ENVIRONMENTAL RESEARCH
LETTERS

PURPOSE-LED PUBLISHING™

LETTER • OPEN ACCESS

Prey dynamics as a buffer: enhancing copepod resilience to ocean alkalinity enhancement

To cite this article: Amrita Bhaumik *et al* 2025 *Environ. Res. Lett.* **20** 024058

ENVIRONMENTAL RESEARCH
LETTERS

LETTER

Prey dynamics as a buffer: enhancing copepod resilience to ocean alkalinity enhancement

Amrita Bhaumik[1,*], **Giulia Faucher**[2], **Merle Henning**[1], **Cédric L Meunier**[1] and **Maarten Boersma**[1,3,4]

[1] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Biologische Anstalt Helgoland, Kurpromenade, 27483 Helgoland, Germany
[2] Biological Oceanography, GEOMAR Helmholtz Centre for Ocean Research Kiel, 24148 Kiel, Germany
[3] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Wadden Sea Station, Sylt, Hafenstraße 43, 25992 List, Germany
[4] FB2, University of Bremen, Bremen, Germany
[*] Author to whom any correspondence should be addressed.

E-mail: amrita.bhaumik@awi.de

Keywords: ocean alkalinity enhancement, carbon dioxide removal, negative emission technology, environmental impacts, copepod

Supplementary material for this article is available online

OPEN ACCESS

RECEIVED
8 October 2024

REVISED
6 January 2025

ACCEPTED FOR PUBLICATION
15 January 2025

PUBLISHED
4 February 2025

Abstract

Ocean alkalinity enhancement (OAE) aims to counteract climate change by increasing the ocean's carbon storage capacity through the addition of alkaline substances into seawater. However, this process alters seawater chemistry, increasing total alkalinity and pH, which can directly influence marine organisms' metabolic activities or indirectly impact them through changes in prey availability and quality. This study disentangled the OAE-driven factors that might influence zooplankton physiology. We assessed the direct effects of altered chemistry on the copepod, *Temora longicornis*, and the indirect effects through changes in the phytoplankton prey, *Rhodomonas salina*. We cultured the prey under OAE conditions and used it to feed copepods to investigate the indirect effects. We found that OAE negatively impacted prey growth but improved its nutritional quality, thereby offsetting the direct negative impact of OAE on the copepod. These findings regarding OAE's impact on prey-predator dynamics contribute to a deeper understanding of how OAE may influence zooplankton communities.

1. Introduction

While rapid reductions in CO_2 emissions are essential to limit global warming below 2 °C, climate models suggest achieving this goal will require the parallel application of carbon dioxide removal (CDR) approaches using negative emission technologies [1, 2]. Gigatons of atmospheric CO_2 need to be removed and either utilized or safely stored [3]. Oceans, which have sequestered one-fourth of anthropogenic CO_2 emissions since industrialization, could significantly contribute as CO_2 sink if their buffering capacity is restored [4, 5]. CO_2 not only dissolves in seawater but also reacts to form carbonic acid (H_2CO_3), which dissociates into bicarbonate (HCO_3^-) and hydrogen (H^+) ions. The HCO_3^- further breaks into carbonate (CO_3^{2-}) and H^+, reducing seawater pH and buffering capacity, causing Ocean Acidification (OA) and negatively affecting marine calcifying organisms [6], and reducing seawater's buffering capacity to take up more atmospheric CO_2 [7].

Ocean alkalinity enhancement (OAE) emerges as a promising CDR method that can be scaled to enhance seawater's buffering capacity and remove substantial amounts of atmospheric CO_2 without further acidifying the seawater [8]. OAE involves adding alkaline substances to increase seawater's total alkalinity (TA) [9]. These substances release proton acceptors that bind with proton donors' H^+, neutralizing acidity and shifting carbonate chemistry equilibrium towards HCO_3^- and CO [32–10]. Among various alkalizing substances, slaked lime ($Ca(OH)_2$) is notable for its worldwide availability [11], rapid dissolution[12], and low toxicity[13]. When added to seawater, slaked lime dissociates into calcium ions (Ca^{2+}) and hydroxide ions (OH^-) which react with

the H^+, leading to a pH increase. The remaining H^+ reacts with the dissolved CO_2 to form HCO_3^-, and CO_3^{2-}, thereby increasing seawater's TA. This process reduces the seawater's partial pressure of CO_2 (pCO_2), creating an imbalance between oceanic and atmospheric pCO_2 levels. Thus, the diffusive processes to equilibrate with the atmosphere foster the ocean's CO_2 uptake capacity [10]. During this equilibration, seawater pH remains elevated, with timescales varying from months to years depending on the physicochemical characteristics of the OAE application area [14]. In this CDR method, the elevated pH before equilibration may pose risks to marine life [15].

Several computational studies have assessed the efficiency of OAE as a CDR method [7, 16–20], with a recent focus on its ecological safety, particularly regarding phytoplankton [21–24]. However, studies on higher trophic levels like zooplankton, the most abundant metazoans globally, remain underexplored [25–27]. Copepods, which dominate zooplankton biomass, are globally distributed, with calanoid copepods contributing up to 80% [28]. Furthermore, copepods contribute significantly to the carbon flux and nutrient cycling by producing carbon- and nutrient-rich fecal pellets, molting exoskeletons, and performing diel vertical migration [29–31]. While alterations in copepod physiology can affect their roles in ecological and biogeochemical processes, no data currently exist on OAE's impacts on their metabolic activities, such as respiration and grazing. Moreover, copepods' metabolic activities are highly linked to their prey, the phytoplankton. Since OAE might directly affect the quantity or availability and nutritional quality of the prey[15], these changes can further indirectly affect the metabolic rates of the copepod.

In this study, we used a slaked lime-simulated OAE approach to manipulate seawater chemistry and study both the direct and indirect effects of OAE on the physiology of *Temora longicornis*, a calanoid copepod species prevalent in the northern hemisphere throughout the years [32, 33] and key prey for commercially relevant fish [34]. Since this copepod cannot store energy reserves, it constantly depends on the availability of high-quality prey [33] and could be sensitive to OAE-mediated seawater chemistry changes.

OAE likely affects phytoplankton growth by limiting carbon availability, due to lower pCO_2, leading to reduced prey availability for copepods with potential impacts on their metabolic rates. However, changes in carbon availability may also alter phytoplankton's elemental composition [35]. OAE-caused reduced carbon availability might result in phytoplankton with lower carbon-to-nutrient ratios, enhancing the nutritional quality of prey for copepods. To disentangle these potential OAE effects on *T. longicornis*, we carried out three sets of experiments aimed at separating the influence of seawater chemistry, prey availability, and prey quality changes on the copepods. In ***Experiment 1***, we investigated the direct impact of OAE-induced carbonate chemistry changes. In ***Experiment II***, we assessed the combination of the direct impact of OAE-induced carbonate chemistry changes and the indirect impact of OAE-influenced prey quality changes. In ***Experiment III***, we assessed the combination of the direct impact of OAE-induced carbonate chemistry changes and the indirect impact of OAE-influenced prey quality and availability changes.

2. Material and methods

2.1. Experimental design

Two trophic levels were considered to explore both the direct effects of OAE and the indirect effects through varying availability and quality of the prey, the cryptophyte *Rhodomonas salina* (Wislouch) (D. R. A. Hill and R. Wetherbee, 1989), on the physiology of the copepod *Temora longicornis* (Müller O. F., 1785). To disentangle these effects, three experiments were conducted to measure copepod's metabolic responses across six different TA levels.

In *Experiment I*, the direct effects of OAE on copepods were assessed by feeding them prey cultured in natural seawater without alkalinity manipulation (figure 1(A)). *Experiments II* and *III* aimed to investigate the further impacts of both direct and indirect OAE effects through altered prey conditions. In these experiments, the prey was cultured at the same six TA levels as the copepods. *Experiment II* focused on assessing the influence of prey quality by feeding copepods a consistent cell density of prey from corresponding TA cultures to ensure uniform food quantity across TA levels (figure 1(B)). *Experiment III* investigated the combined effects of prey availability and quality by feeding copepods equal volumes of prey cultures from corresponding TA levels, reflecting differences in food availability due to varying algae growth at each TA level (figure 1(C)).

