Acute and Chronic Effects of Roundup QuickPro (73.3% Glyphosate Salt) on *Halodule wrightii* and *Halophila johnsonii* Seagrass in Closed Tank Systems

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Background / Fundamental inquiries

Glyphosate is currently one of the most widely used herbicides, with direct herbicidal application used used for weed-control in residential gardening and commercial agricultural practices (Saunders et al. 2015). In high concentrations glyphosate has been detected in runoff for up to 4 months, causing concern for non-target species (Edwards et al. 1980). Little is known of glyphosate transportation and even less is known about its potential affects on nontarget aquatic species. This study was conducted on seagrasses collected from the Loxahatchee River Estuary (Fig. 1) to document the effects of glyphosate, a hydrophilic highly polar molecule, that has been overlooked in the marine environment (Mercurio et al. 2014). This study is aimed to understand if glyphosate will affect the two most dominant seagrass in the southern Indian River Lagoon (IRL), *H. johnsonii* and *H. wrightii* (Metz et al. 2020), at two levels: (1) **acute direct exposure** of low concentrations observed in watersheds, and (2) **indirect chronic high concentrations**.

Methods

- Roundup QuickPro, an IPA salt formulation of 73.3% concentrated glyphosate salt.
- 12 tanks were assigned: **Direct acute** (n=4), **Indirect chronic** (n=4), **or Control** (n=4).
- Each tank was drained for one hour to simulate a low tide event, during which they were treated according to group:
  - **Direct, acute**: Application of 112 mg Roundup QuickPro™ (15mL of 7.5 g/L solution), sprayed directly onto Halodule wrightii and Halophila johnsonii during a simulated low tide event (LTE).
  - **Indirect, chronic:** Application of 3.74 g Roundup QuickPro™ salt, poured into the water while tanks were drained for the LTE (125mg/L).
  - **Control group:** The control was only lowered for the LTE. See Table 1 for concentration levels of the groups.
- The two parameters we analyzed in this experiment were mortality and health score.
  - Mortality was assessed using shoot count and calculated using:

$$\left(\frac{Starting\ value - final\ value}{|starting\ value|}\right) * 100$$

  - Health score is a simple categorization used as a proxy for chlorophyll concentration in the blades of seagrass. Seagrass *sp.* in tanks scored either a 1, 2, or 3 (Table 2).
- Differences between groups over time were tested using mixed model analyses of variances (ANOVAS) as well as a pairwise function to compare P values within the groups in Rstatix package in Rv.4.

Table 1. Concentrations of Roundup QuickPro herbicide applied to treatments vs. manufacturers recommended concentration.

	Dosed (g/L)	Sustained (g/L)	Area (g/m²)
Manufacturer Recommended	11.2	(N/A)	0.121
Direct (acute)	7.48	0.00374	0.081
Indirect (chronic)	0.125	0.125	NA
Control	0.00	0.00	0.00

Table 2. Criteria to be met by the majority of grass (per spp.) in each tank to receive the tanks respective score

Score	Seagrass Health Score Criteria
1	Dull colored and non-rigid or clear (2/3 to whole blade brown)
2	Green and non-rigid or more extensive browning of the blades (~ 1/8-2/3 of the blade brown)
3	Green and rigid; very slight browned (~1/8 or less of the blade)

Results

- There were significant differences between treatments measured by mortality (*H. johnsonii,* ANOVA, p< 0.001, *H. wrightii,* ANOVA , p<0.01) and health score (p<0.001 for both *H. johnsonii* and *H. wrightii*).
- The post hoc tests revealed no significant differences in seagrass mortality between Direct and Control tanks and significant differences in mortality were measured in indirect tanks compared to direct or control tanks (*H. Johnsonii*, t-Test, p<.0001 and *H. wrightii*, t-test, p<.01).
- Unlike mortality, there were significant differences between controls and both direct and indirect treatment tanks for health score (Direct: *H. johnsonii,* t-test, p<0.1 and *H. wrightii,* t-test, p<0.01; Indirect: p<0.0001 for both *H. johnsonii* and *H. wrightii*).

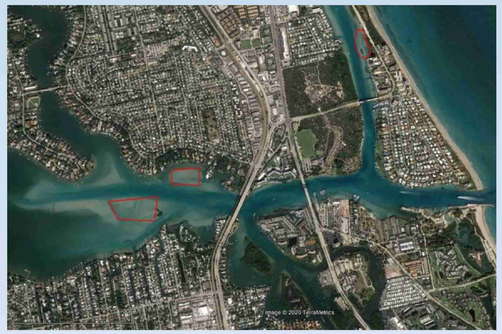

Figure 1. Map showing the locations of the three seagrass beds (black boxes) in the Loxahatchee River Estuary (LRE); 12 seagrass/ sediment cores were collected from each seagrass bed (total n=36).

