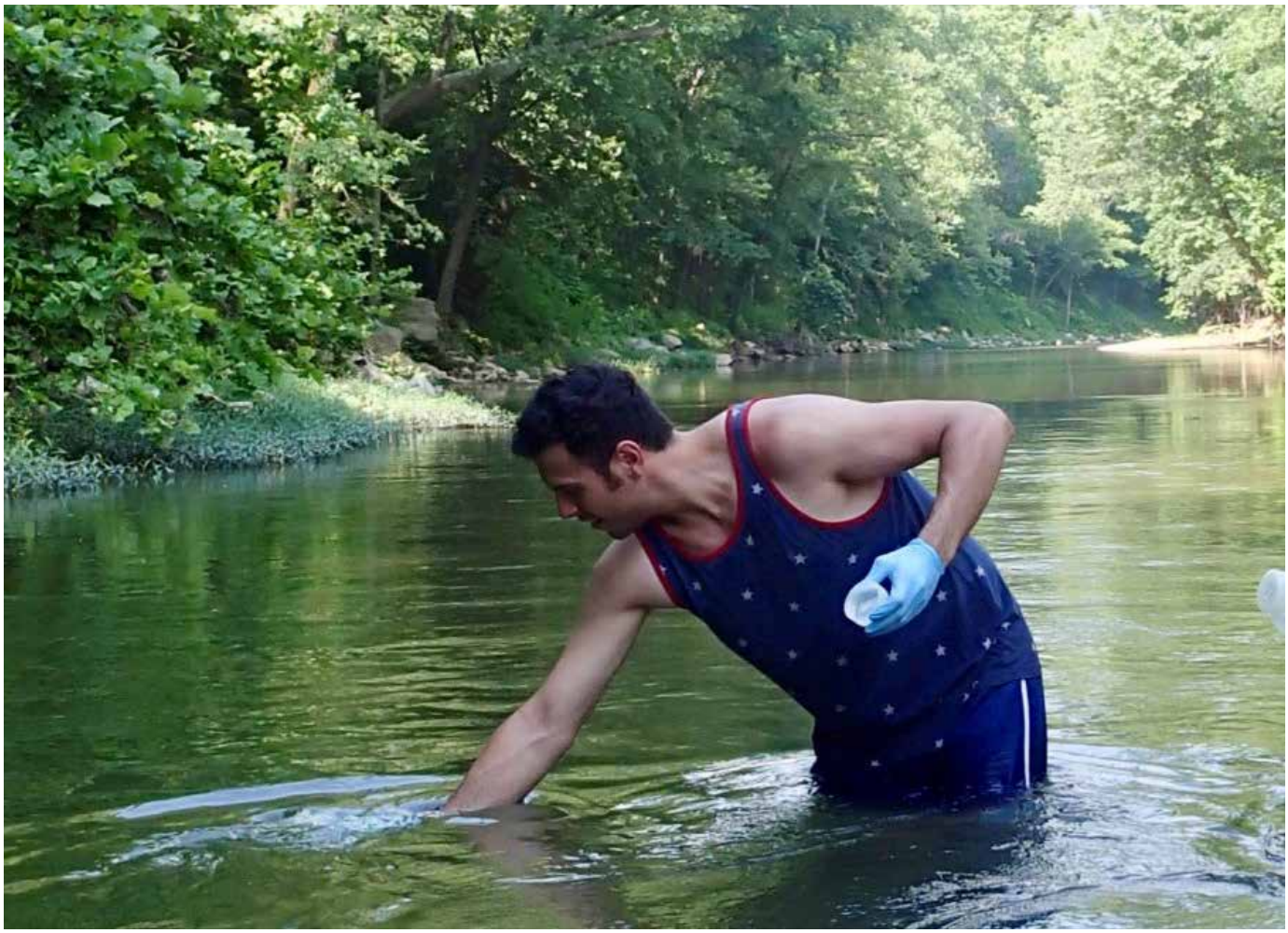
ENDANGERED SPECIES GRANT—INDIANA
Detection of Mudpuppies Using eDNA



A Purdue biologist collects water samples from an Indiana stream to analyze for mudpuppy eDNA. (Photo by Nick Burgmeier)

CURRENT STATUS

Final year of a two-year project

FUNDING SOURCES AND PARTNERS

Endangered Species Grant Program (E19R1)
Purdue University

PROJECT PERSONNEL

Dr. Rod Williams, Principal Investigator, Purdue University
Dr. Obed Hernández-Gómez, Co-Principal Investigator, Purdue University
Jenny Sutherland, Technician, Purdue University

