Labyrinthula protists on Seagrass in the Loxahatchee River Estuary?

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Background

'Seagrass wasting disease' describes the persistent abundance of protists (*Labyrinthula* spp) which are known to create lesions on seagrass blades and destroy plant tissue leading to decreased photosynthesis and seagrass mortality. Previous studies have identified the presence of pathogenic species of *Labyrinthula* associated with seagrass wasting disease in Florida Bay (Duffin et al. 2021) and have identified 2 pathogenic *Labyrinthula* species in the Indian River Lagoon (Lohan et al. 2020). Here we sought to establish if any *Labyrinthula* species (**Figure 1**) were present in mixed species seagrass beds in the Loxahatchee River Estuary (**Figure 3**).

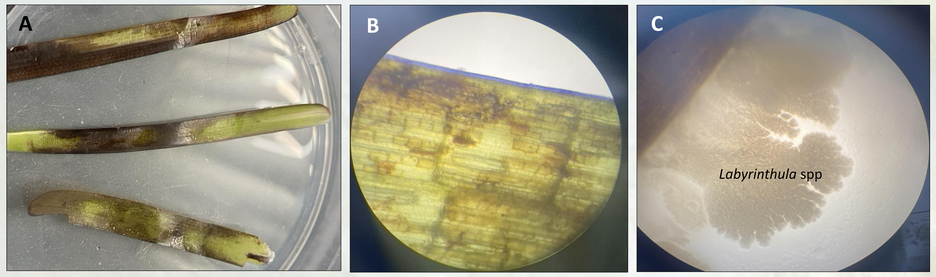

Figure 1: **A** *Thalassia testudinum* collected from Loxahatchee River Estuary. **B** *Thalassia testudinum* under light microscope. **C** *Labyrinthula* spp cultured from *Thalassia testudinum* collected from Loxahatchee River Estuary.

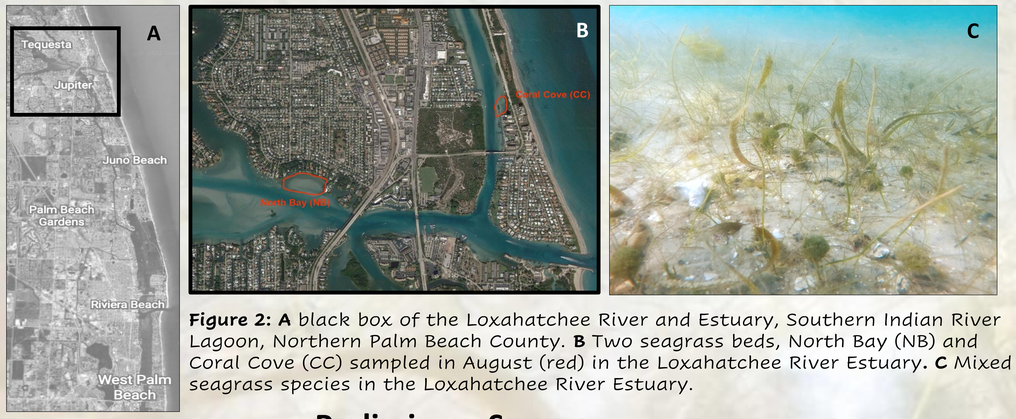

Figure 2: **A** black box of the Loxahatchee River and Estuary, Southern Indian River Lagoon, Northern Palm Beach County. **B** Two seagrass beds, North Bay (NB) and Coral Cove (CC) sampled in August (red) in the Loxahatchee River Estuary. **C** Mixed seagrass species in the Loxahatchee River Estuary.

Preliminary Survey

Two sampling events were conducted in August and November 2021 from two mixed species seagrass beds (**Figure 2A**) in the Loxahatchee River Estuary (**Figure 2B**). Individual blades of each seagrass species (*Thalassia testudinum, Syringodium filiforme, Halodule wrightii, Halophila ovalis* (previously *johnsonii*), and *Halophila decipiens*) were collected by Loxahatchee River District staff and processed at the University of North Florida (UNF). Analyses were primarily focused upon *Labyrinthula* **culturing and microscopy** (August 2021) in addition to **quantitative polymerase chain reaction (qPCR)** technology (November 2021) to identify any pathogenic species (following methods previously established by Duffin et al. 2020).

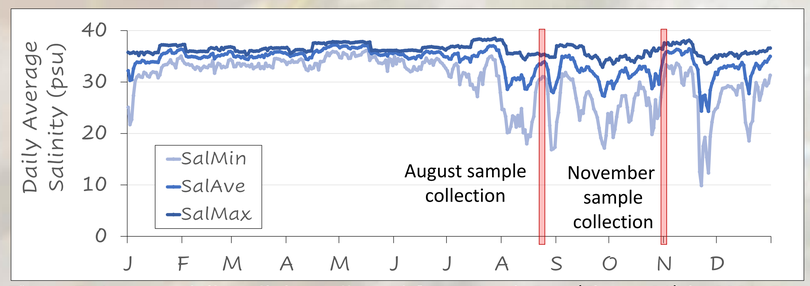

Figure 7: Average daily salinity collected from North Bay (Figure 2A) in 2021. Data was collected every 15 minutes using a deployed YSI data sonde (https://loxahatcheeriver.org/river/datasonde/).

