# Fish Health News You Can Use

**Brought to you by the Pacific Region Fish Health Program**

**Fall 2023 Edition**

## This Issue (#13)



## Next Edition:

A look into the future. We are planning a theme edition building onto this issue's article on pain and fish welfare. Stress, handling, anesthesia, euthanasia and more.


**Contact Us**

Andrew Goodwin
Pacific Region Fish Health Program Manager
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/office/pacific-region-fish-health-program

## The Aeromonas Bacteria Family

More than in any other group of bacteria, the taxonomy of the aeromonads is always confusing. This is unfortunate because this group includes many important fish pathogens, and some species in this genus are nearly ubiquitous in the environment. The simplest way to look at this genus is to break it into motile (swimming) and non-motile species, and to then break the non-motile species down into those that produce a brown pigment in culture and those that do not. The motile species are everywhere and cause disease when fish are injured or stressed, especially at warm temperatures. In the non-motile species, the ones that make brown pigment cause furunculosis and those that don't cause ulcers. That sounds easy, but the reality is that some furunculosis bacteria don't make the brown pigment, some that do make the pigment cause ulcers, and there are species that clearly don't fit into the right categories for motility. To make matters even worse, the motile varieties that are ubiquitous in the environment are often subdivided into a bewildering number of species that no two papers agree on. Fortunately, our colleagues at WADDL have some very sophisticated instrumentation (and sophisticated microbiologists!) that can reliably put these bacteria into three groups.

*Figure 1: A culture tube with agar growth medium. Brown pigment produced by Furunculosis bacteria (Aeromonas salmonicda salmonicida) has stained the top part of the agar brown.*

1) **Aeromonas bacteria that cause furunculosis:** Non-motile and make brown pigment (usually). These bacteria have been found in many species of freshwater and marine fish, but the strains that are important to us cause furunculosis disease in salmon, especially when high temps and low flows are an issue. We have also found them in Pacific lamprey but not associated with disease. Furunculosis is relatively easy to diagnose and, in an emergency, treatable with medicated feed.

*Figure 2: A juvenile coho (left) with typical furunculosis. In the right, the same disease in an adult lake trout. Both fish have raised skin lesions that superficially resemble "furuncles", the boils that sometimes occur in the skin of mammals.*

2) **Aeromonas bacteria that cause ulcer diseases:** Non-motile, no pigment (usually). These bacteria cause skin ulcer diseases in salmonids and in many other species of fish. I have not seen a case on a R1 hatchery, but these bacteria are very important in other places and in other

species. To make matters more complicated, they are exceedingly difficult to isolate and grow very slowly in culture. Ulcer diseases caused by these bacteria are also quite tricky to treat and antibiotic resistance is quite common, especially in strains that circulate in koi.

*Figure 3: A koi with skin ulcers caused by Aeromonas salmonicida achromogenes. As you can tell by the subspecies name, these bacteria do not produce a brown pigment.*

3) U**biquitous environmental Aeromonas bacteria that cause septicemia:** Motile, no pigment (usually). Unlike the previous two groups, these bacteria can live free in the environment and only attack fish when the fish host has been injured or compromised by stress, injury, other diseases, or poor environmental conditions. They are often even a part of the skin and intestinal flora of healthy fish. Treatment focuses on correcting environmental conditions. The rapid emergence of antibiotic resistance generally makes medicated feeds useless.

*Figure 4: A typical motile Aeromonas infection in the tail of a white bass. These lesions often start as infections of skin injuries that get infected by normally free-living bacteria from the environment.*

Being able to reliably put the Aeromonas bacteria into these three groups is enormously important because the management of diseases caused by the three groups are so different. With the furunculosis bacteria (our group 1), we are going to try to find ways to reduce temperature and improve Density Index and Flow Index, and treat with medicated feed if necessary. With the motile Aeromonas (group 3) we are very unlikely to consider antibiotics and will instead try to identify the environmental problem that is predisposing fish to infection by these normally benign bacteria. With group 2 (the ulcers), we are probably going to convene an emergency HET meeting to discuss a response to what is, to us, an exotic disease!

*Figure 5: A channel catfish with a systemic motile Aeromonas infection caused by a very virulent strain that first appeared about 15 years ago. This strain is highly contagious, spreads quickly even in healthy fish, and has a very high mortality rate. It may have been introduced from Asia.*

## Aquatic Animal Health Plans

The new(ish) FWS Fish Health Policy (FHP) requires that HET-like teams put together Aquatic Animal Health Plans (AAHPs) for every FWS-operated hatchery. The final description of AAHPs, (in the Handbook document that is cited in the FHP) seem onerous, but there are three things for hatchery staff to keep in mind:

- A lot of the information in the AAHP will be really useful during changeovers in staff (at the hatchery, PRFHP, HET) to carry on institutional memory.
- Most of the work is being done by the PRFHP; the rest of the HET serves as reviewers.
- Along with the bits and pieces mandated in the Handbook, there is one especially useful section that motivated our Region's support for AAHPs. This section identifies the

diseases of importance to the hatchery, how they will be avoided, and how they will be managed should they occur.

