Alkalinity Cycling And Carbonate Chemistry Decoupling in Seagrass Mystify Processes of Acidification Mitigation

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Research Article

Keywords: seagrass, acidification, alkalinity, carbonate chemistry, phytoremediation

Posted Date: April 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-419885/v1>

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1 **Alkalinity cycling and carbonate chemistry decoupling in seagrass mystify processes**
2 **of acidification mitigation**

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11
12 **Abstract**

13 The adverse conditions of acidification on sensitive marine organisms has led to the
14 investigation of bioremediation methods as a way to abate local acidification. This
15 phytoremediation, by macrophytes, is expected to reduce the severity of acidification in
16 nearshore habitats on short timescales. Characterizing the efficacy of phytoremediation
17 can be challenging as residence time, tidal mixing, freshwater input, and a limited
18 capacity to fully constrain the carbonate system can lead to erroneous conclusions. Here,
19 we present *in situ* observations of carbonate chemistry relationships to seagrass habitats
20 by comparing dense (DG), patchy (PG), and no grass (NG) *Zostera marina* pools in the
21 high intertidal experiencing intermittent flooding. High-frequency measurements of pH,
22 alkalinity (TA), and total-CO₂ elucidate extreme diel cyclicality in all parameters. The DG
23 pool displayed frequent decoupling between pH and aragonite saturation state (Ω_{arg})
24 suggesting pH-based inferences of acidification remediation by seagrass can be
25 misinterpreted as pH and Ω_{arg} can be independent stressors for some bivalves. Estimates
26 show the DG pool had an integrated ΔTA of 550 $\mu\text{mol kg}^{-1}$ over a 12 h period, which is

~60 % > the PG and NG pools. We conclude habitats with mixed photosynthesizes (i.e., PG pool) result in less decoupling between pH and Ω_{arg} .

Introduction

The myriad biophysical factors that modify estuarine carbonate chemistry often transcend the effects of atmospheric CO₂ hydrolysis in seawater (ocean acidification), where the effects of groundwater flux, fluvial inputs, enhanced biological metabolism, eutrophication, upwelling, and tidal pumping interact in complexity resulting in coastal acidification¹⁻⁴. The synthesis of these processes is a long-term pH variability that is estimated to be ~20x greater than the open-ocean, where the increasing baseline of dissolved CO₂ magnifies the frequency and duration of carbonate chemistry extremes resulting in impeded growth and development of calcifying organisms^{1,5-9}. The deleterious socioeconomic implications of acidification has led to policy initiatives aimed at utilizing phytoremediation (i.e., photosynthetic CO₂ uptake) by seagrass and kelp to locally mitigate acidification events¹⁰⁻¹². This assumes that photosynthesis by macrophytes can reduce the dissolved inorganic carbon (TCO₂)—simultaneously raising pH—during daylight hours when photosynthetic rates are high relative to heterotrophic respiration in seagrass beds and kelp forests. Initial research on this topic found that daytime reduction of TCO₂ by seagrass is capable of increasing pH on short time scales, however residence time, depth, and enhanced community metabolism in nearshore seagrass habitats were found to dampen mitigation or exacerbate extreme conditions offering only minor, temporary, refuge from acidification^{9,13-17}. These equivocal conclusions of carbonate chemistry variability in seagrass habitats may partially explain the contrasting correlations between bivalve growth and proximity to seagrass patches

51 ^{18,19}. Notwithstanding the emerging complexity of phytoremediation, it is clear that
52 further studies are needed to investigate the habitat specificity as it relates to biological
53 communities and the physicochemical oceanographic dynamics of seagrass and kelp
54 ecosystems from an acidification context.

55 To quantify phytoremediation by seagrass the carbonate system needs to be
56 properly constrained as organismal sensitivities to acidification are specific to individual
57 parameters (e.g., pH and CaCO_3 saturation state Ω)—acidification is a multi-stressor ^{20,21}.
58 Complicating matters is the nuance of carbonate chemistry variability in coastal margins
59 where the potential for specific parameters to diverge from co-varying positive
60 correlations (i.e., pH and Ω) is high, a phenomenon referred to as a decoupling of the
61 carbonate system ^{1,22}. Previous studies have examined seagrass phytoremediation via
62 autonomous pH and O_2 sensors complemented by periodic discrete sampling of TCO_2 or
63 TA (total alkalinity) ^{9,15,17,18,23}, however logistics including site accessibility, timing of
64 tidal cycles, and ability to conduct high-frequency sampling often precludes fully
65 constraining the carbonate system; an inherently necessary step in properly identifying
66 occurrences of decoupling and quantifying phytoremediation.

67 To date, most phytoremediation analyses from observations and modelling rely
68 heavily on positive correlations between pH and seagrass density as a means to identify
69 the potential for acidification mitigation ^{13,16,17}. Conclusions regarding acidification
70 amelioration are, thus, limited by assumptions of a coupled carbonate system and an
71 opacity to detail the multi-stressor component. In this study, we characterize the
72 carbonate chemistry of adjacent pools in the high intertidal defined as being dense,
73 patchy, or devoid of seagrass (*Zostera marina*). We measured multiple carbonate

chemistry parameters (pH, TCO₂, and TA) in each pool over a 17-d period at high frequency to identify tidal and diurnal patterns of carbonate chemistry change as a function of the presence and abundance of seagrass. Our analysis highlights the frequency of carbonate chemistry decoupling of pH and Ω_{arg} as it relates to seagrass density and TA variability. If this decoupling is common in seagrass beds, then previous estimates of phytoremediation are potentially overestimated due to assumptions about positive correlations among parameters (e.g., pH, Ω_{arg}) despite the use of a robust TA-salinity relationship in calculating carbonate chemistry variables.

