Supplementary Material

Organic Alkalinity modulates pH from the Sea-Surface Microlayer during a mesocosm study

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This supplementary material provides additional details supporting the analysis for this manuscript. It includes quality assurance and control tests for DIC, TA, and OA measurements, attempts to establish an OA-free baseline using artificial seawater (ASW), and a comparison between directly measured OA and indirect estimates derived from DIC–pH pairs. To contextualise the bloom evolution, chlorophyll-a dynamics (Bibi et al., 2025) are summarized, alongside supplementary figures showing the co-variation of NDIC, NTA, and pH parameters of the marine carbon system across phases. Together, these sections provide methodological validation and additional results that complement the main text, ensuring transparency and reproducibility of the OA dataset.

S1. Analytical QA/QC: CRM and substandard results.

To evaluate DIC and TA performance we analysed replicate samples of CRM batch 209. DIC averaged 1945.68 ± 1.57 µmol kg^{-1} (precision of 0.08%) versus the certified 2060.05 µmol kg^{-1}, and TA averaged 2243.65 ± 2.81 µmol kg^{-1} (precision of 0.13%) versus the certified 2210.40 µmol kg^{-1} in Table S1. Precision targets were met, yet the CRM comparisons revealed biases, so we applied CRM-based scaling factors of 1.058 for DIC and 0.985 for TA to place all measurements on the certified scale. Multiple substandard (SB) analysis were performed to achieve precision in OA measurements. The SB OA of 48.88 ± 0.38 µmol kg^{-1} shows a high precision of 0.78% of this method (Table S1). Nevertheless, precision was controlled with a SB and only proceeded with discrete samples when the substandard's short-term SD was successfully achieved <3 µmol kg^{-1}. For OA, a correction factor was not possible to compute, since there is no CRM for this parameter. For this reason, the same correction factor for TA measurements was applied to all the OA discrete samples.

Table S1. Example of daily routines of five replicates samples of CRM and substandard OA (SB OA). CRM Batch 209 (DIC= 2060.05 ± 0.36 µmol·kg^{-1}; TA= 2210.40 ± 0.43 µmol·kg^{-1}) provide average measured, standard deviation (SD), and relative standard deviation (%RSD) for precision and the resultant correction factor (CF) only for DIC and TA. SB OA reported the best precision of these parameters methods.

Sample No.	DIC [µmol·kg^{-1}]	TA [µmol·kg^{-1}]	SB OA [µmol·kg^{-1}]
1	1944.81	2242.52	48.35
2	1946.37	2241.67	48.93
3	1943.7	2240.9	49.43
4	1945.66	2247.51	48.83
5	1947.85	2245.65	48.86
Average	1945.68	2243.65	48.88
SD	1.57	2.81	0.38
Presicion (%RSD)	0.08	0.13	0.78
CF	1.06	0.99	-

S2. Artificial Seawater Blank Trials for Organic Alkalinity-free baseline attempts.

In the absence of an OA-certified reference material to assess the accuracy of the OA method, we established a zero-OA baseline with dissolved organic carbon DOC-free artificial seawater (ASW). ASW (S ≈ 34) was prepared by dissolving 38 g Tropic Marin Pro Reef Salt in 1 L ultrapure water, then passing through pre-conditioned cartridges (1 g, Varian) to minimize residual DOC (MeOH conditioning, controlled flow, discard initial eluate). Treated ASW was analyzed on a Shimadzu total organic carbon analyzer to verify DOC removal (instrument precision 2.6–3.7%, accuracy 0.18–4.5%) (Sugimura and Suzuki,

1988). We adapted the Bradshaw and Brewer (1988) matrix-matched approach to a DOC-free seawater matrix for the calibration of OA. ASW served as the operational zero for OA back-titrations accuracy: any alkalinity detected in the second titration, after correcting for inorganic contributors (Eq. 1) at measured Temperature, Salinity, and total hydrogen-ion concentration scale, was required to be indistinguishable from zero within uncertainty. Parallel to accuracy, precision is one key component of validating the back titration method. using a laboratory standard (North Sea seawater: UV-treated, 2 µm-filtered, $HgCl_2$-poisoned, homogenized, and bottled). Each analytical day began with duplicate substandard titrations; long- and short-term controls demonstrated precision of $\leq \pm 3$ µmol kg^{-1} before proceeding to measure the mesocosm samples.