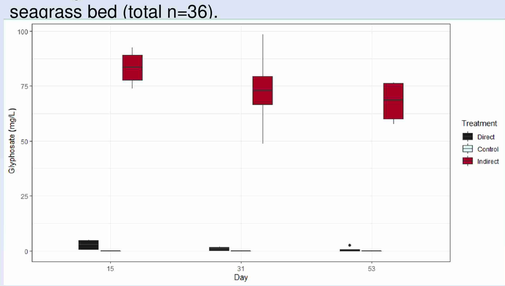

Figure 2. Measured glyphosate concentrations in tanks throughout the study.

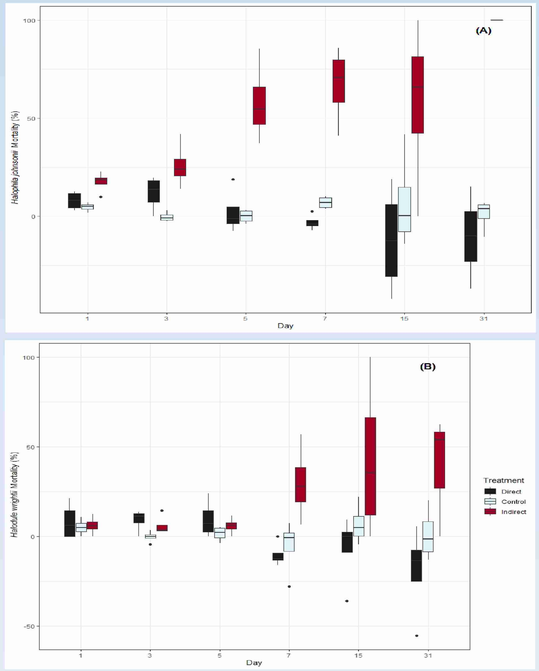

Figure 3. Mortality, as measured by changes in shoot counts, between treatments during the experiment for (a) H. johnsonii and (b) H. wrightii. Positive values represent decreasing shoot counts and negative values reflect increasing shoot counts

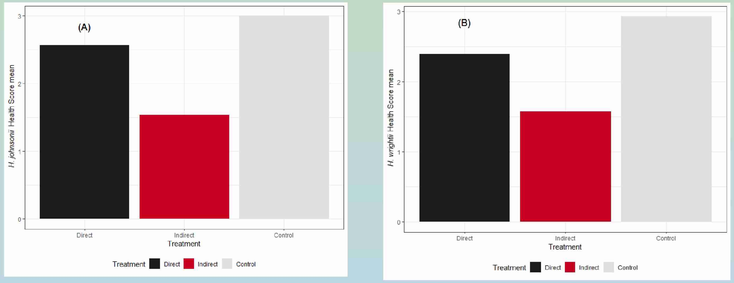

Figure 4. Qualitative assessment showed both direct and indirect application of glyphosate degraded seagrass health for (a) H. johnsonii and (b) H. wrightii. Table 2 shows criteria used to categorize seagrass health, a proxy for chlorophyll concentrations in seagrass blades. A higher score represents healthy seagrass.

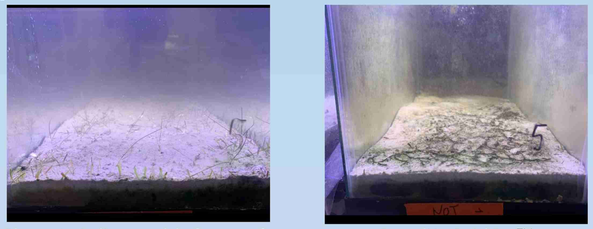

Photo. An indirect tank before vs. after exposure to RoundUp QuickPro™

Discussion

- This study provides evidence of seagrass mortality with high doses of indirect application of the 'water soluble' herbicide Roundup QuickPro™ (73.3% glyphosate salt).
- When applied indirectly at high concentrations, glyphosate persisted in tanks for 53 days (Fig. 2).
- Seagrass mortality was highest in indirect sustained exposure tanks at high concentrations for both species of grass (Figure 3a). Direct acute exposure at low concentrations did not significantly differ from controls.
- Direct, acute exposure to glyphosate appears to have resulted in marginal increases in seagrass shoot counts (Figure 3), and degraded seagrass health (Figure 4). Some toxicants can produce a hormesis-like response in plants, due to a temporary reallocation of carbon through the shikimic pathway (e.g., Cedergreen and Olsen et al. 2010). Direct acute exposure at low concentrations to *H. wrightii* may provide an example of this (Fg. 2B) and warrants further study.
- Future directions of this work would be to establish an Ld_{50} and assess how that Ld_{50} may fluctuate when coupled with other stressors that seagrass face in the environment (e.g., low light, elevated temperatures, etc.), in addition to understanding the low dosage impacts of glyphosate on seagrass which were shown to promote growth.

Acknowledgements

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