish health problem. One tank has carbon filter. Healthy fish are placed in both tanks. Tanks can be static (aerated) for short term experiments or flow-through for longer term.

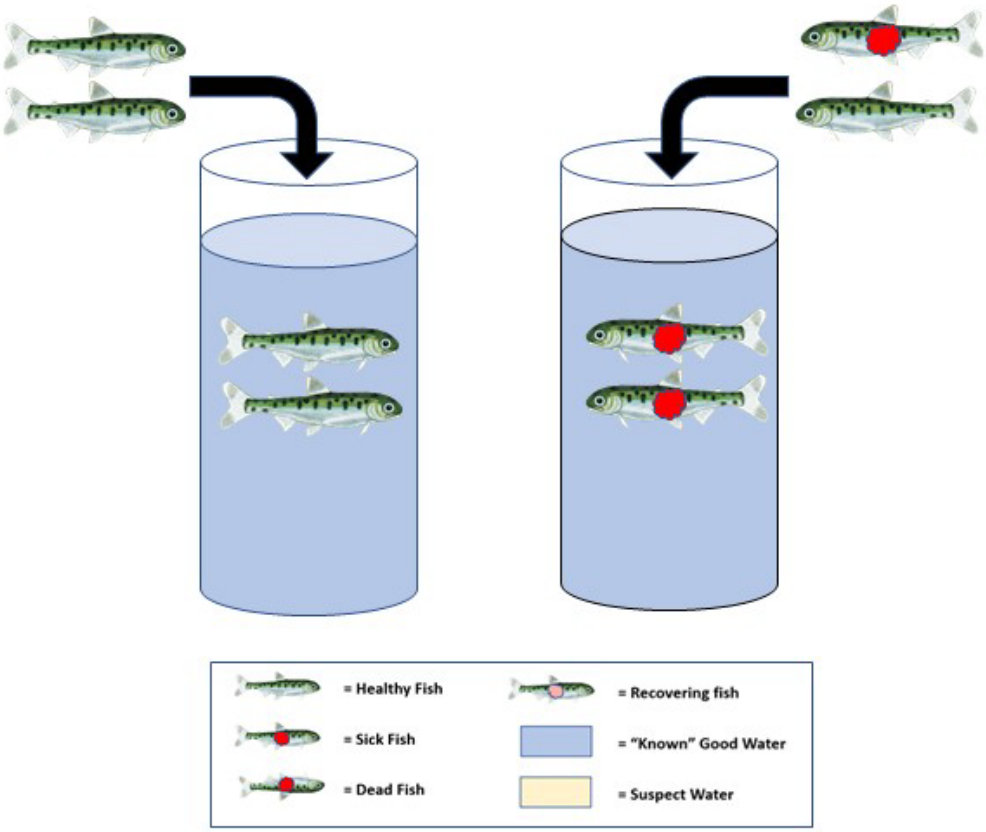
Result: The healthy fish in the carbon-filtered tank stay healthy while the fish in the unfiltered water get sicker and die.

What does it tell us?



What does it tell us?

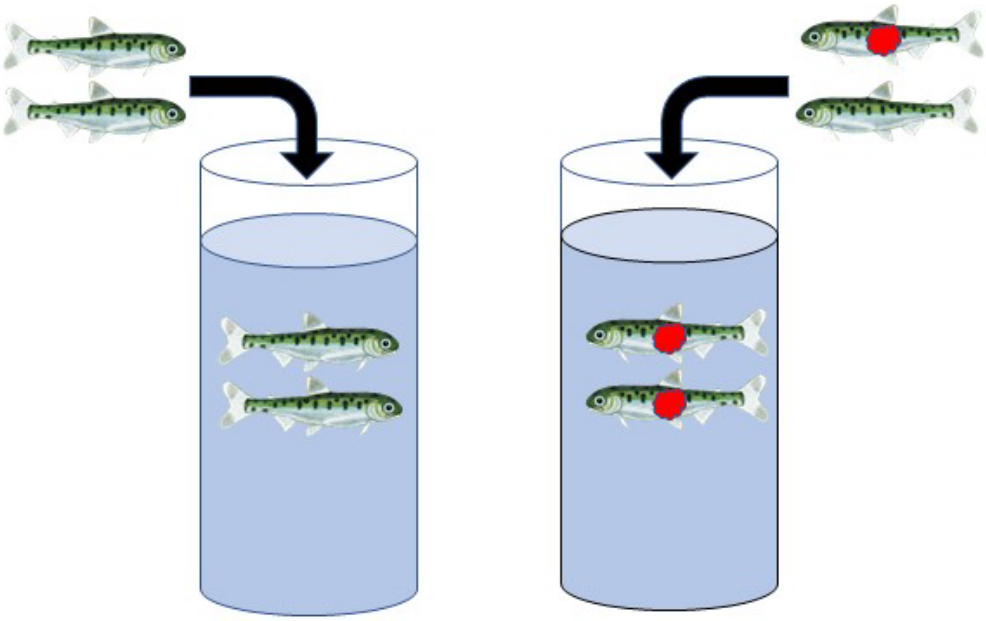
This assay result implies that the fish health problem may be the result of a toxin that is effectively removed by the carbon filter. What does it mean if the fish die in both tanks? It may still be a toxin, but one that is not removed by the carbon.



Setup: Two tanks, both filled with water that "known" to be good. Tanks can be static (aerated) for short term experiments or flow-through for longer term. Healthy fish from another source are put into one tank. A mix of sick fish and those healthy fish are put in the second tank.

