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SHORT COMMUNICATION

RESUSCITATION OF MICROALGAE FROM MISSISSIPPI RIVER PLUME SEDIMENTS[§]

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INTRODUCTION

It is widely known that many pelagic and benthic microalgae (microscopic single-cell primary producers) can persist during periods of poor light or cold by forming spores, cysts, or low-energy vegetative cells (Garrison 1981, Kremp and Anderson 2000). In shallow coastal systems, senescent microalgae which are buried in the sediments can be reintroduced into the water column due to the passage of powerful storms which can completely mix the water column and suspend sediment (Dzwonkowski et al. 2017, Zang et al. 2018). Individual microalgal cells ranging from 5 – 50 μm sink 0.2 – 2.0 m/d (Smayda 1971). Thus, in a 20 m water column, a resuspension event could keep a microalgal cell in the pelagic environment up to 10 d prior to sinking to the benthos.

The physical transport of sedimentary microalgal material into the water column could also alter the diversity of the phytoplankton community if microalgae resuscitate and persist, and therefore potentially modify the carbon flow. In a subtropical lake system, sediment microalgae have been shown to rejuvenate after reintroduction into surface waters (i.e. euphotic zone conditions) on the scale of 7 – 28 d (Waters et al. 2005). These authors noted that sediments up to 120 cm in depth had microalgae which were able to resuscitate; material from the deeper depths would have been buried for several decades (Waters et al. 2005). Thus, senescent sedimentary microalgae have the potential to decouple regional phytoplankton dynamics by introducing material (e.g., organic matter) which may have been physiologically inactive for periods corresponding to 100s or 1000s of microalgal generations.

Microalgae buried in sediments may represent a pool of organic material which is resistant to diagenetic transformation after burial. Among microalgae, diatoms are responsible for a significant quantity of organic matter burial (e.g., Parsons and Dortch 2002, Rabalais et al. 2004). However, their silica shell may also be used as substrate for precipitation of secondary clays, i.e. reverse weathering (Presti and Michalopoulos 2008, Rahman et al. 2017). This process is an important sedimentary sink for many elements (e.g., Fe, Al, K, Mg, Si) and produces CO_2 .

We examined the resuscitation of sediment microalgae from 2 sites in the northern Gulf of Mexico (GOM), a subtropical region prone to extreme weather. Northern GOM tropical storms have been observed to erode up to 13 cm of seabed sediment into the water column (Zang et al. 2018); such events provide the physical forcing necessary to resuspend sedimentary microalgae. Using field sediments, we conducted a mesocosm-based sediment bioassay experiment. Our objectives were to test whether sedimentary microalgae could be resuscitated in a marine environment and if so, to quantify the temporal evolution of such a resuscitation and the physical characteristics of the rejuvenated microalgae (e.g., diatoms).

MATERIALS AND METHODS

Sediment was collected using a multicorer during the Coastal Louisiana Silicon Cycling (CLASiC) cruise, 4 – 13 May 2017, aboard the *R/V Pelican*. Two deployments were conducted at bottom depths of ~30 m (6 May, MC4) and ~50 m (10 May, MC5) on the Louisiana Shelf (Figure 1A). Both sites were ~32 – 35 km west of the Mississippi River mouth and its plume, (Figure 1A); these are also locations of seasonal bottom-water hypoxia (Rabalais et al. 2004). Upon recovery, the multicorer tubes were extruded and sectioned at 1 – 4 cm intervals, depending on depth. Each section was placed into plastic bags which were immediately sealed. These sediments were stored at 4°C in the dark and transported to Dauphin Island Sea Lab (AL, USA) at the same temperature. Sediments were stored in this manner for approximately 3 weeks prior to the experiment.

In the laboratory, sediment sections were homogenized, and subsamples taken to determine water weight (range 42 – 82% among all depths). Coastal Alabama seawater was filtered (0.2 μm pore) and amended with nutrients (10 μM nitrate, 1 μM phosphate). Wet sediment, corresponding to 1.5 – 2 g of dry mass after correcting for water weight, was added to 500 mL bottles (triplicates) with the amended filtered seawater; 3 additional bottles with amended filtered seawater but no sediment were used as controls. All bottles were placed in an outdoor mesocosm and submerged below the surface of the water to

[§]The first author led analysis of these data as part of the Dauphin Island Sea Lab's (DISL) Research Experience for Undergraduates (REU) program in 2019, and the second author conducted this research as part of the DISL REU program in 2017.

