Viability of coastal fish larvae under ocean alkalinity enhancement: from organisms to communities

Silvan Urs Goldenberg[1], Ulf Riebesell[1], Daniel Brüggemann[1], Gregor Börner[2], Michael Sswat[1], Arild Folkvord[3,4], Maria Couret[5], Synne Spjelkavik[6], Nicolás Sánchez[1], Cornelia Jaspers[6], Marta Moyano[7,8]

1 Biological Oceanography, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany
2 Institute of Marine Ecosystem and Fishery Science, University of Hamburg, Große Elbstraße 133, Hamburg 22767, Germany
3 Department of Biological Sciences, University of Bergen, 5020 Bergen, Norway
10 4 Institute of Marine Research, 5817 Bergen, Norway
5 Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, Unidad Asociada ULPGC-CSIC, Campus de Taliarte, 35214 Telde, Gran Canaria, Canary Islands, Spain.
6 Centre for Gelatinous Plankton Ecology & Evolution, Technical University of Denmark, DTU Aqua, 2800 Kongens Lyngby, Denmark
15 7 Norwegian Institute for Water Research (NIVA), Økernveien 94, 0579 Oslo, Norway
8 Centre for Coastal Research, University of Agder, Postbox 422, 4604 Kristiansand, Norway

Correspondence to: Silvan Urs Goldenberg (sgoldenberg@geomar.de)

20 **Abstract.** Ocean alkalinity enhancement (OAE) stands as a promising carbon dioxide removal technology. Yet, this solution to climate change entails shifts in water chemistry with unknown consequences for marine fish that are critical to ecosystem health and food security. With a laboratory and mesocosm experiment, we show that early life stages of fish can be resistant to OAE. We examined metabolic rate, swimming behavior, growth and survival in Atlantic herring (*Clupea harengus*) and other temperate coastal fish species. Neither direct physiological nor indirect food web-mediated impacts of OAE were apparent. This was despite non-CO_2-equilibrated OAE (ΔTA = +600 µmol kg^{-1}) that induces strong perturbations (ΔpH = +0.7, 25 pCO_2 = 75 µatm) compared to alternative deployment scenarios. Whilst our results give cause for optimism regarding the large-scale application of OAE, other life history stages (embryos) and habitats (open ocean) may prove more vulnerable. Still, our study across ecological scales (organism to community) and exposure times (short- to long-term) suggests that some fish populations, including key fisheries species, may be resilient to the carbonate chemistry changes under OAE.

1. Introduction

30

Ocean alkalinity enhancement (OAE) is being proposed as a carbon dioxide removal technology to help limit global warming to 1.5 °C (Renforth and Henderson, 2017; Rogelj et al., 2018). This ocean-based solution accelerates a natural process – rock weathering – by dissolving basic minerals in seawater. As alkalinity increases, so does the capacity of seawater to store CO_2 from the atmosphere. The storage may be scalable, safe and cost-effective (Lackner, 2002). Besides generating negative 35 emission 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). While OAE indeed seems promising from a technological and economic perspective, an understanding of the potential environmental risks and side-effects is lacking (Gattuso et al., 2021; Nawaz et al., 2023; Bach et al., 2019). Research is needed to evaluate the ecosystem safety of OAE and guide its potential large-scale implementation.

OAE modifies the carbonate system in seawater including known drivers of biological processes. Added alkalinity reduces 40 CO_2 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). Applied from stationary facilities or ships, the perturbation would initially be localized. A pH of up to 9 may be reached (Bach et al., 2019; Hartmann et al., 2023). Over the timeframe of days to years, this 'OAE plume' would mix with

surrounding water masses and absorb CO_2 from the atmosphere (equilibration), alleviating the perturbation (He and Tyka, 2023). Therefore, whilst at a global scale sequestering gigatons of CO_2 involves minor changes in water chemistry, species communities at deployment sites could experience OAE as a stressor. In this sense, studying coastal regions is particularly relevant. 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.

Fishes are essential for the stability of coastal ecosystems and global food security (FAO, 2022). Physiological processes in fish are highly sensitive to the concentrations of bicarbonate, CO_2 and H^+ (Tresguerres et al., 2020), which are all altered under OAE. To maintain homeostasis of body fluids and proper functioning of cells and tissues in variable environments, fish have evolved a sophisticated acid-base regulation (Perry and Gilmour, 2006). They are hence thought to cope well with mild changes in pH (i.e. H^+ concentration) and to be generally less sensitive than marine invertebrates (Melzner et al., 2009). Whether fishes can still compensate under scenarios of OAE that far exceed natural pH variability (Bach et al., 2019; Carstensen and Duarte, 2019; Hofmann et al., 2011) is, however, uncertain. Are there additional energetic costs for acid-base regulation that channel resources away from growth and reproduction? What are the pH thresholds beyond which physiological functions fail and threaten population viability?

The related field of ocean acidification has taught us several lessons on the pH sensitivity of fishes. While they were mostly found to be robust to acidification, some ecosystems, species, life stages and traits were surprisingly vulnerable (Cattano et al., 2018; Esbaugh, 2018; Nagelkerken and Connell, 2015). Physiological and behavioral impacts were observed related to metabolic rate, otolith calcification, sensory functioning, lateralization, swimming, foraging, predator avoidance and habitat selection. An OAE plume could entail a similar magnitude of change in H^+ concentration than what may impair fishes under end-of-century acidification (Bach et al., 2019; Hartmann et al., 2023). Unlike for global acidification, however, the exposure of fishes to OAE would be abrupt and spatially inconsistent lowering the chances for phenotypic buffering and genetic adaptation (Sunday et al., 2014; Wong and Candolin, 2015; Blewett et al., 2022). An investigation into the shorter-term responses of fishes to OAE is thus needed.

OAE may prove particularly challenging for early life stages, a bottleneck for most fish populations. At the organism-level, larvae are highly sensitive to their abiotic environment (Melzner et al., 2009; Cattano et al., 2018) but lack the locomotory capacity to avoid unfavorable water masses. This may facilitate direct physiological effects of OAE (Brownell, 1980; Parra and Yufera, 2002). 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_2 limited primary production, giving rise to indirect effects (Ockendon et al., 2014; Goldenberg et al., 2018). Only by combining these different ecological scales will we achieve a mechanistic understanding of impacts on larvae growth and survival and ultimately recruitment success.