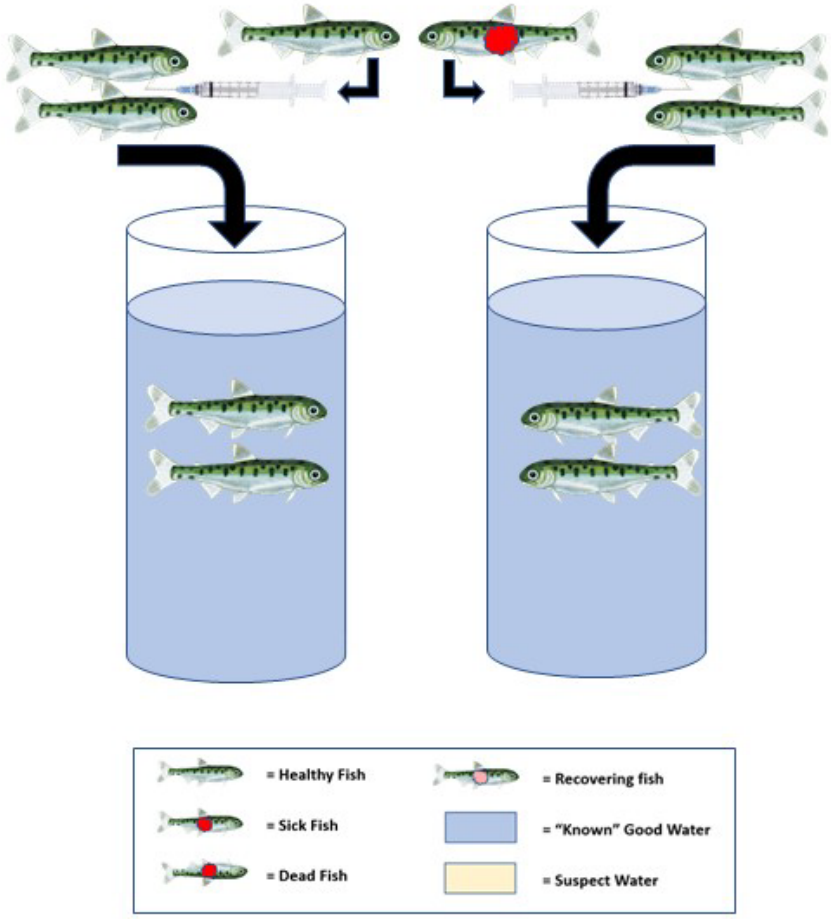
Result: The healthy fish in the tank by themselves stay healthy. The healthy fish put on with sick fish get sick.

What does it tell us?



What does it tell us?

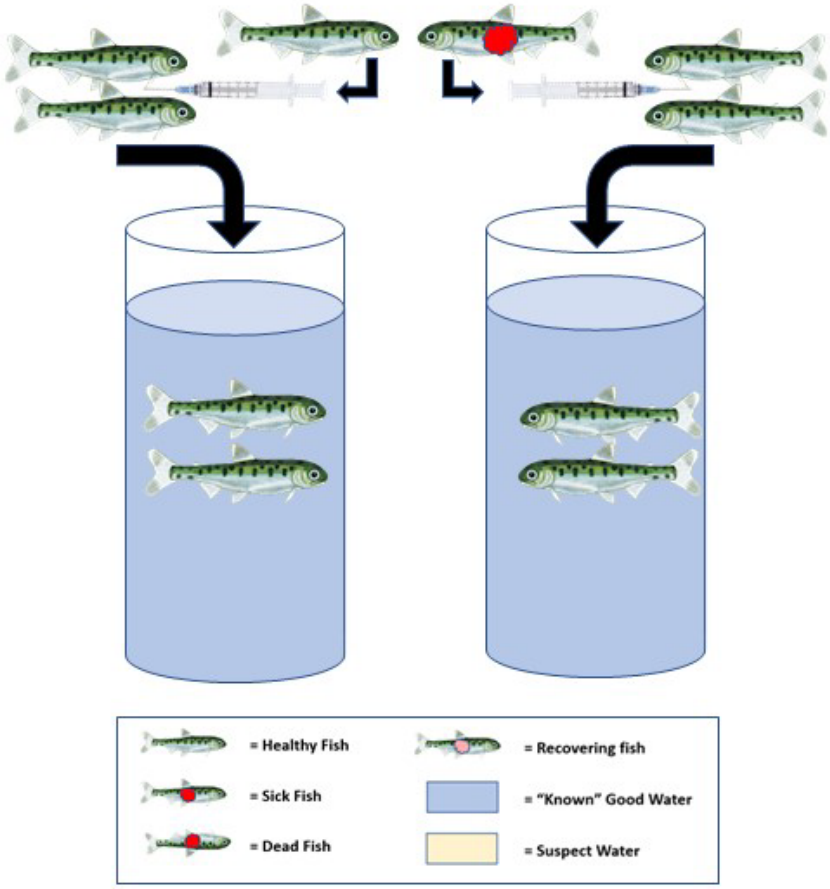
This assay result implies that the fish health problem is an infectious disease that can spread from fish to fish. Why is the tank on the left important? It is the negative control tank. For a valid assay result, these fish must be just the same as the fish on the right with the only difference being the exposure to sick fish. If these left-tank control fish die, it means that you've messed up your assay.



Setup: Two tanks, both filled with water that "known" to be good. Tanks can be static (aerated) for short term experiments or flow-through for longer term. Healthy fish from another source are put into both tanks. Fish in the tank on the left are injected with homogenized tissues of healthy fish. Fish in the tank on the right are injected with homogenized tissues of sick fish

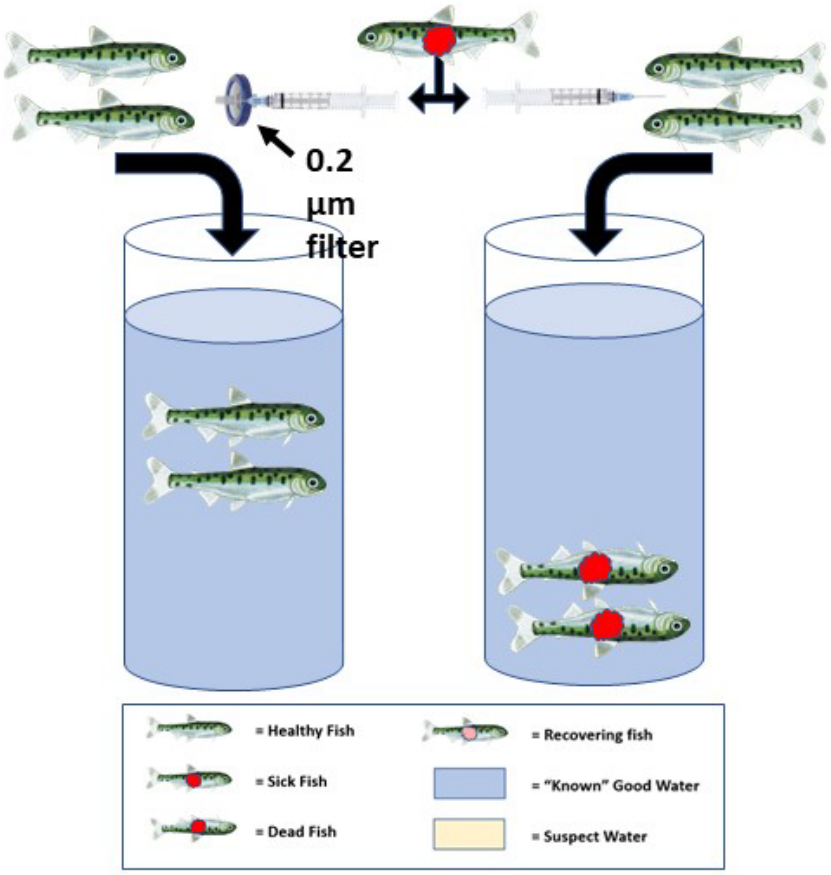
Result: The healthy fish injected with healthy fish stay healthy. The healthy fish injected with sick fish stay healthy.

