Gas exchange, carbon flows, and ecosystem metabolism over a temperate seagrass meadow

Kayleigh E. Granville
Charlottesville, VA

B.S. Environmental Science, University of Connecticut, 2019

A Dissertation presented to the Graduate Faculty of the University of Virginia in Candidacy for the Degree Doctor of Philosophy

Department of Environmental Sciences
University of Virginia
April 2024

Committee:
Peter Berg (advisor)
Karen McGlathery
Patricia Wiberg
Scott Doney
Lisa Colosi-Peterson
# Table of Contents

**Abstract**  
5

**Acknowledgements**  
6

**Introduction**  
7  
1. Dissertation outline  
9  
2. Literature cited  
10

**Chapter 1: A high-resolution submersible oxygen optode system for aquatic eddy covariance**  
14  
1. Introduction  
14  
2. Materials and procedures  
17  
2.1. New O₂ measuring system  
17  
2.2. Response time evaluation  
18  
2.3. System precision  
20  
2.4. Time delay  
20  
2.5. Temperature correction bypass  
20  
2.6. Sunlight sensitivity  
21  
2.7. Stirring sensitivity  
22  
2.8. Field test  
22  
3. Results  
24  
3.1. Response time evaluation  
24  
3.2. System precision  
25  
3.3. Time delay  
26  
3.4. Temperature correction bypass  
27  
3.5. Sunlight sensitivity  
28  
3.6. Stirring sensitivity  
29  
3.7. Field test  
30  
4. Discussion  
32  
5. Comments and recommendations  
34  
6. Literature cited  
35  
7. Acknowledgements  
39

**Chapter 2: Gas transfer velocities and CO₂ fluxes vary diurnally over a temperate seagrass meadow**  
40  
1. Introduction  
41  
2. Methods  
43  
2.1. Study site  
43  
2.2. Regional wind analysis  
44  
2.3. Upside-down aquatic eddy covariance  
45  
2.4. \( k_{600} \) model analysis  
46  
2.5. Diurnal CO₂ flux estimates  
46  
3. Results  
48
3.1. Regional wind analysis 48
3.2. $k_{600}$ model analysis 49
   3.2.1. Peak seagrass density deployments 49
   3.2.2. Ground-truthed in situ $k_{600}$ 50
3.3. Diurnal CO$_2$ fluxes 53
3.4. Fair-weather CO$_2$ fluxes 55
4. Discussion 56
   4.1. Regional wind speed analysis 56
   4.2. $k$ value analysis 57
      4.2.1. In situ trends in wind speed 57
      4.2.2. $k_{600}$ model analysis 57
   4.3. Diurnal variability in CO$_2$ fluxes 59
   4.4. Effect of storms on CO$_2$ fluxes 60
5. Conclusions 61
6. Literature cited 62

**Chapter 3: Seasonal trends in air-water CO$_2$, CH$_4$, and N$_2$O fluxes over a temperate seagrass meadow** 68
1. Introduction 69
2. Methods 71
   2.1. Study site 71
   2.2. Sampling design 73
   2.3. Storm periods 74
   2.4. Dissolved gas fluxes 74
   2.5. Bubble traps 76
   2.6. Data analysis 77
3. Results 77
   3.1. Environmental variables 77
   3.2. Storm periods 79
   3.3. Dissolved CO$_2$ and CH$_4$ fluxes 80
   3.4. Ebullitive CH$_4$ and N$_2$O fluxes 82
   3.5. Total CH$_4$ fluxes 82
   3.6. N$_2$O flux estimates 83
   3.7. Growing season estimates 85
   3.8. Fair-weather fluxes 85
4. Discussion 86
   4.1. Overview of in situ fluxes 86
   4.2. Dissolved CO$_2$ fluxes 87
   4.3. Total CH$_4$ fluxes 88
   4.4. CH$_4$ ebullition 89
   4.5. N$_2$O fluxes 90
5. Conclusions 91
6. Literature cited 92
Chapter 4: At the intersection of science and education: The process of scientists and educators co-developing authentic learning experiences for K-16 students

1. Student views of science
2. Responding to local community needs
3. Best practices for developing authentic science learning experiences
   3.1. Take a scholarly approach to teaching and learning
   3.2. Build a mutually-beneficial partnership between scientists and educators
   3.3. Use pedagogical practices to develop authentic science learning experiences
   3.4. Prioritize sensemaking
   3.5. Help students put scientific conclusions into a real-world context
4. Outcomes
5. Literature cited

Conclusions

Literature cited

Funding acknowledgement
Abstract

Blue carbon ecosystems such as seagrass meadows are widely regarded as carbon sinks that can partially mitigate the effects of anthropogenic climate change. However, the carbon sequestration potential of seagrass meadows is poorly constrained on local and regional scales due to methodological uncertainties and variability in biogeochemical processes such as air-water greenhouse gas exchange of carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O). In this dissertation, I describe several projects that evaluate biogeochemical processes in a seagrass meadow in South Bay, a shallow coastal lagoon in the Virginia Coast Reserve (VCR), and educate the community about blue carbon ecosystems. 1) A new oxygen optode system for aquatic eddy covariance was rigorously evaluated over the full range of oxygen saturation states in the lab and in the field. The system was well-suited for aquatic eddy covariance and the protocols developed established a new standard for future systems. This work is increasingly relevant as aquatic eddy covariance can be used to derive seagrass metabolism estimates and estimates of air-water gas exchange. 2) The diurnal variability in the gas transfer velocity and CO$_2$ flux was evaluated using high-frequency data during peak seagrass density. South Bay was primarily a CO$_2$ sink during the day and a source overnight, following the diurnal pattern of seagrass photosynthesis and respiration. The gas transfer velocity was best predicted with empirical parameterizations based on wind speed. 3) Air-water CO$_2$ and CH$_4$ fluxes were derived throughout the seagrass growing season. South Bay was a moderate CO$_2$ sink and was a consistent source of CH$_4$ that increased throughout the growing season. CH$_4$ flux pathways were also assessed. Dissolved and ebullitive CH$_4$ fluxes were quantified, and CH$_4$ flux from plant-mediated transport was also identified. Finally, our knowledge of coastal processes in the VCR is leveraged to 4) develop a series of best practices for scientists co-developing authentic science learning experiences with teachers using evidence-based practices.
Acknowledgements

It has been an honor and a privilege to be a graduate student in the Department of Environmental Sciences, and there are many people who have helped and supported me over these last five years. First and foremost, my advisor, Peter Berg, for his wisdom and guidance, and for the many opportunities he has helped pursue throughout multiple projects and an entire pandemic. I would also like to thank the rest of my committee, Karen McGlathery, Scott Doney, Pat Wiberg, and Lisa Colosi-Peterson, for their invaluable feedback, ideas, and support that has helped shape this work. To my other collaborators, Markus Huettel, Bongkeun Song, Cora Baird, and Charles Carlson, and my Sea Grant mentor, Stef Simpson: I have learned so much from each of you. You have been inspiring to work with and this experience would not have been the same without you – thank you. To my undergraduate advisor, Ashley Helton, thank you for helping me get to the starting line, and for all of the advice since then. I have received so much help and support from so many other members of the department, especially Meg Miller, the members of the LTER lagoon group, and the Graduate Student Association. Thank you for everything.

Thank you to my labmates and my shoremates: Carly LaRoche, Ieva Juska, Kylor Kerns, Luke Groff, Carolyn Ewers Lewis, Amelie Berger, Martin Volaric, Kinsey Tedford, Libby Bieri, Elise Turrietta, Lauren Brideau, Paola Granados, and Emily Riffe. A million thank you’s to Carly, my partner in crime in South Bay. And thank you to my wonderful friends and fellow scientists who I started this journey with, Marion McKenzie, Madeline Miles, and Zoe Bergman – I could not have done it without you.

I am eternally grateful to the staff at the UVA Coastal Research Center. To Sophia Hoffman, Tom Burkett, Jonah Morreale, and Buck Doughty, thank you for all the boat trips, feedback, and laughs. To Donna, thank you for all the hard work you do and for being a great neighbor in 2021. To Cora, thank you for being an incredible mentor in so many ways.

I am incredibly grateful for all of the unwavering love and support I have received on this journey from my family and friends, especially my partner, David Csordas, and of course, our cats, Adaira and Sidra. To David, thank you for being my rock, confidante, and biggest supporter.
Introduction

Atmospheric concentrations of greenhouse gases such as carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O) have been rising since the start of the industrial era due to increases in anthropogenic emissions (IPCC 2023). The ocean is a significant global carbon reservoir and, in recent decades, has taken up ~25% of anthropogenic CO$_2$ emissions annually (Wanninkhof et al. 2013; Devries 2022). In the coastal ocean and estuaries, physical processes such as mixing and biological processes such as photosynthesis facilitate carbon storage, sequestration, and export to the open ocean for burial (Dai et al. 2022; Resplandy et al. 2024). Coastal vegetated ecosystems, specifically salt marshes, mangroves, and seagrass meadows, are regarded as “blue carbon ecosystems” for their ability to sequester carbon for long periods of time, potentially increasing the amount of anthropogenic greenhouse gas emissions stored in the coastal ocean (Prentice et al. 2020; Macreadie et al. 2021). However, blue carbon ecosystems can also emit CO$_2$, CH$_4$, and N$_2$O, which can partially offset their uptake capacity (Banerjee et al. 2019; Al-Haj and Fulweiler 2020; Rosentreter et al. 2021b). CO$_2$, CH$_4$, and N$_2$O fluxes over seagrass meadows in particular are poorly constrained due to a lack of local and regional data and uncertainties in flux methodologies (Rosentreter et al. 2023).

Seagrasses are flowering marine angiosperms that form meadows off the coast of all continents except Antarctica, in both subtidal and intertidal areas (Dunic et al. 2021; Macreadie et al. 2021). Seagrass meadow ecosystems provide nursery habitat for marine species, increase water quality by trapping sediment and preventing resuspension, and store organic carbon (Corg) in their sediments and biomass (Lawson et al. 2007; Zhu and Wiberg 2022; do Amaral Camara Lima et al. 2023). Evidence suggests that seagrasses may be able to moderate water column pH and that they may be able to keep pace with sea level rise due to sediment deposition (Hendriks et al. 2014; Ricart et al. 2021a; b). In seagrass meadows, oxygen and carbon dioxide are biologically coupled through photosynthesis and respiration (Berg et al. 2019, 2022). Thus, the balance of gross primary production and respiration derived from high-frequency measurements of the benthic O$_2$ flux can serve as a proxy for carbon flows (Duarte et al. 2010; Rheuban et al. 2014a; Berger et al. 2020).
Aquatic eddy covariance is a groundbreaking flux technique that derives high-quality benthic O₂ fluxes from in situ velocity and O₂ concentration measurements. The technique, which is described in detail in Chapter 1, resolves fluxes at a high temporal resolution (15-60 minutes) and integrates fluxes over broad spatial scales (10-100 m²) (Berg et al. 2003, 2022; Rheuban and Berg 2013). The quality and robustness of O₂ flux measurements has been improved over time by technological advances in the meters and sensors used to measure O₂ (Berg et al. 2016). Upside-down aquatic eddy covariance is an emerging technique that is modeled after aquatic eddy covariance. This approach, detailed in Chapter 2, directly measures O₂ fluxes across the air-water interface (Berg and Pace 2017; Long and Nicholson 2018; Berg et al. 2020).

The benefits of seagrass meadows and other blue carbon ecosystems are of particular importance to coastal communities, as these ecosystems can provide buffering from storm surges and floods, increased revenue through recreation and tourism, and can provide nursery ground for fishers and watermen (do Amaral Camara Lima et al. 2023). These ecosystem services can be especially valuable because coastal communities are more likely to be socially vulnerable, i.e., more susceptible to harm from environmental hazards, compared to non-coastal communities (Harris et al. 2022). Coastal communities are also likely to have higher populations of people from minoritized groups. Seagrass meadows are declining worldwide and are vulnerable to increased heating events as a result of increased atmospheric greenhouse gas concentrations (Aoki et al. 2021; Dunic et al. 2021). Educating coastal communities about coastal vegetated ecosystems in this context is an essential strategy to improve support for restoration and conservation practices.

The Eastern Shore of Virginia (ESVA) is located on the east coast of the United States on the Delmarva peninsula, which is located between the Chesapeake Bay and the Atlantic Ocean (see Ch. 2, Fig. 1, Ch. 3, Fig. 1). The ESVA is a socially vulnerable and historically-underserved area (U.S. Census, 2020). The research conducted in this dissertation occurred in the Virginia Coast Reserve (VCR), a system of shallow coastal lagoons and barrier islands located between the Delmarva peninsula and the Atlantic Ocean. The VCR is the longest stretch of coastal wilderness on the east coast of the United States and is a pristine coastal ecosystem with minimal anthropogenic influence. It is the location of a long-term ecological research site (VCR-LTER),
and many VCR-LTER scientists spend a significant amount of time living on the ESVA and interacting with the local community. It is also the site of a successful *Zostera marina*, or eelgrass, meadow. Large-scale restoration efforts were initiated in 2001, and after twenty years, the project has resulted in the restoration of 3,612 benthic ha of eelgrass (Orth et al. 2006, 2020; McGlathery et al. 2012). The VCR was also chosen as the site of the world’s first eelgrass carbon crediting project (Needelman et al. 2018; Oreska et al. 2020). The carbon crediting project uses the verified carbon standard (VCS) to translate carbon stored in ecosystems into monetary credits, which are sold in the voluntary carbon market to fund further restoration and conservation initiatives (Needelman et al. 2018). When greenhouse gas fluxes are not measured, carbon crediting projects must take a conservative deduction in the number of credits that can be sold (Needelman et al. 2018).

The majority of the work reported in this dissertation was conducted in South Bay, one of the shallow coastal bays located in the VCR. South Bay is an ideal location to evaluate ecosystem metabolism, and the instruments used to measure it, because it is the site of over 12 years of high-quality seagrass metabolism measurements derived from benthic aquatic eddy covariance (Berger et al. 2020; Berg et al. 2022). It is also the central area of the seagrass restoration project, and the location of a significant body of work measuring carbon stocks and biogeochemical processes related to gas fluxes (Greiner et al. 2013; Oreska et al. 2018; Aoki and McGlathery 2018; Berg et al. 2019).

1 Dissertation outline

**Chapter 1** details a thorough evaluation of a new O₂ measuring system for aquatic eddy covariance in the laboratory and field. The evaluation considered specific field conditions of environments where aquatic eddy covariance has been used. The results of our evaluation show that the system is well-suited for aquatic eddy covariance at the benthic interface, but not upside-down aquatic eddy covariance. Chapter 1 also outlines a set of guidelines, or best practices, for evaluating new systems that occur in the future.

**Chapter 2** constrains air-water CO₂ fluxes over South Bay via a methodological evaluation and fine-scale CO₂ flux measurements. Wind speed, one of the primary drivers of the air-water CO₂ flux, was evaluated locally and regionally. The upside-down aquatic eddy covariance technique
was used to derive *in situ* measurements of gas transfer velocity \((k)\), the primary source of uncertainty in CO\(_2\) flux measurements. The *in situ* \(k\) values were used to determine the most appropriate wind-based empirical relationship to estimate \(k\) at our site. Finally, air-water CO\(_2\) fluxes were derived for 5-7 days per year in July of 2021, 2022, and 2023 with the improved methods for measuring with speed and \(k\).

**Chapter 3** builds on the improved methodologies of Chapter 2 and provides estimates of air-water CO\(_2\), CH\(_4\), and N\(_2\)O fluxes over South Bay throughout the seagrass growing season of 2022 and 2023. For CH\(_4\) and N\(_2\)O, fluxes were measured via the dissolved, ebullitive, and plant-mediated transport pathways.

**Chapter 4** outlines a series of best practices for scientists and educators to use to translate research into authentic science learning experiences for students. The results of this work included implementing a lesson plan and field experience for high school students on the ESVA. The project followed a scholarly approach to teaching and learning.

**2 Literature Cited**


Orth, R. J., J. S. Lefcheck, K. S. Mcglathery, and others. 2020. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services.


Chapter 1: A high-resolution submersible oxygen optode system for aquatic eddy covariance


Abstract

The aquatic eddy covariance technique is increasingly used to determine oxygen (O₂) fluxes over benthic ecosystems. The technique uses O₂ measuring systems that have a high temporal and numerical resolution. In this study, we performed a series of lab and field tests to assess a new optical submersible O₂ meter designed for aquatic eddy covariance measurements and equipped with an existing ultrafast optical fiber sensor. The meter has a 16-bit digital-to-analog-signal conversion that produces a 0-5 V output at a rate up to 40 Hz. The device was paired with an acoustic Doppler velocimeter for velocity measurements. The combined meter and fiber-optic O₂ sensor’s response time was significantly faster in O₂-undersaturated water compared to in O₂-supersaturated water (0.087 vs. 0.14 s), but still sufficiently fast for aquatic eddy covariance measurements. The O₂ concentration signal was not sensitive to variations in water flow or light exposure. However, the response time was affected by the direction of the flow. When the sensor tip was exposed to a flow from the back rather than the front, the response time increased by 37%. The meter’s internal signal processing time was determined to be ~0.05 s, a delay that can be corrected for during post-processing. In order for the built-in temperature correction to be accurate, the meter should always be submerged in the same body of water as the fiber-optic sensor. In multiple 21 – 47 h field tests, the system recorded consistently high-quality, low-noise O₂ flux data. Overall, the new meter is a powerful option for collecting high-quality aquatic eddy covariance data.

1 Introduction

O₂ flux measurements at the sediment-water interface are typically used to quantify benthic ecosystem health and as a proxy for carbon cycling in aquatic environments such as
seagrass meadows, oyster reefs, and mudflats (Gazeau et al. 2005; Glud 2008; Eyre et al. 2011; Berg et al. 2022). However, representative in situ fluxes, determined under naturally varying environmental conditions, are difficult to obtain due to various limitations of standard flux methodologies (Cook et al. 2007; Glud 2008). Benthic flux chambers separate an enclosed area from the surrounding environment, which excludes in situ hydrodynamics and disturbs in situ light conditions (Tengberg et al. 2004; Polsenaere et al. 2021; Amo-Seco et al. 2021). In situ microelectrode profiling and subsequent profile interpretations exclude the effects of local macrofauna (Archer and Devol 1992; Glud 2008) and may produce artefacts in permeable sediments due to interactions with bottom flows (Huettel and Gust 1992). Laboratory sediment core incubations introduce additional artefacts by removing the sample from the natural environment (Khalil et al. 2013).

Over the last two decades, the aquatic eddy covariance (AEC) technique (Berg et al. 2003) has improved the quality of benthic O$_2$ flux measurements for many ecosystems, including seagrass meadows (Lee et al. 2017; Berger et al. 2020; Koopmans et al. 2020), oyster and mussel reefs (Attard et al. 2019b; Volaric et al. 2020), macroalgal and Maerl beds (Attard et al. 2019a; Polsenaere et al. 2021), permeable sands (Chipman et al. 2016; Merikhi et al. 2021), freshwater systems (McGinnis et al. 2008; Koopmans and Berg 2015), and coral reefs (Long et al. 2013; de Froe et al. 2019). It has also recently been used for upside-down measurements of gas exchange at the air-water interface (Berg and Pace 2017; Long and Nicholson 2018; Berg et al. 2020). The technique measures in situ O$_2$ fluxes without altering light and flow conditions or excluding the sampling area from the surrounding environment. It accounts for small-scale spatial variability and benthic heterogeneity by integrating the flux over an area of 10-100 m$^2$ (Berg et al. 2007; Rheuban and Berg 2013). Deployments typically last for 24-72 hours, and fluxes are usually resolved over time intervals of 15 min to 1 h. The technique derives fluxes from simultaneously-measured in-situ water column velocities and associated water column O$_2$ concentrations. The velocities are measured at rates between 8-64 Hz by an acoustic Doppler velocimeter (ADV), while O$_2$ concentrations are recorded near the ADV measuring volume by a fast-responding O$_2$ measuring system (Berg et al. 2022).

Fast-responding O$_2$ measuring systems used for AEC include systems based on microelectrodes, fiber-optic optodes, or micro-planar optodes. Thin Clark-type glass
microelectrodes typically have short response times (< 0.3 s) but consume O\textsubscript{2}, which makes their signal sensitive to current velocity changes (stirring sensitivity) (Reimers et al. 2016; Attard et al. 2016). Their fragile sensor tips (outer tip diameter 10-200 µm) break more easily than other sensors, resulting in a loss of data (Chipman et al. 2012; Attard et al. 2014). Fiber-optic optodes also typically have short response times (< 0.3 s), and use luminescence quenching by O\textsubscript{2} to quantify O\textsubscript{2} concentrations, a process that does not consume O\textsubscript{2} (Chipman et al. 2012; Koopmans et al. 2020). While some studies report no or negligible stirring sensitivity (Holtappels et al. 2015; Chipman et al. 2016), others have shown that fiber-optic sensors can exhibit stirring sensitivity (Berg et al. 2017). Although fiber-optic sensors are less susceptible to signal drift and typically have a more robust tip (e.g. outer tip diameter 430 µM) than microelectrodes (Chipman et al. 2016), they will experience a loss of signal strength over time due to bleaching of the fluorophore that coats the tip. Biofouling of both fiber-optodes and microelectrodes can cause erroneous signals (Huettel et al. 2020), and collisions with debris can lead to signal spikes, sensor defects and data loss (Koopmans et al. 2020, 2021). To increase robustness and thus, deployment success, some studies use multiple microelectrodes or fiber-optic optodes (Attard et al. 2014, 2019b; Merikhi et al. 2021). An alternative to these sensing systems is the micro-planar optode sensor, which is more robust because of its larger tip (8 mm diameter) that does not break and is rarely affected by floating debris (Berg et al. 2016; Amo-Seco et al. 2021). However, its relatively large tip can disturb the current flow, so recorded data may be compromised when the current flow comes from behind the sensor (Huettel et al. 2020). The micro-planar optode must be placed farther away from the ADV so that its tip does not disturb velocity measurements (Berg et al. 2016).

Due to their complexities, O\textsubscript{2} measuring systems used for AEC must undergo rigorous testing under well-defined lab and field conditions to assess performance, reliability, and potential limitations (McGinnis et al. 2011; Chipman et al. 2012; Berg et al. 2016). Typically, these O\textsubscript{2} measuring systems are tested in the lab in water that is close to atmospheric saturation or O\textsubscript{2}-undersaturated. These conditions are indeed representative of some ecosystems where AEC has been used, such as unvegetated muddy sediments at depths below the photic zone (Berg et al. 2009; Attard et al. 2014; Amo-Seco et al. 2021). However, the water column of ecosystems dominated by photosynthesizing vegetation such as seagrass meadows (Rheuban et al. 2014b; Berger et al. 2020) or macroalgal canopies (Attard et al. 2019a; Volaric et al. 2019) can be highly
supersaturated with O\textsubscript{2} during the daytime. To best represent such field conditions, new O\textsubscript{2} measuring systems for AEC should be evaluated under both O\textsubscript{2}-undersaturated and O\textsubscript{2}-supersaturated conditions.