Here, we study the sensitivity of coastal fish larvae to OAE at both the organism- and community-level (Fig. 1). In temperate Western Norway, we employed two contrasting experimental approaches during the main spawning season in spring. First, in a controlled laboratory setting, we examined direct physiological effects on a major fisheries species, Atlantic herring (*Clupea harengus*) (FAO, 2022). Second, with large-scale mesocosms, we assessed the viability of a fish assemblage in the presence of indirect effects mediated via the plankton food web. Non-CO_2-equilibrated OAE was applied up to 600 µmol kg^{-1} of added alkalinity (Fig. 1), entailing strong perturbations in pH (increase by 0.7 units) and pCO$_2$ (decrease by 325 µatm). This intense yet realistic employment scenario (Bach et al., 2019; Hartmann et al., 2023; Renforth and Henderson, 2017) may reveal the maximum impact potential of OAE and facilitate the identification of response pathways in fish. Our findings on the resilience of fish larvae help establish an environmentally safe operating space of OAE for carbon dioxide removal.
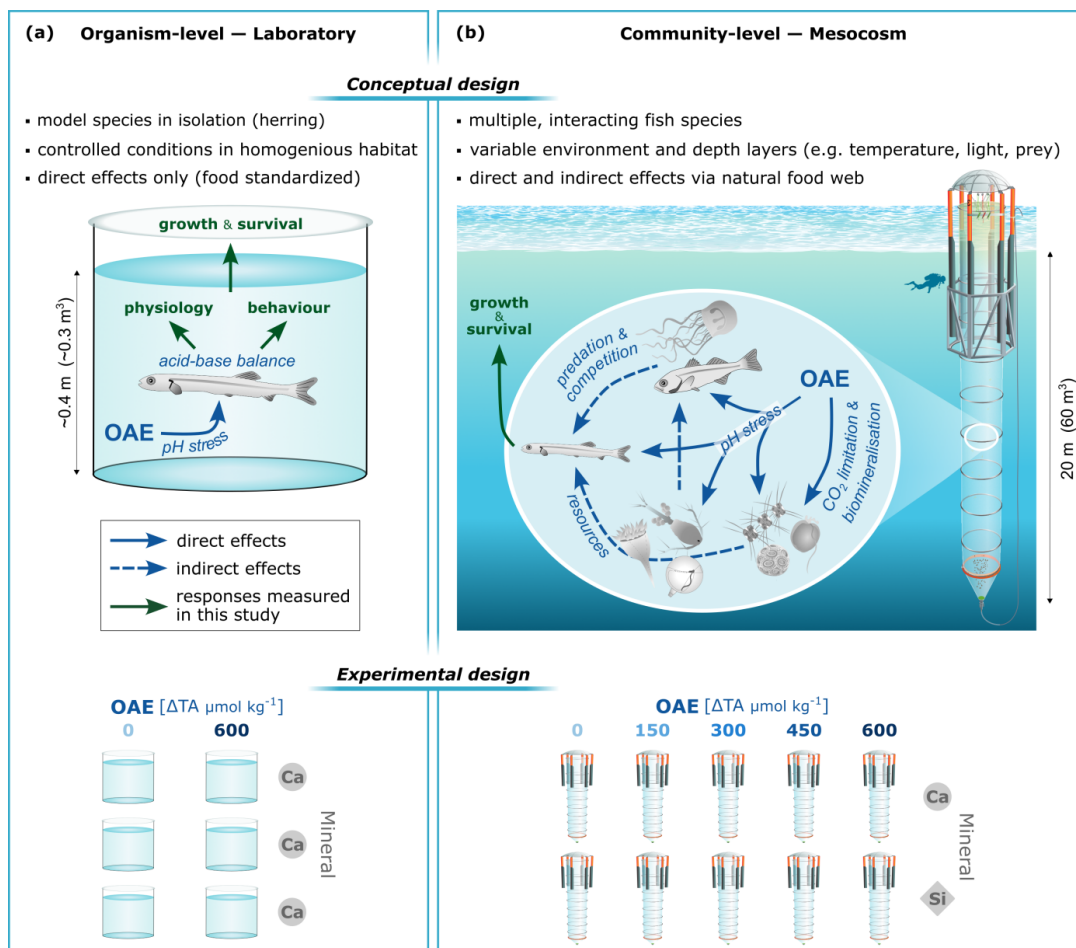
Figure 1: OAE could affect fish larvae at different levels of ecological complexity. We address these by combining a laboratory **(a)** and mesocosm **(b)** experiment. The focus is on pure alkalinity as driver of biology during OAE, independent of the mineral used (Ca: calcium-based, Si: silicate-based). 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/).

2 Methods

2.1 Study System

Experiments took place in 2022 at the University of Bergen (laboratory) and the nearby Espegrend Marine Station in Raunefjord (mesocosm). This is a temperate mesotrophic region (60 °N) characterized by a seasonal plankton succession. Here, spawning in many fish species coincides with the spring bloom. The subsequent growth and survival of larvae is vital for a balanced ecosystem and profitable fisheries. We studied the local assemblage of planktonic larvae, comprised of various demersal and pelagic fish species. 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). Herring larvae have proven to be ideal model organisms for the study of human impacts (Sswat et al., 2018a; Folkvord et al., 2015).

2.2 Laboratory experiment

2.2.1 Experimental setup

100 To investigate direct effects of OAE, herring larvae were reared under controlled environmental conditions (Fig. 1a). Following Folkvord et al. (2020), two wild-caught females (~356 g in size) and males (~297 g) were strip spawned onto plastic plates in two crosses. Fertilization success was 92%. Eggs were incubated at 7.1 ± 0.1 °C and a salinity of 33.4 ± 0.1 (mean ± SD). After hatching 17 days later (24th April), larvae were transferred to six aerated experimental tanks. Each tank held 0.25-0.4 m^3
105 fjord water with an estimated 1800 larvae at the start. Temperature was maintained at 8.5 ± 0.2 °C, oxygen levels at $88.5 \pm 5.2\%$ and salinity at 33.8 ± 0.7. Artificial light mimicked the local diurnal light cycle. The larvae were fed *ad libitum* with a diverse assemblage of zooplankton including nauplii and copepodites, regularly collected from the nearby fjord (Folkvord et al., 2015). Prey densities were closely monitored in the tanks and restocked daily to maintain high levels (2000 prey L^{-1}). Algae cultures were also added daily (2 L, *Rhodomonas* sp. and *Isochrysis* sp.).

2.2.2 OAE application

On day 18 post-hatch, alkalinity was enhanced (ΔTA) in three larval tanks by 600 µmol kg^{-1}, while the remaining three served as untreated controls (Fig. 1a). For this, one liter of alkaline solution that simulated calcium-based OAE was prepared with NaOH, CaCl$_2$ and Milli-Q water. The solution was then poured slowly into the center of each tank, where the current created by the aeration system assured rapid mixing. During the experiment, the equilibration of CO_2 with the atmosphere was slowed
115 down through aeration with CO_2-reduced air (pCO_2 = ~100 µatm) using CO_2-scrubbers (absorption via $Ca(OH)_2$ and NaOH). To further minimize gas exchange with the laboratory room, tanks were covered by a transparent lid. The control tanks were instead treated with pure Milli-Q water and ambient air.