What does it tell us?



What does it tell us?

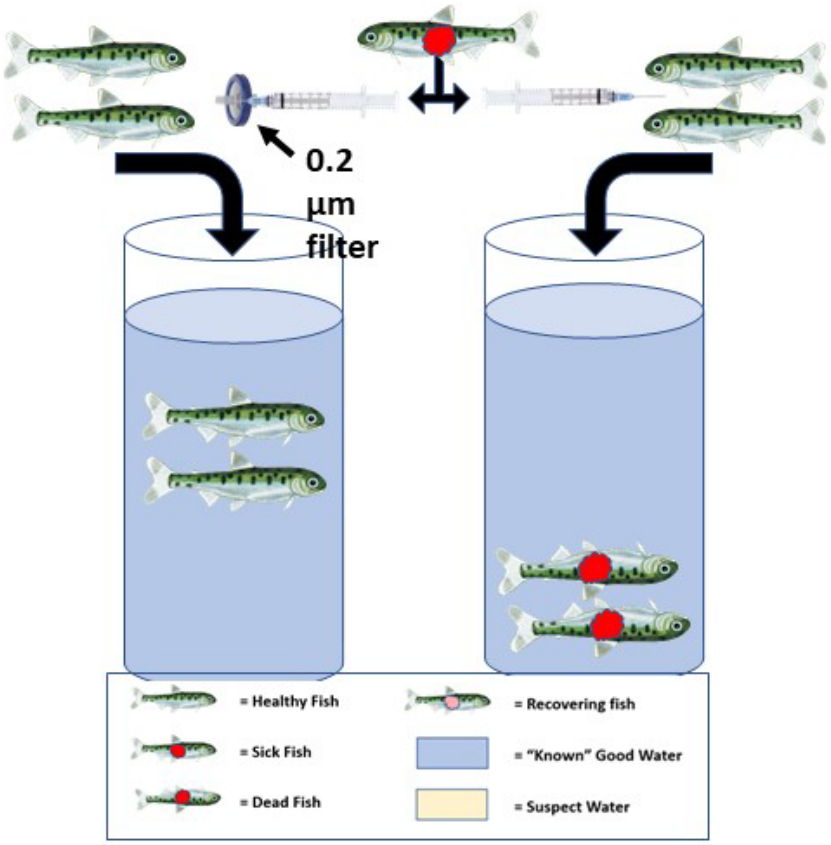
This assay result implies that the fish health problem is not an infectious disease that can spread from fish to fish. If the fish that are injected with sick fish do die, then an infectious disease is a real possibility. Note that this works well for bacteria, viruses, and fungi, but not for parasites that have complex life cycles and are not spread directly from fish to fish. The negative control fish (left tank) are particularly important because even a homogenate from a healthy fish can sometimes kill other healthy fish.



Setup: Two tanks, both filled with water that "known" to be good. Tanks can be static (aerated) for short term experiments or flow-through for longer term. Healthy fish from another source are put into both tanks. Fish in both tanks are injected with homogenized tissues of sick fish, but for the left tank the homogenate has been filtered at 0.2 µm prior to injection.

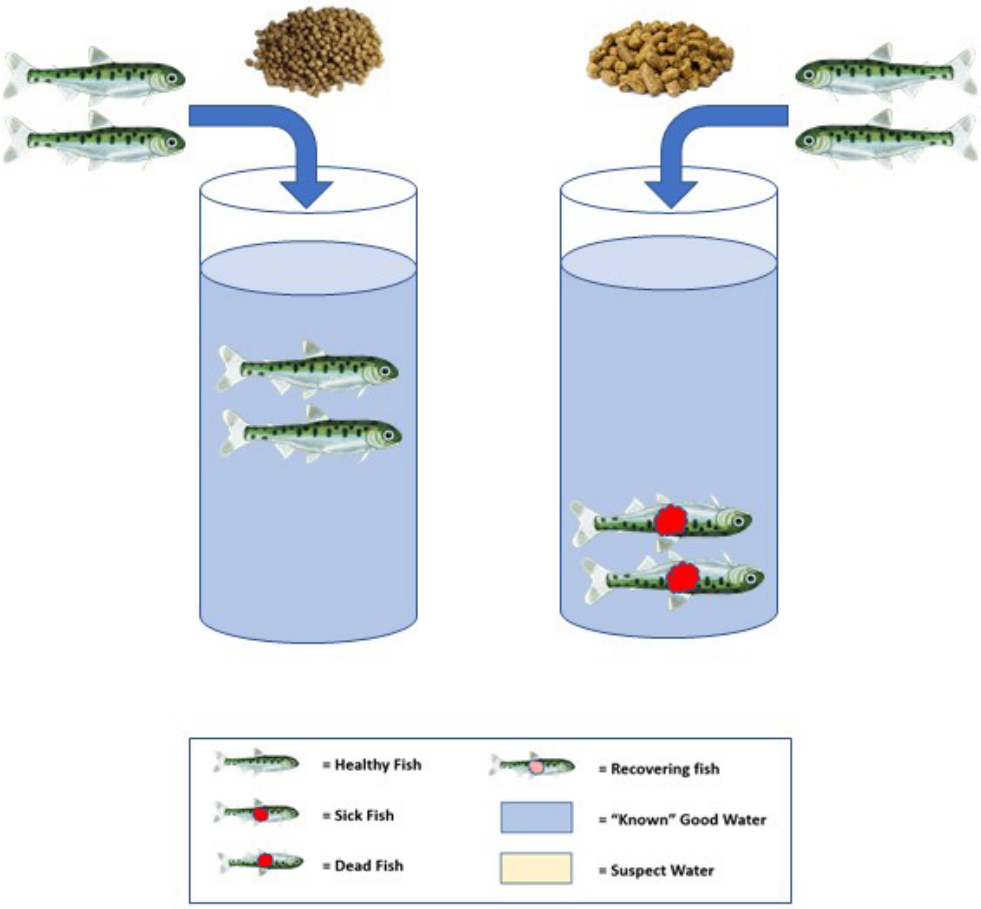
Result: The healthy fish injected with filtered homogenate stay healthy but the unfiltered group gets sick.

What does it tell us?



What does it tell us?

