

## Reviewer 1

The authors present the findings from an ocean alkalinity experiment (OAE) addressing the response of larval herring. Two separate experiments were carried out: small-scale experiment on individual larval herring response, and a large mesocosm experiment designed to examine community level interactions in the context of OAE. The authors report non-significant effects of OAE on herring larvae in both experiments. This manuscript provides some baseline evidence addressing the importance of identifying potential negative impacts of OAE. Several aspects of the data analysis and experimental design need additional details and clarification before publication.

We are grateful to both reviewers for their very thoughtful and thorough feedback. Its implementation improved the quality and clarity of our manuscript significantly.

While addressing the reviewers' comments, we became aware of a shortcoming in the laboratory experiment. The pCO<sub>2</sub> concentration in the 'control' fish tanks was above what one may consider 'ambient'. It appears that respiration in the tanks was not entirely compensated for by the aeration system, leading to an accumulation of CO<sub>2</sub> and thus acidification. With that we lose our 'control' treatment for the test of OAE. The laboratory results cannot be contrasted to the ones from the mesocosm. We removed the laboratory part from the manuscript.

This re-orientation did not alter the principle take-home message of the study. Changes were required at a more detailed level, however. Specifically, the 'mineral' treatment of our mesocosm experiment is now properly integrated. Before, it had been restricted to the method section to allow for a straight forward combination of mesocosm and laboratory experiments. Judging from the reviewers' comments, this neglect of the mineral treatment had been confusing. So, in a sense, the now uncompromised focus on the mesocosm improves clarity. In the revised manuscript, the structure and data presentation match the experimental design exactly.

### General comments

1) The introduction appears to focus more on pH and pH variability rather than OAE. As pH was not held constant during these experiments, the authors should shift the focus of the introduction to alkalinity specific effects. This could take the shape of effects of added Ca with OAE and osmoregulation. The introduction also lacks details about the concerns of OAE and the reactions that occur. More attention needs to be given to this to build context.

The increase of alkalinity itself is not affecting biology. Also, changes in osmolarity are minor compared to the osmolarity of seawater and have thus been considered negligible as stressor (stated in line 168). Instead, biology is expected to be most strongly driven by shifts in associated variables (e.g. pH, pCO<sub>2</sub>, ΩCa). These are inseparable from alkalinity and thus an intrinsic element of OAE. In our opinion, an understanding of their effects is what is required to evaluate environmental safety of OAE. We give the associated increase in pH particular attention as it may matter most for fish physiology. Please note as well that the non-CO<sub>2</sub> equilibrated OAE approach tested here entails particularly strong shifts in carbonate chemistry (pH, pCO<sub>2</sub>, ΩCa), which makes their study especially relevant.

We state the above more clearly in the revised manuscript:

"Biological processes are not affected by alkalinity itself but through the associated changes in various ions and molecules (Bach et al., 2019)."

The general explanations around OAE including chemical reactions, associated changes in carbonate chemistry and the various implementation scenarios of OAE are already presented several times in great detail in the existing literature (e.g. cited here: Renforth and Henderson 2017, Bach et al. 2019). In contrast, information (theoretical and empirical) on the potential consequences for fish is not yet available. To increase the value of our manuscript (via complementarity), we prefer keeping the general introduction on OAE short so that more focus can be placed on introducing the questions that matter more specifically for fish.

2) What is the reason for choosing such a moderate delta TA (600  $\mu\text{mol kg}^{-1}$ ). Predictions of real applicable scenarios estimate higher TA (See Renforth and Henderson 2017).

In the last paragraph of the introduction (lines 79-82), we classify our maximum delta TA of 600 as relatively high and provide references for it: Bach et al., 2019; Hartmann et al., 2023; Renforth and Henderson, 2017. What matters here is whether an equilibrated ( $\text{CO}_2$  added together with alkalinity) or non-equilibrated ( $\text{CO}_2$  sequestration happens afterwards) OAE application is used. We here test the non-equilibrated application, which is the basic approach to OAE. This is stated in the aims paragraph of the introduction (line 79) and the treatment section of the methods (line 118) and context is given in the introduction (lines 42-45) and discussion (lines 324-327).

The non-equilibrated application involves much more drastic increases in calcium carbonate oversaturation at any given delta TA than the equilibrated one. In other words, abiotic precipitation is occurring sooner. Our choice of maximum delta TA was meant to (just) avoid precipitation. The difference between these two approaches and the risk of abiotic precipitation is a central topic in the references cited, including Renforth and Henderson 2017.

Our original manuscript obviously lacked clarity here. The 'OAE application' section in the methods of the revised manuscript now includes a thorough explanation.

" $\Delta\text{TA}$  was applied non- $\text{CO}_2$  equilibrated (Bach et al., 2019). This economically more feasible approach, only adds alkalinity and  $\text{CO}_2$  sequestration occurs afterwards through natural equilibration with the atmosphere. In the alternative pre- $\text{CO}_2$  equilibrated approach, the to-be-sequestered  $\text{CO}_2$  would instead be added together with the alkalinity leading to milder changes in seawater chemistry. We restricted  $\Delta\text{TA}$  to 600  $\mu\text{mol kg}^{-1}$  to avoid abiotic precipitation of calcium carbonate, which would signify a loss in alkalinity and thus a nonsensical OAE scenario (Hartmann et al., 2023)."

3) Since pH was not held constant, this should be considered as a random effect in the mixed model. Despite the finding of nonsignificant effects with the current model, the added random effect will shift the distribution of your df giving you a more accurate representation of  $\Delta\text{TA}$  as a predictor variable.

