

## Effects of slow-release nutrients on eelgrass (*Zostera marina* L.) morphometrics and water quality

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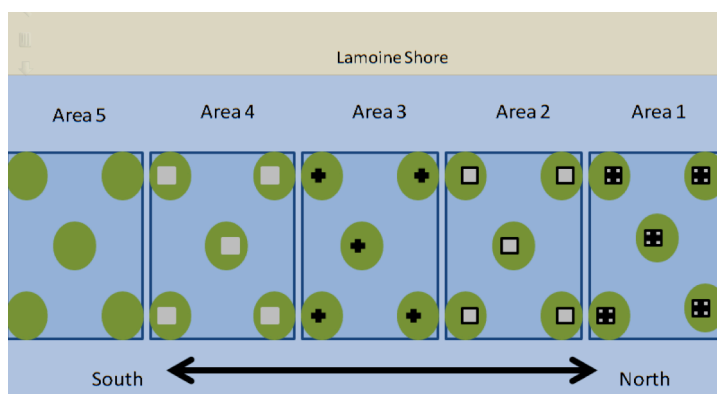
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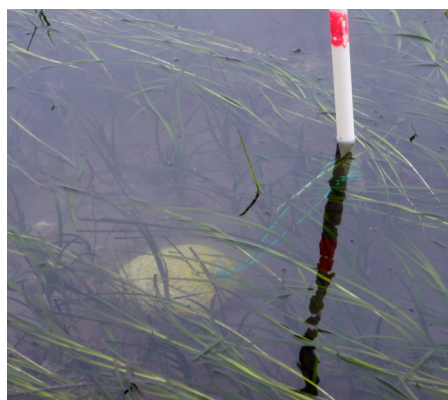
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We applied slow-release nutrient treatments at the sediment surface of existing eelgrass patches in order to determine their effect on plant growth and water quality in Frenchman Bay, ME. The nutrient addition did not seem to adversely affect water quality in the study area; in addition, there were no same-season effects of nutrient addition on plant growth. Potential long-term effects will be investigated in summer 2013.

*Zostera marina* L. (eelgrass) beds are productive estuarine habitats that provide many benefits to the greater coastal ecosystem; however, *Z. marina* is disappearing rapidly from Frenchman Bay<sup>1</sup>. Nitrogen and phosphorous availability has been cited as a limiting factor for *Z. marina* growth and has led to the study of nutrient addition, especially as it relates to eelgrass restoration<sup>2</sup>. The benefits of nutrient addition to sediments are documented as a possible restoration aid because of the tolerance of *Z. marina* to sediment nutrient enrichment and the positive growth response of the plant in nutrient-enriched sediments in mesocosm experiments<sup>2</sup>. We investigated the effects of slow-release nutrient treatments (various combinations of nitrogen, phosphorus, and iron) on water quality and eelgrass morphometrics in pre-existing *Z. marina* patches in Frenchman Bay.



**Figure 1.** Experimental design in Berry Cove, Lamoine. Symbols represent the following nutrient treatments applied to each of five *Zostera marina* patches per area: Area 1 (N+P+Fe), Area 2 (N+P), Area 3 (Fe), Area 4 (gypsum brick), Area 5 (control).



**Figure 2.** Slow-release nutrient brick in a *Zostera marina* patch in Area 2 (N+P). Nutrient bricks were placed in plastic mesh bags, tied to PVC marker poles and nestled into sediment to ensure placement and minimize rhizome disturbance.

This *in situ* experiment was located in Berry Cove, Lamoine (44°27.25'N x 68°19.75'W). We identified 25 distinct *Z. marina* patches for this experiment, five patches for each of four treatment areas and one control area, with an effort to randomize distance from shore as well as patch shape and size (Fig 1). Nutrient bricks, the slow-release

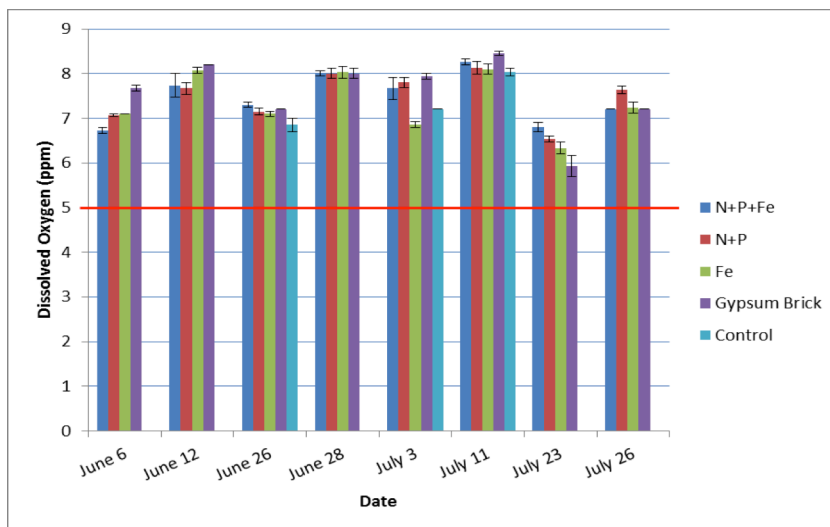
source of nitrogen and phosphorous, consisted of a mix of 900 g of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  (gypsum), 110 g of Portland Ordinary Cement, 230 g of  $\text{CH}_4\text{N}_2\text{O}$  (urea), and 230 g of  $(\text{NH}_4)_2\text{HPO}_4$  (di-ammonium phosphate). Each brick weighed approximately 1470 g. A 25 cm, 145 g iron spike was used as a slow-release source of iron in surface sediments.