Let's look at this last bulleted item (diseases of importance) and how it will address three categories of disease:

1. *Exotic diseases that are not known to be present on the facility but might arrive through some plausible pathway and would cause significant problems if they did.* This would include pathogens like IPN virus or whirling disease. The best protection against these is surveillance and biosecurity. Diseases in this category may carry a heavy policy and regulatory burden so AAHP details may include quarantine and eradication measures.
2. *Diseases known to be widespread throughout the PNW, but that can be avoided through careful husbandry or effective diagnosis and treatment.* This would include diseases like bacterial gill disease and furunculosis.
3. *Diseases that can't be avoided by any practical means and must therefore be managed to minimize fish losses.* This would include bacterial kidney disease in spring Chinook and common parasites present in hatchery water supplies (ich, C. shasta…).

The PRFHP staff have been working on this project and have completed early drafts for some hatcheries. Our plan is to complete several in FY 2024, representing the PSOP, Leavenworth, and Lower Columbia Complexes, and to complete this project in FY 2025. We look forward to sharing these drafts with our HET colleagues and working to produce final versions and the required annual updates.

> **Aquatic Animal Health Policy**
>
> 713 FW 1
>
> Aquatic Animal Health Policy Introduction
>
> Exhibit 1
>
> Glossary for the Aquatic Animal Health Policy
>
> 713 FW 2
>
> Aquatic Animal Health Operations

## Reporting Drug and Chemical Use

Speaking of the New Fish Health Policy…

Remember that it requires reporting of all aquatic animal drug and chemical use by the FWS. AADAP is developing a database for FAC to use. It is still a work in progress. The reporting responsibility falls on the drug and chemical users (hatcheries, refuges, etc.) but the PRFHP will provide all the support we can. We are encouraging AADAP to include hatcheries and other users groups in the development of the database.

*Figure 6: Blue Plastic Chemical Drums*

# Fish, Pain, and Animal Welfare

Do fish feel pain, anxiety, and fear the same way that humans do? To make a long story short, the answer is unknown and probably unknowable. Because of that uncertainty, the PRFHP staff are always going to give fish the benefit of the doubt and recommend that we treat fish with the same level of welfare concern that we apply to mammals. Basically, if it wouldn't be okay for your dog, it isn't okay for a fish. This level of concern might be appropriate, or it might be overkill…why don't we know?

To understand the problem, we'll start out by talking about pain. In humans, pain starts with a message through your nervous system that tissue is being damaged. That message causes pain, a strong emotional response that is very unpleasant and motivates you to avoid situations where that response might be triggered. It is nature's way of making sure that you work hard to protect yourself from injuries, and that you will only voluntarily accept an injury when the perceived benefit outweighs the penalty of the pain emotion. A strong pain emotion motivates you to prevent unnecessary damage to your body and is of great benefit.

Things are not as simple in fish. We know that they have sensors for tissue damage just like humans, that those sensors send messages to the brain when damage occurs, and that the fish will then avoid whatever is causing the damage. The big question is whether this avoidance involves a very unpleasant pain emotion like it does in humans, or are these damage messages just part of a hard-wired reflex loop that triggers avoidance without pain?

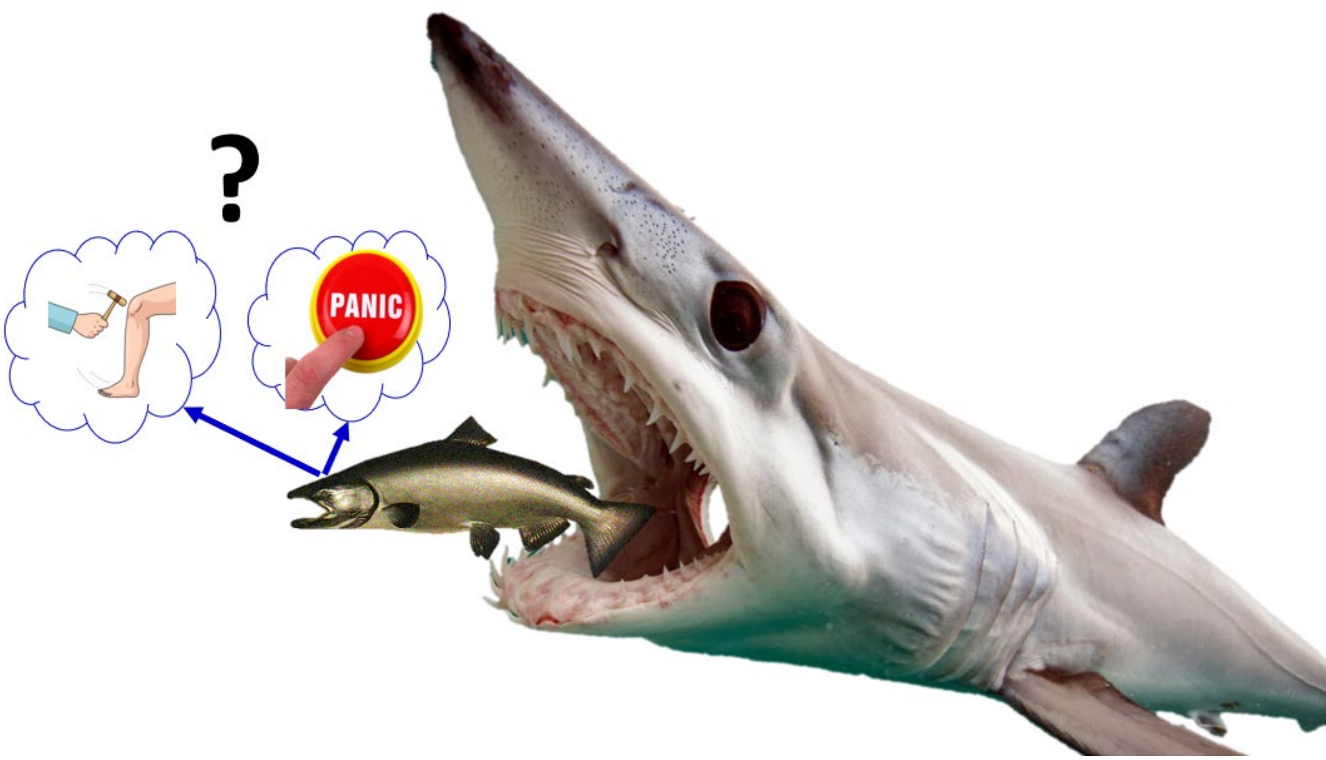



*Figure 7: Do fish feel emotions like fear and pain, or is everything just reflex? How can we tell?*