Our fish responses represent a temporal integration of abiotic and biotic conditions. This is especially the case for variables like growth and survival but also physiology and behavior to some extent. Including current pH as covariate would not reflect this lag in response. The lag is unknown and will differ between variables. There could even be effects in the history of the larvae that are irreversible. Then, strictly speaking one cannot stop at pH but needs to consider other carbonate chemistry variables that are modified by delta TA like  $\text{pCO}_2$  and  $\Omega\text{Ca}$ . Our experimental design is not able/meant to disentangle their effects. Instead, our non- $\text{CO}_2$  equilibrated deltaTA levels should be seen as 'OAE scenarios' that entail a certain carbonate chemistry (pH,  $\text{pCO}_2$ ,  $\Omega\text{Ca}$  etc.).

To note, temporal variability in carbonate chemistry is less of a topic in the revised manuscript that is based on the mesocosm only. These variables were relatively stable in the mesocosm. The revised manuscript shows the temporal development of not only of pH but also  $\text{pCO}_2$ .

4) Why was it chosen to have no replicates for the mesocosm experiment? I understand seeing no mineral effect in the lab experiment as potential justification but it was never mentioned that Si was used during the lab experiments. How do you know Si didn't carry a random effect. This needs to be explained and justified.

The revised manuscript is less confusing in this respect as it only includes the mesocosm design without compromises. The mineral treatment is now fully integrated in all data analyses and their interpretation. In the 'OAE application' section, we also added an explanation regarding the choice of a gradient design for  $\Delta\text{TA}$ .

"The gradient design with non-replicated treatment levels (Riebesell et al., 2023) was preferred for  $\Delta\text{TA}$  to allow for a more informative study of biogeochemical processes that were also part of the multidisciplinary mesocosm project

(e.g. Ferderer et al., 2024). For analysis,  $\Delta TA$  could then be tested as continuous explanatory variable in the sense of linear regression.”

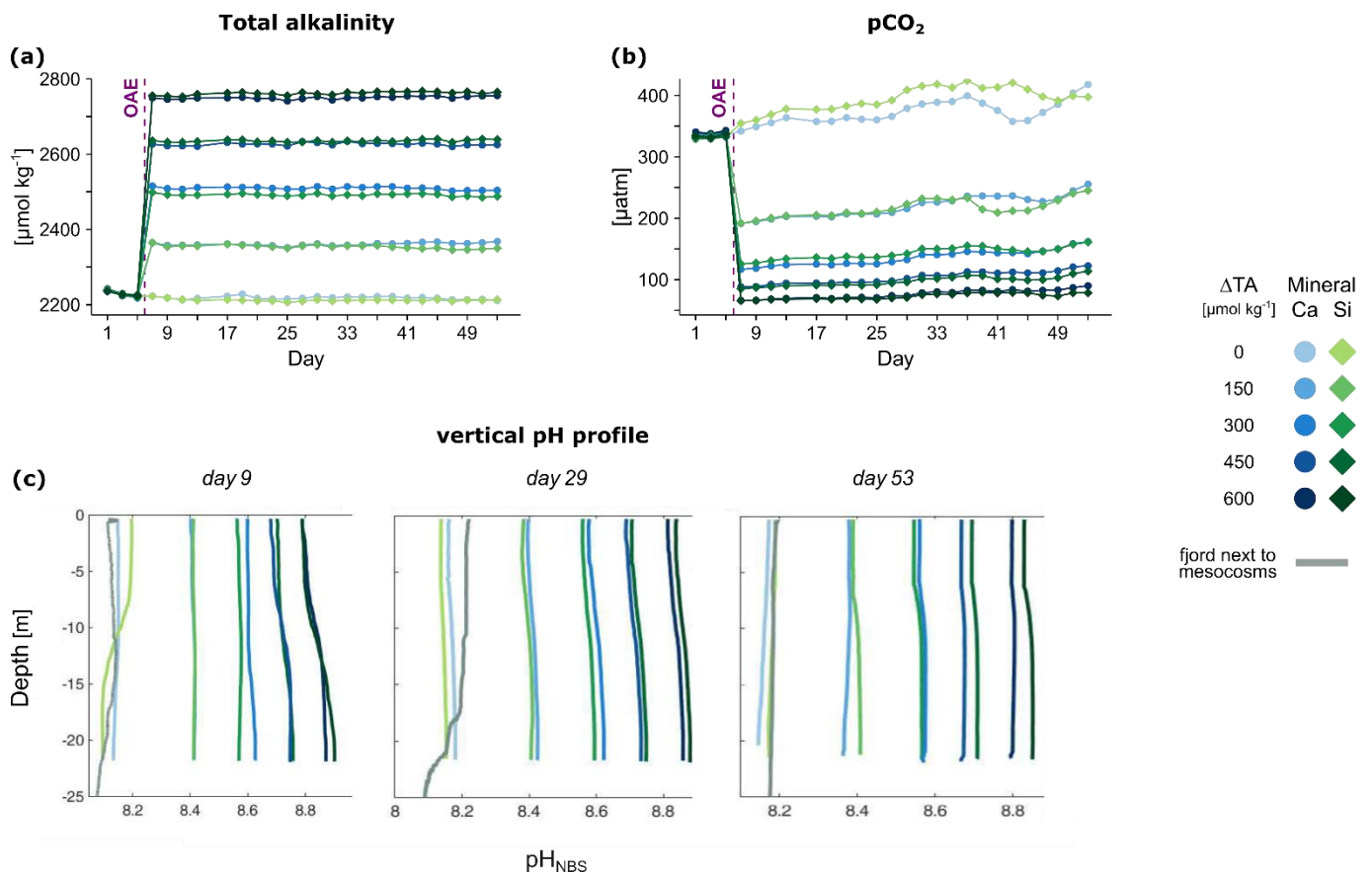
5) There is no timeseries of delta\_TA just pH. A delta TA timeseries needs to be shown. If this information is not available, then report and justify why not. Where was pH measured in the mesocosms, surface, depth? If stratification was strong, there could have been a pH gradient as well, particularly just from the temperature effect from 8 to 15 C. This needs to be address? Again, pH is a covariate for the mesocosm experiment. Discuss this.