Table S2. Organic alkalinity (OA) concentration of the artificial seawater (ASW) under three pre-treatments. Initial ASW, freshly prepared; Neutral, pH-adjusted to seawater working pH without irradiation; Neutral and UV-A, pH-adjusted then UV-A irradiated. OA (µmol kg^{-1}).

OA-free Treatment	OA [µmol kg^{-1}]
Initial ASW	47.00
Neutral	36.97
Neutral and UV-A	42.81

ASW were analysed to seek an "OA-free" zero point for the back-titration method, on the premise that should contain negligible dissolved OM, so OA should be minimal. Over three days measured OA in ASW was testing this idea. Table S2 shows the three different treatments done to achieve the zero point OA: Freshly prepared initial ASW; the same ASW adjusted to the working pH of seawater with strong acid and/or base and without irradiation; and ASW same as before but irradiated with UV-A light to photochemically oxidise dissolved OM. The concentration of OA-ASW change significantly. Initial ASW had OA 47.00 µmol kg^{-1}. For the second method the OA was lower with a concentration of 36.97 µmol kg^{-1}. For the last treatment with UV-A light, OA was 42.81 µmol kg^{-1}. ASW did not yield an OA-free zero. UV-A irradiation only partly reduced OA, implying incomplete DOM removal and possible formation of new acid–base active products. Thus, residual dissolved OM contributes measurable alkalinity and blank conditioning might affect OA corrections.

S3. Bloom Context and Marine Carbon System Dynamics

As reported in detail by Bibi et al. (2025), chlorophyll-a (Chl-a) dynamics characterised the progression of the induced bloom and are included here as supplementary reference to contextualise carbonate system changes (Figure S1). On the pre-bloom phase (18 to 26-May), concentrations remained low at 2.20 ± 0.91 µg L^{-1}. At bloom phase, Chl-a increased sharply, reaching a maximum of 10.62 ± 1.23 µg L^{-1} around 02-Jun, coinciding with the dominance of coccolithophores (*E. huxleyi*). This peak was short-lived, as concentrations declined rapidly toward the end of the bloom and stabilised at low levels (2.37 ± 1.51 µg L^{-1}) during the post-bloom phase. In parallel, pH evolved from being consistently higher in the SML than in the ULW during the

pre-bloom, to convergence during bloom maximum, and then to small alternating anomalies at the post-bloom phase. Overall, Chl-a trends provided the temporal framework for bloom initiation, biomass maximum, and decay, against which the coupled behaviour of DIC, TA, pH, and OA was being interpreted.

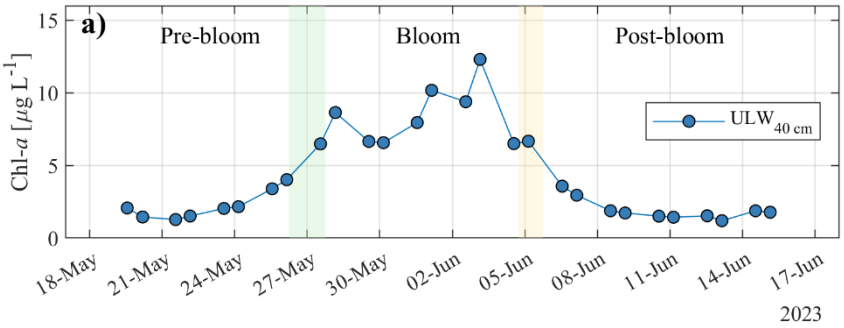

Figure S1: Chlorophyll-a across bloom phases. Data collected only from the ULW. Shaded bands indicate the onset of bloom (green) and the transition to bloom decay (yellow).