There is some evidence that fish experience pain differently than mammals.  We can argue that the smaller and simpler brains of fish seem much better suited to reflex responses than they do to complex emotions like pain.  More importantly, we can look at the differences in behavior between an injured mammal and an injured fish.  In general, an injured mammal will respond in ways that we readily recognize as a pain response. They will avoid using the damaged body part, curl up in a corner, and display many of the other behaviors that we ourselves would exhibit when hurt.  Fish are different.  It seems like injured fish will continue to carry on as normal to the greatest extent allowed by the mechanical limitations imposed by the injury. In other words, while fish certainly work to avoid injury, once an injury occurs it does not seem to have the emotional impact that we see in mammals.

If injured fish just keep on carrying on, does this mean that the fish isn't feeling the pain emotion and that injury avoidance is a simple reflex?  Unfortunately, there is no way to know.  We cannot rule out the possibility that fish do experience the pain emotion but that they don't communicate the distress in a way that we understand as mammals.  It is hard enough to try to understand the emotions of our own families: what chance do we have of understanding fish feelings?

The question of pain perception in fish has been debated for decades. There are hundreds of papers, protocols, positions, and scientific meetings that explore this controversial issue. Unfortunately, there is no way to measure fish emotions, or to even tell if fish brains use emotions to motivate self-protective behaviors.  It seems like the answer is unknown and unknowable. So what do we do?  The only ethical answer is to give fish (and frogs, and reptiles…) the benefit of the doubt.


*Figure 8: A selection of books about fish welfare.  Fish are also included in most books about animal welfare.*

The best approach is to think of pain and distress in fish the same way that you would in your dog.  Ignore the lack of empathy between humans and fish that comes so easily with our companion animals, admit that the final answer to fish and pain is probably unknowable, and treat fish with the same concern that you would a cat, dog, or horse.  Not only is this the ethical thing to do, but in many situations the avoidance of pain and distress pays off as better growth and survival.  A win for fish and for people.

We in the PRFHP are very proud of the concern for fish welfare that is a daily part of FAC work in this Region. If you have any animal welfare concerns, please let your PRFHP contacts know so that we can help you to find better solutions.

## Prebiotic, Probiotics, and Fish Health

First, a couple of definitions with a fish spin.

**Probiotic:** Living microorganisms put into, or onto, fish to improve growth or disease resistance. As an example, in humans, yoghurt is full of live bacteria that when consumed are thought to become part of the gut flora and provide health benefits.

**Prebiotic:** Non-living ingredients, usually digestible by bacteria and not by fish, that provide food for gut bacteria and thereby enhance the growth of those bacteria in a way that is intended to improve growth or disease resistance in the fish. In short, they are feed ingredients found to promote the growth of beneficial gut bacteria. Prebiotic feed ingredients can also directly stimulate activity in immune system cells, triggering a non-specific immune response.

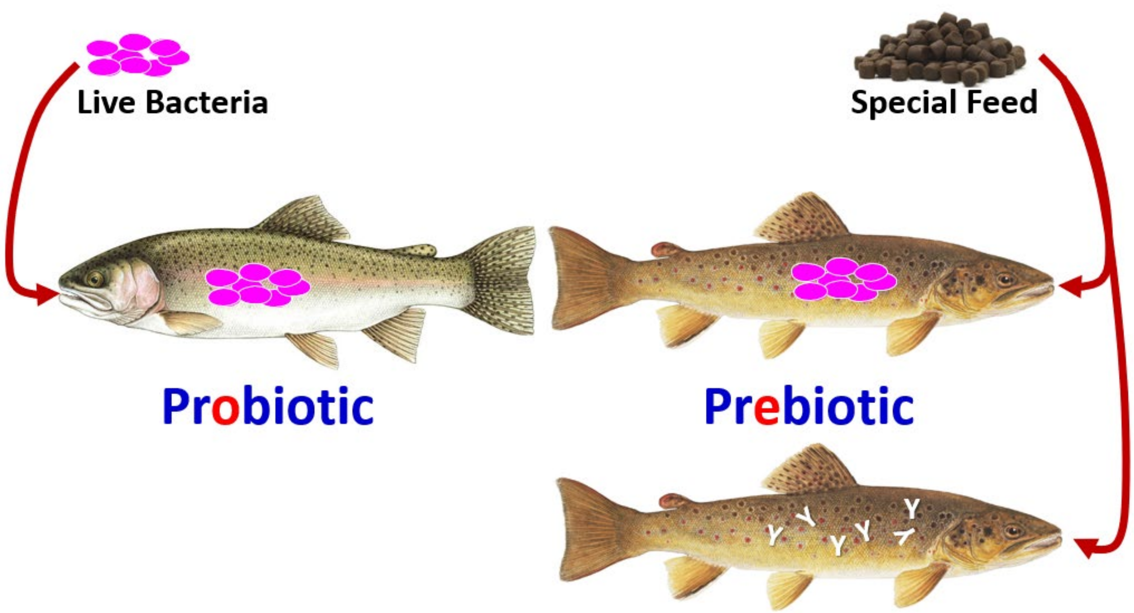



*Figure 9: Probiotics are live bacteria, like yoghurt. Prebiotics are either foods for bacteria, or substances that may be immunostimulants.*

Both probiotics (alive) and prebiotoics (food for bacteria) are designed to produce the growth of beneficial bacteria, usually in the gut. So how might these additional gut bacteria help the fish?

