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_2 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_2 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. The updated title and figures are provided as supplement at the end of this document.

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 replicated 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 umol 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)4) in seawater well above natural levels. This macro-nutrient favors silicifying diatoms over other primary producers (Sarthou 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 ΔTA 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 ΔTA 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, ΔTA 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 ΔTA (continuous), mineral (categorical) and their interaction (ΔTA × mineral) as explanatory variables …"

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

"We restricted ΔTA to 600 µmol 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 ΔTA is at the upper limit of the 'safe' range of non-CO2 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. ΔTA 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 ΔTA 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 CO2'.
Line 34: Again, storage seems like not the nest word to use for OAE CO2 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_2 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, CO2 and H+.

We prefer to not extent 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+ 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+ 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) where 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 10 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 $\log_{10}$-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 ΔTA (continuous), mineral (categorical) and their interaction (ΔTA × 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 ΔTA 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 ΔTA 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.

Appendix

title: Viability of fish larvae under ocean alkalinity enhancement in coastal plankton communities

all figures of the revised manuscript:

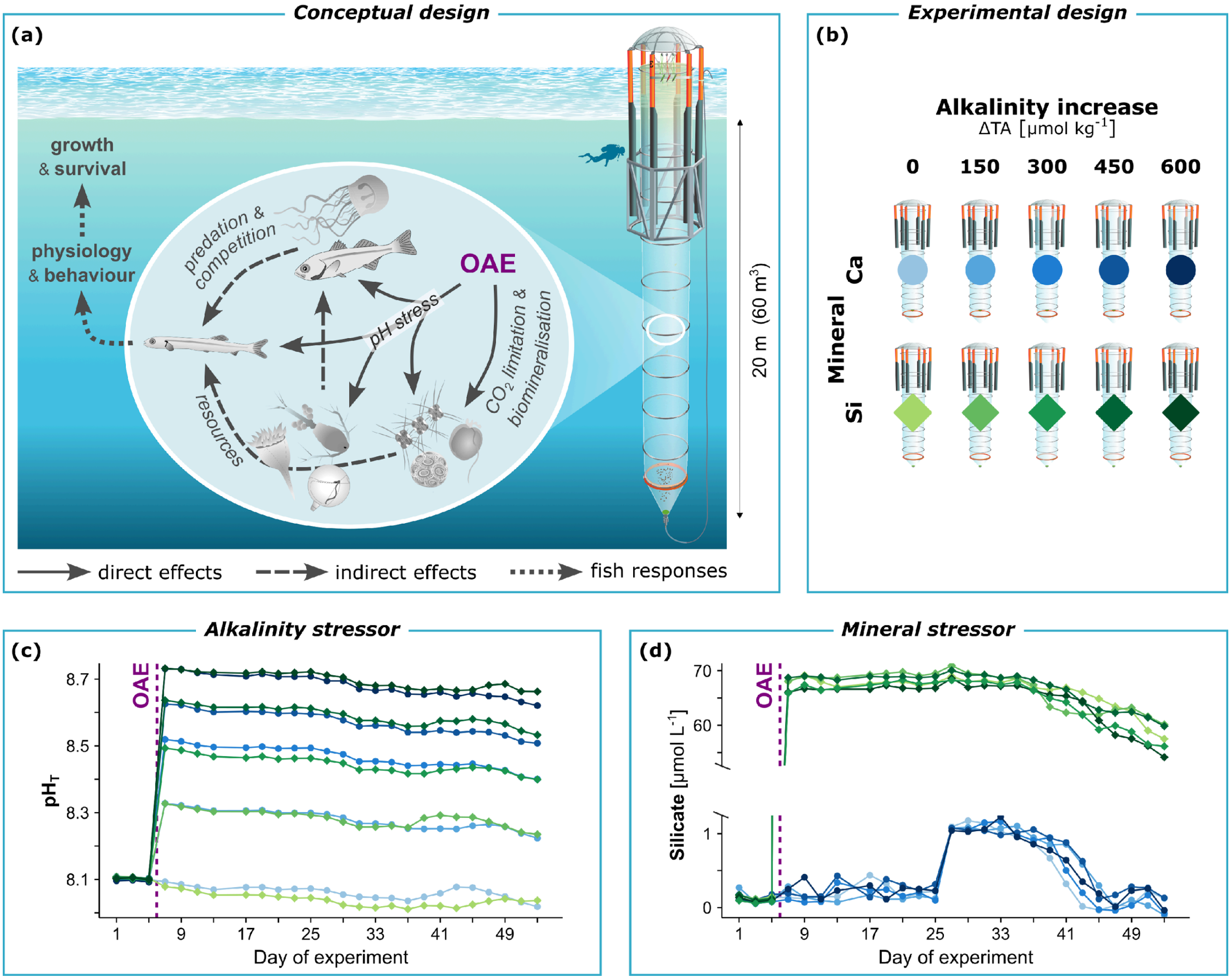

Figure 1: Conceptual and methodological framework of our mesocosm study on non-CO_2 equilibrated OAE. **a)** Potential pathways of change in fish in natural plankton communities. **b)** Water chemistry manipulations to simulate different scenarios of OAE. Using 10 mesocosm units, we tested increases in total alkalinity (ΔTA) under calcium-based (Ca) or silicate-based (Si) mineral addition. **c)** OAE-induced shifts in pH and **d)** silicate availability, as measured in each mesocosm throughout the experimental period. Mesocosm symbol from Rita Erven, GEOMAR, and organism symbols partly from Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

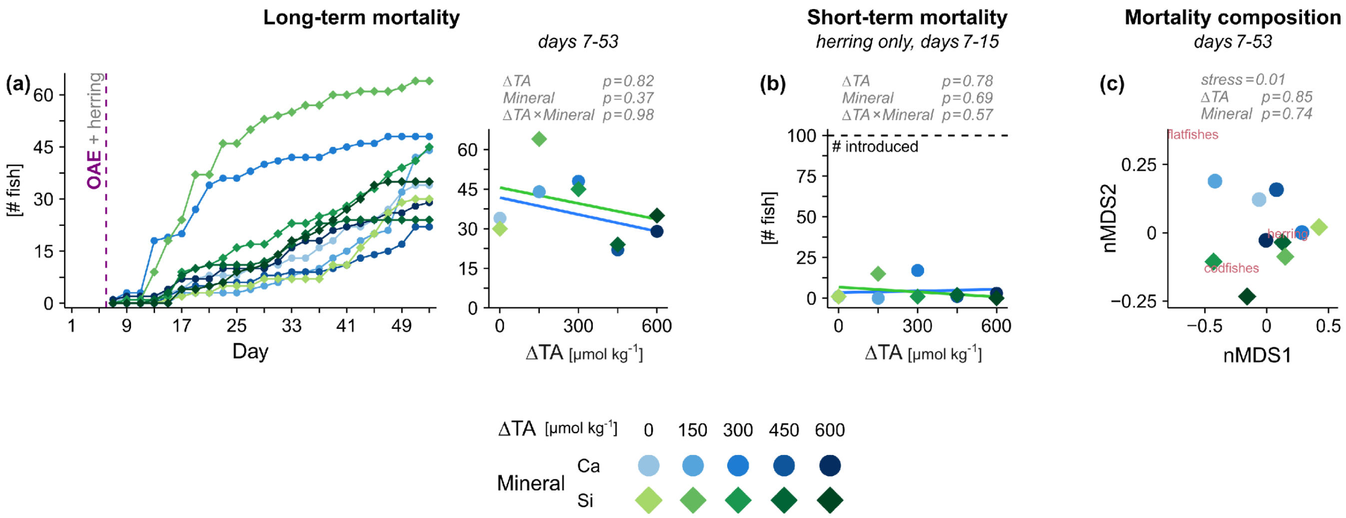

Figure 2: Fish mortality under OAE monitored via the sediment trap. **a)** Cumulative mortality over time across all species and **b)** immediately following the OAE perturbation for herring. **c)** Differences in taxonomic composition between mesocosms via non-metric multidimensional scaling (nMDS). Statistical tests in grey (details in Table S1).