2.2. Seawater chemistry alteration and measurement

To achieve target seawater TA levels, stock solutions of sodium hydroxide (NaOH) (Merck) and calcium chloride ($CaCl_2$) were prepared in Milli-Q water and added to UV-sterilized, filtered (0.2 μm) natural seawater. The CO2SYS program[36] was used to calculate the required stock solution volumes for six TA levels, increasing by 250 μmol l^{-1} increments, resulting in ΔTA levels of 0, 250, 500, 750, 1000, and 1250 μmol l^{-1}. The study was conducted at the Helgoland Roads long-term observation site (54°11′N, 07°54′E) in the southern North Sea (figure S1) [37]. During the study, the average TA of natural seawater was 2314 ($\pm$16.23) μmol l^{-1}. Therefore, the highest achieved TA level reached 3531

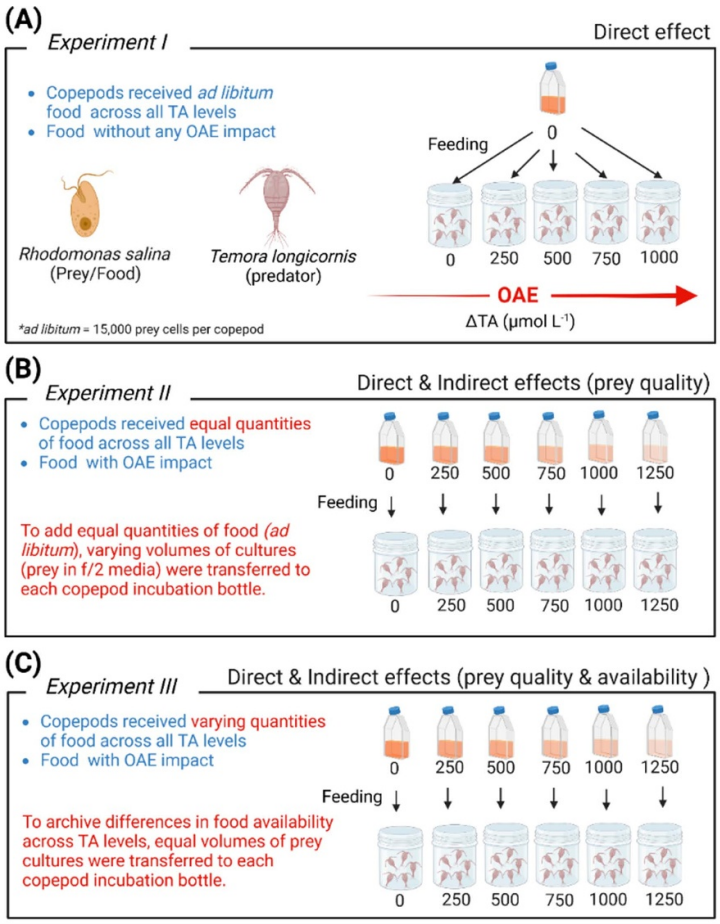

Figure 1. Graphical representation of the experimental setup. (A) Experimental setup for *Experiment I*, assessing the direct effect of OAE-induced carbonate chemistry changes on copepods. (B) Experimental setup for *Experiment II*, combining the indirect effect of OAE-driven prey quality changes with the direct impact of carbonate chemistry changes on copepods. (C) Experimental setup for *Experiment III*, combining the indirect effects of OAE-driven prey quality and availability changes with the direct impact of carbonate chemistry changes on copepods. All setups were maintained for four days, and copepods' metabolic rates were measured on day five. Created with BioRender.com.

($\pm$51.26) μmol l^{-1}. We simulated the slaked lime ($Ca(OH)_2$)-induced alkalinity enhancement, where 1 mole of $Ca(OH)_2$ removes 2 moles of CO_2 and produces 2 moles of HCO_3^- (equations (1) and (2)),

$$CaCl_2 + NaOH \rightarrow Ca(OH)_2 + 2NaCl \quad (1)$$

$$Ca(OH)_2 + 2CO_2 \rightarrow Ca^{2+} + 2HCO_3^-. \quad (2)$$

Post-manipulation, TA, and pH were measured, while temperature and salinity were recorded earlier to calculate the stock solution volumes. TA samples were filtered with non-pyrogenic sterile 0.2 μm filters (Sartorius) and stored at 6 °C until analysis. TA was determined by titration with 0.1 M sulfuric acid within an 855 Compact Titrosampler (Metrohm), and pH was measured with a probe (WTW MultiLine® Multi 3630 IDS). Additional carbonate chemistry parameters (e.g., *p*CO2, DIC) were calculated from the TA, pH, temperature, and salinity using the CO2SYS program, with stoichiometric equilibrium constants from Lueker *et al* [38] and default settings for other constants.

2.3. Copepod sampling and laboratory maintenance

Copepods were collected at the Helgoland Roads long-term observation site during spring 2023 (March–April) (figure S1), using an Apstein plankton net (150 μm mesh). Samples were transported in a cooling box with seawater to maintain the sampling site's temperature. In the laboratory, active *T. longicornis* at copepodite stages IV and V were picked under a stereomicroscope (Olympus SZX16) and transferred to 5 L bottles (~100 copepods per bottle) containing UV-sterilized, filtered (0.2 μm) natural seawater. Copepods were placed in a temperature-controlled room at 6 °C to replicate the sampling site conditions. Copepods were incubated for one day with adequate food, with a density of 15 000 prey cells per copepod, which is considered *ad libitum* food for the copepod's copepodite life stages. The next day, stock solutions were added to the copepod incubation bottles to achieve the desired TA levels. TA and pH were measured post-manipulation. The copepods were incubated at six TA levels for four days before measuring their respiration and grazing rates on the

fifth day. During incubation, the water was stirred gently with a glass rod three times daily to keep the prey suspended.

2.4. Culture and laboratory maintenance of copepod's prey

The prey, cryptophyte *Rhodomonas salina*, was cultured in f/2 media prepared with UV-sterile-filtered (0.2 μm) natural seawater in a temperature-controlled room at 18 °C, with a 12:12 h light/dark cycle at a photon flux of 180 μmol m^{-2} s^{-1} [39]. This culture was used to feed the copepods during the incubation period and the grazing experiment for each of the TA levels in *Experiment I*. During *Experiments II* and *III*, the prey was cultured also in six TA levels under the same temperature and light conditions in 600 ml culture flasks, which were sealed with lids fitted with filters. All cultures were maintained in triplicate sets for six days following the TA manipulation. As in *Experiment I*, these cultures were used to feed the copepods at corresponding ΔTA levels during the four-day incubation period in *Experiments II* and *III* and also used on the fifth day for the grazing experiment.

2.5. Copepod's feeding regime

In *Experiments I* and *II*, the copepods were provided with *ad libitum* prey incubated in each TA level. In these two experiments, the food quantity remained consistent across all TA levels, but the quality differed. In *Experiment I*, copepods received uniform-quality food across all TA levels because the prey was cultured under uniform conditions. In *Experiment II*, the copepod received varying qualities of food because the prey was cultured under different TA levels, leading to variation in its elemental composition. In *Experiment III*, the quantity of prey for copepods remained the same only at ΔTA 0 μmol l^{-1}, while it varied across the other TA levels due to the differing growth rates of the prey across TA levels. As a result, copepods in *Experiment III* received varying quantities and qualities of food across the TA levels.

Every day, we measured the prey cell density (*R. salina* cell numbers/volume of culture media) to calculate the required volume of prey culture to feed the copepods in *ad libitum*. In *Experiment I*, we had only a single culture at ΔTA 0 μmol l^{-1}. In *Experiment II*, the cell densities of prey were estimated daily for each ΔTA level to determine the required culture volume needed to provide the same quantity of prey to the copepods for every treatment. Therefore, we added different volumes of cultures to reach the *ad libitum* food for the copepod in each TA level. In *Experiment III*, the cell density of prey at ΔTA 0 μmol l^{-1} was measured daily to calculate the required volume to feed the copepods at the other five TA levels (from ΔTA 250 to ΔTA 1250 μmol l^{-1}). The estimated volume of prey culture from ΔTA 0 μmol l^{-1} was taken from each TA level to feed the copepods at the corresponding TA levels. Hence, each treatment received the same volume with different prey quantities, along with different prey quality.

2.6. Assessment of variation in Prey's quality & availability with increased TA

2.6.1. Prey's growth rate estimation

The cell density of each prey culture at different TA levels was documented every day by obtaining cell counts using the CASY particle counter (Schärfe System, Reutlingen, Germany). After six days of incubation, the growth rate (μ) was calculated using the following equation,

$$\mu\left(d^{-1}\right) = \frac{\left(\ln\left(N_1\right) - \ln\left(N_0\right)\right)}{t}.$$

Here, N_0 and N_1 are the number of cells at time t_0 and t_1, and t is the difference in time (d), in this case 6 d, between t_0 and t_1 samples.

