OPTIMIZING FECAL DNA COLLECTION AND STORAGE TECHNIQUES FOR NONINVASIVE GENETIC SAMPLING OF NEOTROPICAL OTTERS

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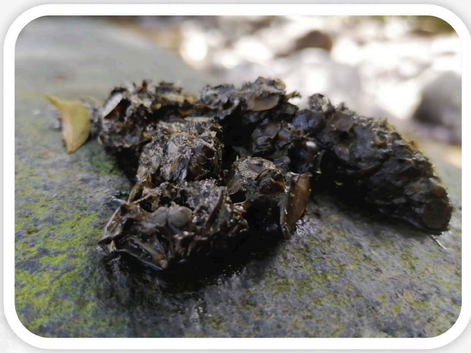
INTRODUCTION

- Molecular analysis from non-invasively collected fecal samples has revolutionized the field of conservation biology providing valuable information on ecological parameters for cryptic and endangered species such as Neotropical otters (*Lontra longicaudis*).
- Otter fecal samples obtained in tropical countries are frequently exposed to warmer temperatures, and higher humidity.[1,2]
- Previous studies on this species have used diverse storage collection methods (e.g., Silica gel, RNA buffer, EtOH 96%). Yet, success rate of fecal DNA amplification for this species is considered low (<50%).[3,4,5,6]
- Collection and storage methods for fecal DNA must be optimized, especially when working in tropical regions where samples are exposed to warmer temperatures that can accelerate DNA degradation.

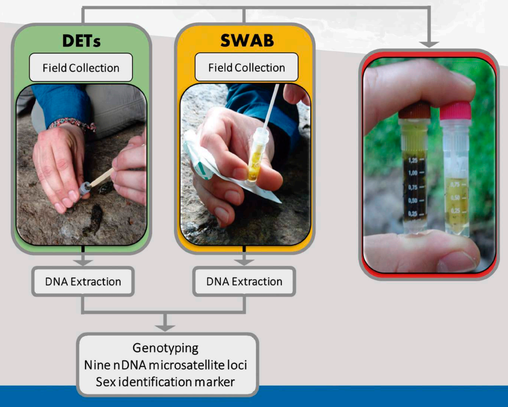
OBJECTIVE

- Optimize fecal DNA collection techniques and maximize the success of PCR amplification rates for the species.



METHODS

- Otter samples were collected during two sampling periods (2021-2022) along canals, lakes, and streams within the TNP.
- Fresh feces (< 24 hours old) and anal jellies were collected in duplicate through two storage techniques:
  - Collection of ~ 200 ul of fecal material and storage in DETs buffer.
  - Fecal external swabbing stored in ATL buffer.
- Amplification success was estimated by the total number of positive amplifications of each sample, divided by the total number of loci attempted across two PCR replicates.
- We classified a positive amplification as any sample with an amplification success ≥40% because these samples will likely result in a finalized individual ID genotype.



STUDY AREA

- Tortuguero National Park (TNP).
- TNP is located on the northeast Caribbean coast of Costa Rica.
- Area of 769.37 km2 of which 63% are marine and 37% terrestrial land.[7]
- It is characterized by the presence of a large number of interlinked canals, lakes, streams, and swamps.
- At TNP, humid tropical forests predominate.7
  - It receives up to 6,400 mm of rain per year.
  - Annual average temperature ranges between 25° to 30° C
  - Annual average humidity: 87%

