OUR ANGRY INNER FISH

Can a Humble Minnow Reveal Why Some People Become Violent?

U.S. Army Corps of Engineers; Engineer Research and Development Center (ERDC)
OVERVIEW

Researchers at U.S. Army Corps of Engineers Engineer Research and Development Center are using fish as a model to understand if exposure to common environmental pollutants can trigger extreme aggression in susceptible people.

U.S. Army Corps of Engineers; Engineer Research and Development Center (ERDC) - Vicksburg, Mississippi

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IMAGE INFO
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A simple plus sign-shaped maze made of clear acrylic sits inside a tank measuring five feet square. Together, they’re perched atop a table that has another tank of water below it. A video camera is mounted above the contraption so that its unblinking eye peers straight down to take in the whole scene.

In early spring 2018, scientists will pump 40 gallons of water from the floor tank into the maze and start dropping in little inch-long fish into an enclosure at the end of one of the plus sign’s arms. The researchers will exit into the adjoining room and wait for the fish—informally called zebrafish, but officially classified as Danio rerio—to settle in. When everybody’s ready to begin, they’ll pull on a length of fishing line running through a hole in the wall and a pulley down to the gate. The fish will swim where it wants in the maze while camera and researchers on the other side of the wall watch.

Besides the apparatus and the whining sound of a water pump circulating tank water, the bedroom-sized lab is unremarkable. But the voices of a handful of biologists and ecologists making it ready betray excitement and earnestness about the path of discovery they are about to embark on.

“There’s a lot we can learn from these fish that can be relevant to higher-level vertebrates and even humans,” says Christa Woodley, a U.S. Army Corps of Engineers (ACE) research biologist working with the animals.

In one test, a projector below the maze will fool the test subject into thinking a school of zebrafish are swimming into the plus sign arm on the left. Will it decide to follow its virtual schoolmates to the left, or go in another direction? How quickly will it make that choice?

Later, the fish will be put into a tiny flume—another acrylic 1.5-gallon tank and pump system that acts like an endless swimming pool—so that researchers can see how they move. How long will they be able to swim against the current before tiring out? Will some opt out of the experiment altogether?

Such questions may seem trivial to those unacquainted with the work going on here at the ACE’s Engineer Research and Development Center (ERDC) in Vicksburg, Mississippi. But just down the road from the now quiet lab, the aquatic test subjects that will be the stars of this show are being reared. And soon, the maze and the fish tested within it will be at the center of a fascinating inquiry into the ecology of violence.

Researchers here are using the fish as a model to understand if exposure to common environmental pollutants can trigger extreme aggression in susceptible people.

**Breeding rage**

Untold acts of violence are constantly erupting around the world and on our televisions. Amass shooting that erases the future for five random people in Northern California; extremists spewing words of hatred and intolerance; a man head-butting and breaking the nose of a journalist asking him questions he doesn’t like; road rage ending in shootings and assaults across the country during the course of a single weekday.

Though famed Harvard psychologist Steven Pinker argues that humanity has steadily become less violent over history, it sure doesn’t feel like it. Armchair analysts offer a number of causes for the violence that tears at the fabric of societies around the globe—racial bias, drugs, a family history of violence, resource scarcity, toxic stress, poor treatment for mental illness, among many other candidates. But what if our environment and genetics play as much a part in how much we fight as our psychology and the stresses exacerbated by our modern world?

That’s exactly the question that the ACE team is trying to answer. Science has already uncovered how some people are born with gene variants that have been linked to higher rates of depression and extreme aggression. A malfunctioning gene called monoamine oxidase, or MAO has been known since at least 1993 to induce impulsive aggression in humans and rats. Now the group is trying to figure out if industrial pollutants commonly found in our
environment might just play a role triggering or amplifying aggression in those people.

But scientists can’t test out a hunch like this on actual people. So instead, they’re turning to an unlikely source—the zebrafish, an animal that is considered a model organism for research because its neurobiology, though simpler than higher animals, offers scientifically relevant similarities. For the Vicksburg team, the fact that the zebrafish has the same genes linked to depression and aggression in humans—MAO and two more that have shown an association, CDH13 and SLC6a4a—made it a natural choice.

Genes are the blueprints that cells use to build proteins. Variants of the three target genes in the study cause the animal’s cells to produce fewer of the proteins, which can eventually translate to behaviors like extreme aggression and depression. But these variants don’t naturally occur often enough in regular zebrafish to make a study practical. So the researchers are having transgenic fish made that have been modified to be carriers of the desired variants.

