

# Development of Environmental DNA Probe for Early Detection of Bullseye Snakehead, *Channa marulius*

Pamela J. Schofield<sup>1</sup>, Mohammad Jubair<sup>2</sup>, Mary Brown<sup>1</sup>, Margaret Hunter<sup>1</sup>

<sup>1</sup>US Geological Survey, Gainesville FL, USA; <sup>2</sup>Cherokee Nation, Gainesville FL, USA

## Summary

- Invasive bullseye snakehead, *Channa marulius*, was first identified in Tamarac, Florida in 2000.
- Since the initial population was discovered, it has spread to cover more than 1,000 km<sup>2</sup>.
- It threatens to invade important natural areas such as the Water Conservation Areas (WCA), Loxahatchee National Wildlife Refuge and Everglades National Park.
- We have developed an environmental DNA (eDNA) assay to calculate snakehead detection and occurrence estimates.
- The species-specific eDNA assay was validated on both quantitative and state-of-the-art digital PCR.
- Water samples were collected in areas where snakehead were present and with unknown presence.
- This project addresses a goal of the USGS invasive species program to develop tools, technology, and information to prevent, contain, control and manage invasive species.

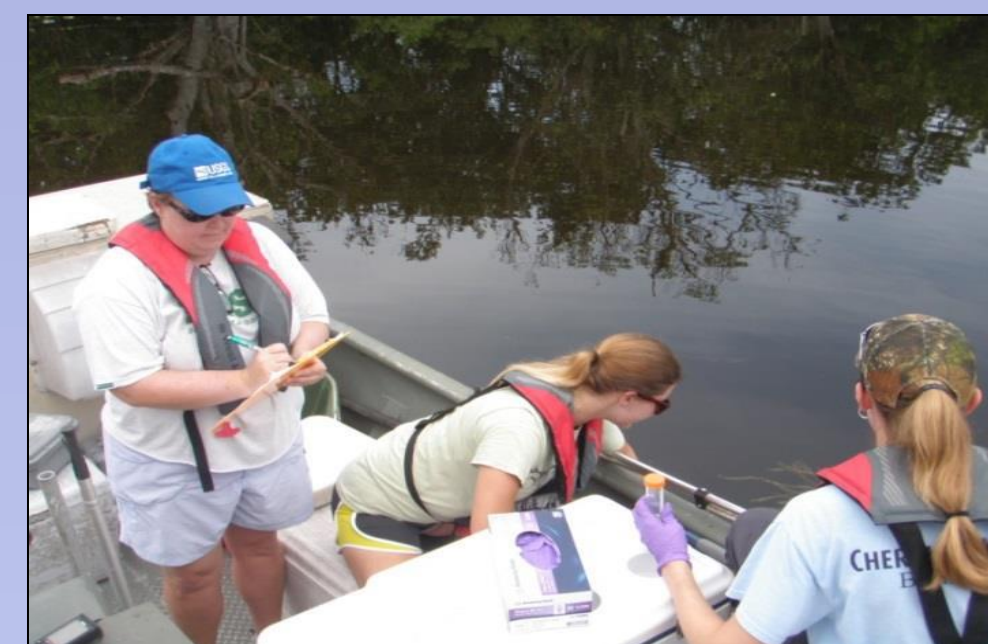
## Genetic Analysis

### Assay development

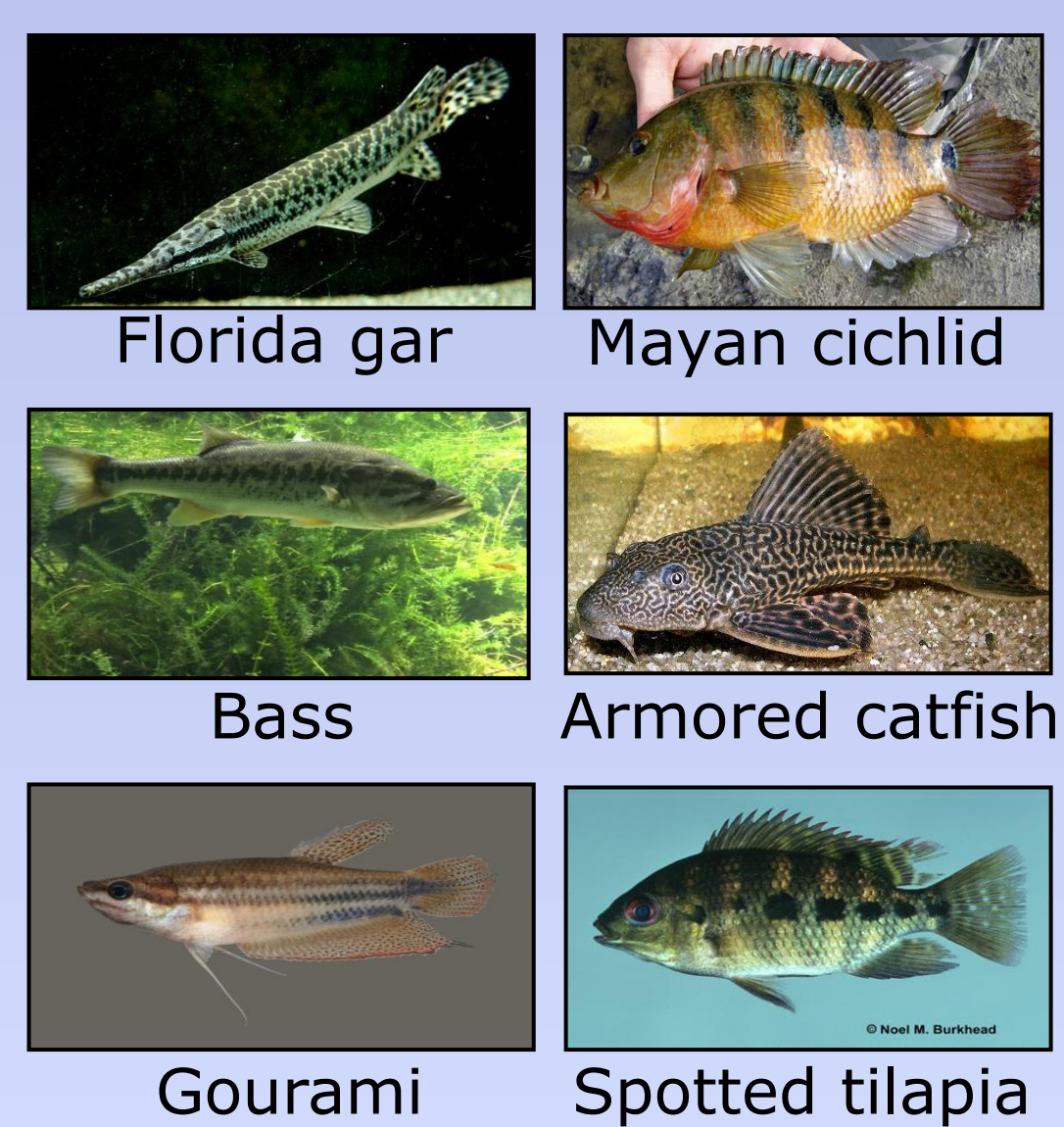


30 snakehead finclips

### Environmental DNA analysis



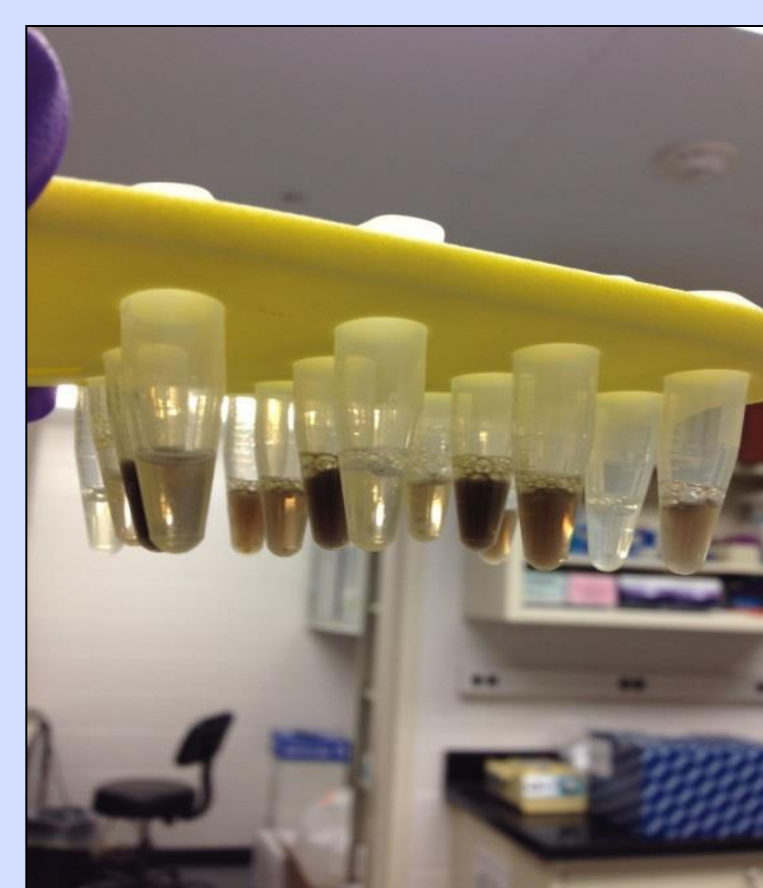
Sterile water collection



The species-specific snakehead assay was designed to exclude common species co-occurring in the environment



