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Effects OF Oil-Contaminated Sediments On Submerged Vegetation: An Experimental Assessment Of Ruppia Maritima

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Effects of Oil-Contaminated Sediments on Submerged Vegetation: An Experimental Assessment of *Ruppia maritima*

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Abstract

Oil spills threaten the productivity of ecosystems through the degradation of coastal flora and the ecosystem services these plants provide. While lab and field investigations have quantified the response of numerous species of emergent vegetation to oil, the effects on submerged vegetation remain uncertain. Here, we discuss the implications of oil exposure for *Ruppia maritima*, one of the most common species of submerged vegetation found in the region affected by the recent Deepwater Horizon oil spill. We grew *R. maritima* in a range of manipulated sediment oil concentrations: 0, 0.26, 0.53, and 1.05 mL oil /L tank volume, and tracked changes in growth (wet weight and shoot density/length), reproductive activity (inflorescence and seed production), root characteristics (mass, length, diameter, and area), and uprooting force of plants. While no statistical differences were detected in growth, plants exhibited significant changes to reproductive output, root morphology, and uprooting force. We found significant reductions in inflorescences and fruiting bodies at higher oil concentrations. In addition, the roots growing in the high oil were shorter and wider. Plants in medium and high oil required less force to uproot. A second experiment was performed to separate the effects of root morphology and oiled sediment properties and indicated that there were also changes to sediment cohesion that contributed to a reduction in uprooting forces in medium and high oil. Given the importance of sexual reproduction for these plants, oil contamination may have substantial population-level effects. Moreover, areas containing buried oil may be more susceptible to high energy storm events due to the reduction in uprooting force of foundation species such as *R. maritima*.

Introduction

Coastal seagrasses and submerged aquatic vegetation are historically abundant throughout coastal regions worldwide [1–3]. Despite their widespread distribution, numerous threats such as disease, physical disturbance (such as boat propeller scars and hurricanes), eutrophication, sediment input, overharvesting of top predators leading to an increase in herbivores, and global

climate change have all been posited to have reduced the areal coverage of submerged vegetation to historically low levels [4–5]. In Louisiana (USA), for example, there was a 50–75% decline in macrophyte coverage between 1954 and 1992 [6–8].

The estuaries of the northern Gulf of Mexico (GoM) are relatively productive areas [9–10] because of a diverse mix of submerged and emergent vegetation. These structured habitats provide refuge for the young of many commercially and recreationally important nekton species [11–13], contribute to the forage base of consumers [14–15], buffer coastlines from high energy events [16–18], and improve water quality and clarity to potentially mitigate some of the negative effects of eutrophication [19–21].

The 2010 Deepwater Horizon (DWH) oil spill in the GoM has the potential to greatly exceed all other regional threats to coastal vegetation. Approximately 4.9 million barrels of oil [22] were released over the course of 87 days, prompting a number of protective measures to keep oil out of vital wetland areas including: application of chemical dispersant both at depth and aerially to increase the surface area of oil and enhance microbial degradation, opening river diversions to increase freshwater discharge and possibly keeping oil offshore, burning oil at the water's surface, and mobilizing cleanup crews and local fishermen to place oil protection booms and remove oil from coastlines [23–24]. Despite these efforts, there was oiling of 1,773 km of shoreline, over 60% of which occurred along the Louisiana coast [24].

Experimental studies conducted to date have focused on the oil's impacts to emergent vegetation such as *Spartina alterniflora*, *S. patens*, *Juncus roemerianus*, and *Phragmites australis*. The results of oil exposure experiments have been highly variable and species-specific [25], with some species, such as *S. patens* [26–27] more susceptible than others. Some species of emergent vegetation, such as *P. australis* [28], *Sagittaria lancifolia* [26,29], and in a few cases *S. alterniflora* [30–34], appears to be resilient to the toxic effects of oil. Physical smothering of plant tissue reducing photosynthesis, application of oil to soils, and repeated, heavy exposure seems to have a large impact on plant productivity [28,35]. The effects of oil on submerged vegetation (such as seagrasses and other subtidal, coastal species), however, remain untested, perhaps because many researchers assume that the oil floated over grassbeds. Moreover, many submerged grasses are ephemeral and field studies to understand disappearance of these grasses are difficult to design without controlled, manipulative experimentation.

Findings from previous spills such Ixtoc-I [36], Exxon Valdez [37–38], and the *Florida* barge in Buzzards Bay, MA [39], as well as this spill [40–41], indicate that oil can persist in the environment buried in the sediment where minimal weathering occurs [42] and continue to affect coastal flora and fauna long after the spill [24,39,43]. Additional evidence suggesting that Macondo oil may be buried in nearshore environments is that marshes in Barataria Bay, Louisiana (USA) were re-oiled with remobilized Macondo oil after Hurricane Isaac in September 2012 [24,44]. We hypothesize, therefore, that oiled sediments will influence submerged vegetation and influence assessments and restoration efforts of benthic areas [28]. Here, we present the results of a manipulative experiment to determine the effects of varying concentrations of oil within sediments on one cosmopolitan species of submerged vegetation found throughout the northern GoM.