Results

Timeseries of pool carbonate chemistry

Observations recorded in dense grass (DG: 62 % of pool area), patchy grass (PG: 26 % of pool area), and no grass (NG: 0 % of pool area) pools at the head of Jakolof Bay, AK (Fig. S1), displayed robust hourly changes in TA, TCO₂ and pH that depicted an increasing dynamic range correlated with residence time from 15 – 24 June (Fig. 1). Immersion time (i.e., period pools were flooded) decreased from 3.85 to 2.60 h while depth of overlying water at high tide decreased from 1.18 to 0.30 m during the spring to neap tidal transition increasing the magnitude of carbonate chemistry change (Fig. 1). Over the 17-d period the ΔTA ($\mu\text{mol kg}^{-1} \text{ h}^{-1}$) range was greatest in the DG pool with a maximum value 43 % greater than the PG pool, and 26 % greater than the NG pool (Table S1). The ΔTCO_2 maximum rate in the DG pool was double that of its ΔTA and only 23 % and 48 % greater than the PG and NG pools, respectively (Table S1). Immediately following each flood cycle when pools were emersed and retained an average depth of 6 cm, the TA, TCO₂, and pH_T signals were approximately equal to

ocean measurements and similar to normative estuarine systems. This is despite extremely low salinity (Fig. S2) at the surficial layer of the incoming flood tide which lowered ocean TA only at the surface.

Estimated pH_T calculated from TCO_2 and TA in all pools were robustly different from direct pH_T measurements (Fig. 1c–e). Measured values were consistently higher where the mean of $\text{pH}_T - \text{pH}_{T_est} (\pm \text{SD})$ was 0.38 ± 0.35 for DG, 0.22 ± 0.26 for PG, and 0.33 ± 0.23 for NG pools. The NG pool flooded at a height 0.21 m higher than DG and PG pools resulting in an extended emersion period and complete evaporation of the pool on 26 June resulting in anomalous measurements after the 24th as robust deviations became present (Fig. S2). The PG pool had the greatest overall pH_T range from 7.64 to 9.25 while the daily extremes were slightly less from 7.64 to 9.14, and 7.65 to 9.08 for the DG and NG pool, respectively. The daily increase in pH_T occurred concomitantly with temperature that ranged from ~ 12.4 to 26.6 (Fig. S2), which would thermodynamically decrease pH_T by ~ 0.225 units over the TA and salinity values observed due to the positive correlation between temperature and carbonic acid dissociation constants.

Hourly rates of change during emersion

High-frequency sampling of carbonate chemistry and ancillary parameters (O_2 , temperature, salinity, and nutrients) characterize a diel modulation for all pools with increases in TA and TCO_2 at night ($\text{PAR} < 100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and decreases during the day (Fig. 2a–c). Hourly nighttime increases in TA and TCO_2 for DG and PG pools were 42.13 and 48.50 ($R^2 = 0.99$ and 0.98), and 44.99 and 173.08 ($R^2 = 0.99$ and

0.92) $\mu\text{mol kg}^{-1} \text{ h}^{-1}$, respectively. The ratio at which TA and TCO_2 increased for the DG (1.07) and PG (1.14) pools was ~ 1 whereas the NG pool had a TA: TCO_2 ratio of 1.84. TA decreased linearly for all pools at a rate $\sim 51.0 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ ($R^2 = 0.98$) for DG and NG pools, and $32.3 (R^2 = 0.97) \mu\text{mol kg}^{-1} \text{ h}^{-1}$ for the PG pool. TCO_2 decreased fastest in the PG pool resulting in higher pH_T earlier in the day compared to DG and NG pools, and a more rapid shift in carbonate chemistry speciation from HCO_3^- to CO_3^{2-} . Supersaturation of CaCO_3 (Ω_{arg}) persisted in all pools for the entire emersion period (~ 21 h) regardless of PAR levels (Fig. 2d–f). If partitioned by the processes that are known to modify TCO_2 , it is estimated that the predominate mechanism driving variability was biological respiration and photosynthesis compared to CaCO_3 precipitation or dissolution, which outpaced changes in $[\text{O}_2]$ that peaked in late morning.

Alkalinity drawdown and associations with seagrass

Logistical curve fits to ΔTA as a function of emersion time was greatest in the DG pool reaching a maximum of $\sim 550 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ (RMSE = 82.3) around 10.5 h compared to the NG (RMSE = 46.4) and PG (RMSE = 58.0) pools with ΔTA maxima of $\sim 300 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ at 8.5 h (Fig 3a). At maximum ΔTA , DG was significantly different than both PG and NG pools represented by nonoverlapping model bounds. The ΔTA per proportion of seagrass cover over the same emersion period appeared greater in the PG pool relative to the DG pool, however, the large RMSE (DG = 133.3 and PG = 215.5) bounds suggests these two pools are undistinguishable (Fig. 3b).

Carbonate chemistry decoupling

Aragonite saturation state and $[O_2]$ relationships with pH_T varied by pool for the entire timeseries. A decoupling of a consistent positive correlation between pH_T and Ω_{arg} was observed in the DG and PG pools which displayed $\Omega_{arg} < 1.5$ across a pH_T range 7.63 – 9.10, with a greater proportion of low Ω_{arg} values at high pH_T in the DG pool relative to the PG pool (Fig. 4). The $[O_2]$ in the DG and PG pools had a gaussian distribution with an RMSE of 51.4 and 55.7, respectively, where $[O_2]$ peaked at a $pH_T \sim 8.8$ and then began to decrease. This trend was not present in the NG pool, and a linear relationship was observed in the ocean signal.