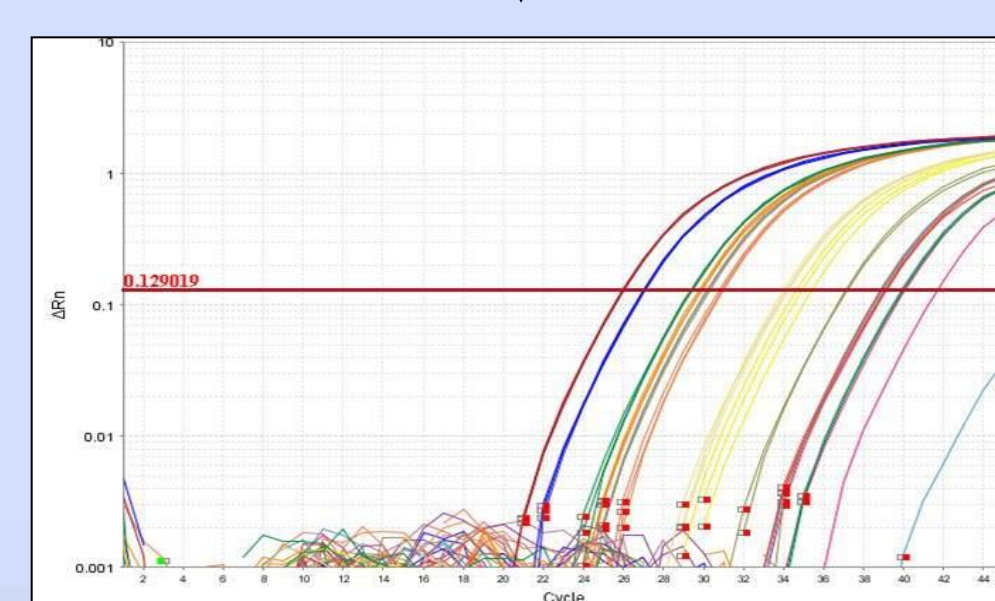
Filter water to concentrate eDNA



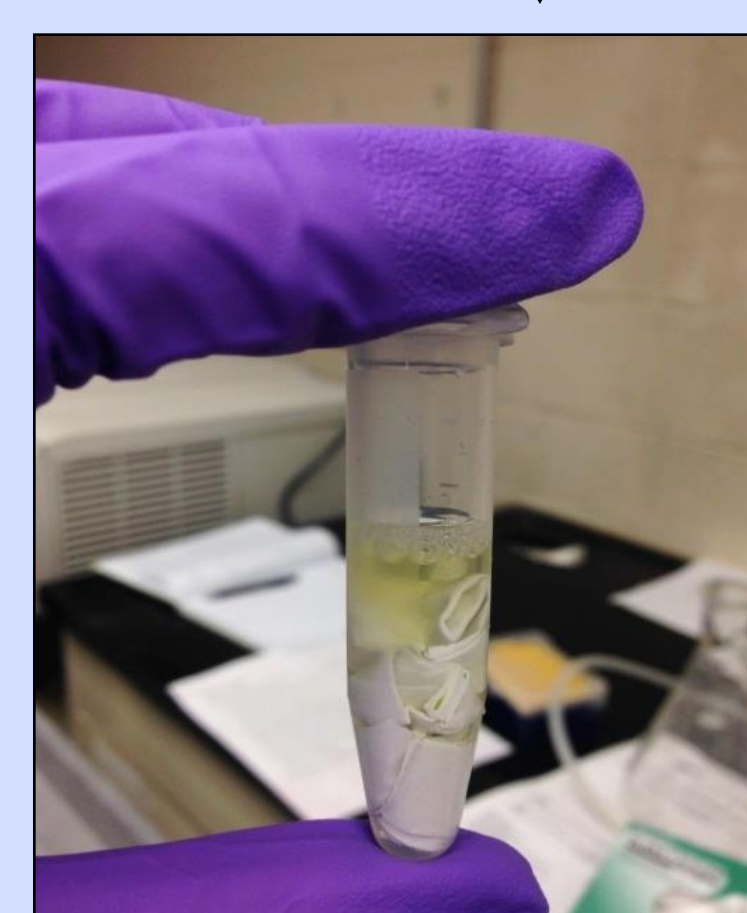
Extract DNA from tissue

Cytb PCR and sequencing

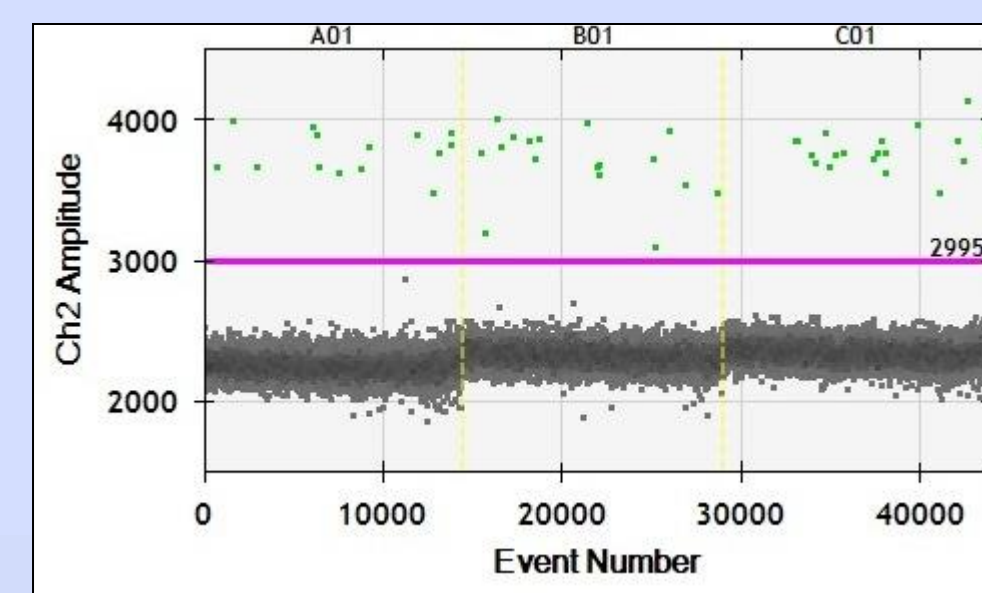
### Validation and optimization of the assay



Quantitative PCR



Extract eDNA from filter



Digital Droplet PCR to determine Concentration in sample replicates

### Calculate detection and occurrence estimates

## Results

Table 1: Snakehead eDNA water samples collected in south Florida from 2015-2017

Timeline	Region	No. of H <sub>2</sub> O samples	Conc. (molecules/uL)*
2015-2016	Palm Beach Broward	n=20	1.245 (0.080-5.695)
2016	Loxahatchee NWR interior	n=12	0.176 (0.069-0.678)
2015-2017	Miami-Dade Collier	n=20	0.061 (0.076-0.266)

\*Given range excludes zeros

Table 2: Occurrence and detection probabilities for snakehead eDNA in the three regions studied

Region	Occ	95% CI	Occ <sub>Ave</sub>	95% CI	Det	95% CI
Palm Beach Broward	0.792	0.537-0.979	0.581	0.457-0.712	0.669	0.545-0.782
Loxahatchee NWR interior	0.379	0.119-0.712	0.354	0.174-0.581	0.465	0.246-0.690
Miami-Dade Collier	0	0	0	0	0	0

Occurrence: Proportion of sites occupied by a species  
 Occ<sub>Ave</sub>: Probability of eDNA occurrence in a single water sample, given its presence.  
 Detection: Probability of detecting a species during a survey, given its presence

## Conclusions

- The developed assay was successful in detecting snakehead DNA from water samples collected in southeast Florida
- Palm Beach and Broward water samples had positive eDNA detections for snakehead, whereas Miami-Dade and Collier were not positive
- Loxahatchee NWR interior had few positive detections and low concentrations
- Environmental DNA can be used to further provide occurrence information to assist in prevention, control and management of invasive this species

### Acknowledgements

This study was funded by the USGS Invasive Species program. We thank Kelly Gestring and Murray Stanford of the Florida Fish and Wildlife Conservation Commission (FWC) for their support of our field work. Jeff Kline (Everglades National Park) provided many thoughtful comments that helped shape the project. Dr. Jason Ferrante, Gaia Meigs-Friend and Jessica Schulte assisted with laboratory analyses.