The application of non-CO_2-equilibrated OAE was successful. In each tank, total alkalinity (TA) was measured three times over the treatment period following the principles in Schulz et al. (2023). Water was filtered (PES, 0.2 µm) into glass bottles
120 and stored at 4 °C until analysis on the following days via titration (Metrohm 862 Compact Titrosampler with Aquatrode Plus with PT1000) calibrated against certified reference material. pH was measured daily directly in the tanks with a handheld probe (WTW 3320 with sensor 662-1305, Xylem Analytics). Following the OAE manipulation, TA increased abruptly from 2519 ± 5 to 3082 ± 175 µmol kg^{-1} (mean ± SD) with corresponding increase in pH of ~0.7 units (Fig. 2a). Over the following 18-day test period, pH steadily decreased back towards ambient levels due to gas exchange and dilution by feed water. Our
125 experiment may thus represent a scenario where larvae are exposed to a plume of intense OAE that subsequently mixes with ambient seawater and equilibrates with the atmosphere, slowly alleviating the perturbation.
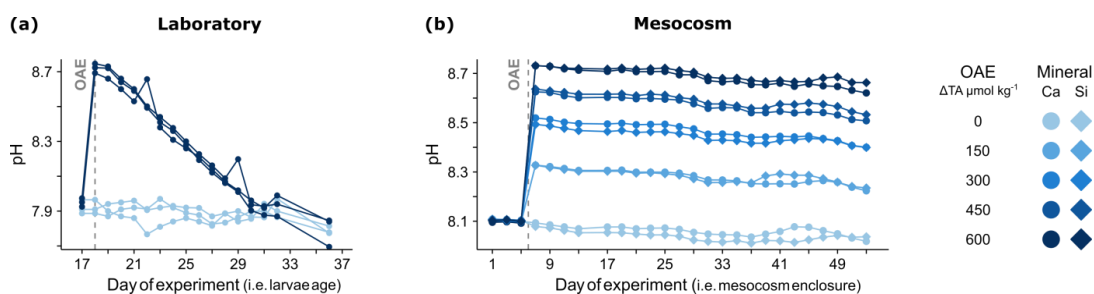
Figure 2: Development of pH in each larvae tank **(a)** and mesocosm unit **(b)**.

2.2.3 Measured variables

130 The sensitivity of herring to OAE was assessed through different traits and at different exposure times (i.e. larvae ages). As basic physiological trait, routine metabolic rate was measured on five larvae per tank following Berg et al. (2020). Larvae were placed individually in glass vials (4 or 20 mL) before dawn to assure empty guts. Oxygen consumption was tracked for 1.5 h in darkness using a 4-channel fiber-optic oxygen meter (FireSting O_2, PyroScience GmbH). Then, larvae were anesthetized (0.1 g L^{-1}) followed by euthanizing (overdose) with MS222, photographed and freeze-dried to obtain dry mass. Finally, mass-135 specific routine metabolic rate was estimated. See supplementary information for further methodological details.

Swimming behavior while hunting for live prey was assessed directly in the tanks following Illing et al. (2018). Herring larvae are pause-travel predators that swim in bouts and pause to search for and target prey. An observer tracked 10 larvae per tank for 2 min, one larva at a time. Then, total time spent moving, pause frequency and duration of each pause were computed. Growth was also assessed regularly for ten larvae per tank via measures of standard length on euthanized larvae. At the end of 140 the experiment, longer-term survival was evaluated by counting all larvae remaining in the tanks.

2.3 Mesocosm experiment

2.3.1 Experimental setup

Our second approach studied a fish assemblage of multiple species under close to natural conditions (Fig. 1b). As part of plankton communities in mesocosms, larvae were not only exposed to potential direct but also indirect effects of OAE. On 145 May 7th, ten floating KOSMOS mesocosms (Riebesell et al., 2013) were moored from RV ALKOR nearby Espegrend research station at a bottom depth of ~60 m (map in Spisla et al. (2022)). The units consisted of 20 m long and 2 m wide transparent polyurethane bags that terminated in a 2 m long sediment trap. The lower end of the bags was extended to depth, while open at the bottom, to enclose ~60 m^3 of seawater including any planktonic organisms present. Then, the bottom was closed isolating the bags from the surrounding fjord water and marking the start of the experiment (day 0). Wall growth was removed regularly 150 using brushes from the outside and a cleaning ring from the inside. Environmental conditions were assessed with CTD casts in two-day intervals. Average salinity was at ~33 and photosynthetically active radiation reduced to ~30% of surface radiation. Oxygen saturation stayed consistently above 100%. Temperature increased from 8.8 to 15.4 °C over the course of spring. These physiochemical conditions varied across the stratified water column from surface to depth.

A diverse, multi-trophic plankton food web was enclosed in the mesocosms. Larger organisms were removed at the start using 155 a net with a mesh size of 3 mm that spanned the width of the mesocosms. The resulting community comprised of primary producers (e.g. diatoms and coccolithophores), grazers (e.g. copepods) and predators (e.g. jellyfish) as in previous experiments at this locality (Schulz et al., 2017; Spisla et al., 2022). Larvae of various fish species were also present including codfishes (mostly Atlantic cod *Gadus morhua*), gobies (mostly *Pomatoschistu* spp.) and flatfishes (mostly *Limanda* spp. and *Microstomus* spp.). This naturally occurring fish assemblage was supplemented on day 6 with 95 herring larvae per mesocosm 160 (25 days post-hatch, 15 ± 1 mm). Herring had been reared together with the fish of the laboratory experiment. Primary production was initially low as the experiment began shortly after the spring bloom with little inorganic nutrients remaining (Ferderer et al., 2023). To also consider bloom conditions in our study of OAE, nutrients were added on day 25 to all mesocosms leading to an increase in nitrate, phosphate and silicate of 3.8 ± 0.2, 0.20 ± 0.01 and 0.98 ± 0.06 µmol L^{-1}, respectively.

2.3.2 OAE application

165 We tested a gradient in alkalinity enhancement from 0 to 600 µmol kg^{-1} using two different mineral sources (Fig. 1b). Half of the mesocosms simulated calcium-based (Ca) and the other half silicate-based (Si) OAE to address two contrasting mineral

addition scenarios (Renforth and Henderson, 2017; Bach et al., 2019). The Ca-based treatment only introduces elements that are already abundant in seawater and are thus unlikely to impact marine life beyond the alkalinity effect. The Si-based treatment, on the other hand, adds a limiting nutrient with the potential to alter food webs.

The OAE manipulation was conducted on day 6 following the principles in Riebesell et al. (2023). NaOH was used as alkalizer with a proportional amount of $CaCl_2$ to simulate quick or hydrate lime (Ca-based) and $MgCl_2$ to simulate olivine (Si-based) (Ferderer et al., 2023). To separate the effects of alkalinity and silicate and to prevent mineral precipitation, Na_2SiO_3 was added in equal amounts (75 µmol L^{-1}) to all five Si-based mesocosms. A special distribution device enabled a homogeneous injection of the OAE solutions into the mesocosms (Riebesell et al., 2013).