Cultures & Microscopy (August 2021)

- 20 blades of seagrass were cultured for each species for 7-days (**Figure 3A**) and presence of *Labyrinthula* spp observed (**Figure 3B**).
- *Labyrinthula* spp prevalence ranged from 38% to 48% by site (**Figure 4**) and was most prevalent in Turtle Grass (Tt=*Thalassia testudinum*) (**Figure 5**).

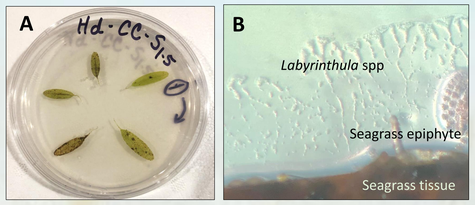

Figure 3: **A** *Halophila decipiens (Hd).* **B** Light microscopy image of cultured *Lybyrinthula* spp

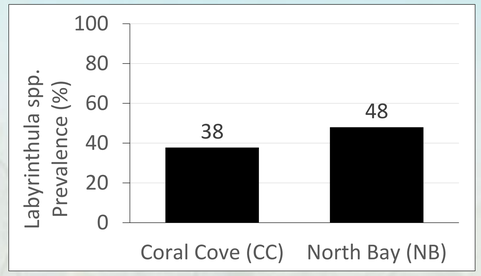

Figure 4: *Labyrinthula* spp cultured by site.

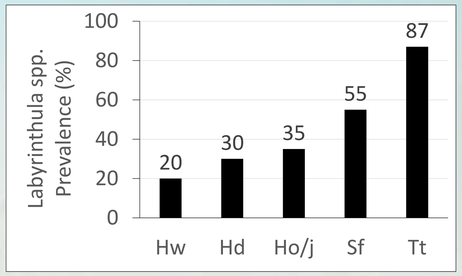

Figure 5: *Labyrinthula* spp cultured by species.

qPCR (November 2021)

- We examined *Thalassia testudinum (*Turtle Grass) due to the high prevalence of *Labyrinthula* spp cultured (**Figure 5**).
- Out of the 13 samples analyzed in triplicate, none contained known pathogenic *Labyrinthula* species (**Figure 6A, B**).

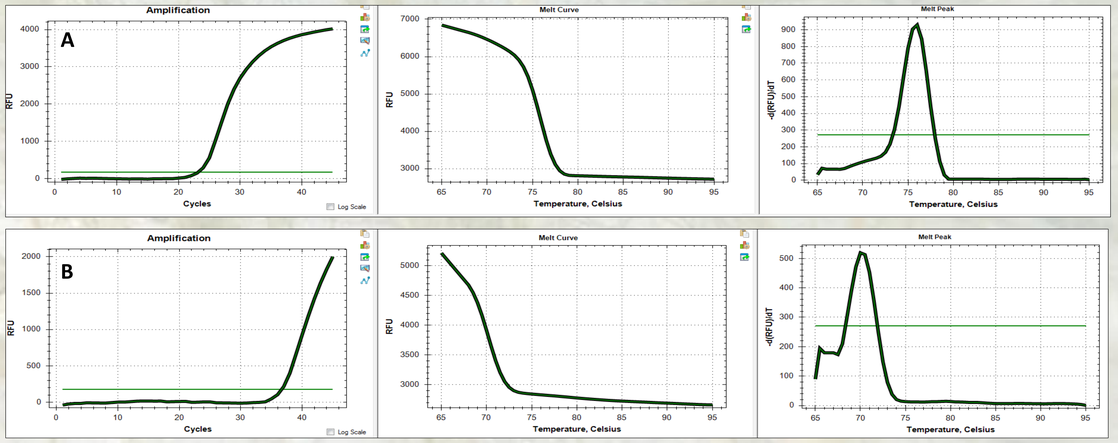

Figure 6A Q-PCR **positive control** showing the presence of an amplification curve (left) and a melt peak at about 76 degrees (right), indicating the presence of pathogenic *Labyrinthula* species.

Figure 6B Q-PCR **negative sample result different from the positive control** showing amplification curve (left) and melt peak at about 70 degrees (right), indicating the absence of pathogenic *Labyrinthula* species.

Discussion

- Semi-quantitative culture-based observations indicated 43% prevalence of *Labyrinthula* spp across two sites, which is 'moderate' compared to other studies in Florida (Lohan et al., 2020).
- *Labyrinthula* are known to be seasonal, and/or affected by prior abiotic regime (see Duffin et al. 2021), which may explain why the first samples collected in August, after a 6-month period of high salinity (**Figure 7**), were high in *Labyrinthula* yet no pathogenic species were detected roughly 2 months later when average minimum daily salinity decreased in November.
- Given the limited number of samples collected, future work should broaden the sampling size, considering additional larger scale spatial and temporal aspects, linking the presence of protists under stress.
- These results bring to question:
  - (1) If unknown pathogenic *Labyrinthula* species are present yet remain uncharacterized?
  - (2) What is the role of prevalent non-pathogenic *Labyrinthula* present on seagrass?
  - (3) How variable is *Labyrinthula* spp prevalence over space and time?
- Subtle positive correlations between seagrass diversity and *Labyrinthula* diversity have been previously identified (Lohan et al. 2020), potentially suggesting diversity driven parasite transmittance. Therefore, future investigations should consider *Labyrinthula* prevalence and seagrass disease susceptibility with potential host-parasite transmissions in mind.

Acknowledgements

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References

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