Evaluating the bioextractive capacity of a South Florida native macroalgae, *Agardhiella subulata*, for use in integrated multitrophic aquaculture

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Introduction

- As a mass production industry, finfish aquaculture can discharge high concentrations of dissolved inorganic nutrients – particularly nitrogen and phosphorus – into the environment that can cause eutrophication and ecosystem collapse
- Removal of introduced nutrients from wastewater can require specialized equipment, skilled labor, and high monetary input
- **Integrated multitrophic aquaculture (IMTA)** systems combine the culture of finfish species with cultures of bioextractive macroalgae or suspension feeders to organically remove introduced nutrients from effluent water while producing an additional marketable biomass from the filtering organisms[4]
- IMTA systems have not been broadly explored in the Gulf of Mexico or Caribbean Regions, particularly in the context of compatible native species for efficient nutrient reduction and growth within the system
- *Agardhiella subulata* is a South Florida-native red macroalgae species (Fig. 1a) chosen for use in this project due to its compatibility for year-round tank culture in the region[2] and potentially high bioextractive nutrient capabilities[3]

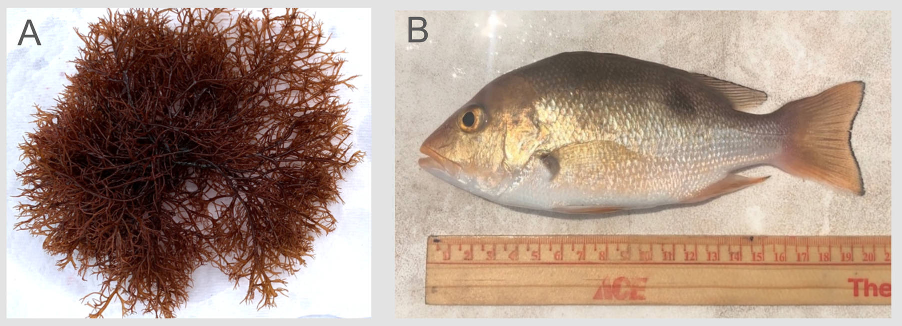

Fig. 1. A) *A. subulata*, a South Florida-native red macroalgae used as the secondary extractive species in the project, B) Juvenile American red snapper (*Lutjanus campechanus*) used as the primary fed, nutrient-producing species in the IMTA project

We aim to quantify the nitrogen and phosphorus bioextractive capabilities of *A. subulata* in an IMTA system with American red snapper (*L. campechanus*) (Fig. 1b) to assess its potential uses as a marketable biofilter for sustainable marine aquaculture practices

Methodology

- Culture system (Fig. 2) contained one primary tank of juvenile *L. campechanus* culture and six secondary tanks of *A. subulata* culture
- Three secondary tanks were supplied with control water from Biscayne Bay and were considered nutrient starved, while the other three were supplied with experimental *L. campechanus* effluent water
- Two identical, 15-day trials were run from March 8-April 7, 2020
- Every third day during each trial consisted of an identical sampling procedure:
  - *A. subulata* tissue samples collected from each tank and frozen for later elemental analysis
  - Temperature, dissolved oxygen concentration, pH, and irradiance for each tank were recorded
  - Water samples from inflow and outflows of each tank tested for concentration of NH_3, NO_3^-, NO_2^-, and PO_4^{3-} using colorimetric seawater tests
  - Recorded biomass (kg) from each algae tank

Fig. 2. (Left) culture system used for IMTA trials with three tanks used for experimental growth (EXP 1-3) and three tanks used for control growth (CON 1-3) with the primary *L. campechanus* culture in the round tank in the rear. (Right) 60-gallon macroalgae culture tank with centered aeration to reduce self-shading.