There are several possibilities:

1) They may provide vitamins or nutrients of value to the fish.

2) They could outcompete less-desirable bacteria that might be associated with disease or other adverse effects.

3) They may trigger an immune response, without causing disease themselves, and thereby help protect the fish from pathogens.

*But do they work?* There are some reasons for doubt:

1) Probiotics (living organisms) are sometimes organisms not normally found in a healthy fish gut. Can they survive? Do they have benefits? Is changing fishes' gut flora a good idea?

2) Probiotics can themselves cause disease in compromised hosts or provide antibiotic resistance genes to pathogenic bacteria

3) Prebiotics (non-living nutrients to support bacteria) might support the growth of good bacteria, bacteria that cause harm, or nothing at all. There is no control.

Things get even more complicated when we look at probiotic bacteria or prebiotics that are intended to function as immunostimulants. If a probiotic or prebiotic can induce an up-regulation of the fish's immune response, it obviously means that the fish has the inherent genetic capacity to do that up-regulation. If fish have that capacity, we must stop and think about why the fish have instead evolved to keep immunity at a lower level and only upregulate it when it is needed to fight a disease. This is the strategy that developed through millions of years of evolution and natural selection. There must be an advantage to that strategy and, by forcing constant upregulation with a pre or probiotic, we are interfering with the fine-tuned regulation chosen through natural selection.

So, what are the potential problems associated with constantly upregulated immune function?

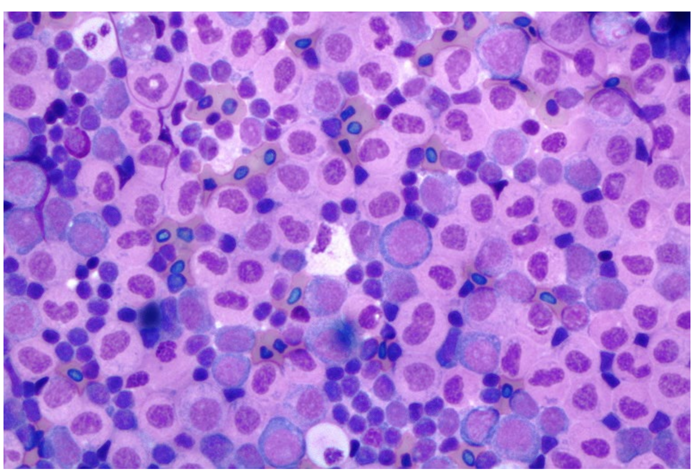


*Figure 10: A stained imprint of a fish kidney. Many of these cells are immature white blood cells waiting to be called into action to fish an infection.*

1) The obvious answer is that keeping the immune system turned up high all the time is metabolically expensive. Immunity is very energy intensive and unnecessary upregulation wastes energy.

2) An upregulated immune system is more likely to lead to unintended consequences like trying to fight off innocuous organisms. It might even trigger autoimmune diseases.

3) We might also upregulate one part of the immune system at the expense of another part and destroy the delicate balance that the fish rely on to stay healthy.

So what does the science say?  There are hundreds of papers that look at pre- and probiotics in fish and other animals.  Many of them are studies that show beneficial effects, especially in disease resistance. However, it is important to note that most of these studies are short term. Other work shows that the effects of immunostimulant pre- or probiotics are transient, and that fish re-regulate their immune function back to the baseline level within a few weeks even if the pre-or probiotic is still being used. Other studies show that lengthy stimulation can result in suppression. In addition, while laboratory studies often look encouraging, positive results in ponds are rare.

The literature in fish, like the literature in human medicine, has some tantalizing findings but it is clear that pre- and probiotics are not a silver bullet.  Commercial pre- and probiotics in fish foods seem unlikely to cause significant harm, but the benefits are largely unclear: pre- and probiotic use is no substitute for good husbandry.

## Timely and Accurate: How ELISA Assays Happen at WADDL

Every fall, the Washington Animal Disease Diagnostic Laboratory (WADDL)in Pullman, Washington, conducts about 4,000 ELISA assays to support the Pacific Region's efforts to manage BKD by culling eggs from heavily-infected Spring Chinook salmon broodfish.  In order for our Spring Chinook programs to be successful, the ELISA assays must be conducted with speed and accuracy to produce timely and reliable reports that PRFHP staff and hatchery managers can depend on for culling decisions. This is how it all works.

The PRFHP staff collects kidney samples at spawning.  Samples and spawns must all be clearly numbered so that the correct eggs can be culled when the ELISA results become available. Samples are rushed to shipping hubs for overnight delivery to WADDL.  Once at WADDL, samples are logged into the Laboratory Information Management System (LIMS) where they each receive a unique specimen identifier. The sample receiving staff also check to ensure that the temperature of the arriving samples is within spec and that the accession form submitted with the samples matches the samples that were received.  If there are questions or concerns, the submitter is contacted immediately. After the samples have been received and logged in, the "BKD ELISA Surveillance" test is ordered, and they are sent up to the Aquatic Health Laboratory for testing.

