Gone fishing. In Trinidad, a mark-recapture study of guppies is testing the connections between evolution and ecology.

Downloaded from www.sciencemag.org on August 24, 2012

The Great Guppy Experiment

A tiny fish is showing how ecological and evolutionary forces interact to shape the world we live in

GUANAPO RIVER VALLEY, TRINIDAD AND TOBAGO—David Reznick is on a first name basis with the hundreds of guppies living in a stretch of the Taylor River, a tropical mountain stream in Trinidad's Northern Range. Ditto for populations of these tiny fish in three other nearby streams, all headwaters of this Caribbean island's Guanapo River. For the past 4 years, this evolutionary biologist from the University of California, Riverside, and a cadre of dedicated interns and colleagues have made monthly treks up these streams to follow the lives of the guppies. They have learned how fast each fish grows, where it spends its time, and, thanks to genetic tests, who its descendents are.

This intimacy, along with detailed studies of the streams themselves, is helping to answer a key biological question: What is the interplay between ecology and evolution in shaping how both a species and its environment change through time? "They are looking at everything from the changes in the guppies up to the ecosystem," says Stephen Ellner, an ecologist at Cornell University. Others are also looking for these sorts of effects (see sidebar, p. 906). But "there's been nothing like it with such thoroughness and detail."

But the pioneering, $5 million "eco-evo" experiment hasn't always flowed smoothly. An unexpected guppy baby boom forced Reznick's team to scramble to find more work space and help, and the researchers have had to cope with flash floods and voracious army ants. And now that results are starting to come in, the project is running low on money. Nonetheless, "it's already a classical study," Ellner says.

Rapid evolution

One morning this past June, Reznick steered a Suzuki Jeep along a steep, winding road into his study site at the top of the Guanapo watershed. Just 2 years ago, the condition of the road—which saves an hour's walk—was so bad that researchers and local residents spent 3 days resurfacing part of it with pitch to make it passable. Now, the entire road is sorely in need of repair, and Reznick worries that it won't last much longer.

Reznick, 60, has been doing research in Trinidad for nearly 35 years. In 1978, he was a fourth-year graduate student at the University of Pennsylvania studying mosquito fish in New Jersey when he heard another scientist talk about the island's guppies. Soon, Reznick had persuaded his thesis committee to let him travel here for a project that changed the trajectory of his career. "I always wanted to work in the tropics," says Reznick, who still has a childlike enthusiasm about the island's plants and animals, particularly frogs and snakes. Many evenings after the work is done, he heads into the bush, tracking down the peeps and croaks of tree frogs.

He wasn't the first scientist to be captivated by Trinidad's guppies—or the possibility of using the island's steep mountain streams as a natural laboratory. Beginning in the late 1940s, independent biologist Caryl Haskins helped bring ecological genetics to life by exploring how ecological conditions influenced color variations among Trinidad's guppy populations. He discovered that, as one moves upstream along the rivers and their tributaries, the fish communities get simpler. Lower reaches have guppies and other fish, such as cichlids, that hunt guppies. But these predators can't make it upstream past waterfalls, so middle stream reaches tend to harbor just guppies and killifish, an omnivore that only sometimes eats guppies. And in the headwaters, Haskins found that not even guppies were present, just killifish, which are able to hop out of the water and climb up seemingly impassable falls. Most importantly, Haskins showed that male guppies from the

CREDIT: COURTESY OF ANDREW BURNESS

Published by AAAS

downstream, high-predation communities had duller colors than fish in the upstream, low-predation stretches, presumably to be less attractive to predators.

During the 1970s, Haskins's findings prompted John Endler, now at Deakin University, Geelong Waurn Ponds Campus, in Australia, to try follow-on experiments: He took some of those downstream, dull guppies and moved them upstream into a low-predation environment. Within about five generations, the fish evolved brighter color patterns.

Such findings helped add heft to conceptual work done in the 1960s by ecologist David Pimentel of Cornell University. At the time, the fledging field of evolutionary ecology considered ecology to be the template that shaped evolution. Pimentel had a different idea. He proposed that ecology and evolution were like two actors in a play, constantly interacting and influencing each other. For example, he suggested that as organisms increased in number in a given environment, they consumed local resources, reducing what was available to support population growth. This change altered the selection pressures exerted by the environment, which in turn altered the organism's evolutionary course. Pimentel's experiments with house flies and parasitoid wasps bore out his idea, but "he didn't gain any traction at all," Reznick says.

