South end of Lake Bacalar with the sinkhole Cenote Azul.
PHOTO CREDIT: Manuel Elías-Gutiérrez

RESIDENT OR INVASIVE SPECIES? ENVIRONMENTAL DNA CAN PROVIDE RELIABLE ANSWERS

by NATALIA V. IVANOVA[1], MARTHA VALDEZ-MORENO[2] and MANUEL ELÍAS-GUTIÉRREZ[2]

[1] Centre for Biodiversity Genomics, University of Guelph, Guelph, Canada
[2] El Colegio de la Frontera Sur, Chetumal, México

Environmental DNA can be successfully applied to identify vertebrates in a tropical lake improving our capacity to map and monitor species.

Monitoring life within large bodies of water – those species that should and shouldn't live there – can be very expensive and time consuming. To overcome these limitations, efforts in many temperate regions employ methods that use environmental DNA (eDNA), enabling effective and targeted detection of invasive and resident endangered species. Our study is the first to demonstrate that eDNA-based monitoring can be successfully applied to target the whole fish community in a tropical freshwater system and its adjacent wetlands.

Between 1980 -1990, eDNA was the term introduced to define particulate DNA and it was used to detect and describe microbial communities in marine sediments and phytoplankton communities in the water column[1]. However, eDNA is presently defined as the genetic material left behind by eukaryotic organisms in the environment, reflecting a rise in the use of eDNA for the detection of vertebrate and invertebrate species in aquatic systems[1]. The popularity of using eDNA has increased following the development of next-generation sequencing, advances in quantitative PCR (qPCR), and the growth of DNA barcodes libraries such as the Barcode of Life Data System (BOLD), providing a quicker and more taxonomically comprehensive tool for biodiversity assessments.

Lake Bacalar is the largest epicontinental habitat in Mexico's Yucatan Peninsula, and it is renowned for its striking blue color, clarity of the water, and for the world's largest occurrence of living stromatolites, a calcareous mound built up of layers of lime-secreting cyanobacteria. Due to the presence of sediments derived from karst limestone, it represents the world's largest fresh groundwater-feed ecosystem. The northern part of Lake Bacalar is connected to a complex system of lagoons and the southern part has an indi-

rect connection to the sea via a wetland system that connects with Hondo River and enters Chetumal Bay. This river has been heavily impacted by the discharge of organic waste and pesticides, by vegetation clearing, and by the introduction of invasive fish such as tilapia and the Amazon sailfin catfish (*Pterygoplichthys pardalis*)[2-4], first detected in 2013[4]. The Amazon sailfin catfish is a serious threat to the fragile stromatolite ecosystem due to its burrowing habits and competition with local fish. The impact of declining water quality and the rise of invasive species on the native fish fauna needs to be carefully monitored in aid of conservation efforts of Lake Bacalar.

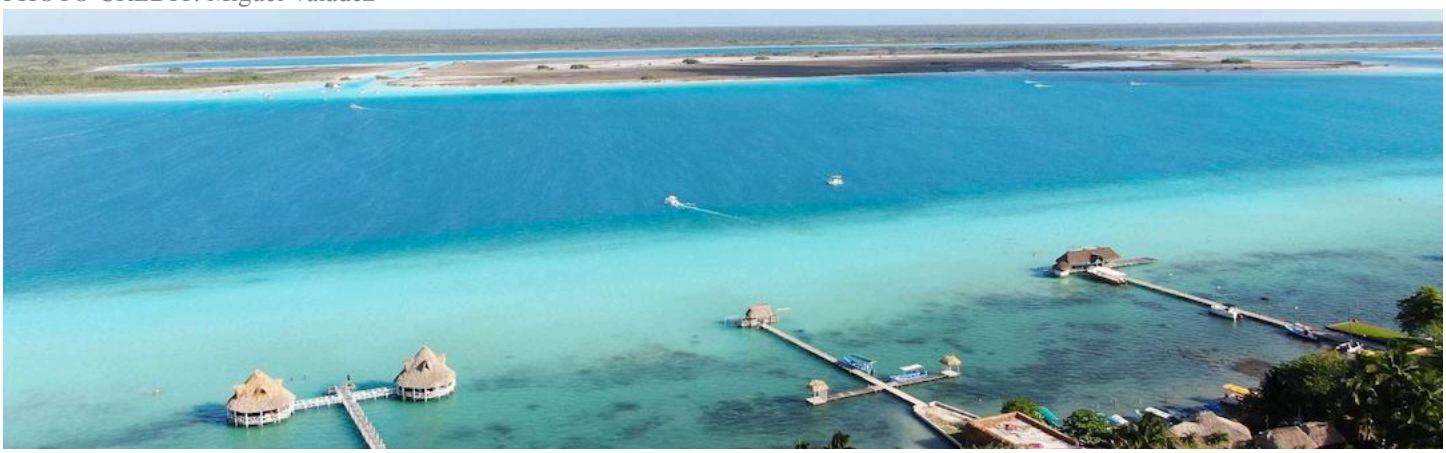
A team of researchers from the Instituto Tecnológico de Chetumal and El Colegio de la Frontera Sur sampled eight localities in December 2015, and January and April 2016. After each of 14 sampling events, water and sediment samples were immediately placed on ice before transportation to the lab in Chetumal. To minimize eDNA degradation, we filtered water samples within seven hours of collection. All filters and sediments were stored at -18°C before being transported on ice from Chetumal to the Centre for Biodiversity Genomics in Guelph, Canada, where DNA extraction was undertaken.