Discussion

The extreme diel cyclicity of TA observed in this study is unprecedented for nearshore macrophyte habitats in temperate locations and refutes assumptions of its invariability and strong correlation with salinity, a relationship often used to constrain the carbonate system. The modulation of pH_T , TCO_2 , and TA in each of the pools exceeds those that would be derived based on any two of the carbonate chemistry parameters and exemplifies an uncoupled system likely overlooked by previous studies^{15–17,23,24}. Extreme decoupling of the carbonate system was present in the DG bed only where pH_T ranged from 7.65 to 9.19 while maintaining a salinity > 26 and $\Omega_{arg} < 1.5$ —a threshold at which acute stress occurs in certain bivalve larva²⁵. The two seagrass pools (DG, PG) displayed fundamental differences as it relates to extremes in carbonate chemistry variability, characteristic of previous findings detailing an exacerbation of extremes in seagrass habitats⁹. Despite abundant filamentous macroalgae and observed

microphytobenthos (Fig. S1), the NG pool exhibited a reduced magnitude of variability and maintained a mostly positive correlation between pH_T and Ω_{arg} . The OC signal experienced more modest decoupling, however this was due to the freshwater lens at the surface upon the incoming flood tide. While phytoremediation may appear present during occasions with extremely high pH_T and Ω_{arg} , the reduction of TCO_2 and bioavailable carbon for calcification remained extremely low, potentially impeding organismal calcification,^{25–27}, inducing a result opposite of phytoremediation. While these conclusions are based on *in situ* timeseries sampling without replication, the consistent behavior and difference between each pool and autocorrelation over the tidal period gives confidence in our conclusions.

Model estimates of daytime ΔTA as a function of residence time suggest that the mixed autotroph PG pool resulted in a lower integrated TA decrease but a faster rate of TCO_2 drawdown shifting the distribution of carbonate chemistry speciation to limited CO_2 availability earlier in time (Fig. 5). Based on our results, we hypothesize that higher photosynthetic rates by non-seagrass photosynthesizers (e.g., microphytobenthos) in mixed seagrass communities can raise pH and drawdown TCO_2 faster leading to a more rapid increase in TA: TCO_2 due to the TCO_2 uptake physiology by those autotrophs^{14,28,29}. This is counter to other assertions suggesting greater seagrass density (leaf area index) leads to a greater potential of acidification remediation¹³; it is clear though, that a more rigorous characterization of the carbonate system is needed to address the efficacy of mitigation and potential decoupling of the system.

The effects of extreme TA variability relate to changes in the TA: TCO_2 ratio which influence CaCO_3 saturation state as well as the sensitivity of carbonate chemistry

parameters (e.g., pH and PCO_2)³⁰. The TA:TCO₂ ratio ranged from 1.05 to 1.80 with highs correlated to longer residence times during neap tidal periods that affected the duration of immersion and emersion. Studies that have previously recorded such changes in TA identify benthic flux or CaCO₃ dissolution as the driver of diel variability, however the attributes of the sediment in those habitats were permeable, medium sand or coarse, and rich in CaCO₃—conducive conditions for enhanced diffusive and advective efflux from porewater^{31–33}. While decreases in TA have been found as a result of calcification by epibionts or by seagrass leaves themselves, those scenarios occurred in tropical environments with waters extremely high in CaCO₃, or in temperate nearshore waters where riverine inputs carried high [TCO₂] and [TA] where Ω_{arg} was found to be > 10 in the submerged aquatic habitats; however, neither reported diel cycling of TA or found the unique TA:TCO₂ ratios observed here^{34,35}. In this study, the sediment for all pools was muddy and interspersed with gravel. Porewater profiles at 1, 2, and 3 cm depths depicted [TCO₂] > [TA_{carb}] and were consistently higher than the concentrations in the overlying water (Fig. S3). Given this orientation, efflux of TA and TCO₂ from porewater should be persistent, particularly as the concentration gradient would increase during the day as TA and TCO₂ decreased in the pools.

If we assume the solute exchange between porewater and overlying water was the mechanism for observed diel TA variability, we can estimate an integrated benthic flux at night in the DG pool of 4.4 mmol m⁻² (~ 6 h) and ~ 10 mmol m⁻² (~ 12 h) during the day, with daytime rates for the PG and NG pools slightly lower at ~ 5.5 mmol m⁻². These rate estimates are similar to those reported in tropical environments where TA flux occurs concomitantly with dissolution in permeable sediments³¹, however, there was no

evidence of high CaCO_3 in the muddy sediments at this site, nor calcifying epiphytes—
epiphytic growth overall was surprisingly minimal. The linear changes in TA over the 21
h period would assume a diffusive flux with a likely stagnant boundary layer because
advective processes that can enhance flux rates were limited to bio-irrigation and-
turbation—which visually appeared minimal—due to lack of other forces (e.g., wave,
current, tide). The characteristics of TA variability were expressed as linear rates of
change during the day and at night, whereas TCO_2 changes were nonlinear during the day
and linear at night. At night TA and TCO_2 increased $\sim 1:1$ in the DG and PG pools,
which exemplified a possible scenario of CaCO_3 dissolution (2:1 change) coupled with
respiration that would change TA: TCO_2 0:1. If plausible, this would have to occur in
superstrated waters (Ω_{arg} was > 1) where the ΔO_2 roughly matches the estimated—
respiration only— ΔTCO_2 . This was not the case, however, as the ΔTCO_2 was $< \Delta\text{O}_2$ after
3 h (ratio of 0.55 and 0.44, respectively) and mixed after 6 h with a ratio of 0.75 and 2.0
in the DG and PG pools, respectively. Denitrification-nitrification processes could
account for this excess O_2 lowering the community respiration quotient in seagrass
communities³⁶, however, the $[\text{NO}_x]$ and $[\text{NH}_4^+]$ were inconclusive correlatives for which
to draw any such conclusions (Table S1). In addition, when PO_4^{3-} and SiO_2 were
measured, correlations with TA were not found. These speculations for TA variability of
course assume ΔTCO_2 could be partitioned by precipitation/dissolution, of which there is
no strong evidence. This further complicates the identification of processes responsible
for TA variability and, thus, provokes the speculation of non-dissolution mechanisms.