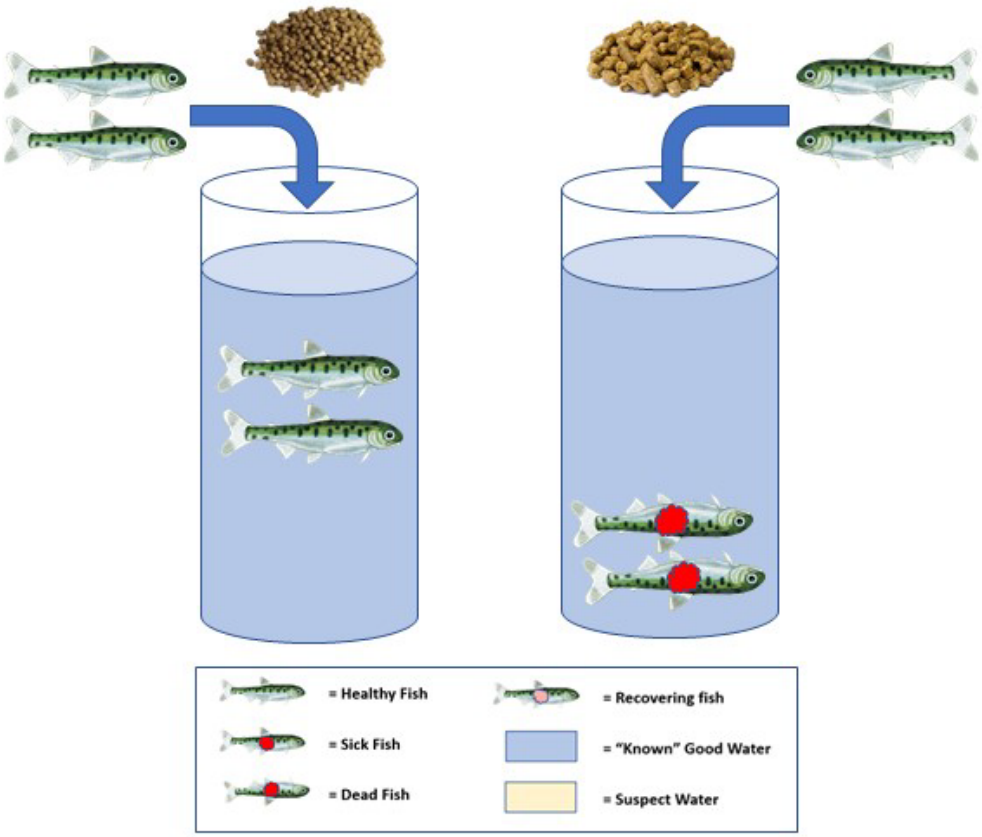
This assay actually gives us a hint about the nature of the infectious disease. Most viruses are very small and easily pass through a 0.2µm filter. Bacteria and fungi are filtered out. The result above is evidence that the disease is not viral. If the fish in both tanks die, it is probably a virus.



Setup: Two tanks, both filled with water that "known" to be good. Tanks can be static (aerated) for short term experiments or flow-through for longer term. Healthy fish from another source are put into both tanks. Fish in the tank on the left are fed a feed assumed to be good and the fish on the right are fed a suspect feed.

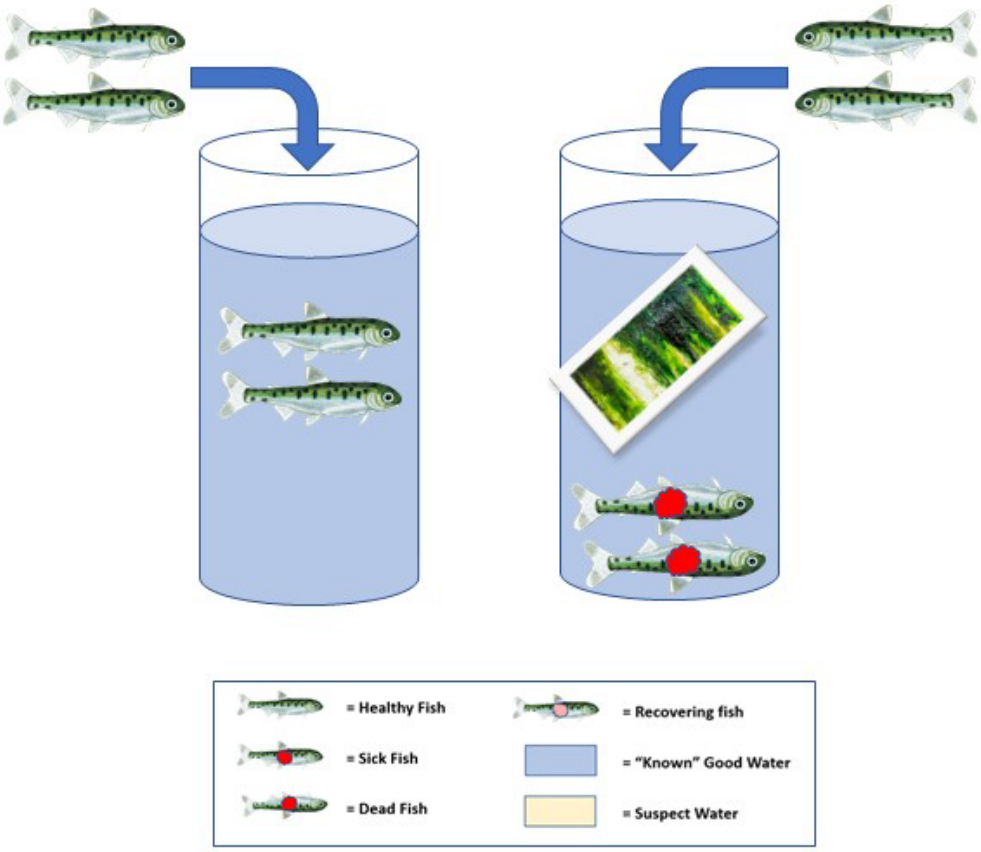
Result: The healthy fish with good feed stay healthy while the fish on the suspect feed sick.

What does it tell us?



What does it tell us?