2.6.2. Prey's photochemical efficiency estimation

The photochemical efficiency of photosystem II (F_v/F_m) was measured using the FastAct System and FastPro8 software (Chelsea Technologies Group). The samples were kept in the dark for at least 20 minutes at room temperature before measurement (Schreiber *et al* [40]). After TA manipulation, F_v/F_m measurements were taken for each culture from day one to day four. The mean F_v/F_m value was then calculated using the measurements obtained from triplicate cultures at each TA level. The following formula was used to calculate the F_v/F_m,

$$F_v/F_m = \left(F_m - F_0\right)/F_m$$

F_m and F_0 are the maximum and minimum fluorescence of the samples.

2.6.3. Prey's elemental composition analysis

The particulate Carbon (C), Nitrogen (N), and Phosphate (P) were measured to assess the elemental composition of prey. On day five, known quantities of prey from each TA level were filtered onto pre-combusted (500 °C for 24 h) glass microfiber filters (Whatman GF/F, 25 mm diameter). For C and N measurements, the filters were transferred to 6-well plates and dried (60 °C for at least 24 h). The dried filters were then folded in aluminium foil and stored in a desiccator until analyzed using a Vario Micro cube CHN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). For the P measurement, the filters were preserved in the freezer at −20 °C for subsequent analysis. The P content was determined as orthophosphate following acidic oxidative hydrolysis with 5% H_2SO_4 [41]. P levels were measured using an autoanalyzer (Thermo Scientific Multiskan® Spectrum) at an absorbance of 880 nm. The C:N, C:P, and N:P ratios were calculated as molar ratios.

2.7. Analysis of copepods' metabolic activity

In all experiments, after four days of incubation, the copepods' respiration and grazing rates were measured on the fifth day. Respiration rate was assessed by measuring O_2 consumption using a non-invasive optical fluorescence-based 24-channel oxygen respirometer (oxygen meter-SDR SensorDish Reader, PreSens Precision Sensing GmbH, Regensburg, Germany) [42] and gas-tight glass vials with a volume of 2.7 mL containing an O_2 sensor type PSt5 (PreSens, Regensburg, Germany). The O_2 consumption rate was determined by monitoring the decrease in dissolved O_2 concentration in seawater over time, detected by the SensorDish Reader, following the Schoo *et al* [35].

Three sets of glass sensor vials, in triplicate, were prepared to measure the copepods' respiration and grazing rates. (1) The first set of vials contained only filtered seawater from each TA level, which served as blanks, to detect any microbial respiration. (2) The second set of vials contained only prey in seawater for each TA level. This set was used as a control to quantify grazing rate and monitor the O_2 production or consumption by prey. (3) The third set of vials contained copepods and their prey in the filtered seawater from each TA level. The prey was added following the feeding regime outlined in the previous section. The initial density of added prey served as the basis for calculating grazing rates. Ten visually healthy and active copepods were carefully transferred from the 5 L incubation bottles to the first and second sets of vials. The experiment ran for approximately nine hours to attain a substantial reduction in O_2 concentration. The respiration rate was adjusted by subtracting the O_2 concentration obtained from the first set of vials and was also adjusted with the O_2 concentration obtained from the second set of vials.

After the O_2 content measurement, all vials containing prey were thoroughly mixed, and subsamples were taken to recount the cell numbers. Cell counting was conducted with the CASY particle counter (Schärfe System, Reutlingen, Germany). The grazing and ingestion rates were calculated using Frost's equations [43] and normalized to copepods' biomass (μg carbon) to determine weight-specific feeding rates. The respiration rate was similarly normalized to obtain weight-specific values.

2.8. Statistical analysis

A simple linear regression model was used to analyse the relationship between response variables (phytoplankton growth, elemental composition, copepod respiration, grazing rate, and photochemical efficiency), and TA levels as the continuous predictor. This model aimed to detect significant changes in the response variables due to TA levels. Additionally, a piecewise regression model was applied with a fixed breakpoint at ΔTA 500 μmol l^{-1}, determined through visual inspection of the scatterplot. This divided the data into two distinct linear segments, allowing the exploration of how the relationships differ before (from ΔTA 0–500 μmol l^{-1}) and after (from ΔTA 500–1250 μmol l^{-1}) the breakpoint. The significance level for all statistical tests was set at $p < 0.05$. Before fitting the models, normality, and homogeneity of variance were tested with the Shapiro–Wilk and Levene tests. Data sets were log-transformed if necessary to meet these assumptions. These tests were conducted using the '*car*' and '*stats*' packages. Simple linear regression analysis was performed using the '*lm*' function, while piecewise linear regression was conducted using the '*segmented*' package in RStudio (version 4.3.1, R core Team 2023). Data visualization was done using the '*ggplot2*' package [44].

2.9. OAE impact on seawater chemistry

The targeted changes in seawater carbonate chemistry were achieved. We observed a significant linear relationship between pH ($R^2 = 0.96$; $p < 0.05$) and pCO_2 ($R^2 = 0.97$, $p < 0.05$) with ΔTA. The shift in carbonate chemistry speciation increased the pH and decreased the pCO_2 with increasing TA (figures 2(B) and (C)).

2.10. OAE alters prey availability and nutritional quality

In *Experiments II* and *III*, copepods were fed prey cultured at different ΔTA levels. On day one, average cell densities ranged from 3.7 * 104–4.9 * 104 cells ml^{-1} across all TA levels. By day six, the average cell density at ΔTA 0 μmol l^{-1} had increased to 9.2 * 105 cells ml^{-1}, whereas at ΔTA 1250 μmol l^{-1} was nearly half, at 4.8 * 105 cells ml^{-1} (figure 3(A)). The density reduction was also documented in the prey growth rate, which showed a significant negative simple linear relationship with ΔTA ($R^2 = 0.82$, $p < 0.05$). However, the piecewise linear regression analysis revealed no significant relationship between growth rate and ΔTA in segment 1 (ΔTA $\leqslant$ 500 μmol l^{-1}). In segment 2 (ΔTA $\geqslant$ 500 μmol l^{-1}), the relationship remained significant ($R^2 = 0.77$, $p < 0.05$) (figure B).

The stoichiometry of prey was also affected by elevated TA. The C:P ($R^2 = 0.62$, $p < 0.05$) and N:P ($R^2 = 0.46$, $p < 0.05$) ratios decreased with increasing TA. Additionally, piecewise linear regression analysis indicated no significant relationship in segment 1; however, in segment 2, both C:P ($R^2 = 0.76$, $p < 0.05$) and N:P ratios ($R^2 = 0.58$, $p < 0.05$) showed significant relationships with increasing TA. Both ratios decreased significantly as TA increased (figures 4(E) and (F)). Conversely, no significant relationship was observed in the C:N ratios ($R^2 = 0.12$, $p > 0.05$) with TA (figure 4(D)). These changes in prey elemental composition are linked to Carbon (C) and Nitrogen (N) concentrations in prey cells, which significantly decreased with increasing TA (figures 4(A) and (B)).

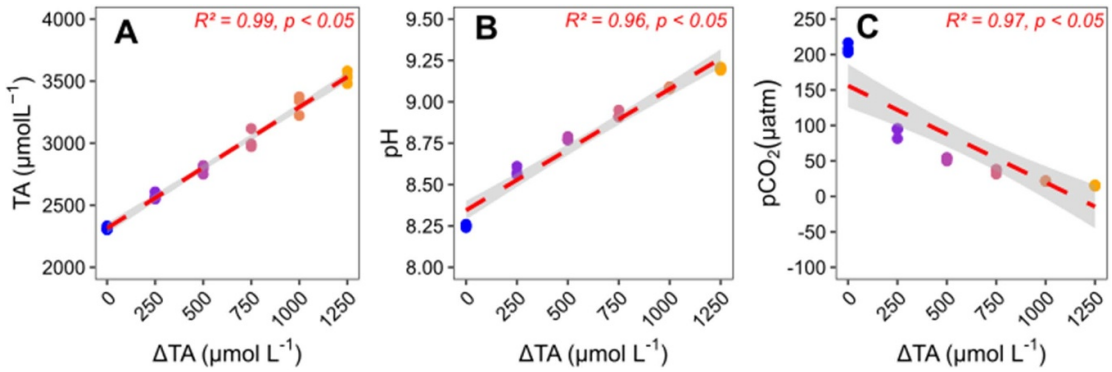

Figure 2. Influence of increased TA on seawater carbonate chemistry. (A) and (B) show the measured TA and pH values with increasing ΔTA. (C) shows the calculated pCO_2 values with increasing ΔTA. All Plots display the triplicate data sets. The dashed lines represent the linear regression fitted through the triplicate dataset, with red lines indicating the statistically significant ($p < 0.05$) relationship. The grey bands display the 95% confidence intervals.

