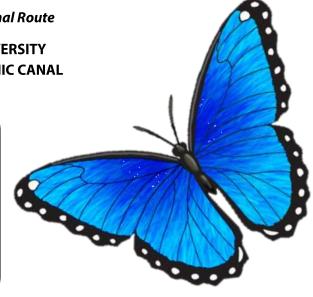
Saving Species along Nicaragua's Inter-Oceanic Canal Route

INVESTIGATION AND CONSERVATION OF BIODIVERSITY ALONG THE ROUTE OF NICARAGUA'S INTER-OCEANIC CANAL

Key messages:

- Forest patches are a refuge for fauna living in areas threatened by habitat destruction.
- The construction of a deep water port would be highly damaging to local ecosystems.
- To guarantee the preservation of local ecosystems, independent, transparent studies must be carried out by scientific experts and their trained teams of students and researchers.



Nicaragua has abundant natural resources, including the largest and best conserved tropical forests and ecosystems of the Central American lowlands, the majority of which have yet to be studied. Approximately 7% of the world's biodiversity can be found in Nicaragua which comprises only 0.13% of the global landmass. This biodiversity, found among Nicaragua's diverse ecosystems, native species and indigenous communities, is under threat from over-exploitation, deforestation, pollution and other human activities.

The recent inter-oceanic canal construction project represents an enormous threat to the region's biodiversity and ecosystems. The construction of the canal will cause irreversible damage to biodiversity, resulting in loss of multiple habitats, alterations in the food chain, deforestation, extinction of endemic species; impacting water resources and the activities of the indigenous, farming and ranching communities. Biodiversity provides society with goods and services due to its productive, recreational, visual and ecological values. Its conservation must be prioritized, guaranteeing the sustainable use

of resources in harmony with the wellbeing of the community and ecosystems.

Our society is currently struggling with the great challenge of protecting and conserving our biodiversity. This must be achieved with the involvement of many actors from civil society, government and universities. The involvement of universities, in particular is crucial for research and providing essential information for decision-makers. Research on biological systems must begin with determining which species are appropriate conservation strategies for the ecosystem as a whole.

Keeping in mind the role that must be assumed by educational systems in protecting Nicaraguan biodiversity, the Molecular Biology Center (CBM) of the University of Central America (UCA) has advocated to preserving flora and fauna in various local ecosystems to safeguard Nicaraguan biodiversity's genetic information.

For many years, the CBM-UCA has promoted the creation of a biorepository of Nicaraguan species which includes preserved tissues and genomic DNA. In consideration of the possible effects of the construction of the inter-oceanic canal and its subprojects, the CBM-UCA carried out a research project entitled INVESTIGATION AND CONSERVATION OF BIODIVERSITY ALONG THE ROUTE OF NICARAGUA'S INTER-OCEANIC CANAL.

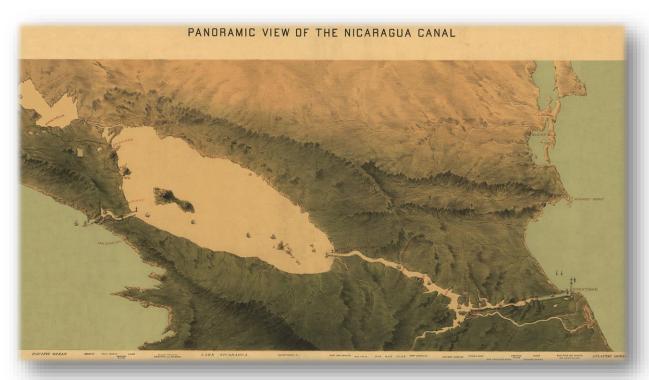
The goal of the project was to collect a variety of organisms found in the habitats that will suffer from the impacts of canal construction and preserve the tissues and DNA for future research. An additional goal of the project was to identify which species among those collected which are classified as threatened and those which require immediate protection.

On the eve of the sixth Great Mass Extinction, it is our responsibility, as guardians of our

planet, to protect nature in all its manifestations and valuable resources for the benefit of future generations.