1) Yes, we agree with the need for a full presentation of the carbonate chemistry data. The revised manuscript includes a supplementary figure with the temporal development of TA and  $pCO_2$  as well as a vertical pH profile. pH of time is in the main text.

TA is not shown in the main text as it corresponds to the intended target values already provided via the experimental design and figure legends. Unless there is precipitation, TA is stable during OAE.

The variability in pH across depth was small compared to the shift in baseline caused by the TA manipulation itself. An elaborate discussion is not needed. We added a corresponding sentence in the main text in the “OAE application” section:

“These OAE-induced shifts in carbonate chemistry were present across depth (as exemplified via pH, Fig. S1c).”



“Figure S1: Further assessment of carbonate chemistry. Development of a) total alkalinity (TA) and b)  $pCO_2$  in each mesocosm unit. c) Depth-dependent variability in pH. Sampling days at the beginning, middle and end of the treatment period serve as examples. These pH measures were taken in situ via CTD with a potentiometric pH sensor (NBS scale) and are hence slightly higher than the spectrometric measures (total scale) shown in figure 1c.”

2) In the revised manuscript, we provide a more detailed explanation about how water was sampled:

“Carbonate chemistry and inorganic nutrients were monitored in two-day intervals based on depth-integrated water samples. For this, samplers equipped with a pressure-controlled motor (5 L, Hydro-Bios, Kiel, Germany) were lowered

from the surface to the bottom of the mesocosms to collect water evenly across the water column. The resulting samples represented mesocosm averages.”

6) For the NMDS plots, it would be better to examine larval herring survival or biomass to other assemblages, chl a measurement, temp and OAE rather than changes to whole assemblages of other fish. If you also want to examine the effect of different fishes, then examining the different environmental variables would be useful. Could be useful to also show correlations with other variables to explain the lack of effect of OAE.

1) We decided to not analyse the final fish assemblage from the perspective of the herring as they were not dominant amongst the fishes (see Fig. S3). Instead we took a more neutral stand considering all fish groups equally. The question we address with this multivariate analysis – is there evidence for an OAE-driven shift in the composition of the fish assemblage? - is basic but valid. Under different circumstances (high herring survival dominating the fish assemblage), we would agree with the analysis strategy recommended by the reviewer.

2) Our study is based on an experiment with only 10 independent measurements (via 10 mesocosms). Unfortunately, that means we are limited in the number of predictors that can/should be included in the model due to overfitting. This is true for both our multivariate and univariate analyses. We hence restrict ourselves to the predictors of our experimental design, as only these test cause and effect. Following *deltaTA*, the next predictor is *Mineral* and the interaction *deltaTA x Mineral* (see Table S1). With that we have come close to the limit of our data structure. Again, under different circumstances (more independent measurements), we would agree with the reviewer in also considering (correlation) other abiotic and biotic predictors.

Line comments:

39: List some biological processes specifically

This first sentence is the topic sentence of the paragraph. The arguments come after. Most of our paragraphs follow this assert-justify style of writing.

In this case, the next sentence (lines 39-43) lists the biological processes:

“Added alkalinity reduces CO<sub>2</sub> as carbon source for primary producers (Hansen, 2002), rises calcium carbonate saturation which facilitates calcification (Renforth and Henderson, 2017) and increases pH concerning the acid-base balance of organisms (Tresguerres et al., 2020; Pörtner, 2008).

45: provide reference for gigaton estimate and scale of plume/ deployment mass of material

In the revised manuscript, we reference Renforth and Henderson (2017) here. This general review on OAE covers precisely these points. Further, aspects are covered by He and Tyka (2023) and Bach et al. (2019) that we cite already throughout the paragraph.

46: This line doesn't make much sense. “because of this coastal systems are relevant”. Provide more description/context.

The context is given in the subsequent sentence.

“They are not only most attractive economically for OAE deployment given the proximity to mineral and energy sources (He and Tyka, 2023) but also hotspots of biodiversity and ecosystem services.”

56: What are these thresholds. Provide more discussion here. Where these things even specifically addressed in the study?

We mention “energetic costs” and “thresholds” (that we indeed don't study specifically), as these are key processes that can cause altered growth and survival (which we study) in the context of stressors in general. Specifics about

thresholds for fish under OAE and the underlying mechanisms are not yet described in the literature, given the novelty of the field. In the revised manuscript, we adopted a more neutral formulation of this content:

“There could be additional energetic costs for acid-base regulation that channel resources away from growth and reproduction or pH thresholds beyond which physiological functions fail and threaten population viability.”

78: indirect effects can be explained better.

“Indirect effects” were introduced in the preceding paragraph

lines 70-72: “At the community-level, larvae are tightly controlled by resources, competition and predation (Houde, 2008). OAE could change these food web interactions, for example via species-specific pH sensitivities, expanding calcifiers or CO<sub>2</sub> limited primary production, giving rise to indirect effects (Ockendon et al., 2014; Goldenberg et al., 2018).”

and explained again graphically in the same paragraph via figure 1b (line 75).

105: Is this 1800 larvae in total, so evenly distributed across all treatments?

In the revised manuscript, this sentence now only relates to the larvae rearing for the mesocosm introduction, given that the laboratory experiment was removed. We made sure to be clearer this time:

“After hatching 17 days later (24th April), a total of 2400 larvae were distributed amongst two ~500 L rearing tanks.”

106: Was temperature maintained and continuously monitored in the aquaria?

We clarified this in the revised manuscript:

“Temperature was continuously adjusted to match that of the outside fjord and averaged  $6.5 \pm 0.8$  °C.”

115: Explain better. Was this a column filled with soda lime ?