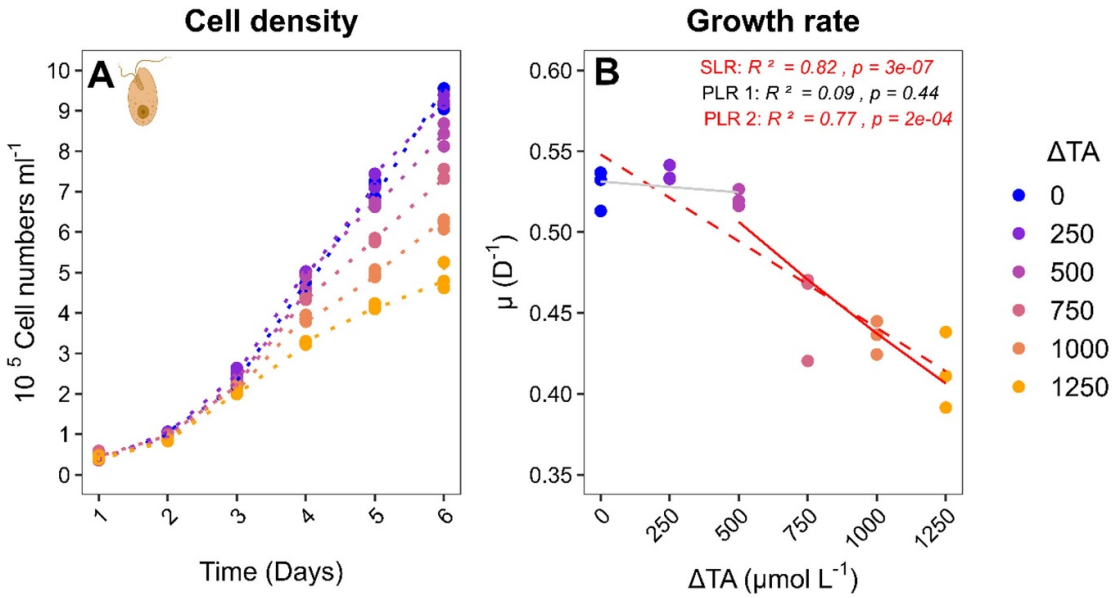

Figure 3. Impact of OAE on the prey availability for copepod T. longicornis. (A) shows the growth rate response of prey *R. salina* to elevated ΔTA. The dashed black line in (A) represents simple linear regression (SLR) fitted to the triplicate dataset, while the solid black line indicates the piecewise linear regression (PLR). The first segment, PLR 1, covers ΔTA 0–500 μmol l^{-1}, and the second segment, PLR 2, spans ΔTA 500–1250 μmol l^{-1}. R^2 and p-values were calculated for each segment, with statistical significance ($p < 0.05$) highlighted in red. (B) displays the temporal change of *R. salina* cell density.

The F_v/F_m levels remained relatively constant on day one, but significant variations appeared from day two onwards, with significant linear relationships on the third ($R^2 = 0.22$, $p < 0.5$) and fourth ($R^2 = 0.48$, $p < 0.5$) days (figures 5(A) and (B)). Additionally, temporal variations in F_v/F_m were observed, and statistically significant linear relationships were noted at ΔTA levels of 750, 1000, and 1250 μmol l^{-1} ($R^2 = 0.85, 0.89, 0.69$; $p > 0.05$) (figures S3 and (D)–(F)). Conversely, at ΔTA 0 and 250 μmol l^{-1}, the F_v/F_m was significantly increased with time ($R^2 = 0.52, 0.49$; $p > 0.05$) (figures S3, (A) and (B)). No significant relationship of F_v/F_m was observed at ΔTA 500 μmol l^{-1} over four days of OAE exposure ($R^2 = 0.08$, $p > 0.5$) (figures S3 and (C)).

2.11. OAE impact on Copepod's metabolic rates

A significant simple linear regression ($R^2 = 0.39$, $p < 0.05$) and significant piecewise linear regression ($R^2 = 0.58$, $p < 0.05$) were observed for the respiration rate in segment 1 in *Experiment I*, where the rate declined with increasing TA (figure 6(A)). In *Experiments II* and *III*, no significant relationship between respiration rate and ΔTA was observed (figures 6(B) and (C)).

Copepod's grazing rate showed no significant linear relationship with ΔTA in *Experiment I*, where only the direct effect of OAE was present (figure 6(C)). Similarly, in *Experiment III*, no significant relationship was observed when both prey quality and availability indirect factors were combined with

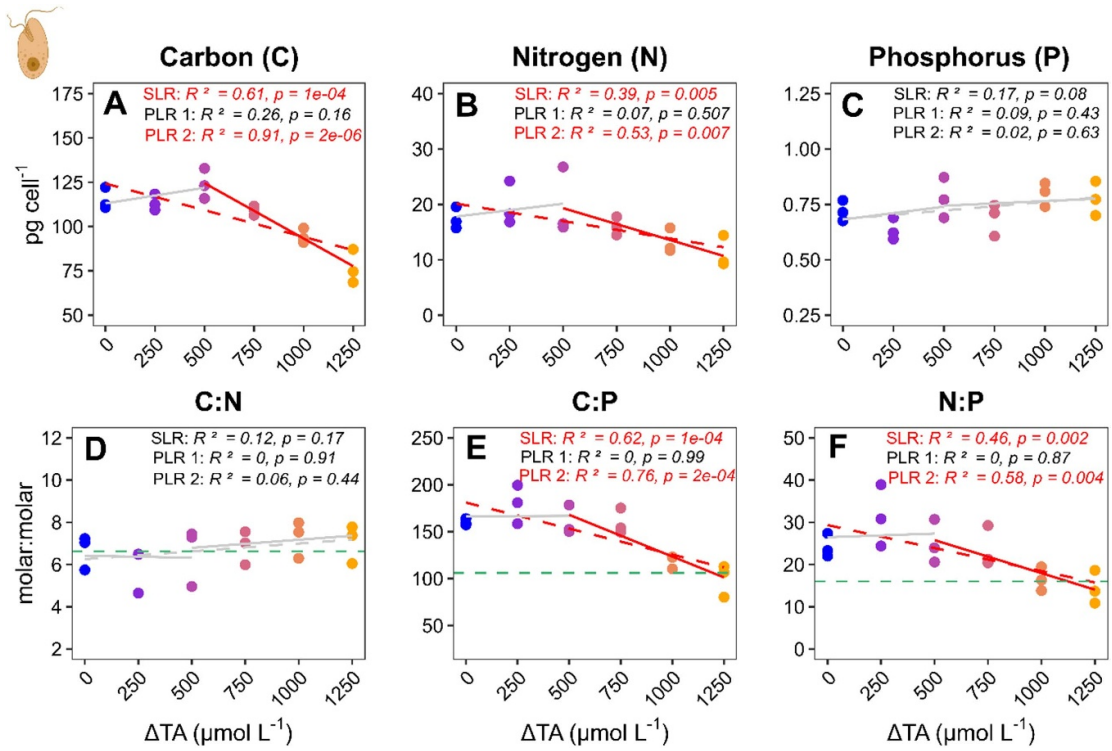

Figure 4. Impact of OAE on prey quality for copepod T. longicornis. Each plot illustrates the change in the elemental composition of prey *R. salina* with increasing ΔTA. The dashed line represents the simple linear regression (SLR) fitted to the triplicate dataset, while the solid line indicates the piecewise linear regression (PLR), divided into two segments. The first segment, PLR 1, covers ΔTA 0–500 μmol l^{-1}, and the second segment, PLR 2, spans ΔTA 500–1250 μmol l^{-1}. R^2 and p-values were calculated for each segment, with statistical significance ($p < 0.05$) highlighted in red. The dashed green lines indicate the Redfield ratio (C:N:P = 106:16:1), representing the standard for the optimal elemental composition in marine ecosystems.