Methods

We performed a series of experiments to determine the impacts of oil on a common submerged macrophyte species, widgeon grass (*Ruppia maritima* L. sensu lato, hereafter referred to as *Ruppia*). In Experiment 1, we exposed plants to various experimental doses of oil under greenhouse conditions and measured the consequences to plant growth, reproduction, morphology, and uprooting forces. In Experiment 2, uprooting force was tested independent of plants. The

effect of oil on sediment cohesion was tested by burying inert substances (plastic beads) in the various oil concentrations and measuring the force needed to remove beads from the sediment.

Study Species

Ruppia is among the most widespread species of submerged vegetation found in GoM estuaries [45–47], especially in Louisiana [7,48–49]. *Ruppia* is thought to be very tolerant of fluctuating environmental conditions and is found in areas highly variable in salinity. As a result, the distribution of *Ruppia* is thought to be governed by competition with other submerged macrophytes [45,50]. *Ruppia* is used as a food source by a variety of herbivores from benthic invertebrates [51] to fishes [52] and waterbirds [53] and serves as habitat for numerous nekton species [49].

Experiment 1: Oil Effects on *Ruppia maritima*

Experimental Design. We used *Ruppia* and sediments collected from Lake Pontchartrain near Lacombe, Louisiana (30.258, -89.948). No permits were required for the described study, which complied with all relevant regulations. In addition, no private land was accessed and this study involved no threatened or endangered species. Whole plants were placed in a cooler with an airstone and transported to the Louisiana State University Greenhouse Facility in Baton Rouge, LA, where the experiment took place. Approximately 1 L of sediment (3 cm) was placed in each 19 L tank, before adding a homogenous layer of oil (1 of 4 randomly selected levels: 0, 5, 10, or 20 mL; hereafter referred to as none, low, medium, and high, respectively), and then another 1 L (3 cm) of sediment was added. These concentrations were chosen because it represents a range of concentrations used in previous experimental studies [54–55]. The oil used in this experiment was obtained from the Marlin Platform of the Dorado field and contains an almost identical toxicity/chemistry as MC252 oil from the Deepwater Horizon drilling platform. We used unweathered oil in this experiment to represent a potential worst-case scenario. Each treatment was replicated 12 times. At the conclusion of the experiment, a sediment sample was taken from one tank in each treatment to verify oil concentrations using the GCMS methods described in Turner et al. [56–57].

Before planting, whole *Ruppia* plants were spun in a salad spinner for approximately 60 spins to remove epiphytes and potential herbivores. Any remaining epiphytes were removed by hand without damaging the plant [48]. Three individual plants were then planted in each tank to approximately 3 cm depth. Dechlorinated 10 psu seawater, mixed using Instant Ocean ® salt mix, filled tanks to their 19 L capacity. The surface water temperatures ranged between 23–31°C during the experiment. All tanks contained an airstone, 10% of the water was changed every other day, and water was added daily to account for evaporation.

The tanks were planted/harvested on 4 different dates and each treatment was represented equally on each date). Tanks were planted from July 24, 2014 through August 6, 2014 and the experiment was terminated after 31–33 days.

Growth and Reproductive Activity. Several indicators of growth were measured before and after the experiment. Before planting, we measured the wet weight of plants on an Ohaus ® Model TS400S balance (± 0.01 g). Shoot density was determined as the number of separate, vertical stems ascending from the rhizome. The length of each shoot was measured (± 1 mm), and reproductive activity determined by counting the number of inflorescences and fruiting bodies on each plant. At the conclusion of the experiment, all plants were again placed in the salad spinner and these same variables were quantified. The data were standardized to a per day basis in the analyses to account for the slight variation in trial length. Proportional change was calculated as the $(X_{final}-X_{initial})/X_{initial}$, where X represents one of the aforementioned response variables.

Root Morphology. We hypothesized that roots may show oil-induced effects, because roots were in closest proximity to the oil layer. Therefore, a more detailed analysis of roots was determined for one randomly selected plant in each tank at the conclusion of the experiment. Three randomly selected roots from each plant were removed using a scalpel for measurement. Root length was measured by securing the specimen to a board with rubber bands. The total length of an individual root was measured using a digital caliper (± 0.1 mm) and digital planimeter (Calculated Industries Scale Master® Classic v3.2 Model 6020). A digital micrometer (Starrett® IP67) secured to a Bunsen burner stand with clamps was used to measure root diameter at three points along the root to the nearest (± 0.01 mm). Mass was measured by weighing each root on a digital scale (Mettler Toledo AB104S; ± 0.1 mg). Root cross-sectional area was calculated by assuming that the roots were cylindrical and using the formula for the area of a circle [$A = \prod(d/2)^2$]. Volume was calculated by multiplying the cross-sectional area by the length of the root.

Uprooting Force. We measured the force needed to uproot one randomly selected plant in each tank at the end of the experiment using a Lyman® digital pull gauge, a technique used in previous studies [58–60]. A clip, tied to a line to the gauge, was placed at the base of each plant and vertical pressure exerted until the plant was removed from the sediment. The maximum amount of force (± 0.02 N) was recorded.

Experiment 2: Soil Cohesion

A second experiment was conducted to identify the oiling effects on sediment cohesion independent of plants. This experiment was conducted with identical treatments and conditions, with the exception that instead of plants, five 12 mm plastic beads, each attached to a separate monofilament line, were placed in the oiled layer in each tank. At one week intervals, the force needed to remove one bead from the sediment in each tank was measured using the Lyman® digital pull gauge as before.