The principle objects of this project were the following:

- 1. Collect and identify genomic DNA of flora and fauna in various sites along the route of the inter-oceanic canal.
- 2. Train students in sample collecting and photographing flora and fauna.
- 3. Create an online open access database containing all the information on the collected species.
- 4. Identify vulnerable and/or threatened species according to the UICN Red List and make this information available to Nicaraguan authorities, and the national and international communities.



The Canal, an old story: a proposed route of the canal, c. 1870, which followed the southern route. Source: Wikipedia

Methodology

The project was launched in September 2015 with a series of activities and the formation of a team of biology experts to lead the individual work groups. In order to conduct a precise and verifiable study, the species collection focused on birds, mollusks and insects, the areas of expertise of the three biologists leading the work groups. In regard to insects, butterflies were selected due to their ecological importance within ecosystems.

Once the taxa of interest were established, theoretical and practical training workshops were held to prepare students and local investigators in techniques of capture, management, collection and preservation of species. The area around Brito in the department of Rivas, in Nicaragua's coastal southern Pacific region, Brito River Basin #70, was selected for the study. This zone, consisting of dry tropical forest, was selected because an inter-oceanic canal and subprojects are scheduled to be built here, the first construction effort being a deep water port. The individual collection sites were Playa Brito, Tacotal and San Diego (Figure 1).

The methods of capture varied according to the species being collected. Fermented fruit and hand nets were used to capture butterflies (Figure 2a). In the majority of cases, the specimens were sacrificed for their future taxonomic identification in the laboratory. Individual samples were also taken of legs and antennae. A small team accompanied by an expert in lepidoptera taxonomy returned to the site for a secondary collection during the rainy season in August 2016.

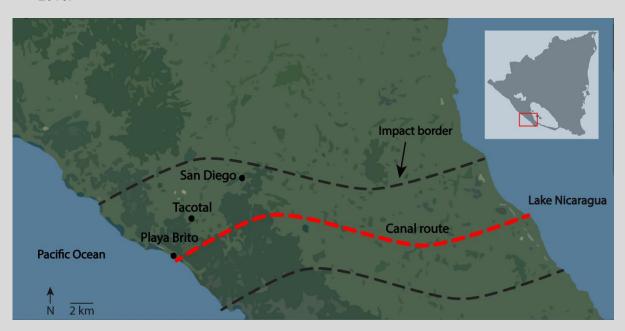


Figure 1. Collection sites selected for the study along the current canal route.

Bird collection was carried out by placing mist nets close to trees within the forest (Figure 2b), at 6 a.m. and removing them at 4:00 p.m., with nets inspected every 40 minutes. Blood and feather samples were taken from captured birds, before releasing the birds into their habitat. Bird observation was also carried out in order to identify bird species present in the area which might not have been present in the mist nets.

Mollusks collection was carried out in various habitats including: rocky zones, fallen trees, leafy litter, humus, swampy areas and small pools of water (Figure 2c). Mollusks were collected by hand and preserved in jars with alcohol.

All samples (birds, butterflies and mollusks) were then transported to the Center for Molecular Biology and stored in the center's biorepository for future genetic analysis.

The purpose of the biorepository is to support the study and conservation of Nicaraguan biodiversity through the creation of a tissue and genomic archive of the country's flora and fauna. Samples in the biorepository are identified by traditional methods and molecular analysis. Additional corresponding data is added to each record (e.g. common name, scientific name, collection site, geographic coordinates, DNA sequence) and entered into a data base which includes photographs and/or videos of each species. Tissue and DNA samples are preserved and labeled for future analysis.

Example of species found during the study



Trogon melanocephalus



Danaus eresimus ssp. montezuma

Collection methods



B. Mist network to capture birds







C. Searching for molluscs

Figure 2. Collection methods used in the study.

Results

Birds. Thirty-nine bird species were identified, 25 of which through observation and 6 through capture in mist nets. The bird species identified reflect a mix of low to medium tolerance to habitat loss. The species observed are classified by the International Union for Conservation of Nature (IUCN) Red List as of "Least Concern". No birds were found listed by IUCN as Near Threatened, Vulnerable, Endangered or worse.

Mollusks. Some 376 mollusk samples were collected, representing 39 species and 20 families. Within the species found, 31 were land based, 4 were fresh water species and 4 were interestuarine. The 3 most common species were Beckianum beckianum, Bulimulus corneus and Lamellaxis gracilis. None of the species of mollusks found in the area of study are listed as being at risk according to the IUCN Red List.