We achieved the intended alkalinity enhancement and associated shifts in carbonate chemistry. Depth-integrated water samples were taken at two-day intervals to measure TA (as in laboratory experiment) and pH (spectrophotometer). The manipulation created a clean gradient in TA from 2215 ± 5 to 2756 ± 7 µmol kg^{-1} (Ferderer et al., 2023) and pH from 8.05 ± 0.02 to 8.69 ± 0.03 (Fig 2b). Across this gradient, pCO$_2$ dropped from 384 ± 22 to 75 ± 6 µatm and ΩCa rose from 3.2 ± 0.2 to 11.7 ± 0.2. In contrast to the smaller laboratory tanks, equilibration with the atmosphere was negligible throughout the experimental period given the large depth and stratified water column of the mesocosms. Perturbations hence remained relatively stable over 48 days of OAE; optimal conditions for the emergence of food web effects and their propagation to fish.

2.3.3 Measured variables

We monitored the fish assemblage closely. Mortality through processes other than predation was tracked over the entire experiment. For this, the sediment trap was sampled in two-day intervals via a tube connected to the surface (Fig. 1b) and immediately screened for dead fish. This method had proven successful in previous campaigns, especially in colder climates where fish carcasses disintegrate slowly (Spisla et al., 2022). At the end of the experiment, all live fish were caught with a net (1 mm mesh) spanning the width of the mesocosm. Fish were anesthetized (0.1 g L^{-1}) followed by euthanizing (overdose) with MS222 and preserved at -80 °C. Finally, fish were weighed (wet mass) and assigned to broad taxonomic groups. Predation on herring was estimated as the difference between the individuals initially introduced and the ones found later in the sediment trap and final sampling.

The wider plankton community was also monitored to better understand potential indirect effects on fish. Primary producer biomass and photosynthetic capacity was approximated every two days via chlorophyll a. For this, depth-integrated water samples were filtered and analyzed on a fluorometer (Ferderer et al., 2023). Metazoan zooplankton was sampled every four days across the entire water column using a net with 55 µm mesh size (Apstein, Ø 17 cm) (Spisla et al., 2022). After preservation in 70% ethanol, copepods (main larval prey) and hydrozoans (potential larval competitors and predators) were counted under the microscope.

2.4 Data analysis

For the laboratory, the responses of herring were tested based on measurements of individual larvae. Linear mixed models were employed with *OAE* as categorical fixed effect (ΔTA 0 vs. ΔTA 600) and larvae rearing *tank* as random effect. A separate analysis was conducted for each larval age.

For the mesocosm, fish responses were instead assessed using sums or averages across all individuals. Linear regressions were employed with OAE as continuous explanatory variable (ranging from ΔTA 0 to ΔTA 600). To align with the laboratory experiment, we only focused on *alkalinity* as OAE driver. This simplification was acceptable given that the thereby omitted factor *mineral* showed no effect (Table S1). Unexplained variability was particularly high at the level of single taxa (Fig. S1) 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. Finally, we assessed possible changes in taxonomic composition under OAE using non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity and Mantel tests (Oksanen et al., 2022).

210 All analyses were conducted at a significance level of $\alpha = 0.05$ with R version 4.0.5 (R Core Team, 2021). 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. Per capita sizes of mesocosm fish were log 10 transformed. Some outlier individuals in the laboratory trials on physiology and behavior were excluded based on interquartile range.

3 Results

3.1 Organism-level responses in the laboratory

Our single-species test under controlled conditions suggests that Atlantic herring larvae may cope with OAE. Larvae under OAE maintained a similar routine metabolic rate than larvae under ambient conditions (Fig. 3a). Their activity pattern during hunting of live prey was also unaffected by OAE, including the time they spent swimming and how often and how long they paused (Fig. 3b). The absence of an OAE effect on metabolic rate and activity was consistent across exposure time (i.e. larval

220 age). To note, the pH perturbation slowly alleviated and reached ambient levels 13 days after OAE application (age 31, Fig. 2a). From then onwards, our data tested for the lasting after-effects of elevated pH.

Larvae developed normally despite OAE. Growth of treated larvae was indistinguishable from that of untreated ones (Fig. 3c) and averaged at 0.41 mm day^{-1}. Larval size structure was also maintained (Fig. 3d). Ultimately, similar numbers of larvae survived under OAE compared to ambient conditions until the end of the experiment 19 days after OAE application (Fig. 3e).

225 This final assessment was based on 1800 individuals per treatment tank feeding on natural zooplankton prey. The estimated survival of 98 %, which corresponds to 99.95 % average daily survival, can be considered extremely high, given the vulnerability of this life stage, and suggests an optimal rearing environment. In conclusion, we found no evidence for a direct effect of OAE on herring larvae across different responses at the organism-level, from basic physiology and behavior to growth and survival.
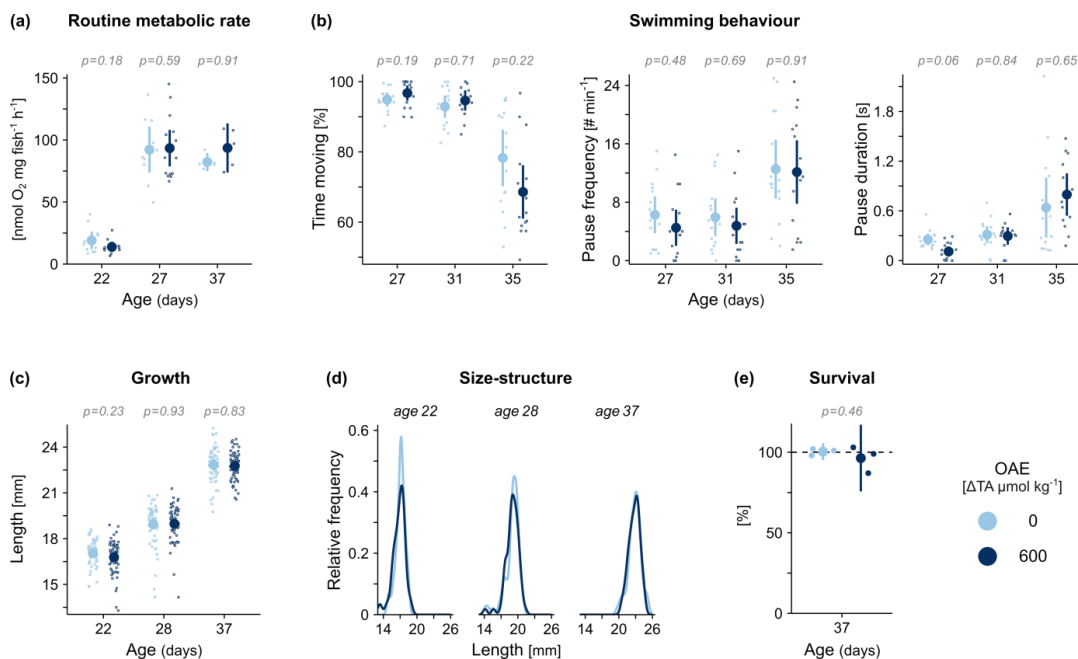
Figure 3: Organism-level responses of herring larvae to OAE, under controlled laboratory conditions. Basic physiology **(a)** and behavior **(b)**, growth via size-at-age **(c)**, size distribution **(d)** and long-term survival at the end of the experiment **(e)**. Shown are individual larvae (a-c) or treatment tanks (e) (small points) and averages (large points) with ±95% confidence intervals. At each age (days post-hatch), linear models (in grey) test for an OAE effect (details in Table S2). Larvae had been exposed to OAE since day 18.