To complement how the marine carbon system evolved across bloom phases, we plotted salinity-normalised DIC (NDIC) against pH, with total alkalinity (NTA) as the colour scale and phase clusters identified (Figure S2). This approach allowed us to visualise how NDIC and pH shifted relative to NTA during the pre-bloom, bloom, and post-bloom periods. The pre-bloom cluster (blue) grouped at high NDIC (2000 to 2250 µmol kg^{-1}) and high NTA (>2200 µmol kg^{-1}), with comparatively low pH (8.27 to 8.49). During the bloom (green), the cluster shifted toward lower NDIC (1600 to 1900 µmol kg^{-1}) and intermediate NTA (2150 to 2250 µmol kg^{-1}), while pH rose sharply (8.54 to 8.81). In the post-bloom (yellow), NDIC stabilised at intermediate values (1600 to 1800 µmol kg^{-1}), NTA further decreased to 2050 to 2150 µmol kg^{-1}, and pH moderated to 8.41 to 8.54. These phase-dependent cluster movements show a consistent trajectory: from a buffered, high-NDIC and high-NTA and low-pH state in the pre-bloom, through bloom-driven carbon removal and pH elevation at lower NTA, to a post-bloom recovery with stabilised but altered carbonate chemistry.

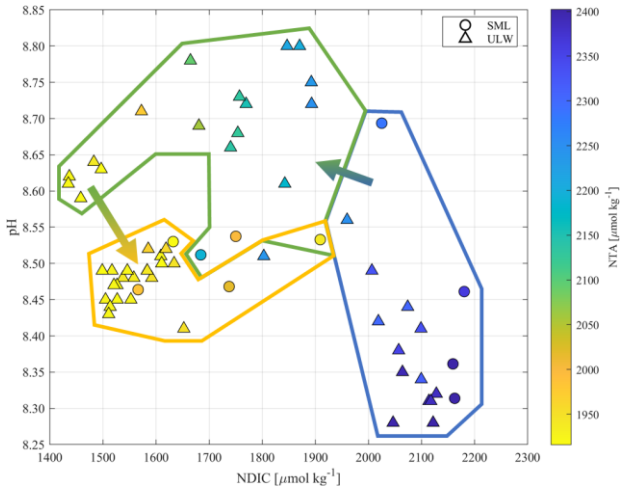

Figure S2: NDIC vs pH, with NTA clustering the pre-bloom phase in blue, the bloom phase in green, and the post-bloom phase in yellow.

S4. Direct vs Indirect Organic Alkalinity.

We directly measured the concentration of OA (OA_{meas}), by closed-cell back-titration as described above, and we assessed its reliability by comparing it with an indirect OA calculation (OA_{cal}) as it has been done before (Sharp and Byrne, 2020; Song et al., 2023). To compute OA_{cal} we first computed the theoretical inorganic TA (TA_{cal}) from the measured DIC-pH pairs using the CO2SYS v3.0 software (Pierrot, 2021). Then we subtracted the TA_{cal} to the directly measured TA (TA_{meas}), method described above, to get the OA_{cal} across layers and bloom phases. This approach allowed us to cross-validate OA measurements by contrasting two independent methods: a direct titration-based technique and an indirect calculation constrained by carbon system parameters. The comparison is critical in dynamic systems such as phytoplankton blooms, where organic matter composition and concentration can shift rapidly. By evaluating the consistency between both approaches, OA_{meas} vs OA_{cal}, we aimed to assess measurement robustness and to identify whether bloom-related changes in organic matter affected the reliability of OA_{cal}. This dual verification strengthens confidence in the OA dataset and provides insights into the limitations of indirect approaches when applied to environments strongly shaped by biological activity and variable organic matter pools.