Statistical Analyses

Assumptions (normality and homogeneity of variance) were tested before analysis, and data were transformed if the assumptions were not satisfied. The daily proportional change in wet weight, stem number, stem length, inflorescences, and fruiting bodies were analyzed using a General Linear Model with oil treatment (none, low, medium, and high) as a fixed factor and planting date as a random factor [61]. Root measurements (mass, length, diameter, and area) and uprooting force, both attained only at the conclusion of the experiment, were also analyzed using this approach. The bead experiment was analyzed using a repeated measures analysis of variance. All values were considered significant at p≤ 0.05 and Tukey's post hoc test conducted when significant differences were detected. Statistical analyses were performed in MINITAB v13.

Results

Experiment 1: Oil Effects on *Ruppia maritima*

Chemical analyses of a subset of tanks verified the presence of numerous compounds associated with oil contamination (S1 and S2 Figs). These concentrations are within the range of those found in field conditions after the DWH oil spill [56–57].

Growth and Reproductive Activity. *Ruppia* grew in all treatments, with little differences found in growth (Table 1). Plants grew from an initial biomass of approximately 0.31 g to an average of 1.09 g over the course of the experiment. This growth rate is a daily proportional

Table 1. Results of a General Linear Model analysis of the various comparisons of growth, reproduction, and uprooting in the four different oil treatments. Statistically-significant values are in **bold**.

Response Variable	Transformation	Oil Treatment		
		F	df	p
Wet weight	Fourth Root	0.81	3	0.494
Shoot density	N/A	0.42	3	0.737
Stem length	N/A	0.88	3	0.461
Inflorescences	N/A	4.51	3	**0.008**
Fruiting bodies	N/A	3.93	3	**0.015**
Uprooting	N/A	15.6	3	**<0.001**

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change of approximately 0.07, with no significant difference among oil treatments (Fig 1A). Likewise, stem density per plant increased from around 3.7 shoots initially to over 5 at the end of the experiment, which is a daily proportional change of approximately 0.17, with a tendency of decreasing density with increasing oil, although this trend was not significant (Fig 1B). Stem length was highly variable and not significant across treatments, with average tank stem length increasing from 15.0 cm to 21.7 cm over the experiment duration. This translates to a 69% increase in shoot length with an average growth rate of 0.2 cm per day.

Reproductive output, unlike growth, did differ significantly across oiling treatments (Table 1). The presence of fruiting bodies on plants significantly declined with oil concentration (Table 1, Fig 2A). Specifically, no oil significantly differed from medium (p = 0.019) and high oil (p = 0.034), while reproductive output in the low oil treatment was not different from any of the other treatment levels (p> 0.05). At the conclusion of the experiment, the number of fruits on plants changed from an approximately zero fruits found in all treatments to 12.42 in no oil, 7.92 in low oil, 4.5 in medium oil, and 5.13 in high oil. Expressed as the proportional change per day, this decrease was approximately 1.1, 0.75, 0.4, and 0.45 in none, low, medium, and high oil treatment, respectively. Likewise, inflorescences significantly differed across oiling treatments (Table 1, Fig 2B). With an average initial number of 1.15 flowers per tank, *post hoc* tests indicate that there were no differences between none, low, and medium oiling (p> 0.05), but flowering in the low oil treatment differed from the flowering in the high oil treatment (p = 0.006). The highest incidence of flowering was found in the low oil treatment, with a daily proportional change of around 0.4, decreasing to 0.31 in the no oil treatment and 0.25 in the medium treatment, and the lowest 0.20 in the high oil treatment. This translated to 3.6, 5.0, 3.2, and 2.6 inflorescences per plant in the none, low, medium and high oil treatments, respectively, at the conclusion of the experiment.