One problem was that most biologists thought evolution happened too slowly to observe in nature, so they didn't try. "People didn't see evolution as a contemporary process," Reznick says. But in 1978, when Reznick heard Endler describe his work translocating guppies in Trinidad's streams, he had an epiphany: "His system was perfect for studying rapid evolution." The Trinidad guppies potentially reproduced quickly enough—producing up to four generations per year—to exhibit evolutionary changes in just a few years.

Soon, Reznick had convinced his thesis committee to let him go to the island to test one theory: that fish exposed to an abundance of predators should evolve to mature faster, at a smaller size, and put more resources into producing more young more often. He spent 21 days collecting fish from 20 locations, bringing five groups of guppies back to the lab. Ultimately, he showed that the higher-predation guppies evolved as predicted, and that the changes were due to fundamental genetic shifts and not just short-term exposure to a new and different environment.

The Trinidad studies weren't the only ones suggesting that evolution could happen on an observable time scale. Over the course of their several-decade study, Peter and Rosemary Grant of Princeton University had observed rapid changes in the size and shape of the beaks of Darwin's finches on the Galápagos Islands. Beaks varied from year to year, depending on how weather, particularly droughts, changed the types of seeds available for birds to eat. Soon, Reznick—who was by then a researcher at the Academy of Natural Sciences of Philadelphia in Pennsylvania—was following up with more studies in Trinidad that involved transplanting guppies into new stream environments and observing the changes over 4 to 11 years. The studies provided "an experimental test of evolution in nature," he says.

Guppy hunter. For 35 years, David Reznick has used isolated stretches of Trinidad's tropical streams as natural laboratories to test ideas about evolution by transferring and studying guppies.

CREDITS: E.PENNISI/SCIENCE

While Reznick was chasing his guppies, Cornell University ecologists Nelson Hairston and Ellner were following evolution from a different perspective. In the lab, they set up a model ecosystem that included microscopic freshwater organisms called rotifers and either one or two strains of their algal prey. In 2004, they monitored this enclosed environment, tracking the rise and fall in the abundances of the rotifers and algae. The rotifers preferred to eat one strain of algae, and so over time they changed the relative abundance of the two strains. Ultimately, the algae population "evolved" to have the genetic background of the less tasty strain. That shift, in turn, changed the ecology of the system by altering predator-prey dynamics. The experiment neatly demonstrated Pimentel's concept: "Eco" and "evo" acted together to stage an unfolding biological play. Observers "couldn't explain the [ecological] dynamics without understanding what's going on with the evolution in the system," says David Post, a community ecologist at Yale University.

Reznick wondered whether he might be able to document similar eco-evo interactions among transplanted guppies in his natural streams. In 2005, he decided to pull out all the stops to try to find out. He recruited ecosystem experts, geneticists, theorists, and population biologists to help develop a proposal

Downloaded from www.sciencemag.org on August 24, 2012

Published by AAAS

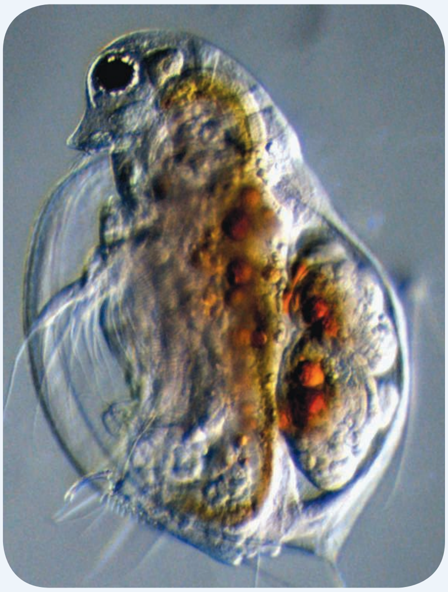
Eco-Evo Effects Up and Down the Food Chain

A decade ago, few ecologists factored evolution into their studies. How species changed over time was important, but it happened too slowly to be worth considering as they sought to understand ecosystem processes today. That attitude, however, is changing. Using guppies living in natural streams (see main text, p. 904) and other organisms, researchers are exploring links between evolution and ecology in a number of different settings, documenting interconnections that extend down to genetic changes. "It's a very dynamic field," says Andrew Hendry, an evolutionary biologist at McGill University in Montreal, Canada. "Everyone is getting involved."

In one notable example, David Post is focusing on how the alewife, a fish that lives in lakes in eastern North America, shapes and is shaped by its freshwater ecosystem. The community ecologist from Yale University and his colleagues have shown how these so-called eco-evo effects can ripple across a food web in unexpected ways. "It's one of the best examples of how ecology and evolution interact in a contemporary time frame," Hendry says.

