All eyes on zebrafish

Arlene Weintraub

As zebrafish research continues to grow, labs and facilities are expanding their technical repertoire.

At New York University School of Medicine (NYUSM), about 60 tanks teeming with zebrafish are stacked on shelves, library-style, in a room that's 100 square feet—about the size of your average walk-in closet. A half-dozen or so separate scientific projects are going on here simultaneously, says David Schoppik, Assistant Professor in the departments of Otolaryngology and Neuroscience & Physiology. The Schoppik lab, which specializes in studying balance disorders, does most of their animal husbandry in this space, but all of that will change later this year when NYUSM opens a new zebrafish facility with ten times the capacity of the existing space devoted entirely to the finned creatures.

NYUSM's upsized facility is a testament to the growing realization of the value of zebrafish in scientific research, says Schoppik, who first became enchanted with the thumbnail-sized fish when he was a post-doc at Harvard in the late-2000s. "Everything from studying basic development to answering questions about how the brain works" is being done in zebrafish models, he says. "I really saw the breadth of possibilities presented by the zebrafish."

NYUSM is far from alone in expanding its use of zebrafish in medical research. Zebrafish facilities are being built or expanded at leading medical research institutions across the world. A decade ago, the fish were mainly used to study embryonic development, brain circuitry and cardiovascular health, but the advent of sophisticated transgenic models has given rise to research on a range of disorders, including diabetes, cancer and kidney disease.

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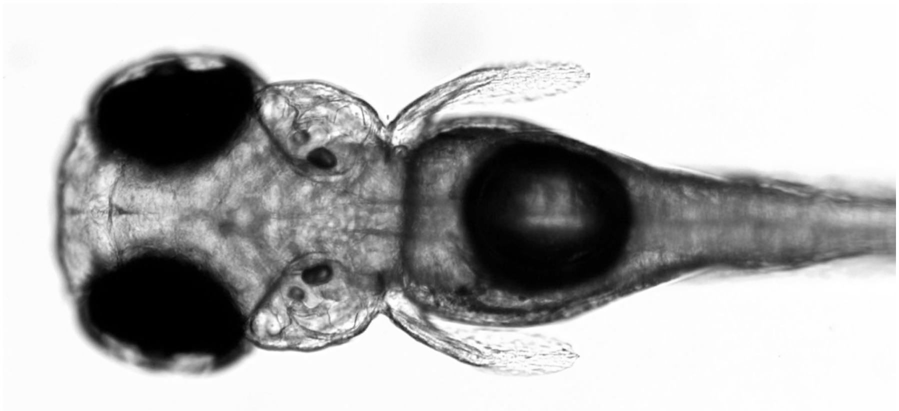

Schoppik lab

THE EYES HAVE IT | Image of a four-day-old zebrafish larva.

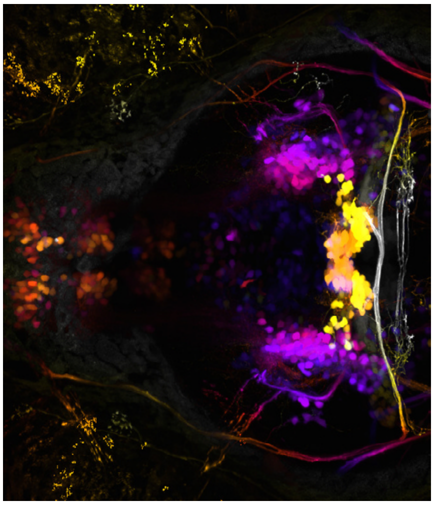
Between 2008 and 2015, the proportion of zebrafish projects vs. all research that was awarded R01 project grants from the National Institutes of Health (NIH) grew 58.8%, far outpacing the growth of projects involving fruit flies, *C. elegans* and frogs. That reflects both the inherent advantages of zebrafish over other model organisms and recent advances in aquatic technology, says Rebekah Rasooly, program director of the NIH's Division of Kidney, Urologic, and Hematologic Diseases.

"Zebrafish have proven to be remarkably useful," Rasooly says. "It is a transparent embryo, so you can watch the zebrafish blood and heart develop, for example, and watch how the heart pumps." The advent of CRISPR/Cas9 editing tools has made it easy to create disease models by genetically altering the fish, she adds.

