Once you've sliced up this little bit of mouse brain, can you do anything with it?
It's a great question. I still haven't gotten to the hard part: analyzing the data. I like to use this Kennedy quote: "We choose to go to the moon in this decade and do the other things, not because they are easy, but because they are hard." We went to the moon! Right? Is analyzing this data that much harder than sending human beings to the moon and coming back?

So now I have two million gigabytes of information. And now we get to the hard part, the really, truly hard part. We have to figure out a way to analyze two million gigabytes of data with some combination of computers and humans.

Here's my current version of that: I teach neuroscience at one local high school, Masconomet Regional High School. I teach the students what a neuron looks like, I teach them how to trace something, and they work on my data. They're free, which is an advantage for me, and it's educational. And so far, they love it.

How much mouse brain have you been able to analyze?
Barely a fraction of the mouse brain. It's so small, it's embarrassing. We're going to get faster, I hope, if I have any chance of making a career out of this. But this is the new reality. And the question is, do we want to face reality or not?

And?
I have to. I can't turn back now.

What if it turns out that the connectome doesn't matter? Maybe everything that makes us human just lies in the genes and how they are turned on and off.
Yes, it could be. My view is not that we have the right answer, but my view is we've got to try. Let's just do it, man! I guarantee we'll find a surprise that's going to change how we think about brains.

WEB EXTRA Watch videos about how Bobby Kasthuri makes detailed images of the brain, and how mapping the way neurons connect could help us understand what makes us who we are, at bu.edu/bostonia.

EMBRYONIC FROGS, AS WELL AS OTHER ANIMALS, CAN LEAVE AN EGG IF DANGER PRESENTS ITSELF / BY CYNTHIA K. BUCCINI

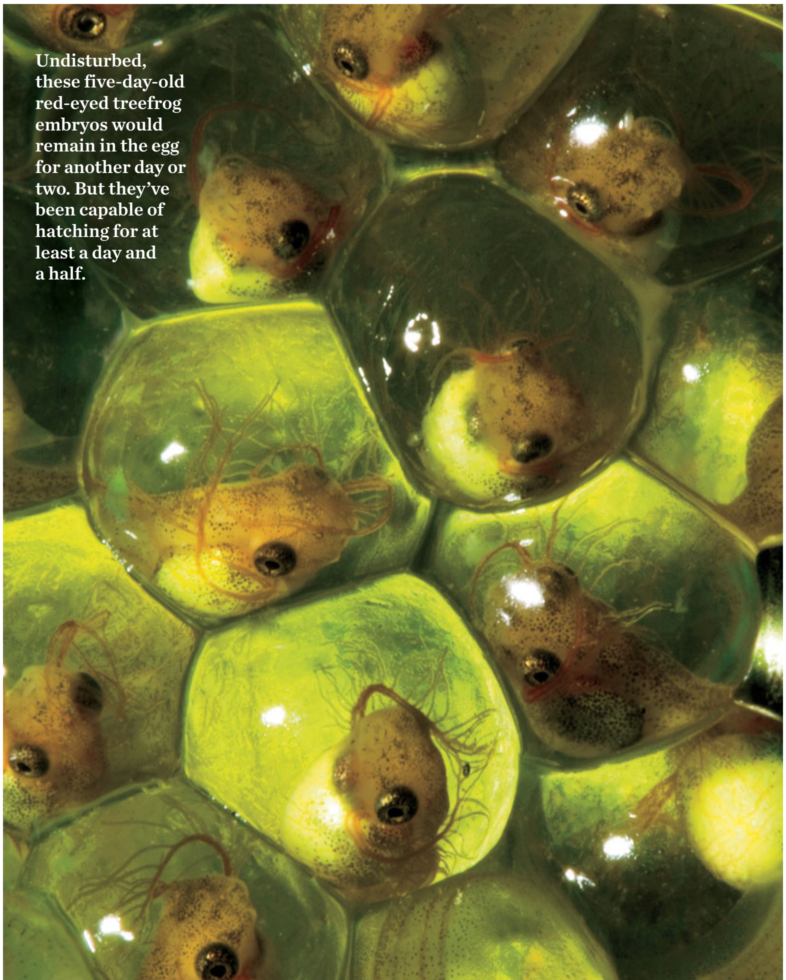
Escape Hatch

As far as housing goes, an egg is a pretty good place for a frog embryo. It offers physical protection and it keeps its inhabitant from drying out.