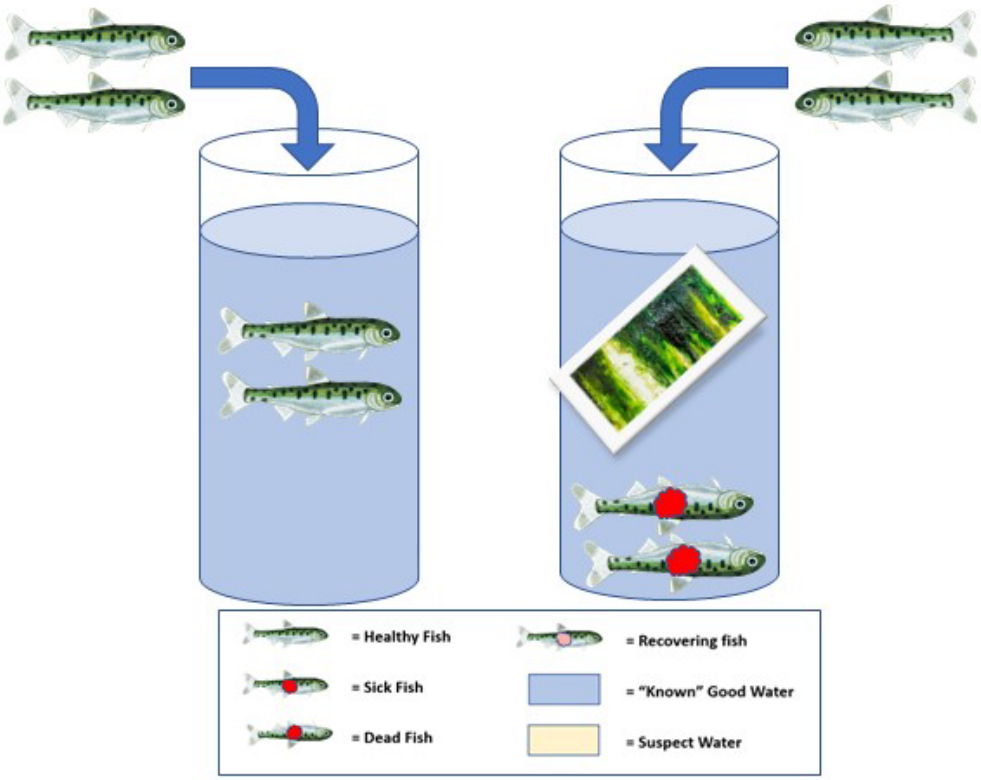
This assay result implies that the fish health problem is feed related. Why is the tank on the left important? It is the negative control tank. For a valid assay result, these fish must be just the same as the fish on the right with the only difference being the exposure to the suspect feed. If these left-tank control fish die, it means that you've messed up your assay.



Setup: Two tanks, both filled with water that "known" to be good. Tanks can be static (aerated) for short term experiments or flow-through for longer term. Healthy fish from another source are put into both tanks. In the tank on the right you add a suspect toxin source (equipment, coatings, algae covered substrates etc.)

Result: The healthy fish on the left stay healthy while the fish exposed to the test materials become sick.

What does it tell us?



What does it tell us?

This assay result implies that the fish health problem is related to whatever you put into the tank on the right. The negative control tank is very important. If these left-tank control fish die, it means that you've messed up your assay.

More thoughts on bioassays

Carefully designed bioassays with good controls can provide very useful diagnostic information when other approaches fail. However, like any other approach, you must keep in mind that while one possible outcome of a particular bioassay might be very helpful, there are probably at least three other possible outcomes that would be noninformative, confusing, or misleading. Of special concern are disease problems that might be caused by two problems working together, like an infectious disease that only happens under environmental conditions that aren't replicated in your bioassay. Bioassays are just one piece of evidence that must fit into the diagnostic puzzle.

Putting it All Together

The following section has real-world examples of fish disease problems that were not diagnosed by standard lab tests. These are an example of lab test limitations, the power of other approaches, and the challenge that new diseases represent.

CASE 1

In the winter, there were catastrophic die-offs of cultured channel catfish. Most losses occurred shortly after severe cold spells. Some of the dead fish had fungal infections, but not all. When fish were dying, they exhibited bizarre "porpoising" behaviors and would often run out on the bank. Among the dead fish were some that had their stomachs everted inside-out in their mouths.

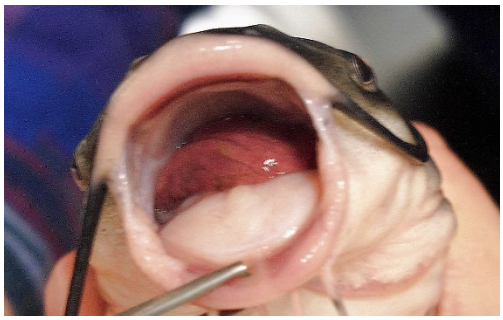


Figure 13: A channel catfish with its stomach inside out in its mouth.

<u>The Clues</u>

No parasites, bacterial diseases, or viruses were detected in lab tests.

Only 10% of the dead fish had serious fungal infections

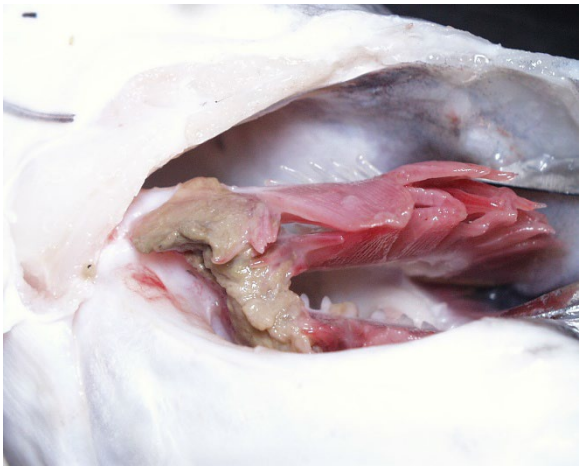


Figure 14: When winter water temps drop more than 10 degrees in 24 hrs, catfish have problems with water and salt balance, and their immune systems are compromised. This predisposes them to saprolegnia (water mold) infections (the tan area on the gills).

No water quality problems were detected.

Algal or other environmental toxins were unlikely because, especially early in the outbreak, only a small percentage of fish were affected.

It occurred in cold weather when fish weren't being fed.

Co-habitation of sick fish with healthy fish didn't cause the healthy fish to get sick.

Plasma from the blood of a sick fish sometimes quickly killed a healthy fish receiving it by injection. Filtering the plasma didn't reduce the effect.

Histopathology yielded nothing helpful. Most tissues were normal except for damage that resulted from the everted stomach or porpoising behavior.

The disease was most common in ponds where tilapia were also present

The disease went for not-even-noticed noticed to widespread and catastrophic in a single season.

It occurred only in a geographically limited area of the Southwest along the Mississippi River.

The leading hypothesis

The best explanation was that the problem was caused by a potent neurotoxin coming from something that the fish were eating. The best guess was botulism. Unfortunately, the same plasma that caused death in healthy catfish had no effect on mice in the standard mouse bioassay test. The mice survived huge injections of the plasma, and actually seemed to thrive on the extra protein. The sudden appearance of the disease also argued against botulism because clostridia bacteria are widespread and there have always been some dead catfish in mid-winter.