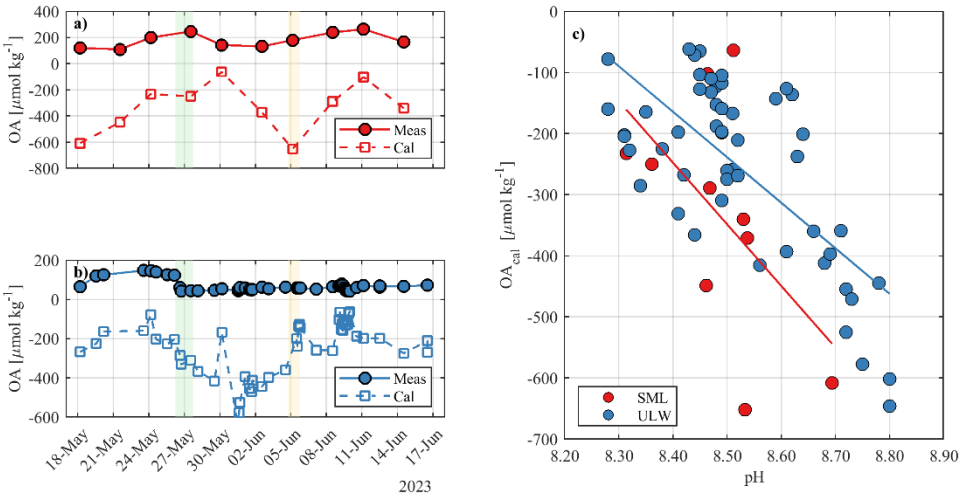

Figure S3: Directly OA_{meas} and indirectly OA_{cal} in the SML and ULW comparison. Shaded bands denote bloom start (green) and bloom end (yellow). Panel (c) plots OA_{cal} against pH for both layers, highlighting how OA_{cal} becomes more negative when pH increase up to 8.80.

To evaluate the reliability of indirect organic alkalinity estimates, we compared OA_{meas} with values calculated from DIC and pH pairs (OA_{cal}) across the SML and ULW (Figure S3). This approach allowed us to assess whether OA_{cal} can reproduce observed dynamics, particularly under the high-pH conditions of the mesocosm. OA_{meas} remained positive throughout the experiment, ranging from ~100 to 350 µmol kg^{-1} in the SML and showing consistent enrichment relative to the ULW. In contrast, OA_{cal} was systematically biased low, frequently yielding negative values and underestimating OA by more than 400 µmol kg^{-1} in the SML and dropping below -600 µmol kg^{-1} in the ULW. Further analysis revealed that OA_{cal} became increasingly negative when pH exceeded 8.50, indicating that TA_{cal} was overestimated at elevated pH. These results demonstrate that OA_{cal} is unreliable under bloom conditions due to nonlinearities in the carbonate system, and confirm that only direct titration provides robust OA estimates in this study.

S5. Supplementary Dataset

Table S3. Daily matched values of salinity-normalised dissolved inorganic carbon (NDIC), total alkalinity (NTA), measured organic alkalinity (OA_{meas}), percentage contribution of OA to TA (%OA to TA), pH, and calculated organic alkalinity (OA_{cal}) in the sea-surface microlayer (SML) and underlying water (ULW) across bloom phases.

		SML					
Phase	**Date**	**NDIC**	**NTA**	**OA_{meas}**	**%OA to TA**	**pH**	**OA_{cal}**
Pre-bloom	18-May	2025.07	2286.05	120.29	5.26	8.69	-537.92
	24-May	2162.42	2409.74	199.81	8.29	8.31	-185.03
	27-May	2159.18	2434.92	246.92	10.14	8.36	-198.86
Bloom	30-May	1684.22	2190.8	142.82	6.52	8.51	-13.99
	02-Jun	1750.19	1998.27	132.04	6.61	8.79	-317.57

	05-Jun	1909.22	1938.82	180.13	9.29	8.53	-592.73
Post-bloom	08-Jun	1737.64	2017.01	238.92	11.85	8.47	-237.40
	11-Jun	1566.27	1984.44	263.99	13.3	8.46	-54.32
	14-Jun	1632.17	1915.44	166.33	8.68	8.53	-285.48

		ULW					
	Date	**NDIC**	**NTA**	**OA_{meas}**	**%OA to TA**	**pH**	**OA_{cal}**
Pre-bloom	18-May	2018.71	2299.64	64.34	2.8	8.42	NaN
	24-May	2080.11	2390.99	142.83	5.97	8.3	NaN
	27-May	2006.48	2328.29	45.12	1.94	8.45	-252.40
Bloom	30-May	1802.92	2241.26	53.64	2.39	8.53	-112.92
	02-Jun	1664.78	2093.42	62.82	3	8.73	-379.29
	05-Jun	1461.83	1922.18	58.34	3.03	8.62	NaN
Post-bloom	08-Jun	1610.63	1916.98	63.78	3.33	8.48	-210.02
	11-Jun	1652.3	1951.34	71.1	3.64	8.45	-151.02
	14-Jun	1633.99	1947.85	66.38	3.41	8.47	-221.76

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