Root Morphology. The root morphology also changed significantly with oil exposure (Table 2, Fig 3). While root mass did not vary (Fig 3A), root length marginally decreased with increasing oil (Fig 3B). Roots were shortest in the high oil treatment (~0.58 mm), and increased in length in the medium (~0.65 mm), low (~0.80 mm), and no oil treatments (~0.83 mm). Conversely, roots were wider with increasing oil concentrations, with the average diameters ranging from 0.34 mm in the no oil treatment to 0.40 mm and 0.35 mm in low and medium treatments, and the largest diameters of 0.43 mm were in the high oil treatment (Fig 3C). This difference was driven by the diameters in the none and high oil treatments (p = 0.027; Table 3). Root area similarly increased across oiling, with areas of 0.09 mm^2 in the none, 0.14 mm^2 in low, 0.11 mm^2 in medium, and 0.15 mm^2 in high oil treatments (Fig 3D). Again, this increase was statistically driven by the significant difference between none and high oil (p = 0.017; Table 3).
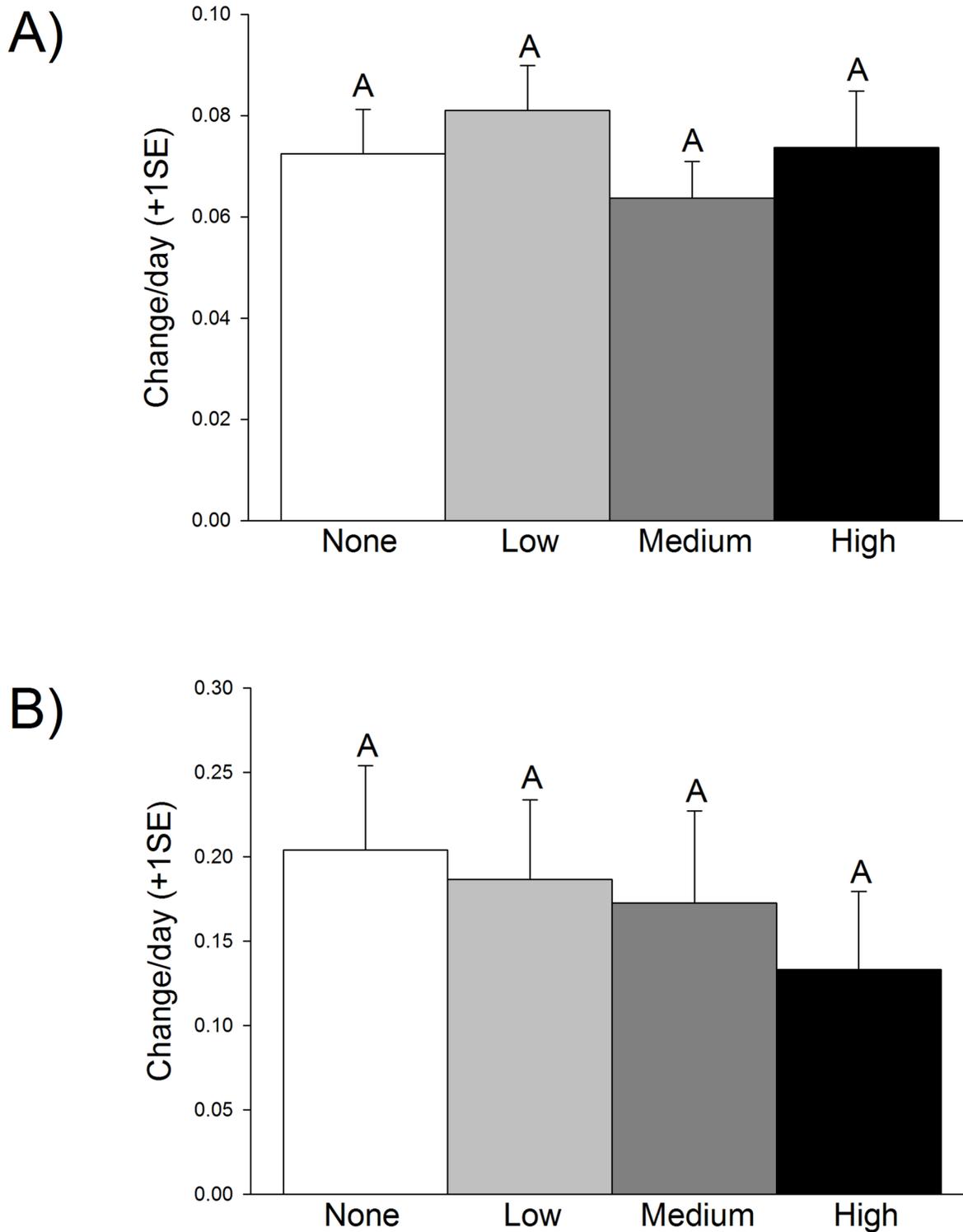
Fig 1. Daily proportional change for (A) wet weight, which increased from an initial average biomass of 0.31 g to 1.09 g, and (B) stem density, which increased from 3.7 to over 5 shoots per tank, for plants grown in the four different oil treatments: none (white), low (gray), medium (dark gray), and high (black). Letters indicate statistically-significant results. There were no statistically-significant differences among treatments (N = 12 per treatment).

