Fishing for Answers in the Neuroscience Laboratory

BY DAVE SHINALL

ONE RESEARCHER AT WESTERN KENTUCKY UNIVERSITY CRUSHES THE OPTIC NERVES OF ZEBRAFISH AND WATCHES THE NERVES GROW BACK.

Another trains zebrafish with colored light and shrimp dinner rewards.

Still another researcher wants to see if growing up in a blue world will have zebrafish seeing red — or green, for that matter.

Western Kentucky University psychology professor, Dr. Joseph Bilotta, hopes his team's work with zebrafish will one day lead to cures for certain forms of blindness and spinal cord injuries. Bilotta heads an elite unit of eager and determined undergraduate and graduate students interested in neuroscience research.

Bilotta and his students take a holistic approach in their investigations that blend behavioral psychology and the physiology of sight.

"We're trying to compare the visual processing at the eye with the visual processing at the brain, and then, the visual processing of the whole animal," Bilotta said.

"For me, that's the most fascinating part," Bilotta said. "To put it all together." In addition, Bilotta points out that the research activity that takes place in the laboratory also provides a place for students to learn about neuroscience and research in general. "For me, teaching and research are the same thing; when I'm in the laboratory, I'm teaching." The laboratory not only produces high quality research, but high quality students. Many of his students have gone on to doctorate programs in psychology and neuroscience as well as medical school. The integration of Bilotta's teaching and research efforts is illustrated by the fact that he has been awarded both teaching awards and research awards. He won the College of Education and Behavioral Sciences award for excellence in research/creative activities in 1996 and excellence in teaching awards in 1993 and 2000. Dr. Bilotta was awarded the University award for excellence in teaching in 1993 as well.

Students working in the laboratory learn that they have to work together and help each other. None of the research in the laboratory can be done by a single person; everyone has to help each other. "Everyone has a project that is their main project; however, when someone needs help collecting data, analyzing data or taking care of the fish, everyone pitches in," Bilotta says. "It is a real team effort."

The centerpiece of his team's current research is optic nerve regeneration.

Graduate student, Angela McDowell, is in charge of the laboratory's neural regeneration project.

"I crush the optic nerves," McDowell said. Then, to measure how the zebrafish neurons grow back, McDowell inserts hair-like microelectrodes into their tiny brains. In a similar project, undergraduate students in the laboratory insert similar electrodes into the retinas of zebrafish larvae, which are about three millimeters in body length.

Unlike humans, when the optic nerves of zebrafish are destroyed, zebrafish optic nerves repair themselves. The repair takes about a month.

Learning how the fish perform this miracle may yield hope for millions of people now visually impaired.

Unlocking the secrets of how zebrafish regenerate crushed optic nerves may also teach doctors how to re-

Dr. Joseph Bilotta points out features of zebrafish to graduate student Angela McDowell.

PHOTO BY SHERYL HAGAN-BOOTH

build damaged spinal cord neurons in people disabled by accidents.

"That's why this research is very hot and very fascinating and on the cutting edge," McDowell said.

As far as doctors repairing humans' crushed optic nerves and severed spinal cords with the fruits of her research, McDowell remains hopeful.

"I think someday we'll be able to do that," she said.

Bilotta, who earned his doctorate in experimental psychology from the City University of New York, has been working on zebrafish visual system development and optic nerve regeneration for about six years at Western.

Zebrafish are ideal for this kind of research.

"It's a great little species for studying developmental neuroscience because as soon as the lights go on in the morning, the female lays the eggs," said Bilotta, who added his school of zebrafish varies from 50 to 60, and one morning produced 1,200 eggs.

"And you know," he said, "the amazing thing is someone had to count all twelve hundred of them. That's another example of how everyone pitches in."

Because zebrafish crank out new generations on a regular basis, mutations, which take several generations to show up, show up rapidly in zebrafish. Plus, Bilotta said, researchers can choose from zebrafish at every stage of development to study, from embryos to those embryos' great-great-great-great-grandparents.

All the work takes place in the Neuroscience Laboratory. The Laboratory, located on the second floor of Western's Tate-Page Hall, consists of three rooms, each the size of a small jail cell.