From north to south, the nutrient treatments for the five study areas were as follows: in Area 1 (N+P+Fe), a nutrient brick was placed in each *Z. marina* patch and an iron spike was driven into the substrate adjacent to the brick; in Area 2 (N+P), a nutrient brick was placed in each patch; in Area 3 (Fe), an iron spike was driven into the sediment of each patch; in Area 4 (gypsum brick), a brick containing gypsum only was placed in each patch; and in Area 5 (control), no treatments were added (Fig 1). The nutrient bricks were nestled into, rather than buried under the sediment so as not to disturb *Z. marina* rhizomes (Fig 2). Our monitoring period began June 5 and extended through August 3, 2012. All treatments were applied June 8, 2012.

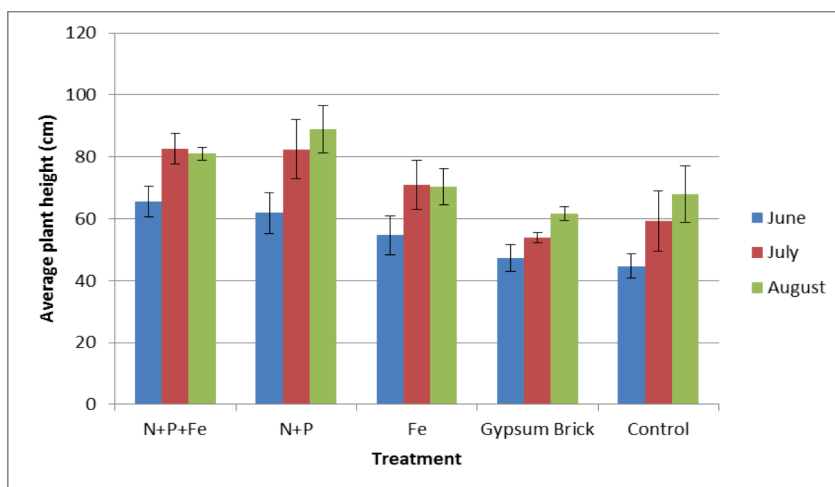
We monitored dissolved oxygen and turbidity in each area to ensure the addition of nutrients did not affect water quality. Dissolved oxygen samples were fixed in the field and analyzed back at the laboratory using the Winkler titration method<sup>3</sup>. Turbidity samples were collected in the field and were analyzed using a LaMotte 2020e turbidimeter (LaMotte Company, Chestertown, MD). Three dissolved oxygen samples and three turbidity samples were collected from treatment areas on each sampling date. We measured *Z. marina* morphometrics including above-ground plant height, shoot density, and patch size (length and width) in each area. Three 0.25 m<sup>2</sup> sinking PVC quadrats were thrown at random into each patch, the number of plants was counted, and aboveground plant height for three random plants was determined using a meter stick. To measure patch size, the widest and longest points on each patch were marked with stakes and measured. The stakes were moved to the outermost *Z. marina* plant each time the patch was measured.

We did not detect any adverse effects of nutrient additions on water quality variables. Across all sampling dates, dissolved oxygen remained above 5 ppm (Fig 3), the level below which organisms become stressed<sup>4</sup>. With the exclusion of high readings for June 26, which we attribute to heavy rainfall, mean turbidity for treatment areas across the seven sampling dates in June and July ranged from 1.18 ( $\pm 0.09$ ) to 1.63 ( $\pm 0.31$ ) nephelometric turbidity units (NTU) ( $\pm$  SE). These data are consistent with turbidity readings taken during these months for Berry Cove in 2011 and 2012, which had a mean of 1.17 ( $\pm 0.16$ ) across four sampling dates, also with three measurements taken on each date.

We did not detect any effects of nutrient additions on plant morphometrics (Figs 4 and 5). Analysis of variance (ANOVA) revealed that the percentage increase in aboveground plant height was not significantly different between treatment areas from June to July ( $F_{4,20}=0.44$ ,  $p=0.78$ ) or from June to August ( $F_{4,20}=1.17$ ,  $p=0.35$ ). Thus, the effects of nutrient additions to the treatment areas could not be detected for aboveground plant height. We used percentage increase in our analysis of plant height because the average plant height



**Figure 3.** Mean ( $\pm$  SE), water column dissolved oxygen (DO) concentration (ppm) at four *Zostera marina* nutrient treatment areas and one control area during the summer 2012 at Berry Cove, Lamoine. Nutrient treatments were applied to five eelgrass patches per treatment area June 8. Three DO measurements per treatment area were taken on each date. Red line demarcates the level below which marine organisms become stressed<sup>3</sup>.