And zebrafish have other inherent attributes that can make them better research subjects than rodents. The average adult zebrafish can reproduce every 10 days and lays between 50 and 300 eggs per mating. Mice, by contrast, generally give birth to no more than 10 pups at a time, and they can only have a few litters in a lifetime, according to the NIH.

"When we mate fish we get eggs right away. If we mate 10 pairs of fish we get up to 3,000 eggs," says Sandra Rieger, assistant professor at MDI Biological Laboratory in Bar Harbor, Maine. "With mice we wait three weeks for a few pups." Zebrafish eggs are laid and fertilized outside the mother's body, making them easy to observe and manipulate. Baby fish move freely just four days after they become embryos, and they reach sexual maturity in three months. Furthermore, 70% of human genes also exist in zebrafish, and they have several physiological and anatomical features that are strikingly similar to what's found in people, including the intestine, pancreas, spinal cord and heart.

Scientists who work with zebrafish marvel at other attributes, including their sociability and hardiness. Having originated in freshwater rivers and ponds in southern Asia, they thrive as residents of home aquariums, or in groups of up to 20 per tank in laboratories. "They can withstand a wide range of water quality



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READY FOR THEIR CLOSE-UP | Image of fluorescently labeled cranial motorneurons in a transgenic zebrafish. *Z*-axis depth is color-coded, with white/yellow being closest to top of fish.

parameters, because they normally live in the Himalayas and the Ganges, in rice paddies," says Edward Jaskolski, Associate Director of Animal Care at the University of Massachusetts Medical School. While it is critical to ensure water quality is closely monitored and kept under strict parameters to improve fertility and reproduction, slight variation in these parameters generally doesn't place the fish in immediate danger as it would other species of fish, he adds.

The ease of caring for zebrafish can result in a significant cost savings. At Boston University, for example, scientists are charged $1.05 per day for the housing of a single mouse, compared to 25 cents for an entire tank of zebrafish.

Emerging zebrafish science

The ability to see inside the developing brains of zebrafish has made them a popular research partner for neuroscientists especially since the development of a genetic line that maintains fish transparency throughout adulthood. The zebrafish brain is useful because it is less complex than the human brain, Schoppik says, but also remarkably similar. "We have hundreds of millions of neurons," he says. "Zebrafish brains have about 100,000 neurons. And all of the major classes of neurons engaged in reflexive behaviors, learning, memory, sleep—all of the things we're really interested in—are present in homologous circuits."

Schoppik and his team use transgenic zebrafish, with much of their research focusing on the neural circuits that stabilize gaze and posture. The brain cells involved in balance reflexes are tagged with fluorescent molecules, allowing his group to watch and record what each cell is doing to produce every movement a fish makes. In a room next door to the fish facility, an imaging system built in collaboration with Elizabeth Hillman's group (Columbia University) allows Schoppik lab members to image neural activity with high spatial and temporal resolution. Similarly, another apparatus allows the group to monitor gaze and posture in fish as they swim freely.

Similar imaging methods are being used to study heart and brain regeneration in zebrafish at the MDI. Zebrafish have been a boon to researchers studying peripheral neuropathy, a painful degeneration of nerve endings that affects 20 million people in the U.S. alone, says Rieger. "We can visualize nerve endings and study the disease process in a living animal" using zebrafish, Rieger says, which would not be possible with traditional rodent models

Creating a model of peripheral neuropathy in zebrafish is quite simple, Rieger says. Simply putting small concentrations of the chemotherapy drug paclitaxel (known commonly as Taxol) into their water causes nerve degeneration similar to peripheral neuropathy in humans. Rieger's team then screens experimental compounds to either prevent degeneration or accelerate regeneration of nerve endings in the fish. They've found two effective compounds so far and are working with the Mayo Clinic to analyze their findings in patient biopsies, she says. They're also able to create a zebrafish model of diabetes—one of the leading causes of peripheral neuropathy—by simply dropping glucose into their water, Rieger says.

At Ghent University in Belgium, zebrafish are proving to be ideal models for studying bone disorders, says Andy Willaert, a scientist in the department of pediatrics and medical genetics. It's difficult to model severe bone disorders in mice and rats, Willaert says, because the symptoms are so crippling they can be lethal. "In zebrafish, because they live in the water, the weight-bearing demand is lower," he says. So even fish that are genetically altered to model bone diseases can survive because their skeleton isn't under intense pressure. What's more, the structure of the skeleton and the functioning of bone cells, such as bone-building osteoblasts, are similar in zebrafish, rodents and humans, he adds.