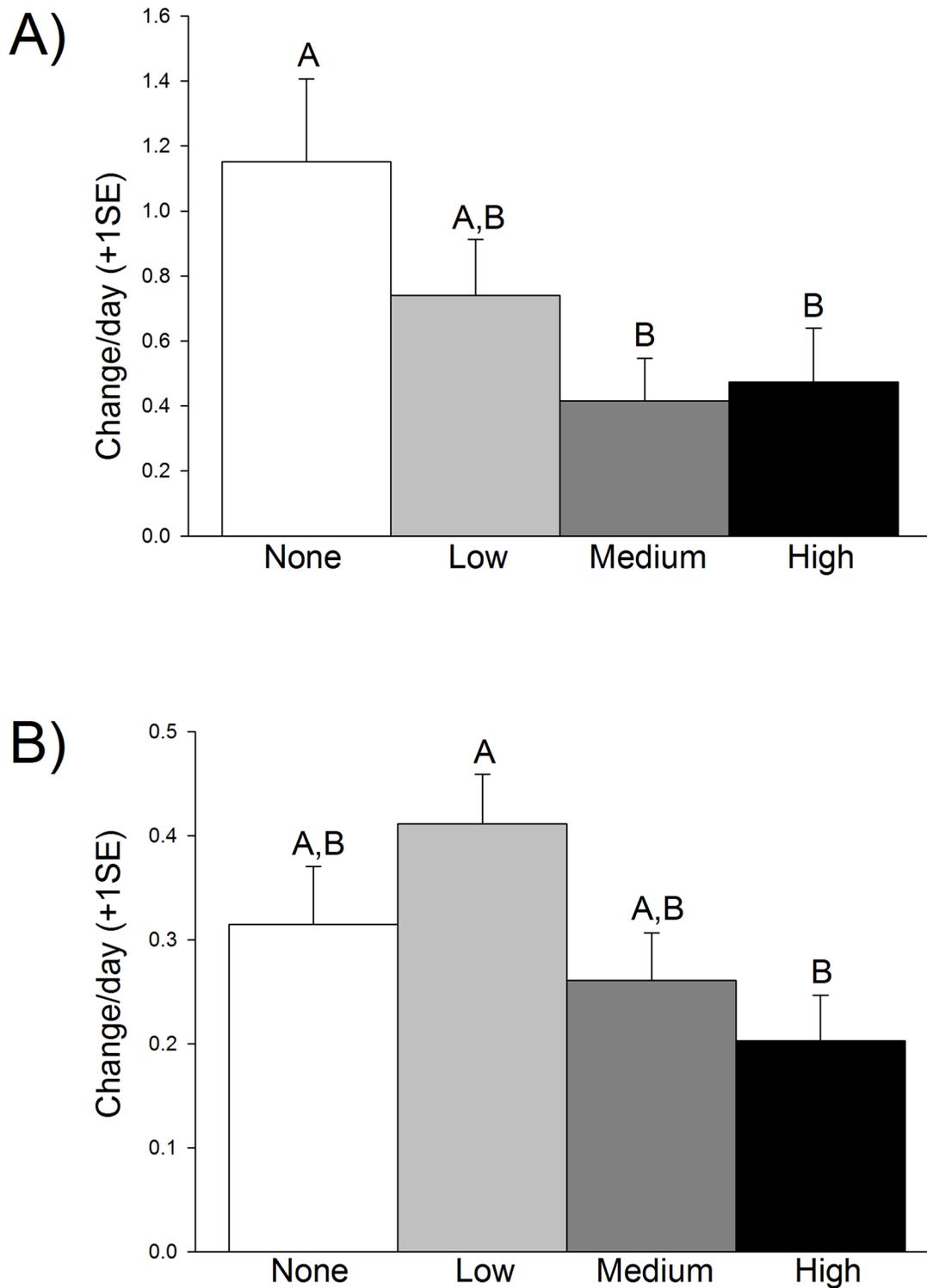doi:10.1371/journal.pone.0138797.g001

Fig 2. Reproductive output, expressed as daily proportional change. Fruiting bodies (A) increased from an average of 0 to 12.42 in no oil, 7.92 in low oil, 4.5 in medium oil, and 5.13 in high oil. Inflorescence production (B) increased from 1.2 flowers per tank to 3.6, 5.0, 3.2, and 2.6 inflorescences per plant in the none, low, medium, and high oil treatments, respectively, at the conclusion of the experiment. Letters indicate statistically-significant results (N = 12 per treatment).

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Table 2. General Linear Model analysis of root morphology. Statistically-significant values are in **bold**.

Response Variable	Transformation	Oil Treatment		
		F	df	p
Mass	Fourth Root	0.5	3	0.686
Length	Fourth Root	2.66	3	0.061
Diameter	N/A	3.59	3	**0.021**
Area	Log	3.95	3	**0.015**