Results

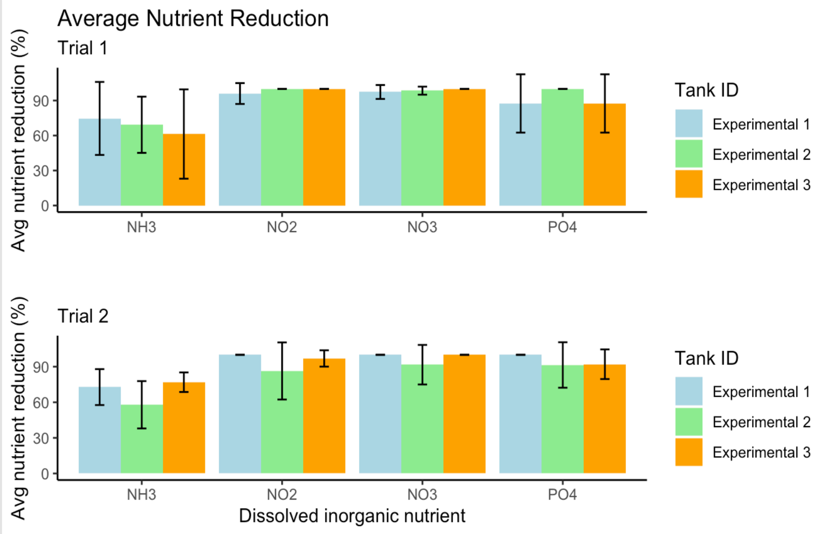

Fig. 3. Average nutrient reduction (%) of each nutrient by tank, by trial. Error bars represent standard deviation from the mean. Control groups were excluded from the figure due to no measurable presence of nutrients in incoming control water throughout either trial.

Table 1. Ranges of nutrients in incoming water throughout trials 1 and 2 as well as the mean percent reduction (% ±SD) of the experimental tanks in each trial for each dissolved nutrient.

Trial	$[NH_3]$ mg L^{-1}	Mean percent reduction (% ±SD)	$[NO_3^-]$ mg L^{-1}	Mean percent reduction (% ±SD)	$[NO_2^-]$ mg L^{-1}	Mean percent reduction (% ±SD)	$[PO_4^{3-}]$ mg L^{-1}	Mean percent reduction (% ±SD)
1	0.04 - 0.67	68.4 ±6.73	0.010 - 0.027	98.6 ±1.34	0.003 - 0.012	98.7 ±2.31	0.00 - 0.20	91.7 ±7.22
2	0.28 - 0.72	69.2 ±10.0	0.000 - 0.030	97.2 ±4.81	0.000 - 0.024	94.4 ±7.14	0.70 – 1.80	94.5 ±4.78

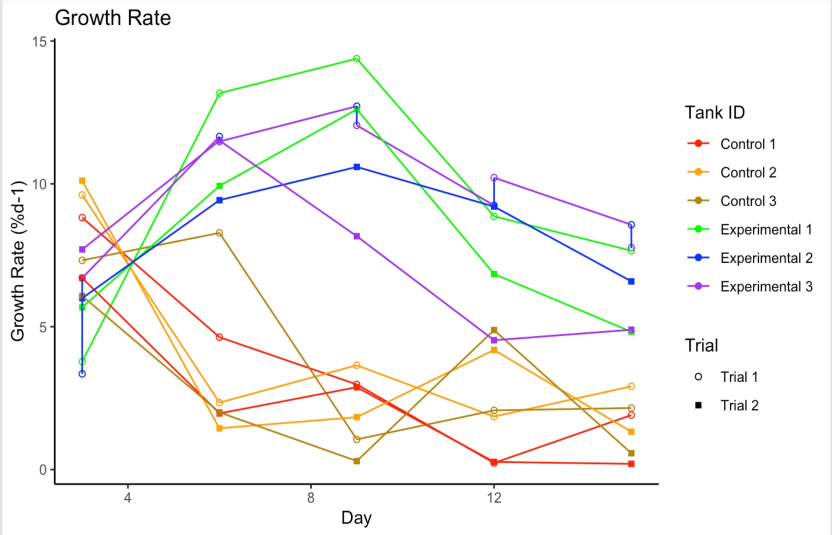

Fig. 4. Growth rate (% d^{-1}) vs. time, with the x-axis representing the growth data collected on every third day of each 15-day trial. Welch two sample t-test results indicate significant differences between control and experimental growth rates of each trial ($t(27.8) = 4.74$, $p = 5.73e-05$; $t(27.6) = 4.94$, $p = 3.36e-05$).