3.2 Community-level responses in mesocosms

Our experiment on fish in natural plankton communities was also not able to detect negative impacts of OAE. Here we studied larvae and young juveniles of different coastal fishes including the introduced herring and naturally enclosed species such as Atlantic cod. In the mesocosms, these fish assemblages were shaped by both environmental conditions and food web interactions.

Mortality was throughout unrelated to OAE. It was assessed via carcasses that had sunk to the sediment trap and thus represented death via processes other than predation. The abrupt changes in water chemistry following OAE application were not lethal to any of the species (Fig. 4a-left), including herring (Fig. 4b). These results indicate that OAE did not impair physiology at the most basic level that leads to an immediate demise. Surviving the first shock, the fishes now had the chance to adjust to the novel water chemistry. At the end of our experiment, mortality still remained unaffected by OAE (Fig. 4a-right) and species-specific effects were not apparent (Fig. 4c). Hence, there was no sign of a physiological or behavioral disruption that rendered fish incapable of foraging for food causing starvation. To note, the unusually high mortality rates in two mesocosms (Ca ΔTA 300 and Si ΔTA 150, Fig 4a, b) were most likely caused by flappy walls that harmed some larvae during wave action.
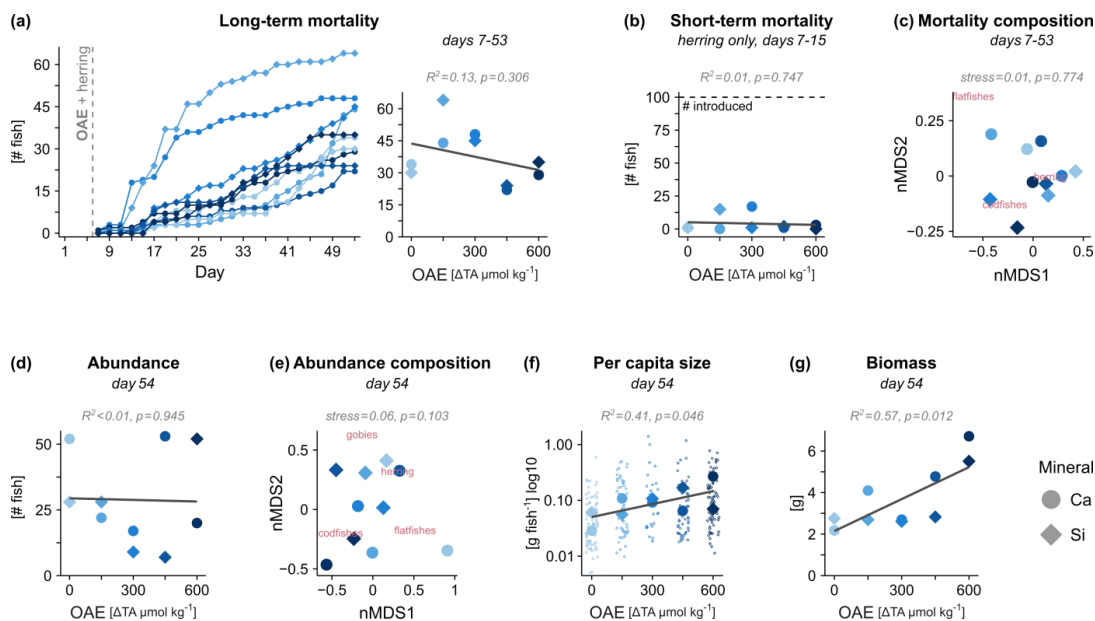
Figure 4: Community-level responses of larvae and young juveniles of various fish species to OAE, under close-to natural conditions in mesocosms. Cumulative mortality over time across all species **(a)** and immediately following the OAE perturbation for herring **(b)** assessed via the sediment trap. Count **(d)**, individual size **(f)** and total biomass **(g)** of all live fish at the end of the experiment. Differences in taxonomic composition between mesocosms via non-metric multidimensional scaling (nMDS) **(c, e)**. Larger points represent mesocosms and smaller points in **f** single individuals. Linear regressions and Mantel tests (in grey) check for an OAE effect (details in Table S3)

The assemblage of live individuals at the end of the 55-day experiment confirmed that OAE was not a dominant stressor for fish. Neither total abundances (Fig. 4d) nor taxonomic composition (Fig. 4e) followed any OAE related pattern. Besides physiological stress and starvation, abundances were also a product of predation by other fish and invertebrate predators. At this point, we have to acknowledge the high variability in some of the mesocosm data, especially at the level of individual fish species, which may have concealed less severe yet ecologically relevant impacts. The only significant responses in our study were that average fish size, and consequently total fish biomass, increased under OAE (Fig. 4f, g). This seemed to be mostly driven by one taxon, the codfishes (Fig. S1).

Finally, we assessed changes in the plankton food web with the potential to affect fish indirectly. Pronounced effects of OAE on lower trophic levels were not apparent, considering primary producers (chlorophyll a, Fig. 5a) and the main prey of fish larvae (copepod zooplankton, Fig. 5b). Invertebrate predators (jellyfish) that compete with fish for food and may prey on small larvae seemed also unaffected by OAE (Fig. 5c). There was no sign of an altered predation rate on herring (Fig. 5d), an estimate that also includes the potential predation from larger piscivorous fish present in the mesocosms (e.g. cod juveniles). Overall, we found no evidence for an increased bottom-up or reduced top-down forcing that could explain the positive effect of OAE on fish biomass.
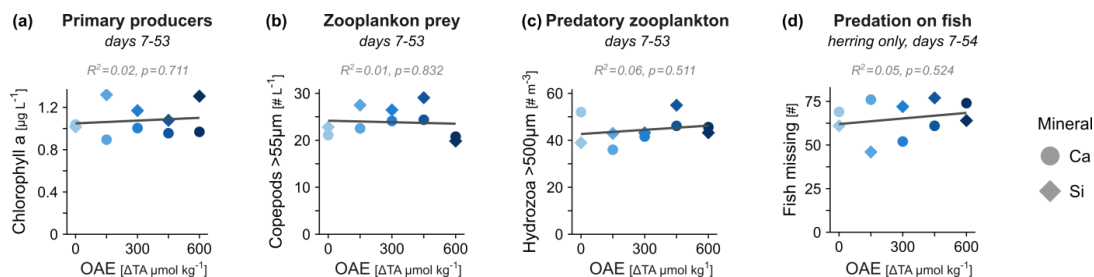
Figure 5: Potential sources of indirect effects of OAE on fish mediated via species interactions. Abundance of other functional groups including **(a)** primary producers, **(b)** invertebrate grazers and **(c)** invertebrate predators. **d)** Predation on herring estimated via missing individuals. Averages across the treatment period are tested with linear regressions (in grey, details in Table S4).