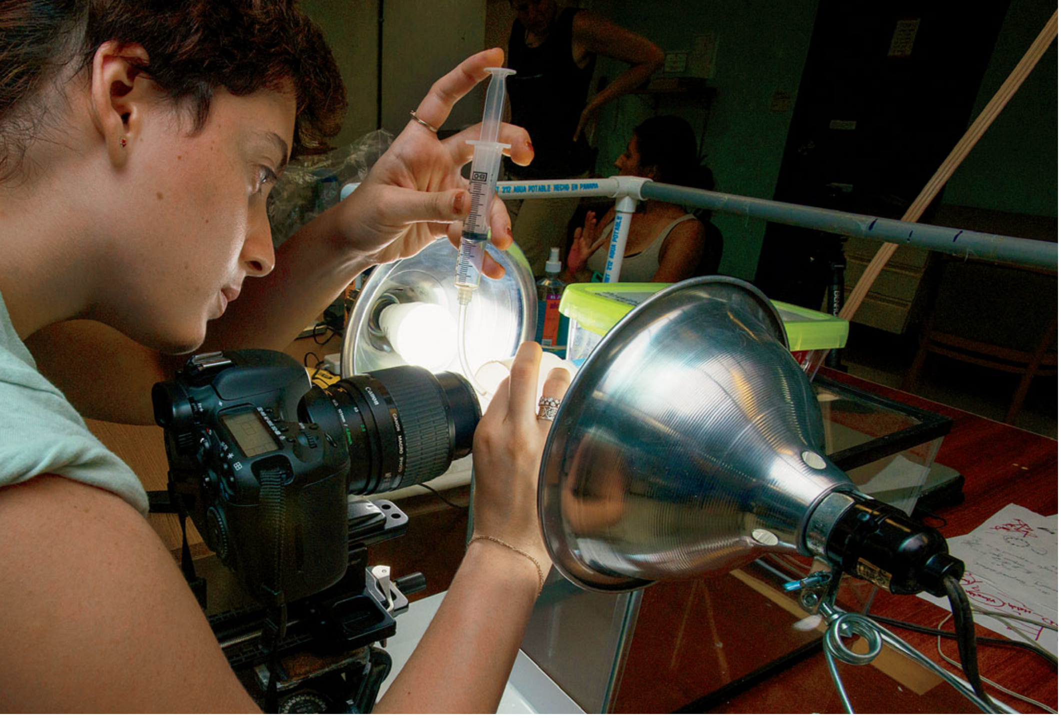
But like many homes with great curb appeal, eggs aren't always the ideal dwelling. For some frog eggs, being packed in a gelatinous mass called a clutch can limit the supply of oxygen. Eggs can be swept away by floods or killed by fungal pathogens, and they make a nice lunch for predators like snakes and wasps. Faced with such peril, it might seem that the eggs have little hope of hatching alive, but red-eyed treefrog embryos have a few survival tricks up their sleeve.

Biologist Karen Warkentin has found that these embryos are capable of assessing a variety of threats in surprising ways, and if danger presents itself, they can choose to hatch days earlier than they typically would. The process is called environmentally cued hatching, and in the last decade researchers have found evidence that it works for all kinds of animals—from flatworms to fishes, from frogs and salamanders to turtles and birds. "Hatching is a controlled, regulated response," says Warkentin, a College of Arts & Sciences associate professor of biology. "At some point, the embryos will decide to do it. It's amazing the information they attend to."

Environmentally cued hatching, says Warkentin, is part of a bigger story, and it has led to a collective shift in thinking about development. Until the 1990s, biologists believed that animals that begin life in an


Undisturbed, these five-day-old red-eyed treefrog embryos would remain in the egg for another day or two. But they've been capable of hatching for at least a day and a half.

K. M. WARKENTIN



SONIA PÉREZ ARIAS (CAS'15) places a newly hatched tadpole into a mechanical rotator.

egg hatch at a fixed time, regardless of what's going on around them. Red-eyed treefrogs, for example, typically hatch at six or seven days. As a frog embryo develops, it releases enzymes through glands in its head that break down the egg membrane, eventually allowing a tadpole to muscle its way out. But recent research by Warkentin and other scientists shows that many animals can scrap those plans. As a result, she says, researchers no longer look at hatching—and development in general—as a process that has a fixed timetable, but rather one that is "fundamentally responsive and contextual."

"Incorporating this plasticity into our thinking about biology is transformative," Warkentin says. "It's changing how we think about how life works."