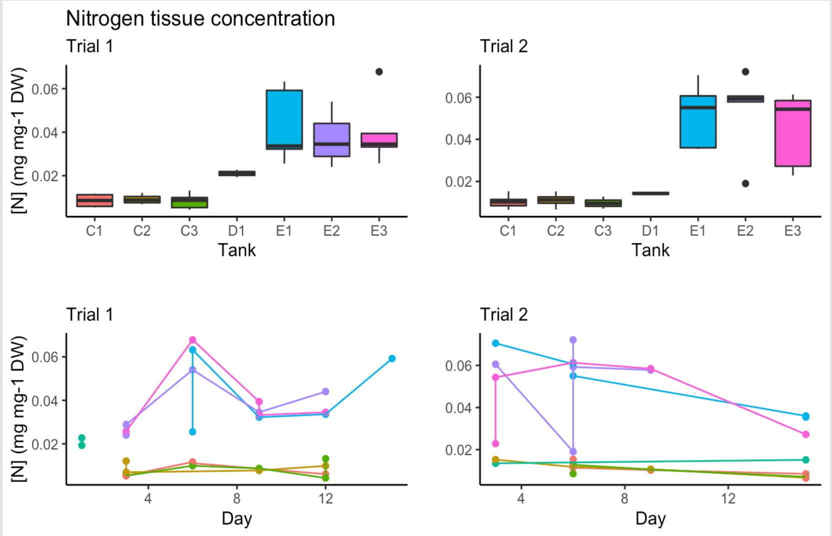

Fig. 5. Nitrogen tissue concentration for samples sent through elemental analysis. (Top) boxplots represent the tissue nitrogen concentration for control tanks (C1-3) and experimental tanks (E1-3), which have an elevated nitrogen concentration in both trials from the levels at the start of the trial (D1, teal). (Bottom) Tissue concentration of groups with time, illustrating the range of values represented in the wide experimental boxplots and narrow control boxplots.

Table 2. Mean values ±SD of aspects measured during elemental analysis. Due to sample size limit on mass spectrometer, $N_{EXP(T1)} = 15$, $N_{EXP(T2)} = 15$, $N_{CON(T1)} = 13$ and $N_{CON(T2)} = 13$.

Parameter	Group	Mean ±SD	p-value
Carbon concentration	T1 Experimental	0.248 ±0.043 mg mg^{-1} DW	NA
	T1 Control	0.240 ±0.016 mg mg^{-1} DW	
	T2 Experimental	0.272 ±0.029 mg mg^{-1} DW	0.017
	T2 Control	0.245 ±0.030 mg mg^{-1} DW	
Nitrogen concentration	T1 Experimental	0.040 ±0.014 mg mg^{-1} DW	6.75e-07
	T1 Control	0.010 ±0.005 mg mg^{-1} DW	
	T2 Experimental	0.050 ±0.017 mg mg^{-1} DW	3.93e-07
	T2 Control	0.011 ±0.003 mg mg^{-1} DW	
C:N	T1 Experimental	7.73 ±1.67	6.31e-06
	T1 Control	33.3 ±14.9	
	T2 Experimental	7.17 ±2.71	6.84e-07
	T2 Control	28.6 ±10.1	
$\delta^{13}C$	T1 Experimental	-17.8 ±1.4‰	0.004
	T1 Control	-19.5 ±1.48‰	
	T2 Experimental	-17.8 ±1.69‰	0.025
	T2 Control	-19.1 ±1.48‰	
$\delta^{15}N$	T1 Experimental	3.90 ±1.61‰	1.19e-05
	T1 Control	-5.07 ±5.40‰	
	T2 Experimental	4.18 ±1.61‰	4.26e-07
	T2 Control	-4.36 ±4.03‰	