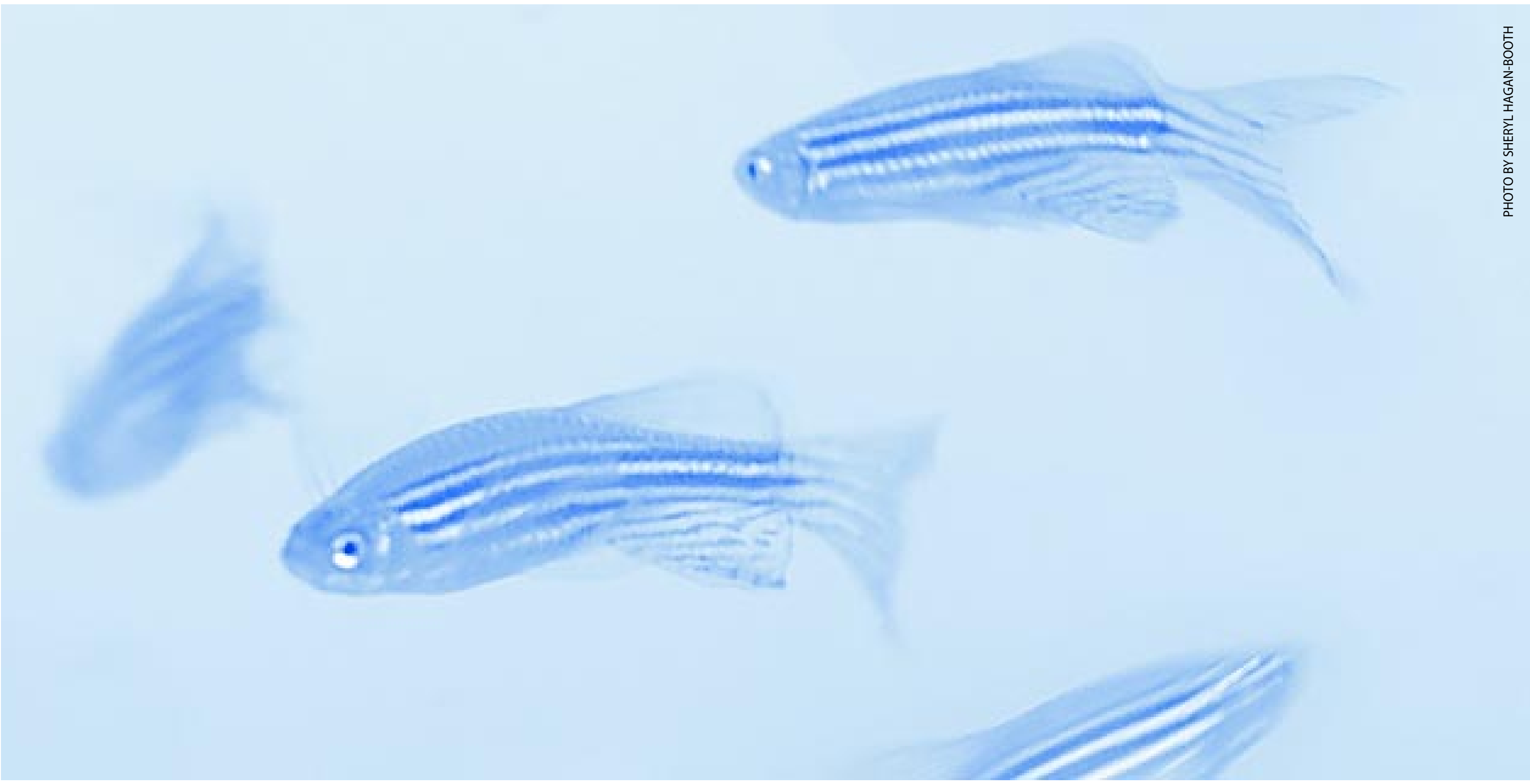
The concrete block walls of one room are lined with racks of aquariums that hold gray-green, black-striped zebrafish of different ages and sizes. They live about a year and grow to nearly an inch.

One covered tank contains 15 baby fish swimming in a cool, blue glow. These zebrafish have never seen any light other than blue light.

"What happens when you're raised in an environment," asked Bilotta, "where you don't need red and green receptors? Will you develop them anyway? Will you develop more blue receptors because that's the light you've been exposed to?"

This project belongs to graduate student Lee Dixon, from Dayton, Ohio. He hopes to learn what color of light the eye needs to develop properly. Dixon estimates the experiment will take another four months to complete.

"I didn't know much about vision to start with," Dixon said, "and I've learned how complex it is and how everything has to work just right for vision to turn out perfectly."



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Angela McDowell gets the equipment prepared for an experiment involving a zebrafish.

University of Washington biologist Susan Brockerhoff echoed Dowling's praise.

"These studies are critical as a framework on which to analyze retinal mutants," wrote Brockerhoff in *Concepts and Challenges in Retinal Biology*.

Another graduate student who works in the Neuroscience Laboratory is Erin Davis. She explores vision's effect on their behavior; for her zebrafish, color has meaning. Davis is working on a project that began several years ago in the laboratory. Davis trains her zebrafish to respond to different colors of light, and for them, color means food.

Davis uses a method pioneered by behavioral science icon B.F. Skinner. Skinner trained lab rats to press a bar in order to receive a food reward.

Davis uses brine shrimp as a reward. So far, Davis has trained her zebrafish to swim toward different colors of light shined into stalls at the end of a small black aquarium.

For the past two years, Davis has continued to improve and modify the procedures of previous students in the laboratory to perfect this technique on zebrafish. She has patiently spent hours each day sitting in a tiny, dark room bribing her frenetic little fish to swim to different colored lights and earn their shrimp dinners.

"I think some people kind of giggle at the fact I go in there and train the fish for two or three hours a day, but I really enjoy it," she said.

'What happens when you're raised in an environment where you don't need red and green receptors? Will you develop them anyway? Will you develop more blue receptors because that's the light you've been exposed to?'

"The research we're doing helps us understand not just how vision works," Dixon explained. Every little building block is a piece of the puzzle, and that picture gets clearer and clearer, and I hope to add a couple of pieces before I leave."

Dixon presented details of his work to an estimated 8,000 members of the Association for Research in Vision and Ophthalmology at the group's Ft. Lauderdale convention last May.

Fellow researchers view the work done by Dr. Bilotta at Western with respect and gratitude.

Grateful colleagues include Harvard University biologist John Dowling, author of books on retinal biology.

"They are clearly at the forefront of such work," Dowling said of Bilotta's work. "My laboratory is interested in visual mutants in zebrafish, and Joe's work has been exceptionally helpful to us."

"I hope to be able to provide the behavioral evidence that zebrafish are an excellent model to use in vision research," she said. "When you think about it, working in the Neuroscience Laboratory, not only do you get to collaborate with current members of the laboratory, but former members as well. The project I am working on now was started by former students."

In addition to the work on visual neuroscience and development, the lab has begun a series of experiments examining the consequences of embryonic exposure to various chemicals or teratogens. For example, they have exposed zebrafish embryos to various doses of ethanol and examined the effects on anatomical, physiological, and behavioral development. The short-term goal of this work is to develop the zebrafish as a model for fetal alcohol syndrome; the long-term goal is to use this information to help prevent this debilitating disorder in humans.