1   **Technical note: Ocean Alkalinity Enhancement Pelagic Impact Intercomparison Project**

2   **(OAEPIIP)**

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14   **Abstract**

16   Ocean Alkalinity Enhancement (OAE) aims to transfer carbon dioxide (CO_2) from the

17   atmosphere to the ocean by increasing the capacity of seawater to store CO_2. The potential

18   effects of OAE-induced changes in seawater chemistry on marine biology must be assessed

19   to understand if OAE, operated at a climate relevant scale, would be environmentally

20   sustainable. Here, we describe the design of the Ocean Alkalinity Enhancement Pelagic Impact

21   Intercomparison Project (OAEPIIP) - a standardised OAE microcosm experiment with plankton

22   communities to be conducted worldwide. OAEPIIP provides funding for participating

23   laboratories to conduct OAE experiments in their local environments. This paper constitutes

24   a detailed manual on the standardised methodology that shall be adopted by all OAEPIIP

25   participants. The individual studies will provide new insights into how plankton communities

26   respond to OAE. The synthesis of these standardized studies, without publication bias, will

27   reveal common OAE-responses that occur across geographic and environmental gradients

28   and are therefore particularly important to determine. The funding available to OAEPIIP and

29   resulting data will be shared to maximise its value and the accessibility. The globally

30   coordinated effort has potential to promote scientific consensus about the potential effects

31   of OAE on diverse plankton communities. Such consensus, through inclusion of the global

32 community, will provide a sounder base to facilitate political decision making whether OAE
33 should be upscaled or not.

34

1. Rationale for the Ocean Alkalinity Enhancement Pelagic Impact Intercomparison Project (OAEPIIP)

37

38 Ocean Alkalinity Enhancement (OAE) is an emerging carbon dioxide removal (CDR) approach
39 (Oschlies et al., 2023, NASEM, 2022) . OAE drives CDR through the introduction of alkaline
40 substances into seawater which shift the carbonate chemistry equilibrium:

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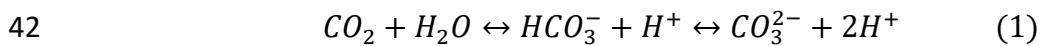
42
$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \qquad (1)$$
43

44 from carbon dioxide (CO_2) on the left to bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}) on
45 the right. The decline in seawater CO_2 concentration lowers the seawater CO_2 partial pressure
46 (pCO_2), thereby enabling an influx of additional atmospheric CO_2, or alternatively, reducing
47 the efflux in cases where the surface ocean is a natural source of CO_2 to the atmosphere. The
48 OAE-induced shift in carbonate chemistry is measurable as an increase in seawater alkalinity
49 – the name-giving feature of OAE. The viability of OAE to serve as a scalable CDR approach
50 critically depends on whether it is environmentally safe. Surface ocean habitats are in focus
51 of the environmental OAE assessment because the surface ocean is where OAE would need
52 to be implemented to enable CO_2 exchange with the atmosphere (Bach et al., 2019).
53   The environmental OAE assessment is only just starting but seems to be evolving in a
54 similar way as environmental assessments of other drivers of environmental change (e.g.
55 ocean acidification) have been set up in the past: Research funding is provided to individual
56 groups, who will perform individual studies in their local environments, seeking novelty. Each
57 of these studies will be valuable and exceeding previous research is central to scientific
58 progress. However, previous research on environmental drivers has also shown that
59 replication of experiments is perhaps equally important as seeking novelty, since replication
60 allows us to reveal re-occurring response patterns across various scales and environments
61 (Benton et al., 2007; Hamm et al., 2022; Stewart et al., 2013). In ocean acidification research
62 for example, an individual study found that carbon to nitrogen (C/N) stoichiometry of
63 plankton communities is increased under high CO_2 conditions due to CO_2 fertilization of the

phytoplankton community (Riebesell et al., 2007). However, replication of the experiment at different locations found that zooplankton communities can strongly modify the response, to the point that the response can be significant in the opposite direction (lower C/N under high CO_2 (Taucher et al., 2021)). Arguably, the crucial progress in this example was understanding of the context-dependency of the C/N response to ocean acidification, which was made possible by replication of a sophisticated experiment across a wide geographical range (Riebesell et al., 2013). Likewise, the intercomparison of climate models via replicated numerical experiments (Dingley et al., 2023) has long been recognised as a cornerstone to the assessment of climate change (Masson-Delmotte et al., 2021), possibly more influential than the output of individual climate models.