Yes, it was. But this information is not relevant any more now that the laboratory experiment is removed.

116: how was PCO<sub>2</sub> measured?

In the revised manuscript, we now include the method for the pCO<sub>2</sub> calculation. To note, this relates to the mesocosm only.

“Carbonate chemistry was assessed following Schulz et al. (2023). TA was measured via titration (Metrohm 862 Compact Titrosampler with Aquatrode Plus with PT1000) calibrated against certified reference material (CRM batch 193) supplied by Prof. Andrew Dickson’s laboratory and pH spectrophotometrically (Dickson et al., 2007). With the temperature and salinity provided by the CTD casts, in situ pH and pCO<sub>2</sub> could then be calculated using CO<sub>2</sub>SYN for Excel with constants from Luecker et al. 2000 and Dickson 1990 (Pierrot et al., 2021).”

118: Show this TA time series and variation across reps.

The full TA development is now shown in supplement for the mesocosm experiment.

123: How was this pH probe calibrated? Tris, NBS buCers? What pH scale (report this throughout)

Thanks for pointing out the lack of pH scale. In the laboratory, pH had been measured potentiometrically on the NBS scale and was then converted to the total scale. In any case, this is not relevant any more now that the laboratory experiment is removed. For the mesocosm experiment, pH scales are now reported throughout the manuscript.

133: So a subsample of 5 larval fish were measured per aquaria. Out of how many total in each aquaria?

Not relevant any more due to removal of laboratory experiment.

152: Show depth gradient in tanks from the CTD data. Where was pH measured. At what depth. Where was TA measured? What depth? Do you have any depth resolution?

The revised manuscript includes a figure with the pH depth gradient measured via CTD. There is no depth data available for TA and also no reason to expect major variability here given the pH profiles. While the pCO<sub>2</sub> depth gradient could in theory be calculated using this information, we believe this to exceed the scope of our manuscript on fish.

We now also include a better description of how water was sampled:

“Carbonate chemistry and inorganic nutrients were monitored in two-day intervals based on depth-integrated water samples. For this, samplers equipped with a pressure-controlled motor (5 L, Hydro-Bios, Kiel, Germany) were lowered from the surface to the bottom of the mesocosms to collect water evenly across the water column. The resulting samples represented mesocosm averages.”

170: Why then were OAE duplicates two different minerals. If Si mineral addition creates reduced nutrient scenarios then having one replicate at one level of delta TA gives you no power. This needs more discussion.

$\Delta$ TA is manipulated as continuous explanatory variable and can so be tested in the sense of regression statistics. Here, the ‘replication’ needed to separate effect from noise is coming from the 5 independent measures along the gradient. Replication of a given level is not required and actually not wanted. Such a design with a continuous explanatory variable is common in ecology (e.g. Quinn and Keough 2002 Experimental design and data analysis for biologists). Its popularity equals that of a factorial design. The statistical calculations underlying these two approaches are the same. We are hence not including a full explanation.

Still, it seems that some more background is needed, which we provide in the ‘OAE application’ section:

“The gradient design with non-replicated treatment levels (Riebesell et al., 2023) was preferred for  $\Delta$ TA to allow for a more informative study of biogeochemical processes that were also part of the multidisciplinary mesocosm project (e.g. Ferderer et al., 2024). For analysis,  $\Delta$ TA could then be tested as continuous explanatory variable in the sense of linear regression.”

The reference ‘Riebesell et al’ includes a section dedicated to design choices in mesocosm studies. It discusses the ‘gradient’ approach and associated statistics.

The ‘Data analysis’ section describes the model structure: “... linear models were employed with  $\Delta$ TA (continuous), mineral (categorical) and their interaction ( $\Delta$ TA  $\times$  mineral) as explanatory variables (type III test).”

174: Explain the effect of NaSiO as means to prevent secondary precip. Discuss the reactions.

There may have been a misunderstanding, sorry. Na<sub>2</sub>SiO<sub>3</sub> provided the silicate for the Si-based mineral treatment. We restructured the sentence:

“Silicate was added in equal amounts of 75  $\mu$ mol L<sup>-1</sup> to all five Si-based mesocosms using Na<sub>2</sub>SiO<sub>3</sub>. This allowed to separate the effects of alkalinity and silicate and prevented mineral precipitation (Ferderer et al., 2024).”

The provided reference is from the same experiment and dedicated to the topic of silicification. It has more information on the risk of secondary Si precipitation.

200: See general comment above about LMM

The laboratory analysis part was removed from the revised manuscript.

216: this should be in the discussion.

The laboratory results were removed from the revised manuscript.

220: If you want to shift the focus to post-exposure effects and discussion then there needs to be some alignment in the discussion about this, despite finding no effect. There are several OA experiments looking at post-exposure effects, these can be used as a contrast.

This is not relevant any more due to the removal of the laboratory experiment. In the mesocosm experiment, the stressor levels are constant throughout the experiment.

227: What is standard herring survival rates for natural communities?

Not relevant any more, as part of laboratory results.

241: How were carcasses differentiated between species?

The revised manuscript includes this information in the method section:

“For this, the sediment trap was sampled in two-day intervals via a tube connected to the surface (Fig. 1a) and immediately screened for dead fish. Carcasses were assigned to either herring, codfishes or flatfishes based on their distinct body shape. This method had proven successful in previous campaigns, especially in colder climates where fish carcasses disintegrate slowly (Spisla et al., 2022).

303: Describe the food-web mediated impacts of OAE.”

The entire paragraph is about the food web-mediated impacts. After the paragraph’s topic sentence (line 303), we first remind about some of the potential effects proposed by the literature (lines 303-306) and then follow with our mesocosm results (lines 306-313).

308: Given you had no real replication of a specific mineral at high TA, an NMDS associating fish biomass with other drivers specific to each mesocosm could help explain this.