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Uprooting Force. The force needed to remove plants from sediment varied significantly across oil treatments (Table 1, Fig 4). *Post hoc* comparisons confirmed that medium and high treatments were not significantly different from each other (p = 0.688), but both were different from low (p< 0.001 in both cases) and no (medium oil vs no oil: p = 0.030, high oil vs no oil: p = 0.001) oil treatments. Significantly less force was needed to remove plants in medium (~1.4 N) and high oil treatments (~1.2 N) than plants in none (~1.7 N) and low oil treatments (~2.0 N).
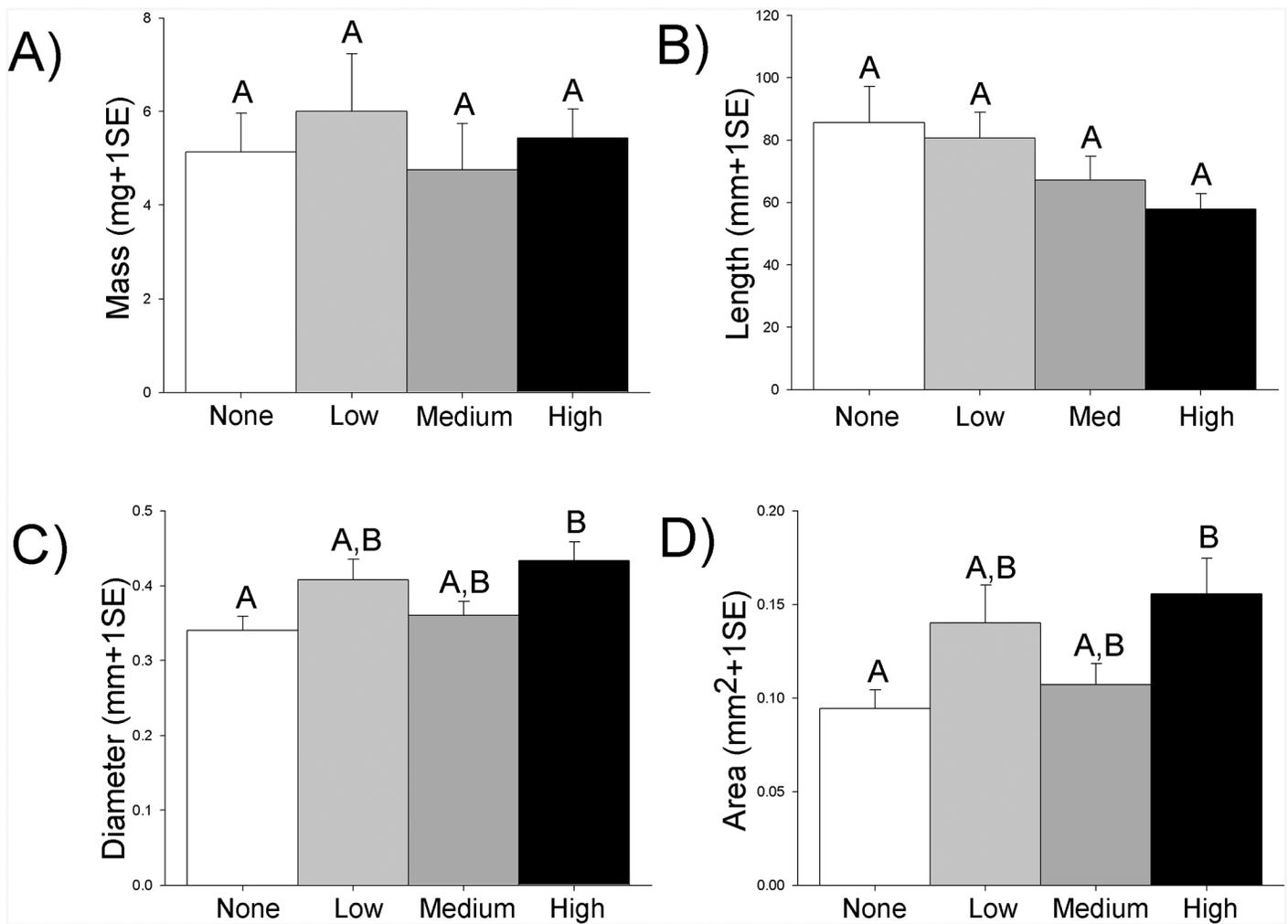

Fig 3. Changes in final root morphology across oil treatments [none (white), low (gray), medium (dark gray), and high (black)] for root mass (A), root length (B), root diameter (C), and root area (D). Letters indicate statistically-significant results (N = 12 per treatment).

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Table 3. Summary of significant changes in the treatment plots compared to control plots for different parameters. Only statistically-significant results are shown. n.s. = not significant.

Parameter	Oil Treatment		
	Low	Medium	High
Wet weight	n.s.	n.s.	n.s.
Stem density	n.s.	n.s.	n.s.
Stem length	n.s.	n.s.	n.s.
Seed production	n.s.	**0.019**	**0.034**
Inflorescence production	n.s.	n.s.	n.s.
Plant uprooting force	n.s.	**0.030**	**0.001**
Inert bead uprooting force	n.s.	**<0.001**	**<0.001**
Root mass	n.s.	n.s.	n.s.
Root length	n.s.	n.s.	n.s.
Root diameter	n.s.	n.s.	**0.027**
Root area	n.s.	n.s.	**0.017**

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Experiment 2: Soil Cohesion

An additional observation indicated that soil strength was significantly altered by oil ($F_{3,279}$ = 106.51; $p \leq 0.001$) (Fig 5), with less force required to uproot beads in medium (~0.33 N) and high oil treatments (~0.33 N) than in the none (~0.93 N) and low oil treatment (~0.91 N). *Post hoc* tests indicated that soil cohesion in the medium and high oil treatments were statistically indistinguishable (p = 0.998), but both were different than low and medium oil treatments (p ≤
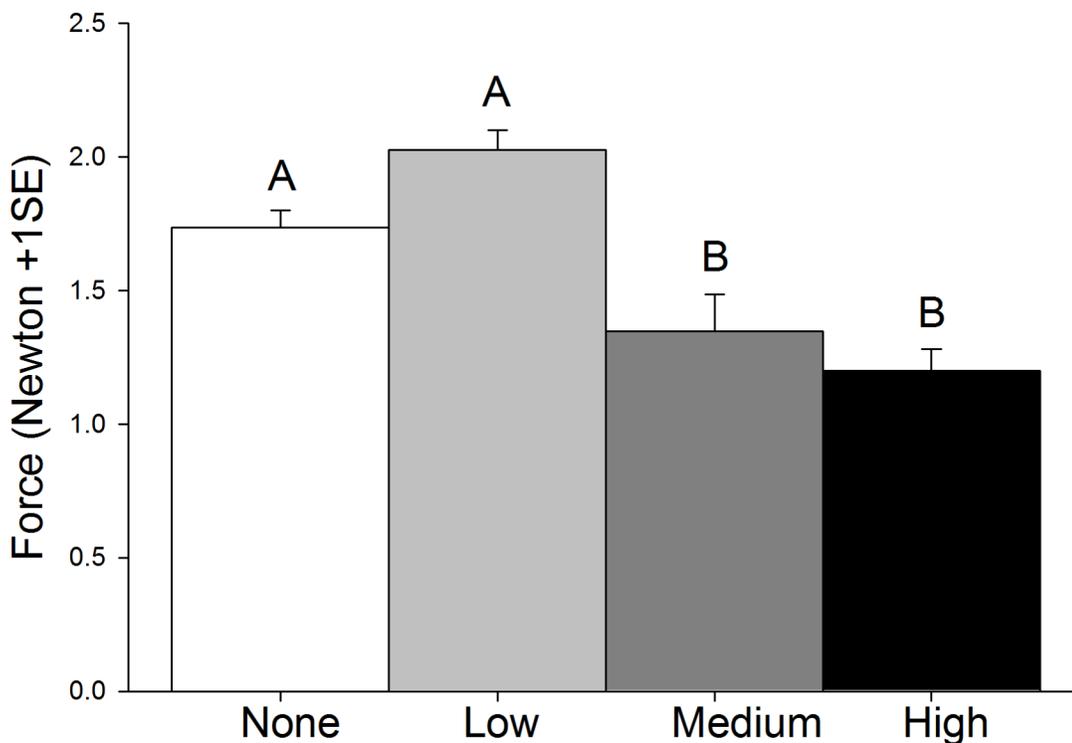