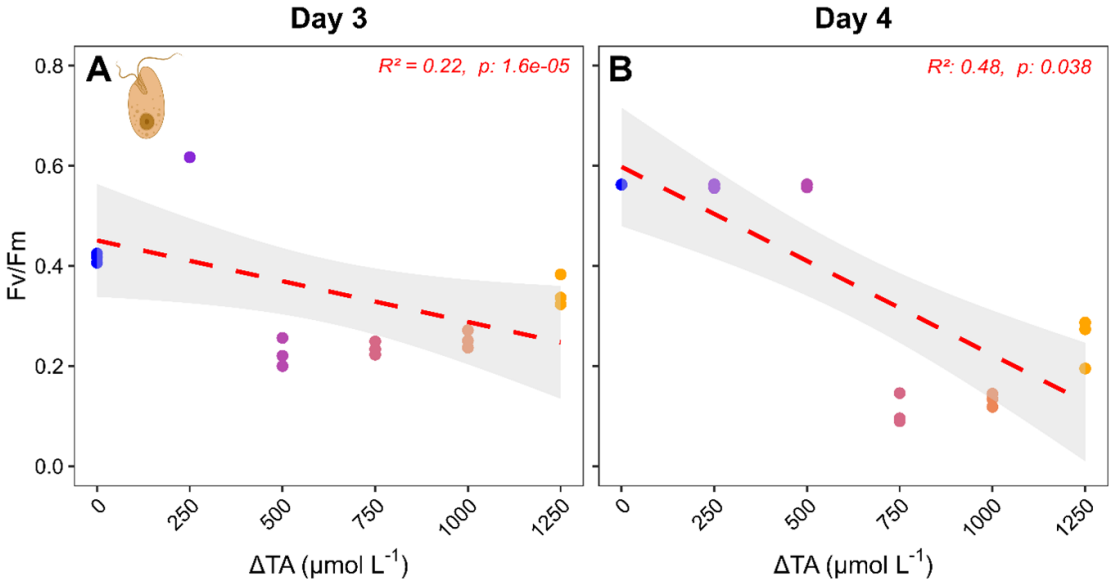

Figure 5. Impact of OAE on the prey's photochemical efficiency (F_v/F_m). (A) and (B) indicate the F_v/F_m change with increasing TA on days 3 and 4. The red dashed lines represent the linear regression fitted through the triplicate datasets and highlight the statistically significant ($p < 0.05$) relationships. The grey bands display the 95% confidence intervals.

the direct effect (figure 6(E)). However, grazing rates obtained from *Experiment II* showed a significant linear relationship with increasing TA (R^2 = 0.3, $p < 0.05$) when different qualities of prey were given (figure 6(D)), thus suggesting that the copepods consume more prey with lower C:P ratios.

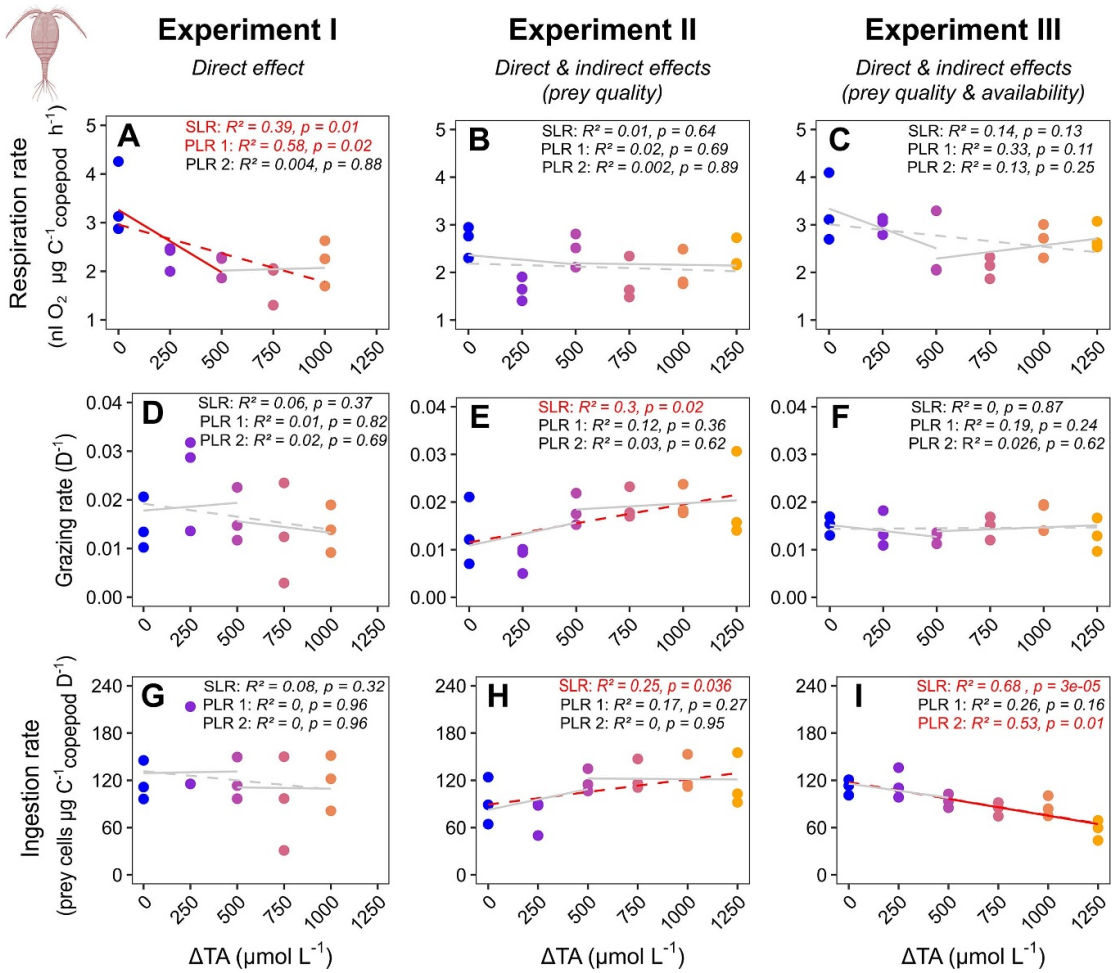

Figure 6. Impact of OAE on the metabolic rates of *T. longicornis*. (A)–(C) display the respiration rate of the copepod with elevated TA. (D)–(F) represent the copepod's grazing rate with increased TA. The dashed line represents the simple linear regression (SLR) fitted to the triplicate dataset, while the solid line indicates the piecewise linear regression (PLR), divided into two segments. The first segment, PLR 1, covers ΔTA 0–500 μmol l^{-1}, and the second segment, PLR 2, spans ΔTA 500–1250 μmol l^{-1}. R^2 and p-values were calculated for each segment, with statistical significance ($p < 0.05$) highlighted in red.

Similar trends were observed for ingestion rates in *Experiments II* and *III*. A non-significant relationship was observed in *Experiment I*, while a significant simple linear relationship ($R^2 = 0.25$, $p < 0.05$) was found in *Experiment II* (figures 6(G) and (H)). In contrast to the grazing rate, the ingestion rate in *Experiment III* exhibited a significant simple linear regression ($R^2 = 0.68$, $p < 0.05$) and significant piecewise linear regression ($R^2 = 0.53$, $p < 0.05$) in segment 2 with ΔTA. The ingestion rate decreased with increasing TA (figure 6(I)).

In *Experiment I*, we observed high copepod mortality in the highest TA treatment (ΔTA 1250 μmol l^{-1}, pH = 9.2). After four days of incubation, we could not find a sufficient number of live active copepods in the incubation bottles at ΔTA 1250 μmol l^{-1} to conduct the experiments to measure respiration and grazing rate. Thus, the data points for both respiration and grazing rates at ΔTA 1250 μmol l^{-1} are missing (figures 6(A) and (D)). However, we did not experience the same during *Experiments II*, and *III*, possibly due to the prey's quality improvement (lower C:P ratios) with increasing TA. Although it was an interesting observation, we do not have quantitative data on the copepod mortality rates due to limited manpower during the experiments.

3. Discussion

3.1. Relevant seawater chemistry changes for copepods and their prey

Studies have reported that increasing TA through OAE has the potential to sequester CO_2 by converting it into other forms of inorganic carbon, thereby enhancing the ocean's buffering capacity [17, 45]. In our experiments, the carbonate chemistry changed as predicted, with increased pH and decreased pCO_2 when we elevated the TA. Since the experiments were conducted during the spring, it is not surprising that we recorded remarkably low pCO_2 (~224 μatm), even in the control treatments. This aligns with prior field studies in coastal waters that reported reductions in pCO_2 down to ~200 μatm or lower, and attributed

these values to heightened primary production during the spring bloom [46, 47]. After OAE application, pCO_2 levels dropped significantly, reaching as low as 15 ($\pm$0.73) μatm in the highest TA treatment. This suggests that applying OAE during a spring bloom considerably reduces CO_2 availability [48]. Such critically low pCO_2 levels can directly impact the phytoplankton growth, which, in turn, affects zooplankton food availability. Bach *et al* [15] already specified that if pCO_2 drops below ~100 μatm, phytoplankton growth can decline based on several previous experimental studies [49–51].