The modulation of TA and TCO_2 at 1:1 suggest HCO_3^- as a potential driver as this
would affect both parameters. If ΔTCO_2 and ΔTA are a function of proportion seagrass

cover, then TA and TCO₂ fluctuate linearly in the DG pool, supporting HCO₃⁻ as the modulator (Fig. S4). Specific seagrass species including *Z. marina* and other macrophytes are known to utilize HCO₃⁻ as a carbon source^{37–39}. Evidence suggests the active uptake of HCO₃⁻ occurs via H⁺ symport in *P. oceanica*, where electroneutrality is likely preserved by a Cl⁻ or NO₃⁻ efflux and Na⁺/H⁺ antiport^{40,41}. The accumulation of HCO₃⁻ in *P. oceanica* aids in the establishment of a robust electronegative potential in the leaves, which is also a phenomenon present in *Z. marina*⁴¹. The accumulation of HCO₃⁻ within the cell wall may occur at a faster rate than the dehydration to CO₂ in the cytoplasm catalyzed by carbonic anhydrase, which could lead to efflux of undehydrated HCO₃⁻ at night when light energy is limiting. This could also explain the disparity between O₂ generation and TCO₂ drawdown during the day as hysteresis can occur on the timescale of hours⁴², which could be further modified by photorespiration⁴³. The evidence here, along with the suggested mechanisms by which electroneutrality is preserved when HCO₃⁻ uptake occurs could explain the decrease in TA observed in these pools when TCO₂ is limiting. Photosynthesis, however, is not presumed to affect TA even if HCO₃⁻ is utilized because it is expected that uptake would be compensated with H⁺ or OH⁻ exchange⁴⁴. More evidence is needed though to determine if this is the case in higher order photosynthesizers because research shows electroneutrality preservation by OH⁻ and H⁺ may be replaced by NA⁺, Cl⁻ and NO₃⁻ in some seagrasses^{39,40,45}. Anomalous to this conclusion would be the observed TA variability in the NG pool. The *Ulva* spp., which was observed in the NG pool, however, can also take up HCO₃⁻ via ion exchange, potentially with OH⁻ or Cl⁻^{38,46}. While further investigation is needed to identify the mechanism of TA variability in these pools, similarly low TA values and cycling have

254 been recorded in enclosed bays with long residence times and abundant seagrass, and
255 even speculation of HCO_3^- as a cause^{33,43,47}. These incidences of low TA and its diel
256 cycling, however, were not assessed from a phytoremediation standpoint, thus the
257 implications hitherto remained unappreciated.

258 The pools in this study are representative microcosms of larger systems,
259 replicating carbonate chemistry variability in seagrass habitats on a magnified scale.
260 Perched estuaries, lagoons, and enclosed bays with long residence times have recoded
261 extremely high pH values similar to those found in this study, including instances of
262 reduced TA: correlated to the proximity of oceanic influence^{43,47,48}. In these systems,
263 biological metabolism and evaporation become the dominate mechanisms that modify pH
264^{5,48,49}. It would be remiss to note, however, that the thermodynamic controls on carbonate
265 chemistry also become enhanced at long residence times as was seen in this study.
266 Observed temperature fluctuations ranged from $\sim 12.5 - 26.5$ °C increasing the carbonic
267 acid dissociation constants resulting in an estimated pH drop of ~ 0.21 units across the
268 recorded pH and TA range (Fig. S2); however, this was superseded by photosynthetic
269 modification of pH. Evaporation appeared to have little effect on the DG and PG pools as
270 salinity remained fairly constant even at extreme temperatures (Fig. S2). Noticeable was
271 the complete evaporation of the NG pool and the rapid salinity increase, suggesting that
272 small variations in the volume of tidal immersion (i.e., depth of overlying water) can
273 modulate the degree to which estuary carbonate chemistry is affected by the slope of the
274 tidal region.

275 Findings here show unequivocal, frequent, decoupling of carbonate chemistry,
276 and without over constraining the system, previous conclusions regarding pH and CO_2

variability within seagrass habitats from a perspective of phytoremediation can be overestimated. This can occur despite a robust TA-salinity relationship or because of a mischaracterization of TA variability if results are derived from discrete samples based on specific sampling times corresponding to tidal period and proximity to macrophyte habitat (above or in canopy), and coupled with pH only monitoring. In accordance with previous studies, findings here show that long residence times and spatial variability along with tidal and current mixing are strong drivers for determining carbonate chemistry variability in seagrass^{13,15}, however, we show that decoupling of the carbonate system in dense seagrass occurs independent of low salinity and is likely related to extreme TA variability caused by a hysteresis of how HCO_3^- cycles within the medium in a carbon limited system.