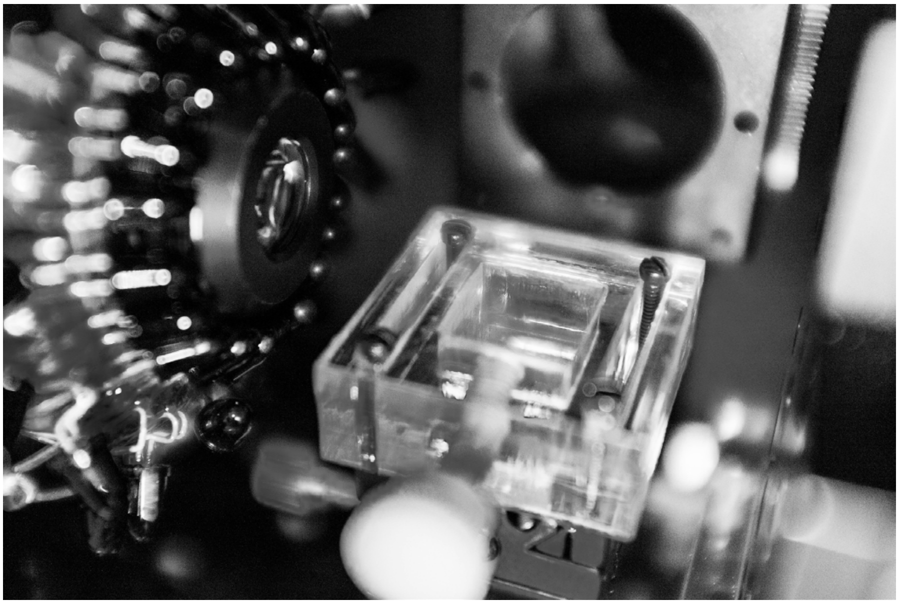
The ability to easily edit zebrafish genomes has made the model increasingly popular in cancer research. For example, at Mount Sinai Medical Center in New York, a team led by assistant professor Samuel Sidi is using zebrafish to study the problem of radiation-resistant tumors. About half of patients with head and neck cancers do not respond to radiation, Sidi says. Many of those patients have mutations in a gene called P53, which normally acts to suppress tumors. So Sidi uses a zebrafish model of mutant P53 to screen novel cancer drugs. "The idea is to restore a normal response to radiation despite the lack of functional P53," Sidi says.

And it's not just adult zebrafish that can be used to screen new drug candidates. Zebrafish embryos, which are much smaller in size, can be genetically altered and then placed in wells similar to those used for high-throughput drug screening of cells or proteins. "Ten years ago, it didn't seem possible to screen lots of compounds in a living organism in a cost-efficient way," says the NIH's Rasooly. "Now a whole range of software and handling tools allow for screening of hundreds and perhaps thousands of compounds with living zebrafish embryos."

Mastering zebrafish logistics

The growth in zebrafish labs presents a challenge to universities and companies more accustomed to working with traditional models, such as rodents. Moving from the cage to the tank is not a straightforward enterprise.

One of the biggest challenges for tank facility managers is maintaining the proper water quality, says Punita Koustubhan, an aquatic facility consultant who advises clients in industry and academia. Koustubhan spent 15 years as a lab manager at Tufts University and Harvard, where she observed that even tiny fluctuations in pH hampered the ability of the fish to



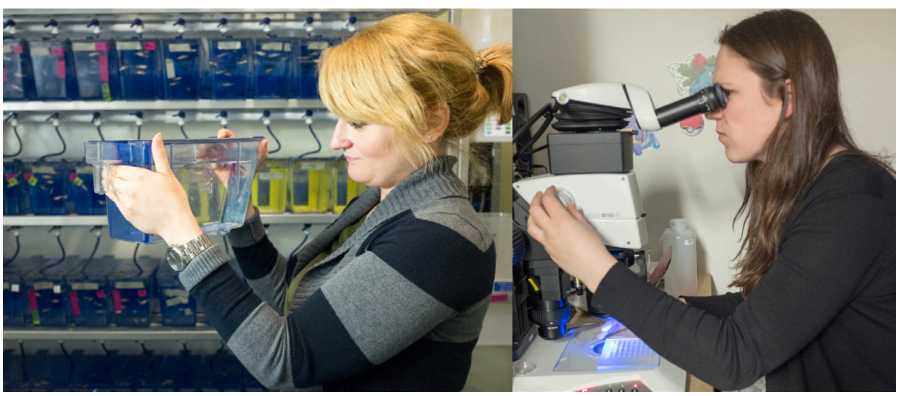
HOMEMADE HIGH-TECH | Microscope and custom-made zebrafish chamber for quantifying eye movements.