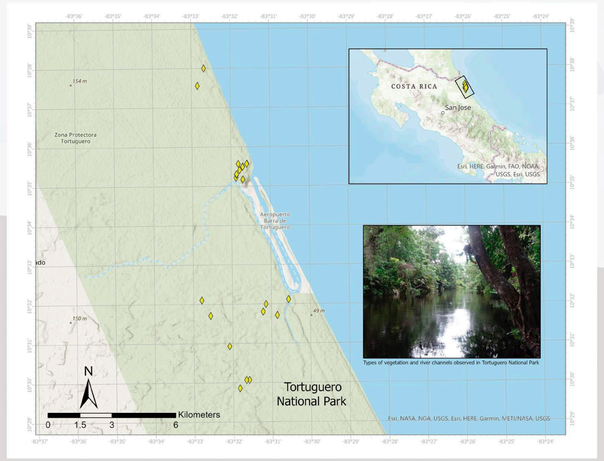
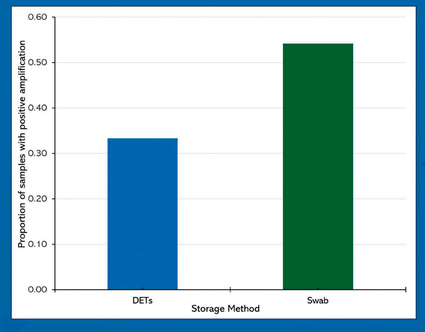
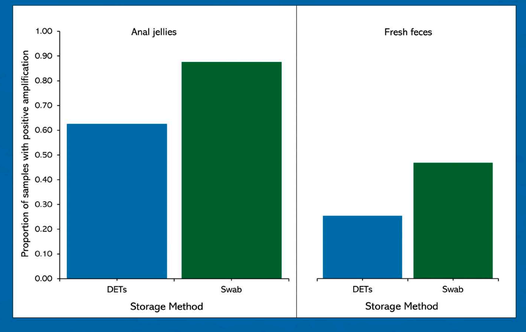


Figure 1. Location of Tortuguero National Park in Costa Rica. Yellow diamonds indicate noninvasive genetic otter collection sites.

RESULTS

- 50 noninvasive genetic otter samples (25 fresh feces/25 anal jellies) were collected in duplicate storage methods (DETs and swabs).
- The swab protocol yielded the highest amplification success rate (54%) for the 2021 otter samples.
- Anal jellies had a higher amplification success rate (Swab 88%, DETs 63%) than fresh feces samples (Swab 46%, DETs 25%) across storage methods.





CONCLUSIONS

- The high amplification success rate in anal jelly samples was consistent with previous genetic otter studies.[8,9]
- This is the first study to show that swab collection methods have substantially higher success rates and should be the preferred collection method for neotropical otters.
- Otter fecal samples likely contain high proportions of PCR inhibitors[9,10] and the swabbing method maximizes the collection of otter DNA while minimizing the amount of inhibitors and prey DNA included in the sample.
- The development of field sampling methods maximizing amplification success of fecal DNA is essential to understanding this semiaquatic mammal's population dynamics to inform future management and conservation plans.

FUTURE DIRECTIONS

- Evaluate the relationship between sampling time of day and amplification success rates.
- Test new loci used in previous Lontra longicaudis research to identify an optimal group of loci for individual ID and gene flow analyses.
- Complete individual ID and gene flow analyses of all collected samples

LITERATURE CITED

[1]Michalski et al. (2011) Molecular Ecology Resources, 11(5); [2]Tirelli et al. (2019) Mammalia, 83(5); [3]Trinca et al. (2013) Biological Journal of the Linnean Society, 109; [4]Trigilia et al. (2015) Hydrobiologia, 768(1); [5]Aristizábal-Duque et al. (2018) Therya, 9(1); [6]Latorre-Cardenas et al. (2020) Conservation Genetics, 21(4); 7National System of Conservation Areas (www.sinac.go.cr); [8]Hájková et al. (2009) Conservation Genetics 10; [9]Mowry et al. (2011) The Journal of Wildlife Management 75(7); [10]Farrell et al. (2000) Molecular Ecology (9).

ACKNOWLEDGMENTS

For funding and others supports we would like to thank Fulbright Fellowship, National Council on Science and Technology (CONACYT), The Rufford Foundation, Sigma Xi Grants in Aid of Research (GIAR) program, Idea Wild, The Explorers Club Grants, as well as UI Department of Fish and Wildlife, and UI Waits Lab group.

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