Post's work follows in the footsteps of two other Yale researchers, John Langdon Brooks and Stanley Dodson. In 1965, they showed the key role that alewives (*Alosa pseudoharengus*), which grow to 25 centimeters, play in determining the makeup of lake zooplankton, particularly *Daphnia*, tiny crustaceans commonly known as water fleas. Typically, alewives are anadromous: They spend their adult lives in the Atlantic Ocean. Each spring, the fish swim up coastal streams from Nova Scotia south to the Carolinas into lakes for a few weeks to mate and spawn. The young spend the summer and fall in fresh water before they head out to sea again.

That cycle has profound implications for a lake's population of *Daphnia*, which are usually the dominant zooplankton. The newly arrived alewives and their young are hungry and feast on the water fleas. "They are a slash-and-burn fish," Post says. *Daphnia* populations are not restored until the following spring, when eggs resting in the lake bottom hatch.



Ripple effect. Both prey and predator, *Daphnia* affect lake food web dynamics.

About 300 years ago, however, the building of dams stranded some alewives in lakes, creating landlocked populations. More than 40 years ago, Brooks and Dodson showed the *Daphnia* had all but disappeared from those lakes. The landlocked alewives were left with smaller prey and, consequently, have evolved smaller mouths and smaller gill rakers inside their mouths that are better suited to catching those prey. That shift itself likely reflects how ecological change imposed by the alewife led to an evolutionary change in the fish.

But Post and postdoctoral fellow Matthew Walsh decided to go a step further: They looked at whether the ecological impact of the alewife on the *Daphnia* had evolutionary consequences for the *Daphnia* as well. Walsh collected eggs from the sediments of lakes with landlocked alewives, as well as lakes that were still connected to the sea and those that had no alewives at all. Then, he raised several generations of *Daphnia* in the lab. He found genetically based differences: *Daphnia* from lakes with anadromous alewives grew faster, matured sooner, and produced many more offspring than *Daphnia* from landlocked or alewife-free lakes. "There was an overall shift in life history evolution," says

Downloaded from www.sciencemag.org on August 24, 2012

and in 2006 won a $5 million grant from the U.S. National Science Foundation's (NSF's) Frontiers in Integrative Biological Research program to carry out the experiments. His plan: to mimic natural migration patterns by transplanting guppies into stream reaches that didn't previously harbor the fish. "I'm taking the results from theory and lab studies and asking, 'Are they important in nature?'" Reznick says.

Eco-evo test bed

At the end of the potholed road, Reznick parks the Jeep and walks along an old plantation trail, overgrown to a narrow path and flanked by tall cocoa and coffee trees. It ends on the banks of the Guanapo River, whose tributaries are at the heart of his eco-evo test bed. Reznick starts sloshing his way upstream. "We wind up walking in the rivers," he says. Boots with studded soles are the shoe du jour. At times the water is chest deep. Some places require the researchers to clamber up small waterfalls, often with two butterfly nets and a backpack full of water bottles in tow. (The bottles are used to take live guppies back to the lab.)

The Taylor is one of four streams that Reznick and his colleagues picked from more than a dozen candidates for their study, which began in 2008. Each has a 100- to 180-meter stretch of relatively flat water between two waterfalls that serve as barriers to fish migration. Prior to the experiment, the stream segments had no guppies, just killifish.

Before seeding each stretch with 40 male and 40 female guppies derived from a high-predation site downriver, ecologists carefully documented the ecosystems. They characterized the killifish and invertebrates, looked at primary productivity, measured the standing algal crop, and even took into account the organic contributions of leaves falling into the stream. At two streams, they also removed some of the overhanging canopy, increasing the amount of available light, potentially an important ecological variable. Then, every month, they began repeating their measurements—and capturing and releasing the guppies in order to monitor changes in both individual fish and the populations as a whole.

It's a laborious process. Three days before the Jeep trip, field manager William Roberts and several interns had trekked up to the Taylor River on a fishing expedition. Using a tape measure, they marked off distinct pools, riffles, and side pools. Then, with butterfly nets, they caught every fish they could see in each section and transferred the fish to marked Nalgene bottles filled with river water for the 2-hour trip back to the lab. The anglers had to stay out of the water to avoid disturbing the stream's ecology, so the netting took some creativity. "You have to contort your body into funny positions," Roberts says. It's not unusual, he says, to find someone draped over a rock reaching into a pool. And, 2 years ago, the collectors had to scramble to rescue their bottled fish from a flash flood that threatened to sweep away their research subjects. "Now we pay

CREDIT: POST LAB/YALE UNIVERSITY

Published by AAAS

Walsh, who is now based at the University of Texas, Arlington. In undammed lakes, the strategy allows *Daphnia* populations to thrive in early spring and deposit plenty of resting eggs before hungry alewives arrive, Walsh and Post reported in 2011.