The Ocean Alkalinity Enhancement Pelagic Impact Intercomparison Project (OAEPIIP) builds upon these insights from previous environmental assessments by establishing a platform that supports replication, while still enabling the pursuit of novelty. In essence, OAEPIIP provides funding for a cost-efficient and standardised OAE experiment, which can be conducted by scientists across the globe (section 2). The experiments will use a microcosm setup to study the response of natural plankton communities to one widely considered OAE implementation strategy, and they will determine the same set of response variables. Each experiment shall be published on an individual basis in a special issue of a peer-reviewed scientific journal under open access with costs largely covered by OAEPIIP (section 3). Individual publication of OAEPIIP experiments gives room to describe novel observations on how plankton communities respond to OAE. All datasets will be shared and synthesized in a meta-analysis. The standardised experimental design facilitates inclusion of individual datasets into the meta-analysis (Harrison, 2011). Likewise, the collection of all datasets, irrespective of their outcomes, avoids publication bias, which is a known problem of meta-analyses (Field and Gillett, 2010). We expect OAEPIIP to promote consensus among scientists concerning the potential environmental side effects of OAE on plankton communities, with significant potential for capacity building (section 4). This paper provides a detailed manual for the OAEPIIP experimental setup and describes its benefits.

2. Experimental infrastructure, operation, and design

2.1. Microcosm setup

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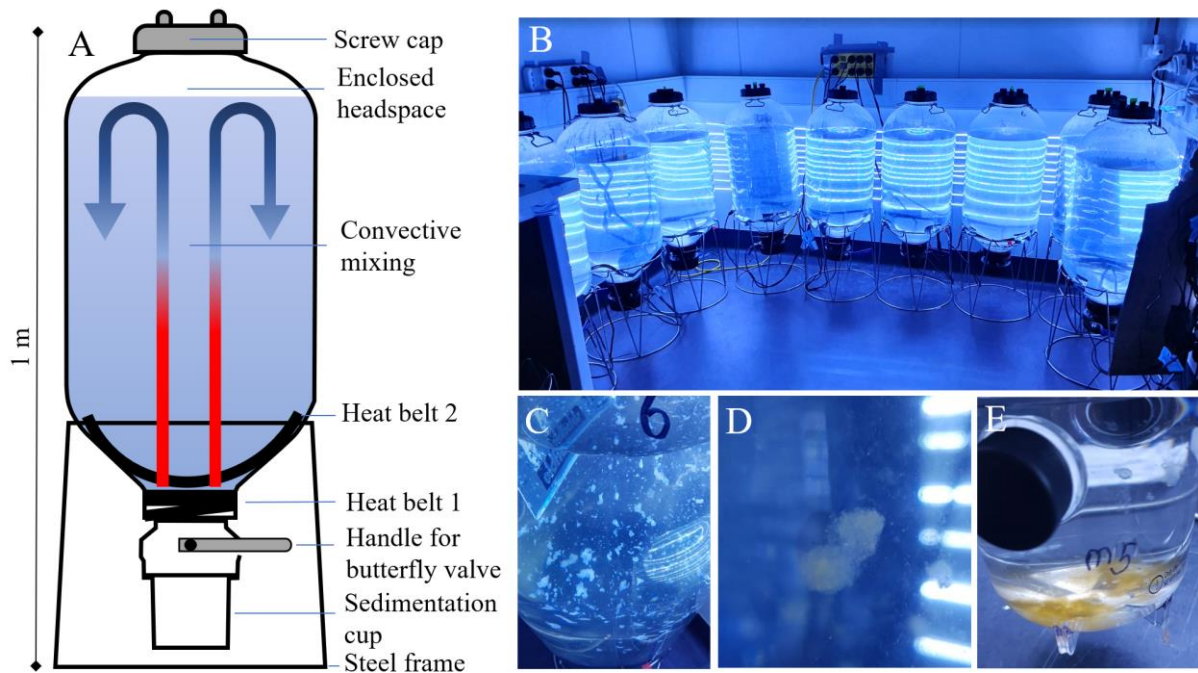
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98 *Figure 1. Microcosm setup. (A) Schematic of the microcosm tanks. The 2 heat belts induce*
99 *convective mixing within the tanks. (B) Arrangement of 9 microcosms in a temperature-*
100 *controlled room in front of a light source. Their position should be changed on a daily basis to*
101 *minimize position-dependent differences in light and temperature over the course of the*
102 *study. (C) A picture of a microcosm, just after NaOH addition. The white flakes are brucite*
103 *particles that need to be dissolved after NaOH addition by stirring the seawater within*
104 *microcosms with a plastic spoon. (D) A close-up of a marine snow aggregate, which frequently*
105 *forms after a phytoplankton bloom. (E) Marine snow aggregates collected in the*
106 *sedimentation cup of the microcosm. Sampling these can be interesting, although this is not*
107 *an essential parameter of OAEPIIP (section 2.6.).*