Fig 4. Difference in the uprooting force required to remove plants grown in four different oil treatments: none (white), low (gray), medium (dark gray), and high (black). Letters indicate statistically-significant results (N = 12 per treatment).

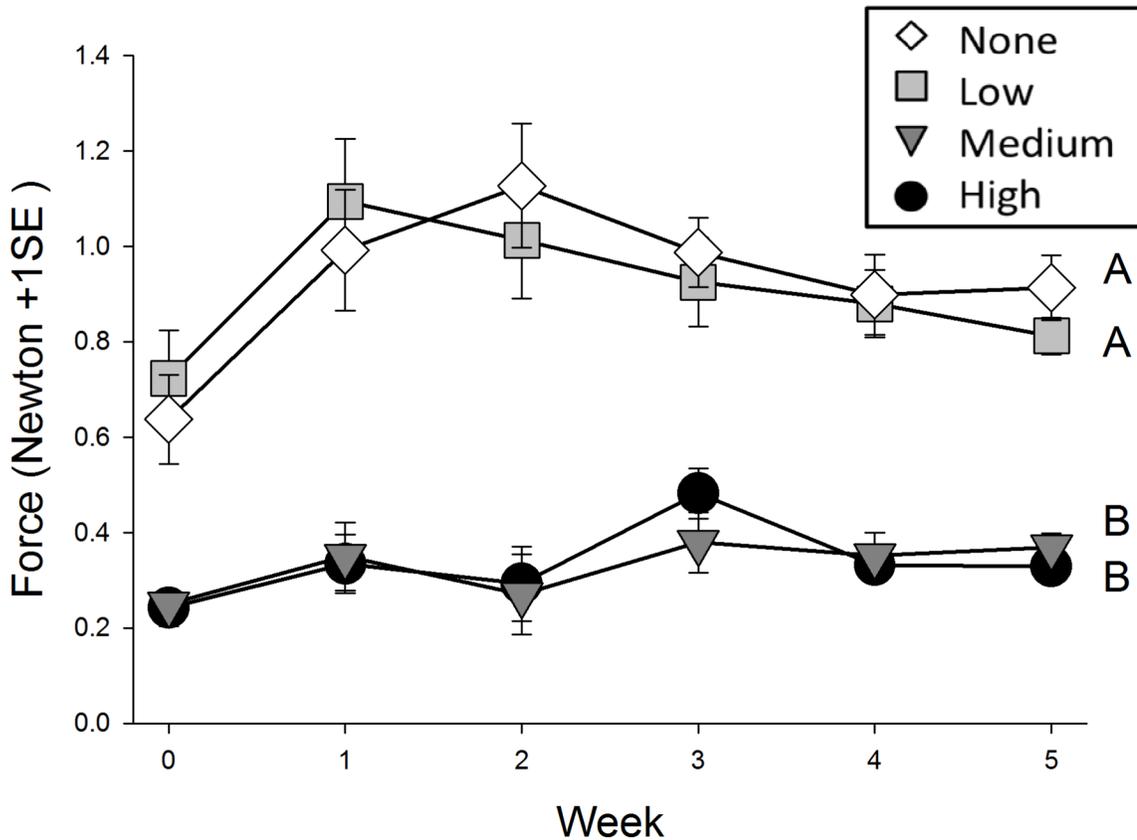doi:10.1371/journal.pone.0138797.g004

Fig 5. Changes in in the uprooting force required to remove inert plastic beads over time buried in the four different oil treatments: none (white diamond), low (gray square), medium (dark gray triangle), and high (black circle). Letters indicate statistically-significant results (N = 12 per treatment at each time interval).

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0.001). Low and medium oil levels were not significantly different from each other (p = 0.98). This trend was consistent over the 5 week course of the experiment.

Discussion

The results of studies of the DWH incident's impacts on GoM ecosystems have occasionally painted a picture of a resilient ecosystem, with a recovery of salt marshes [62], invertebrates [63], and fishes [64–65] in many oil-affected areas. However, other studies have noted the potential for long-term ecosystem alteration through continued exposure to hydrocarbon compounds [56–57] and a legacy of effects from increased erosion in the oiled areas [66]. Buried oil may represent one vector by which this spill may continue to alter the trajectory of ecosystem structure, function, and recovery [24,39,43]. Here, we found that buried oil affects the reproductive output, alters root morphology, and increases potential for erosion of one species of submerged vegetation, *R. maritima*. The implications of this study extend beyond the framework for assessing DWH oil spill impacts, because thousands of oil and natural gas structures currently exist in both offshore and nearshore regions throughout the northern GoM.

