**Task 4.2: Technical memo on assessment of pH in restored eelgrass beds**

**Summary**

The 2012 Washington State Blue Ribbon Panel report on Ocean Acidification and its 2017 addendum recommend the development of “vegetation-based systems of remediation” to “remove carbon dioxide from seawater” and “protect vulnerable young shellfish from acidification” (Action 6.1.1). To test the potential of eelgrass restoration as a remediation tool, the Washington State Department of Natural Resources (WDNR) led a field experiment in 2018 and 2019 in South Puget Sound with support from NTA #2016-0405. WDNR measured pH inside and outside of three restored plots of eelgrass for 18 months. pH dynamics were highly seasonal: lower and less variable in fall and winter, and higher and more variable in spring and summer. During spring and summer, eelgrass appeared to elevate pH at two plots, but not at the third. During fall and winter, eelgrass had no effect on pH. pH was lower in the most recently restored plot than in the more mature plots. Together, these results show that eelgrass restoration has potential as a remediation tool: when eelgrass is most active, in spring and summer, photosynthesis has detectable effects on local pH.

**Introduction**

Seagrass is one of several habitat types that could serve as ocean acidification refugia (Kapsenberg and Cyronak, 2019), where photosynthesis removes enough CO₂ from the water to increase pH and protect CO₂-sensitive organisms, including shellfish. Work to date suggests that eelgrass (*Zostera marina*) may improve average pH, but also exacerbate harmful extremes (Pacella et al. 2018). Field observations are needed to evaluate the comprehensive effects of eelgrass on pH, especially observations that span periods of high biological activity (spring and summer) and periods of low biological activity (fall and winter).

WDNR recognizes eelgrass as critical habitat apart from its potential as refugium, and directs restoration projects where eelgrass has been lost. To develop effective restoration practices, WDNR led a series of experimental transplants in South Puget Sound, where an established model predicted success (Thom et al. 2019). Shoots were transplanted to Joemma Beach State Park to form restoration plots, hereafter ZM1 (transplanted in 2015), ZM2 (2016), and ZM3 (2017) (Fig. 1).

![Fig. 1: Restoration plots at Joemma Beach State Park. ZM1 is in blue, ZM2 in red and ZM3 in orange.](image)
In 2017, WDNR identified the opportunity to use this project to test the effects of eelgrass on pH. For each restoration plot, a nearby unvegetated area was identified as a control: UV1 served as a control plot for ZM1, UV2 for ZM2, and UV3 for ZM3 (Fig. 1). From March 2018 to September 2019, WDNR deployed sensors at all six plots to measure pH and other water quality parameters at 10-minute intervals (Fig. 2).

**Methods**

WDNR used custom-built Durafet-based sensors to measure pH, and commercially available PME MiniDOT loggers to measure dissolved oxygen and temperature, Odyssey Conductivity and Temperature loggers to measure salinity, and PME Cyclops 7 loggers to measure chlorophyll.

Sensors were deployed from 03/07 to 05/07/18, from 05/18 to 08/08/18, from 08/15 to 09/26/18, from 10/23/18 to 02/19/19, from 02/21 to 05/16/19, and from 06/21 to 09/13/19. Between deployments, data were downloaded and sensors cleaned and calibrated as necessary. pH sensors were calibrated before and after every deployment by logging across five temperature steps in Tris-buffered artificial seawater (prepared following SOP 6a, Dickson et al. 2007).

pH was calculated from Durafet voltage using the equation below (adapted from Martz et al. 2010):

\[
pH = \frac{(\text{durafet voltage} - (E^* - E^* T \times T))}{\frac{((R \times (T + 273.15)))}{\ln(10) F)}
\]

Where $E^*$ is the sensor-specific standard potential

$E^* T$ is the standard potential temperature dependence: $-1.101 \text{mV} \times ^\circ \text{C}^{-1}$ (Martz et al. 2010)

$R$ is the gas constant: $8.3145 \text{J} \times \text{K}^{-1} \times \text{mol}^{-1}$

$T$ is the temperature in Celsius, derived from the co-deployed PME MiniDOT sensor

And $F$ is the Faraday constant: $96485 \text{C} \times \text{mol}^{-1}$

Quality control, calculations, visualization and data analysis were performed in R (3.6.1).
Results

In spring and summer, pH showed pronounced daily periodicity, with afternoon maxima and predawn minima (Fig. 3). In fall and winter, pH was flat across the day/night cycle. pH was higher in spring and summer than in fall and winter. Independent of these patterns, pH was higher in eelgrass relative to control plots during the spring and summer at ZM1 and ZM3, but not at ZM2. During fall and winter, there was no difference in pH between eelgrass restoration plots and paired control plots.

Fig. 3: Mean hourly pH by month at eelgrass restoration plots and paired control plots. Upper left, upper right, and lower left panels respectively show data from 2015 (ZM1), 2016 (ZM2) and 2017 (ZM3) restoration plots and paired control plots. Sensors were deployed from March ‘18 to February ‘19. Gray and yellow shading represent night and daylight, using average monthly sunrise and sunset.
Across seasons, pH was higher in the 2015 (ZM1) and 2016 (ZM2) restoration plots than in the 2017 (ZM3) restoration plot (Fig. 4).

Conclusions
Eelgrass appeared to seasonally elevate pH at two of the three restoration plots, demonstrating potential as a tool for remediation. Differences between restoration and control plots were greatest in the afternoon, consistent with the predicted effects of photosynthesis. In some cases, differences persisted across the day/night cycle, raising questions about retention of water in the meadow and the impacts of respiration.

The biological significance of eelgrass effects on pH is unknown, and depends in part on the timing of life history events. Eelgrass appeared to increase pH only in the spring and summer, but this is also the period in which CO$_2$-sensitive bivalve shellfish larvae are most common in Puget Sound. Overall, pH varied more between seasons and between day and night than between eelgrass and unvegetated areas, suggesting that marine organisms experience dramatic differences in pH independent of habitat.

References