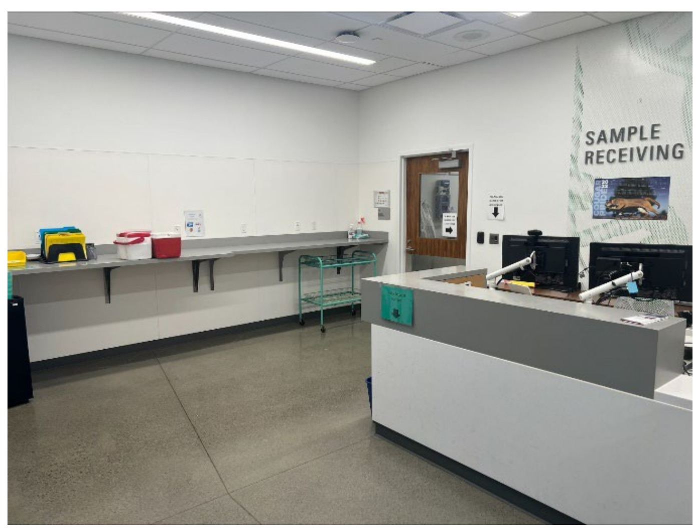



*Figure 11: The sample receiving room at WADDL.*

During the fall spawning season, WADDL has a special crew dedicated to processing the hundreds of BKD ELISA samples that arrive every week. Each member of this crew has gone through quality and safety training and has been specifically trained in the Standard Operating Procedures (SOPs) relevant to the parts of the testing that they are performing. All of the testing is done under the same rigorous third-party accreditation program (AAVLD) that controls almost all work done at WADDL and at other prestigious veterinary diagnostic labs in the US. WADDL is also a founding member in the National Animal Health Laboratory Network, and is approved by APHIS to do fish testing for international trade.

*Figure 12: Austin, Andrew, and Lilly Vo, the WADDL BKD ELISA processing crew*

In order to cope with the fall wave of BKD, ELISA samples, and to ensure the maximum accuracy and repeatability, WADDL has mechanized much of the ELISA assay process. Each sample is inspected to make sure it is suitable for testing, then weighed and diluted before homogenization. The homogenization step is conducted by a machine that shakes the sample tubes with buffer solutions and beads that break down kidney samples.

*Figure 13:The sample prep machine is the MVP of WADDL's Aquatic Health Laboratory during peak ELISA season. It homogenizes samples by shaking beads at 10,000 strokes per minute.*

After a few more processing steps, the sample is ready for the actual ELISA test. These tests are carried out on plastic plates that each have 96 test wells. WADDL uses a robot that loads the plates with samples and numerous controls, incubates them, rinses and replaces reagents for each step in the ELISA, and then sends the plates to a reader. When the test is complete, the reader passes a beam of light through each well and measures how much of the light is absorbed. It is so sensitive that it can accurately measure the color down to where only 1/10,000th of the light beam is able to pass through the sample. The reader is connected to a computer that can directly upload the test results into the LIMS.

*Figure 14: The ELISA Robot. Each of the 8 drawers on the right can incubate an ELISA plate under the specific conditions needed for each step in the assay. To the left is a robot that can remove plates from the incubator, wash them, add new reagents, put them back into the incubator, and then send them to the plate reader when they are done.*

Once there, it is reviewed for quality by another microbiologist and then reported out to PRFHP staff. With this robot, a single WADDL microbiologist can test up to 864 ELISA samples per week.

The skilled staff, training, quality control, accreditation, special facility, and advanced instrumentation are all expensive, but this investment provides the best possible assurance that the test results are accurate and timely.