Please see the discussion above regarding the non-replicated design and the testing of other drivers via correlation.

315: provide more discussion of other metrics used to determine herring tolerance and survival. More discussion needs to be given about why OAE didn’t effect them. What are their osmoregulation strategies, what are the potential, for carry-over effects, etc..

The thorough testing of direct effects of OAE on herring from the laboratory, including metabolic rate and behavior, is not included in the revised manuscript. The focus has shifted to the community level. While these traits still matter for the growth and survival in the mesocosms, they are now not specifically tested any more. We believe that our more general introduction (lines 49-66) and discussion (lines 283-302) on physiology at the organism-level is now appropriate.

The past development of research on other stressors (e.g. ocean acidification) shows a strong preference for laboratory studies on direct effects on physiology. Community-level studies are instead rare because of their large cost. We hope that future OAE research will test and discuss physiological responses in detail and complement our work.

Figure 4 and 5 need a legend for the color.

Color legends now included.

## Reviewer 2

This study investigated the ocean alkalinity enhancement (OAE) effect on fish organismal and community responses under laboratory and mesocosm settings. Overall, no negative effects of OAE were reported. Currently, OAE effects on marine biota remain poorly understood, preventing sustainable implementation of this carbon dioxide removal solution for climate change mitigation. Therefore, the data presented are important for advancing our understanding of the ecological risks associated with OAE. While the manuscript's overall quality is good and the results are highly novel, there are several minor comments listed below that could be addressed to further enhance the quality and clarity of the manuscript.

We are grateful to both reviewers for their very thoughtful and thorough feedback. Its implementation improved the quality and clarity of our manuscript significantly.

While addressing the reviewers' comments, we became aware of a shortcoming in the laboratory experiment. The pCO<sub>2</sub> concentration in the 'control' fish tanks was above what one may consider 'ambient'. It appears that respiration in the tanks was not entirely compensated for by the aeration system, leading to an accumulation of CO<sub>2</sub> and thus acidification. With that we lose our 'control' treatment for the test of OAE. The laboratory results cannot be contrasted to the ones from the mesocosm. We removed the laboratory part from the manuscript.

This re-orientation did not alter the principle take-home message of the study. Changes were required at a more detailed level, however. Specifically, the 'mineral' treatment of our mesocosm experiment is now properly integrated. Before, it had been restricted to the method section to allow for a straight forward combination of mesocosm and laboratory experiments. Judging from the reviewers' comments, this neglect of the mineral treatment had been confusing. So, in a sense, the now uncompromised focus on the mesocosm improves clarity. In the revised manuscript, the structure and data presentation match the experimental design exactly.

### General comments:

The laboratory and mesocosm experiments differ in methodology. Why in the laboratory experiment only Ca OAE was done, contrary to the mesocosm? Why a range of TA was used for the mesocosm but not for the laboratory experiment? Why no replicates in the mesocosm?

This seems peculiar, as to get interchangeable results that could support experiments each other, the same methodology should be used. There is lack of explanation why the decision on using only a TA of 600  $\mu\text{mol kg}^{-1}$  has been used in the laboratory experiment and why this TA has been considered as the highest endpoint in both experiments. Moreover, the lack of replicates in the mesocosm experiment reduced the power of statistical analysis. The authors should provide an explanation of why this was the case.

These issues were resolved by only presenting the mesocosm experiment. We provide more background now so the reader may better understand our treatment and design choices.

1) The mineral treatment is now fully integrated in the theoretical and analytical structure of the manuscript. This includes a new introductory paragraph on why silicate matters for the study of indirect effects of OAE.

"Source minerals for OAE can introduce additional elements that may act as stimulators or stressors for fish. These include trace metals like iron and nickel (Morel and Price, 2003) but also macro-nutrients (Bach et al., 2019). The application of silicate minerals would, for instance, increase silicate concentrations (Si(OH)<sub>4</sub>) in seawater well above natural levels. This macro-nutrient favors silicifying diatoms over other primary producers (Sarhou et al., 2005) with consequences for food web functioning (Sommer et al., 2002). On the one hand, the larger size of diatoms can make primary productivity directly accessible to copepods (Hansen et al., 1994), a primary food source for many fish larvae (Turner, 1984). On the other hand, strong silica cell walls (Pancic et al., 2019), toxic secondary metabolites (Ianora and Miralto, 2010) and low nitrogen content (Goldenberg et al., 2024) may reduce diatom palatability and nutritional value. Silicate minerals may hence cause changes at the bottom of the food web with either positive or negative knock-on effects for fish."



2) The choice of a gradient design for  $\Delta TA$  is now further elaborated on in the 'OAE application' section.

"The gradient design with non-replicated treatment levels (Riebesell et al., 2023) was preferred for  $\Delta TA$  to allow for a more informative study of biogeochemical processes that were also part of the multidisciplinary mesocosm project (e.g. Ferderer et al., 2024). For analysis,  $\Delta TA$  could then be tested as continuous explanatory variable via linear regression."

The reference 'Riebesell et al' includes a section dedicated to design choices in mesocosm studies. It discusses the 'gradient' approach and associated statistics.

With a continuous explanatory variable, the 'replication' needed to separate effect from noise is coming from the independent measures along the gradient (in our case 5). Unlike for a factorial design, replication of a given level is thus not required and actually not wanted. As continuous explanatory variables are common in ecology (e.g. Quinn and Keough 2002 Experimental design and data analysis for biologists), we do refrain from explaining the underlying statistical calculations. A to-the point sentence of our statistical model is given in the 'data analysis' section:

"... linear models were employed with  $\Delta TA$  (continuous), mineral (categorical) and their interaction ( $\Delta TA \times$  mineral) as explanatory variables ..."