The rest of the story

After 2 winters of heavy losses, a brilliant scientist collected toxic plasma samples from catfish, mixed them with antibodies that neutralize specific botulism toxins, then injected them into healthy catfish. All of the injected fish died except for those that got serum mixed with antibodies to Type E Botulism toxin. With that data in hand, the entire story fit together.

During cold spells, a few catfish died from fungal infections. They would settle to the pond bottom where they were colonized by clostridia bacteria that produced Type E Toxin. When it warmed a little, other hungry catfish would feed on the dead fish picking up both the toxin, and the clostridia that produced it. Many fish would feed on a single dead fish. They would be killed by the toxin and all of those fish would then decompose and produce toxins, and be eaten by other fish. A single toxic fish might kill 10 fish that would in turn kill 100 fish that would then kill 1000 fish, then 10,000. It was more common in ponds with tilapia because the tilapia would die in cold weather and set of the chain reaction.

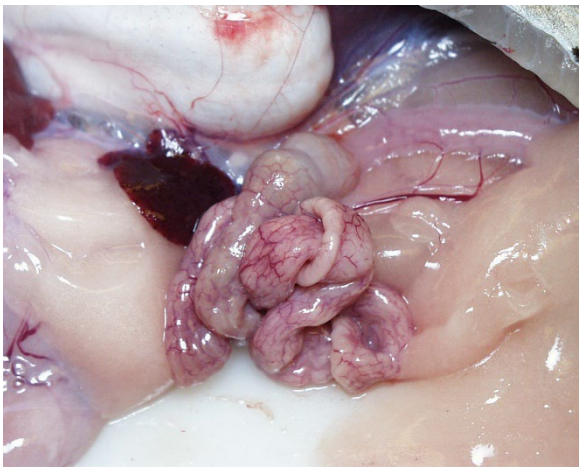


Figure 15: Another spectacular lesion associated with this disease were intestinal intussusceptions. In the center of this picture, the intestine is folded into itself like an inside-out sock, a sign of nerve malfunction.

The mouse bioassays for botulism failed because the toxin produced by these clostridia was so heat sensitive that it was immediately neutralized by the body temperature of the test mice.

It only occurred in the winter because 1) in warmer water other bacteria decomposed dead fish before clostridia could grow, 2) warmer water neutralized the toxin, and 3) because

farmers fed the fish when temperatures were warm.

The Resolution

Farmers quit using tilapia in catfish ponds to reduce the probability of clostridia growing.

Farmers offered feed when a warm spell followed an intense cold spell to encourage catfish to eat the feed rather than their dead brethren

The disease incidence and severity was reduced to where it was no longer a major concern.

The Unresolved Mystery

One of the greatest mysteries, and it still remains, is why this botulism problem went from essentially nonexistent to severe and widespread in a single season. Clostridia are widely distributed and common in the environment and there have always been some losses of catfish during winter cold spells. My best guess is that a new strain of clostridia was imported and spread by humans or migratory birds.

Case 2

Largemouth bass, raised on feeds in earthen ponds, grew beautifully to 1.5 pounds and were then trucked live to seafood markets. During some seasons, there were losses in the ponds and mortality during shipping was high. The farmer noticed that some fish that died were yellow in color.

The Clues

All lab tests for infectious diseases were negative.

Histology of fish revealed massive widespread damage to the liver.

The disease could not be passed to other fish by cohabitation or injection.

There were no correlations between water quality and the disease, but it did have a typical season and effect a specific age of fish.

Fish that survived the sensitive period were not subject to further losses in ponds or during shipping

The fish were fed the floating version of a high quality salmon ration.

Other fish species living in the same ponds and eating the same feed did fine.

The Leading Hypothesis

Stumped… Maybe a toxin? Why only the bass? Why only the 1.5# fish?

The Rest of the Story

Liver histology was performed monthly throughout the life cycle of the fish. As the fish grew, carbohydrate accumulated in the liver cells until they became so swollen and damaged that most of the liver died. After that crisis, the only liver cells left were those that didn't store carbohydrates. These cells would proliferate and produce a new strangely-shaped liver that was not subject to carbohydrate damage. The fish were yellow because their damaged livers were leaking bile into their blood.

Figure 16: Livers of large bass that survived looked cancerous, but the nodules were actually regenerating normal liver cells.

The floating salmon feed was too high in carbohydrates for the strictly piscivorous

largemouth bass. It was especially problematic on this farm because the high heat used in making floating feeds leads to more available carbohydrate than is found in sinking feeds with the same formula. Other fish species in the ponds were unaffected because they were better able to metabolize carbohydrates.

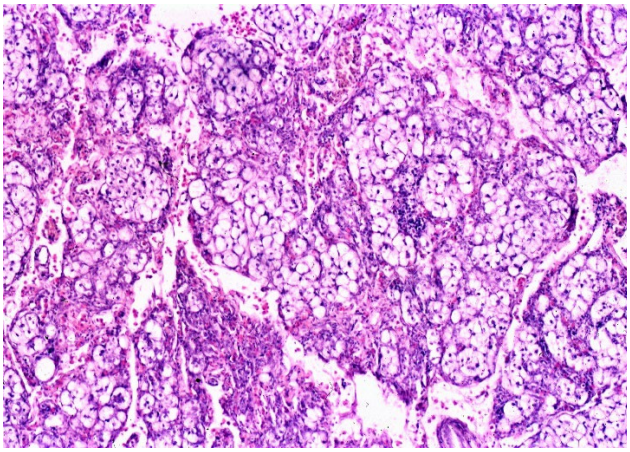


Figure 17: Histology of the liver of a bass fed a diet too high in carbohydrates. The liver cells are transparent and spongy-looking because they are loaded with glycogen, a storage form of carbohydrate.

The Resolution

The farmer's feed manufacturer quickly whipped up a new floating feed formula that was so low in available carbohydrate that it had been previously considered impossible. Switching over to that feed solved the liver problem and the feed was quickly adopted throughout the industry. A side effect of the feed change was that, to be low in carbohydrates, the new feed had to be higher in protein and fat than the previous feed. This gave it a lot more calories leading to bass with a lot of fat. This could have been a disaster, but it turned out that the market for these fish had a strong preference for high fat levels and the new fish were sold at a premium.

Case 4

Large channel catfish raised in earthen ponds suddenly started dying by the tens of thousands, on several farms in two states. The disease seemed to be spreading from farm to farm.