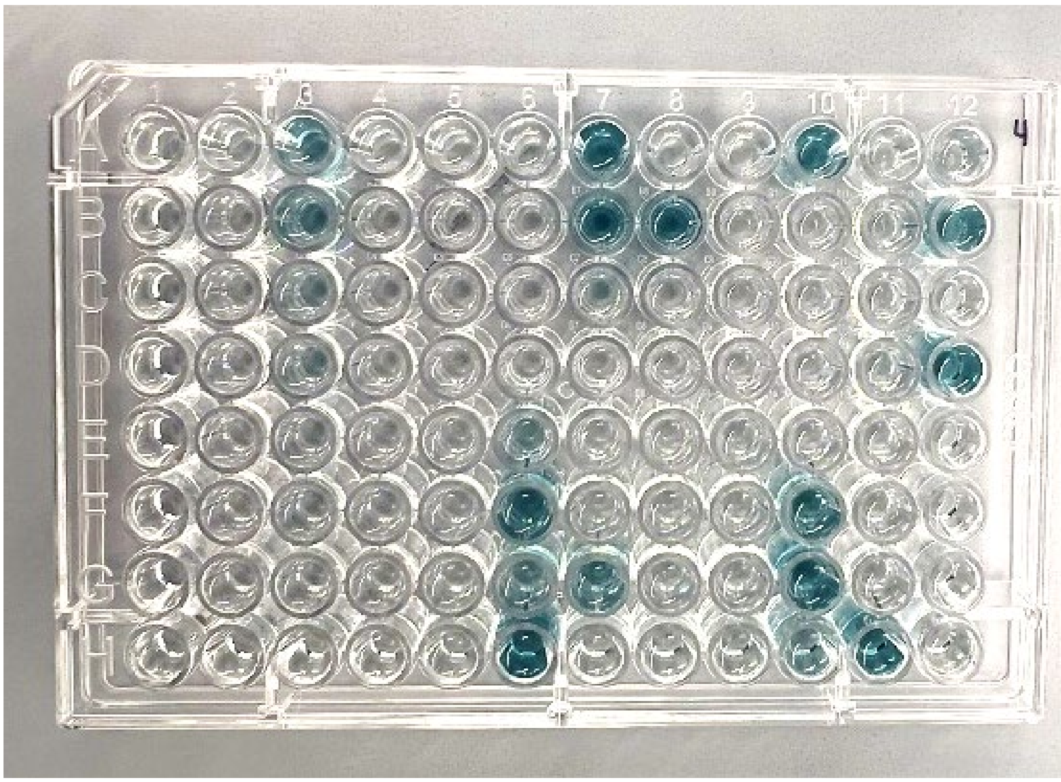


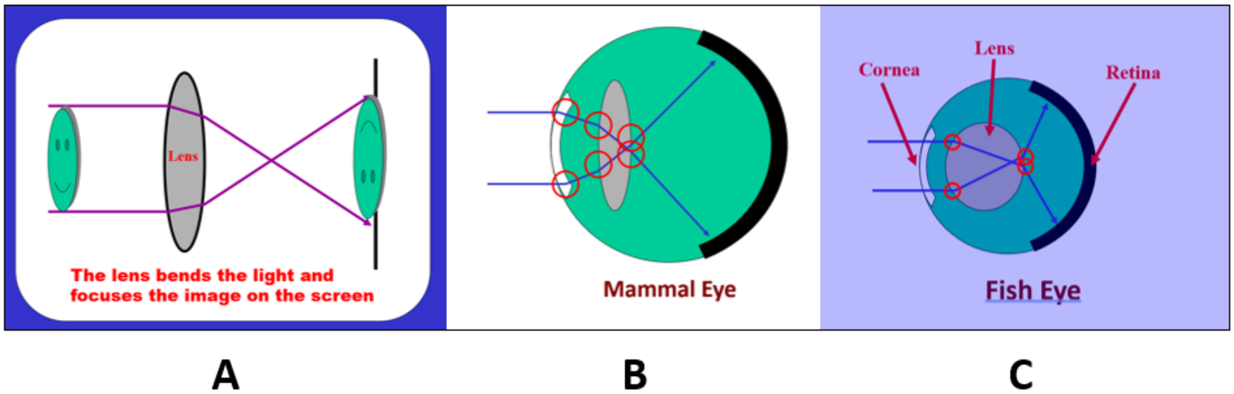

*Figure 15: The robot produces a final result where each well has a green color in proportion to how much BKD protein was in each sample. The reader reads how green each well is and reports its findings to the LIMS.*

## Fun Fish Factoids: Eyes and Ears

Why do fish eyes have spherical lenses? It all comes down to refraction. The lenses in the eyes of land animals are discs, but fish eye lenses are spheres. Light bends when it travels between substances of different density (or more correctly, a different refractive index). In your eyes, light bends when it leaves the air and enters the surface of your eye. It bends again when it enters the eye lens and one more time when it exits the lens. Thus, the light is refracted three times.

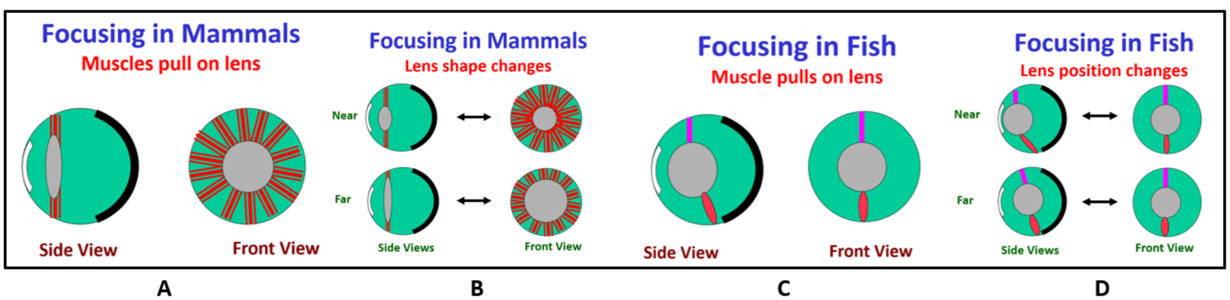
*Figure 14: Fish lens*

Things are different in fish. Because the refractive index of water and the surface of a fish eye are very similar, light traveling through water bends very little as it enters the fish's eye. This means that all of the focusing must take place as the light comes and goes through the eye lens so a much stronger lens is required. That's why fish eye lenses are a nearly perfect sphere.