Materials and Methods

Three shallow, adjacent pools with varying depth on the edges, within 50 m of one another were selected as sample sites and characterized as dense grass (DG), patchy grass (PG), and no grass (NG) with ellipse areas ~ 330.9, 180.0, 339.9 m² with average center depths 5.1, 6.2, and 7.1 cm, respectively, in the high intertidal of Jakolof Bay, AK: 59° 26' 54.09" N, 151° 29' 49.96" W (Fig. S1). TA, TCO_2 , pH_T , O_2 , and salinity samples were collected toward the center of pools at maximum depth at intervals ~ 16 and 8 h for 17 d, with high-frequency sampling occurring every 3 h on 24 June 22:30 AKT during a 21 h emersion period. TA, pH_T and O_2 samples were collected in two separate 150 mL borosilicate bottles (one with optical dot), while measurements for TCO_2 were collected in a 5 mL centrifugal tube and poisoned for preservation with 10 μL of saturated HgCl_2 .

300 Temperature measurements were made in situ with an Omega HH81A digital
301 thermometer, while TA, pH_T, salinity, and O₂, were measured at Kasitsna Bay Laboratory
302 within 30 min of collection. TA was measured using an Apollo SciTech AS-ALK₂ with
303 duplicate titrations performed haphazardly throughout the entire sample collection, and
304 CRMs (certified reference material: batch #181) measured before and after each machine
305 calibrated run. pH_T was measured potentiometrically with a Thermo Scientific ROSS
306 Ultra electrode calibrated at total scale with Tris buffer and corrected with an offset that
307 was derived from a regression of 25 samples between potentiometric and
308 spectrophotometric measurements using a Shimadzu UV-1900: this offset was 0.019
309 units (Fig. S5). O₂ measurements were performed with a PreSens Fibox 4 after each
310 sample was collected and immediately stored in a dark box in the field until measurement
311 ~ 30 min later. Salinity was measured with a YSI 3100 conductivity meter. All TCO₂
312 samples were run at Shannon Point Marine Center, WA, on an Apollo SciTech AS-C3
313 along with several CRMs (batch #179) interspersed after calibration. The uncertainty for
314 measured TA (7.58 ± 8.78 SD) and TCO₂ (6.77 ± 6.32 SD) was the average difference
315 between known CRM and measured CRM across all samples.

316 Estimated pH_T and Ω_{arg} were calculated using CO2SYS (Matlab V1.1) with
317 inputs TA and TCO₂ using the carbonic acid dissociation constants from Lueker et al. ⁵⁰,
318 the bisulfate dissociation constant of Dickson et al. ⁵¹, and the boron constant from
319 Uppström ⁵². Assuming the ΔTCO_2 over the 21 h sampling period was a response to
320 photosynthesis/respiration and CaCO₃ precipitation/dissolution, estimates of partitioned
321 ΔTCO_2 were calculated using the absolute hourly rate of change for TCO₂ and TA during
322 each time point. Where $\Delta\text{TA} \times 0.5$ (based on the alkalinity anomaly) was equal to the

323 ΔTCO_2 as a result of precipitation or dissolution, and the remainder of ΔTCO_2 -
324 $\Delta\text{TA} \times 0.5$ was due to biological metabolism. Proportion of cover was calculated by
325 measuring the length and width of each pool and then using photographed images to
326 define the proportion of pool area covered by *Z. marina* present as a ratio of pool size
327 using ImageJ (v. 1.53a).

328 Logistic curves were fit to each pool correlating ΔTA with emersion time when
329 samples were collected immediately after and before flood tide. This ended up being 12
330 time points for DG and PG pools and 10 for the NG pool (pool evaporated at day 15)
331 during the change in tidal cycle from spring to neap. A conceptual model of carbonate
332 chemistry dynamics for the DG and PG pools was determined by using the estimated
333 values of ΔTA across the entire time series (described above) and the changes in
334 carbonate chemistry speciation derived from the high frequency 21 h sampling period.
335 Since the ΔTA during the 21 h sampling period was integrated into the entire timeseries
336 estimates, the carbonate speciation changes are representative of the expected dynamics
337 that would be visible at other time points.

338 Porewater collectors were assembled using Super Speedfit polypropylene 6.35
339 mm press-connect fittings. T-shaped connectors were fit with two, ~ 3.8 cm length pieces
340 of food grade plastic tubing, with open ends sealed with thermoplastic hot melt adhesive.
341 Two sides of each piece of tubing were punctured five times with a 16-gauge needle,
342 wrapped with a 0.45 micron PES membrane filter and adhered with thermoplastic
343 adhesive to keep out sediment. Three collectors for each pool were buried at 1, 2, and 3
344 cm depths (Fig. S1), ~ 36 h and 3 tidal cycles before the first samples were collected. The

top of each T-connector—which protruded above the sediment—was sealed with a Super Speedfit polypropylene plug.

Prior to collection, 5 mL centrifugal tubes were placed in a glove bag purged of O₂ and filled with N₂. Vials sat in the glove bag with caps off for 1-2 h while being filled with N₂ and shaken haphazardly. The caps of each vial had a 6.35 mm hole drilled in the top and then covered with electrical tape. Caps were secured from inside glove bag and vials then stored and transported in a plastic bag. A 10 mL serological pipette fitted with a 6.35 mm piece of rigid tubing was fit into the top of each press-connect securing a tight connection and porewater slowly extracted. The first 2 mL of water was discarded and 4 – 5 mL transferred to the vial by removing tape temporarily and injecting the collected porewater into the vial. Samples were measured immediate for pH_T using a Thermo Scientific ROSS Ultra electrode calibrated with Tris buffer and reported on the total scale. A 0.019 correction factor derived from a 25-sample regression between potentiometric and spectrophotometric measurements using a Shimadzu UV-1900 was applied. After pH measurement, samples were poisoned with 10 µL of saturated HgCl₂ and capped with a new screw top until TCO₂ was measured at Shannon Point Marine Center, Anacortes, WA, on an Apollo SciTech AS-C3. Samples were collected every 3 d.