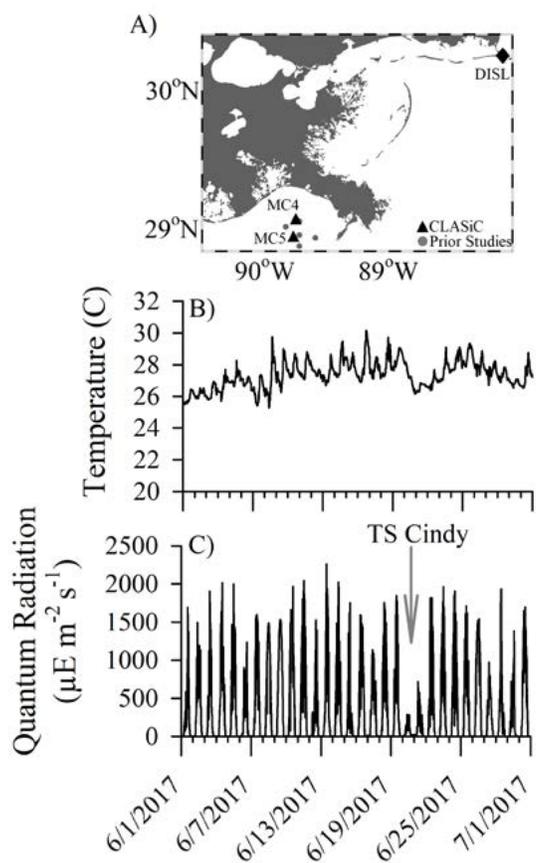


FIGURE 1. Sample core collection locations and environmental data during a month-long mesocosm experiment. A. Sample core (MC4, MC5) collection locations (black triangles) in the northern Gulf of Mexico off Louisiana relative to previously published sediment core analysis of diatom valves and microalgal pigments (grey circles; Parsons et al. 2002, Rabalais et al. 2004). The experiment was conducted at the Dauphin Island Sea Lab (DISL). B. Daily water temperature fluctuations during the month-long experiment. C. Daily quantum radiation fluctuations during the month-long experiment. Data are from the Dauphin Island station within the ARCOS network (arcos.disl.org), approximately 300 m from the experimental mesocosm. The passage of Tropical Storm (TS) Cindy, arrow in panel C, strongly affected the quantum radiation.

reduce the irradiance by ~50% from that at the surface. The mesocosm was continuously cooled with coastal water, drawn directly from an intake ~200 m from the mesocosm. The experiment ran from 1 – 30 June 2017, with subsamples collected every 2 – 3 d (14 time points total). During each subsampling, 5 mL from each bottle was collected and shade adapted for 30 min prior to analysis of *in-vivo* fluorescence using a Turner Trilogy fluorometer. Fluorescence is from both chlorophyll within cells and interfering materials (e.g., chlorophyll degradation products). Subsampling was done at the same time of day (morning ~1000 local time) to avoid potential bias due to diurnal effects (e.g. photoacclimation). Reported *in-vivo* fluorescence values were blank corrected from the control amended filtered seawater treatments without sediment. Once per week, additional 5 mL subsamples were collected, preserved with 0.5 mL of Bouin's Solution and stored at 4°C in the dark for 10 months (McNair et al. 2018).

Preserved samples were imaged using a VS Series benchtop

FlowCam using a 10x objective and 200 μm flow cell in auto-image mode (Fluid Imaging Technologies; Scarborough, ME, USA). While a FlowCam can image particles using a laser trigger setting, and count only those particles which fluoresce, this functionality cannot be used for Bouin's Solution–preserved samples. Additionally, given the vast amount of detrital and lithogenic material, only particles with distinct morphology were able to be identified routinely (e.g., diatoms) during manual classification of the images. A subset of samples from MC5 were analyzed on days 10 and 16 to quantify diatom abundance and determine whether diatom morphology changed over time using cell length as a proxy.