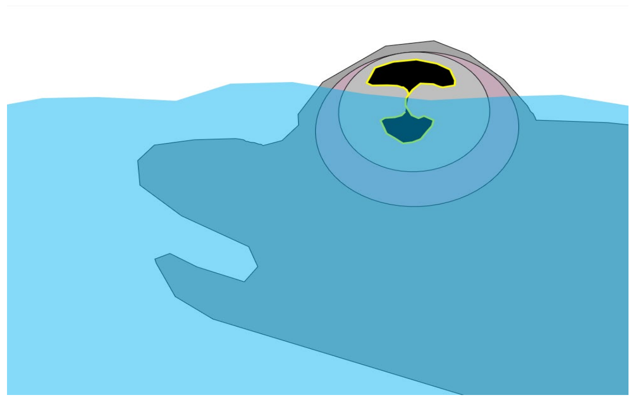

*__A__. Light bends (refracts) when it moves between materials of a different refractive index, like air and a glass lens. __B__. In a mammal eye, refraction happens as light moves from the air into the eye and then entering and exiting the lens (3 times). __C__. In fish, very little refraction happens between the water and the eye surface so a very strong spherical lens is required.*

The spherical shape of fish eye lenses has led to fish using a different focusing method than mammals. In your eyes, there is a ring of muscles around your eye lens that stretch or relax the lens to change its shape so that it can focus on things up close or things far away. The spherical and rigid fish eye lens would be very difficult to stretch, so there is a different solution or focusing. Instead of changing the shape of the lens, fish change its position. The lens hangs from the top of the eye on a tether, and it is moved forward and backward by a small muscle.




*A. In mammal eyes, the lens is disc shaped and surrounded by a ring of muscles. B. To fucus, the muscles stretch or relax to change the shape of the lens. C. In fish, the lens is spherical, hangs from a tether, and is attached to a small muscle below. D. Focus is changed by changing the position of the lens.*

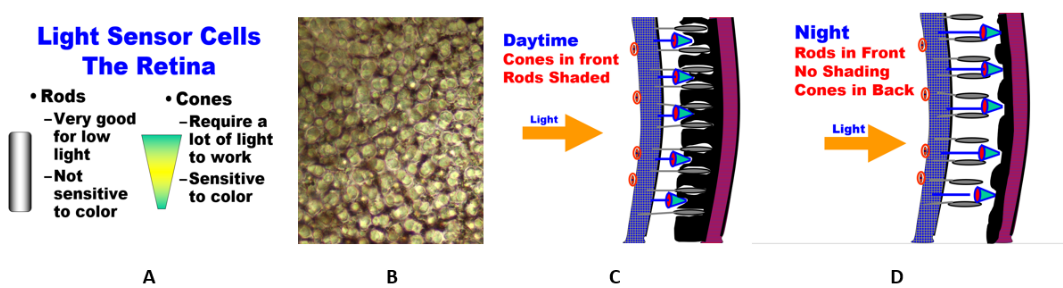
What about amphibious fish? When humans go under water, their vision gets blurry because they no longer have the refraction at the air to eye interface. Likewise, the many species of amphibious fish have similar challenges when they leave the water and suddenly must cope with refraction at the air to eye interface. Fortunately, nature as found two solutions 1) some amphibious fish have flat eye surfaces so that there is no refraction at the eye surface in water or in air (just like a diver's mask), 2) the anableps, or "four-eyed fish," has a figure eight-shaped eye with the top half designed to work in air and the bottom half designed to work underwater. This is especially helpful when the fish is swimming along the surface with the top half of the eye above the water and the bottom half below.



*Anableps, the "4-Eyed Fish". As it cruses the surface, the air adapted part of its eye is in air and the water adapted part is in the water. For more info* [*https://www.calacademy.org/learn-explore/creature-closeups/four-eyed-fish*](https://www.calacademy.org/learn-explore/creature-closeups/four-eyed-fish)

Puzzle question: Humans can see under water by wearing an air-filled dive mask with a flat front surface. What kind of equipment would a typical fish need to be able to see clearly in air?[1]

In humans, we regulate the amount of light getting into our eyes by using our irises to make our pupils larger or smaller. Bony fish can't do that, so, how do fish get along without being able to change the diameter of their pupils? ? They re-arrange their retinas! In low light, fish have their more light-sensitive rod cells (black and white only) in the front of the retina. In bright light, rod cells move to the back, where they are covered by the expansion of a black-pigmented cell layer (natural sunglasses), and the less sensitive cone cells (color vision) move forward. Interestingly, sharks *are* able to change their pupil size.



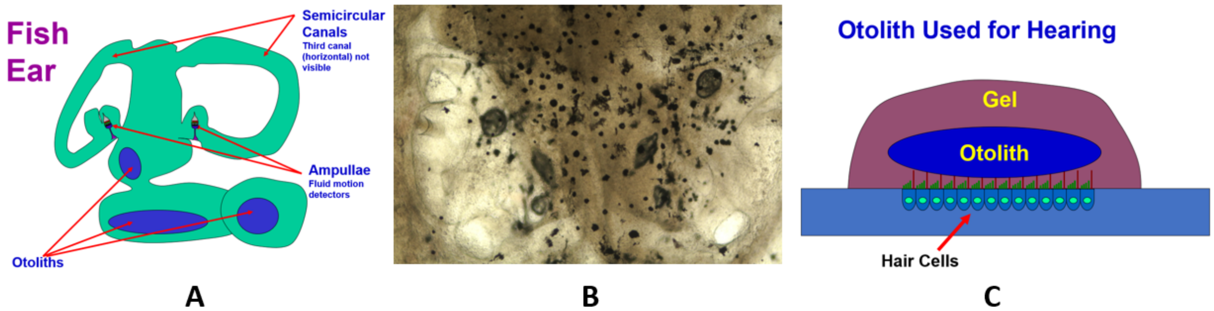

*A. The difference between retinal rods and cones. B. A fish retina. The clear circles are rods and cones. C. In bright light the light passes through nerves and blood vessels and then to the cones. The rods are shaded in a thick pigment layer. D. The same retina at night. Now the rods are forward and the pigment layer has retracted.*

Do fish have ears? It is amazing how many people think that fish don't have ears! Fish do have ears: they hear just fine, and they are not just listening, they are also talking to each other and exchanging information ([https://dosits.org/galleries/audio-gallery/fishes/](https://dosits.org/galleries/audio-gallery/fishes/)). The big difference is that mammals have to collect sound from air and transfer it to fluid in the ear where hearing takes place. This takes a lot of complicated equipment like external ears, ear canals, and ear drums. Fish have it easier. The sound is already in water and it easily passes through their skull to their ears. No fancy gear needed.