3.2. OAE directly affects the abundance and nutritional value of copepods' prey

We investigated the direct OAE impact on prey, focusing on changes in prey's availability and nutritional quality to understand the indirect OAE impact on copepods. We observed a significant negative effect of increased TA on the prey growth rate, attributed to CO_2 limitation and pH increase. Since seawater CO_2 levels are typically below phytoplankton requirements, species employ carbon concentrating mechanisms (CCMs) to elevate internal CO_2 concentrations in response to limited carbon availability [52, 53]. Therefore, OAE-derived pCO_2 reduction during the spring bloom might have heightened the pressure on CCM function, potentially triggering the oxygenase reaction, which requires more energy and reduces the cell's growth rate [53]. Additionally, the OAE-induced high pH levels might have increased the cell's energy costs to maintain pH homeostasis [50], reducing *R. salina*'s physiological efficiency. F_v/F_m or the maximum quantum yield of photochemistry values at higher TA levels also indicated reduced photosynthetic competence, suggesting that prey cells were under stress.

The OAE influence on *R. salina*'s photosynthetic and enzymatic activity likely altered the cell's energy budget, reflected in elemental composition changes. While the C:N ratio remained stable with increasing TA, the C:P, and N:P ratios were decreased, approaching the Redfield ratio in the two highest ΔTA treatments. Prey with a lower C:P ratio is considered more nutritious [54]. *R. salina* was cultured in nutrient-rich f/2 media, therefore, the reduction in the C:P ratio was caused by reduced carbon availability. However, consistent C:N ratios across the TA levels, despite the lower carbon availability, suggest reduced nitrogen solubility at high pH [55].

Previous studies support our findings of lower growth rates in high pH for various phytoplankton and microzooplankton species [55–57]. Taraldsvik and Myklestad [56] observed that a diatom species exhibited a lower growth rate at pH levels above 9, likely due to compromised cells' membrane transport and enzymatic activities. Pedersen and Hansen [57] also reported declining growth rates of three ciliate species at pH 8.8 and 8.9 and a reduced growth rate of a dinoflagellate species at pH 9.2. Hansen *et al* [55] linked a dinoflagellate's reduced growth rate due to high pH of 9.2, while Bach *et al* [50] suggested CO_2 limitation as the main factor. Similar to our findings, Taraldsvik and Myklestad [56] reported a constant C:N ratio in a diatom species with increased pH ranging from 6.5 to 9.4, with decreased organic carbon at pH $>$ 9 and organic nitrogen limitations. In contrast, OA studies reported increased phytoplankton C-to-nutrient ratios at lower pH levels due to higher CO_2 availability, which was linked to the poorer nutritional quality of prey [35, 58] and resulted in reduced zooplankton fitness [58, 59].

Our study suggests that OAE-induced changes in seawater pH and carbon availability directly reduced prey growth rate and altered prey's nutritional quality, resulting in copepods receiving prey of improved quality in reduced quantities. Specifically, we observed a threshold of ΔTA 500 μmol l^{-1}, beyond which prey was significantly impacted. This threshold is crucial because it suggests that changes in prey quality and growth rates were minimal below this point but became pronounced as ΔTA exceeded 500 μmol l^{-1}. This non-linear response implies that the effects of OAE on prey dynamics are not gradual but exhibit a significant shift when the alkalinity surpasses this threshold.

3.3. Improved prey quality indirectly mitigates direct OAE impact on copepods

We investigated the potential impact of OAE on copepods, focusing on the direct impact of carbonate chemistry changes and the indirect impact through prey availability and quality. In *Experiment I*, we observed a significant reduction in copepod respiration rate and a decreasing trend in grazing rate with elevated TA. These findings suggest that copepods struggled to maintain regular metabolic activities under higher alkaline conditions, likely due to physiological stress. The observed high mortality at the highest TA treatment (ΔTA 1250 μmol l^{-1} and pH 9.2) further supports the idea that extreme alkalinity disrupts copepod homeostasis.

The observed reduction in respiration rates in *Experiment I* could be attributed to disruptions in enzymatic activities essential for metabolic processes. Copepod respiration involves phases of metabolic demand for eliminating CO_2 and acquiring O_2, the exchange of these two respiratory gases both internally and externally, and the internal transport of gases between the respiratory surface and the metabolizing protoplasm [60]. Increased pH might disrupt these processes that rely on enzymatic activities, which are often sensitive to pH variations [61]. Altered pH might also have influenced the permeability of the copepod's respiratory membrane, potentially affecting the gas exchange efficiency. Additionally, the copepod's reduced metabolic rates might be the result of physiological stress, as copepods likely shifted

metabolic demands in attempting to adapt to the alkaline conditions.

In contrast, *Experiments II* and *III* demonstrated improved copepod tolerance to elevated TA levels, with no significant reductions in respiration rates. This difference highlights the critical role of prey quality in mitigating the physiological stress induced by OAE. In these experiments, copepods were provided with prey of higher nutritional quality (lower C:P ratios), which likely helped offset the metabolic challenges posed by changes in seawater chemistry.

The interplay between prey quality and availability also influenced copepod grazing and ingestion rates. The grazing rate reflects the proportion of prey removed by the copepods. In *Experiment II*, grazing rates increased significantly with higher TA levels, indicating that copepods consumed more of the high-quality prey to compensate for the stress caused by carbonate chemistry changes. This compensatory behaviour aligns with the idea that copepods can adjust their feeding activity to cope with environmental stressors when sufficient prey is available.

In *Experiment III*, where prey availability was reduced, grazing rates remained stable, but ingestion rates declined significantly with increasing TA. The grazing rate remained constant because copepods maintained their feeding frequency despite the reduced prey availability. However, the ingestion rate, which measures the total amount of prey consumed by individual copepods, declined, as fewer prey encounters resulted from lower prey abundance. This suggests that while copepods maintained their grazing behaviour, the total amount of prey ingested was limited by prey quantity. The improved quality of prey enabled copepods to sustain their feeding frequency and metabolic stability, but the reduced prey availability ultimately constrained their total food intake. Thus, although high prey quality can sustain feeding behavior, prey quantity remains a critical limiting factor for copepod energy acquisition under OAE conditions.

The results also underscore the pivotal role of prey quality in maintaining respiration rates at higher TA levels. Even in *Experiment III*, where prey availability was reduced, the high-quality prey prevented significant reductions in respiration and grazing rates, suggesting that improved quality prey even in lower quantities can mitigate the negative effects of OAE-induced pH changes effectively. These results are supported by earlier studies that reported the importance of prey quality over quantity for copepods' metabolic activities [35, 54, 58, 59, 62, 63]. Overall, this study highlights the complex interactions between prey quality, prey availability, and carbonate chemistry changes in shaping copepod metabolic and feeding responses. By demonstrating the compensatory effects of high-quality prey, our findings provide insights into the potential resilience of copepods to OAE under varying prey conditions.

Previous studies reported the impact of high pH on the survivability and growth of microzooplankton and zooplankton communities, but there is, to the best of our knowledge, no available data on the effects of OAE on copepod physiology to corroborate our findings. Pedersen and Hansen [64] studied the effect of a high pH range, starting from 8 to 9.5 on a natural planktonic community consisting of copepods for two weeks, and reported a slight copepod abundance increase over time at pH 8, but the abundance decreased at pH 8.5, which indicated mortality at higher pH. Similar to our observation of high mortality in the highest treatment at pH 9.2, Pedersen and Hansen [64] also observed that copepods did not survive at pH 9 and 9.5 after 5 days of incubation. Camatti *et al* [27] also reported a significant negative impact on the survivability of a copepod species as a response to long-term exposure (>6 hours) at pH 10 and 11. Camatti *et al* [27] did not observe any negative effects on copepods at pH 9 in shorter exposure times (<6 hours), suggesting that pH 9 may represent a threshold level where copepods can tolerate short-term pH fluctuations.

4. Conclusions

In conclusion, our study demonstrated a direct impact of OAE-induced pH increases on the respiration rate of *T. longicornis*, though this effect was mitigated when combined with the influence of elevated prey quality. Improved prey quality supports the copepod to cope with the physiological stress induced by the carbonate chemistry perturbation and reduced prey availability. Any changes in energy expenditure, such as respiration rate, and energy input, like grazing rate and ingestion rate, can also impact other physiological functions like egg production, development, and growth of copped. These make respiration, grazing, and ingestion rates direct indicators of overall metabolic activity in copepods. Although we did not observe any significant impact on the copepod's respiration and grazing rates when OAE-induced carbonate chemistry changes, and the prey alteration combined (*Experiment III*), we did observe a significant reduction in the ingestion rate with increasing TA, largely attributed to reduced prey availability. While copepods continued to graze at the same frequency, the reduced prey abundance under elevated TA conditions led to fewer encounters with prey, resulting in lower total prey intake. This highlights the critical role of prey quantity in determining ingestion rates, in contrast to grazing rates, which remained unaffected by the prey availability changes. While

improved prey quality was sufficient to compensate for reduced prey availability and helped the copepod maintain its respiration rate, the reduced prey availability still impacted the ingestion rate. The reduction in ingestion rate further emphasizes the importance of both prey quality and prey quantity in regulating copepods' metabolic processes.