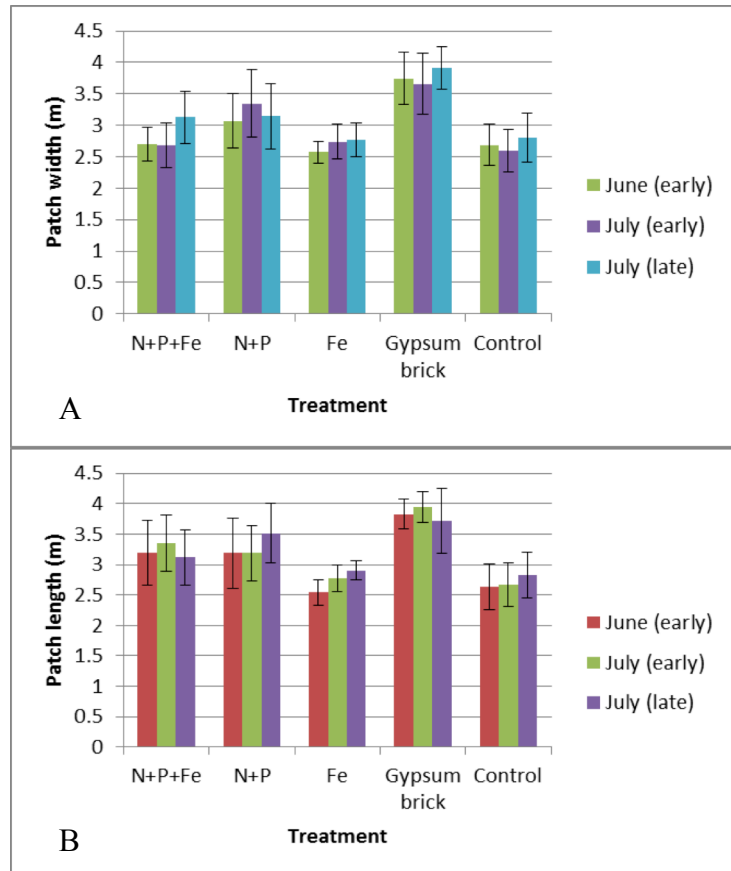
**Figure 4.** Mean ( $\pm$  SE) aboveground plant height for *Zostera marina* at four nutrient treatment areas and one control area during the summer 2012 at Berry Cove, Lamoine. June sampling took place before the application of nutrient treatments on June 8. Five eelgrass patches were sampled within each area.

differed between treatment areas at the start of the experiment. Differences in starting height were revealed in a comparison of mean aboveground plant height (Fig 4). ANOVA showed that shoot density was not significantly different between the five treatment areas in July ( $F_{4,20}=1.47$ ,  $p = 0.25$ ) or in August ( $F_{4,20} = 1.99$ ,  $p = 0.13$ ) after application of nutrient treatments on June 8. In addition, there were no significant differences in shoot density within treated areas between June, July, and August: N+P+Fe ( $F_{2,12}= 0.62$ ,  $p=0.56$ ), N+P ( $F_{2,12}=0.74$ ,  $p=0.50$ ), Fe ( $F_{2,12}=0.60$ ,  $p=0.56$ ), gypsum brick ( $F_{2,12} = 0.05$ ,  $p=0.95$ ). Thus, we did not detect a significant change in shoot density over time as a result of nutrient additions. ANOVA indicated that there were no significant differences in patch width between treatment areas in June ( $F_{4,20}=2.03$ ,  $p=0.13$ ) prior to the application of nutrients, nor were there differences between treatments in early July ( $F_{4,20}=1.31$ ,  $p=0.30$ ) or late July ( $F_{4,20}=1.35$ ,  $p=0.29$ ) following nutrient additions. ANOVA indicated that there were also no significant differences in patch length between treatment areas in June ( $F_{4,20}=1.54$ ,  $p=0.23$ ), early July ( $F_{4,20}=1.97$ ,  $p=0.14$ ), or late July ( $F_{4,20}=0.84$ ,  $p=0.52$ ). It is evident from the data for patch width and patch length that there were also no significant differences within treatment areas between the three sampling dates (Fig 5). There was no significant change in patch width or patch length over time as a result of nutrient additions.

Follow-up studies will include additional measurements of plant height, shoot density, and patch width and length in the coming summer, which will allow us to evaluate whether nutrient additions have a delayed influence on eelgrass growth and spread.

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**Figure 5.** Mean ( $\pm$  SE) width (A) and length (B) of *Zostera marina* patches at four nutrient treatment areas and one control area during the summer 2012 at Berry Cove, Lamoine. June sampling took place before the application of nutrient treatments on June 8. Five eelgrass patches were sampled within each area.