3) Thanks for pointing out the lack of clarity regarding the maximum  $\Delta TA$ . The 'OAE application' section in the methods of the revised manuscript now includes the following statement.

"We restricted  $\Delta TA$  to 600  $\mu\text{mol kg}^{-1}$  to avoid abiotic precipitation of calcium carbonate, which would signify a loss in alkalinity and thus a nonsensical OAE scenario (Hartmann et al., 2023)."

The reference 'Hartmann et al' demonstrates the risks of abiotic precipitation empirically. Other references that we cite in the same paragraph (Bach et al. 2019 and Riebesell et al. 2023) provide further discussion. Our choice of maximum  $\Delta TA$  is at the upper limit of the 'safe' range of non- $\text{CO}_2$  equilibrated OAE according to these references.

Another aspect that seems to be poorly introduced is the species-specific responses. The model species used was Atlantic herring, which as indicated by the authors has been proven resistant to pH fluctuations. It again seems peculiar why this particular species was used for the experiment. From the ecotoxicology point of view, it would make sense to test the OAE effect on the most sensitive species as all fish communities could be protected by regulating the application of OAE based on the most sensitive species results. The authors should address that.

We addressed this concern in the revised manuscript where possible. A thorough elaboration is provided below the respective detailed comments.

Furthermore, in the mesocosm experiment the authors added silicate-based OAE to simulate different mineral addition scenarios. Most of the minerals regarded for usage in OAE would dissolve trace metals into the water and the trace metals aspect is regarded as potentially the most harmful. I wonder why this aspect hasn't been studied by the authors.

We agree with the reviewer that trace metals are critical as well. Both macro-nutrients and trace metals should be thoroughly studied by future OAE research. In the revised manuscript, we acknowledge this more clearly.

Introduction: "Source minerals for OAE can introduce additional elements that may act as stimulators or stressors for fish. These include trace metals like iron and nickel (Morel and Price, 2003) but also macro-nutrients (Bach et al., 2019). The application of silicate minerals would ..."

Conclusion: "With silicate addition, we tested a secondary driver of OAE that is hypothesized to cause major ecosystem shifts (Bach et al., 2019). There are several further drivers, however, such as trace metal toxicity/enrichment (Morel and Price, 2003) or suspended mineral particles (Affandi and Ishak, 2019) that are likely to play a role as well."

With 10 independent mesocosm units we were limited in the number of OAE-associated drivers that could be tested. Since this is the first study on fish under OAE, we chose the ones that we thought are most relevant in our system.  $\Delta$ TA including the associated changes in carbonate chemistry came first, as it is part of all OAE application scenarios. Silicate came second as it is a macro-nutrient with direct consequences for the traditional food web model: diatoms to large grazers to fish. Our mesocosm are ideal to test this community-level question. In the revised manuscript, we added a new paragraph in the introduction to give context for the silicate manipulation (see previous comment). Trace metal research on fish will probably start in the laboratory, as it already has begun for phytoplankton.

To note, we used trace metal free OAE treatments. Only this way drivers were unconfounded. The alternative experimental design, where trace metal concentrations change in parallel to  $\Delta$ TA or silicate, would have been unable to establish cause and effect.

## Specific comments

### Introduction:

Line 32-33: Ocean alkalinity enhancement does not only refer to rock weathering. There are different types of technologies that can increase alkalinity e.g. electrolysis technologies. Therefore this sentence has to be changed accordingly.

Thanks for point this out. We corrected the sentence:

“This ocean-based solution **can be implemented** through the acceleration of a natural process – rock weathering – where alkaline minerals are dissolved in seawater.”

Line 33: Change ‘store Co2’ to ‘sequester CO<sub>2</sub>’.

Line 34: Again, storage seems like not the best word to use for OAE CO<sub>2</sub> removal mechanisms. Also, here the authors introduce a new terminology ‘negative emissions’. I found it confusing because above a term ‘carbon dioxide removal’ has been used. It is not the same therefore authors should stick to carbon dioxide removal terminology.

The section was revised. ‘storage’ and ‘negative emissions’ were replaced.

“As alkalinity increases, so does the capacity of seawater to **take up** CO<sub>2</sub> from the atmosphere. The **sequestration** may be scalable, safe and cost-effective (Lackner, 2002). Besides **removing carbon** to combat climate change, this approach would also counter ocean acidification that is widely recognized as major threat to marine life (Doney et al., 2020).”

Line 49-51: Add examples of how the physiological processes of fishes are sensitive to bicarbonate, CO<sub>2</sub> and H<sup>+</sup>.

We prefer to not extend the introduction on these intra- and extracellular processes. With two paragraphs on fish physiology (lines 54 to 71), we are not able to provide greater detail for each subtopic. It would lead to an unbalanced introduction, especially now that the focus of our manuscript was shifted to responses at the community level.

The intra- and extracellular processes related to acid-base regulation are reviewed in full detail in the references we provide in this paragraph (e.g. Tresguerres et al 2020). To the best of our knowledge, no one has yet tested these in the context of OAE and fish. We hope that future laboratory research on acid-base regulation in fish under OAE will provide a thorough introduction on this topic.

Line 58-62: The authors cite the effects of acidification of different physiological and behavioral responses of fishes. Add some more details about what were those effects, increased or decreased, beneficial vs. negative etc.

Again, we prefer to not extent here to maintain a balanced and concise introduction. The revised manuscript now also includes the introduction of silicate as OAE driver, which increases it to ~1000 words.

Moreover, the reviewer's request is not easily implemented, as under ocean acidification, these responses are not uniform at all. The direction as well as strength of change differs widely between studies, even those conducted on the same species and traits. Giving a few examples may thus be misleading. We instead refer to comprehensive reviews and meta-analyses (Cattano et al., 2018; Esbaugh, 2018; Nagelkerken and Connell, 2015) for those readers who want to know more. The two sentences on ocean acidification (lines 58-62) still serve their purpose: emphasize that pH matters for fish traits and the importance of studying fish under OAE.