RESULTS

Physical conditions during the 30 day bioassay experiment were typical for the region and season. The mesocosm facility draws water from coastal Alabama and water temperatures ranged between 25 – 30°C (Figure 1B). During this period, photon flux densities at the nearby (~300 m) Dauphin Island meteorological station (30.2513°N, –88.0778°E) reached daily maxima between 1500 – 1900 $\mu\text{E}/\text{m}^2/\text{s}$ (Figure 1C). Given that the depth of the incubation bottles attenuated ~50% of the surface light, the microalgae were unlikely to have seen irradiances this high, which could inhibit photosynthesis. During the experiment, Tropical Storm Cindy made landfall on 22 June far west of the study site; however, this still resulted in reduced irradiance during this experiment due to extended cloud cover. All surface hydrographic and meteorological information are publicly available (arcos.disl.org).

Microalgae resuscitated from all core depths, but there was considerable variability. For MC4, microalgae from the shallowest sediment depth (1 – 2 cm) resuscitated the fastest among all samples, with peak fluorescence 4 d post–resuspension (Figure 2A). Peak fluorescence occurred later among deeper core depths from MC4. Specifically, 10–11 cm samples peaked on day 6, while 18–19 cm and 27–31 cm depths both peaked on

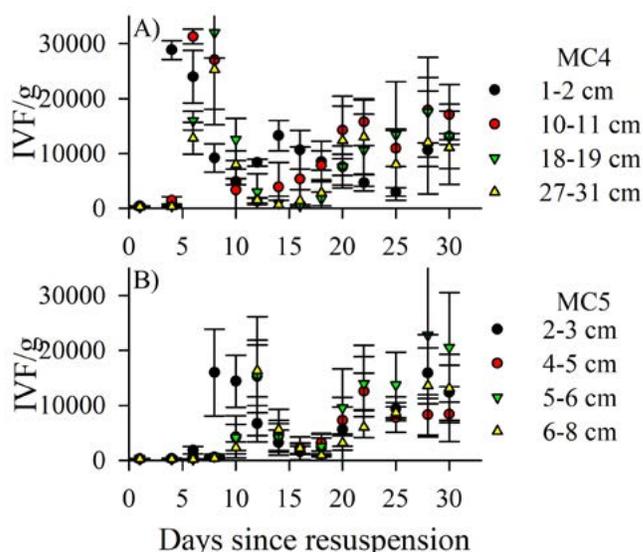


FIGURE 2. Mean (\pm sd) blank-corrected *in-vivo* fluorescence (IVF, normalized to dry mass of sediment added) over time for cores. A. MC4. B. MC5. Symbols denote the depth interval for homogenized sediment for each core.

day 8 (Figure 2A). For MC4, there was a monotonic increase to these peak fluorescence levels followed by a monotonic decline through days 12–14. Beyond 3 weeks, fluorescence values among depths were relatively stable for the duration of the experiment (Figure 2A). As with MC4, microalgae in the shallowest sediments of the MC5 core peaked first; however, this took 8 d, twice as long as MC4 (Figure 2B). Deeper core microalgae in MC5 peaked initially on day 12, followed by a decline among all cores until day 18 (Figure 2B). As with MC4, fluorescence from MC5 microalgae was mostly stable from days 21–30. While these data show varying degrees of resuscitation among depths and cores, the microalgal groups or species which were growing cannot be inferred from these data alone.

FlowCam image data from MC5 on days 10 and 16 suggest the response in the microalgal community varied among depths. Diatoms were below detection in the shallowest core during both sampling days (Figure 3A). Among the deeper core depths, there were no distinguishable changes in diatom abundance with time. These days represent periods of low *in-vivo* fluorescence, suggesting a transition phase between the primary and secondary *in-vivo* fluorescence peaks (Figure 2B). For those diatoms which were observed, the shallower sediment had longer cells, which were typically pennate (opposed to centric) in morphology. Only within the 6–8 cm core treatment were the observed diatoms larger on day 16 than on day 10.