In the natural environment, higher nutritional quality prey grown under elevated TA might support overall copepod density. Our study highlights the complex interplay between seawater chemistry, prey dynamics, and copepod physiology, emphasizing the need for further research on the overall planktonic community to understand the ecological consequences of OAE. The OAE impact on earlier life stages of copepod, specifically the nauplius stages, could be more pronounced, as these stages are known to be more sensitive to environmental changes [25, 65, 66]. Additionally, if the impact of OAE is species-specific, as observed in OA research, species with higher tolerance to high pH may have a competitive advantage, potentially altering the zooplankton community structure. It is crucial to study OAE impacts across copepod species, as metabolic responses could vary depending on species-specific acid-base regulation [67]. Similarly, studies involving diverse phytoplankton species are recommended, as different species may exhibit distinct responses to OAE. Moreover, long-term, multi-generational studies on the entire plankton community are needed to address adaptive responses and the feasibility of continuous or repeated OAE applications in the same deployment area.

Data and materials availability

The data that support the findings of this study will be made openly available soon at the following URL/DOI: https://www.pangaea.de/.

Acknowledgment

We are grateful to Niels Suitner (Universität Hamburg) and Ragna Bergmann (AWI) for assisting with TA and Phosphate measurements and to Julia Haafke (AWI) for measuring the Carbon and Nitrogen content. We would also like to acknowledge Dr. Stephen D Archer (Bigelow Laboratory for Ocean Sciences) for providing the FastAct instrument. This study was supported by the German Federal Ministry of Education and Research (BMBF), Project RETAKE, in the framework of the German Marine Research Alliance (DAM) research mission 'Marine carbon sinks in decarbonization pathways' (CDRmare), Grant No. 03F0895. Additionally, GF was supported by the Carbon to Sea Initiative via the Ocean Alk-Align project. Open Access funding enabled and organised by Project DEAL.

Author contribution

Conceptualization: AB, and MB. Methodology: AB, and MB. Investigation: AB, and MH. Visualization: AB. Supervision: MB, and CM. Writing-original draft: AB. Writing-review & editing: AB, MB, CM, GF, and MH.

Conflict of interest

The authors declare that they have no competing interests.

ORCID iDs

Amrita Bhaumik https://orcid.org/0000-0003-0575-7726
Giulia Faucher https://orcid.org/0000-0001-8930-477X
Merle Henning https://orcid.org/0009-0002-8087-966X
Cédric L Meunier https://orcid.org/0000-0002-4070-4286
Maarten Boersma https://orcid.org/0000-0003-1010-026X

References

[1] Intergovernmental Panel on Climate Change (IPCC) 2023 Oceans and coastal ecosystems and their services *Climate Change 2022—Impacts, Adaptation and Vulnerability* (Cambridge University Press) pp 379–550
[2] Tokarska K B, Stolpe M B, Sippel S, Fischer E M, Smith C J, Lehner F and Knutti R 2020 Past warming trend constrains future warming in CMIP6 models *Sci. Adv.* **6** eaaz9549
[3] Intergovernmental Panel on Climate Change (IPCC) 2023 *Climate Change 2022—Impacts, Adaptation and Vulnerability* (Cambridge University Press)
[4] Von Schuckmann K *et al* 2020 Heat stored in the Earth system: where does the energy go? *Earth Syst. Sci. Data* **12** 2013–41
[5] Friedlingstein P *et al* 2022 Global carbon budget 2022 *Earth Syst. Sci. Data* **14** 4811–900
[6] Guinotte J M and Fabry V J 2008 Ocean acidification and its potential effects on marine ecosystems *Ann. New York Acad. Sci.* **1134** 320–42
[7] Lenton A, Matear R J, Keller D P, Scott V and Vaughan N E 2018 Assessing carbon dioxide removal through global and regional ocean alkalinization under high and low emission pathways *Earth Syst. Dyn.* **9** 339–57
[8] Oschlies A, Bach L T, Rickaby R E M, Satterfield T, Webb R and Gattuso J-P 2023 Climate targets, carbon dioxide removal, and the potential role of ocean alkalinity enhancement *State Planet* **2-oae2023** 1–9
[9] Kheshgi H S 1995 *Sequestering Atmospheric Carbon Dioxide by Increasing Ocean Alkalinity* vol 20
[10] Hartmann J *et al* 2013 Enhanced chemical weathering as a sink for carbon dioxide, a nutrient source and a strategy to mitigate ocean acidification enhanced chemical weathering as a geoengineering strategy to reduce atmospheric carbon dioxide, supply nutrients, and mitigate ocean acidification *Rev. Geophys.* **51** 113–9