In this study, we assessed the suitability of a new fiber-optic O\textsubscript{2} measuring system for AEC. We provide a review of the system’s characteristics and performance in the lab over a broad spectrum of conditions and in the field in a shallow, temperate seagrass meadow.

2 Materials and Procedures

2.1 New O\textsubscript{2} measuring system

The AquapHOx®-LX logger (hereafter termed “AquapHOx”) is a new optical submersible meter designed and optimized for fast response times and high-resolution O\textsubscript{2} readings (PyroScience, GmbH; Aachen, Germany). The AquapHOx has a measuring frequency up to 40 Hz, a 16-bit digital-to-analog signal conversion, and a 0-5 V analog output. To measure O\textsubscript{2} concentrations, the AquapHOx is connected to a manufacturer-calibrated, ultra-fast-responding retractable fiber-optic minisensor (OXR430-UHS-SUB, PyroScience). The AquapHOx automatically compensates for O\textsubscript{2} signal drift due to the temperature dependency of the optode and changes in mean water temperature via a temperature sensor located on the meter’s housing (response time = 0.5 s, accuracy = 0.05°C). The AquapHOx produces analog output signals of both the O\textsubscript{2} concentration and temperature at speeds up to 40 Hz.

For our AEC applications, the AquapHOx was also connected to a standard acoustic Doppler velocimeter (ADV) through a one-cable plug-and-play connection (Vector, Nortek, Norway). The ADV measured velocities (x, y, and z) at 64 Hz, powered the AquapHOx, and recorded the analog O\textsubscript{2} concentration and mean temperature output signals. It is advantageous to record all data on the same data logger to effectively identify and account for time offsets between signals (Berg et al. 2022).

We thoroughly tested the PyroScience system (AquapHOx and O\textsubscript{2} sensor) in the lab and the field (Table 1). In all tests, O\textsubscript{2} concentrations were measured by the PyroScience system at 40 Hz. The ADV recorded velocity data at 64 Hz in the lab experiments and at 16 Hz in the field experiments. For noise reduction, these high-frequency ADV and O\textsubscript{2} signals were reduced to 8
Hz through averaging before eddy fluxes were extracted (Berg et al. 2015, Lorrai et al. 2010, Berg et al. 2009).

Table 1. List of lab and field tests conducted with the PyroScience system.

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response time evaluation: O$_2$ saturation state</td>
<td>Fiber-optic sensor was dipped into a rotating water bath in O$_2$-undersaturated and O$_2$-supersaturated water</td>
</tr>
<tr>
<td>Response time evaluation: Effect of sensor angle</td>
<td>Sensor was dipped into a rotating water bath at three angles relative to the main current flow</td>
</tr>
<tr>
<td>System precision</td>
<td>O$_2$ signal readings from the new system and from reference optodes were compared in a rotating water bath with varying O$_2$ concentrations</td>
</tr>
<tr>
<td>Time delay</td>
<td>The time delay caused by internal signal processing was evaluated</td>
</tr>
<tr>
<td>Temperature correction bypass</td>
<td>The built-in temperature correction was bypassed, and the resulting temperature sensitivity was assessed</td>
</tr>
<tr>
<td>Sunlight sensitivity</td>
<td>The sensor was submerged in a rotating water bath that was exposed to periods of sunlight and darkness</td>
</tr>
<tr>
<td>Stirring sensitivity: water bath</td>
<td>The sensor was submerged in a rotating water bath with varying tangential velocities</td>
</tr>
<tr>
<td>Stirring sensitivity: wave tank</td>
<td>The AEC system was submerged in wave tank</td>
</tr>
<tr>
<td>Field test</td>
<td>The AEC system was deployed for 6 days in a seagrass meadow at the Virginia Coast Reserve Long-Term Ecological Research site</td>
</tr>
</tbody>
</table>

2.2 Response time evaluation

System response time was assessed by measuring the t$_{90\%}$ response time, which is the amount of time a system requires to register 90% of an abrupt change in O$_2$ concentration. To create this abrupt concentration change, the fiber-optic sensor tip was quickly dipped from the air into an O$_2$-undersaturated (<10% saturation) water bath at a perpendicular angle to the main current flow (90°). The water bath (diameter = 30 cm, depth = 20 cm) was continuously stirred with a stir bar to ensure no O$_2$ concentration gradients were present. The stable air and water O$_2$ concentrations, as well as 90% of the change between the two, were marked with dashed lines on a graph (Fig. 1a). The number of time intervals between data points for the signal to get from the
“air-line” to below the “t90%-line” were counted and divided by the ADV frequency (64 Hz). Because the AquapHOx updated its analog signal 40 times per second (40 Hz) while the ADV recorded data at 64 Hz, two data points sometimes occurred side-by-side (Fig. 1). Turbulent frequencies less than 8 Hz typically contribute little to the O2 flux, so these side-by-side points would not compromise flux estimates. Three fiber-optic sensors were used, and each sensor was tested nine times. The entire test was repeated in O2-supersaturated water (~200% saturation) (Fig. 1b).

![Graph](image)

**Fig. 1.** Examples of the t90% response time measured in (a) O2-undersaturated water and (b) O2-supersaturated water. Sensors were dipped vertically from the air (O2 saturation ~240 µmol L⁻¹) into the water. The t90% response time calculation is shown for each set of results.

The effect of sensor angle relative to the main current flow on the t90% response time was also assessed. The response time test was repeated in O2-undersaturated water while the sensor was dipped at two more angles: first, at a 45° angle pointing into the main flow direction (45°), and then at a 45° angle pointing away from the main flow direction (-45°). Response times were compared to the response times determined using perpendicular (90°) dips into O2-
undersaturated water. Differences in t90% response times were analyzed using a one-way ANOVA.

2.3 System precision

We evaluated system precision by comparing the PyroScience system’s O2 concentration readings to those of two reference planar optode systems (t90% = ~30 s) (miniDOT, PME, USA) that are characterized by minimal drift. The fiber-optic sensor and the miniDOTs were submerged in an O2-undersaturated, rotating water bath that was open to the atmosphere. The sensors remained in the water until the O2 concentration in the water bath had re-equilibrated with the atmosphere. This test was repeated in an O2-supersaturated water bath.

2.4 Time delay

Photon collection and subsequent signal processing within the AquapHOx can introduce a time lag between the ADV velocity data and the O2 concentration data. Unless corrected for, such lag time can result in the systematic underestimation of fluxes. To assess this potential time lag, the fiber-optic sensor and the ADV transmitter were simultaneously dipped into an O2-undersaturated water bath that was seeded with particles to ensure adequate backscatter for acoustic velocity measurements. The optical fiber tip was attached to the ADV a few millimeters away from and at the same height as the transmitter to ensure that they penetrated the water surface at the same time. We compared the times that the PyroScience system and the ADV recorded the transition from air into water by analyzing the ADV signal-to-noise ratio (SNR) and the PyroScience system’s O2 concentration reported by the ADV (“counts”). The internal time lag was found by averaging the time difference over 21 trials. To ensure that no time delay existed within the ADV itself, this experiment was repeated where the PyroScience system was replaced by an electric circuit. The circuit was comprised of a conducting wire with a non-insulated ending that was mounted similarly to the optical fiber sensor next to the ADV transmitter, and two 1.5 V batteries that were used as an electric switch that closed when the wire contacted the water surface.

2.5 Temperature correction bypass

Submersible O2 measuring systems are sensitive to changes in water temperature (Gundersen et al. 1998; Berg et al. 2022). As a result, most newer systems have a built-in
temperature correction. The PyroScience system applies the correction using a temperature sensor fixed to the housing of the AquapHOx (Fig. 2b). We quantified the PyroScience system’s sensitivity to temperature changes when the AquapHOx was not submerged in the same body of water as the fiber-optic sensor. The AquapHOx was placed on a lab bench in a room with a constant air temperature and the sensor was submerged in a water-filled flask along with a dual temperature-and-O₂ planar optode sensor (RINKO, JFE Advantech, Japan) (Fig. 2). The flask was capped to ensure a constant molar O₂ concentration and placed on a hot plate with a stir bar (Fig. 2). The RINKO sensor was chosen as a reference because its temperature and O₂ sensors are mounted next to one another (~3 mm apart). Both systems recorded O₂ concentrations and temperatures for 15 minutes at room temperature, and then for 45 minutes as the water was gradually heated. This test was performed in O₂-undersaturated water, O₂-saturated water, and O₂-supersaturated water.

![Fig. 2](image)

Fig. 2. To test the temperature sensitivity of the PyroScience system when the built-in temperature correction was bypassed, (a) the PyroScience sensor and the RINKO sensor were submerged in a water-filled, capped flask while (b) the AquapHOx was placed on the lab bench. The temperature sensor mounted on the AquapHOx is contained in the copper-colored small cylinder located next to the fiber connector.

2.6 Sunlight sensitivity
Some fiber-optic sensors are sensitive to changes in light, which can affect the precision of O$_2$ concentration measurements during the diurnal cycle. We submerged the fiber-optic sensor in an O$_2$-saturated, rotating water bath that was positioned in direct sunlight. O$_2$ concentrations were recorded as the water bath was exposed to alternating 5-minute periods of direct sunlight and complete darkness by placing a black box over the entire setup.

2.7 Stirring sensitivity

To assess stirring sensitivity, the fiber-optic sensing tip was exposed to varying tangential velocities in an O$_2$-saturated, rotating water bath. No O$_2$ concentration gradients were present due to the mixing applied to the bath, so any observed changes in the O$_2$ concentration could be attributed to changes in velocity. The water was rotated at tangential velocities of 0 cm s$^{-1}$, 4 cm s$^{-1}$, and 15 cm s$^{-1}$.

The PyroScience system was similarly evaluated for stirring sensitivity in the presence of wave motion using the setup and data analysis described by Berg et al. (2016). In short, the PyroScience system and the ADV were mounted on a frame used for AEC field measurements (see below) and submerged in a wave tank with seeding particles added to facilitate acoustic flow measurements. Velocities and O$_2$ concentrations were measured by the AEC system.

2.9 Field test

An AEC system comprised of an ADV and the PyroScience system was deployed in a restored eelgrass (Zostera marina) meadow in South Bay, a shallow subtidal lagoon located within the Virginia Coast Reserve Long-Term Ecological Research site (VCR-LTER). The lagoon has a mean water depth of 1.2 m and a tidal range of 1 m (Safak et al. 2015). South Bay is an ideal location to evaluate the PyroScience system because it is the site of 15 years of AEC measurements (Hume et al. 2011; Rheuban et al. 2014b; Berger et al. 2020; Juska and Berg 2022).

Four continuous deployments occurred between June 1$^{st}$, 2021, and June 18$^{th}$, 2021. Deployments ranged from 21 – 47 h in length, resulting in approximately 6 full days of data. In all deployments, the ADV and the AquapHOx were mounted on a thin, light, stainless-steel frame (Fig. 3) (Berg and Huettel 2008). The ADV measured velocity (x, y, z) continuously at 16 Hz in a $\sim$2 cm$^3$ measuring volume located 30 cm above the benthic surface, which is the average
eelgrass canopy height at slack tide. The fiber-optic sensor tip was positioned ~0.7 cm away from the edge of the ADV measuring volume to avoid interfering with the velocity measurements (Fig. 3c). To calibrate O₂ concentrations, two reference miniDOTs were positioned at 2 and 30 cm above the sediment surface. No significant vertical stratification was detected. Photosynthetically-active radiation (PAR) was measured at 5-minute intervals by two planar Odyssey PAR loggers mounted 30 cm above the sediment surface. All instruments were deployed and retrieved during low tide.

Fig. 3. (a) The ADV, AquapHOx, and fiber-optic sensor were deployed on a light stainless steel frame over a seagrass meadow in South Bay, VA, USA. (b) A close-up of the AquapHOx. (c) The submerged ADV head and optical O₂ sensor.

Eddy fluxes were extracted following protocols that have been described in detail by Berg et al. (2022) and others. Briefly, 15-minute O₂ fluxes were extracted from the data using EddyFlux3.2 software (Peter Berg, unpublished). In this process, a standard time shift correction (Fan et al. 1990; McGinnis et al. 2008; Lorrai et al. 2010) and storage correction (Rheuban et al. 2014b) were applied. Flux data with signal spikes, which indicate collisions with or temporary attachment of debris in the flow, were removed. The remaining 15-minute fluxes were binned into hourly fluxes.
3 Assessment

3.1 Response time evaluation

The O2 saturation state of the water had a significant effect on the $t_{90\%}$ response time of the PyroScience system (Fig. 4). The $t_{90\%}$ response time was significantly longer in O2-supersaturated water than in O2-undersaturated water ($p = 0.0015$). Specifically, the mean $t_{90\%}$ response time was $0.088 \pm 0.0034$ s ($n = 27$, mean ± SE) in O2-undersaturated water and $0.12 \pm 0.0067$ s ($n = 27$, mean ± SE) in O2-supersaturated water (Fig. 4).

![Fig. 4. Mean $t_{90\%}$ response times in low-O2 (~10% saturation) and high-O2 (~200% saturation) water when sensors were dipped perpendicularly (90°) into the main current flow. Error bars depict standard error.](image)

The $t_{90\%}$ response time was not significantly different when the sensor was dipped at a 90° angle ($t_{90\%} = 0.088 \pm 0.0034$ s, $n = 27$, mean ± SE) and at a 45° angle ($t_{90\%} = 0.087 \pm 0.0038$ s, $n = 27$, mean ± SE) ($p = 0.91$) in O2-undersaturated water (Fig. 5). However, the $t_{90\%}$ response time was significantly longer when the sensor was dipped at a -45° angle ($t_{90\%} = 0.14 \pm 0.010$ s, $n = 27$) ($p = 0.00001$) (Fig. 5). Thus, the PyroScience system $t_{90\%}$ response time is shortest when the sensor is positioned at a 90° angle or 45° angle facing into the main current flow.
Fig. 5. Mean $t_{90\%}$ response times in O$_2$-undersaturated water for sensors dipped at three different angles: perpendicular to the main current flow (90°), at a 45° angle facing into the flow (“front flow”), and at a -45° angle facing away from the flow direction (“back flow”). Error bars depict standard error.

3.2 System precision

There was a good overall agreement between O$_2$ concentrations measured by the PyroScience system and by the two miniDOTs used as references (Fig. 6). There were no noticeable discrepancies between the sensor readings in O$_2$-undersaturated water or in O$_2$-saturated water (Fig. 6a). In O$_2$-supersaturated water, the PyroScience system reported lower O$_2$ concentrations than the miniDOTs (Fig. 6b). This relatively small concentration difference occurred largely outside of the manufacturer-specified calibration range of the miniDOTs (0-150% O$_2$ saturation, +/- 10 µM accuracy), and thus, is difficult to address.
Fig. 6. The fiber-optic sensor and two reference optodes (miniDOT, PME) were submerged in (a) an O₂-undersaturated and (b) an O₂-supersaturated water bath. During both tests, O₂ concentrations in the water bath re-equilibrated with the atmosphere.

3.3 Time delay

The ADV and electric circuit recorded the dip into water at the same time (Fig. 7a, 7b). Thus, no time delay was detected within the ADV. On the contrary, a distinct time delay was identified between the input and the output sides of the AquapHOx (Fig. 7c, 7d). The average time delay was 0.046 ± 0.0023 s ($n = 21$, mean ± SE). This time delay can be corrected in post-processing by applying a standard time shift correction.
3.4 Temperature correction bypass

When the temperature correction of the O₂ signal was bypassed in the lab, the PyroScience system showed temperature sensitivities comparable to other O₂ measuring systems used for AEC (Berg and Pace 2017; Berg et al. 2022) (Fig. 8). Specifically, when the water temperature was increased, the PyroScience system had a temperature sensitivity of 2.5% per °C in O₂-saturated water, 1.7% per °C in O₂-undersaturated water, and 2.4% per °C in O₂-supersaturated water (Fig. 8).
3.5 Sunlight sensitivity

The fiber-optic sensor’s O\textsubscript{2} concentration readings were not affected by exposure to strong sunlight. The PyroScience system correctly reported consistent O\textsubscript{2} concentrations when the sensor was exposed to periods of strong sunlight and periods of complete darkness (Fig. 9).
Fig. 9. The PyroScience system was evaluated for sensitivities to strong sunlight. There was no significant change in O$_2$ concentration readings when the sensor was exposed to periods of strong sunlight and periods of complete darkness.

3.6 Stirring sensitivity

We detected no measurable stirring sensitivity. When the fiber-optic sensor was submerged in the rotating water bath, concentration readings were not significantly different at different tangential velocities (Fig. 10). The minimal upward drift likely occurs because the water bath was slightly O$_2$-undersaturated with respect to the air at the onset of the test. Similarly, O$_2$ concentration readings recorded by the AEC system in the wave tank were not affected by wave motion.
3.7 Field test

All field deployments produced high-quality AEC data from which benthic $O_2$ fluxes were extracted. In the 43-hour sample deployment, $O_2$ concentrations recorded by the PyroScience system and the two miniDOTs agreed well (Fig 11b). The hourly $O_2$ fluxes derived from these data were well-correlated with PAR (Fig 11c, 11d). The maximum positive $O_2$ flux was 599 mmol $O_2$ m$^{-2}$ d$^{-1}$ and the maximum negative $O_2$ flux was -284 mmol $O_2$ m$^{-2}$ d$^{-1}$ (Fig. 11d). These values are within the range of $O_2$ fluxes previously recorded in South Bay with Clark-type microelectrodes (Berg et al. 2019; Berger et al. 2020). A smaller flux was observed in the middle of day 2 (hour 37), likely due to the effect of a decrease in flow velocity on benthic flux (Fig. 11a, 11d), a relationship that has been documented for seagrass meadows before (Hume et al. 2011; Long et al. 2015).
Fig. 11. An example 43-hour-long AEC deployment over a dense seagrass meadow in South Bay (VCR-LTER). (a) Velocity measured in three directions (x, y, and z) and the mean current velocity. (b) O$_2$ concentrations measured with two miniDOTs and the PyroScience system. (c) Photosynthetically-active radiation (PAR) measured at the average seagrass canopy height. (d) Hourly O$_2$ fluxes extracted from the data in Panel (a) and (b). Positive fluxes represent an O$_2$ flux into the water column. Error bars depict standard error.
Aquatic eddy covariance (AEC) is a cutting-edge technique that is increasingly used to quantify in situ benthic O$_2$ fluxes under naturally varying environmental conditions (Berg et al. 2003, 2022; Long 2021). While the principle of the technique is simple – it relies on rapid measurements of the vertical water velocities and O$_2$ concentrations above the benthic surface – there are challenges associated with the technologies applied to secure such data. In this study, we tested a new optical submersible meter (the AquapHOx®-LX, PyroScience), which was designed and optimized by the manufacturer to fit the criteria for high-quality AEC measurements of O$_2$ fluxes. We present a series of rigorous lab and field tests to evaluate the performance of the AquapHOx. In all tests, the AquapHOx was connected to an ultra-fast-responding fiber-optic O$_2$ sensor (OXR430-UHS-SUB, PyroScience) to measure O$_2$ concentrations and to an acoustic Doppler velocimeter (Vector, Nortek) to provide power to and record the output from the AquapHOx.

The $t_{90\%}$ response time of the PyroScience system was 27% slower when the sensor was dipped into O$_2$-supersaturated water (~200%) compared to into O$_2$-undersaturated water (<10%). Typically, sensor response times are not assessed in O$_2$-supersaturated water, even though O$_2$-supersaturated conditions have been reported in shallow-water environments (Attard et al. 2014, 2019a; Long et al. 2020). For example, Berger et al. (2020) reported frequent O$_2$ saturation levels of over 200% in a temperate seagrass meadow. The substantial difference in response times found here may be caused by the diminishing phosphorescence decay of fiber-optic measuring systems associated with increasing O$_2$ concentrations or the low luminescence at high O$_2$ concentrations which reduces system sensitivity.

Similarly, the dipping angle between the sensor and the water surface affected response time. The sensor response times were, on average, 37% slower when the sensor was dipped at a -45° angle (flow approaching the back of the sensor) compared to a 45° angle (flow approaching the front of the sensor). Dip angles of 45° or 90° (vertical) gave statistically identical response times. The identified difference is presumably due to a thicker diffusive boundary layer between the bulk flow and the O$_2$ sensing coating on the tip at the -45° angle. Regardless of these differences, all identified response times ($t_{90\%} = 0.087-0.14$ s) are well within the range of those reported for other O$_2$ sensors successfully used for AEC ($t_{90\%} = 0.25 – 0.5$ s) (Berg et al. 2016;
Attard et al. 2019b; Long et al. 2019; Koopmans et al. 2020). It should also be noted that such differences will be corrected for if a standard time shift correction (Fan et al. 1990; McGinnis et al. 2008) is applied to the flux calculation. This data processing step is increasingly used in AEC work (Lorrai et al. 2010; Koopmans et al. 2020; Berg et al. 2022). An added advantage of using this correction is that it will also automatically correct for the internal time delay (0.046 s) associated with signal processing in the AquapHOx found in this study.

A fundamental principle of AEC measurements is that recorded variations in O$_2$ concentrations are not attributed to changes in other environmental variables. This condition can be challenging to achieve. For example, some O$_2$ sensors are subject to stirring sensitivity, a phenomenon where changes in velocity alone affect the recorded O$_2$ concentration. Microelectrode sensors are inherently subject to stirring sensitivity due to their internal consumption of O$_2$ (Gust et al. 1987; Holtappels et al. 2015). For unknown reasons, optical sensors are sometimes affected by stirring sensitivity as well (Berg et al. 2017), but not always (Holtappels et al. 2015). These conflicting results still need to be investigated. In this study, which included sensor exposure to both varying currents and wave action in separate tests, the O$_2$ sensors showed no sign of stirring sensitivity. In an independent test, we similarly documented that the O$_2$ sensors did not show any sensitivity to varying light exposure, which is another dynamic environmental variable that can potentially bias the concentration reading.

All electrochemical and optical O$_2$ sensors are sensitive to changes in temperature (Gundersen et al. 1998; Berg and Pace 2017). Typically, when exposed to a constant molar O$_2$ concentration, O$_2$ sensor readings will change 2-3% per °C of temperature change. For that reason, newer O$_2$ measuring systems, including the PyroScience system, have a built-in temperature correction of the O$_2$ signal. We documented that the PyroScience system has a temperature sensitivity that ranges from 1.7–2.5% when this correction is bypassed (Fig. 8). As a result, care must be taken to ensure that the AquapHOx and sensor are submerged in the same body of water at all times during deployments. Even then, the distance between the AquapHOx and the sensor can present a challenge in environments with substantial vertical heat fluxes, such as at the air-water interface or in benthic systems where tides or currents drive pronounced temperature changes (Berg and Pace 2017). In such settings, rapid turbulent fluctuations in temperature associated with the heat flux can be falsely recorded as turbulent fluctuations in O$_2$.
concentration and can bias the O$_2$ flux. The only means to avoid this is to measure the temperature with a fast-responding temperature sensor located right next to the O$_2$ sensor (Berg et al. 2016).