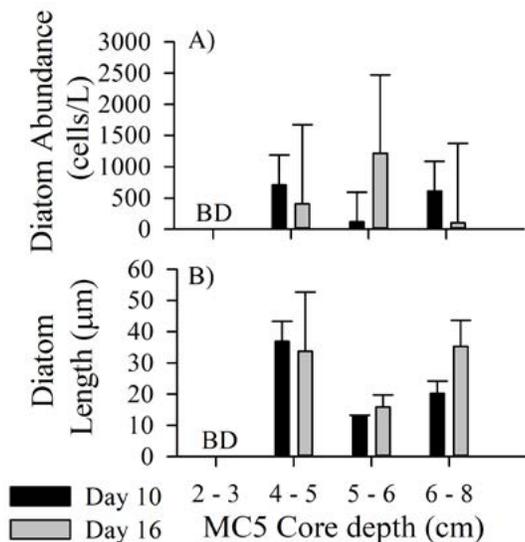


FIGURE 3. Mean (\pm sd) FlowCam-derived diatom observations for experimental days 10 (black fill) and 16 (gray fill) in core MC5. A. Abundance (# cells/L). B. Length (μ m). BD denotes below detection.

DISCUSSION

Microalgae resuscitated upon resuspension. The time lag between the experimental start and a resolvable signal for *in-vivo* fluorescence was on the order of days, and initial peak fluorescence occurred within ~1–2 weeks among all samples. Such a time lag is likely conservative, as this is primarily driven by detection of the signal using our fluorometer and would also vary based on the initial quantity of cells which were able to resuscitate and their growth rates. If deeper sediments had fewer

viable microalgae, this may explain why there was an increasing time lag with depth. Recent methodological advances enable dosing isotopically heavy ^{13}C into the dissolved inorganic carbon pool and examining single-cell assimilation (reviewed by Taylor et al. 2017). Leveraging such methodology in future work may better constrain the time lag between reintroduction of this material and when cellular growth initiates.

In this study, diatoms were confirmed to be among the groups resuscitated. A prior study near our core locations demonstrated that diatom abundances in sediments increased by 3 orders of magnitude from the 1960s to late 1990s (Parsons et al. 2002). Another study from the same core sites reported that diatom and cyanobacteria dominated the microalgal pigments in these sediments (Rabalais et al. 2004). This is consistent with the common observation that both groups can numerically dominate phytoplankton abundances in the water column (e.g. Nelson and Dortch 1996). We posit that these are the two most likely groups to have responded to the resuspension events, and recommend that future experiments examining this process use a more specific analysis (e.g., marker pigments, detailed microscopy) to quantify biomass and net growth rates among taxa, and to determine whether resuscitated species are pelagic or benthic in origin.

The finding that microalgae from all depths resuscitated, albeit at different rates, suggests that cells can persist in a dormant stage for a significant period. Sedimentation rates, derived from the vertical distribution of ^{210}Pb in sediments in the vicinity of MC4 and MC5, infer accumulation between 0.4–0.8 cm/year (Adhikari et al. 2016). Using this range of rates suggests that core material in surface MC4 sediments was buried approximately 1–5 years, whereas deeper sediments could have been buried for 12–78 years. For MC5 the estimated burial age is between 3–20 years. Regional microalgae growth rates are highly variable, ranging from <0.1–3.0/d (Fahnenstiel et al. 1995). However, if we assume a conservative mean growth rate of 0.1/d, equating to a doubling of cells every 7 d, this implies that the deep microalgae in MC4 were buried for 620–4000 generations, while MC5 microalgae persisted for 150–1000 generations.

These data demonstrate that regional microalgae have an exceptional ability to persist in physiologically challenging conditions (e.g., reducing environment, no light) up to 3 orders of magnitude beyond their typical generation time. This also demonstrates that a small fraction of local primary production can be decoupled from the food web over long time scales (e.g., years to decades) and resist alteration by diagenetic processes even after burial. Given the sedimentation times for cell sizes observed in this study (≤ 2.0 m/d, Smayda 1971), the 5–10 d lag in a resolvable *in-vivo* fluorescence signals among all core depths suggest these microalgae could resuscitate in the water column prior to sinking back to the benthos if sediment is disturbed.

In conclusion, we demonstrate that marine sediment microalgae, potentially dormant for hundreds to thousands of generations, can resuscitate upon resuspension into the euphotic

zone on time scales less than 2 weeks. While we confirmed the presence of diatoms among the microalgae, we cannot ascertain whether they were the dominant group; it is likely that other microalgal groups (e.g., cyanobacteria, dinoflagellates) were important, or potentially dominant, contributors to the

resuscitation signal. These results demonstrate a dormant sedimentary source of primary producers exists in the Mississippi River Plume sediments. The biomass associated with these dormant cells and the factors triggering their resuscitation should be considered in future work.

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