Her current research, funded by a five-year National Science Foundation grant, focuses on two of the factors that can trigger early hatching in red-eyed treefrogs: vibrations that disturb the clutches, and a lack of oxygen, or hypoxia. "These are things that lots of eggs over a broad range of organisms—vertebrates and invertebrates—respond to," she says. She is asking an array of questions about how and why the embryo's behavior changes during development—from the animal's first few days to the time it hatches.

Warkentin, who does much of her fieldwork at the Smithsonian Tropical Research Institute in Gamboa, Panama, has been studying red-eyed treefrog eggs for more than 20 years. "She's the leader in this whole area of environmentally cued hatching," says Karen Martin, a professor of biology and the Frank R. Seaver Chair of Natural Science at Pepperdine University. "I think she has framed this question in a way that is very thought-provoking. It makes people think a little bit more about embryos as being agents in their world instead of passive recipients of their genetic programming. They're paying attention and they're responding."

The red-eyed treefrog makes its home in rainforests from Mexico to Colombia. During the breeding season, from May to November in Panama, female frogs lay clutches of eggs—40 or so eggs per clutch—on leaves and other vegetation overhanging ponds and swamps. At six or seven days, the tadpoles emerge and drop into the water.

But there are times when it's better not to wait that long. If the eggs fall into the pond, an accident that will cut off their air supply, embryos can hatch in as little as three days. Warkentin is trying to learn what level of hypoxia induces hatching, how long the embryos take to decide to hatch, and how their oxygen needs and hatching ability change during development.

> If the frog eggs fall into the pond, which will cut off their air supply, embryos can hatch as early as three days.

In the lab, she looks for answers using tiny glass cups just the right size for a dollhouse kitchen. Warkentin places a single frog egg in a cup, and puts the cup in a chamber fronted with a glass microscope slide. She fills the chamber with boiled, degassed water, and videotapes what happens next. In one video, the embryo wriggles around inside the egg in search of oxygen, and failing to find enough air, begins pushing at the egg. Seconds later, it squeezes out.

Warkentin can also manipulate the environment inside the chamber with embryos of different ages, and watch how the frogs behave. "We know what makes them hatch is a lack of oxygen," she says. "They become sensitive to that cue really young and respond to it behaviorally long before they can hatch. So, probably their ability to gather that kind of information is not changing over the hatching period."

Studying an embryo's response to vibrations is a bit more complicated. Gelatinous egg masses are shaky to begin with, and lots of things—both dangerous and benign—can disturb a clutch. Warkentin's earlier research focused on two such disturbances: snake attacks and rainstorms. By recording the vibrations and then playing them back to the embryos, she found that red-eyed treefrog embryos will hatch early in response to shaking by a snake taking a bite out of the clutch, but not to the vibrations of raindrops pelting the eggs.

Working with Greg McDaniel, a College of Engineering associate

SEAN MATTSON/SMITHSONIAN TROPICAL RESEARCH INSTITUTE

WEB EXTRA Watch a video of Karen Warkentin's frog egg experiments at bu.edu/bostonia.

professor of mechanical engineering, to develop and refine the vibration playback system, Warkentin discovered that the embryos are responding to the vibrational patterns and frequencies of each disturbance. "The more different features of the vibration they pay attention to simultaneously, the more it will reduce their risk of mistakes," she says.

That's important, because mistakes—deciding to remain in the egg during a snake attack or to hatch early because of a storm—can be costly. Preemies often die at higher rates; for one thing, they are poor swimmers and less able to escape from aquatic predators. "Clearly, there are costs and risks involved with hatching early," Warkentin says, "but if you're going to die in the egg, you might as well take your chances in the pond."

With the new grant, she hopes to answer more questions about how and why embryo behavior changes during development. "We know they get more 'hatchy' with development, but how exactly does that pattern play out?" she says. "Is the increased response only to certain kinds of stimuli, or is it very general? Does their discrimination of cues change, or their willingness to sample vibrations to get better information, which could be risky?"

Other questions have to do with the frogs' sensory system development. Red-eyed treefrogs start hatching in response to predator attacks *after* they start hatching in response to

A PAIR OF RED-EYED TREEFROGS lay a gelatinous mass of eggs on a leaf overhanging a pond in Gamboa, Panama.

flooding. Warkentin hypothesizes that the frogs feel the vibrations using mechanoreceptors in their inner ears. "Our idea is that the snake- and wasp-induced hatching depends on this mechanosensory system," she says, "and that it's coming online later than the oxygen-sensing system."