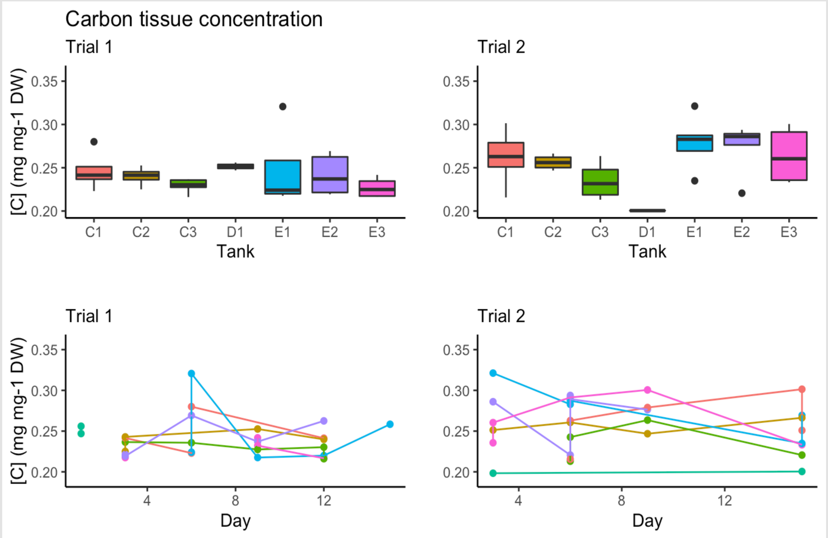

Fig. 6. Carbon tissue concentration for samples sent through elemental analysis. (Top) boxplots represent the tissue nitrogen concentration for the starting algae (D1, teal), control tanks (C1-3) and experimental tanks (E1-3), the two of which showed no significant difference between mean values. (Bottom) Tissue concentration of groups with time, illustrating the range of values represented in the boxplots.

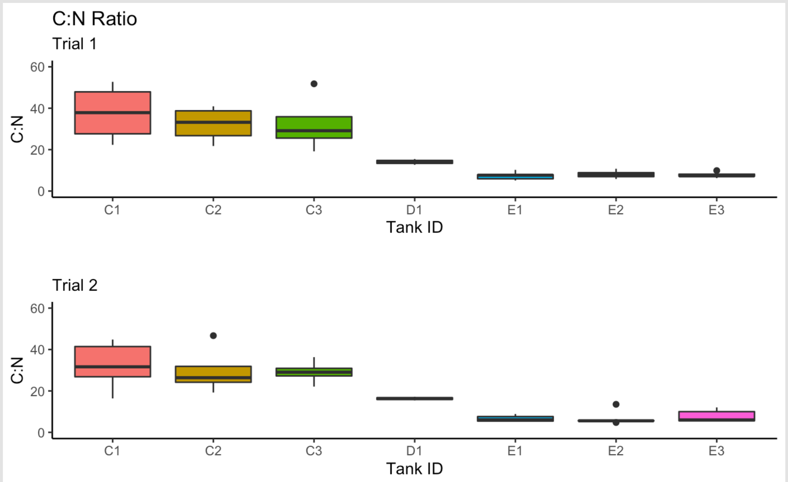

Fig. 7. C:N ratio of control (C1-C3) and experimental (E1-E3) groups as well as starting ratios (D1) for trials 1 (top) and 2 (bottom). Experimental groups showed significantly different mean C:N ratios from control groups.

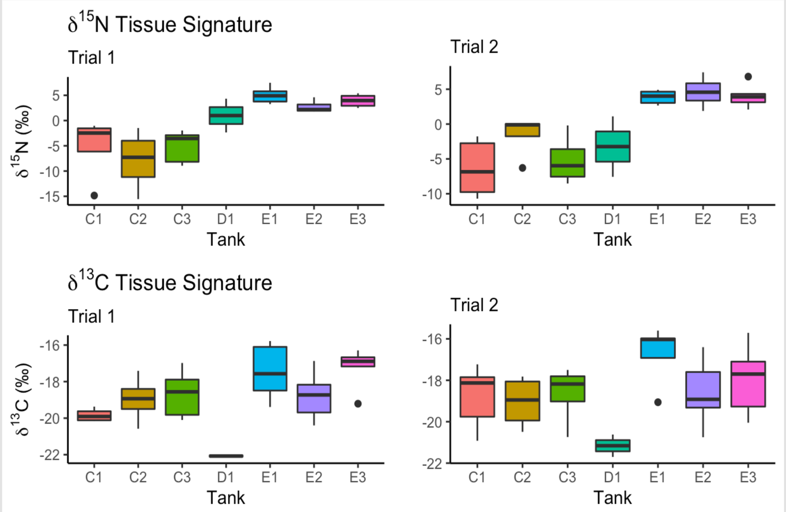

Fig. 8. $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) of tissues samples run through elemental analysis. Significant difference was found between the means of experimental and control groups for $\delta^{15}N$ and $\delta^{13}C$ values in both trials.