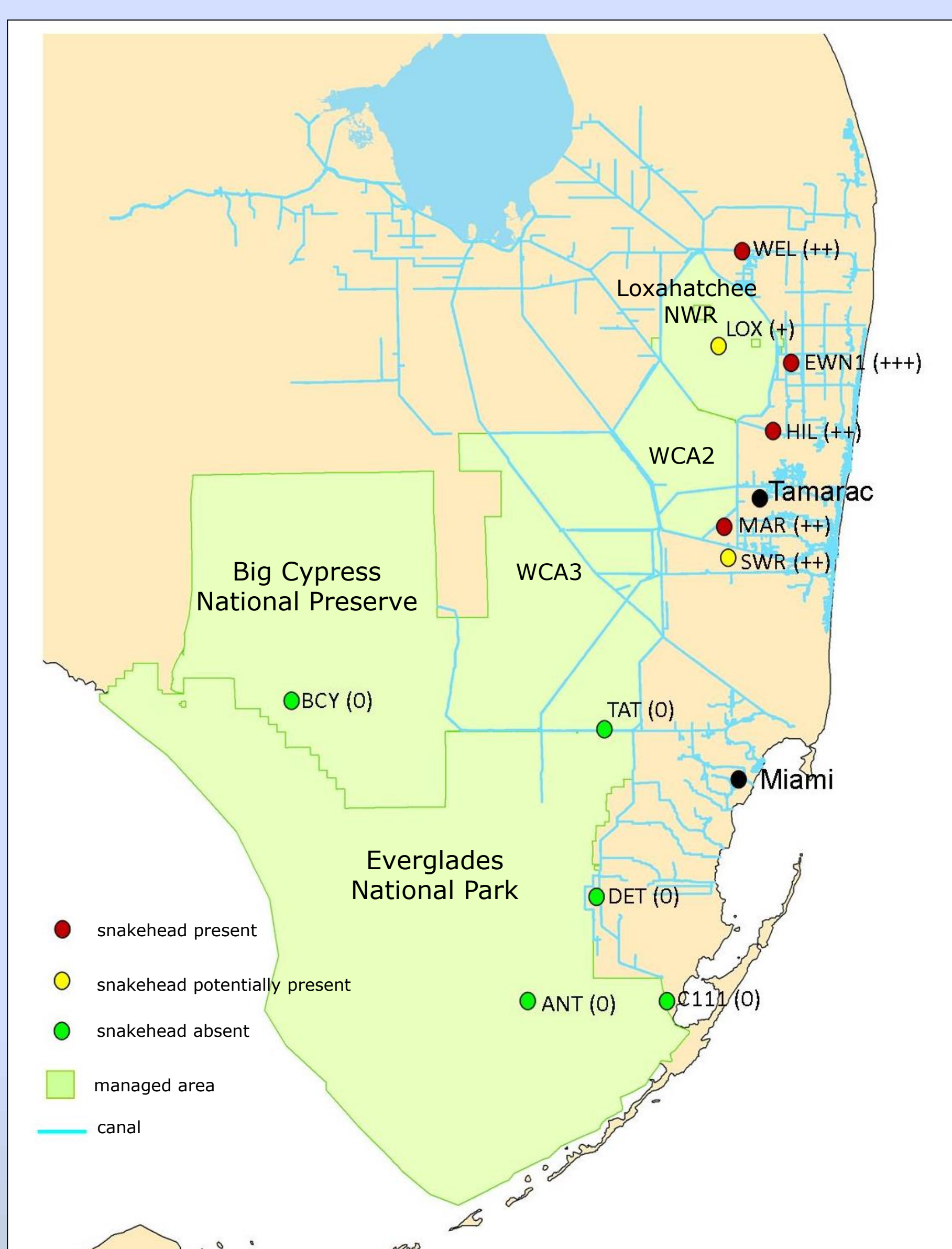


Figure 1: Snakehead eDNA sample locations in South Florida. Positive eDNA detections at low (+), medium (++) and high (+++) concentrations.