The AEC technique requires that data segments corrupted by particle interference or sensor breakage are excluded to reduce the error margin of derived fluxes (Berg et al. 2013; Rheuban and Berg 2013; Attard et al. 2016). The amount of data that is excluded varies with site conditions and the sensor used. Ecosystems with turbid water, floating debris, and frequent high flow rates and wave action such as seagrass meadows sometimes require high percentages of data to be excluded (Rheuban and Berg 2013). For example, no full 24-h data record was collected in South Bay in June 2018 due to breakage of the Clark-type microelectrodes used (Berger et al. 2020). Similarly, and despite using the more robust optical fiber sensors used in this study, 42% of recorded data were excluded from measurements in a eutrophic freshwater embayment (Koopmans et al. 2021). Also, sometimes 50% of the data are excluded when planar optode sensors are used in tidal systems as the larger sensor tip may disrupt velocity measurements (Berg et al. 2016; Amo-Seco et al. 2021). In comparison with these studies, 17% of the recorded data were excluded in our four deployments. Overall, the data were characterized by low noise and less disturbances, and no sensors broke or were compromised by fouling.

The extracted O$_2$ fluxes from our deployments were fully in line with previous flux measurements recorded at our site using microelectrodes (Rheuban et al. 2014a; Berg et al. 2019; Berger et al. 2020). In our example deployment, hourly O$_2$ fluxes ranged from -295 to 590 mmol m$^{-2}$ d$^{-1}$. In comparison, hourly O$_2$ fluxes from the same site ranged from -267 to 359 mmol m$^{-2}$ d$^{-1}$ in June 2015 (Berg et al. 2019) and from -500 to 550 mmol m$^{-2}$ d$^{-1}$ in June 2014 (Berger et al. 2020). Overall, our lab and field tests demonstrate that the PyroScience system resolves high quality O$_2$ fluxes, and is an excellent choice for future AEC measurements.

5 Comments and Recommendations

We recommend that future studies of O$_2$ measuring systems for AEC include an evaluation of t$_{90\%}$ response times at different O$_2$ saturation states, perhaps tailored using site-specific knowledge of where the sensors will be used. Because such tests, to our knowledge, have not been done before, it is unknown if other sensors may show an even stronger O$_2$ concentration level dependency. Although it can be a challenge in shallow water systems, the
AquapHOx and O\textsubscript{2} sensor must be submerged in the same body of water at all times during deployments to ensure an accurate temperature correction of the O\textsubscript{2} signal. Finally, we propose that the test protocols developed during this study should be used as guidelines or as a starting point for future evaluations of new O\textsubscript{2} systems that are going to be used for AEC measurements.

6 Literature cited


Gust, G., K. Booij, W. Helder, and B. Sundby. 1987. ON THE VELOCITY SENSITIVITY (STIRRING EFFECT) OF POLAROGRAPHIC OXYGEN MICROELECTRODES.


7 Acknowledgements

KEG was supported by a graduate research fellowship from Virginia Sea Grant and by the National Science Foundation (NSF) through grants from the Virginia Coast Reserve Long-Term Ecological Research Program (DEB-1832221). PB was supported by the NSF through grants from the Chemical Oceanography Program (OCE-1851424) and the Ocean Technology and Interdisciplinary Coordination Program (OCE-1824144 and OCE-2223204). MH was supported by the NSF through grants from the Chemical Oceanography Program (OCE-1851290). We thank two anonymous reviewers for their insightful feedback to improve the manuscript. We thank PyroScience GmbH for providing the new prototype AquapHOx and fiber-optic sensors for all our tests. Finally, we thank Ieva Juska and Davis Coffey for their help in the field and the lab. The authors report no known conflicts of interest.
Chapter 2: Gas transfer velocities and CO$_2$ fluxes vary diurnally over a temperate seagrass meadow

Conducted with support from: Peter Berg, Carly LaRoche, Scott Doney, and Patricia Wiberg.

Abstract

Seagrass meadows sequester carbon for long periods of time. They can also emit or take up greenhouse gases such as carbon dioxide (CO$_2$). Air-water greenhouse gas fluxes over seagrass meadows are poorly understood primarily due to methodological limitations and lack of local data on fine temporal scales. In this study, we evaluated methodological uncertainties and derived *in situ* air-water CO$_2$ fluxes over South Bay, a shallow coastal bay located in the Virginia Coast Reserve, USA, that has a benthic environment dominated by a temperate seagrass meadow. To address methodological uncertainties, we evaluated the gas transfer velocity, which is the primary source of uncertainty in the CO$_2$ flux estimates. Gas transfer velocities over estuaries and oceans are typically derived from empirical relationships with wind speed as the main driver. We compared wind speeds measured *in situ* and at nearby regional wind stations. All wind stations located in proximity to land features significantly underestimated wind speeds over South Bay, while a wind station located in a nearby estuary (Chesapeake Bay) matched the in situ wind speed measurements well. We then derived *in situ* gas transfer velocities using upside-down aquatic eddy covariance and compared them to multiple wind-based gas transfer velocity models. The best-fitting models for South Bay were originally derived over the ocean and had an intercept of zero. Lastly, we derived hourly air-water CO$_2$ fluxes over South Bay during the peak seagrass density period (early July) for three years. CO$_2$ fluxes exhibited a distinct diurnal pattern that correlated well with photosynthetically active radiation, suggesting that fluxes are driven by photosynthesis and respiration from photosynthetic organisms such as seagrasses and water column microalgae. Overall, South Bay was a small sink of CO$_2$ (-0.60 mmol CO$_2$ m$^{-2}$ d$^{-1}$) during July. During a storm period captured in our measurements, South Bay was a source of CO$_2$. When the storm period was removed, South Bay was a larger sink of CO$_2$ (-4.20 mmol CO$_2$ m$^{-2}$ d$^{-1}$).
m$^2$ d$^{-1}$). Our results underline the importance of evaluating air-water greenhouse gas fluxes over multiple day-night cycles and during multiple weather conditions.

1 Introduction

Coastal vegetated ecosystems such as seagrass meadows contribute to the global carbon cycle by transporting terrestrial carbon to the ocean and by storing and sequestering organic carbon ($C_{org}$) (Bianchi et al. 2018; Fennel et al. 2019; Macreadie et al. 2021). The global carbon sequestration capacity of seagrass meadows is uncertain, with global seagrass extent estimates ranging from 16-165 million ha and $C_{org}$ stock estimates ranging from 1,700 to 21,000 Tg C (Prentice et al. 2020; Macreadie et al. 2021). Local- and regional-scale $C_{org}$ stock variability is partially attributed to uncertainties in the carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) fluxes over seagrass meadows, which either contribute to or partially offset the carbon sequestration potential of the ecosystem, depending on the direction of the flux (Oreska et al. 2020; Ricart et al. 2020; Kim et al. 2022).

Air-water greenhouse gas flux estimates are highly variable over seagrass meadows because common methodologies often do not capture the rapid internal cycling of flux drivers such as wind speed, gas concentrations, and temperatures that have previously been documented in seagrass meadows (Yang et al. 2019; Berg et al. 2019; Van Dam et al. 2021; Rosentreter et al. 2021c). Additionally, variations in ecosystem metabolism due to changes in photosynthetically active radiation (PAR) and oxygen (O$_2$) availability, and these changes can significantly influence CO$_2$ fluxes hourly, daily, and seasonal scales (Ho et al. 2018; Berger et al. 2020; Oreska et al. 2020). CO$_2$ uptake can also be partially offset by CO$_2$ emissions from inorganic carbon burial ($C_{inorg}$) that are facilitated by calcification and carbonate dissolution, especially in meadows that have active bivalve communities or that are located near coral reefs (Saderne et al. 2019; Prentice et al. 2020).

Variability in air-water greenhouse gas flux estimates over estuaries is also partially attributed to uncertainties in existing flux methodologies. Common techniques used to directly measure the in situ air-water gas flux, including cavity ring-down spectrometry (Ollivier et al. 2022) and floating chambers (Rosentreter et al. 2017; Jeffrey et al. 2018), usually only provide several hours of flux estimates on fair-weather days. These measurements are often not representative of fluxes over full tidal, diurnal, and annual cycles, or during poor-weather
periods. Eddy covariance towers deployed over estuaries measure fluxes at fine temporal scales for longer periods but have high start-up and maintenance costs (Van Dam et al. 2021). Upside-down aquatic eddy covariance systems can measure fluxes across the air-water interface as well, but this technique has not yet been applied in a tidal estuary for CO₂ flux measurements (Berg et al. 2017, 2020; Long and Nicholson 2018).

As a result of these shortcomings, air-water gas exchange is frequently estimated by a mass transfer equation such as:

\[ F = k \times (C_w - C_0) \]  

(Eqn. 1)

where \( F \) is the air-water gas flux (mass area\(^{-1}\) time\(^{-1}\)), \( k \) is the gas transfer velocity (length time\(^{-1}\)), \( C_w \) is the bulk concentration of the gas in the water column (mass volume\(^{-1}\)), and \( C_0 \) is the air-equilibrium concentration of the gas at the air-water interface (mass volume\(^{-1}\)) (Garcia and Gordon 1992; Cole et al. 2010; Wanninkhof 2014). The gas transfer velocity is the primary source of uncertainty in this calculation because it describes the effect of air-water boundary layer processes on the flux, which is difficult to measure and is often parameterized as a function of an environmental variable (Wanninkhof 1992; Vieira Borges et al. 2004; Ho et al. 2006; Wanninkhof et al. 2009). These empirical parameterizations are usually presented as \( k_{600} \) or \( k_{660} \) models, where 600 and 660 are the Schmidt numbers for CO₂ at 20°C in freshwater and seawater, respectively (Ho et al. 2011; Wanninkhof 2014). The majority of models used over seagrass meadows or other fetch-limited systems are typically derived from parameterizations of wind speed scaled to 10 m above the ground (\( U_{10} \)) (Frankignoulle and Borges 2001; Borges et al. 2018; Ollivier et al. 2022). A number of well-tested wind-based empirical parameterizations were derived over the open ocean, where wind is the major driver of gas exchange for slightly soluble gases (Nightingale et al. 2000; McGillis et al. 2004; Ho et al. 2006; Wanninkhof et al. 2009). Parameterizations based on current velocity and water depth instead of or in addition wind speeds are also used in estuaries, but these variables are often difficult to constrain, whereas wind speed estimates are generally available from regional wind stations or global models (Wanninkhof 2014; Ho et al. 2016; Rosentreter et al. 2017). There is an implicit assumption that the most appropriate wind data over estuaries are those collected closest to the study area, but this assumption has been challenged because bed stress over the land is greater than over the water due to increased roughness from vegetation (Lawson et al. 2007; Mariotti et al. 2018).
Here, we thoroughly assess air-water CO\textsubscript{2} flux dynamics over a shallow, temperate seagrass meadow (South Bay, Virginia, USA) by evaluating major sources of variability in the flux calculation and by deriving hourly CO\textsubscript{2} fluxes over multiple years. Through a comparison of \textit{in situ} and regional wind speed measurements, we determine the ability of regional wind stations on land to predict \textit{in situ} wind speeds. We conduct an analysis of \textit{in situ} and modelled \( k_{600} \) values at wind speeds \( \leq 4.5 \text{ m s}^{-1} \) using upside-down aquatic eddy covariance. Finally, we used the wind speed comparison and \( k_{600} \) model analysis to report three years of diurnal CO\textsubscript{2} fluxes on during the peak seagrass density period at our site.

2 Methods

**Fig. 1.** Map of the Virginia Coast Reserve (VCR), located between the Atlantic Ocean and the Delmarva peninsula. The study site, SB-152, is located in central South Bay. The regional wind speed stations are located on a channel north of the VCR (Wachapreague, NOAA NDBC WAHV2), at the land-water interface of the Chesapeake Bay (Kiptopeke, NOAA NDBC KPTV2), offshore in the Chesapeake Bay (Rappahannock, NOAA NDBC RPLV2), and on one of the VCR barrier islands (Hog, VCR-LTER).

2.1 Study site

This study was conducted in South Bay (SB-152, 37.261881 N, 75.815125 W), a shallow coastal lagoon in the Virginia Coast Reserve (VCR), located offshore of the Delmarva peninsula in the Mid-Atlantic region of the United States (Fig. 1). South Bay has a tidal range of 1.2 m and an average water depth of 1.0 m below mean sea level, is bordered by a barrier island with a
back-barrier marsh (Wreck Island) to the east, and is connected to the Atlantic Ocean by two inlets at the north and south (Fig. 1) (Reidenbach and Thomas 2018). The meadow has minimal anthropogenic influence and no significant freshwater inputs. Wind is the dominant forcing on circulation and has a strong seasonality, with southerly winds typically occurring in the summer and northerly winds typically occurring in the winter (Fagherazzi and Wiberg 2009; Wiberg et al. 2015). The benthos is dominated by a *Zostera marina* meadow that is the result of a successful seagrass restoration project (McGlathery et al. 2012; Orth et al. 2020). The seagrass meadows exhibit a typical growth pattern for temperate seagrasses and seagrass densities peak around 400 – 550 shoots m\(^{-2}\) in early July (Rheuban et al. 2014b; Berger et al. 2021; Aoki et al. 2021). The average \(C_{\text{org}}\) sequestration rate of the meadow is 1070 t CO\(_2\) yr\(^{-1}\) from 2013-2016 (Oreska et al., 2020). Significant \(C_{\text{inorg}}\) burial has not been observed (Saderne et al. 2019; Oreska et al. 2020).

Our primary site is in the central area of the seagrass meadow (SB-152, Fig. 1), which is also the site of over a decade of ecosystem metabolism measurements conducted with the aquatic eddy covariance technique (Berg et al. 2003; Berger et al. 2020).

### 2.2 Regional wind analysis

Regional and *in situ* wind speeds were compared for 5-7 days per month in June, July, August, and October of 2022. *In situ* wind speeds and directions over South Bay were measured at a 1-minute resolution with a compact anemometer (WindLog data logger, RainWise). The compact anemometer was mounted at SB-152 on a 5 m PVC pole that was driven into the sediment. Wind speeds were rescaled to a 10 m height (\(U_{10}\)) following (Mariotti et al. 2018):

\[
U_{10} = \frac{u_z \log \left( \frac{10}{z_0} \right)}{\log \left( \frac{z}{z_0} \right)}
\]  
(Eqn. 2)

where \(z\) is the height of the wind sensor above the water (m) and \(z_0\) is the roughness coefficient (0.002 m). Changes in \(z\) were accounted for with water depth measurements that were taken at 1-minute intervals with a water level data logger (HOBO U20-04, Onset). *In situ* \(U_{10}\) measurements were averaged to 1-hour intervals to reduce noise.

Wind speed and direction measurements were collected from four geographically distinct regional wind stations that have previously been used to estimate wind over the VCR. 6-minute resolution measurements were collected over a channel (“Wachapreague”, NOAA NDBC
WAHV2; 37.608 N, 75.686 W), at the land-water interface of the Chesapeake Bay (“Kiptopeke”, NOAA NDBC KPTV2; 37.165 N, 75.988 W), and at an open-water site in the Chesapeake Bay (“Rappahannock”, NOAA NDBC RPLV2; 37.538 N, 76.014 W) (Fig. 1). 1-hour resolution measurements were collected over a barrier island in the VCR (“Hog” Island, VCR-LTER, 37.45052 N, 75.6668 W) (Fig. 1) (Porter and Williams, 2024). All wind speed measurements were rescaled to U_{10} (Eqn. 2) and averaged to a 1-hour resolution for comparison with our in situ wind speed measurements.

Hourly wind speed and direction estimates were also collected from a global climate and weather reanalysis product (ERA5). The data occur in a latitude-longitude grid of 0.25 degrees. The wind field site was located offshore of the Chesapeake Bay.

2.3 Upside-down aquatic eddy covariance

High-frequency air-water O_2 fluxes were derived using the upside-down aquatic eddy covariance technique (UAEC) (Berg and Pace 2017; Long and Nicholson 2018; Berg et al. 2020). Briefly, the technique measures the three-dimensional current velocity field using an acoustic Doppler velocimeter (ADV) equipped with a cabled sensor head positioned upward (Vector, Nortek AS), and water column O_2 concentrations using a fast-responding O_2 sensor that is standard for aquatic eddy covariance at 16 Hz ~10 cm below the air-water interface (Berg et al. 2003, 2022; Berg and Pace 2017). An important consideration for UAEC is that turbulent heat fluxes at the air-water interface, unlike those typically found in the benthic environment, can be substantial (Berg et al. 2020). Fast-responding O_2 sensors are inherently sensitive to temperature changes, so O_2 sensor readings must be corrected with parallel high-speed temperature measurements to prevent temperature bias in the O_2 eddy flux calculation (Berg et al. 2020; Granville et al. 2023). Thus, we used a fast-responding dual O_2-temperature planar optode sensor that measured O_2 and temperature at the same point in the water column for this study (RINKO, JFE Advantech). We also independently measured water column O_2 concentrations and temperatures with stable oxygen optodes with a 1-minute resolution (miniDOT, PME). The UAEC system was mounted to a stainless steel pole with a custom mounting device that allowed the system to slide up and down the pole with tidal height. Two buoys and a rudder held the system at a fixed point beneath the water surface, oriented into the main current. O_2 fluxes were extracted from the eddy covariance data, cleaned, and binned into 15-minute intervals following
protocols described in detail in Berg and Pace (2017) and Berg et al. (2022). The UAEC system measured high-quality O₂ fluxes when wind speeds were ≤ 4.5 m s⁻¹. For the purpose of this analysis, we selected a 4-hour deployment period when wind speeds consistently met this condition (August 26th, 2021, 02:45 – 06:45 AM EDT).

We then used Equation 1 to calculate in situ $k$ values for O₂ ($k_{O₂}$), where $F$ was the O₂ flux measured by the UAEC system, $C_w$ was the water column O₂ concentration measured with the stable optode, and $C_0$ was the air-equilibrium concentration of O₂ derived within the UAEC analysis software (Peter Berg, unpublished). The $k_{O₂}$ values were converted to $k_{CO₂}$:

$$\frac{k_{O₂}}{k_{CO₂}} = \left(\frac{Sc_{O₂}}{Sc_{CO₂}}\right)^n$$  \hspace{1cm} (Eqn. 3)

where the value of -0.5 was used for $n$ in low-wind systems, and $Sc_{O₂}$ and $Sc_{CO₂}$ are the Schmidt numbers for O₂ and CO₂, respectively (Cole et al. 2010). $Sc_{O₂}$ and $Sc_{CO₂}$ were derived from in situ water temperature measurements collected with the miniDOTs, a standard salinity estimate for South Bay (31 ppt), and well-established solubility coefficients for O₂ and CO₂ (Weiss 1974; Jähne et al. 1987; Garcia and Gordon 1992; Wanninkhof 2014).

$k_{CO₂}$ values were also normalized to $k_{600}$ for comparison with the $k_{600}$ models:

$$k_{600} = k_{CO₂} \times \left(\frac{600}{Sc_{CO₂}}\right)^n$$  \hspace{1cm} (Eqn. 4)

2.4 $k_{600}$ model analysis

The in situ $k_{600}$ values from the August 2021 deployment were compared to values derived from several empirical relationships to determine the best-fitting model for our site (Table 1). We selected $k_{600}$ and $k_{660}$ relationships that had been designed for seagrass meadows or previously been established for use over shallow fetch-limited estuaries comparable to our site. All $k_{660}$ models were normalized to $k_{600}$ assuming the Schmidt numbers were scaled to $n = -0.5$ for low wind systems (Eqn. 4). A $k_{600}$ model used to derive air-water CO₂ fluxes was chosen through this analysis and scaled to $kCO₂$.

2.5 Diurnal CO₂ flux estimates
Air-water CO₂ fluxes were derived for 3 days in 2021 (10:00 EDT on July 14th – 10:00 EDT on July 17th), 5 days in 2022 (08:30 EDT on July 6th – 12:30 EDT on July 11th), and 6.5 days in 2023 (16:00 EDT on July 4th – 09:00 EDT on July 11th). These deployments coincided with peak seagrass density. We measured photosynthetically active radiation (PAR) with a LI-192 underwater quantum sensor (miniPAR, PME) and water column temperature and O₂ concentrations with a stable O₂ optode (miniDOT, PME) once per minute at mid-canopy height (30 cm above the seafloor). Salinity was measured with a salt water conductivity logger (HOBO U24-002-C, Onset) in 2023 and a pH sensor in 2022 (SeapHOx V2, SeaBird Scientific). Salinity measurements were unavailable in 2021 and for three days in 2022, so a standard value for South Bay was used (31 ppt). In 2022 and 2023, in situ wind speeds were measured with the compact anemometer as described above. In situ wind speed data were not available for 2021, so wind data were collected from Rappahannock (NOAA NDBC RPLV2), the best-fitting regional wind station (see below). We counted seagrass shoot densities by hand during each deployment.

The air-water CO₂ flux was calculated every minute following:

\[ F_{CO_2} = K_0 \cdot k_{CO_2} \cdot (p_{CO_2w} - p_{CO_2a}) \]  
(Eqn. 5)

where \( F_{CO_2} \) is the air-water CO₂ flux (mmol m⁻² h⁻¹), \( K_0 \) is the gas solubility based on temperature and salinity (mmol m⁻³ atm⁻¹), \( k_{CO_2} \) is the gas transfer velocity at the air-water interface (m h⁻¹), and \( p_{CO_2w} \) and \( p_{CO_2a} \) are the partial pressures of CO₂ in the water column and overlying air, respectively (atm) (Wanninkhof 1992; Mu et al. 2014). \( p_{CO_2w} \) was measured continuously (every 2 seconds) with an autonomous, submersible infrared sensor (miniCO₂, Pro Oceanus Systems Inc.). Atmospheric CO₂ mole fractions were retrieved from the NOAA Carbon Tracker Near-Real Time (CT-NRT.v2023-5, Jacobson et al. 2023) and converted to \( p_{CO_2a} \) using barometric pressure measured over the VCR (Porter and Williams, 2024) and water vapor pressure, which was derived from the in situ temperature and salinity measurements using the RStudio “seacarb” package (Weiss and Price 1980; Mu et al. 2014). \( K_0 \) was derived from the 1-minute in situ temperature and salinity data and a modified form of Henry’s law (Weiss 1974; Wanninkhof 2014). \( k_{CO_2} \) was derived using a best fitting \( k_{600} \) model determined by the k600 model analysis (see below). The \( k_{600} \) equation was converted to \( k_{CO_2} \) by using in situ temperature and salinity data to derive \( Sc_{CO_2} \) throughout the deployment (Eqn. 4). The 1-minute fluxes were summed to hourly values for analysis. All analyses were conducted in RStudio,
primarily with the “tidyverse” package (v3.3.3, R Core Team). All mean values are reported as mean ± standard deviation.