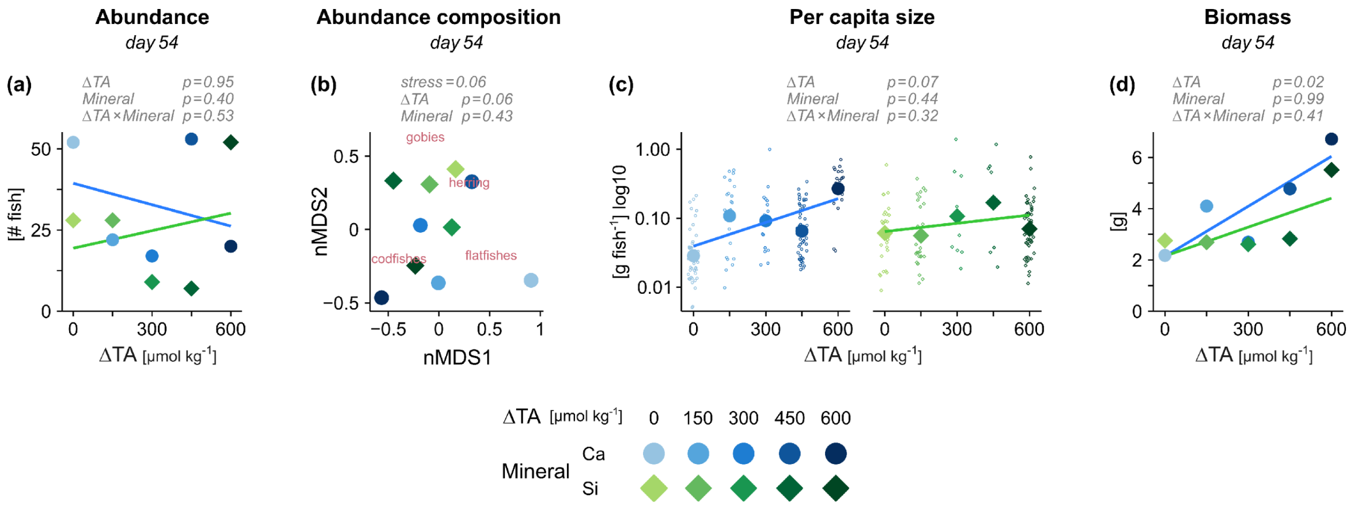

Figure 3: Fish growth and survival under OAE, assessed via the assemblage of live fish at the end of the experiment. **a)** Abundance, **c)** individual size and **d)** total biomass across all fish taxa. **c)** Differences in taxonomic composition between mesocosms via non-metric multidimensional scaling (nMDS). Larger points represent mesocosms and smaller points in **c** single individuals. Statistical tests in grey (details in Table S1).

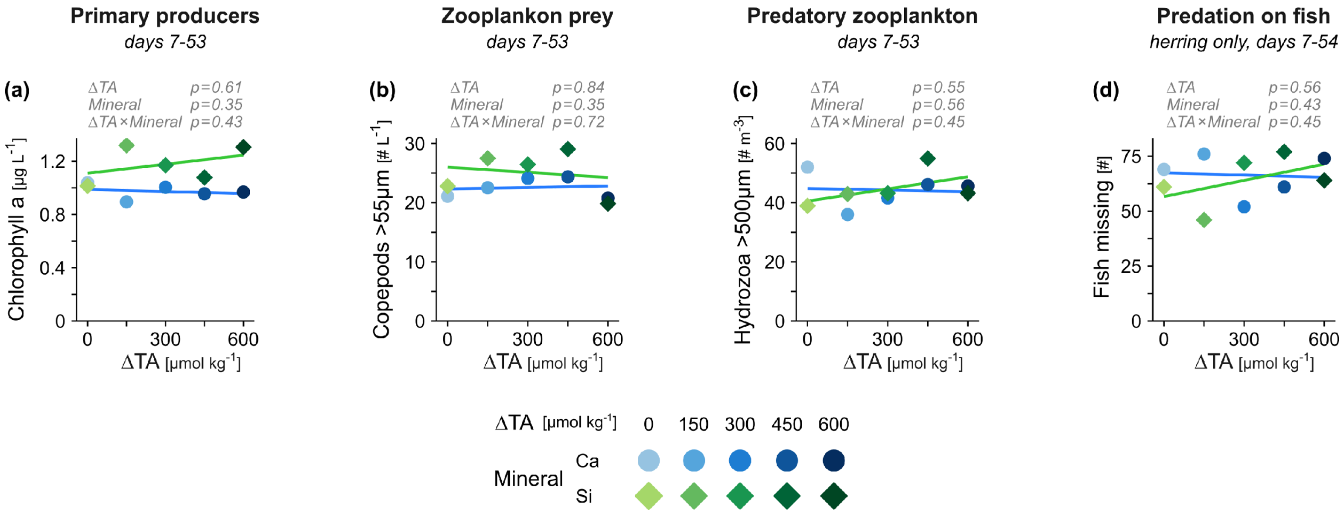

Figure 4: Potential sources of indirect effects of OAE on fish mediated via species interactions. Abundance of other functional groups including (a) primary producers, (b) invertebrate grazers and (c) invertebrate predators. d) Predation on herring estimated via missing individuals. Averages across the treatment period are tested (in grey, details in Table S2).