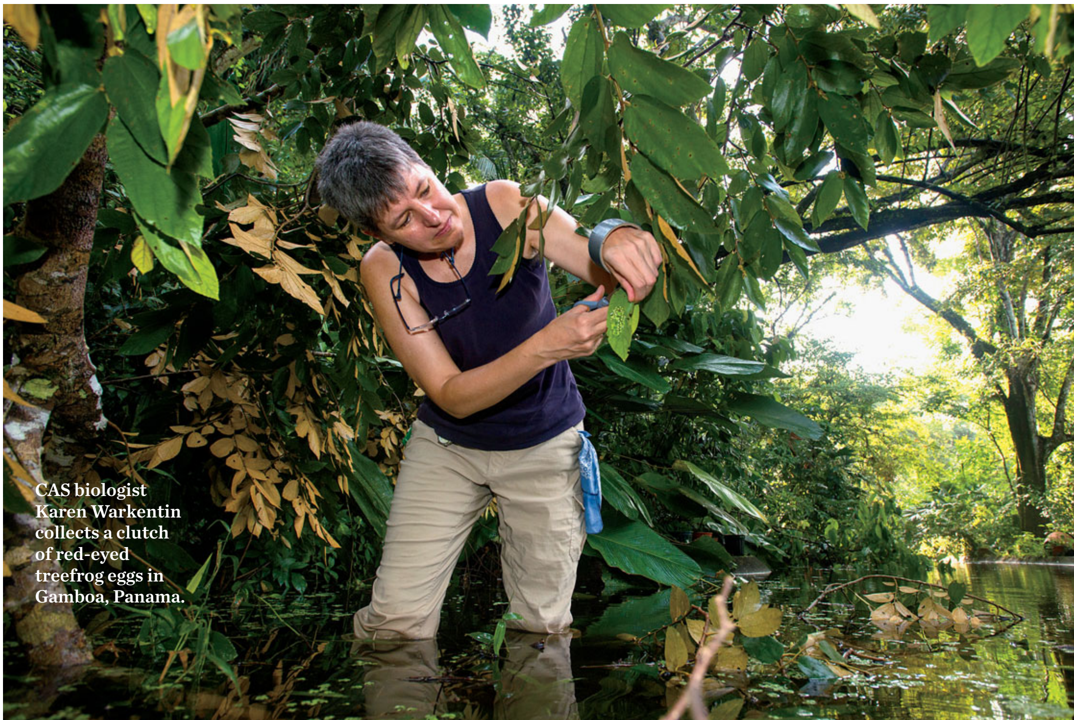
To test her hypothesis, she worked with McDaniel and an ENG undergraduate to build a tadpole rotator, a mechanical device fitted with a tube about the size of a plastic coffee stirrer. She places a newly hatched red-eyed treefrog tadpole inside the tube, along with some water, rotates the tube in increments of 15 degrees, and photographs the tadpole each time.

The tadpoles that have inner ear mechanoreceptors will rotate their eyes opposite to the way Warkentin rolls their bodies. It's called the vestibulo-ocular reflex, and tadpoles that haven't yet developed the mechanoreceptors don't have this ability.

Warkentin, along with Sonia Pérez Arias (CAS'15), a biology student in the Undergraduate Research Opportunities Program (UROP), put the rotator to work in summer 2014 in Panama. The results so far show that at the beginning—when the frogs are hatching in flooding but not escaping from snakes—they don't roll their eyes. Her hypothesis: the frogs' ability to flee from snakes is limited by their sensory development. "This is a work in progress," she says, "but from Sonia's UROP project, we see there is a big change in the vestibulo-ocular reflex from three to four to five days. It looks like the change in responsiveness to snake attacks matches up with the changes in the reflex."

She'll continue the tests this summer. "This is a critical step in understanding how escape hatching, and embryo decisions, work," Warkentin says. "The sensory system is how embryos—or any animal—get information about the world. So, I think that these embryos are—during the period of time when they are able to hatch but are usually still in the egg—basically gaining a whole new sensory modality, a new channel of information flow....Then they can make decisions or show behavioral responses based on that new stream of information.

"It's going to be fascinating. It will be so much fun to test these ideas."



CAS biologist Karen Warkentin collects a clutch of red-eyed treefrog eggs in Gamboa, Panama.

K. M. WARKENTIN (TOP); SEAN MATTSON/SMITHSONIAN TROPICAL RESEARCH INSTITUTE (BOTTOM)