3 Results

3.1 Regional wind analysis

Throughout the 2022 deployments, in situ wind speeds over South Bay were not significantly different than wind speeds measured at the open water station (Rappahannock) or reported by the ERA5 reanalysis (Table 1). The in situ wind speeds were under-estimated by an average of 33% by the land-water interface station (Kiptopeke), 43% by the channel station (Wachapreague) and 68% by the barrier island station (Hog) (Table 1). Time series of the July 2022 data are shown to demonstrate the variability across stations (Fig. 2, Table 1). The empirical relationship between $U_{10}$ and $k_{600}$ is often represented by a quadratic or power function, so using these stations to estimate in situ wind speeds over South Bay would significantly under-estimate $k_{600}$ and the flux calculation (Ho et al. 2011).

Table 1. The number of observations (n), mean, and standard deviation (sd) of hourly wind speeds scaled to a height of 10 m ($U_{10}$, m s$^{-1}$) reported for the in situ station, regional stations, and the ERA5 reanalysis for the 2022 study period (June, July, August, and October, 2022). For each regional station and the ERA5 reanalysis, the mean percent deviation from the in situ wind station (%) was provided, as well as the p value resulting from the pairwise Wilcoxon rank-sum tests with the in situ wind station (significance level p > 0.05).

<table>
<thead>
<tr>
<th>Site</th>
<th>n</th>
<th>Mean</th>
<th>Sd</th>
<th>Percent difference</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Bay (in situ)</td>
<td>485</td>
<td>4.99</td>
<td>2.36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rappahannock</td>
<td>485</td>
<td>4.79</td>
<td>2.48</td>
<td>3.8</td>
<td>0.19</td>
</tr>
<tr>
<td>Kiptopeke</td>
<td>485</td>
<td>3.34</td>
<td>1.71</td>
<td>33</td>
<td>3.7 * 10^{-29}</td>
</tr>
<tr>
<td>Wachapreague</td>
<td>485</td>
<td>2.84</td>
<td>2.16</td>
<td>43</td>
<td>2.3 * 10^{-47}</td>
</tr>
<tr>
<td>Hog</td>
<td>485</td>
<td>1.60</td>
<td>0.88</td>
<td>68</td>
<td>5.6 * 10^{-16}</td>
</tr>
<tr>
<td>ERA5 Reanalysis</td>
<td>485</td>
<td>4.85</td>
<td>2.42</td>
<td>2.7</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Fig. 2. The July 2022 time series of *in situ* wind speeds (South Bay) was compared to wind speeds reported at the following wind stations: (a) Rappahannock, (b) Kiptopeke, (c) Hog, and (d) Wachapreague, as well as by the (e) ERA5 reanalysis product. All wind speeds were converted to $U_{10}$. See Fig. 1 for wind station locations.

3.2 $k_{600}$ model analysis

3.2.1 Peak seagrass density deployments

During the peak seagrass density deployments that occurred in 2021, 2022, and 2023, the mean wind speed was $U_{10} = 4.95 \pm 2.01$ m s$^{-1}$ (mean ± standard deviation). The relationship between $k_{600}$ and wind speed during these deployments was derived for each wind-based empirical relationship (Fig 3a, Table 2). The relative standard deviation of the mean $k_{600}$ across all wind speeds revealed that $k_{600}$ model variability was greatest at low wind speeds, ranging from 123 % when $U_{10} = 0.33$ m s$^{-1}$ to 50.9 % when $U_{10} = 3.50$ m s$^{-1}$ (Fig. 3b). $k_{600}$ model variability was lowest when $U_{10}$ ranged from 7.0 – 11.1 m s$^{-1}$, and the minimum relative standard deviation (18.3 %) occurred when $U_{10} = 9.36$ m s$^{-1}$ (Fig. 3b). The relative standard deviation increased again at the highest wind speed 43.6 % at $U_{10} = 13.70$ m s$^{-1}$ (Fig. 3b).
The $k_{600}$ models in Table 1 and *in situ* $U_{10}$ measurements were used to derive *in situ* $k_{600}$ values during peak seagrass density. (b) The relative standard deviation of the mean $k_{600}$ value across measured $U_{10}$.

3.2.2 Ground-truthed *in situ* $k_{600}$

The upside-down aquatic eddy covariance technique produced high-quality O$_2$ fluxes for four hours (August 26$^{th}$, 2021, 02:45 – 06:45 EDT) when wind speeds were low. The mean wind speed at the Rappahannock station was $U_{10} = 3.03 \pm 0.63$ m s$^{-1}$. Current velocity decreased throughout the deployment and had a mean value of $4.90 \pm 2.22$ cm s$^{-1}$ (Fig. 4a). The deployment occurred during low tide and the mean water depth was $0.87 \pm 0.0082$ m. The O$_2$ flux was negative throughout the deployment, showing that the water column was a net sink of O$_2$ (Fig. 4c). The gas transfer velocity of O$_2$ ($k_{O_2}$) was positively correlated with both current velocity and wind speed (Fig. 4, Fig. 3). Similarly, a positive correlation between wind speed and current velocity ($r = .64$, $p = .0055$, $n = 17$) was identified with a Spearman’s rank-order correlation test using the Hmisc R package (v5.1-1, Harrell, 2023). When $k_{O_2}$ was converted to $k_{600}$, simple linear regressions showed that wind speed explained 58.84% of the variation in $k_{600}$ and velocity explained 80.63% of the variation.
Fig. 4. (a) Current velocity measured in three directions (u, v, and w) and the mean current velocity (vmean), as well as (b) water column O\textsubscript{2} concentrations ([O\textsubscript{2}]) and percent O\textsubscript{2} saturations ([O\textsubscript{2}]\textsubscript{sat}) were measured with the upside-down aquatic eddy covariance technique and used to derive (c) 15-minute air-water O\textsubscript{2} fluxes. Negative values represent uptake by the water column. (d) The gas transfer velocity of O\textsubscript{2} (k\textsubscript{O\textsubscript{2}}) was derived from the O\textsubscript{2} fluxes.

The in situ k\textsubscript{600} values derived from UAEC ranged from 1.08 cm h\textsuperscript{-1} to 5.71 cm h\textsuperscript{-1} and had a mean value of 2.64 ± 1.46 cm h\textsuperscript{-1} during the August 2021 deployment (Fig. 5a). A Kruskal-Wallis rank-sum test identified significant differences between the k\textsubscript{600} models (chi-squared = 203.97, p = 2.2 \times 10\textsuperscript{-16}) using the R stats package (v4.3.2, R Core Team, 2023). Pairwise Wilcoxon rank-sum tests conducted with the stats R package revealed that the k\textsubscript{600} values derived from in situ data were not significantly different from those calculated by the following models: Nightingale et al. (2000) (N00, p = 0.14), Ho et al. (2006) (H06, p = 0.88), Ho et al. (2011) (H11, p = 0.92), and Wanninkhof (2014) (W14, p = 0.92) (n = 17) (v4.3.2, R Core Team, 2023). The relative root-mean-square-error (rRMSE) between the in situ k\textsubscript{600} values and each k\textsubscript{600} model was derived using the Metrics R package (v0.1.4, Hamner and Frasco, 2018), where a lower rRMSE represents a better goodness-of-fit of the model (Table 2) (Ho et al. 2011, 2016). The rRMSE results are in agreement with the results of the rank-sum tests.
The positive correlation between $k_{O_2}$ and current velocity indicates that current velocity partially drove the variability in gas transfer velocity at our site (Fig. 4a, 4d). However, while the models that included current velocity and water depth (B04c, B04a, and H16) replicated the variability in our in situ data, they consistently over-estimated the magnitude (Fig. 5a, Table 2).

![Graph](image)

**Fig. 5.** (a) $k_{600}$ values derived from Rappahannock wind speed measurements and wind-based empirical parameterizations were compared to in situ $k_{600}$ values from upside-down aquatic eddy covariance measurements. (b) The mean and relative standard deviation of the $k_{600}$ models across deployment wind speeds. The method described by each code is given in Table 2. The in situ measurements are denoted by the code “SB152”.

**Table 2.** For each empirical parameterization, the analysis code, the reference, and the original $k_{600}$ or $k_{660}$ model equation is provided. All equations were standardized to $k_{600}$ for our analysis. $U_{10}$ is wind speed scaled to a height of 10 m above the water surface (m s$^{-1}$), $v$ is current velocity (cm s$^{-1}$), and $h$ is water depth (m). The mean $k_{600}$ (m s$^{-1}$) during the August 2021 upside-down aquatic eddy covariance deployment, as well as the relative root-mean-square-error (rRMSE, %) of the in situ and modeled data, is shown for each parameterization.

<table>
<thead>
<tr>
<th>Parameterization</th>
<th>Parameterization</th>
<th>Mean $k_{600}$</th>
<th>rRMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wind speed parameterizations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N00</strong> Nightingale et al. 2000</td>
<td>$k_{600} = 0.333U_{10} + 0.222U_{10}^2$</td>
<td>3.13 ± 0.264</td>
<td>0.685</td>
</tr>
</tbody>
</table>
**Multi-variable parameterizations**

<table>
<thead>
<tr>
<th>Code</th>
<th>Reference</th>
<th>Equation</th>
<th>CO₂ Value</th>
<th>Wind Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M01</td>
<td>McGillis et al. 2001</td>
<td>$k_{660} = 3.3 + 0.026U_{10}^3$</td>
<td>4.15 ± 0.128</td>
<td>1.26</td>
</tr>
<tr>
<td>RC01</td>
<td>Raymond and Cole 2001</td>
<td>$k_{600} = 1.58e^{0.3U_{10}}$</td>
<td>3.99 ± 0.188</td>
<td>1.12</td>
</tr>
<tr>
<td>M04</td>
<td>McGillis et al. 2004</td>
<td>$k_{660} = 8.2 + 0.014U_{10}^3$</td>
<td>8.66 ± 0.0691</td>
<td>4.20</td>
</tr>
<tr>
<td>B04</td>
<td>Vieira Borges et al. 2004</td>
<td>$k_{600\text{wind}} = 1.0 + 2.58U_{10}$</td>
<td>8.82 ± 0.395</td>
<td>4.28</td>
</tr>
<tr>
<td>H06</td>
<td>Ho et al. 2006</td>
<td>$k_{600} = (0.266 ± 0.019)U_{10}^2$</td>
<td>2.54 ±0.256</td>
<td>0.598</td>
</tr>
<tr>
<td>J08</td>
<td>Jiang et al. 2008</td>
<td>$k_{600} = 0.314U_{10}^2 - 0.436U_{10} + 3.99$</td>
<td>5.67 ± 0.235</td>
<td>2.16</td>
</tr>
<tr>
<td>W09</td>
<td>Wanninkhof et al. 2009</td>
<td>$k_{660} = 3 + 0.1U_{10} + 0.064U_{10}^2 + 0.011U_{10}^3$</td>
<td>4.47 ± 0.135</td>
<td>1.44</td>
</tr>
<tr>
<td>H11</td>
<td>Ho et al. 2011</td>
<td>$k_{600} = (0.262 ± 0.022)U_{10}^2$</td>
<td>2.50 ± 0.252</td>
<td>0.603</td>
</tr>
<tr>
<td>W14</td>
<td>Wanninkhof 2014</td>
<td>$k_{600} = 0.251U_{10}^2$</td>
<td>2.51 ± 0.253</td>
<td>0.601</td>
</tr>
<tr>
<td>D23</td>
<td>Dobashi and Ho 2023</td>
<td>$k_{600} = 0.143U_{10}^2$</td>
<td>1.37 ± 0.137</td>
<td>1.12</td>
</tr>
</tbody>
</table>

3.3 Diurnal CO₂ fluxes

Hourly CO₂ fluxes ranged from -2.00 mmol m⁻² h⁻¹ to 5.51 mmol m⁻² h⁻¹ and had a mean value of -0.01 ± 0.842 (Fig. 6a). Here, negative fluxes represent uptake by the water column and positive fluxes represent emission by the water column. The mean $k_{CO₂}$ value was 11.1 ± 7.36 cm h⁻¹. Mean wind speeds were $U_{10} = 4.95 ± 2.01$ m s⁻¹. Water column $p_{CO₂}$ concentrations and temperatures, components of the CO₂ flux equation, exhibited a negative relationship across all years. Changes in the flux magnitude generally followed the sinusoidal pattern of water column pCO₂ and the inverse of the sinusoidal patterns of water column temperature and dissolved O₂ (Fig. 6).

A storm occurred in 2022 from July 9th at 17:00 to July 10th at 08:00 (Fig. 6a). Flux magnitudes were approximately 2-5 times greater than the average flux during the storm period and reached the maximum positive CO₂ flux value ($CO₂_{max} = 5.51$ mmol m⁻² h⁻¹) (Fig. 6a). During the storm period, the water column temperature oscillations were disrupted, and
temperatures decreased from 26.70 °C to 21.7 °C, which was the minimum value recorded (Fig. 6a). pCO₂ concentrations increased and reached the maximum value (pCO₂max = 1032 µatm) that was well above the average pCO₂ value of 342 ± 176 µatm (Fig. 6b). Wind speeds also increased during the storm period (Fig. 2) and reached the maximum value for the 2022 deployment (U₁₀max = 11.1 m s⁻¹). The maximum negative CO₂ flux (CO₂min = -2.00 mmol m⁻² h⁻¹) occurred on July 14th, 2021, at 20:00 EDT due to a high wind event that occurred within one hour (U₁₀ = 13.70 ± 2.40 m s⁻¹, mean ± standard deviation). The increased negative flux likely did not persist like the 2022 storm flux increase because there were no noticeable changes to other variables that were used to derive the flux, such as water column pCO₂ concentrations and temperature (Eqn. 5, Fig. 6).

Fig. 6. Hourly time series data of (a) CO₂ fluxes, water column temperatures, (b) water column pCO₂ concentrations, and water column O₂ concentrations during the peak seagrass density deployments that occurred in July of 2021, 2022, and 2023. The time of “00:00” on the x axis denotes midnight of each day. Negative CO₂ fluxes represent an uptake of CO₂ by the water column.
3.4 Fair-weather CO₂ fluxes

All hourly CO₂ fluxes were binned by the hour of the day (Fig. 7). Fluxes followed a diurnal sink-source cycle where South Bay was a source of CO₂ from 01:00 – 11:00 EDT and a sink of CO₂ from 11:00 – 24:00 EDT (Fig. 7). The cumulative daily sum was -0.60 mmol CO₂ m⁻² d⁻¹. To illustrate the potential bias in flux measurements that would occur from only measuring fluxes during fair-weather periods, we compared the full dataset of hourly average CO₂ fluxes to the non-storm period (referred to as the “fair-weather dataset”), in which the 2022 storm period was removed (Fig. 7). The fair-weather dataset followed a similar diurnal source-sink cycle as the full dataset, but the magnitude and duration of the negative CO₂ flux was greater (Fig. 7). The magnitudes of the positive CO₂ fluxes were significantly smaller (Fig. 7). The cumulative daily sum of the fair-weather dataset was -4.20 mmol CO₂ m⁻² d⁻¹, significantly overestimating the CO₂ sink capacity. The significant decrease in the negative flux magnitude one hour after last light (21:00) is partially attributed to the high negative flux (-0.66 mmol CO₂ m⁻² d⁻¹) that occurred for one hour in 2021 and to the 2022 storm, which primarily occurred overnight (Fig. 6). South Bay was a greater CO₂ source during the storm period compared to the fair-weather period. The decrease is also partially attributed to the length of the datasets, which consistently ended in daytime, resulting in more measurements during the day than the night (Fig. 7).
**4 Discussion**

4.1 Regional wind speed analysis

Wind speeds measured at stations located at or near a land-water interface are assumed to be representative of estuarine waters, even though the surface roughness over land is greater than over water due to shear stress from land and vegetation (Lawson et al. 2007; Mariotti et al. 2018). In this study, wind speeds measured over a barrier island, a channel, and at the land-water interface were surprisingly not representative of wind speeds measured over South Bay (Fig. 2, Table 1). These stations consistently underestimated wind speeds. Similar results were reported in the Mississippi Delta estuary, where the underestimation of bay wind speeds by land stations was greatest when the winds were oriented from the direction of the land (Mariotti et al. 2018). To appropriately represent wind speeds over bays such as South Bay with land stations, a
boundary layer correction is needed when the wind blows from the direction of land (Schwing and Blanton 1984; Hsu 1986). This is particularly important for gas exchange estimates because the relationship between wind speed and gas transfer velocity \(k\) is generally reported with a quadratic or power function, so an underestimation in wind speeds would result in a significant underestimation of \(k\). In turn, this would then result in an underestimation of the derived gas flux (Eqn. 5). Ultimately, a bias in the wind speed of \(\geq 1\) m s\(^{-1}\) can bias the CO\(_2\) flux by 43% or greater (Wanninkhof et al. 2002, 2009).

Our study suggests that the absence of land is more important than site proximity when selecting a regional wind station to represent an estuary (Mariotti et al. 2018). Wind speeds measured at Rappahannock, the wind station located in the Chesapeake Bay, were not significantly different to those measured over South Bay (Fig. 2a, Table 1). Wind speed estimates derived offshore of the Chesapeake Bay with the ERA5 analysis were also not significantly different from the \textit{in situ} station or from Rappahannock (Fig. 2e, Zhu et al. \textit{in prep}). Overall, regional wind stations located over water or fine-scale models such as the ERA5 reanalysis likely provide the most appropriate estimates of wind speeds over estuaries.

4.2 \(k\) value analysis

4.2.1 \textit{In situ} trends in wind speed

Recently, there has been an enhanced effort to constrain gas transfer velocities \(k\) in estuaries (Van Dam et al. 2019; Rosentreter et al. 2021c; Dobashi and Ho 2023). Different empirical wind speed parameterizations can produce highly variable \(k\) values in estuaries, partially because of the lack of observational data over the ocean when \(U_{10} < 4\) m s\(^{-1}\), a value that estuarine wind speeds are often at or below (Wanninkhof et al. 2009; Ho et al. 2018; Dobashi and Ho 2023). During the peak seagrass density deployments in 2021, 2022, and 2023, we observed the most variability in the wind speed \(k\) models when \(U_{10} \leq 5\) m s\(^{-1}\) (Fig. 3b). This is particularly relevant for selecting a \(k\) model for our site because \(U_{10} \leq 5\) m s\(^{-1}\) for 60 % of the total period of our CO\(_2\) flux estimates (Fig. 6, Fig. 7). During our \(k\) model analysis period in August 2021, wind speeds ranged from 0.3 – 4.5 m s\(^{-1}\) \((U_{10})\), capturing the majority of this variability (Fig. 5).

4.2.2 \(k\) model analysis
The in situ $k_{600}$ values derived from upside-down aquatic eddy covariance varied with wind speed (Fig. 3, Fig. 5). In other vegetated estuaries, variability in $k_{600}$ values indicated that gas transfer velocity is driven by multiple processes, such as wind speed, current velocity, and water depth (Ho et al. 2016; Rosentreter et al. 2017). Thus, we evaluated parameterizations that included current velocity and water depth (B04c, B04a, H16) in addition to the wind speed parameterizations during the $k_{600}$ model analysis (Table 2). The current velocity and water depth parameterizations were representative of variability in $k_{600}$ values, but consistently overestimated the magnitude (Fig. 5). This may be because wind-driven waves and currents are recognized as a dominant forcing in South Bay, which indicates that wind speed and current velocity are correlated (Lawson et al. 2007; Fagherazzi and Wiberg 2009). In this study, we also observed a correlation between wind speed and velocity. Due to this correlation, including both wind speed and velocity in parameterizations may overestimate the gas transfer velocity at our site.

Ultimately, the best fitting models in our study (N00, H06, H11, and W14) were wind speed parameterizations that were derived over the ocean and had an intercept of zero (Fig. 5, Table 2). These findings are at odds with the established concept that gas transfer velocity does not approach zero at low wind speeds and rather asymptotes at a finite value due to environmental forcings such as buoyancy, chemical enhancement, and physical mixing (Mcgillis et al. 2001; McGillis et al. 2004; Wanninkhof et al. 2009). However, our results are in agreement with a study conducted in a tidal river estuary that found that parameterizations derived from the open ocean with an intercept of zero were better predictors of $k_{600}$ compared to parameterizations derived over estuaries or over the ocean with a nonzero intercept (Ho et al. 2016). In contrast, the parameterizations with nonzero intercepts derived over either the open ocean (M01, M04, J08, and W09) or estuaries (R01 and B04) overestimated $k_{600}$ at the low wind speeds in our study, although the curves of M01 and W09 suggest that they may better predict $k_{600}$ over South Bay when $U_{10} \geq 5$ m s$^{-1}$ (Fig. 5). This phenomenon may be explained by the aforementioned lack of $k_{600}$ measurements over the ocean at low wind speeds, or by the influence of current velocity on gas transfer velocity at our site (Wanninkhof et al. 2009; Ho et al. 2016).
Surprisingly, $k_{600}$ was consistently underestimated by an empirical wind speed parameterization derived over a different seagrass meadow (D23) (Dobashi and Ho 2023). The Dobashi and Ho (2023) parameterization was determined in the early growing season, whereas our $k_{600}$ analysis occurred in August after seagrass senescence. High seagrass densities are known to attenuate flow, and it is possible that their parameterization may fit our site better during the early or peak growing season periods (Reidenbach and Thomas 2018; Zhu et al. 2021). Potential seasonality in the drivers of gas transfer velocity in shallow vegetated ecosystems have not been evaluated, to our knowledge.

Differences in $k_{600}$ within ecosystems have been reported in mangrove forests, where a uniform $k_{600}$ value derived at one site (Ho et al. 2016) underestimated CO$_2$ fluxes at a different site 60-70 %. The presence of submerged, floating, and emergent vegetation can similarly affect gas exchange by reducing flows, facilitating direct gas exchange across the sediment-water or plant-water interface, and disrupting the air-water boundary layer (Ho et al. 2018; Zhu et al. 2021).

We determined that there were four models that fit our site best because they produced $k_{600}$ values that were not significantly different from the in situ values: Nightingale et al. (2000), Ho et al. (2006), Ho et al. (2011), and Wanninkhof et al. (2014) (Table 2, Fig. 5). We chose Nightingale et al. (2000) (N00) as the empirical parameterization for our CO$_2$ flux estimates because this parameterization considered local measurements in fetch-limited environments. Wanninkhof (2014) (W14), a widely used model, was also one of our best fitting models. We selected N00 over W14 because W14 was recommended for regional to global wind speed estimates of $k$. Our analysis was limited to low and low-intermediate wind speeds. In the future, it would be informative to assess gas transfer velocities over consecutive full tidal cycles and during stormy periods.