Butterflies. In total, 123 butterfly specimens were collected belonging to 34 species. None of the species of butterflies found in the area of study are listed as being at risk according to the IUCN Red List.

Discussion

The composition of bird species found in the study area is similar to that of other areas along Nicaragua's Pacific region. Net capture was low (<1 bird/100 net hours). This can be explained by the fact that these forests generally tend to have relatively poor undergrowth due to frequent intrusions by cattle and the resulting damage to and destruction to bird habitats. The close proximity to roadways also has a negative effect on tropical bird diversity. Sections of the study area that still conserve forest cover constitute a patchwork of remnant forest surrounded by road and agricultural areas.

Generalist bird species were close to 50% of the bird species found in areas with forest cover during this study. This means that land fragmentation has favored generalist bird species areas.

Land fragmentation is a product of economic activities such as cattle ranching which creates large expanses of pasture land and the destruction of the local forests. Frugivore bird species such as the Psittacidae were observed in the study; these birds do not adapt to loss of natural habitat and the most sensitive to the destruction of the remnants of forest still present in the area.

The structure of the mollusk community observed could be likened to that suggested by González, et al. (2010), in which the association of land gastropod species is categorized according to their preference for: 1) undisturbed land; 2) slightly modified land; 3) moderately altered; 4) altered; and 5) cosmopolitan. This is important in that mollusks are excellent indicators of the status of a given ecosystem. Due to their role as bio-indicators, their conservation status must be monitored. In neighboring Costa Rica, 42% of the registered animal extinctions belong to mollusk species, the majority of them pertaining to freshwater and land species. As mollusks have limited means of mobilization, they are unable to migrate elsewhere in the event of habitat destruction; it is therefore mandatory that their habitats be protected.

Data obtained regarding butterflies revealed that the conservation status of the ecosystems observed is good for hosting various species of butterfly. For example, the species *Memphis forreri* and *Myscela pattenia* found in the area are associated with well conserved tropical forest remnants.

Storage of specimens in the CBM biorepository is of great importance due to the possible and likely destruction of habitats and loss of species resulting from the construction of the inter-oceanic canal. In addition, a record of the biodiversity found in the different ecosystems, a principle objective of the bio-repository, is of great value as it allows us to permanently store the genetic information of the species found in the area. An individual's DNA is the primary material of its morphological and physiological characteristics. Genomics is indispensable in many areas of research and development, including the search for new pharmaceutical and antibiotics. Understanding the genetic relationships between individuals of different populations

will also provide improved tools for their conservation. With these in mind, a future project will be carried out to study the population genetics of the butterflies and mollusks observed and sampled to determine if the populations in the different habitats are related, which will provide us with more information on local ecosystem fragmentation.

Conclusion

The study area is comprised of the presence of patches of secondary forest growth. The variety of species found highlights the importance of these patches as refuges for the fauna living in this area and affected by habitat destruction resulting from cattle farming.

The construction of a deep water port of the magnitude designed by HKND Group, the concession grantee of inter-oceanic canal through Nicaragua, along with extensive accompanying infrastructure, would be highly damaging to local ecosystems.

The project, Saving Species along Nicaragua's Inter-Oceanic Canal Route, achieved the proposed objectives. Students and researchers were trained in fauna sampling and handling techniques. The resulting trained human resource body is of great value for carrying out future studies in other areas to be affected by the inter-oceanic canal, as well as general studies on local biodiversity. A total of 505 specimens were collected, identified and stored in a biorepository and genetic analyses will be performed on all specimens in the near future. The resulting database from this and future studies is open to all researchers and authorities who wish to access this information.

The Molecular Biology Center is committed to the conservation and protection of Nicaragua's biodiversity. The Center firmly

believes that in order to guarantee the preservation of local ecosystems, independent, transparent studies must be carried out by scientific experts and their trained teams of students and researchers. Faced with the imminent ecological disturbances resulting from the construction of the inter-oceanic canal, the CBM will continue to lead research that provides critical knowledge to actors and institutions responsible for decision making in an effort to reduce anticipated damage to regional ecosystems and biodiversity.

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