4. Discussion

Our study suggests that larvae of some temperate coastal fishes may be viable under ocean alkalinity enhancement (OAE) for carbon dioxide removal. Negative impacts of OAE were not apparent despite our thorough investigation across response types (physiology to survival), ecological scales (organism to community) and timeframes (short- to long-term exposure). Early life stages from these fish populations may recruit successfully and continue to support fisheries in regions of OAE deployment.

Physiology and behavior of the tested fish larvae seemed resistant to the direct effects of OAE. Functional impairment, as reported in some cases for the opposing stressor ocean acidification (Cattano et al., 2018; Esbaugh, 2018; Nagelkerken and Connell, 2015), could not be observed. 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 OAE 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). At least for Atlantic herring, this result should come as no surprise given the species' tolerance to ocean acidification (Sswat et al., 2018a; Maneja et al., 2015; Franke and Clemmesen, 2011, but also see Frommel et al., 2014). Whilst our larvae experiments did not demonstrate adverse effects of OAE, other life history stages may prove more vulnerable, in particular reproductive cells and early embryos that lack specialized regulatory tissue (Melzner et al., 2009; Dahlke et al., 2020).

Ecosystems and fish species may differ in their sensitivity to OAE. We only know of two comprehensive studies on the viability of fish larvae under increased pH (Brownell, 1980; Parra and Yufera, 2002). Five species were investigated here in an aquaculture context. After only 24 h exposure to pH above ~8.5, the authors reported reduced first-feeding success and, in one species, even lethality. Interestingly, high pH was considerably more detrimental than low pH (Brownell, 1980; Parra and Yufera, 2002), further emphasizing the impact potential of OAE. Given these acute sensitivities that contrast our finding of tolerance, we can infer a species-specific effect of OAE on fish larvae. OAE could be more stressful for fishes that have adapted to stable environmental conditions, such as in the open ocean (Blewett et al., 2022; Hofmann et al., 2011), or for fishes that are already challenged physiologically, like in warm tropical waters (Vinagre et al., 2016; Pörtner, 2008). Our results at the organism-level are clearly only a first step towards assessing the safety of OAE for fish populations.

Once fish survive the water chemistry perturbation itself, time would be given for food web mediated impacts of OAE. In this context, several mechanisms were proposed with the potential to lower the quantity and quality of food for higher trophic levels. These include slowed primary production (Hansen, 2002), proliferation of heavily armored calcifiers (Renforth and

Henderson, 2017) and pH stress in invertebrate prey (Melzner et al., 2009). Yet, in our mesocosms, we could not find evidence for these indirect effects of OAE on fish, not even after long-term exposure. However, what we did observe was a positive effect of OAE on fish biomass. This unexpected result was not easily explained through major changes elsewhere in the community. Neither food availability seemed enhanced nor the potential for competition with or predation by jellyfish reduced.

310 Altered intraguild predation — larger fish eating smaller ones — seemed not to be responsible either. With that we explored some of the more obvious bottom-up and top-down processes that can propagate environmental change to fish (Sswat et al., 2018b; Goldenberg et al., 2017; Spisla et al., 2022). These findings emphasize the complexity of species interactions that will require careful consideration in the emerging research field of OAE.

Whilst more subtle responses may have been overseen, our study fishes seemed able to maintain basic physiological and

315 ecological capabilities under OAE. This is a significant first step towards the classification of OAE as a stressor, since under these circumstances fish populations are given an opportunity to adjust. At the organismal-level, individuals may avoid the OAE plume, acclimate physiologically (Pörtner, 2008) or simply persevere until mixing or equilibration alleviates the perturbation (He and Tyka, 2023). At the community-level, behavioral plasticity may enable individuals to react to changing resources and predation (Wong and Candolin, 2015; Mcmeans et al., 2016), while the selection of robust phenotypes over

320 generations allows for genetic adaptation (Sunday et al., 2014). The basic survivability demonstrated in our study is a prerequisite for these compensatory mechanisms that could improve population resistance and recovery.

Over geological time scales, natural rock weathering and the resulting alkalization of the ocean has been buffering earth's climate. An artificial acceleration of this process could be accomplished through different scenarios, each one causing a distinct perturbation of seawater (Bach et al., 2019; Renforth and Henderson, 2017; Hartmann et al., 2023). We focused on changes in

325 carbonate chemistry before CO_2-equilibration of the alkalized water. Our inability to detect severe impacts here may classify the alternative, pre-CO_2-equilibrated application as especially safe, given its stable CO_2 partial pressure and smaller increases in pH and calcium carbonate saturation. While not subject of our study, secondary drivers of OAE including trace metal toxicity/enrichment (Morel and Price, 2003) or suspended mineral particles (Affandi and Ishak, 2019) are likely to play a role as well. A large-scale intervention such as OAE will inevitably bring about some level of environmental change, not just via

330 the manipulation itself but also supporting activities on land and at sea. OAE has to be seen in relation to other carbon-dioxide removal technologies and the environmental, economic and social harm caused by unrestrained climate change. Our study on temperate coastal species helps to develop such a risk-benefit analysis towards the preservation of fish in marine ecosystems and the livelihoods they support.

Ethics

335 Fish research was approved by the Norwegian Animal Research Authority (NARA) via the University of Bergen (laboratory, FOTS ref 29008) and University of Agder (mesocosm, FOTS 28931).

Data availability

The raw data supporting the conclusions of this article are being made available via PANGAEA. This process takes time and the DOIs are not ready yet. In the meanwhile, editors and reviewers can access the data here:

340 https://drive.google.com/drive/folders/1yCOyxDavylXheQff1L2WIll1jdvKsnP7?usp=sharing

Competing interests

The authors declare that they have no conflict of interest.

Author contributions

SUG, MM, UR, MS, GB and AF conceptualized the study, all authors conducted the experiments, SUG and MM analysed the
345   data, SUG wrote the manuscript with particular input from MM and DB, and all authors revised the manuscript.

Acknowledgments

We thank the Espegrend Marine Biological Station and the University of Bergen for providing facilities and technical support.
Further, we thank the whole KOSMOS team, including Andrea Ludwig for logistics, Jana Meyer for fish sediment sampling,
Kai Schulz and Julieta Schneider for their expertise in carbonate chemistry, and Leila Kittu and Anna Groen for chlorophyll
350   filtrations. This study was funded by the European Union's Horizon 2020 research and innovation program via the projects
OceanNETS (869357) and AQUACOSM-plus (871081). Additional funding was provided through the German Research
Foundation (THRESHOLDS, MO 2873/3-1), the Center for Coastal Research at the University of Agder and Villum Fonden
(25512).

References

355   Affandi, F. A. and Ishak, M. Y.: Impacts of suspended sediment and metal pollution from mining activities on riverine fish
populationa review, Environmental Science and Pollution Research, 26, 16939-16951, 10.1007/s11356-019-05137-7, 2019.

Bach, L. T., Gill, S. J., Rickaby, R. E. M., Gore, S., and Renforth, P.: CO_2 Removal With Enhanced Weathering and Ocean
Alkalinity Enhancement: Potential Risks and Co-benefits for Marine Pelagic Ecosystems, Frontiers in Climate, 1,
10.3389/fclim.2019.00007, 2019.