### 4.3 Diurnal variability in CO$_2$ fluxes

Hourly CO$_2$ fluxes varied in magnitude and direction, demonstrating the rapid internal cycling of $C_{\text{inorg}}$ that occurs in seagrass meadows (Fig. 6a). The inverse sinusoidal relationship of water column $p_{\text{CO}_2}$ and O$_2$ concentrations shown here and in previous work (Berg et al. 2019) suggests that the hourly-scale variability in the CO$_2$ fluxes is driven by patterns in photosynthesis.
and respiration (Fig. 6b). The differences between the water column concentration patterns and the variability in the CO₂ flux suggests that a significant amount of this metabolism is occurring in the water column, which is in agreement with the findings of Berg et al. (2019). The time periods in which South Bay was a CO₂ sink primarily occurred during the day, and the time periods in which South Bay was a CO₂ source primarily occurred overnight (Fig. 6, Fig. 7). This suggests that daytime measurements will overestimate the total CO₂ sink capacity of seagrass meadow ecosystems. We find that these patterns are interannually persistent, except for the storm period. Averaged over all deployments, the hourly CO₂ fluxes followed a diurnal source-sink pattern with PAR and the cumulative daily sum was -0.60 mmol CO₂ m⁻² d⁻¹, indicating that South Bay is in metabolic balance during peak seagrass density (Fig. 7). Our results are in line with an analysis of decadal ecosystem metabolism in South Bay determined from benthic O₂ fluxes that found similarly that the seagrass meadow was in metabolic balance (Berger et al. 2020).

4.4 Effect of storms on CO₂ fluxes

The maximum positive CO₂ flux recorded in this study occurred during the storm period in 2022 (Fig. 6a). During this period, there was a corresponding increase in wind speed that was documented in situ over South Bay, as well as at the Chesapeake Bay station, the channel station, and the barrier island station (Fig. 2). A decrease in water temperature and an increase in water column pCO₂ concentrations also occurred during the storm period. Using water level data collected at the NOAA Wachapreague station (NOAA NDBC WAHV2), we also identified a storm surge during the 2022 storm period where the difference between the predicted astronomical tide and the measured water levels was > 0.2 m (NOAA NDBC WAHV2) (Zhu and Wiberg, 2022; Castagno et al., 2018). By measuring all components of the flux equation (Eq. 5) on temporal scales ≤ 1 min, we captured the effect of rapid changes in k_CO₂ from wind speed, K₀ from temperature, and ΔpCO₂ from water column pCO₂ on the CO₂ flux (Fig. 6). The maximum negative CO₂ flux recorded in this study occurred in 2021 when the mean wind speed was U₁₀ = 13.70 ± 2.40 m s⁻¹, but this flux did not persist for more than an hour and no significant change in temperature was identified. We also did not identify a storm surge during this period, so this flux was not included as a storm period.
During storm periods, there is increased resuspension of suspended sediments that may facilitate increased gas exchange from the sediment to the water column and then to the atmosphere (Zhu and Wiberg, 2024; Zhu et al., 2021). Increased wind speeds, whitecapping, and rainfall may also contribute to a thinning of the air-water boundary layers, further facilitating gas exchange (Wanninkhof et al., 2009; Ho et al., 2000). The relationship between wind speed and gas exchange should be studied further during storm periods to better constrain the effect of rain on gas fluxes.

Storms in particular are not well-documented in existing greenhouse gas flux measurements over estuaries. When the 2022 storm was removed to demonstrate the difference between determining fluxes during fair-weather and all-weather conditions, we observed an overestimation of the daily CO$_2$ sink capacity of South Bay (Fig. 7). Storm events over the VCR occurred for 5 % of the total time between August 2008 to December 2020, but storm events may increase in the future due to sea level rise and potential changes to the intensity and frequency of storms from climate change (Lin et al. 2019; Kulp and Strauss 2019; Zhu and Wiberg 2022). The frequency, intensity, and duration of storms should be taken into consideration when fluxes are scaled temporally and spatially.

5 Conclusions

Measuring air-water CO$_2$ fluxes on fine temporal scales is essential for disentangling diurnal and inter-annual patterns. In this study, we performed a regional wind speed analysis, an analysis of in situ and modelled $k_{600}$ values, and diurnal CO$_2$ flux patterns over South Bay, a shallow coastal bay with a temperate seagrass meadow dominating the benthos. The regional wind speed station that gave measurements closest to those recorded at our site was located in the Chesapeake Bay. All stations located in proximity to land underestimated wind speeds over South Bay, which would ultimately underestimate both the $k$ value, and thus, the CO$_2$ flux. The results of the analysis of in situ and modeled $k_{600}$ values determined that the best fitting $k_{600}$ models were wind speed parameterizations that were derived over the ocean and had a zero intercept. In estuaries, the most appropriate $k_{600}$ model is likely site-specific and should be determined using in situ $k_{600}$ data when possible, or alternatively, by thoroughly evaluating site
characteristics such as water depth, current velocity, and wind speed. We applied the regional wind speed analysis and the $k_{600}$ model analysis and derived air-water hourly CO$_2$ fluxes during the peak seagrass density period (early July) for three years. The magnitude and direction of the CO$_2$ flux varied on hourly and diurnal time scales, but the daily average showed that South Bay was in metabolic balance and was a minor CO$_2$ sink. We compared the fair-weather CO$_2$ fluxes to the full CO$_2$ flux dataset, which included the storm period, and found that the meadow was a significantly larger CO$_2$ sink during the fair-weather period. During the fair-weather period, multiple components of the flux equation (water column pCO$_2$ and temperature) followed predictable patterns. During the storm period, these components deviated from their predictable patterns and largely drove the increase in CO$_2$ flux.

6 References


Chapter 3: Seasonal trends in air-water CO₂, CH₄, and N₂O fluxes over a temperate seagrass meadow

Conducted with support from: Peter Berg and Bongkeun Song.

Abstract

Blue carbon ecosystems such as seagrass meadows are widely regarded as carbon sinks that can help mitigate the effects of climate change. However, the carbon balance in seagrass meadows is complex and can, for example, be affected by emissions of greenhouse gases such as carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O). Although the importance of measuring these fluxes is widely recognized, there is still a lack of reliable flux data due to internal site variability and low sampling frequencies, especially for temperate seagrass meadows. We addressed these knowledge gaps by measuring air-water CO₂, CH₄, and N₂O fluxes throughout the seagrass growing season over a temperate coastal lagoon with a substrate dominated by a Zostera marina meadow (South Bay, Virginia, USA). Here, we report CO₂, CH₄, and N₂O fluxes derived during the early seagrass growing season (April-June), peak seagrass density (July), seagrass senescence (August) and post-senescence (October) across two years. To constrain the variability attributed to the different flux pathways, we measured dissolved CO₂ and CH₄ fluxes continuously with autonomous underwater sensors, and ebullitive and plant-mediated fluxes of CH₄ and N₂O using floating bubble traps. Dissolved N₂O fluxes were estimated from point measurements of water column N₂O concentrations. South Bay was a consistent source of total (dissolved and ebullitive) CH₄ to the atmosphere, but the magnitude varied both seasonally and between emission pathways. We observed distinctly elevated CO₂ and CH₄ emissions during two storm periods. South Bay was a consistent sink of total N₂O throughout the growing season. We observed an overall uptake of CO₂-equivalent gases by South Bay during the early growing season (-0.5 ± 0.03 g CO₂-e m⁻² d⁻¹, mean ± standard deviation) and outgassing during seagrass senescence (0.9 ± 0.1 g CO₂-e m⁻² d⁻¹) and post-senescence (0.8 ± 0.04 g CO₂-e m⁻² d⁻¹). During peak seagrass density, South Bay was in metabolic balance and fluxes were not significantly different from zero (0.002 ± 0.1 g CO₂-e m⁻² d⁻¹). Summarized over the whole study period (April – October), South Bay is a small source of CO₂-equivalent gases (0.24 ± 0.1 g CO₂-e m⁻² d⁻¹).
1 Introduction

Carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) are greenhouse gases that contribute significantly to atmospheric warming (IPCC 2023). Coastal vegetated ecosystems, which are known to sequester carbon through primary production and store carbon through sediment deposition and organic matter burial, emit or take up these greenhouse gases (Marbà et al. 2015; Serrano et al. 2018; Garcías-Bonet et al. 2019; Rosentreter et al. 2023). Recent studies suggest that coastal vegetated ecosystems are an overall net sink of CO$_2$-equivalent (CO$_2$-e) gases (CO$_2$, CH$_4$, and N$_2$O) on the global scale, but that the total CO$_2$-e uptake is offset by up to 40% by air-water CO$_2$-e emissions (Al-Haj and Fulweiler 2020; Rosentreter et al. 2021a, 2023). However, local measurements of CO$_2$, CH$_4$, and N$_2$O fluxes are known to vary spatially and temporally, and flux estimates are particularly uncertain in seagrass meadows (Wells et al. 2018; Al-Haj and Fulweiler 2020; Asplund et al. 2022). Increasing evidence shows that seagrass meadows are smaller CH$_4$ sources that salt marshes and mangroves, and that temperate seagrass meadows may be sinks of N$_2$O (Al-Haj et al. 2022; Ollivier et al. 2022; Rosentreter et al. 2023).

The dynamics of CH$_4$ production, consumption, and emissions from seagrass meadows are complex. In anaerobic sediments, the majority of CH$_4$ is produced through methanogenesis, a form of anaerobic respiration in which oxidized carbon such as CO$_2$ is used as a terminal electron acceptor. In seagrass meadows, methanogenesis primarily occurs via methylotrophic methanogenesis, a non-competitive form of methanogenesis in which CH$_4$ is formed from the degradation of methylated compounds such as dimethylamine (DMA), dimethyl sulfide (DMS), and methanol (Schorn et al. 2022; Brodersen et al. 2024). Hydrogenotrophic methanogenesis, in which CO$_2$ is reduced to CH$_4$ via H$_2$, and acetoclastic methanogenesis, can also occur in seagrass meadow sediments when there is enough organic matter to meet the demands of sulfate-reducing bacteria (SRBs), but SRBs routinely outcompete methanogens for hydrogen and acetate substrates (Berghuis et al. 2019; Rosentreter et al. 2021b; Schorn et al. 2022). The CH$_4$ that is produced may also be removed from the sediments via anaerobic oxidation with sulfate (SO$_4^{2-}$) or other terminal electron acceptors (Whiticar 2020). The CH$_4$ that is not removed is usually transported into the water column via diffusion. The role of oxic CH$_4$ production and consumption in the water column is unclear in coastal environments, but understood to be important (Schubert et al. 2006; Bogard et al. 2014; Tang et al. 2014; Günthel et al. 2020).
is evidence that CH$_4$ is produced in the oxic water column by methanogenesis and by processes such as the bacterial degradation of dimethylsulfoniopropionate (DMSP) precursors from phytoplankton metabolism (Tang et al. 2014; Upstill-Goddard and Barnes 2016; Matoušů et al. 2017). (Damm et al. 2010; Whiticar 2020). A fraction of dissolved CH$_4$ can be removed by methanotrophic bacteria via aerobic oxidation of O$_2$ (Tang et al. 2014; Upstill-Goddard and Barnes 2016; Matoušů et al. 2017).

CH$_4$ can also be emitted from the sediment to the water column as bubbles when dissolved CH$_4$ becomes supersaturated in sediment porewater, when current-induced bottom shear stress increases, or when water pressure increases at low water depths (Joyce and Jewell 2003; McGinnis et al. 2006; Long and Nicholson 2018). This process, known as the ebullitive flux, allows CH$_4$ to escape the microbial oxidation filter as bubbles rise and exchange gases with the water column before escaping to the atmosphere (Whiticar 2020). The air-water ebullitive flux from the water column over seagrass meadows is highly uncertain, but increasing evidence suggests that the relative contribution of the ebullitive flux is an important component of the total flux (Oreska et al. 2020; Al-Haj et al. 2022; Rosentreter et al. 2023).

Seagrasses may also play a direct role in CH$_4$ production-consumption dynamics. Plant-mediated transport, in which gases are transported between the sediments and water column through the plant aerenchyma, is an important CH$_4$ production pathway in other vegetated ecosystems that has not been studied in seagrass meadows (Al-Haj and Fulweiler 2020; Schorn et al. 2022; Vroom et al. 2022). When seagrasses are submerged, plant-mediated transport would likely occur as ebullition from the seagrass blades. Bubble formation on seagrasses has been observed as a result of internal pressurization in the aerenchyma, external pressurization from low water depths, or O$_2$ supersaturation in the water column (Wilson et al. 2012; Long et al. 2020). Ebullition of O$_2$ has been measured from these bubbles, and was found to be a significant component of the whole system O$_2$ flux (Long et al. 2020). Potential CH$_4$ ebullitive fluxes from the sediment-water and plant-water interfaces are not captured by dissolved gas measurement techniques, and they may have a significant effect on the balance of air-water greenhouse gases over systems such as seagrass meadows.
Plant-mediated transport and ebullition have also been suggested as potentially significant pathways of N$_2$O exchange in seagrass meadows, but these pathways are understudied (Al-Haj et al. 2022). N$_2$O is mainly produced through nitrification and incomplete denitrification, and consumed via complete denitrification when dissolved inorganic nitrogen contents are low (Bange et al. 1996; Murray et al. 2015). Water column O$_2$ supersaturation can stimulate nitrification, and “hotspots” of denitrification have been observed where seagrass is present (De Wilde and De Bie 2000; Aoki and McGlathery 2018). Additionally, denitrification may be stimulated when methanotrophs consume O$_2$ during aerobic CH$_4$ oxidation (Whiticar 2020).

Here, we report air-water CO$_2$, CH$_4$, and N$_2$O fluxes measured over a temperate seagrass meadow during four distinct time periods throughout the seagrass growing season. Hourly dissolved CO$_2$ and CH$_4$ fluxes were derived based on in situ measurements over multiple diurnal cycles, and point N$_2$O fluxes were derived from daytime water samples. These fluxes were compared to ebullitive CH$_4$ and N$_2$O fluxes derived on daily time scales. Here, the ebullitive flux is considered representative of the sediment-water column ebullitive flux and the potential plant-mediated ebullitive flux.

2 Methods

2.1 Study site

The Virginia Coast Reserve (VCR) is a system of coastal lagoons and barrier islands located between the Delmarva peninsula and the Atlantic Ocean. This study was conducted in South Bay, a shallow coastal lagoon that is bordered by a barrier island (Wreck Island) to the east and is connected to the Atlantic Ocean by two inlets to the north and south of the island (Fig. 1a). South Bay has a mean water depth of 1.0 m below sea level and a tidal range of 1.2 m (Reidenbach and Thomas 2018). Wind-driven waves are the predominant forcing on sediment suspension in South Bay (Lawson et al. 2007). Southerly winds are dominant in the summer and northerly winds occur in the winter (Fagherazzi and Wiberg 2009). The water column is well-mixed with no significant stratification (Berger et al. 2020).
Fig. 1(a). Our study site was located in central South Bay (denoted as “SB152”), a shallow coastal bay in the Virginia Coast Reserve. The Virginia Coast Reserve is located between the Eastern Shore of Virginia and the Atlantic Ocean on the east coast of the United States. (b) Autonomous underwater sensors and (c) floating bubble traps deployed at the site. Map created with R Studio (v4.3.2, R Core Team, 2023) with the “leaflet” package (Cheng et al. 2024).

The benthic environment is South Bay is dominated by a restored eelgrass (*Zostera marina*) meadow. The South Bay seagrass meadow is in metabolic balance on annual time scales (Berger et al. 2020). The VCR is located at the southern geographical limit of the temperature range for *Z. marina* and as a result the seagrass meadows frequently experience heat stress (Aoki et al. 2020; Berger et al 2020). Seagrass densities increase from late spring to early summer, and peak-summer shoot densities (≥400 shoots m$^{-2}$) typically occur in June or July (Oreska et al. 2017; Zhu et al. 2021). Senescence, a seasonal loss of seagrass biomass, occurs in August due to high water temperatures (Orth and Moore 1986; Rheuban et al. 2014a). After senescence,
seagrass shoot densities increase slightly before gradually decreasing during the fall and winter to minimum densities of 50-100 shoots m$^{-2}$ (Reidenbach and Thomas 2018; Zhu et al. 2021).

2.2 Sampling design

We conducted four deployments per year. In 2022, the deployments occurred from June 1$^{st}$-7$^{th}$, July 6$^{th}$-11$^{th}$, August 16$^{th}$-22$^{nd}$, and October 6$^{th}$-12$^{th}$. In 2023, the deployments occurred April 11$^{th}$-19$^{th}$, July 4$^{th}$-11$^{th}$, August 9$^{th}$-14$^{th}$, and October 5$^{th}$-11$^{th}$. Due to instrumentation issues, the fluxes measured during each deployment varied. Continuous dissolved CO$_2$ fluxes and ebullitive CH$_4$ fluxes were derived during all deployments except June 2022, while continuous dissolved CH$_4$ fluxes were derived during all 2023 deployments. Ebulitive N$_2$O fluxes and point estimates of dissolved N$_2$O fluxes were derived during all 2022 deployments.

During all deployments, several environmental variables were measured at 1-minute intervals. Water column temperatures and O$_2$ concentrations were measured at the average seagrass canopy height (30 cm above the sediment surface) with stable O$_2$ optodes (miniDOT, PME). Photosynthetically active radiation (PAR) at the average seagrass canopy height was measured with a LI-192 underwater quantum sensor (miniPAR, PME). Wind speed and direction were measured with a compact anemometer (WindLog, Rainwise Windlogger) that was mounted on a PVC pole approximately 5 m above the sediment surface. Water depth was measured with a water level data logger (HOBO U20-L, Onset Brands), and used to scale wind speed measurements to 10 m ($U_{10}$) following methods described in detail in Granville et al. (in prep) (Chapter 2). Salinity was measured continuously in June and July of 2022 with a pH sensor (SeapHox V2, Seabird Scientific) (LaRoche et al., in prep), and in July, August, and October of 2023 with a salt water conductivity logger (HOBO U24, Onset Brands). Continuous salinity data were not available in August 2022, October 2022, and April 2023, so point salinity measurements were taken with a conductivity meter every 1-2 days (YSI Pro30, Xylem). Hourly barometric pressure data were collected from a station located on a barrier island within the VCR, Hog Island (Porter and Williams 2024). The average seagrass shoot density (shoots m$^{-2}$) was obtained for each deployment by counting seagrass shoots by hand within 5-10 replicate 0.25 m$^2$ quadrats thrown randomly within the sampling site (McGlathery et al. 2012; Berger et al. 2020). To increase the robustness of our growing season seagrass density dataset, average
seagrass shoot density counts obtained by the same methodology from May 27th, 2021, to August 8th, 2021 were included in our analysis.

2.3 Storm periods

Storm periods that occurred during each deployment period were identified using wind speed and water level thresholds. Previous work at the VCR has identified major storm surge events as periods when wind speeds were >11 m s\(^{-1}\) and when the difference between measured and predicted water levels was > 0.2 m (Castagno et al., 2018; Zhu and Wiberg, 2022). We used a more conservative wind speed threshold, \(U_{10} > 8.0\) m s\(^{-1}\), which was determined by evaluating wind speeds before, during, and after two known storms that occurred at the site that are discussed below (see Section 3.2). This lower threshold seeks to account for smaller storm events that have been shown to have an impact on flux magnitudes (Granville et al. in prep) (Chapter 2).

We collected astronomical tide predictions and hourly measured water levels from the NOAA NDBC station at Wachapreague for all deployments. Time periods when wind speeds were > 8.0 m s\(^{-1}\) and the difference between measured and predicted water levels was > 0.2 m were classified as storm periods. Time periods where one or both variables were below the respective threshold were classified as “fair-weather” periods.

To determine the likelihood of storms affecting air-water gas fluxes throughout the year, we assessed the amount of time this threshold was crossed in 2022 and 2023 using tide and water level data from Wachapreague and wind speed data from the NOAA NDBC station Rappahannock, which was shown to be representative of wind speeds measured over South Bay (Granville et al. in prep) (Chapter 2). The likelihood of storms was determined for each month during the deployment, and the percentage of storm periods and fair-weather periods per month was used to scale our total monthly flux estimates so that the storm periods were not overrepresented in the total dataset.

2.4 Dissolved gas fluxes

Continuous dissolved CO\(_2\) and CH\(_4\) fluxes were derived following methods described in detail in Granville et al. (in prep) (Chapter 2, Section 2.5). Briefly, standard mass transfer equations were used to estimate 1-minute dissolved CO\(_2\) fluxes (Chapter 2, Eqn. 5) and CH\(_4\)
fluxes (Chapter 2, Eqn. 1). Continuous water column pCO$_2$ and pCH$_4$ were measured with autonomous underwater sensors (miniCO$_2$ and miniCH$_4$, Pro Oceanus Systems Inc.). Atmospheric pCO$_2$ was derived from atmospheric xCO$_2$ mole fractions collected by NOAA CarbonTracker Near-Real Time (CT-NRT.v2023-5, Jacobson et al. 2023) and barometric pressure collected by the VCR-LTER (Porter and Williams 2024). CarbonTracker Near-Real Time data were unavailable for all CH$_4$ deployments and for the July, August, and October 2023 CO$_2$ deployments. For these deployments, monthly marine surface averages of xCO$_2$ and xCH$_4$ mole fractions were used (NOAA GML). The gas transfer velocity was derived from in situ $U_{10}$ using Nightingale et al. (2000), an empirical relationship where wind speed is the primary driver that was determined to be a best fit at our site (Chapter 2), and scaled to CO$_2$ and CH$_4$ using the Schmidt numbers of each gas (Wanninkhof 2014). Dissolved CO$_2$ and CH$_4$ fluxes were summarized to hourly values for analysis. Dissolved N$_2$O fluxes were not measured continuously because, to our knowledge, a sensor that could continuously measure the low levels of pN$_2$O at our site was not available.

