Application of Computed Tomography (CT) imaging in tropical seagrasses: Quantifying aerenchyma tissue critical for gas exchange

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BACKGROUND

Seagrasses are ecologically important marine plants that form habitat for many marine species. Tropical seagrasses require internal gas conduits to transfer oxygen from photosynthesis and the water column to belowground tissues. Traditional approaches to examine internal airspace, or aerenchyma, require cross-sectioning and labor-intensive techniques. Here, we examine a new approach to quantify aerenchyma in three tropical seagrass species using Computed Tomography (CT) and 3-D image analyses.

HYPOTHESES

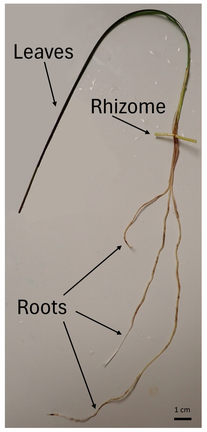
- The percent aerenchyma varies across species and tissue type (leaf, rhizome, and root).
- CT imagery combined with image analysis can provide rapid and reliable data on seagrass percent aerenchyma across species without physically sectioning tissue.

METHODS

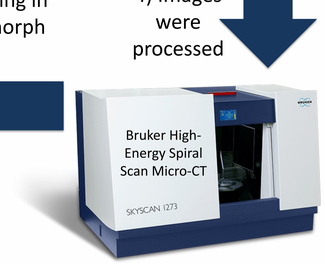
Dataviewer → Slicermorph → inPixio → ImageJ → SigmaPlot (ANOVA) → CTVox (video)

2) Whole plant prepped for CT scanning

3) Tissue types were embedded in wax for higher resolution CT scanning

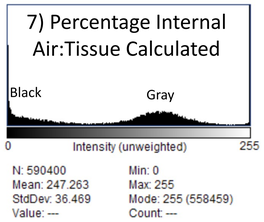


4) Images were processed



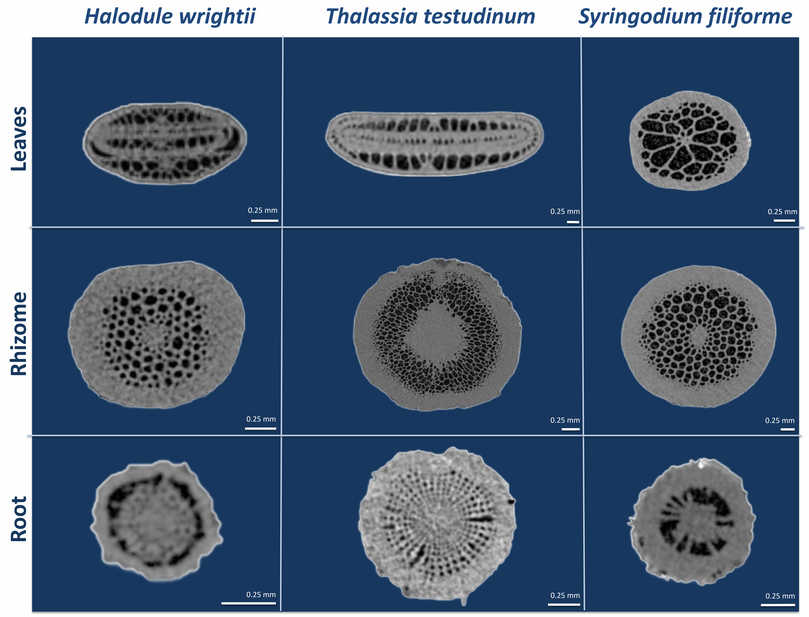
5) Virtual Cross-sectioning in Slicermorph

6) Black and white values were calculated from Virtual Cross-sections

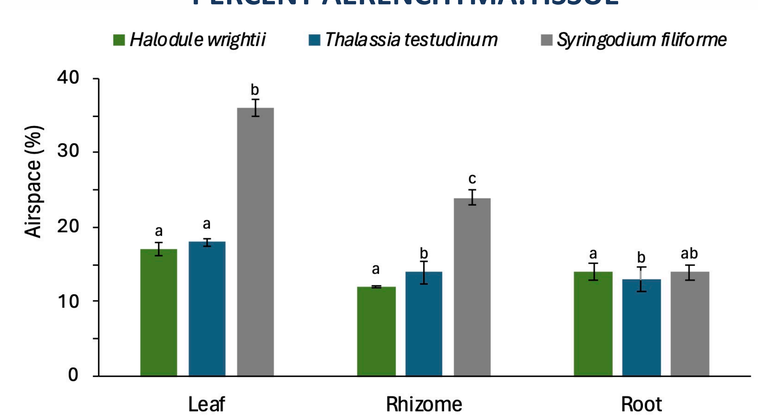


RESULTS

IMAGE-GENERATED CROSS-SECTIONS



PERCENT AERENCHYMA:TISSUE



- *S. filiforme* leaves contained a significantly higher ratio of aerenchyma:tissue than *H. wrightii* and *T. testudinum* leaves ($p<0.01$), while *H. wrightii* and *T. testudinum* percent leaf aerenchyma was not different ($p = 0.15$).
- Airspace was significantly different between all species in the rhizome ($p<0.01$).
- Aerenchyma percentage was significantly different in root tissues between *H. wrightii* and *T. testudinum* ($p<0.05$); however, root resolution was low and further scans are required to support these results.

DISCUSSION

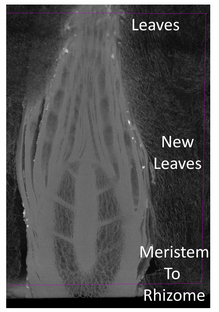
SEAGRASS AERENCHYMA

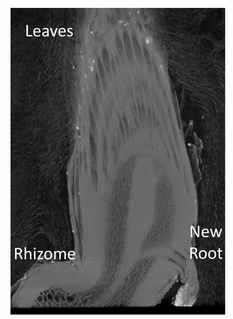
- Seagrass internal tissue was 15-35% airspace allowing for efficient transfer of O_2 in the plants.
- *Syringodium filiforme,* with a round leaf structure, had the highest percentage of airspace, suggesting a relationship between form aerenchyma.
- The slow-growing late successional species, *T. testudinum*, had greater tissue:airspace area, indicating a different strategy to survive in anaerobic sediments.

COMPUTED TOMOGRAPHY SEAGRASS RESEARCH

- The CT imaging was successful in producing high resolution cross-sections without physical thin sectioning, a new technique for botanical research.
- Our Lab's future CT seagrass research goals are:
  - Optimize CT scan resolution – particularly for roots
  - Add replicate scans for each species
  - Optimize image analysis by using AI software
  - Examine transition zones between tissues (e.g., leaves to rhizome, rhizome to roots) to analyze gas transport throughout the entire plant (see below).

CHECK OUT QR CODE BELOW FOR SEAGRASS VIDEO CT SCAN IN ACTION TO BACH!





ACKNOWLEDGMENTS

We would like to acknowledge FAU's OURI for awarding us an Undergraduate Research Grant, Dr. Koch for mentoring and assisting us, the FAU High Imaging Lab for use of the micro-CT scanner, and Jamie Knaub for assisting us in everything CT.