Line 62: This sentence sounds confusing. OAE is supposed to counteract ocean acidification and in this sentence, it reads like it is going to cause the same change in H<sup>+</sup> concentrations as acidification. Correct accordingly.

True, this could be written more clearly. We revised the sentence:

“An OAE plume could entail a similarly large change in H<sup>+</sup> concentration, **albeit in the opposite direction**, to that which may impair fishes under end-of-century acidification (Bach et al., 2019; Hartmann et al., 2023).”

Materials and methods:

Line 96: Add that the primary focus of the laboratory experiment was put on the Atlantic herring. In the mesocosm experiment, more species were studied.

This is resolved now that the manuscript only presents the mesocosm study.

Line 117: Specify what was the amount of pure Milli-Q water added to control tanks.

The OAE manipulation in the laboratory is not part of the manuscript any more.

Figure 2 – it is hard to distinguish the Mineral type on plot b, the shapes are not distinct enough. Maybe make one of them empty. Use the same pH scale on graphs a and b so it is easier to compare.

The revised manuscript has a different color gradient for each of the mineral symbols (blue vs. green). This should allow for an easy distinction. Please view the 'figure' file attached to the answers to the reviewers' comments.

Line 132: Were larvae only placed in the vials to clear their guts for 1.5 h? Is this a standard procedure? If yes, add a reference.

This description of the laboratory tests has been removed from the manuscript.

Line: 163: What are those nutrient concentrations based on? On local data? If yes, add a reference.

We included an explanation with references in the revised manuscript:

“During past experiments, this level of fertilization induced phytoplankton blooms in mesocosms (e.g. Schulz et al., 2017) that corresponded in intensity to those observed in the natural environment (Paulino et al., 2018).”

Line: 193-194: Were there different depths from which the primary producer's biomass was measured? The term 'depth-integrated water samples' is not clear. Please specify.

In the revised manuscript, we explain what we mean by ‘depth-integrated water samples’. The explanation is given at first mention, so with the sampling of carbonate chemistry.

“Carbonate chemistry and inorganic nutrients were monitored in two-day intervals based on depth-integrated water samples. For this, samplers equipped with a pressure-controlled motor (5 L, Hydro-Bios, Kiel, Germany) were lowered from the surface to the bottom of the mesocosms to collect water evenly across the water column. The resulting samples represented mesocosm averages.”

Line 199: Change to ‘For the laboratory experiment,’

Line 202: Change to ‘For the mesocosm experiment,’

Not applicable any more due to removal of laboratory experiment.

Line 208: Were the data normally distributed or transformed for the nMDS analysis?

Line 210: Provide information on which R package has been used for the statistical analysis.

Line 212: Why only per capita sizes of fish were log<sub>10</sub> transformed? Provide an explanation.

1) Our **non-metric** multidimensional scaling (nMDS) analysis uses Bray-Curtis dissimilarity. It is based on ranks and does hence not require normal distribution (e.g. Quinn and Keough 2002 Experimental design and data analysis for biologists). These data were not transformed.

2) The main R packages had been referenced in the original manuscript: multivariate analysis (Oksanen et al., 2022) and assumptions (Lüdecke et al. 2021) in main text and linear mixed models with the detailed statistics table in the supplement (Bates et al. 2015; Kuznetsova et al. 2017). We see that these references are not immediately recognizable as R packages. Still, they correspond to how the authors want to be cited and are at the right place.

3) We added an explanation as to why fish size had been transformed.

Below is the revised data-analysis section. We separate the univariate and multivariate analyses into two paragraphs. We hope the information is now presented more clearly.

“To assess the responses of fish and other functional groups to OAE, replicate measures within mesocosms (i.e. individuals or sampling days) were first summed or averaged. This provided one independent measure per mesocosm. Per capita size of fish had been log<sub>10</sub>-transformed to reduce the influence of large but rare individuals on the calculated average. The subsequent analyses were conducted at a significance level of  $\alpha = 0.05$  with R version 4.0.5 (R Core Team, 2021).

For univariate responses, linear models were employed with  $\Delta TA$  (continuous), mineral (categorical) and their interaction ( $\Delta TA \times \text{mineral}$ ) as explanatory variables (type III test). Unexplained variability was particularly high at the level of single taxa (Fig. S2) likely due to random differences in starting numbers and sizes. We therefore restricted our main analyses to the whole fish assemblage. Here, across all fishes, some of the variability was compensated for by competition for limited food resources. Model assumptions were addressed following Lüdecke et al. (2021). Normality of residuals was checked with normal Q-Q plots and homogeneity of variance with residual versus fitted plots.

Potential shifts in the taxonomic composition of the fish assemblage were assessed using multivariate analyses based on Bray-Curtis dissimilarity (vegan package, Oksanen et al. 2022). Differences in composition between mesocosms were illustrated via non-metric multidimensional scaling (nMDS). Mantel tests with Pearson correlation were employed to test for  $\Delta TA$  and permutational multivariate analysis of variance (adonis2 function) to test for mineral based on 999 permutations.”

Results:

Line 224: I would add that there was a larger spread in the OAE application on larvae survival than in the control (Fig. 3e).

This laboratory result is not part of the manuscript any more.

Figure 4: The figure lack of color legend.

Color legends now included.

Line 264: The increase in biomass seems to be not only driven by cod but also herring according to Fig. S1. Update accordingly.

The reviewer may have misinterpreted figure S1. For herring, there is no trend for the relationship between  $\Delta TA$  and abundance or biomass. It's a flat line. Please, also note that cod had ~5 times higher biomass than herring and thus more potential to affect the total biomass of the community.