We sequenced short fragments (<200 bp) of the cytochrome *c* oxidase I (COI) gene on Ion Torrent PGM or S5 platforms. In total, we recovered eDNA sequences from 75 species of vertebrates including 47 fishes, 15 birds, seven mammals, five reptiles, and one amphibian. Although all species are known from this region, six fish species represent new records for the study area, while two require verification (*Vieja fenestrata* and *Cyprinodon beltrani /simus*), because their presence is unlikely in this ecosystem. While there were species (two birds, two mammals, one reptile) only detected from sediments, water samples recovered a much higher diversity (52 species), indicating better eDNA preservation in the slightly alkaline Bacalar water. Because DNA from the Amazon sailfin catfish was not detected, we used a mock eDNA experiment that confirmed our methods were effective.

Water sampling between stromatolites.
PHOTO CREDIT: Miguel Valadez

Interesting findings include the detection of rare species, such as an anteater *Tamandua mexicana*, which was detected by both PGM and S5 instruments from a river sample (Juan Sarabia), and migratory birds, such as warbler *Oreothlypis peregrina* known to overwinter in the Yucatan Peninsula.

Docks in front of Bacalar town.
PHOTO CREDIT: Miguel Valadez



Our study indicates that eDNA can be successfully applied to monitor vertebrates in a tropical oligotrophic lake as well as more eutrophic (higher primary production) wetlands and can aid conservation and monitoring programs in tropical areas by improving our capacity to map occurrence records for resident and invasive species.

Our next step is to convince Mexican and international stakeholders to implement these methodologies and establish a permanent biomonitoring system for this and other pristine freshwater ecosystems found in the Yucatan Peninsula. This work is necessary to detect effects of climate change, declining water quality, and the increasing tourism activities in this region.

References:

1. Díaz-Ferguson EE, Moyer GR (2014) History, applications, methodological issues and perspectives for the use of environmental DNA (eDNA) in marine and freshwater environments. *Revista de Biología Tropical* 62: 1273-1284. DOI: 10.15517/RBT.V62I4.13231
2. Wakida-Kusunoki AT, Luis Enrique Amador-del Ángel (2011) Aspectos biológicos del pleco invasor *Pterygoplichthys pardalis* (Teleostei : Loricariidae) en el río Palizada, Campeche, México. *Revista Mexicana de Biodiversidad* 82: 870-878
3. Alfaro REM, Fisher JP, Courtenay W, Ramírez Martínez C, Orbe-Mendoza A, Escalera Gallardo C, et al. (2009) Armored catfish (Loricariidae) trinational risk assessment guidlines for aquatic alien invasive species. Test cases for the snakeheads (Channidae) and armored catfishes (Loricariidae) in North American inland waters. Montreal, Canada: Commission for Environmental Cooperation. pp. 25-49.
4. Schmitter-Soto JJ, Quintana R, Valdéz-Moreno ME, Herrera-Pavón RL, Esselman PC (2015) Armoured catfish (*Pterygoplichthys pardalis*) in the Hondo River basin, Mexico-Belize. *Mesoamericana* 19: 9-19.

Online:

https://ibol.org/barcodebulletin/research/resident-or-invasive-species-edna-can-provide-reliable-answers/