Clues

The disease seemed to be spreading from pond to pond and farm to farm

Fish had bright pink/skins and internal organs

Figure 18: The pink color of the skin of this catfish resulted from damage to blood vessels in the skin.

The only disease organisms found was Aeromomas hydrophila, it was present on huge numbers in pure culture from affected fish.

Aeromonas hydrophila is a common bacterium found free living in the environment, and on the skin and in the gut of healthy fish. When we isolate it from fish, we consider it to be a secondary infection.

Hypothesis

A new disease virulent strain of Aeromonas hydrophila

Rest of the story

Attempts to reproduce the disease by exposing healthy catfish to the Aeromonas isolate revealed no differences between the disease isolate and the bacteria commonly found in secondary infections.

While there are many strains of Aeromonas hydrophila, all of the isolates from sick fish shared the same biochemical characteristics (what they eat and what they secrete).

The correlation between the unique lesions (pink skin and organs), heavy mortality, and the single strain of Aeromonas was rock solid.

DNA sequencing revealed that all of the disease isolates were very similar, and closely related to Aeromonas associated with a serious fish disease problem in China.

Resolution

A new Aeromonas strain was introduced. It had the capability of being the primary cause of fish disease rather than just appearing as a secondary problem when fish were compromised by other health problems.

The Mystery

In controlled disease challenge experiments, the new Aeromonas strain is indistinguishable from common and ubiquitous Aeromonas strains that catfish see every day. The correlation between the unique new strain and the new disease in natural infections in ponds is so solid that there is no doubt that the bacterium is responsible, but scientists cannot explain why the disease cannot be reproduced in the lab.

Other Cool Cases

For decades, rainbow trout have been afflicted by "strawberry disease" that appears as read blotches on the fish's skin. Despite its commercial importance and a determined research effort, the cause was unknown. Recently, scientists have found a correlation between strawberry disease a primitive type of bacteria that do not grow on agar plates and are extremely difficult to detect by histopathology.

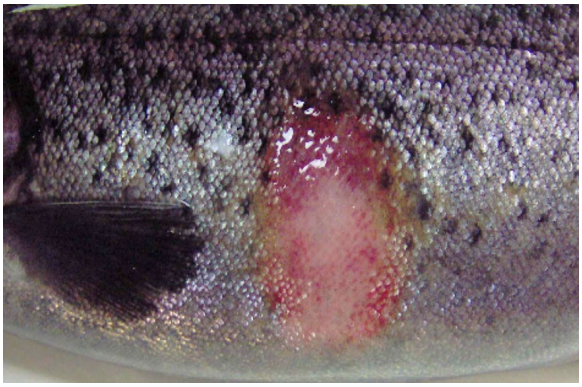


Figure 19: Strawberry disease lesions on a trout.

Channel catfish in ponds sometimes experience summer outbreaks of catfish anemia. The disease afflicts large numbers of fish in seemingly random ponds. Some farms are more prone to the problem than others and the disease almost always occurs in mid-summer. Losses can be severe. The disease has a sudden onset and the loss of red blood cells is so pronounced that the blood of afflicted fish may look like water with a slight pinkish cast. Despite decades of effort, the disease cannot be produced in controlled settings and the cause is completely unknown.

Figure 20: The gills of this catfish are a pale pink because there are almost no red blood cells in circulation.

Farmed golden shiner minnows experience die-offs, especially in hot weather, that don't seem to be tied to any bacteria or parasite infections. Virus cultures though often detected a virus associated with the fish losses. There was an

excellent correlation between the disease and the virus and experimental exposures showed that the virus propagated to much higher levels in fish stressed by heat or crowding. Problem solved? No! The virus is an aquareovirus. This virus family often percolates along at low levels in healthy animals without causing disease. When a fish is stressed, its immune system is compromised and the aquareovirus responds by replicating at a higher level. The correlation between detection of the virus and the disease is strong, but there is no cause and effect. The best evidence for this conclusion is that the virus can sometimes be found at high levels in apparently-healthy fish, and that experimental stresses often lead to high virus levels but no disease. Interestingly, sequencing the RNA of the golden shiner virus revealed that it is a very close relative of the Chinese Grass Carp Aquareovirus. This virus causes major losses in grass carp culture in China and may be the source of the golden shiner virus in the USA.

Figure 21: Th red muscle and bulging eyes seen in this golden shiner are often attributed to GSV, but there is almost no correlation between the presence of the lesions and the virus.

Many kinds of fish, including salmon, are sometimes hosts for the single-celled intestinal parasite hexamita (now more properly known as Spironucleus). Hatcheries, especially in cooler months, sometimes have raceways where feed consumption drops and some fish cease feeding altogether. They become thin, and eventually die. Microscopic examination of gut contents reveals hexamita, and the levels are highest in raceways that are feeding the least and in fish that have stopped feeding altogether. Thus, we have an excellent correlation between disease and the parasite. The problem though is that, like the golden shiner virus, hexamita burbles along at low levels in healthy fish and then explodes in fish that have been immunocompromised by another disease. This always leaves us with a tough chicken-or-the-egg question: “Are fish off-feed because of the hexamita, or are the hexamita exploding because the fish are off feed?’ There is no way to tell, but we suspect that the hexamita is very often secondary to another problem. This leads to some very difficult treatment questions” “Do we treat a secondary problem when it is being caused by something else? Given that we sometimes see high levels in healthy fish, do we treat at all? We are going to explore treatment decisions in the next Fish Health News, but (spoiler alert) in hexamita we often make the treatment decision based on the severity of the intestinal damage that we see by histology.

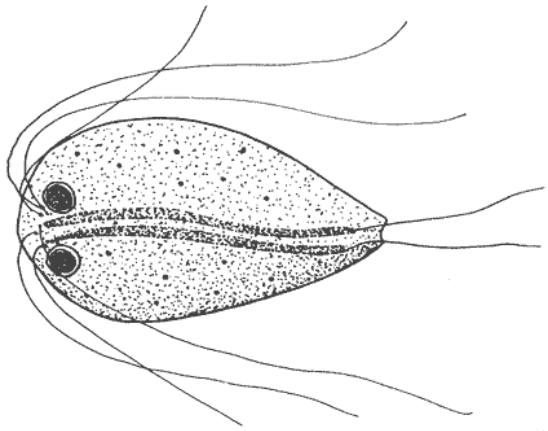


Figure 22: Spironucleus (hexamita) is a tiny flagellated intestinal parasite.