360   Berg, F., Andersson, L., and Folkvord, A.: Respiration rates of herring larvae at different salinities, and effects of previous
environmental history, Mar. Ecol.-Prog. Ser., 650, 141-152, 10.3354/meps13318, 2020.

Blewett, T. A., Binning, S. A., Weinrauch, A. M., Ivy, C. M., Rossi, G. S., Borowiec, B. G., Lau, G. Y., Overduin, S. L.,
Aragao, I., and Norin, T.: Physiological and behavioural strategies of aquatic animals living in fluctuating environments,
Journal of Experimental Biology, 225, 10.1242/jeb.242503, 2022.

365   Brownell, C. L.: Water quality requirements for first-feeding in marine fish larvae. II. pH, oxygen, and carbon dioxide, J. Exp.
Mar. Biol. Ecol., 44, 285-298, 10.1016/0022-0981(80)90159-8, 1980.

Carstensen, J. and Duarte, C. M.: Drivers of pH variability in coastal ecosystems, Environmental Science & Technology, 53,
4020-4029, 10.1021/acs.est.8b03655, 2019.

Cattano, C., Claudet, J., Domenici, P., and Milazzo, M.: Living in a high CO_2 world: a global meta-analysis shows multiple
370   trait-mediated fish responses to ocean acidification, Ecological Monographs, 88, 320-335, 10.1002/ecm.1297, 2018.

Dahlke, F. T., Wohlrab, S., Butzin, M., and Pörtner, H. O.: Thermal bottlenecks in the life cycle define climate vulnerability
of fish, Science, 369, 65-70, 10.1126/science.aaz3658, 2020.

Doney, S. C., Busch, D. S., Cooley, S. R., and Kroeker, K. J.: The Impacts of Ocean Acidification on Marine Ecosystems and
Reliant Human Communities, in: Annual Review of Environment and Resources, Vol 45, edited by: Gadgil, A., and Tomich,
375   T. P., Annual Review of Environment and Resources, 83-112, 10.1146/annurev-environ-012320-083019, 2020.

Esbaugh, A. J.: Physiological implications of ocean acidification for marine fish: emerging patterns and new insights, Journal
of Comparative Physiology B-Biochemical Systems and Environmental Physiology, 188, 1-13, 10.1007/s00360-017-1105-6,
2018.

FAO: The State of World Fisheries and Aquaculture 2022, FAO, Rome, 10.4060/cc0461en, 2022.

380   Ferderer, A., Schulz, K. G., Riebesell, U., Baker, K. G., Chase, Z., and Bach, L. T.: Investigating the effect of silicate and
calcium based ocean alkalinity enhancement on diatom silicification, Biogeosciences Discuss., 2023, 1-28, 10.5194/bg-2023-
144, 2023.

Folkvord, A., Vollset, K. W., and Catalán, I. A.: Differences in growth and survival between cod *Gadus morhua* and herring
Clupea harengus early stages co-reared at variable prey concentrations, J. Fish Biol., 87, 1176-1190, 10.1111/jfb.12783, 2015.

385   Folkvord, A., Lakso, E., Laupsa, M., Meier, S., Musialak, L. A., and Sundby, S.: Swimbladder filling in herring larvae: effects
of food oil on the water surface, Marine Biology Research, 16, 446-457, 10.1080/17451000.2020.1837882, 2020.

Franke, A. and Clemmesen, C.: Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.), Biogeosciences, 8, 3697-3707, 10.5194/bg-8-3697-2011, 2011.

Frommel, A. Y., Maneja, R., Lowe, D., Pascoe, C. K., Geffen, A. J., Folkvord, A., Piatkowski, U., and Clemmesen, C.: Organ damage in Atlantic herring larvae as a result of ocean acidification, Ecological Applications, 24, 1131-1143, 10.1890/13-0297.1, 2014.

Gattuso, J.-P., Williamson, P., Duarte, C. M., and Magnan, A. K.: The potential for ocean-based climate action: negative emissions technologies and beyond, Frontiers in Climate, 2, 37, 10.3389/fclim.2020.575716, 2021.

Goldenberg, S. U., Nagelkerken, I., Ferreira, C. M., Ullah, H., and Connell, S. D.: Boosted food web productivity through ocean acidification collapses under warming, Glob. Change Biol., 23, 4177-4184, 10.1111/gcb.13699, 2017.

Goldenberg, S. U., Nagelkerken, I., Marangon, E., Bonnet, A., Ferreira, C. M., and Connell, S. D.: Ecological complexity buffers the impacts of future climate on marine consumers, Nature Climate Change, 8, 229-233, 10.1038/s41558-018-0086-0, 2018.

Hansen, P. J.: Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession, Aquatic Microbial Ecology, 28, 279-288, 10.3354/ame028279, 2002.

Hartmann, J., Suitner, N., Lim, C., Schneider, J., Marin-Samper, L., Aristegui, J., Renforth, P., Taucher, J., and Riebesell, U.: Stability of alkalinity in ocean alkalinity enhancement (OAE) approaches -consequences for durability of CO2 storage, Biogeosciences, 20, 781-802, 10.5194/bg-20-781-2023, 2023.

He, J. and Tyka, M. D.: Limits and CO2 equilibration of near-coast alkalinity enhancement, Biogeosciences, 20, 27-43, 10.5194/bg-20-27-2023, 2023.

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., Paytan, A., Price, N. N., Peterson, B., Takeshita, Y., Matson, P. G., Crook, E. D., Kroeker, K. J., Gambi, M. C., Rivest, E. B., Frieder, C. A., Yu, P. C., and Martz, T. R.: High-frequency dynamics of ocean pH: a multi-ecosystem comparison, PLoS One, 6, 10.1371/journal.pone.0028983, 2011.

Houde, E. D.: Emerging from Hjort's shadow, Journal of Northwest Atlantic Fishery Science, 41, 2008.

Illing, B., Moyano, M., Berg, J., Hufnagl, M., and Peck, M. A.: Behavioral and physiological responses to prey match-mismatch in larval herring, Estuar. Coast. Shelf Sci., 201, 82-94, 10.1016/j.ecss.2016.01.003, 2018.

Lackner, K. S.: Carbonate chemistry for sequestering fossil carbon, Annual Review of Energy and the Environment, 27, 193-232, 10.1146/annurev.energy.27.122001.083433, 2002.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., and Makowski, D.: performance: an R package for assessment, comparison and testing of statistical models, Journal of Open Source Software, 6, 10.21105/joss.03139, 2021.

Maneja, R. H., Frommel, A. Y., Browman, H. I., Geffen, A. J., Folkvord, A., Piatkowski, U., Durif, C. M. F., Bjelland, R., Skiftesvik, A. B., and Clemmesen, C.: The swimming kinematics and foraging behavior of larval Atlantic herring (*Clupea harengus* L.) are unaffected by elevated pCO2, J. Exp. Mar. Biol. Ecol., 466, 42-48, 10.1016/j.jembe.2015.02.008, 2015.