Nutrient NO_x, NH₄⁺, PO₄³⁻, and SiO₄ samples were collected in each pool every 3 h during the 21 h sampling period starting 24 June 2019. Water samples were collected with a 60 mL syringe and filtered through a GFF with a particle retention rate of 1.2 µm into a 20 mL scintillation vial. Samples were frozen at – 4 °C within 30 min of collection until analysis. Samples were processed on a SmartChem multi-element analyzer at Oregon State University.

References

1. Waldbusser, G. G. & Salisbury, J. E. Ocean Acidification in the Coastal Zone from an Organism's Perspective: Multiple System Parameters, Frequency Domains, and Habitats. *Annu. Rev. Mar. Sci.* **6**, 221–247 (2014).
2. Duarte, C. M. *et al.* Is Ocean Acidification an Open-Ocean Syndrome? Understanding Anthropogenic Impacts on Seawater pH. *Estuaries Coasts* **36**, 221–236 (2013).
3. Johnson, Z. I. *et al.* Dramatic Variability of the Carbonate System at a Temperate Coastal Ocean Site (Beaufort, North Carolina, USA) Is Regulated by Physical and Biogeochemical Processes on Multiple Timescales. *PLOS ONE* **8**, e85117 (2013).
4. Baumann, H. & Smith, E. M. Quantifying Metabolically Driven pH and Oxygen Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries Coasts* **41**, 1102–1117 (2018).
5. Carstensen, J. & Duarte, C. M. Drivers of pH Variability in Coastal Ecosystems. *Environ. Sci. Technol.* **53**, 4020–4029 (2019).
6. Clark, H. R. & Gobler, C. J. Diurnal fluctuations in CO₂ and dissolved oxygen concentrations do not provide a refuge from hypoxia and acidification for early-life-stage bivalves. *Mar. Ecol. Prog. Ser.* **558**, 1–14 (2016).

- 393 7. Mangan, S., Urbina, M. A., Findlay, H. S., Wilson, R. W. & Lewis, C. Fluctuating
394 seawater pH/pCO₂ regimes are more energetically expensive than static pH/pCO₂
395 levels in the mussel *Mytilus edulis*. *Proc. R. Soc. B Biol. Sci.* **284**, 20171642 (2017).
- 396 8. Hauri, C., Gruber, N., McDonnell, A. M. P. & Vogt, M. The intensity, duration, and
397 severity of low aragonite saturation state events on the California continental shelf.
398 *Geophys. Res. Lett.* **40**, 3424–3428 (2013).
- 399 9. Pacella, S. R., Brown, C. A., Waldbusser, G. G., Labiosa, R. G. & Hales, B. Seagrass
400 habitat metabolism increases short-term extremes and long-term offset of CO₂ under
401 future ocean acidification. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 3870–3875 (2018).
- 402 10. Washington State Blue Ribbon panel on Ocean Acidification. *Scientific summary of*
403 *ocean acidification in Washington State marine waters: Washington shellfish*
404 *initiative Blue Ribbon panel on ocean acidification*. (U. S. Dept. of Commerce,
405 National Oceanic Atmospheric Administration, 2012).
- 406 11. Nielsen, K. *et al.* *Seagrass and Kelp as an Ocean Acidification Management Tool in*
407 *California – Ocean Science Trust*. <http://www.oceansciencetrust.org/projects/sav/>
408 (2018).
- 409 12. Ekstrom, J. A. *et al.* Vulnerability and adaptation of US shellfisheries to ocean
410 acidification. *Nat. Clim. Change* **5**, 207–214 (2015).
- 411 13. Kowek, D. A. *et al.* Expected limits on the ocean acidification buffering potential of
412 a temperate seagrass meadow. *Ecol. Appl.* **28**, 1694–1714 (2018).
- 413 14. Miller, C. A., Yang, S. & Love, B. A. Moderate Increase in TCO₂ Enhances
414 Photosynthesis of Seagrass *Zostera japonica*, but Not *Zostera marina*: Implications
415 for Acidification Mitigation. *Front. Mar. Sci.* **4**, (2017).

- 416 15. Cyronak, T. *et al.* Short-Term Spatial and Temporal Carbonate Chemistry Variability
417 in Two Contrasting Seagrass Meadows: Implications for pH Buffering Capacities.
418 *Estuaries Coasts* **41**, 1282–1296 (2018).
- 419 16. Unsworth, R. K. F., Collier, C. J., Henderson, G. M. & McKenzie, L. J. Tropical
420 seagrass meadows modify seawater carbon chemistry: implications for coral reefs
421 impacted by ocean acidification. *Environ. Res. Lett.* **7**, 024026 (2012).
- 422 17. Hendriks, I. E. *et al.* Photosynthetic activity buffers ocean acidification in seagrass
423 meadows. *Biogeosciences* **11**, 333–346 (2014).
- 424 18. Greiner, C. M., Klinger, T., Ruesink, J. L., Barber, J. S. & Horwith, M. Habitat
425 effects of macrophytes and shell on carbonate chemistry and juvenile clam
426 recruitment, survival, and growth. *J. Exp. Mar. Biol. Ecol.* **509**, 8–15 (2018).
- 427 19. Groner, M. L. *et al.* Oysters and eelgrass: potential partners in a high $p\text{CO}_2$ ocean.
428 *Ecology* **99**, 1802–1814 (2018).
- 429 20. Waldbusser, G. G. *et al.* Ocean Acidification Has Multiple Modes of Action on
430 Bivalve Larvae. *PLOS ONE* **10**, e0128376 (2015).
- 431 21. Hurd, C. L. *et al.* Ocean acidification as a multiple driver: how interactions between
432 changing seawater carbonate parameters affect marine life. *Mar. Freshw. Res.* **71**,
433 263–274 (2020).
- 434 22. Hales, B., Suhrbier, A., Waldbusser, G. G., Feely, R. A. & Newton, J. A. The
435 Carbonate Chemistry of the “Fattening Line,” Willapa Bay, 2011–2014. *Estuaries*
436 *Coasts* 1–14 (2016) doi:10.1007/s12237-016-0136-7.
- 437 23. Ricart, A. M. *et al.* Coast-wide evidence of low pH amelioration by seagrass
438 ecosystems. *Glob. Change Biol.* **n/a**,.