regenerate their tissue—a process that's widely studied by diabetes researchers. If a lab technician was slow to change out a filter, levels of ammonia or nitrates changed, which impacted appetite levels in the fish, Koustubhan observed.

Older systems use probes to measure and correct water quality, but "they're not sensitive enough to detect a small change," she says. "There were repercussions that all pointed back to the water quality. I realized we had to keep everything in strict parameters." Newer systems come with more sophisticated water-quality probes, which include built-in mobile capabilities that allow lab managers and researchers to get alerts via cell phone when something is awry and needs to be fixed. Some problems can even be corrected remotely, Koustubhan says, by switching on or off a valve from any computer with an Internet connection.

With new technologies, lab managers can now fend off problems that were unavoidable in older systems. Jaskolski recalls that in the early days of zebrafish research at his facility, a small leak caused the water level to drop slowly in one reservoir, which prompted one pump to suck up air along with the water. That touched off a rash of 'gas bubble disease'—a condition similar to the bends in scuba divers. "We lost 50 percent of the fish in that system and there wasn't much available to prevent that," Jaskolski says.

Now the University of Massachusetts' system monitors gas saturation in the



TEAM EFFORT | Basak Rosti (left) and Katherine Harmon (right) of the Schoppik lab manage and work with zebrafish to understand the neurobiology of balance.

water, sends out alarms and automatically shuts the system down when they deviate from proper ranges. "That gives us a lot of peace of mind," Jaskolski says.

Artemia time

Several universities have been experimenting with different strategies to improve the care and welfare of zebrafish, with the aim of improving the quality and throughput of research. Because zebrafish are natural predators, several schools raise live bait, such as artemia or rotifers, in separate tanks that they feed periodically to the zebrafish.

Susan Farmer, Assistant Director for Aquatics in the animal resources program at the University of Alabama at Birmingham, says giving zebrafish at least one live feed per day makes a visible difference. "That's a natural behavior, and the fish seem very excited about the live food," Farmer says. "I think they know that at the end of the day everybody gets artemia, because I see increased schooling activity near the feeding hole close to artemia time."

Even small steps in aquatic husbandry can make a big difference in how actively zebrafish breed. At the University of Alabama, for example, a study showed that simply putting red marbles at the bottom of the tank can increase breeding activity, Farmer says.

Christian Lawrence, a fish biologist at Boston Children's Hospital, noticed that zebrafish prefer to spawn in shallow water, so he and a colleague developed a breeding tank that stimulates spawning and egg production on demand based on the ability to change water depth. "We were able to basically turn on and off spawning and collect 10,000 embryos in 10 minutes, all fertilized at the same time," Lawrence says. The invention was patented, and Tecniplast purchased the license for commercialization.

Other new technologies that have come to market over the last few years include robotic feeding systems, cameras that allow scientists to observe their fish from remote locations, and "swim tunnels," where the fish swim against a current, allowing researchers to observe the effects of exercise on the cardiovascular system in real time.

Fulcrum Automation & Control Technologies of Beachwood, OH, recently introduced DanioData, a mobile app that's designed to streamline the process of tracking fish. It's a considerably more complex task than tracking rodents is, says Dave Weintraub, DanioData's principal architect. "You can store half a million fish in a 50-by-30 foot room," Weintraub says. "If you take two fish and cross them, you create 200 offspring. That forces you to redistribute those fish into multiple tanks, and to do continual genotyping to maintain lines. There's a lot of work involved." DanioData's product incorporates notifications that remind scientists when to genotype and redistribute fish, and it's all managed with QR codes on tanks that they can scan with their smart phones.

The explosion in new technologies is fueling the growing interest in zebrafish science, Lawrence says. "The scale and sophistication of the automation has taken off. That's driving the growth."