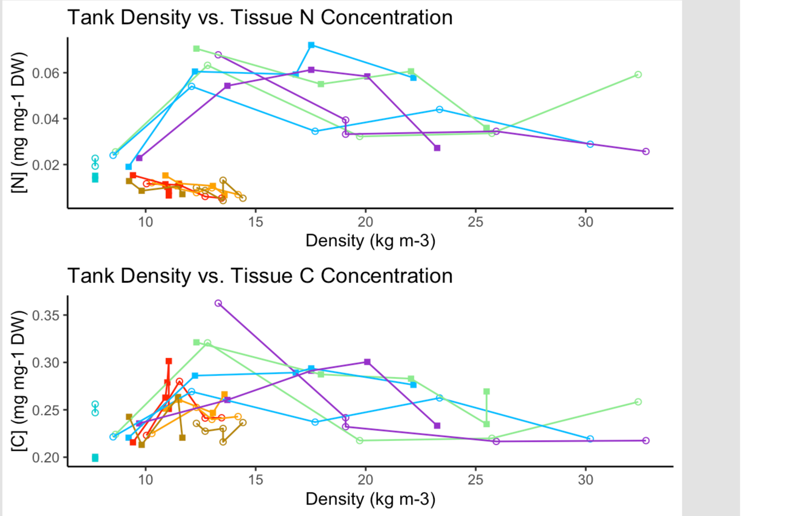

Fig. 9. Tank density (kg m^{-3}) vs. tissue nitrogen and carbon concentration (mg mg^{-1} DW) in both trials 1 and 2. Summarized elemental analysis data used is shown in Table 2.

Conclusions

- The results of this project confirm that ***A. subulata* would be an ideal candidate for regional IMTA projects** due to high nutrient bioextractive and growth capabilities
  - **Reduced mean values of 68% of NH_3, 97% of NO_3^-, 96% of NO_2^-, and 93% of PO_4^{3-}** from effluent water (Fig. 3, Table 1)
  - Based on *L. campechanus* feed composition, *A. subulata* was able to absorb up to 16.7 kg P from effluent water between the two trials
  - Mean N and C tissue concentrations (Fig. 5-6), $\delta^{13}C$, and $\delta^{15}N$ values (Fig. 8) of experimental groups were significantly different than those of control groups in both trials (Table 2)
  - Experimental groups also maintained lower C:N ratios (Fig. 7, Table 2) that are optimal for downstream use as feed for model organisms[1] compared to control groups
  - Mean growth rates of experimental groups (Trial 1: 9.44 ±3.27%d^{-1}, Trial 2: 7.90 ±2.56%d^{-1}) were significantly different from those of control groups (Trial 1: 3.99 ±3.03 %d^{-1}, Trial 2: 2.98 ±2.88%d^{-1}) in both trials (Fig. 4)
- Growth and subsequent nutrient reduction exhibited signs of **density-dependence**
  - Both experimental tissue growth and percent nutrient reduction peaked at stocking densities of around 17-20 kg m^{-3} in each trial (Fig. 9)
  - This point of growth illustrates the existence of a carrying capacity for the system, and represents the density at which biomass should be harvested in commercial systems in order to retain the most efficient growth and nutrient reduction in *A. subulata*
- Future steps:
  - Establish market value of *A. subulata* to determine the revenue gained per kg of algae produced
  - Longer-term projects to get an idea of *A. subulata*'s seasonal viability
  - Investigate correlations between nutrient reduction and flow rates to determine idea ratios for long-term reduction efficiency of the system

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