BACKGROUND AND OBJECTIVES

The common mudpuppy (*Necturus maculosus*) is a fully aquatic salamander that historically occurred throughout much of the eastern United States. Mudpuppies occupy streams, rivers and lakes, and feed primarily on aquatic invertebrates and small fish. Declines in abundance are suspected, allegedly from siltation, water pollution, collection for the pet trade, and angling mortality. Because of their sensitivity to environmental degradation, mudpuppies are often seen as important indicators of water quality and the overall health of aquatic ecosystems. As a consequence of suspected declines, mudpuppies maintain a status of special concern in most states in which they occur. In addition, mudpuppies are the only known host for salamander mussel (*Simpsonaias ambigua*) larvae, a mollusk that is also experiencing population declines throughout its range. In Indiana, mudpuppies and salamander mussels are listed as species of special

Six positive (triangles) and 40 negative (dots) detections of mudpuppy eDNA in 13 sites with unknown mudpuppy presence (blue), and three sites with mudpuppy records (gold) in Indiana. Mudpuppy eDNA was also detected in the Detroit River in Michigan.

concern due to population declines throughout the state. As a result, surveys of mudpuppy populations are essential to assess the current distribution of this vital salamander throughout its historic range in Indiana.

Recent records of mudpuppies in Indiana were obtained by teams of biologists flipping large shelter or nest rocks in streams and rivers, deployment of baited minnow traps, or incidental encounters by anglers or biologists. This work is labor-intensive, time-consuming, logistically challenging, and invasive. For example, lifting large rocks is potentially dangerous for both researchers and mudpuppies (mudpuppies can be injured or killed during such sampling). Further, although care is taken to maintain the original orientation of its shelter rock, each animal's micro-environment is altered. Minnow traps are time intensive, and detection of mudpuppies is low. Therefore, there is a need for new techniques to facilitate non-invasive detection of mudpuppies in the environment. Such a development could prevent further disturbance of populations already stressed by habitat degradation and other human influences.

The goal of our project is to provide insights into the current distribution of mudpuppies throughout the state of Indiana using environmental DNA (eDNA). Organisms expel genetic material into the environment through shed cells, feces, decomposition, and other exogenous processes. Use of eDNA methodologies allows for the detection of a species' DNA in environmental samples such as water, thus providing an efficient and non-invasive way to assess a species' presence in the environment. We developed a DNA sequencing-based assay to detect mudpuppy eDNA in water samples. We applied this assay to evaluate the presence of mudpuppies throughout Indiana rivers with both known and unknown densities of mudpuppies.

METHODS

We first tested mudpuppy-specific sequencing polymerase chain reaction (PCR) primers for their ability to amplify mudpuppy DNA. We then validated the specificity of primers in silico and in the laboratory using banked DNA collections. We used these primers to develop a new DNA-sequencing based assay for the detection of mudpuppy eDNA in in water samples collected from rivers throughout Indiana.

Our sampling regime included streams with known and unknown presence of mudpuppies. Rivers with recent mudpuppy records included the Detroit River in Michigan and Buck Creek, Otter Creek, and Little Graham Creek in Indiana. Rivers with unknown mudpuppy presence included streams that historically have been occupied by mudpuppies, but recent surveys have not confirmed their presence. These streams included the Eel River, Tippecanoe River, Wildcat Creek, Big Pine Creek, Coal Creek, Sugar Creek (Parke County), Big Raccoon Creek, Little Walnut Creek, Sugar Creek (Johnson County), Flatrock River, Laughery Creek, Fish Creek, and the St. Joseph River.

For all streams, except the Detroit River and Buck Creek, we sampled water from three equally spaced locations in the mainflow of the stream. The Detroit River and Buck Creek were sampled directly downstream from a site with known mudpuppy presence. River water was vacuum-pumped through micro pore filters to capture any cells suspended in the water column. The filters were subjected to established DNA-extraction protocols to release DNA from captured cells. Products of the extraction step were used in amplicon sequencing library preparation for sequencing on an Illumina MiSeq machine at the Purdue University Genomics Core Facility. Sequencing results were evaluated for amplification of mudpuppy eDNA through bioinformatic tools, and sequence identity of positive samples was confirmed against genetic-sequence databases.

RESULTS

Our sequencing assay successfully detected mudpuppy eDNA in samples with 96% consistency among replicates. At the four sites with recent mudpuppy records, mudpuppy eDNA was detected in the Detroit River, Buck Creek, and two sites in Otter Creek; it was not detected in Little Graham Creek. At the 13 sites with unknown mudpuppy presence, mudpuppy eDNA was detected only in the Big Piney River (two sites) and Fish Creek (one site). Methodology for our sequencing-based eDNA assay will be made available to other researchers and agencies through publication in the scientific literature.

COST: $92,845 FOR THE COMPLETE TWO-YEAR PROJECT