This ecologically induced evolution in turn has another ecological effect. The spring population explosion of *Daphnia* takes a serious toll on the algae the water fleas eat, in turn shaping overall ecosystem function, Walsh and his colleagues reported in the 23 May issue of the *Proceedings of the Royal Society B.* Walsh grew *Daphnia* in large 56-liter tubs stocked with algae and monitored the growth of both the algae and the *Daphnia*, as well as the primary productivity of the tubs. In the tubs with *Daphnia* from lakes that harbored seasonal alewives, there was a rapid and sharp decline in the phytoplankton population that also caused the clarity of the water to improve. At the same time, primary productivity dropped by 32%. Those changes did not occur in tubs with water fleas from landlocked and alewife-free lakes. "More and more studies are showing that evolution can have strong effects on ecology," says Patrik Nosil, an ecologist at the University of Sheffield in the United Kingdom. Whether these ecological changes in turn affect evolution in the phytoplankton remains to be determined, Walsh says.

Opportunist. Chain pickerel have moved offshore in lakes with landlocked alewives.

Meanwhile, Post and postdoc Jakob Brodersen have now looked in a different direction along the food web. Chain pickerel are a native predator in eastern North American lakes, lurking close to shore to catch other fish. For a year, Post and his colleagues intensely sampled 10 lakes, three with landlocked alewives, three with seasonal alewives, and four with none. To their surprise, they found pickerel in the middle of landlocked lakes, far from their usual shoreline lairs. These fish were not just passing through, either. They tended to have a deeper body and a slender head compared to their counterparts close to shore, and their stomachs were full of alewives. Carbon-isotope ratios in the pickerels' tissues, which can differ depending on whether the fish has an offshore or inshore diet, indicated that these pickerel are offshore residents, Post reported last month at the First Joint Congress on Evolutionary Biology in Ottawa. That's important because it suggests that the change in the alewives' life history—to a landlocked population—has rippled out to affect the pickerel.

Voracious youngsters. In lakes, young alewives devour all the *Daphnia*.

"We believe they are undergoing a novel niche shift," Post says. Pickerel probably don't hang out in the middle of lakes with seasonal alewives, he notes, because the prey disappear each fall. But in landlocked lakes, there appears to be an advantage to heading out to the lake's middle: Offshore pickerel had a higher fat content than inshore pickerel, suggesting they have found a better way of making a living.

Hendry says the alewife system is a "particularly elegant example" of "how evolutionary and ecological effects cascade throughout the food web." **–E.P.**

more attention to the weather," Roberts says.

Once the collecting was done, the researchers hauled the now-25-kilogram packs back to the lab, a covered veranda in the back of the house where they live. There they transferred the fish to a series of aquaria lining a wall. Now, however, processing the fish is delayed. As rain pours down outside, army ants invade the lab, covering the floors and walls in black streams and devouring termites that have flown into the room the night before as part of their breeding migration. An unlucky gecko that strays into the ants' path is also gobbled up. By midmorning, the ants are gone without a trace and the interns set up an assembly line.

Reznick, eager to help, anesthetizes each fish and then hands it off to an intern, who puts it under a microscope to check for identifying tattoos. (When a guppy reaches 14 millimeters, the researchers inject two microscopic dots of colored plastic under its skin. There are 12 colors and eight possible injection points, creating enough combinations to give thousands of fish a unique tattoo.) Fish that aren't yet marked get a tattoo, and workers take three scales for DNA sequencing. They weigh and photograph the fish, and add information on any distinguishing characteristics to a master data sheet. There is a sense of urgency, as the researchers try to minimize their handling of the fish and get them through the process quickly before they wake up. Finally, the researchers are ready for a return trip to the Taylor, where they will release the fish into the same sections where they were caught.