McMeans, B. C., McCann, K. S., Tunney, T. D., Fisk, A. T., Muir, A. M., Lester, N., Shuter, B., and Rooney, N.: The adaptive capacity of lake food webs: from individuals to ecosystems, Ecological Monographs, 86, 4-19, 10.1890/15-0288.1, 2016.

Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Portner, H. O.: Physiological basis for high CO2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?, Biogeosciences, 6, 2313-2331, 10.5194/bg-6-2313-2009, 2009.

Morel, F. M. M. and Price, N. M.: The biogeochemical cycles of trace metals in the oceans, Science, 300, 944-947, 10.1126/science.1083545, 2003.

Nagelkerken, I. and Connell, S. D.: Global alteration of ocean ecosystem functioning due to increasing human CO2 emissions, Proc. Natl. Acad. Sci. U. S. A., 112, 13272-13277, 10.1073/pnas.1510856112, 2015.

Nawaz, S., Lezaun, J., Valenzuela, J. M., and Renforth, P.: Broaden research on ocean alkalinity enhancement to better characterize social impacts, Environmental Science & Technology, 57, 8863-8869, 10.1021/acs.est.2c09595, 2023.

Ockendon, N., Baker, D. J., Carr, J. A., White, E. C., Almond, R. E. A., Amano, T., Bertram, E., Bradbury, R. B., Bradley, C., Butchart, S. H. M., Doswald, N., Foden, W., Gill, D. J. C., Green, R. E., Sutherland, W. J., Tanner, E. V. J., and Pearce-Higgins, J. W.: Mechanisms underpinning climatic impacts on natural populations: altered species interactions are more important than direct effects, Glob. Change Biol., 20, 2221-2229, 10.1111/gcb.12559, 2014.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro

Cunha, E., Smith, T., Stier, A., Ter Braak, C., and Weedon, J.: vegan: community ecology package (R package version 2.6-4), 2022.

440 Omar, A. M., Skjelvan, I., Erga, S. R., and Olsen, A.: Aragonite saturation states and pH in western Norwegian fjords: seasonal cycles and controlling factors, 2005-2009, Ocean Sci., 12, 937-951, 10.5194/os-12-937-2016, 2016.

Parra, G. and Yufera, M.: Tolerance response to water pH in larvae of two marine fish species, gilthead seabream, *Sparus aurata* (L.) and Senegal sole, *Solea senegalensis* (Kaup), during development, Aquaculture Research, 33, 747-752, 10.1046/j.1365-2109.2002.00713.x, 2002.

445 Perry, S. F. and Gilmour, K. M.: Acid-base balance and CO2 excretion in fish: unanswered questions and emerging models, Respiratory Physiology & Neurobiology, 154, 199-215, 10.1016/j.resp.2006.04.010, 2006.

Pörtner, H. O.: Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view, Mar. Ecol.-Prog. Ser., 373, 203-217, 10.3354/meps07768, 2008.

R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, 450 Austria. URL https://www.R-project.org/, 2021.

Renforth, P. and Henderson, G.: Assessing ocean alkalinity for carbon sequestration, Reviews of Geophysics, 55, 636-674, 10.1002/2016rg000533, 2017.

Riebesell, U., Basso, D., Geilert, S., Dale, A. W., Kreuzburg, M., and Meysman, F.: Mesocosm experiments in ocean alkalinity enhancement research, State of the Planet Discussions, 2023, 1-21, 10.5194/sp-2-oae2023-6-2023, 2023.

455 Riebesell, U., Czerny, J., von Brockel, K., Boxhammer, T., Budenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R., and Schulz, K. G.: Technical Note: A mobile sea-going mesocosm system - new opportunities for ocean change research, Biogeosciences, 10, 1835-1847, 10.5194/bg-10-1835-2013, 2013.

Rogelj, J., Popp, A., Calvin, K. V., Luderer, G., Emmerling, J., Gernaat, D., Fujimori, S., Strefler, J., Hasegawa, T., Marangoni, G., Krey, V., Kriegler, E., Riahi, K., van Vuuren, D. P., Doelman, J., Drouet, L., Edmonds, J., Fricko, O., Harmsen, M., Havlik, 460 P., Humpenoder, F., Stehfest, E., and Tavoni, M.: Scenarios towards limiting global mean temperature increase below 1.5 °C, Nature Climate Change, 8, 325–332, 10.1038/s41558-018-0091-3, 2018.

Schulz, K. G., Bach, L. T., and Dickson, A. G.: Seawater carbonate system considerations for ocean alkalinity enhancement research, State Planet Discuss., 2023, 1-24, 10.5194/sp-2023-12, 2023.

Schulz, K. G., Bach, L. T., Bellerby, R. G. J., Bermúdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel, A., Ludwig, 465 A., Meyerhöfer, M., Larsen, A., Paul, A. J., Sswat, M., and Riebesell, U.: Phytoplankton blooms at increasing levels of atmospheric carbon dioxide: experimental evidence for negative effects on prymnesiophytes and positive on small picoeukaryotes, Frontiers in Marine Science, 4, 10.3389/fmars.2017.00064, 2017.

Spisla, C., Taucher, J., Sswat, M., Wunderow, H., Kohnert, P., Clemmesen, C., and Riebesell, U.: Ocean acidification alters the predator - prey relationship between hydrozoa and fish larvae, Frontiers in Marine Science, 9, 10.3389/fmars.2022.831488, 470 2022.

Sswat, M., Stiasny, M. H., Jutfelt, F., Riebesell, U., and Clemmesen, C.: Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and CO2, PLoS One, 13, 10.1371/journal.pone.0191947, 2018a.

Sswat, M., Stiasny, M. H., Taucher, J., Alguero-Muniz, M., Bach, L. T., Jutfelt, F., Riebesell, U., and Clemmesen, C.: Food web changes under ocean acidification promote herring larvae survival, Nat. Ecol. Evol., 2, 836-840, 10.1038/s41559-018- 475 0514-6, 2018b.

Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., and Reusch, T. B. H.: Evolution in an acidifying ocean, Trends in Ecology & Evolution, 29, 117-125, 10.1016/j.tree.2013.11.001, 2014.

Tresguerres, M., Clifford, A. M., Harter, T. S., Roa, J. N., Thies, A. B., Yee, D. P., and Brauner, C. J.: Evolutionary links between intra- and extracellular acid-base regulation in fish and other aquatic animals, Journal of Experimental Zoology Part 480 a-Ecological and Integrative Physiology, 333, 449-465, 10.1002/jez.2367, 2020.

Vinagre, C., Leal, I., Mendonça, V., Madeira, D., Narciso, L., Diniz, M. S., and Flores, A. A. V.: Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms, Ecological Indicators, 62, 317-327, 10.1016/j.ecolind.2015.11.010, 2016.

Wong, B. B. M. and Candolin, U.: Behavioral responses to changing environments, Behavioral Ecology, 26, 665-673, 485 10.1093/beheco/aru183, 2015.