If fish don't have ear drums, how to they hear? In mammals, the eardrum sloshes fluid in a spiral shaped tube (the cochlea) where it causes the cilia on hair cells to wave back and forth and send sound messages to the brain. Fish don't have the spiral tube. In fish, ear bones (otoliths) rest on lawns of hair

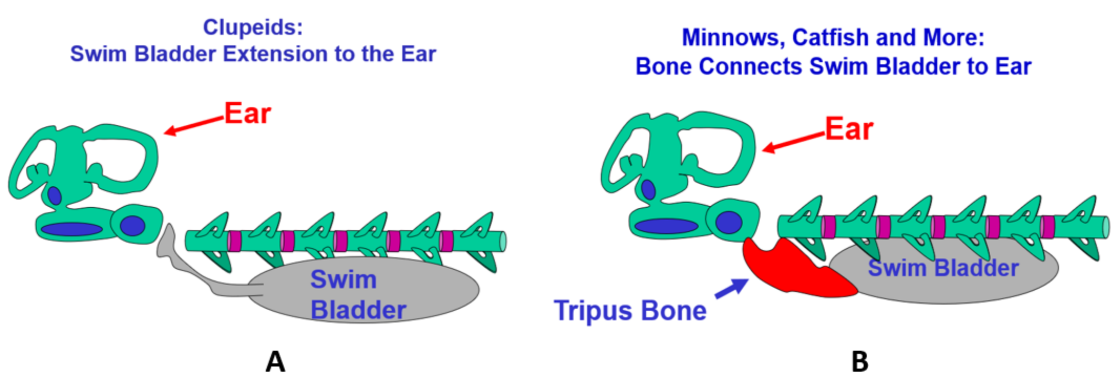
[1]Puzzle answer: A dive mask full of water.

cells. Sound vibrations cause the otolith to move back and forth bending the hair cells and sending sound messages to the brain.




*A. A fish ear. There are three semicircular canals that the fish uses for balance. The canals are in all 3 plains of motion and are equipped with sensors that detect fluid movement as the fish changes orientation. B. A view down through the skill of a catfish fry. The three gray patches on each side of the head are the otoliths. The large light areas to the outside of the otoliths are the rest of the ear. Small black spots are pigment cells in the skin. C. An otolith sitting on a bed of hair cells (a macula). Sound vibrates the otolith back and forth bending the hair cells. The grey is a jelly like substance that makes sure that the otolith stays in the right orientation*

To improve the sensitivity of their hearing, some fish have extensions from their swim bladders (bones or tubes) that extend forward to contact the ears. Underwater sounds compress the sides of the fish, which then compresses the swim bladder and sends pressure waves to the ears. It is a built-in sound amplifier.




*A. The ear, spinal cord, and swim bladder in a shad. Sound vibrates the swim bladder and an extension carries those vibrations to the ear. B. Minnows, catfish, and many other species of fish have a bones that carry vibrations to the ear. Both adaptations increase the ability of the fish to hear faint sounds.*

How do fish don't have lungs (well, most fish…) or larynxes, make sound? They have a lot of different tricks. Some have muscles to vibrate their swim bladders. Others, like catfish, have bumps in their pectoral spine joints that they can ratchet back and forth to make sounds. Some fish grind pharyngeal (throat) teeth. There are even some herrings that make sounds by expelling gasses out of their vents.

How do fish know which way is up? This seems like a silly question but imagine that you are underwater in scuba gear. Close your eyes, spin around and end over end a few times, then try to guess which way is up without opening your eyes. You won't be able to do it. You have semicircular canals in your ears that help you balance (fish do too), but the only reliable absolute information about which way is up comes from visual cues and the feeling of gravity. Underwater in the dark, those cues aren't available so humans can't tell which way is up. So, why aren't fish all upside down and sideways at night? Well, otoliths aren't just for hearing. Bony fish have three otoliths in each ear that are mounted over beds of hair cells. They work for hearing, but also for figuring out which way is up. If the fish is right side up, the otoliths are sitting on the hair cells bending them. If the fish is upside down, the otolith falls off the hair cells and the fish senses that it is inverted. Having three otoliths adds some nuance.


***A***. *Ear anatomy in a stained section seen under a microscope. Otoliths, embedded in jelly, rest on a macula covered with hair cells. This structure is used for hearing and for sensing which way is up.* ***B***. *An otolith sitting on a bed of hair cells (a macula). It loses contact with the hair cells when the fish turns upside down. The grey is a jelly like substance that makes sure that the otolith stays in the right orientation. Otoliths do double duty for hearing and to sense which way is up.*

## Free Bonus Picture

*Freshwater Fish Lice, Argulus sp. These are common parasites of freshwater fish. It is rare for infestations to get so severe that treatment is needed, but their large size (up to several millimeters) makes them easy to spot as they scurry across the skin of a fish. The orange eyes of this species make it especially intimidating.*