We found that the growth of *Ruppia* was resilient to the toxic effects of oil, with plants growing throughout the range of oil concentrations tested here. With many oil and natural gas structures throughout the area, as well as natural seeps, it is possible that resilient plants have already been selected for, and plants are previously adapted to, these conditions. Culbertson

et al. [39], however, found that after four decades of exposure, *S. alterniflora* in Buzzards Bay still exhibited long-term impacts in areas where buried oil persists, with lower above- and below-ground biomass in oiled areas indicating that adaptation to these conditions has not occurred to date.

The reduction in reproductive output under high oil conditions may have consequences for some populations of *Ruppia*. Dunton [45], for example, found that some populations are entirely dependent on the seed bank as a mechanism for new growth, while other populations may maintain vegetative mechanisms of dispersal. Similarly, Cho and Poirrier [7] took monthly core samples from four areas containing *Ruppia* in Louisiana and found spatial and temporal variability in the amount of root and rhizome biomass that persisted throughout the winter, suggesting some reliance on sexual reproduction may be present for some populations. The impact of oil on seed germination and the seed bank remains the focus of future study.

Ruppia flowers opportunistically under favorable conditions [45] and this may explain the increase in flowering in unoiled and low oiled treatments. Conversely, some plants are known to increase flowering as a response to stress as a means of enhancing potential resistance to stressors through genetic recombination [59,67–69]. The decrease in *Ruppia* flowering at high oil levels could, therefore, indicate that the plant is less stressed and perhaps even using oil as a nutritional reserve. The consequences of these trends in reproduction require further investigation.

In several instances, oil has been hypothesized to be a carbon source for organisms. For example, carbon isotopes have been used to demonstrate the incorporation of depleted signatures into plankton [70] and deep sea nekton [71], because oil contains a depleted signature of approximately -27 $\delta^{13}C$. Lin and Mendelssohn [26] found that oil stimulated growth of *S. lancifolia* at concentrations up to 24 L m^{-2}. While we found no differences in growth for *Ruppia*, the decrease in root length and increase in area may indicate that plant roots are able to use oil as a source of nutrition, thus making it unnecessary to grow deeper. Alternatively, it is possible that the redox conditions created by the oil layer [72] may influence this lack of vertical growth, although several studies [73–75] indicate that oxygen transported to the rhizomes and roots creates an oxic microzone in sediments around roots in submerged grasses.

Reductions in uprooting force indicate a vulnerability to physical forces for not only *Ruppia*, but all plants living in oil contaminated areas. While some of this is due to the change in root morphology, the second experiment, designed to tease apart the effects of root morphology and sediment cohesion, indicate that significant changes to the soil occur at the medium and high levels of oil used here. Plants, even in the medium and high oil treatments, required more force to uproot than do the inert beads, however, indicating that both root morphology and sediment properties are important in this trend. To date, this is the first study (that we are aware of) to demonstrate these findings experimentally. However, a number of field studies have indicated that areas where oil came ashore experienced enhanced erosion [62,66], and this may be due not only to the toxicity to vegetation, but also to changes in sediment properties.

We acknowledge several deficiencies in the current study that should be improved with additional research. *Ruppia* was grown under optimal greenhouse conditions with no epiphytes or herbivores and it is possible that the addition of multiple stressors may have non-additive effects on plants [4]. Moreover, the amount of buried oil in oil-affected areas remains unknown and the subject of future work, but early estimates indicated that a large amount of oil (~2 million barrels) released at the wellhead is unaccounted for [76]. A portion (4–31%) of this has been found in sediments surrounding the accident site, and it has been hypothesized that the rest may be heterogeneously distributed in sediments throughout the region [77]. Additional testing of sediments in field locations should continue to search for this oil, perhaps under

newly-accreted sediment. Field verification in reproductive trends and uprooting of plants in oiled areas, as well as how buried oil affects other less tolerant vegetation, could shed light on the generality of these results. Finally, we used unweathered oil in this experiment, although it is possible that the weathered oil that reached the coastline could have a different effect. It is important to note, however, that many of the chemical constituents of oil presumed to precipitate quickly, such as naphthalenes, have been found in marsh ecosystems [56–57]. It is our hope that these findings further our understanding of how this tragedy affected coastal fauna and flora and contribute positively to the continued conservation of coastal ecosystems in the northern GoM.

Supporting Information

S1 Fig. Alkane Hydrocarbon Concentrations. The concentration of alkane petroleum hydrocarbons ($\mu g\ g^{-1}$) measured at the end of the experiment.
(TIF)

S2 Fig. Aromatic Hydrocarbon Concentrations. The concentration of aromatics petroleum hydrocarbons (ng g^{-1}) measured at the end of the experiment.
(TIF)

Acknowledgments

Author Contributions

Conceived and designed the experiments: CWM. Performed the experiments: CWM LOH RET. Analyzed the data: CWM LOH RET. Contributed reagents/materials/analysis tools: CWM LOH RET. Wrote the paper: CWM LOH RET.

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