Guppy boom

Early on, the grand guppy experiment almost became a victim of its own success. At first the numbers were manageable—populations in each stream grew to about 300 the first year. But by 2009, one stream had 1600 fish and by 2010, it had 2600. Populations in other streams were also exploding. Reznick got a panicked call from Andrés López-Sepulcre, the postdoc in charge of the census. "We didn't have the means to deal with that scale of fish," Reznick recalls. But they scrambled to hire more people and developed the high-speed production line. Now, the team has dossiers on 30,000 fish (about 15% of which are currently alive). For each, "We have a personal history, where it lives, who it lives with, what its weight gain is," Reznick says.

The rich database is giving the researchers a detailed look at how the eco-evo script is playing out for the Trinidad guppies. The guppy population explosion, for example, meant fish numbers in the test streams reached densities 10 times higher than those in the high-predation stream where the guppies originated. The denser populations led to changes in the amount and type of available food, and within three generations, the fish had begun to shift to different reproduction and growth patterns. For example, instead of growing fast and maturing young, as guppies in high-predation streams do, males are now older and larger

CREDITS: (LEFT) POST LAB/YALE UNIVERSITY; (RIGHT) GEORGE GRALL/GETTY IMAGES

Downloaded from www.sciencemag.org on August 24, 2012

Published by AAAS

at maturity. “We can see them changing their environment and evolving,” Reznick says. “The question is whether they are imposing selection on themselves and helping to transform the great-grandchildren into low-predation guppies.”

The fish are also affecting the ecology of their new homes. In the test streams, certain invertebrates, such as midges and mayflies, have become less abundant. Also, it seems that adding guppies increased primary productivity a little. And guppies have reduced the number of small killifish, as they eat or outcompete the newborns. “Guppies are in the process of changing their environment in all four streams,” Reznick says.

To help clarify how the fish influence stream ecology, Reznick’s team has also set up 16 small artificial streams. “We put in different combinations of guppies from low- and high-predation streams and ask how the ecosystem changes over time,” Reznick explains.

As the stream ecosystems shift, the new environments favor guppies with different traits, such as body shape and coloration, from those of their ancestors. To distinguish which of these traits are genetically based and which are influenced by the environment, once a year the researchers collect young guppies from each test stream and their source river. They bring these populations back to the lab to be raised in identical aquarium environments for two generations (essentially controlling for environmental factors). One finding from such studies is that the overhanging canopy appears to be playing a role: In streams with an intact canopy, and thus more limited light and plant growth, “we can see the evolution of male age and size at maturity occurs more quickly,” Reznick says. The males are maturing later in life and at larger sizes than those living in the two streams where researchers pruned the canopy.

The DNA taken from the scales is also enabling the scientists to glean details about reproductive success. Geneticist Paul Bentzen of Dalhousie University in Halifax, Canada, is using DNA markers to build guppy pedigrees that identify who mated with whom and the offspring. What they know so far is that there was a wide variation in reproductive success among the founding 40 females. After 4 months, numbers of their surviving young ranged from 0 to 16. Males also varied in their success.

By looking for shifts in how many offspring each male and female produces, Reznick hopes to detect eco-evo effects. Theory suggests that if eco-evo forces are at work, then selection should be weak at first—most females should be able to reproduce successfully—and become stronger over time, as the guppies modify their environment by using up resources. At that point, most females should have no offspring and a few should have many.

Reznick is convinced the guppy project will eventually put the spotlight on such compelling eco-evo examples. But he’s worried the story might go unfinished. The NSF program that is funding the work has ended, and Reznick is uncertain how he will continue the monthly mark-and-recapture studies beyond 2012. He’s already started using personal funds and frequent-flyer miles to stretch budgets. It’s an unfamiliar situation for the researcher, who has been continuously funded by NSF since 1978. He’d like to continue his work in Trinidad for another decade, estimating that’s how long it will take to nail everything down. “We know that guppies are ecosystem engineers,” he says, but “we’re only partway to showing that the way guppies adapt to their environment is part of that impact.”

Other researchers also worry that funding issues could bring the curtain down on the Trinidad project too early. “Things are going to start happening in the next 5 to 10 years,” predicts field manager Roberts. “It’s been going on for such a long time, it would be kind of a waste to stop what we are doing.”

–ELIZABETH PENNISI

No easy task. Monthly, researchers hike up to test streams to capture all the guppies (*inset*) there. They bring the live fish to the lab to weigh and photograph, then put them back into the streams.

CREDITS: (TOP LEFT) PIERSON HILL; (TOP RIGHT) CATHRYN HENDERSON; (BOTTOM) E. PENNISI/*SCIENCE*; (INSET) PAUL BENTZEN

Downloaded from www.sciencemag.org on August 24, 2012

Published by AAAS