In June, July, August, and October of 2022, water column CH$_4$ and N$_2$O concentrations were assessed via the headspace equilibration technique to obtain point estimates of air-water CH$_4$ and N$_2$O fluxes (Hudson 2004; Helton et al. 2014; Borges et al. 2018). Briefly, we collected 80-100 ml of water in 120-ml glass serum bottles that had previously been crimp-capped, weighed, and evacuated of air (Helton et al. 2014). The serum bottles were held approximately 1-5 cm below the water surface and a 20 g needle was inserted through the septum to collect the water samples (Helton et al. 2014). Samples were poisoned with mercuric chloride (HgCl$_2$) to prevent microbial activity and stored septum-side-down, underwater, and on ice until analysis. At the time of analysis, the serum bottles were injected with a known volume of helium and shaken vigorously before a sample volume of gas was extracted. The gas samples were analyzed for CH$_4$ on a gas chromatograph fitted with a flame ionization detector (GC-FID) that used ultra-high purity He as the carrier gas (7890A GC, Agilent), and for N$_2$O on a gas chromatograph fitted with an electron capture detector (GC-ECD) that used a P5 mixture as the make-up gas (GC-8A Series, Shimadzu Corporation). The limit of detection (LOD) for CH$_4$ and N$_2$O was determined daily from known standards and gas concentrations were determined from the known standards compared to the peak areas of the samples. Samples were run in triplicate and averaged. When the relative percent difference of the samples was greater than 5%, we assessed the triplicates
and removed outliers, assuming a sampling error associated with a syringe or serum bottle leak. The CH$_4$ samples were consistently below the LOD, so they were not analyzed further. For the N$_2$O samples, water column N$_2$O concentrations were derived following Henry’s law, which assumes that the gas components in the serum bottle headspace and liquid phase are in equilibrium (Hudson 2004; Helton et al. 2014). N$_2$O fluxes were derived following the standard procedure for CO$_2$ and N$_2$O (Chapter 2) (Weiss 1974; Weiss and Price 1980; Mu et al. 2014).

2.5 Bubble traps

We designed floating bubble traps to measure ebullitive CH$_4$ and N$_2$O emissions at the air-water interface (Fig. 1c). For this study, we defined ebullitive emissions as emissions of bubbles occurring at both the sediment-water and plant-water interfaces. Nine bubble traps were deployed across three floating foam boards. On each board, three 24-cm diameter funnels were inserted into pre-drilled holes and fitted with a 100 ml syringe with a luer lock tip. The connection between the funnel, board, and syringe was sealed with 3M 5200 marine adhesive sealant and reinforced with strings that connected the funnel and the syringe to the board. The floating boards were deployed in an east-west transect 15 m apart. Each floating board was weighed down with three 5 lb dive weights to keep the boards steady at the air-water interface, and anchored to a 10-m-tall PVC pole with floating polypropylene line. This allowed the bubble traps to rise and fall with changes in water depth.

Multiple bubble trap deployments that were 24-72 h in length occurred during each deployment. At the start of each bubble trap deployment, each bubble trap was fitted with two stopcocks with luer connections to prevent leakage. The air was evacuated from each trap via a syringe connected to the uppermost stopcock. At the end of each bubble trap deployment, the gas volume in the syringes was recorded and a 12 ml gas sample was extracted. The gas samples were injected into 12 ml Exetainers (Labco) that had previously been flushed for 5 minutes with inert He gas (Sturm et al. 2015). The bubble trap samples were analyzed for CH$_4$ on the GC-FID and for N$_2$O on the GC-ECD following the procedure described in the dissolved gas fluxes section. CH$_4$ fluxes were calculated following the equation:

$$F_{CH_{4\text{Bubble}}} = \frac{[CH_{4}] \cdot V}{A \cdot t_d}$$

Eqn.
where $F_{CH4Bubble}$ is the combined ebullitive and plant-mediated CH$_4$ flux ($\mu$mol m$^{-2}$ d$^{-1}$), $[CH_4]$ is the CH$_4$ concentration in the trap ($\mu$mol m$^{-2}$), $V$ is the gas volume in the bubble trap (m$^3$), $A$ is the surface area of the funnel (m$^2$), and $t_d$ is the deployment time in days (Barbosa et al. 2021).

2.6 Data analysis

All analyses were conducted in R Studio version 4.3.2, primarily with the ‘tidyverse’ package (R Core Team 2023). Simple linear regressions were conducted to determine correlations between dissolved CO$_2$, CH$_4$, and N$_2$O fluxes and their potential environmental drivers. Bubble-mediated fluxes were grouped by bubble trap deployment and then by month. Pairwise Wilcoxon rank-sum tests with a Benjamin-Hochberg correction were conducted to determine significant differences in bubble-mediated CH$_4$ fluxes between months. CO$_2$-equivalent fluxes of dissolved and bubble-mediated CH$_4$ and N$_2$O were derived by converting CH$_4$ and N$_2$O fluxes on the 100-year time horizon. We used global warming potentials reported by IPCC AR6 and used the non-fossil origin potential for CH$_4$. On the 100-year time horizon, CH$_4$ and N$_2$O fluxes have a global warming potential equivalent to 27.2 kg CO$_2$ and 273 kg CO$_2$, respectively (IPCC 2023). For reference, on the 20-year time horizon, CH$_4$ fluxes have a global warming potential equivalent to 80.3 kg CO$_2$ and the global warming potential of N$_2$O fluxes remains equivalent to 273 kg CO$_2$ (IPCC 2023).

3 Results

3.1 Environmental variables

The average wind speeds, dissolved O$_2$ concentrations, and atmospheric pressures varied significantly between deployments and no clear trends were observed on the monthly scale (Table 1). Water column temperature varied significantly between all deployments, except for October 2022 and April 2023 (Table 1). Seagrass shoot densities varied inter-annually and throughout the growing season, peaking annually in early July (Fig. 2). Seagrass shoot densities significantly increased with increasing water temperatures ($R^2 = 0.91$, $p = 0.0015$).
Table 1. Each deployment was assigned a period: growing season (GS), peak seagrass density (PD), senescence (SC), or post-senescence (PS). Average values (mean ± standard deviation) are reported each deployment for wind speed scaled to a height of 10 m ($U_{10}$, m s$^{-1}$), depth (m), photosynthetically active radiation (PAR, µmol m$^{-2}$ s$^{-1}$), water column temperature (°C), salinity (ppt), seagrass shoot density (shoots, shoots m$^{-2}$) and atmospheric pressure ($P_{atm}$, mbar).

<table>
<thead>
<tr>
<th>Period</th>
<th>$U_{10}$</th>
<th>Depth</th>
<th>PAR</th>
<th>$O_2$</th>
<th>Temperature</th>
<th>Salinity</th>
<th>Shoots</th>
<th>$P_{atm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2022</td>
<td>5.36 ± 2.2</td>
<td>1.32 ± 0.3</td>
<td>224.3 ± 260.8</td>
<td>245.7 ± 50.4</td>
<td>25.6 ± 1.8</td>
<td>31.3 ± 0.3</td>
<td>315 ± 17.3</td>
<td>1012.0 ± 3.9</td>
</tr>
<tr>
<td>July 2022</td>
<td>5.85 ± 2.2</td>
<td>1.16 ± 0.4</td>
<td>176.3 ± 257.0</td>
<td>239.7 ± 70.7</td>
<td>26.1 ± 2.7</td>
<td>30.3 ± 2.0</td>
<td>410 ± 70.6</td>
<td>1013.9 ± 1.5</td>
</tr>
<tr>
<td>Aug. 2022</td>
<td>4.58 ± 2.2</td>
<td>1.22 ± 0.4</td>
<td>196.3 ± 232.2</td>
<td>227.5 ± 64.3</td>
<td>25.7 ± 1.7</td>
<td>31.9 ± 1.3</td>
<td>241 ± 63.8</td>
<td>1016.4 ± 2.5</td>
</tr>
<tr>
<td>Oct. 2022</td>
<td>4.06 ± 2.5</td>
<td>1.03 ± 0.5</td>
<td>209.3 ± 328.6</td>
<td>260.1 ± 30.5</td>
<td>18.1 ± 1.4</td>
<td>31.5 ± 0.4</td>
<td>97.6 ± 26.6</td>
<td>1020.3 ± 3.4</td>
</tr>
<tr>
<td>April 2023</td>
<td>5.09 ± 1.5</td>
<td>1.00 ± 0.4</td>
<td>281.1 ± 374.9</td>
<td>286.2 ± 46.7</td>
<td>17.7 ± 2.2</td>
<td>31.7 ± 0.3</td>
<td>172 ± 24.8</td>
<td>1012.5 ± 5.0</td>
</tr>
<tr>
<td>July 2023</td>
<td>4.04 ± 1.6</td>
<td>1.40 ± 0.5</td>
<td>129.9 ± 164.7</td>
<td>266.4 ± 80.6</td>
<td>28.6 ± 0.9</td>
<td>28.9 ± 2.2</td>
<td>491 ± 61.4</td>
<td>1010.3 ± 2.4</td>
</tr>
<tr>
<td>Aug. 2023</td>
<td>4.86 ± 1.8</td>
<td>1.25 ± 0.4</td>
<td>192.1 ± 283.8</td>
<td>255.4 ± 119.2</td>
<td>28.0 ± 1.7</td>
<td>29.7 ± 0.8</td>
<td>425 ± 43.4</td>
<td>1010.4 ± 2.6</td>
</tr>
<tr>
<td>Oct. 2023</td>
<td>5.28 ± 2.5</td>
<td>1.44 ± 0.3</td>
<td>70.0 ± 142.7</td>
<td>251.6 ± 72.7</td>
<td>20.0 ± 2.3</td>
<td>30.5 ± 0.8</td>
<td>116 ± 8.29</td>
<td>1010.7 ± 3.4</td>
</tr>
</tbody>
</table>
Fig. 2. Seagrass shoot densities measured throughout the growing season were plotted by the month and the day of data collection. Shoot densities were counted biweekly from late May – early August of 2021 and during each deployment in 2022 and 2023.

We defined four time periods throughout the seagrass growing season from existing estimates of *Z. marina* biomass changes and measured seagrass shoot density counts and water temperatures (Fig. 1) (Rheuban et al. 2014b; Reidenbach and Thomas 2018). The early growing season (GS) occurred from April to the end of June. The peak seagrass density period (PS) occurred in early July. The senescence period (SC) occurred from late July through August and represented the annual die-back and sloughing-off of seagrass leaves. The post-senescence period (PS) occurred from September to the end of October (Table 1, Fig. 2). Each deployment was then grouped into one of these time periods (Table 1).

3.2 Storm periods

The field site was primarily affected by two storms during this study. In July 2022, a consecutive storm period occurred from July 9th, 2022, at 17:00 EDT to July 10th, 2022, at 08:00 EDT (Chapter 2). The storm threshold ($U_{10} > 8 \text{ m s}^{-1}$ and storm surge > 0.2 m) was also crossed from 13:00-14:00 on July 9th, from 11:00-14:00 on July 10th, and during the 16:00 hour on July 10th. CO$_2$ fluxes and ebullitive CH$_4$ fluxes were measured during this storm. In October 2023, remnants of post-tropical cyclone Phillipe passed over the study site from October 7th, 2023, at 10:00 EDT to October 8th, 2023, at 03:00 EDT. During the October deployment, the storm threshold was also crossed at 09:00, 10:00, and 19:00 on October 8th. The storm period threshold was further validated by other *in situ* conditions. The maximum wind speed during the October 2023 storm period was $U_{10} = 12.5 \text{ m s}^{-1}$, which was significantly higher than the October 2023 deployment average of $U_{10} = 5.28 \pm 2.5 \text{ m s}^{-1}$ (Table 1). The wind direction shifted from predominantly north/northwest (20-30°) to predominantly west/northwest (290-300°), suggesting that winds were blowing in the anticlockwise direction. Anticlockwise winds are expected for low pressure systems that form in the northern hemisphere. Barometric pressure gradually decreased from 1022.1 mbar at the start of the deployment to 1006.2 mbar during the storm period, indicating the approaching low pressure center of the system. Barometric pressure stabilized around the deployment average (1010.7 ± 3.4 mbar, Table 1) by midnight on October 8th. In addition to the two major storms, the deployment periods were above the storm threshold for eleven hours in August 2022, five hours in April 2023, and two hours in July 2023, but these
hours were not always consecutive. The analysis of annual Rappahannock wind speeds and water levels and tide predictions at Wachapreague showed that storm periods occurred for 13.2% of the total year in 2022 and 16.0% of the total year in 2023.

3.3 Dissolved CO₂ and CH₄ fluxes

South Bay was a consistent sink of atmospheric CO₂ in the early growing season (April) on an hourly scale (Table 2, Fig. 3a). During the peak seagrass density period (July), South Bay was a minor CO₂ sink that was not significantly different from zero (Table 2, Fig. 3a). South Bay was an overall source of CO₂ to the atmosphere during senescence (August) and post-senescence (October) (Table 2, Fig. 3a). During all deployments, the maximum negative CO₂ flux occurred in the afternoon (Fig. 3a). July and August demonstrated a pattern of the maximum positive CO₂ flux occurring around dawn (Fig. 3a). Dawn also corresponded with the minimum negative CO₂ flux in April (Fig. 3a).

Dissolved CH₄ fluxes were always positive, indicating that South Bay was a consistent source of dissolved CH₄ to the atmosphere throughout the growing season. The daily dissolved CH₄ flux decreased significantly from April to July (Table 2, Fig. 2b). Dissolved CH₄ fluxes then increased significantly from July to August, and again from August to October (Table 2, Fig. 2b). Unlike CO₂, dissolved CH₄ fluxes did not exhibit a clear diurnal pattern (Fig. 2). Simple linear regressions revealed that water column pCO₂ and pCH₄ were strongly negatively correlated during and after seagrass senescence (August $R^2 = -0.95$, October $R^2 = -0.95$, $p < 0.05$). pCH₄ was also strongly positively correlated with water column temperatures during these periods (August $R^2 = 0.95$, October $R^2 = 0.97$, $p < 0.05$), but was not correlated with temperature during the early growing season or during peak density.
Fig 3. (a) Full dataset of mean dissolved CO\textsubscript{2} and (b) mean dissolved CH\textsubscript{4} fluxes measured in April, July, August, and October deployments in 2023. Fluxes are represented as the hourly average flux per hour of the day (0-24). Note the different scales for CO\textsubscript{2} and CH\textsubscript{4}. Positive fluxes represent a source to the atmosphere and negative fluxes represent uptake from the atmosphere. Error bars depict standard error.

Table 2. The daily mean ± standard deviation of the dissolved CO\textsubscript{2} fluxes, dissolved CH\textsubscript{4} fluxes, bubble mediated CH\textsubscript{4} fluxes, total CH\textsubscript{4} fluxes (dissolved + bubble mediated) are shown for each full deployment and for the fair-weather dataset which excludes the storm periods (mmol m\textsuperscript{-2} d\textsuperscript{-1}). Total CH\textsubscript{4} fluxes are expressed as CO\textsubscript{2}-equivalent fluxes on a 100-year time horizon (CH\textsubscript{4} CO\textsubscript{2}-e), as well as the combined CO\textsubscript{2} and CH\textsubscript{4} fluxes (Total CO\textsubscript{2}-e) in units of g CO\textsubscript{2} m\textsuperscript{-2} d\textsuperscript{-1}. “July: fair-weather” and “October: fair-weather” depict the July and October datasets when the storm periods were removed in units of mmol m\textsuperscript{-2} d\textsuperscript{-1}.

<table>
<thead>
<tr>
<th>Period</th>
<th>Dissolved CO\textsubscript{2}</th>
<th>Dissolved CH\textsubscript{4}</th>
<th>Bubble-mediated CH\textsubscript{4}</th>
<th>Total CH\textsubscript{4}</th>
<th>CH\textsubscript{4} CO\textsubscript{2}-e</th>
<th>Total CO\textsubscript{2}-e</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full dataset</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>-11.8 ± 6.2</td>
<td>0.1 ± 0.05</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.03</td>
<td>0.1 ± 0.01</td>
<td>-0.5 ± 0.1</td>
</tr>
<tr>
<td>July</td>
<td>-0.4 ± 12.9</td>
<td>0.04 ± 0.02</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.03</td>
<td>0.2 ± 0.01</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>August</td>
<td>12.7 ± 14.4</td>
<td>0.8 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>1.04 ± 0.1</td>
<td>0.5 ± 0.06</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>October</td>
<td>4.5 ± 9.9</td>
<td>2.3 ± 1.6</td>
<td>0.06 ± 0.05</td>
<td>2.4 ± 0.6</td>
<td>1.02 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td><strong>Fair-weather dataset</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July: fair weather</td>
<td>-4.5 ± 5.7</td>
<td>0.038 ± 0.02</td>
<td>0.2 ± 0.07</td>
<td>0.21 ± 0.01</td>
<td>0.1 ± 0.004</td>
<td>-0.1 ± 0.03</td>
</tr>
<tr>
<td>October: fair weather</td>
<td>0.9 ± 5.4</td>
<td>1.5 ± 0.8</td>
<td>0.03 ± 0.02</td>
<td>1.5 ± 0.4</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.04</td>
</tr>
</tbody>
</table>
3.4 Ebullitive CH$_4$ and N$_2$O fluxes

Daily ebullitive CH$_4$ fluxes were variable across months and years, and were most variable during the summer months (Fig. 4a). In both 2022 and 2023, ebullitive CH$_4$ fluxes were smallest in October (Fig. 4a). When the 2022 and 2023 fluxes were averaged, July CH$_4$ fluxes were not significantly different from August fluxes ($p = 0.092$) (Fig 4b), but were significantly greater than April ($p = 0.00047$) and October ($p = 1.0 \times 10^{-7}$).

Daily ebullitive N$_2$O fluxes were significantly smaller than ebullitive CH$_4$ fluxes in all months and exhibited less variability (Fig. 4b). The majority of the variability in the ebullitive N$_2$O fluxes occurred in April. Ebullitive N$_2$O fluxes were smallest in October (Fig. 4b).

![Fig. 4 (a) Bubble-mediated CH$_4$ and (b) N$_2$O fluxes were averaged by day within each deployment month in 2022 and 2023. Here, the bubble-mediated flux includes the ebullitive fluxes across the sediment-water and plant-water interface. Note that CH$_4$ fluxes are shown here in units of mmol m$^{-2}$ d$^{-1}$ and N$_2$O fluxes are shown in units of µmol m$^{-2}$ d$^{-1}$.](image)

3.5 Total CH$_4$ fluxes

The daily dissolved and bubble-mediated CH$_4$ fluxes were binned by month to produce the daily total CH$_4$ flux (Fig. 5). The bubble-mediated flux was 54% of the total CH$_4$ flux in April, 89% in July, 22% in August, and 2.5% in October (Fig. 5). Total CH$_4$ fluxes were not
significantly different in April and July, even though the ratio of dissolved to bubble-mediated fluxes changed between months (Fig. 5).

![Graph showing total daily CH₄ flux by month]

**Fig. 5.** The total daily CH₄ flux was averaged by month. The total CH₄ flux is the sum of the bubble-mediated CH₄ flux and the dissolved CH₄ flux.

### 3.6 N₂O flux estimates

Water column N₂O concentrations were below the detection limit of the gas chromatograph (0.1 ppm N₂O) in all deployments. Atmospheric N₂O concentrations over South Bay were consistently above the detection limit. To estimate maximum possible air-water N₂O fluxes for South Bay, we assigned all water column N₂O concentrations to the detection limit and derived the flux. The estimated dissolved N₂O flux was negative in all months. N₂O fluxes were not significantly different in July and August (-0.005 ± 0.002 mmol m⁻² d⁻¹, mean ± standard deviation) (Fig. 6, Table 3). N₂O fluxes were greatest in June (-0.01 ± 0.002 mmol m⁻² d⁻¹) (Fig. 6, Table 3). Ebullitive N₂O fluxes were negligible in comparison to the dissolved fluxes and comprised < 1% of the total N₂O flux in every deployment (Fig. 4b).
Fig 6. Estimates of the maximum possible dissolved N₂O flux from point samples in June (n = 9), July (n = 6), August (n = 4), and October (n = 9) 2022. A negative flux indicates uptake by the water column. b). Dissolved N₂O concentrations increase with increasing water temperatures.

Table 3. The total (dissolved + ebullitive) N₂O flux is given for each month in mmol m⁻² d⁻¹ (mean ± standard deviation). Each month was also categorized into one of the growing season periods. N₂O fluxes were converted into CO₂-e (g m⁻² d⁻¹) for comparison. Finally, the N₂O CO₂-e fluxes were combined with the CO₂ and CH₄ CO₂-e fluxes from Table 2 by period.

<table>
<thead>
<tr>
<th>Month</th>
<th>Period</th>
<th>N₂O flux</th>
<th>N₂O CO₂-e N₂O</th>
<th>Total CO₂-e by period</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>GS</td>
<td>-0.01 ± 0.002</td>
<td>-0.1 ± 0.02</td>
<td>-0.5 ± 0.03</td>
</tr>
<tr>
<td>July</td>
<td>PD</td>
<td>-0.005 ± 0.002</td>
<td>-0.06 ± 0.03</td>
<td>0.89 ± 0.1</td>
</tr>
<tr>
<td>August</td>
<td>SC</td>
<td>-0.005 ± 0.002</td>
<td>-0.06 ± 0.02</td>
<td>0.07 ± 0.1</td>
</tr>
<tr>
<td>October</td>
<td>PS</td>
<td>-0.007 ± 0.0005</td>
<td>-0.09 ± 0.006</td>
<td>1.1 ± 0.04</td>
</tr>
</tbody>
</table>
3.6 Growing season estimates

Our existing data were used to interpolate average daily fluxes for the entire growing season (April – October). To estimate each gas flux for months during the growing season that were not included in this analysis, a standard linear interpolation was conducted between each existing data point with the function “approx” in the “stats” package. On average, the interpolated flux estimates suggest that South Bay is a modest CO$_2$ sink (-0.34 ± 8.2 mmol CO$_2$ m$^{-2}$ d$^{-1}$), a source of ebullitive CH$_4$ (0.19 ± 0.08 mmol CH$_4$ m$^{-2}$ d$^{-1}$), a source of dissolved CH$_4$ (0.59 ± 0.7 mmol CH$_4$ m$^{-2}$ d$^{-1}$), and a small sink of N$_2$O (-0.007 ± 0.002 mmol N$_2$O m$^{-2}$ d$^{-1}$). In CO$_2$-equivalents, this amounts to 0.24 g CO$_2$-e m$^{-2}$ d$^{-1}$.

3.7 Fair-weather fluxes

The “fair-weather” dataset, comprised of time points where wind speeds and storm surges did not meet the threshold to qualify as storm periods, was compared to the full dataset (Fig. 7, Table 2). In the fair-weather dataset, the magnitude of the CO$_2$ sink increased in July and the magnitude of the CO$_2$ source decreased in October (Table 2). The variability in the flux was reduced for both months (Table 2). The magnitude of the dissolved CH$_4$ flux also decreased in the fair-weather dataset, but the decrease was not significant (Table 2). Bubble-mediated CH$_4$ fluxes significantly decreased during the July 2022 storm period (p = 0.004) and during the October 2023 storm period (p = 0.015), and ebullitive fluxes were greater in the fair-weather dataset as a result (Table 2).
Fig. 7. (a) Fair-weather dataset of mean dissolved CO$_2$ and (b) mean dissolved CH$_4$ fluxes measured in April, July, August, and October deployments in 2023. Positive fluxes represent a source to the atmosphere and negative fluxes represent uptake from the atmosphere. Error bars depict standard error.