- 439 24. Buapet, P., Gullström, M. & Björk, M. Photosynthetic activity of seagrasses and
440 macroalgae in temperate shallow waters can alter seawater pH and total inorganic
441 carbon content at the scale of a coastal embayment. *Mar. Freshw. Res.* (2013).
- 442 25. Waldbusser, G. G. *et al.* Saturation-state sensitivity of marine bivalve larvae to ocean
443 acidification. *Nat. Clim. Change* **5**, 273–280 (2015).
- 444 26. Comeau, S., Carpenter, R. C. & Edmunds, P. J. Coral reef calcifiers buffer their
445 response to ocean acidification using both bicarbonate and carbonate. *Proc. R. Soc.*
446 *B-Biol. Sci.* **280**, 20122374 (2013).
- 447 27. Jury, C. P., Whitehead, R. F. & Szmant, A. M. Effects of variations in carbonate
448 chemistry on the calcification rates of *Madracis auretenra* (= *Madracis mirabilis*
449 sensu Wells, 1973): bicarbonate concentrations best predict calcification rates. *Glob.*
450 *Change Biol.* **16**, 1632–1644 (2010).
- 451 28. Raven, J. A., Giordano, M., Beardall, J. & Maberly, S. C. Algal evolution in relation
452 to atmospheric CO₂: carboxylases, carbon-concentrating mechanisms and carbon
453 oxidation cycles. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 493–507 (2012).
- 454 29. Vieira, S., Cartaxana, P., Máguas, C. & Marques da Silva, J. Photosynthesis in
455 estuarine intertidal microphytobenthos is limited by inorganic carbon availability.
456 *Photosynth. Res.* **128**, 85–92 (2016).
- 457 30. Egleston, E. S., Sabine, C. L. & Morel, F. M. M. Revelle revisited: Buffer factors that
458 quantify the response of ocean chemistry to changes in DIC and alkalinity. *Glob.*
459 *Biogeochem. Cycles* **24**, GB1002 (2010).

- 460 31. Cyronak, T., Santos, I. R., McMahon, A. & Eyre, B. D. Carbon cycling hysteresis in
461 permeable carbonate sands over a diel cycle: Implications for ocean acidification.
462 *Limnol. Oceanogr.* **58**, 131–143 (2013).
- 463 32. Burdige, D. J. & Zimmerman, R. C. Impact of sea grass density on carbonate
464 dissolution in Bahamian sediments. *Limnol. Oceanogr.* **47**, 1751–1763 (2002).
- 465 33. Chou, W.-C. *et al.* A Unique Diel Pattern in Carbonate Chemistry in the Seagrass
466 Meadows of Dongsha 1 Island: implications for ocean acidification buffering.
467 *Geophys. Res. Lett.* (In review).
- 468 34. Su, J. *et al.* Chesapeake Bay acidification buffered by spatially decoupled carbonate
469 mineral cycling. *Nat. Geosci.* **13**, 441–447 (2020).
- 470 35. Enríquez, S. & Schubert, N. Direct contribution of the seagrass *Thalassia testudinum*
471 to lime mud production. *Nat. Commun.* **5**, 3835 (2014).
- 472 36. Martin, S. *et al.* Comparison of *Zostera marina* and maerl community metabolism.
473 *Aquat. Bot.* **83**, 161–174 (2005).
- 474 37. Invers, O., Zimmerman, R. C., Alberte, R. S., Pérez, M. & Romero, J. Inorganic
475 carbon sources for seagrass photosynthesis: an experimental evaluation of
476 bicarbonate use in species inhabiting temperate waters. *J. Exp. Mar. Biol. Ecol.* **265**,
477 203–217 (2001).
- 478 38. Sand-Jensen, K. & Gordon, D. M. Differential ability of marine and freshwater
479 macrophytes to utilize HCO_3^- and CO_2 . *Mar. Biol.* **80**, 247–253 (1984).
- 480 39. Larkum, A. W. D., Davey, P. A., Kuo, J., Ralph, P. J. & Raven, J. A. Carbon-
481 concentrating mechanisms in seagrasses. *J. Exp. Bot.* **68**, 3773–3784 (2017).