After the ERDC team verifies that they have the needed number of fish with altered aggression genes to conduct their study, they’ll start exposing some of the zebrafish to a pollutant called benzo(a)pyrene, a ubiquitous cancer-causing hydrocarbon produced when fossil fuels and other organic matter is burned. This study architecture will let them create three groups of test subjects—a control group of unmodified wild-type zebrafish, a group of mutants left unexposed to the pollutant and an exposed mutant group. Then they’ll put the three sets of animals through behavior, decision-making and physiological tests to see if the mutants show signs of heightened aggression compared to regular fish, and if pollutant-exposed mutants show even greater signs of aggression.

“These animals are a good model for humans, so we’re modifying these target genes in the fishes’ brains and then exposing them to chemicals to see how that impacts
their aggression levels,” says Ed Perkins, a senior research scientist who has worked at the ERDC for more than two decades. “We’re hoping this work shines a light on us.”

Perkins and his wife, Natalia Vinas, who has been an ERDC research biologist for a decade, are two of the co-principal investigators on the project, which has gained the informal title of “The Angry Fish Study.” Together and separately, the two have spent their professional lives exploring the scientific frontiers of ecology and ecotoxicology, a field of study that tries to understand how substances in the environment affect organisms.

Vinas and Perkins share matching tattoos—a spiral shape that they saw in Utah that is thought to depict growth and change. The two balance each other out professionally. Perkins studied painting alongside genetics during college and says that creativity is fundamental to his personality. “In art and science, I’ve always been fascinated by the questions of what makes us, us and how does that change over time,” he says. Vinas, meanwhile, says her creativity falls within the strict lines of her scientific pursuits and her moral obligation to environmental conservation. “I’m interested in neurotoxicity and how that impacts behavior,” she says. “I’m a believer in conservation and protecting the environment and wildlife.”

**Clues About Our Own Violent Nature**

Perkins and Vinas hypothesize that the mutant fish will exhibit different behavior from their wild-type counterparts. They also think that fish carrying the mutant aggression genes that are also exposed to pollutants that further impact the functioning of these genes will magnify the behavioral impact. Among the things they’ll be looking for are increased aggressive displays, along with circling, striking, biting and chasing, all commonly accepted indicators of a bullying-like superdominance that is equivalent to human violence. Other tests will challenge the fish alone and in groups to make various decisions, they say. The choice they make and the time it takes them to make it will be measured.

Research ecologist David Smith, another co-principal investigator, says the entire project is unique and he’s interested to see the results.

“I wonder if an aggressive fish means faster group decision-making,” he says while looking into the plus-sign-shaped maze. “If a fish responds aggressively in a consistent way, we can ask if that behavior decreases the time it takes the group to make a decision or does it make everybody around the aggressive fish crazy and slow the time it takes to make a decision.”

Other tests could yield even more insights. The flume swimming trial, for instance, could yield insights into how physiology and behavior interact. Woodley posits that higher testosterone that the more aggressive fish will produce could lead to increased muscle mass that helps them swim longer. Or, going the other way, their angry disposition might make the fish choose not to take part in the test altogether.

By investigating fish behavior and physiology this way, the team should be able to tease out if more aggressive behavior is seen in fish with just the mutation or just the pollutant exposure. They’ll also be able to tell if a combination of the mutation plus the pollutant affect violent behavior more than either of the factors by themselves.

Such data could lead to a novel understanding into the molecular and environmental underpinnings of violence. If the team sees a strong signal that mutants exposed to pollutants show significantly more aggressive behavior, it would shine a light on how contaminants in our environment could help to trigger violence at the cellular level. That would be the first step to creating a diagnostic tool to tell who among us are more sensitive to environmental stressors that could cause aggression or who is experiencing the effects of exposure. It could also, in the distant future, result in therapies that counteract our own violent proclivities and rules to limit exposure to these chemicals.

“We know there’s something here in this study, in these zebrafish, that we will be able to translate into something useful for the world,” says Woodley.

Smith draws an even finer point on it. “If we can understand how molecules in our environment interact to change behavior in this way, if we can figure out a cause and effect, then we might be able to mitigate or enhance the aggressive response,” he says.
The study is looking at an element of biology called epigenetics, a phenomenon in which the functioning of a gene within an organism’s DNA changes but the DNA itself doesn’t change. This alteration, which is inherited by subsequent generations, can be induced by external factors in the environment like chemicals or stress.

“What we’re doing is asking how the environment changes how we behave,” says Vinas. “We want to see if chemicals have epigenetic and behavioral effects if a person has the genetic background for it. Extreme aggression could be caused by the chemicals you’re exposed to.”

Perkins says some are calling this line of research molecular psychiatry—the search for how genes and ecological factors like chemical exposure play a role in behavior.

“There is now a lot of evidence that epigenetics has a role in changing our behavior—evidence that epigenetics may be involved in people who are overly aggressive,” he says. “The work we’re doing could help us understand how traumas could initiate behavioral impacts.”