4 Discussion

4.1 Overview of in situ fluxes

Air-water gas fluxes varied seasonally over a temperate seagrass meadow located in a shallow lagoonal estuary (South Bay). South Bay was a source of CO$_2$-equivalent gases (CO$_2$-e) during and after seagrass senescence, and was a sink of CO$_2$-e gases in the early growing season. During the peak seagrass density period in July, CO$_2$-e gas fluxes were not significantly different from zero, despite high variations in the flux magnitudes throughout the day (Fig. 3). This gradual shift from a sink to a source of CO$_2$-e gases over time shows the importance of measuring fluxes throughout the seagrass growing season before scaling fluxes up for global accounting projects (Rosentreter et al., 2021b; Saunois et al., 2020). Other temperate seagrass meadows have been reported to be either net sinks or negligible sources of CO$_2$-e gases (Al-Haj et al. 2022; Ollivier et al. 2022). On average, South Bay was a minor source of CO$_2$-equivalent gases during our study period (0.24 g CO$_2$-e m$^{-2}$ d$^{-1}$); however, our study included nighttime and
storm period fluxes that were more representative of overall conditions at our site. Similarly to studies conducted in other coastal vegetated systems, our study did not encompass the wintertime period, in which fluxes are likely to be reduced compared to the growing season (Rosentreter et al., 2023). On the coastal shelf, CO₂ uptake increases during the winter (Dai et al., 2022).

In this study, we defined a storm threshold using established protocols and \textit{in situ} conditions during two storm periods, one in July 2022 and the other in October 2023. We found that dissolved air-water CO₂ and CH₄ fluxes increased significantly during the storm periods due to increases in wind speed and water column gas concentrations, and decreases in temperatures, while ebullitive CH₄ fluxes decreased significantly during storm periods (Fig. 7). These altered water column gas concentrations are likely partially driven by increased sediment resuspension from wind-waves during the storm period, which can facilitate the transport of dissolved gases from the sediment to the water column (Zhu and Wiberg, 2024, 2022). Whitecapping, rainfall, and bubble entrainment likely enhance gas exchange as well during storms (Wanninkhof et al., 2009; Ho et al., 2006). South Bay was above the storm threshold for 13.2 % of the year in 2022 and 16.0 % of the year in 2023, which demonstrates the importance of evaluating fluxes during all potential weather conditions.

4.2 Dissolved CO₂ fluxes

South Bay was a consistent sink of air-water CO₂ fluxes during the early growing season in April (Fig. 3). During the peak seagrass density period in July, we found that South Bay was in metabolic balance and CO₂ fluxes were not significantly different from zero. South Bay shifted to an overall CO₂ source in August during senescence, likely due to increased water column temperatures that have been shown to increase CO₂ outgassing in other seagrass meadows by reducing CO₂ solubility (Fig. 3) (Burkholz et al., 2020; Weiss, 1974). South Bay remained a CO₂ source after senescence, but the magnitude of the source decreased, likely due to decreases in water temperatures, air temperatures, and seagrass biomass (Rheuban et al., 2014b; Reidenbach and Thomas, 2018). Respiration and gross primary production in seagrass meadows peak with seagrass densities, so this result suggests that the air-water CO₂ flux is primarily driven by ecosystem metabolism of the benthos and the water column during this time of year (Berger et al., 2020; Oreska et al., 2018). Additionally, we observed a decrease in PAR in October, which
could be attributed to increased sediment resuspension from the reduced seagrass biomass (Nardin et al., 2018; Zhu et al., 2021).

Our continuous flux methodology may explain why South Bay is a modest CO₂ sink compared to other studies where measurements were only collected during the day (Banerjee et al., 2019; Burkholz et al., 2020). Importantly, South Bay was primarily a CO₂ source during the nighttime hours and primarily a sink CO₂ during the afternoon hours, which suggests that exclusively measuring daytime CO₂ fluxes, especially in the afternoon, will lead to an overestimation of the magnitude of the CO₂ sink (Rosentreter et al., 2023). These results are in agreement with a study conducted over a temperate seagrass meadow in Australia, where a diurnal time series flux was -0.46 ± 0.18 mmol CO₂ m⁻² d⁻¹ and it was determined that daytime spatial sampling overestimated the sink capacity of their meadow by 96% (Ollivier et al., 2022).

4.3 Total CH₄ fluxes

The dissolved and ebullitive CH₄ fluxes exhibited different patterns throughout the growing season (Fig. 5, Fig. 6). In contrast to CO₂, dissolved CH₄ fluxes were low in April and July, increased significantly during senescence, and then peaked during the post-senescence period (Fig. 3). Ebullitive CH₄ flux patterns varied slightly by year, but generally increased throughout the growing season from April to July and then decreased during and after senescence in August and October (Fig. 4a, Fig. 5). Other studies that measured benthic ebullitive CH₄ emissions also found that CH₄ emissions were highest when biomass was greatest (Oreska et al., 2020; Bahlmann et al., 2015). Total CH₄ fluxes increased throughout the growing season, which aligns with the findings of a study that measured fluxes across the air-water interface with cavity ring-down spectrometry and found that CH₄ emissions were highest when biomass was lowest (Fig. 5) (Ollivier et al., 2022). Our mean total CH₄ flux (0.78 mmol CH₄ m⁻² d⁻¹) is within the range of the mean air-water fluxes reported by other studies in seagrass meadows and a recent global synthesis (0.104 ± 0.039 mmol CH₄ m⁻² d⁻¹, mean ± SE) (Eyre et al., 2023; Burkholz et al., 2020). Our total flux was approximately five times greater than the average CH₄ flux measured at our site with benthic chambers (0.137 mmol CH₄ m⁻² d⁻¹), but our ebullitive flux was similar (0.19 ± 0.08 mmol CH₄ m⁻² d⁻¹, mean ± standard deviation) (Oreska et al., 2020).
We observed the highest CH$_4$ emissions during and after seagrass senescence, when seagrasses can often experience heat stress from high temperatures (Berger et al., 2020; Aoki et al., 2021). This stress likely causes seagrasses and other organisms to produce increased amounts of methylated compounds, which then increases the amount of substrate available for methylotrophic methanogenesis to proceed (Schorn et al., 2022; Berghuis et al., 2019). Increased substrate for methylotrophic methanogenesis is likely one reason why dissolved CH$_4$ fluxes significantly increased from July to August. After senescence, O$_2$ produced by seagrass photosynthesis is reduced (Berger et al., 2020), in turn reducing the potential for CH$_4$ to be removed via oxidation in the seagrass rhizospheres or the water column (Ollivier et al., 2022; Rosentreter et al., 2021a; Brodersen et al., 2024). Additionally, methylotrophic methanogenesis can occur in living or dead seagrass tissues, so supply of biodegradable substrate for methanogenesis persists throughout the fall and autumn and leads to increased fluxes during this time (Schorn et al., 2022; Vroom et al., 2022). We did not identify a significant diel pattern in the dissolved CH$_4$ fluxes, but we observed moderately heightened CH$_4$ fluxes overnight in April, July, and August. This may occur because CH$_4$ consumption by methanotrophs living on submerged plants increases during the day, which would reduce available CH$_4$ concentrations in the water column (Fig. 3b) (Vroom et al., 2022).

The diel and seasonal dynamics of methanogenesis and methanotrophy may indirectly influence CO$_2$ fluxes. pCO$_2$ and pCH$_4$ were strongly negatively correlated in August and October. This phenomenon indicates that aerobic CH$_4$ oxidation to CO$_2$ is occurring in the water column above the seagrass meadow, resulting in a removal of some CH$_4$ from the system and potentially increasing CO$_2$ concentrations during and after senescence (Whiticar, 2020). Thus, the enhanced CO$_2$ flux shown in August and October may be a result of reduced CH$_4$ concentrations from CH$_4$ oxidation.

4.4 CH$_4$ ebullition

We found that ebullition was a significant component of the total CH$_4$ flux, contributing 54% of total emissions in April, 89% in July, and 22% during senescence (Fig. 6). Ebullition was a minor component of the total CH$_4$ emissions (2.5%) in October, after senescence. Similarly to lakes, a major percentage of sediment-water column ebullition may occur in the summer because
ebullition increases with increasing temperatures and sedimentation rates (DelSontro et al., 2016; Praetzel et al., 2021). The decrease in the ebullitive flux during and after seagrass senescence also suggests that plant-mediated transport is also an important ebullitive transport pathway (Fig. 6) (Schorn et al., 2022). In lakes, pressure gradients are established in the lacunar system of submerged plants with high photosynthetic activity, such as *Potamogeton amplifolius*, due to O\textsubscript{2} accumulation from photosynthesis (Vroom et al., 2022; Whiticar, 2020). This gradient causes a pressurized flow of CH\textsubscript{4} from the roots to the shoots that follows a diel pattern with photosynthesis and contributes 19-29 % of the total daytime emission of CH\textsubscript{4} from the benthic system (Vroom et al., 2022). Plant-mediated transport of CH\textsubscript{4} has not been explicitly studied in seagrass meadows, but the pathway has been suggested as a potential mechanism for CH\textsubscript{4} emissions by recent studies (Al-Haj et al., 2022; Schorn et al., 2022). Plant-mediated transport of photosynthetic O\textsubscript{2} is known to occur in seagrass meadows because the ebullition of O\textsubscript{2} from *Z. marina* has been observed, primarily during daytime low tides when the pressure differences between the water column and the gases in the seagrass aerenchyma would be greatest (Long et al., 2020). O\textsubscript{2} comprised < 40% of the total gas in the observed bubbles, which suggests that ebullition of other gases, such as CH\textsubscript{4}, is occurring in *Z. marina* meadows (Long et al., 2020). Overall, measuring diffusion, sediment-water column ebullition, and plant-mediated ebullition is important for constraining the total CH\textsubscript{4} flux in seagrass meadows.

4.5 N\textsubscript{2}O fluxes

Similarly to other seagrass meadows, South Bay is a net sink of N\textsubscript{2}O across the air-water interface (Al-Haj et al. 2022; Ollivier et al. 2022). The concept of seagrass meadows as sinks of N\textsubscript{2}O on the global scale is relatively new and is contrary to mangroves and salt marshes, which are sources of N\textsubscript{2}O (Rosentreter et al., 2021b; Eyre et al., 2023). Previous studies have shown that the benthos in South Bay emits N\textsubscript{2}O, as sediment-water column N\textsubscript{2}O fluxes measured with benthic chambers were 3.65 μmol m\textsuperscript{-2} d\textsuperscript{-1} (Oreska et al., 2020). Average air-water N\textsubscript{2}O fluxes measured by this study were -7.0 ± 2.0 μmol m\textsuperscript{-2} d\textsuperscript{-1}, which is about five times larger than the global average for *Z. marina* reported by a recent synthesis (Fig. 4b, Fig. 6, Table 3) (Eyre et al., 2023). It is likely that the N\textsubscript{2}O produced by the benthic environment is removed from the system through burial, retention in biomass, and denitrification (Aoki et al., 2020). In other oligotrophic seagrass meadows and mangrove forests, N\textsubscript{2}O consumption in the water column has been
observed when NO$_3^-$ concentrations are below 5 µmol L$^{-1}$ (Murray et al., 2020; Wells et al., 2018). We identified air-water ebulitive N$_2$O emissions in this study, but they were consistently small (approximately 5.0 * 10$^{-3}$ µmol m$^{-2}$ d$^{-1}$) and had a negligible effect on the total N$_2$O uptake (Fig. 5) (Al-Haj et al., 2022).

The negative N$_2$O fluxes observed here are an important consideration for greenhouse gas accounting. Although the N$_2$O flux data in this study were limited by available methodologies, N$_2$O uptake offset CH$_4$ emissions by 100% in the early growing season, 40% during peak seagrass density, 18% during seagrass senescence, and 13% during post-senescence (Table 3). Our dissolved N$_2$O flux measurements were obtained from point samples collected during the day and as a result, do not capture potential diel variations in the dissolved N$_2$O flux. However, the N$_2$O percent saturation in the water column was lower overnight compared to the day in a similar system, suggesting that the magnitude of the negative N$_2$O flux may increase overnight in South Bay, and that our daytime fluxes may be an underestimate of the total negative flux (Ollivier et al., 2022). In the future, more measurements of nighttime and wintertime dissolved N$_2$O fluxes would help establish a clearer picture of the potential for N$_2$O uptake to offset CH$_4$ emissions in temperate seagrass meadows.

5 Conclusions

Overall, our results show the importance of evaluating local-scale, in situ fluxes of CO$_2$, CH$_4$, and N$_2$O on diurnal and seasonal scales. South Bay was primarily a source of dissolved CO$_2$ overnight in all months except April, and dissolved CH$_4$ fluxes were highest overnight in all months except October (Fig. 3). Thus, measuring daytime fluxes exclusively would have underestimated the emission of CO$_2$-equivalent gases from our site. Throughout the growing season, CH$_4$ was a consistent source to the atmosphere and N$_2$O was a consistent sink, while CO$_2$ shifted from a sink to a source throughout the growing season (Fig. 3, Fig. 5, Fig. 6). In April, negative CO$_2$ and N$_2$O fluxes were greater than CH$_4$ emissions and South Bay was a net CO$_2$-e sink. In July, South Bay was in metabolic balance and net CO$_2$-e was not significantly different from zero. During seagrass senescence and post-senescence, South Bay was a source of CO$_2$-e gases that was partially offset by negative N$_2$O fluxes. These shifts in the overall CO$_2$-e flux throughout the growing season are critical to consider when scaling fluxes for global estimations.
6 Literature Cited


R Core Team: R: A Language and Environment for Statistical Computing, 2023.


Chapter 4: At the intersection of science and education: The process of scientists and educators co-developing authentic learning experiences for K-16 students

This chapter was published in Limnology and Oceanography: Bulletin, a magazine-style journal, after receiving an invitation to contribute from the journal editors.


Abstract

Students lose interest in science as they progress from elementary to high school. There is a need for authentic, place-based science learning experiences that can increase students’ interest in science. Scientists have unique skillsets that can complement the work of educators to create exciting experiences that are grounded in pedagogy and science practices. As scientists and educators, we co-developed a lesson plan for high school students on the Eastern Shore of Virginia, a historically-underserved coastal area, that demonstrated realistic scientific practices in students’ local estuaries. After implementation of the lesson plan, we observed that students had a deeper understanding of ecosystem processes compared to their peers who had not been involved, were enthusiastic about sharing their experiences, and had a more well-rounded ability to think like a scientist than before the lesson plan. We share our experiences and five best practices that can serve as a framework for scientists and educators who are motivated to do similar work. Through collaboration, scientists and educators have the potential to bolster student science identities and increase student participation in future scientific endeavors.

1 Student views of science

Picture a scientist. Who do you see? From the time students enter kindergarten around age five to the time they graduate from twelfth grade around age eighteen, these students, hereafter referred to as K-12 students, are unlikely to identify themselves as people who can, or should, be scientists (Kang et al. 2019). This lack of “science identity” may be because classroom science experiences represent science as a difficult subject focused on memorizing complex facts and deep knowledge (Wade-Jaimes et al. 2023). Classroom experiences often do
not relate science to the skills that scientists typically consider most important, such as
developing questions through logical thinking and curiosity, learning about and finding solutions
to problems, and collaborating with others (Wade-Jaimes et al. 2023). This disconnect likely
contributes not only to a lack of student science identity, but also to the decrease in science
identity as students’ progress from primary to secondary school (Miller et al. 2018; Wade-Jaimes
et al. 2023). This lack of student science identity was analyzed by a meta-analysis of the Draw-
A-Scientist studies, in which K-12 students in the U.S. were asked to draw a picture of a scientist
(Miller et al. 2018). Older students tended to draw scientists indoors with laboratory coats and
eyeglasses, which was likely due to the stereotypical conceptions of scientists that students
learned in and out of the classroom. Further, these studies demonstrated that a strong science
identity was particularly difficult for girls and students of color to develop and maintain. 79% of
all students drew a Caucasian scientist (Miller et al. 2018). The percentage of girls who drew
male scientists increased from 30% at the age of six to 75% at the age of sixteen, whereas the
percentage of boys who drew male scientists increased from 83% to 98% over the same age
range (Miller et al. 2018).

Students who were able to develop and maintain a strong science identity were more
likely to identify with science, technology, engineering, and math (STEM) careers (Kang et al.
2019). A lack of strong science identity, especially in girls and students of color, may contribute
to the broader lack of diversity and representation in STEM fields in the U.S, including marine
and coastal sciences (Fry et al. 2021; Wade-Jaimes et al. 2023). From 1973-2016, less than 8% of
ocean science Ph.D. degrees in the U.S. were awarded to students who identified as American
Indian or Alaska Native, Black or African American, and Hispanic or Latino (Bernard and
Cooperdock 2018). From 2010-2019, the combined number of undergraduate and graduate
degrees in marine sciences awarded to Black or African American and Hispanic students in the
U.S. was less than 1% of the total marine science degrees awarded (Harris et al. 2022).

Representation in the STEM educational system is correlated with diversity of the U.S.
STEM workforce (Fry et al. 2021). Black or African American and Hispanic workers remain
underrepresented in STEM fields when compared to their percentages in the total U.S.
population, while the representation of women varies by field (Fry et al. 2021). According to the
UNESCO Institute for Statistics (UIS), although women now earn a majority of both
undergraduate and advanced degrees, they make up less than 30% of the research and development workforce worldwide (https://uis.unesco.org/en/topic/women-science, last accessed Nov. 30, 2023). Increasing diversity and representation in STEM fields is important for continued scientific advancement, but the path to doing so is long, complex, and requires multiple approaches (Bernard and Cooperdock 2018; Fry et al. 2021). Providing support for students to develop and maintain science identities during both their K-12 and undergraduate educations (grades 13-16, hereafter referred to together as K-16) may help increase participation in science fields (Kang et al. 2019; Fry et al. 2021).

Scientists can contribute to this work. While authentic science activities done in classrooms can facilitate a positive change in student attitudes towards science, many teachers are not trained to develop these activities (Wade-Jaimes et al. 2023). As scientists, we have unique skills that can be leveraged to support student science identity and increase their environmental literacy. As environmental scientists, we also tend to have a deep understanding of our focal research areas and research sites that can be shared to benefit the local community. When we work together with educators, we can co-develop authentic science learning experiences for students that accurately reflect the experiences and identities of scientists. This is an emerging field of research, but there is evidence to suggest that experiences such as these can positively impact student science identities and within and beyond the classroom (Kang et al. 2019; Wade-Jaimes et al. 2023).

We - a high school ocean sciences teacher, a marine science graduate student, and our collaborators - co-developed a lesson plan for high school students based on the coastal ecosystems of Virginia (VA). Our goals were to: 1) demonstrate the process scientists follow during research projects from start to finish, 2) deepen students’ scientific understanding of local ecosystems, 3) increase students’ science identity in a historically-underserved area, and 4) increase data literacy by guiding students through the use of publicly-available data and resources. Our resulting lesson plan was implemented in a high school classroom in 2022 and can be accessed online (https://doi.org/10.6084/m9.figshare.24738627.v1, last accessed Dec. 5, 2023). Here, we share insights from our collaboration to offer our view on best practices for others venturing into the co-production of authentic science learning experiences for K-16 students (Fig. 1).
2 Responding to local community needs

Coastal and shoreline communities in the U.S. tend to be more diverse in their ethnic and racial identities than non-coastal areas, and are also more likely to be described as socially vulnerable, overburdened, and underserved (Harris et al. 2022). Our team works and does research on the Eastern Shore of Virginia (ESVA), a coastal community located on the Delmarva peninsula and separated from mainland VA by the Chesapeake Bay. To the east of the ESVA are the shallow lagoons that comprise the longest stretch of coastal wilderness on the east coast of the U.S, hereafter referred to as the Virginia Coast Reserve (VCR). When compared to the state of VA, the ESVA has a higher percentage of persons in poverty, a lower median household income, and a smaller percentage of high school and college graduates (https://www.census.gov/quickfacts/, last accessed Oct. 30, 2023). In 2022, the ESVA had one of the lowest environmental justice scores across the Chesapeake Bay watershed, indicating a high social vulnerability, environmental burden, and health vulnerability (UMCES 2023).

ESVA locals have a unique cultural heritage and relationship to both the Chesapeake Bay and the VCR, locally known as the ‘seaside’. Watermen, waterwomen, fishers, and aquacultural farmers have worked on the ESVA for generations. As scientists working on the ESVA, we wanted to develop a learning experience that expanded on this knowledge in a scientific context, while prioritizing the needs of students on the ESVA.

3 Best practices for developing authentic science learning experiences

Developing an authentic science learning experience for students can seem daunting. Fortunately, there are many tools and structures available to support this endeavor. Here, we share five best practices that helped us navigate and simplify the process, as well as several examples from our own work (Fig. 1).
We identified five best practices for scientists and educators to use to co-develop authentic science learning experiences for K-16 students. Here, they are divided between the pre-planning and co-development stages of our work.

3.1 Take a scholarly approach to teaching and learning

Before reaching out to a teacher, the scientists reviewed the scholarly literature on student science identity and on pedagogy, or the theory and practice of teaching. This practice added nuance to our understanding of student learning, and provided context for how evidence-based educational practices can be used to teach authentic and impactful science (Alegado and Lewis 2018; Fick and Arias 2019; Kang et al. 2019; Wade-Jaimes et al. 2023). We also sought other available resources, including a short course developed by the University of Virginia School of Education and the Coastal Research Center designed to help scientists improve their mentoring skills by introducing the fundamentals of learning. A similar course is available online for free through the Center for the Integration of Research, Teaching, and Learning (https://www.stemteachingcourse.org/, last accessed Nov. 20, 2023). This initial effort helped us identify the pedagogical practices and support structures that we used to develop the lesson plan,
which are described below as a part of the third best practice. Even scientists without plans to incorporate formal teaching into their careers can benefit from these foundations, as they are relevant to the mentorship and informal learning scenarios that often arise throughout the scientific endeavor.

To identify curriculum concepts relevant to our work, we also reviewed the Virginia K-12 Standards of Learning (SOLs), which are publicly available online and provide standardized expectations for student learning concepts and curriculum framework (https://www.doe.virginia.gov/teaching-learning-assessment/instruction, last accessed Apr. 18, 2022). The science SOLs reflected a transition in classroom science from a focus on the scientific method to a focus on science practices. There were several authentic science practices in the SOLs that we believed scientists could uniquely support: supporting students throughout the scientific process, providing students with opportunities to explore science-related careers and interests, and helping students develop their scientific curiosity, creativity, and collaboration skills. These skills are critical for future generations of scientists and are generally not adequately supported in classroom science (Wade-Jaimes et al. 2023).

Doing this preparation allowed us to create a lesson plan pitch that was tailored specifically towards student and teacher needs on the ESVA. Our understanding of pedagogical practices helped convey a willingness to think about the tools and challenges of teaching science in the classroom, which is becoming increasingly relevant to teachers. Throughout the process of developing the lesson plan, we were able to share a vocabulary and a way of thinking with the teacher we worked with.