Another very difficult area is toxins produced by bluegreen algae (cyanobacteria). In the southeast, farmers sometimes experienced catastrophic losses of catfish raised in 10-20 acre earthen ponds. Mortality could approach

100% and fish could go from healthy and feeding to dead in just a few hours. The sudden onset and complete mortality made algal toxins the first hypothesis, but determining which algae and which toxin was involved, and figuring out what to do about it, was a lot tougher. There are many species of algae that make toxins and many types of toxins. Some toxins cause liver damage, others nerve damage, and some are powerful skin irritants. To make matters even more complicated, there is huge variation in toxin production within species and many only produce toxins under certain conditions, or only release stored toxins under certain conditions. The only possible approach for solving the catfish losses was to monitor algal species and density in dozens of ponds weekly, for months, in hopes that the algae could be caught in the act. After months of work, a specific bluegreen algae was found that bloomed to high densities and sometimes crashed in a synchronized way.

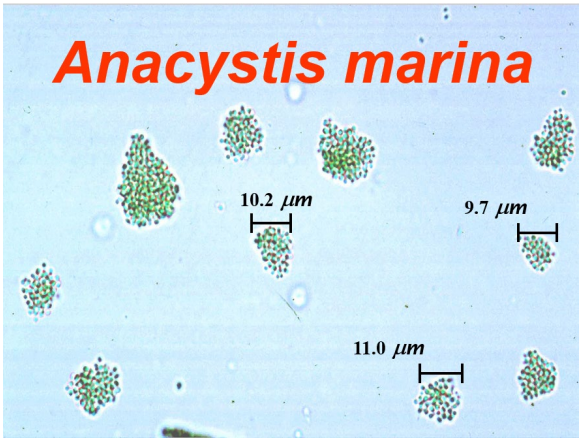


Figure 23: The unimpressive algae that caused massive fish kills.

The catfish losses occurred when the algae became stressed, produced toxin, then released it when the cells died. The algal die-offs were so complete that investigating algal populations after a catfish die off was useless because the problem algae were essentially gone. The rest of the story: The algae was only growing in a region that had salinities of 2-4 parts per thousand (1/10 seawater) in their ponds. A massive monitoring program was begun in hope that we could head off disasters by managing algal blooms when the potentially toxic algae started to dominate. Soon after the program was up and running, a massive tropical storm dropped two feet of rain on the region in a single day. The salt-loving algae almost disappeared even though many ponds remained in its optimum salinity range. In the many years since, problems with that algae have not returned.

Figure 24: A classic bucket bioassay used to conform the role of this algae in fish kills.

As evidence of how complex algae toxins can be, another research group published a paper about the problem that mis-identified the algae and the toxin. The liver toxin "microcystin" was blamed but other research showed that catfish were quite resistant to microcystin toxin, and that microcystin produced obvious liver lesions that were not seen in these cases. The potent neurotoxin that was causing the problem remains un-identified and un-described.

Figure 25: A catfish pond hit by a toxic algae bloom. Note the deep green color of the water.

Back in the ornamental fish world, there is a skin disease called Hikkui that hits expensive koi. The disease progresses slowly for years causing unattractive damage to the surface of the fish and ruining their value as show animals. Despite years of study, the cause is unknown.

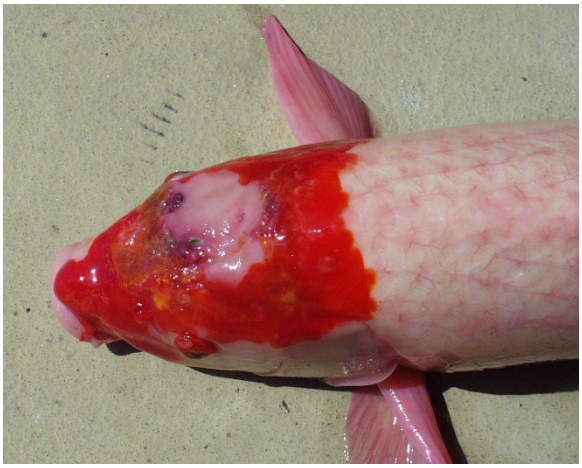


Figure 26: Hikkui has, over several years, caused an irregular pink patch in the red skin of the head of this koi. The disease is disfiguring, but not life threatening.

Hikkui is not the only mysterious koi disease. Another that is completely unsolved is "laying down disease" (not to be confused with the "Koi Sleepy Disease Virus"). Sometimes when koi are harvested from earthen ponds and put in tanks indoors, the farmer will check on the fish several hours after harvest and find them laying at the bottom of the tank. Bang the tank and they all pop up, swim normally, and even feed. A few minutes later they are all back on their sides on the bottom. The cause of those behavior is an underinflated swim bladder. It takes a lot of energy for underinflated fish to stay up in the water so when they get tired, they sink and roll over. If the stress doesn't lead to fatal bacterial infections, the fish recover in 4-6 weeks. Oddly, putting the fish back into an earthen pond leads to a much quicker resolution of the symptoms. Nobody has been able to produce this behavior in controlled circumstances and the cause of the disease is unknown.

Figure 27: Laying down disease also happens in several strain of fancy goldfish.

Figure 28: Aphanizominon algae cells associated with this case.

Algae was collected and concentrated and fed to healthy fish where it triggered the disease within a few hours. Examination of fish from the pond revealed that they were full of partially digested algae while the healthy fish

were full of feed. The sick fish were those that were feeding on a floating scum of the algae.

Figure 29: A very heavy bloom with a floating scum of Aphanizominon bluegreen algae.

A great graduate student, Scott Snyder, came up with a solution that prevented further losses. The farmer turned on all of the paddlewheel aerators and thus prevented the scum from forming so the catfish could no longer feed on the algae.

Figure 30: Paddlewheel aerators preventing the formation of an algal scum that catfish could feed on.

Conclusions

Collecting samples, submitting them to a testing lab, and reading reports is relatively simple, but this is just a small part of the puzzle involved in diagnosing and managing infectious and non-infectious diseases in fish. Even perfectly-performed lab tests can miss the cause of infectious and noninfectious diseases, or mislead us by finding apparent pathogens that aren't the real problem. Our Region's fish health professionals earn their place in the FWS by collecting all kinds of epidemiological information, consulting with scientists and other experts, conducting bioassays and experiments, and making sure that all of the pieces fit into a comprehensive explanation of the cause of a fish health problem. Then they use that information to determine if there is a safe and legal treatment to stop further losses and improve fish welfare. Finally, they develop plans to prevent similar problems in the future. To do that all successfully requires a broad and comprehensive knowledge of infectious and non-infectious diseases, test methods, water quality, toxicology, physiology, chemistry, drug and chemical regulations, pharmacology, and husbandry, combined with an inquisitive nature, puzzle solving skills, and the ability to inspire the confidence necessary to gain support for treatment recommendations and husbandry changes.