[11] Caserini S, Storni N and Grosso M 2022 The availability of limestone and other raw materials for ocean alkalinity enhancement *Glob. Biogeochem. Cycles* **36** e2021GB007246
[12] Renforth P, Baltruschat S, Peterson K, Mihailova B D and Hartmann J 2022 Using ikaite and other hydrated carbonate minerals to increase ocean alkalinity for carbon dioxide removal and environmental remediation *Joule* **6** 2674–9
[13] Flipkens G, Blust R and Town R M 2021 Deriving nickel (Ni(II)) and chromium (Cr(III)) based environmentally safe olivine guidelines for coastal enhanced silicate weathering *Environ. Sci. Technol.* **55** 12362–71
[14] He J and Tyka M D 2023 Limits and CO_2 equilibration of near-coast alkalinity enhancement *Biogeosciences* **20** 27–43
[15] Bach L T, Gill S J, Rickaby R E M, Gore S and Renforth P 2019 CO_2 removal with enhanced weathering and ocean alkalinity enhancement: potential risks and co-benefits for marine pelagic ecosystems *Front. Clim.* **1** 476698
[16] Butenschön M, Lovato T, Masina S, Caserini S and Grosso M 2021 Alkalinization scenarios in the mediterranean sea for efficient removal of atmospheric CO_2 and the mitigation of ocean acidification *Front. Clim.* **3** 614537
[17] Wang H, Pilcher D J, Kearney K A, Cross J N, Shugart O M, Eisaman M D and Carter B R 2023 Simulated impact of ocean alkalinity enhancement on atmospheric CO_2 removal in the bering sea *Earths Future* **11** e2022EF002816
[18] Fakhraee M, Li Z, Planavsky N and Reinhard C 2022 Environmental impacts and carbon capture potential of ocean alkalinity enhancement *Research Square* (https://doi.org/10.21203/rs.3.rs-1475007/v1)
[19] Keller D P, Feng E Y and Oschlies A 2014 Potential climate engineering effectiveness and side effects during a high carbon dioxide-emission scenario *Nat. Commun.* **5** 3304
[20] Hauck J, Köhler P, Wolf-Gladrow D and Völker C 2016 Iron fertilisation and century-scale effects of open ocean dissolution of olivine in a simulated CO_2 removal experiment *Environ. Res. Lett.* **11** 024007
[21] Ferderer A, Chase Z, Kennedy F, Schulz K G and Bach L T 2022 Assessing the influence of ocean alkalinity enhancement on a coastal phytoplankton community *Biogeosciences* **19** 5375–99
[22] Gately J A, Kim S M, Jin B, Brzezinski M A and Iglesias-Rodriguez M D 2023 Oceanography Coccolithophores and diatoms resilient to ocean alkalinity enhancement: a glimpse of hope? *Sci. Adv.* **9** eadg6066
[23] Oberlander J L, Burke M E, London C A and Macintyre H L 2024 Assessing the impacts of simulated ocean alkalinity enhancement on viability and growth of near-shore species of phytoplankton
[24] Ramírez L, Pozzo-Pirotta L J, Trebec A, Manzanares-5 Vázquez V, Díez J L, Arístegui J, Riebesell U, Archer S D and Segovia M 2024 Ocean alkalinity enhancement (OAE) does not cause cellular stress in a phytoplankton community of the sub-tropical Atlantic Ocean
[25] Pedersen S A, Hansen B H, Altin D and Olsen A J 2013 Medium-term exposure of the North Atlantic copepod Calanus finmarchicus (Gunnerus, 1770) to CO_2-acidified seawater: effects on survival and development *Biogeosciences* **10** 7481–91
[26] Gim B-M, Hong S, Lee J-S, Kim N-H, Kwon E-M, Gil J-W, Lim H-H, Jeon E-C and Khim J S 2018 Potential ecotoxicological effects of elevated bicarbonate ion concentrations on marine organisms *Environ. Pollut.* **241** 194–9
[27] Camatti E, Valsecchi S, Caserini S, Barbaccia E, Santinelli C, Basso D and Azzellino A 2024 Short-term impact assessment of ocean liming: a copepod exposure test *Mar. Pollut. Bull.* **198** 115833
[28] Hammill E, Johnson E, Atwood T B, Harianto J, Hinchliffe C, Calosi P and Byrne M 2018 Ocean acidification alters zooplankton communities and increases top-down pressure of a cubozoan predator *Glob. Change Biol.* **24** e128–38
[29] Hernandez-Leon S 2004 A global assessment of mesozooplankton respiration in the ocean *J. Plankton Res.* **27** 153–8
[30] Castellani C and Altunbaş Y 2014 Seasonal change in acclimatised respiration rate of Temora longicornis *Mar. Ecol. Prog. Ser.* **500** 83–101
[31] Juul-Pedersen T, Nielsen T, Michel C, Friis Møller E, Tiselius P, Thor P, Olesen M, Selander E and Gooding S 2006 Sedimentation following the spring bloom in Disko Bay, West Greenland, with special emphasis on the role of copepods *Mar. Ecol. Prog. Ser.* **314** 239–55
[32] Halsband C and Hirche H 2001 Reproductive cycles of dominant calanoid copepods in the North Sea *Mar. Ecol. Prog. Ser.* **209** 219–29
[33] Kreibich T, Saborowski R, Hagen W and Niehoff B 2011 Influence of short-term nutritional variations on digestive enzyme and fatty acid patterns of the calanoid copepod Temora longicornis *J. Exp. Mar. Biol. Ecol.* **407** 182–9
[34] Ringuette M, Castonguay M, Runge J A and Grégoire F 2002 Atlantic mackerel (Scomber scombrus) recruitment fluctuations in relation to copepod production and juvenile growth *Can. J. Fish. Aquat. Sci.* **59** 646–56
[35] Schoo K L, Malzahn A M, Krause E and Boersma M 2013 Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore *Mar. Biol.* **160** 2145–55
[36] P D E, W D W R and L E 2011 MS excel program developed for CO_2 system calculations *Carbon Dioxide Information Analysis Center*
[37] Dummermuth A *et al* 2023 Marine stations helgoland and sylt operated by the Alfred Wegener institute helmholtz centre for polar and marine research *J. Large-scale Res. Facil.* **8**
[38] Lueker T J, Dickson A G and Keeling C D 2000 *Ocean pCO Calculated from Dissolved Inorganic Carbon, 2 Alkalinity, and Equations for K and K : Validation Based on 1 2 Laboratory Measurements of CO in Gas and Seawater at 2 Equilibrium* vol 70
[39] Guillard R R L and Ryther J H 1962 Studies of marine planktonic diatoms: i. cyclotella nana hustedt, and detonula confervacea (cleve) gran *Can. J. Microbiol.* **8** 229–39
[40] Schreiber U B W N, Bilger W and Neubauer C 1995 Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis *Ecophysiology of Photosynthesis* pp 49–70 (Springer)
[41] Anon Andreae-Determination_As_Sb_Ge-Methods_Seawater_Analysis_1999
[42] Harris R *et al* eds 2000 *ICES Zooplankton Methodology Manual* (Elsevier)
[43] Frost B W 1972 Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod calanus pacificus *Limnol. Oceanogr.* **17** 805–15
[44] Wickham H 2016 *Getting Started with Ggplot2* pp 11–31
[45] Hartmann J, Suitner N, Lim C, Schneider J, Marín-Samper L, Arístegui J, Renforth P, Taucher J and Riebesell U 2023 Stability of alkalinity in ocean alkalinity enhancement (OAE) approaches—consequences for durability of CO_2 storage *Biogeosciences* **20** 781–802
[46] Thomas H, Bozec Y, Elkalay K, De Baar H J W, Borges A V and Schiettecatte L-S 2005 *Controls of the Surface Water Partial Pressure of CO_2 in the North Sea* vol 2
[47] Honkanen M, Müller J D, Seppälä J, Rehder G, Kielosto S, Ylöstalo P, Mäkelä T, Hatakka J and Laakso L 2021 The diurnal cycle of pCO_2 in the coastal region of the Baltic Sea *Ocean Sci.* **17** 1657–75
[48] Paul A, Haunost J, Goldenberg M, S U, Hartmann J, Sánchez N, Schneider J, Suitner N and Riebesell U 2024 Ocean alkalinity enhancement in an open ocean ecosystem: biogeochemical responses and carbon storage durability
[49] Sett S, Bach L T, Schulz K G, Koch-Klavsen S, Lebrato M and Riebesell U 2014 Temperature modulates coccolithophorid

sensitivity of growth, photosynthesis and calcification to increasing seawater pCO_2 *PLoS One* **9** e88308
[50] Bach L T, Riebesell U and Schulz K G 2011 Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore Emiliania huxleyi *Limnol. Oceanogr.* **56** 2040–50
[51] Bach L T, Riebesell U, Gutowska M A, Federwisch L and Schulz K G 2015 A unifying concept of coccolithophore sensitivity to changing carbonate chemistry embedded in an ecological framework *Prog. Oceanogr.* **135** 125–38
[52] Raven J A, Beardall J and Sánchez-Baracaldo P 2017 The possible evolution and future of CO_2-concentrating mechanisms *J. Exp. Bot.* **68** 3701–16
[53] Moreno H D, Rokitta S, Tremblay N, Boersma M, Groß E, Klip H C L, Wiltshire K H and Meunier C L 2024 Higher temperature, increased CO_2, and changing nutrient ratios alter the carbon metabolism and induce oxidative stress in a cosmopolitan diatom *Limnol. Oceanogr.* **69** 121–39
[54] Schoo K L, Aberle N, Malzahn A M, Boersma M and Browman H 2012 Food quality affects secondary consumers even at low quantities: an experimental test with larval european lobster *PLoS One* **7** e33550
[55] Hansen P, Lundholm N and Rost B 2007 Growth limitation in marine red-tide dinoflagellates: effects of pH versus inorganic carbon availability *Mar. Ecol. Prog. Ser.* **334** 63–71
[56] Taraldsvik M and Myklestad S 2000 The effect of ph on growth rate, biochemical composition and extracellular carbohydrate production of the marine diatom skeletonema costatum *Eur. J. Phycol.* **35** 189–94
[57] Pedersen F M and Hansen P J 2003 Effects of high pH on the growth and survival of six marine heterotrophic protists *Mar. Ecol. Prog. Ser.* **260** 33–41
[58] Meunier C L, Algueró-Muñiz M, Horn H G, Lange J A F and Boersma M 2017 Direct and indirect effects of near-future pCO_2 levels on zooplankton dynamics *Mar. Freshwater Res.* **68** 373–80
[59] Malzahn A M and Boersma M 2012 Effects of poor food quality on copepod growth are dose dependent and non-reversible *Oikos* **121** 1408–16
[60] Wolvekamp H P and Waterman T H 1960 Respiration *Metabolism and Growth* (Elsevier) pp 35–100
[61] Freese D, Kreibich T and Niehoff B 2012 Characteristics of digestive enzymes of calanoid copepod species from different latitudes in relation to temperature, pH and food *Comp. Biochem. Physiol.* B **162** 66–72
[62] Morata N and Søreide J E 2015 Effect of light and food on the metabolism of the Arctic copepod Calanus glacialis *Polar Biol.* **38** 67–73
[63] Boersma M and Kreutzer C 2002 Life at the edge: is food quality really of minor importance at low quantities? *Ecology* **83** 2552–61
[64] Pedersen F and Hansen P 2003 Effects of high pH on a natural marine planktonic community *Mar. Ecol. Prog. Ser.* **260** 19–31
[65] Pedersen S A, Håkedal O J, Salaberria I, Tagliati A, Gustavson L M, Jenssen B M, Olsen A J and Altin D 2014 Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates *Environ. Sci. Technol.* **48** 12275–84
[66] Cripps G, Lindeque P and Flynn K J 2014 Have we been underestimating the effects of ocean acidification in zooplankton? *Glob. Change Biol.* **20** 3377–85
[67] Isari S, Zervoudaki S, Saiz E, Pelejero C and Peters J 2015 Copepod vital rates under CO_2-induced acidification: a calanoid species and a cyclopoid species under short-term exposures *J. Plankton Res.* **37** 912–22