3.2 Build a mutually-beneficial partnership between scientists and educators

After we reviewed the scholarly literature and curriculum standards, we reached out to a high school teacher on the ESVA with a lesson plan pitch and an invitation to collaborate. We discussed ways to make the collaboration beneficial to both scientists and educators. As scientists who work at the Virginia Coast Reserve Long-Term Ecological Research site (VCR-LTER), we wanted to communicate our scientific work to students on the ESVA in a way that would support their development both as scientists and as stewards to their local estuaries. As an educator at Broadwater Academy, a private school on the ESVA, the teacher was also interested in providing more hands-on field and data analysis experiences for high school students who were close to
graduation. For these reasons and because high school students typically have diminished science identities, we decided that an upper-level high school class would benefit the most from our efforts (Miller et al. 2018).

The teacher had been teaching an environmental sciences course for high school students in grades 11 and 12 that was dual-enrollment with a nearby college. The dual-enrollment status increased the rigor of the class compared to other high school classes. In previous years, part of this rigor had come from a field experience that required travel and an overnight stay. However, overnight travel had been cancelled due to COVID-19 restrictions. The teacher had a specific need for a replacement activity that would be equally rigorous. As a group, we modified the original pitch from the scientists to meet this need. Our resulting lesson plan had two classroom sessions and a field experience. For the field experience, the class used established scientific methods and existing equipment from the VCR-LTER and Broadwater Academy to collect data at sites in both the Chesapeake Bay and the VCR.

Although our priority was to provide an authentic learning experience for this classroom, we were also interested in making our work accessible for other students on the ESV A and beyond. The lesson plan has been shared online and can be implemented by other scientists and educators or used as a guide for developing similar work ([https://doi.org/10.6084/m9.figshare.24738627.v1](https://doi.org/10.6084/m9.figshare.24738627.v1), last accessed Dec. 5, 2023). Because the lesson plan was developed for a high school class on the cusp of the college level, it can easily be scaled up or down for other high school and college classrooms. The structure of the lesson plan can also be used as a guide to develop a similar local learning experience with two other estuaries or bodies of water. We found that developing a high-quality, local learning experience had the potential to be more inclusive for students who may have experienced cost barriers or other obstacles for trips that required extensive travel. Support from scientific institutions can also make these experiences possible for classrooms and schools who lack the specific materials to conduct such work independently.

A cornerstone in our collaboration was to establish a foundation of trust and recognize our shared and individual areas of interest and expertise. As scientists, our background research on pedagogy and curriculum standards helped us understand student needs, while our own expertise helped us translate the scientific processes outlined in the SOLs into practices. The
teacher had an established relationship with the VCR-LTER and some experience in biology and environmental sciences that enabled him to scale our work for high school students. As a private school educator, he had the capacity and resources to work on this learning experience. Two of our team members were ESVA locals and the others had spent extensive periods of time on the ESVA, which helped us incorporate local perspectives into our work. Working with an experienced teacher with the skills and capacity for lesson design helped make co-development and sharing feasible.

3.3 Use pedagogical practices to develop authentic science learning experiences

Our research into pedagogy helped us identify useful structures for developing authentic science learning experiences. Here, we share two pedagogical practices that were essential to our work: ‘backward design’ and ‘scaffolding’.

‘Backward design’ is a method for building learning experiences that support focal learning outcomes (Michael and Libarkin 2016). In backward design, the learning experience is shaped to provide students with the necessary knowledge, experience, and data to reach an understanding of a topic. After a discussion of potential scientific concepts and practices, the teacher determined that a focal outcome of understanding the drivers of coastal ecosystem health would be best for his class. This framework simultaneously tied our lesson plan to other concepts that students were learning and expanded the class syllabus. Following backward design, we first outlined the claims, or outcomes, about ecosystem health that we wanted students to be able to make at the end of the lesson plan. We then determined the investigative questions and student learning goals using our expected claims, science learning priorities, and the VA SOL priorities.

Once the questions, goals, and outcomes of the lesson plan were established, we continued with this ‘backward design’ approach and worked together to determine the topics that would lead students to our expected claims. We familiarized ourselves with relevant work on ecosystem health in the area by reviewing the Chesapeake Bay Ecosystem Health Report Card, a freely available, annual report that uses publicly-available data to translate economic, ecological, and societal indicators into letter grades (https://ecoreportcard.org/report-cards/chesapeake-bay/, last accessed Nov. 30, 2023). We then reviewed the publicly-available VCR-LTER data catalog to identify variables that overlapped with the report card and that would be reasonable for students to measure (https://www.vcrlter.virginia.edu/cgi-bin/browseData.cgi, last accessed Nov.
Whenever possible, we aligned the environmental variables discussed in the lesson plan with both the report card and the VCR-LTER long-term datasets. Backward design was a useful way to determine what content would help students understand ecosystem health in the Chesapeake Bay and VCR and what content needed to be removed as extraneous or too high-level.

Scaffolding is a classroom teaching technique in which instructors add support for students to enhance their learning. These supports systematically build on prior experiences and knowledge students gain as they learn (Fick and Arias 2019). Less and less support is given over time as students master new concepts or material. The scaffold essentially fades away as the learner becomes skilled enough to no longer need the guidance. Using this technique is a way to ensure the educational content can support students who may be at different learning levels. Throughout the lesson plan development, the teacher provided critical insight into areas where high school students would need additional support through scaffolding to understand specific concepts and ideas.

The lesson plan’s introduction to ecosystem health is an example of how we used scaffolding to model the beginning of the scientific process. First, the students were given a prompt: “What are some ways we might be able to tell if a coastal ecosystem is healthy or not?” After the students individually answered the prompt, the class discussed their answers and the teacher guided the discussion towards measurable variables. In addition to helping the teacher understand individual students’ background knowledge, the practice of sharing out allowed the class to establish a common knowledge baseline. Next, the teacher connected elements of the class discussion to definitions of ecosystem health and environmental drivers. Here, we used the anecdote of a car as a scaffold to explain how environmental variables could drive ecosystem health. In the anecdote, the person driving the car was an analogy for an environmental variable, and the car itself was an analogy for ecosystem health. Just like the driver of the car would determine where the car was going, the environmental variable would determine the health of the ecosystem. The students were already familiar with the role of drivers in cars, so this scaffold was an effective way to explain that measuring environmental drivers was necessary for understanding ecosystem health.
We felt that the learning experience would be more authentic if we guided the students through the process of selecting the environmental drivers that they wanted to measure to evaluate ecosystem health in the Chesapeake Bay and the VCR instead of telling them. To simulate this experience, we grouped the variables we had chosen for the field experience into four driver categories: physical, biogeochemical, biodiversity, and human impacts (Fig. 2a). The purpose of grouping the drivers was to demonstrate that different variables were inter-connected. For the category titles, we had intentionally chosen words that either matched vocabulary the students were familiar with from previous classroom activities or that could be easily explained using the students’ background knowledge. During the lesson plan, the teacher wrote the four categories on the board (Fig. 2b). The class used the introductory exercise and the categories as scaffolds to brainstorm environmental drivers (Fig. 2b). After the brainstorm, the teacher showed the list of variables that the students would actually measure (Fig. 2a). The class talked about what the students had missed, as well as additional variables the students had mentioned that the class would not measure. We used this as an opportunity to discuss how equipment availability could influence a scientists’ methods. These exercises gave students a better opportunity to think critically about their study objective and take ownership of their research methods. More detailed explanations of the exercises outlined here can be found in the lesson plan (https://doi.org/10.6084/m9.figshare.24738627.v1, last accessed Dec. 5, 2023).
3.4 Prioritize sensemaking

If you’ve ever heard a student summarize a science class as ‘we looked at water today’ or ‘we used lasers in science class’, then you’ve likely experienced the outcome of a science experience that did not focus enough on sensemaking. Sensemaking, or the process of interpreting and synthesizing the results, fitting them into a real-world context, and discussing them with classmates, is often not prioritized in traditional science classrooms and labs. Instead, time is spent following protocols to do an experiment. The lack of sensemaking can leave students feeling frustrated with or disconnected from their experience (Wade-Jaimes et al. 2023). Prioritizing sensemaking takes a lot of work and careful support, but when done well, it can cultivate student science identity by helping students understand and remember the ‘findings’ of their investigation. Much of our planning involved adapting scientific practices of data analysis and presentation into activities that were authentic but suitable for high school students. Two of our activities are described below.

In advance of the lesson plan, we created time series plots of publicly-available VCR-LTER water quality data, including water temperature, salinity, nitrate, and dissolved oxygen...
During the field experience, students used VCR-LTER water quality sampling protocols to measure these variables, among others. After the field experience, students drew their datapoints on top of the plots we had made, which allowed us to use the plots as a scaffold for understanding the students’ water quality data in the context of the broader water quality patterns in the VCR. Our water temperature plot from this activity is shown as an example (Fig. 3a). This exercise served as a way for students to put their data in a real-world context and learn about local water quality trends.

We also used the students’ data to demonstrate that qualitative data can be valuable and that science can be communicated in multiple ways. In the field, students had circled adjectives that described inner and outer leaves on seagrass shoots to evaluate how the leaves changed visually over time. We entered all words circled by each student into a free online word cloud generator and generated word clouds for the inner and outer seagrass leaves (Fig. 3b, 3c). Essentially, the students were able to see a summary of what adjectives the class agreed and disagreed on. They were given a chance to discuss and explain their individual reasoning, which allowed them to practice collaboration with their peers and apply appropriate vocabulary. This activity is an example of how an accessible visual representation of data can support student sensemaking.
Fig. 3. Examples of the data analysis done in the sensemaking portion of the lesson plan. (a) Before the lesson plan, the teacher plotted a time series of measured water temperature at the students’ field site in the VCR in blue. During the lesson plan, students plotted their measured water temperature datapoint from the VCR on top of the plot for comparison, which is represented here by a horizontal line at 27 °C. Long-term data were provided by a publicly-available VCR-LTER dataset. In a separate activity, the adjectives used to describe the characteristics of (b) inner and (c) outer seagrass leaves by individual students were summarized with word clouds. Word clouds, or diagrams of words, where the size of each word is positively correlated to the number of times the word is entered into the generator, are a visual tool that students are generally familiar with.

3.5 Help students put scientific conclusions into a real-world context

Like sensemaking, the practice of drawing meaningful conclusions is generally not prioritized in classroom settings. We dedicated intentional time for students to relate their field experiences and results back to real-world science and outreach efforts by asking them to explore the online Chesapeake Bay Ecosystem Health Report Card outreach tool
The 2022 report card gave both the Chesapeake Bay and the Chesapeake Bay Watershed a ‘C’ for overall ecosystem health (UMCES 2023). We learned that the report card was an excellent way to challenge student perceptions of ecosystem health across spatial scales. Many students were used to seeing more pristine areas of the Chesapeake Bay, and were surprised that the letter grades were so low. The report card structure was an ideal scaffold, because K-16 students could easily conceptualize the differences between letter grades. The intentional overlap between the variables in their data and the report card helped students relate their data to the report’s conclusions. Since this type of report card does not exist for the VCR, this exercise was also a scaffold that encouraged students to think about what a VCR report card would look like. Overall, this exercise had the additional broader impact of demonstrating how data can be communicated with the public.

To conclude the lesson plan, we asked students to make evidence-based claims about 1) whether the Chesapeake Bay or the VCR was healthier and 2) which driver was the most important indicator of ecosystem health. Students followed the Claim, Evidence, Reasoning, and Rebuttal framework, where they backed up their claims with evidence from their dataset, explained why the evidence fit their claim, and then challenged their claims by providing counter-evidence and counter-reasoning (Alegado and Lewis 2018). We expected students’ claims to match the ones we had identified during our backward design planning. Instead, the students’ claims were more nuanced and showed a deeper understanding of the scientific concepts than we had anticipated. Several students challenged the investigative questions and used their data to argue either that one variable was not enough to determine ecosystem health in a whole estuary, or that ecosystem health was likely variable inside both estuaries. We were positively surprised and impressed by the complexity and maturity of their claims. Ultimately, their claims showed that the lesson plan helped the students practice authentic science while increasing their scientific understanding of the local estuaries – the major goal of our work.

4 Outcomes

Through collaborative work, we co-developed a lesson plan that gave students a far better understanding and appreciation of their local ecosystems. Students were able to practice the scientific process, interact with real scientists, and learn how to use publicly-available data and
resources. Although this work could be further developed through repetitive use and formal evaluations of how student sentiments about science and science identity changed over time, our observations of the participating class suggest that this lesson plan met our goals.

This learning experience likely strengthened students’ science identities and deepened their connections to their local environment (Kang et al. 2019; Wade-Jaimes et al. 2023). After completing the lesson plan, the teacher observed that students had a deeper understanding of the scientific process. Their confidence of ecosystem processes in the Chesapeake Bay and the VCR was demonstrated by their nuanced claims about how ecosystem health should be evaluated. Beyond their local estuaries, the students in this class also seemed to have a firmer grasp of scientific concepts and practices compared to their peers. Specifically, the students were able to better explain how multiple fields – biology, chemistry, ecology, and physics – were connected and influenced one another in the natural world. This may be because our lesson plan was structured to follow the scientific process instead of focusing on the scientific method that is historically taught in classrooms (Wade-Jaimes et al. 2023). The teacher observed that these students had a more well-rounded ability to think like a scientist compared to students who were just given a dataset to analyze. Later, some students in this class placed third in a regional competition of the National Sciences Bowl (the Blue Crab Bowl), which the teacher partially attributed to their ability to connect to the ecosystem on a deeper level after this lesson plan. Overall, the teacher believed that the lesson plan experience and the ownership of a dataset empowered students’ science identity, regardless of whether or not students pursued a career in STEM.

Working with scientists during the field experience may have helped students deconstruct some of the common stereotypes about scientists (Miller et al. 2018; Wade-Jaimes et al. 2023). Many students had been unaware that the University of Virginia and other research centers maintained field stations on the ESV A. It was valuable for them to see that a university was a part of their community, and that university scientists were interested in contributing to student learning. After the lesson plan, students were passionate about sharing their findings and experiences with other peers outside of class, which helped foster a collaborative and enriching learning environment.
Teachers and scientists can co-produce authentic science learning experiences that are strongly needed in today’s classrooms. When we collaborate, our combined skill sets and perspectives can positively impact student science identities and potentially shape the next generation of scientists. Our lesson plan can be modified to fit other local ecosystems and can be scaled for high school and college classrooms (https://doi.org/10.6084/m9.figshare.24738627.v1, last accessed Dec. 5, 2023). The best practices outlined here can also serve as a guide to help scientists meet teachers where they are and work with them to translate our science in a way that is meaningful for everyone.

5 Literature cited


UMCES. 2023. 2022 Chesapeake Bay & Watershed Report Card.

Conclusions

Blue carbon ecosystems such as seagrass meadows play a significant role in global carbon cycling and the mitigation of greenhouse gases, but the dynamics of these fluxes are poorly understood on diurnal, tidal, and seasonal scales (Al-Haj & Fulweiler, 2020; Rosentreter et al., 2023). This dissertation evaluates tools used to measure fluxes across the air-water interface, provides a thorough accounting of air-water greenhouse gas flux magnitudes and pathways in a temperate seagrass meadow, and outlines best practices for communicating complex scientific concepts to students. Throughout this work, we show the importance of collecting high-quality and high-frequency in situ data when constraining gas fluxes. The majority of the work in this dissertation was conducted in South Bay, a shallow coastal bay located in the Virginia Coast Reserve (VCR) that has a benthic environment dominated by a *Zostera marina* meadow. This dissertation builds on the work of previous scientists who constrained ecosystem metabolism and gas fluxes in situ in South Bay (Berg et al., 2019; Berger et al., 2020; Oreska et al., 2020).

There is a growing body of literature that describes the aquatic eddy covariance technique and applies it to various ecosystems to estimate ecosystem metabolism and carbon flows (Berg et al., 2003, 2022; Berger et al., 2020). As the use of this technique becomes more widespread, the evaluation of new potential O\textsubscript{2} sensing systems is critical to ensure accurate flux estimates (Berg et al., 2016; Chipman et al., 2012). This dissertation builds on previous work that tests new sensors by evaluating a new O\textsubscript{2} optode system (meter and fiber-optic sensor) for aquatic eddy covariance in various conditions that represent common field environments (Chapter 1) (Granville et al., 2023). The rigorous evaluation protocol identified here can serve as a guideline for tests of future systems. We found that the new O\textsubscript{2} sensing system had a fast response time that was well within the range of sensors currently used for aquatic eddy covariance. In the field, O\textsubscript{2} fluxes measured using the new system were in agreement with fluxes measured at our site using established systems. The data were less noisy compared to other systems and no sensors broke or were fouled during our deployments. Overall, we found that the O\textsubscript{2} sensing system is suitable for aquatic eddy covariance. However, it is not an ideal choice for upside-down aquatic eddy covariance. O\textsubscript{2} concentrations measured by O\textsubscript{2} sensors are inherently sensitive to temperature changes, and the distance between the O\textsubscript{2} sensor and the temperature sensor, located on the meter, indicates the potential for heat fluxes to bias the O\textsubscript{2} concentration readings at the
air-water interface, where heat fluxes are greater than at the benthic interface (Berg & Pace, 2017). At the air-water interface, a more suitable system is the dual O$_2$-temperature planar optode, in which the O$_2$ and temperature sensors are both located at the tip of the sensor.

Gas exchange across the air-water interface is poorly constrained in temperate seagrass meadows due to a lack of high-frequency *in situ* measurements and uncertainties in flux methodologies (Al-Haj & Fulweiler, 2020; Rosentreter et al., 2021). In this dissertation, we built on established flux measurement techniques to constrain air-water CO$_2$ exchange over diurnal and tidal cycles (Chapter 2) (Berg et al., 2020; Wanninkhof, 2014). We compared measured and modeled gas transfer velocities ($k$) because $k$ is the primary source of uncertainty in the flux equation, and we evaluated local wind speeds, which are recognized as a primary driver of $k$ (Ho et al., 2006; Wanninkhof et al., 2009). Wind speeds that were measured at a station in the Chesapeake Bay with no proximity to land were representative of wind speeds over South Bay, while stations located in the VCR in proximity to land under-estimated wind speeds over South Bay. We derived *in situ* $k$ values using the upside-down aquatic eddy covariance technique and compared them to modeled values derived from multiple empirical parameterizations based on wind speed, current velocity, and water depth. From this comparison, we identified four best fitting models that were most appropriate to use at our site when *in situ* data were not available. The best fitting models were wind-based models with an intercept of zero that were originally derived over the open ocean. This result varies from other vegetated ecosystems, which shows the importance of evaluating gas transfer velocity on a site-specific basis (Dobashi & Ho, 2023; Ho et al., 2016). Using a best-fitting model, appropriate wind speed measurements, and high-frequency autonomous underwater sensors, we derived hourly air-water CO$_2$ fluxes over South Bay for multiple days in July of 2021, 2022, and 2023, when the seagrass meadow was at peak seagrass density. CO$_2$ fluxes varied by year and by hour of the day, with the maximum positive CO$_2$ fluxes, which represent outgassing to the atmosphere, occurring at dawn, and the maximum negative CO$_2$ fluxes, which represent uptake by the water column, occurring in the afternoon (Fig. 7, Chapter 2). During a thunderstorm, CO$_2$ outgassing significantly increased, indicating the importance of evaluating fluxes over multiple weather conditions. On average, South Bay was in metabolic balance in July.
The seasonality of air-water CO$_2$, CH$_4$, and N$_2$O fluxes is poorly understood in seagrass meadows, especially on local scales (Al-Haj et al., 2022; Oreska et al., 2020). This dissertation provides an accounting of air-water CO$_2$, CH$_4$, and N$_2$O fluxes over a temperate seagrass meadow from April to October by expanding on the methods of Chapter 2 and measuring fluxes throughout the seagrass growing season and during storm periods. We found that CO$_2$ and CH$_4$ fluxes South Bay were strongly influenced by the seagrass growing season. South Bay was an overall sink of CO$_2$ during the early growing season and shifted to an overall source of CO$_2$ during seagrass die-off in August. In July, during the peak seagrass density period, South Bay was in metabolic balance, as CO$_2$ fluxes were not significantly different from zero. Similarly to other seagrass meadows, South Bay was an overall source of CH$_4$ (Eyre et al., 2023; Rosentreter et al., 2023). We evaluated both dissolved and ebullitive CH$_4$ fluxes and found that ebullition, which includes plant-mediated transport and sediment-water column ebullition, was a significant component of the total air-water CH$_4$ flux (Schorn et al., 2022; Vroom et al., 2022). Overall, total CH$_4$ fluxes increased throughout the growing season, although the proportion of dissolved to ebullitive fluxes changed over time. South Bay was a sink of total and dissolved N$_2$O (Rosentreter et al., 2023). Unlike CH$_4$, we found that N$_2$O ebullition was negligible and was fully offset by the dissolved N$_2$O fluxes. Overall, South Bay was a minor source of CO$_2$-equivalent gases during this period, and CO$_2$ and N$_2$O fluxes partially offset the total CH$_4$ emissions.

Communicating complex scientific topics such as gas exchange to the public is a difficult but critical task, especially in coastal areas that are at a high risk of potential harm from environmental hazards (Harris et al., 2022). Increasing scientific literacy in students, who generally lose the ability to identify with scientists and science as they progress through their K-12 education, is one way to make this task easier (Miller et al., 2018). The observed lack of science identity in students partially attributed to a lack of authentic science learning experiences in classrooms (Wade-Jaimes et al., 2021, 2023). Environmental scientists have a unique skill set that can be leveraged alongside teachers to develop authentic science learning experiences that connect students to their local environment. In this dissertation, we co-developed an authentic science learning experience for students on the Eastern Shore of Virginia and outlined a series of best practices that were important for our work for scientists and educators who want to do similar work (Chapter 4) (Granville et al., 2024). The best practices are 1) take a scholarly approach to teaching and learning, 2) build a mutually-beneficial partnership between scientists...
and educators, 3) use pedagogical practices to develop authentic science learning experiences, 4) prioritize sensemaking, and 5) help students put scientific conclusions into a real-world context. We observed several positive impacts on the students after the science learning experience, including an improved ability to connect complex scientific concepts across fields and a deeper understanding of the organisms and processes in their local environment. This work can be used as a guideline for other scientists who want to connect with their communities in similar ways.

**Literature cited**


119


Funding acknowledgement

Kayleigh E. Granville was supported by a Virginia Sea Grant Graduate Research Fellowship, a Jefferson Scholars Foundation Dissertation Year Fellowship, and research and teaching assistantships from the Department of Environmental Sciences at the University of Virginia. This work was also funded by the National Science Foundation (NSF) through grants to the Virginia Coast Reserve Long-Term Ecological Research Program (DEB-1832221) and through grants awarded to Peter Berg (OCE-1851424, OCE-1824144, and OCE-2223204).