**Good For The Army, Good For Society**

What could scientists do with the knowledge that certain pollutants play a role in causing violent behavior or depression? Diagnostics for screening purposes, targeted gene therapies and new regulations to control contaminant discharge into the environment would all be fair game to make huge impacts on the broader world.

What, though, would compel the Army, an organization known more for using aggression than looking for ways to diminish it, to spend about $1.5 million over the course of three years on the Angry Fish Study?

The answer lies in the unique mandate of the Corps of Engineers. The service has one foot in the civilian world and another in the military. It is responsible for building and maintaining some of the country’s most important civilian infrastructure—bridges, dams, waterways and others—along with U.S. military installations around the world. For their installation work, ACE engineers strive to build facilities that are safer for the personnel stationed on them and that minimize damage to the environment. Since the zebrafish study could lead to revealing that some service members are genetically predisposed to pollutant sensitivity, it could eventually provide tools to shield them from unwanted exposure.

There also may be an element to this basic research, Perkins says, that could be useful in understanding and treating post-traumatic stress disorder. According to the U.S. Department of Veterans Affairs, up to 20 percent of veterans who served in Operation Iraqi Freedom and Operation Enduring Freedom have PTSD in a given year.

“With the zebrafish, we can rapidly understand the impacts of environmental stressors on fish embryos, and this often can be a good predictor of impacts on people,” Perkins says. “This work also provides a relatively straightforward model to understanding of aspects of the human brain that you can’t necessarily get from other approaches.”

**Into the Little Beasts’ Chambers**

More than 100 of the transgenic, potentially rage-filled fish are already swimming around on the ERDC campus. To see them, Perkins walks up a flight of metal stairs painted yellow outside a nondescript white building. Stepping inside, the walls to the left are like the sandwiched aluminum and insulating material of a restaurant’s walk-in refrigerator. These are the outer walls of the zebrafish testing lab’s three environmental chambers. Each measures about 600 square feet and all are kept at a balmy 83 degrees Fahrenheit, a perfect endless summer for the generations of fish sitting in tanks on racks in the middle of each of the chambers. In the first two chambers, a few hundred wild-type fish amble about in small, pristinely clean plastic tanks arranged in rows on racks similar in size to those found in a library. The animals seem to be unaware of their captivity and are uninterested in the scientists moving around purposefully outside.

Dozens of fish slip effortlessly around their enclosures in the third chamber, indistinguishable from those in the other two rooms. With bold horizontal, blue stripes running the length of their bodies, these zebrafish would not be out of place in any pet shop’s fish section. But these are all part of the transgenic cohort, and this chamber is designated a quarantine zone. With protocols that include that every visitor steps on a squishy mat soaked with a solution made to kill bacteria and viruses that could
destroy the valuable genetically modified organisms inside.

In this chamber, the silvery striped fish aren’t just another pretty face. The ACE scientists are hoping that a solid proportion of them have the rage gene in their brain, a variant of the MAO gene that expresses less protein and causes heightened levels of aggression. The genetic modification process that the transgenic fish supplier uses isn’t a sure thing, so Vinas and fellow research biologist Mitch Wilbanks, a specialist in zebrafish husbandry, hope that 30 or 40 of them are carriers.

“You can’t tell physically that they’re different, but usually they’re a bit more anxious than wild fish—swimming around more,” says Vinas.

Indeed, when the two walk in to take a look at the mutants, many bunch up at the top of their tank, a possible sign that they are behaving differently from their wild cousins in the other chambers. “Usually, they’re swimming throughout the water column in their tanks,” says Wilbanks. “I don’t know that they’re stressed, but they are acting different.”

They won’t know if the fish are acting quantitatively differently until the fish are positively identified as altered-gene carriers by lab tests and then put through their paces with Smith’s team. None of that can happen until the team gets approval from the Institutional Animal Care and Use Committee, a body that oversees animal research.

Vinas expected that clearance to come in the ensuing days or weeks. Standing in front of the little shiny creatures, though, she became philosophical about their critical place in her work. She says she tries to never forget that they are conscious beings that are being forced to sacrifice themselves in the pursuit of human knowledge.

“They’re really amazing animals. I feel bad about using them for research, but they have so many similarities to us,” she says inside chamber three while gazing at the
transgenic group. “But they’re still animals. They still feel pain. We’re using them, but we don’t have to be mean to them. They learn. They recognize human faces. They get scared. They’re social, so they like to be together—they’re more complex than you think.”

Vinas and her team take solace in their pursuit of something worthy. And like many of the human heroes in service to the Army, the lives of these zebrafish are forfeit for something bigger than themselves. Perhaps one day the things that the ERDC researchers learn from the fish will contribute to creating a more peaceful and healthy world.
TOGETHER, WE’RE INVENTING THE FUTURE