- 482 40. Rubio, L. *et al.* Direct uptake of HCO_3^- in the marine angiosperm *Posidonia oceanica*
483 (L.) Delile driven by a plasma membrane H^+ economy. *Plant Cell Environ.* **40**, 2820–
484 2830 (2017).
- 485 41. Fernández, J. A., García-Sánchez, M. J. & Felle, H. H. Physiological evidence for a
486 proton pump and sodium exclusion mechanisms at the plasma membrane of the
487 marine angiosperm *Zostera marina* L. *J. Exp. Bot.* **50**, 1763–1768 (1999).
- 488 42. Berg, P. *et al.* Dynamics of benthic metabolism, O_2 , and $p\text{CO}_2$ in a temperate
489 seagrass meadow. *Limnol. Oceanogr.* **64**, 2586–2604 (2019).
- 490 43. Buapet, P., Rasmusson, L. M., Gullstrom, M. & Bjork, M. Photorespiration and
491 Carbon Limitation Determine Productivity in Temperate Seagrasses. *Plos One* **8**,
492 e83804 (2013).
- 493 44. Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A. & Dickson, A. G. Total
494 alkalinity: The explicit conservative expression and its application to biogeochemical
495 processes. *Mar. Chem.* **106**, 287–300 (2007).
- 496 45. García-Sánchez, M. J., Jaime, M. P., Ramos, A., Sanders, D. & Fernández, J.
497 Sodium-Dependent Nitrate Transport at the Plasma Membrane of Leaf Cells of the
498 Marine Higher Plant *Zostera marina* L. *Plant Physiol.* **122**, 879–886 (2000).
- 499 46. Drechsler, Z. & Beer, S. Utilization of Inorganic Carbon by *Ulva lactuca*. *Plant*
500 *Physiol.* **97**, 1439–1444 (1991).
- 501 47. Ribas-Ribas, M. *et al.* Effects of upwelling, tides and biological processes on the
502 inorganic carbon system of a coastal lagoon in Baja California. *Estuar. Coast. Shelf*
503 *Sci.* **95**, 367–376 (2011).

48. Omarjee, A., Taljaard, S., Weerts, S. P. & Adams, J. B. The influence of mouth status on pH variability in small temporarily closed estuaries. *Estuar. Coast. Shelf Sci.* **246**, 107043 (2020).
49. McCutcheon, M. R., Saryk, C. J. & Hu, X. Characteristics of the Carbonate System in a Semiarid Estuary that Experiences Summertime Hypoxia. *Estuaries Coasts* (2019) doi:10.1007/s12237-019-00588-0.
50. Lueker, T. J., Dickson, A. G. & Keeling, C. D. Ocean $p\text{CO}_2$ calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2 : validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. *Mar. Chem.* **70**, 105–119 (2000).
51. Dickson, A. Thermodynamics of the Dissociation of Boric-Acid in Synthetic Seawater from 273.15-K to 318.15-K. *Deep-Sea Res. Part -Oceanogr. Res. Pap.* **37**, 755–766 (1990).
52. Uppström, L. R. The boron/chlorinity ratio of deep-sea water from the Pacific Ocean. *Deep Sea Res. Oceanogr. Abstr.* **21**, 161–162 (1974).

Acknowledgments

We would like to thank Edwin (Ted) Grosholz and J. Jay Stachowicz for providing feedback and comments to previous iterations of this manuscript. We would also like to thank Brooke Love and Gene McKeen at Shannon Point Marine Center and George Waldbusser at Oregon State University for use of analytical instrumentations to process samples.

Author Contributions: CAM and ALK designed the study. CAM performed all sample collection, processing, and data analysis. CAM led the writing with editing and contributions from ALK.

Additional Information: The authors report no conflicts of interest.

Figures

Figure 1. Timeseries of measured TA (a), TCO₂ (b), pH_T and estimated pH_T (c-e) in dense grass (DG), patchy grass (PG), no grass (NG), and ocean (OC). Shaded blue region is predicted tide and shaded grey is night when PAR < 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Dashed line indicates tidal height when pools become immersed and tidal height at when samples were taken (blue dots). Three-hour sampling occurred for a period of 24 h starting 24 June 22:30.

Figure 2. Alkalinity and TCO₂ (left y-axis) during 21 h emersion sampling period for dense grass (a), patchy grass (b), and no grass (c) pools. The log concentration of CO₂, HCO₃⁻, and CO₃²⁻ are marked as grey on the right y-axis with colored pH_T isoclines. Absolute values of ΔTCO₂ and ΔO₂ during same emersion period where the total ΔTCO₂ was estimated based on proportion of change due to biological respiration/photosynthesis or CaCO₃ precipitation/dissolution for dense grass (d), patchy grass (e), and no grass (f). Isoclines are Ω_{arg}. Note: The NG pool during this period began to experience increased salinity due to evaporation reducing confidence in the displayed values.

Figure 3. The ΔTA and ΔTA per proportion of seagrass cover as a function of emersion time for dense grass (DG) and patchy grass (PG) in panels (a) and (b), and no grass (NG) pool in panel (a). Highlighted region is the RMSE of modeled logistical fit.

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Figure 4. All sample points of Ω_{arg} (a-d) and O_2 concentration (e-h) correlated to pH_T for dense grass (DG), patchy grass (PG), no grass (NG), and ocean (OC). Color bar is the measured TA:TCO₂ ratio for each point in the timeseries. Gaussian fits applied to DG pool with an RMSE of 51.39 and 55.68 for the PG pool. Open circles in panels (c) and (g) indicate measurements taken after 24 June during NG pool evaporation.

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Figure 5. Conceptual model plots of ΔTA as a function of residence time and the concomitant change in carbonate chemistry speciation (shaded in grey) for dense grass (green) and patchy grass (yellow) based on measurements presented in Figs. 3 and 4. Dashed lines on plots indicate specific time points at which TA decreases, rate of ΔTCO_2 , and carbonate chemistry speciation. Potential processes modulating TA are depicted in stoichiometric equations with hypothesized processes modulating TA based on this study shown with purple arrows and text box.

Figures

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