Discussion:

Line 289-291: So herring is overall resistant to ocean acidification. I wonder why this species has been used as a model organism. The results could be different for more sensitive species. Provide information on why this species has been used in the context of OAE. The information provided in Methods, line 96-98 does not mention the OAE aspect.

Following, some discussion about how more sensitive species might have responded differently to the OAE should be added here. Expand why such contrasting results were found between the current study and the other ones.

Line 294-302: Related to the above, the species-specific responses should be more pronounced. Expand this aspect.

In the methods and discussion of the revised manuscript, we contrast the supposedly less pH sensitive herring to the supposedly more pH sensitive cod. Cod was the most prominent naturally enclosed species in our mesocosms and is the second most studied species (after herring) under ocean acidification in this ecosystem.

A general discussion on the underlying physiological reasons for species-specific effects of OAE is difficult. There are no other studies on OAE and fish yet. The two older pH studies that we mention are not on OAE, i.e. are not based on an alkalinity increase. At this point, the purpose of our paragraph on species-specific effects is to emphasize that our results are not to be generalized and to encourage future OAE studies on other species. Throughout our discussion, we also emphasize the potential for life-stage or trait specific effects and the need to study further drivers of OAE.

1) In the revised methods, we gave more background regarding the choice of herring and its sensitivity to pH:

“A particular focus was put on Atlantic herring. This small pelagic fish supports the largest fishery in Norway and the fourth largest in the world with an annual yield of ~1.7 Mt (Fao, 2022). As such, it holds a key role as trophic intermediary in transferring energy from zooplankton to larger predators (Toresen et al., 2019). Herring larvae had served us as model organisms during past laboratory (Sswat et al., 2018a; Maneja et al., 2015) and mesocosm work on ocean acidification (Spisla et al., 2022; Sswat et al., 2018b). These studies suggested a certain tolerance of herring to pH decreases that already impacted larvae of other species such as cod (Stiasny et al., 2016).”

We understand the reviewer's argument of testing the species most sensitive to acidification. However, we also think that economic, ecological and academic importance matter. This latter argument holds regardless of the species' response to acidification. A tolerance here does not automatically imply a tolerance to alkalization. The potential food web-mediated effects differ as well between these stressors. In fact, for the test of indirect effects (mesocosm), selecting a highly sensitive species could be problematic as it may succumb to the direct effects of OAE before the food web had a chance to act upon it.

Please note as well that many mesocosm studies on fish fail methodologically because of lack of experience with the study species. These are expensive tests involving 50+ researchers that cannot simply be repeated. Working with a species for which there is maximum experience is often the only way.

2) In the revised discussion, we added a comparison of herring and cod. Based on ocean acidification research, cod is supposed to be a more pH sensitive species.

“During the first days of OAE exposure in our mesocosms, fish mortality was not elevated suggesting an absence of severe direct effects on their physiology. In the local ecosystem, natural pH variability peaks at ~8.35 (Omar et al., 2016) and thus remains well below the ~8.7 of our highest alkalinity scenario. The fishes were hence challenged by an instantaneous pH increase that considerably exceeded what their populations should have been pre-adapted to in this system. The ability to compensate despite the severity and abruptness of the perturbation confirms a powerful machinery for acid-base regulation in these vertebrates (Tresguerres et al., 2020). This result may have been expected for herring based on its tolerance to moderate levels of the opposing stressor ocean acidification (Sswat et al., 2018a; Maneja et al., 2015; Franke and Clemmesen, 2011). However, with cod that can experience high larvae mortality under similar conditions (Stiasny et al., 2016), we also included a supposedly more pH sensitive species. Interestingly, both species – herring and cod – can develop major organ damage at more extreme levels of acidification (Frommel et al., 2014; Frommel et al., 2012). We could have missed minor damages caused by OAE that did not pose an immediate threat to larval survival. Other life history stages may prove more vulnerable to OAE, in particular reproductive cells and early embryos that lack specialized regulatory tissue (Melzner et al., 2009; Dahlke et al., 2020).”

Line 296: What does it mean: ‘Five species were investigated here’. Seems like the authors are referring to another study so should use the word ‘here’.

corrected:

“We only know of two comprehensive studies on the viability of fish larvae under increased pH (Brownell, 1980; Parra and Yufera, 2002). These investigated five species in an aquaculture context. After only 24 h exposure to pH above ~8.5, the authors reported reduced first-feeding success ... ”

Line 303: Why tropical species would be more resistant to OAE, provide explanation and mechanisms.

This statement is meant as an encouragement to study other ecosystems, as OAE may interact with major environmental drivers. It also points out again that our results should not be generalized. We believe that our discussion is not the right place to go into depth on the potential temperature dependency of OAE effects. The two references we provide provide context for those readers who want to know more.

Line 303 – 313: Authors should look for other than feeding reasons for the positive effect of OAE on biomass. Maybe those treatments were different in terms of environmental parameters (e.g. nutrients). Those data are not provided.

In the revised manuscript, we include a statement on element cycling. These data are provided in the preceding article that we cite.

“The biogeochemical perspective including inorganic nutrients (nitrate, phosphate and silicate) and export of matter (Ferderer et al., 2024) provides also no clues as to how alkalization may have facilitated the build-up of fish biomass.”

Line 337: The aspect of toxicity coming from trace metals dissolved from minerals should be addressed more here. The trace metals effect is potentially more stressful than solely changes in pH.

In the revised manuscript, the potential introduction of trace metals via mineral is now stated upfront in the introduction

“Source minerals for OAE can introduce additional elements that may act as stimulators or stressors for fish. These include trace metals like iron and nickel (Morel and Price, 2003) but also macro-nutrients (Bach et al., 2019).”

and we acknowledge trace metals again in the conclusion. We do not go into detail, as we believe our study is not the right place for this. We also do not want to rank OAE drivers in terms of their stressor potential. They should all be studied by the research community.