108

109 OAEPIIP utilizes the microcosm setup developed by Ferderer et al. (2022), as it is cost-effective
110 and relatively easy to set up and operate. The microcosms are 55L Polyethylene terephthalate
111 (PET) tanks (FermZilla), which were originally designed for home brewing (Fig. 1), and
112 available worldwide (Table S1). It is important that all OAEPIIP participants purchase the same
113 type of microcosm incubators so that comparability can be established between individual
114 studies (details on the availability of FermZilla tanks are provided in Table S1). The tanks are
115 mounted on steel frames and have 120 and 70 mm openings at the top and bottom,
116 respectively. The bottom opening is equipped with a butterfly valve and a sedimentation cup,

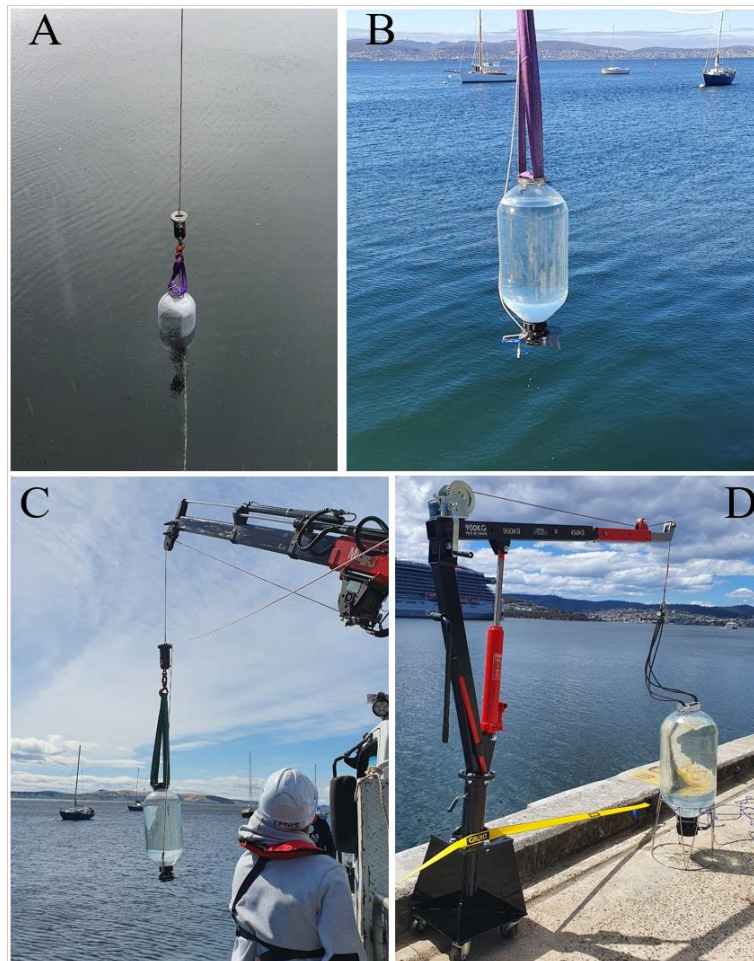
117 used for the collection of settling material. The butterfly valve has a handle so that the
118 sedimentation cup can be isolated from the water column.

119 The crucial steps for setting up the microcosms, their filling, and their operation are listed in
120 Table S1, illustrated in Figs. 1 and 2 and Videos S1 (https://doi.org/10.5446/66751) and S2
121 (https://doi.org/10.5446/66753), and briefly described here. OAEPIIP experiments occupy
122 approximately 9 m^2 in a temperature-controlled room with a cooling capacity of roughly 6°C
123 below the temperature aimed for in the microcosm study (e.g., to 14°C if the desired
124 experimental water temperature is 20°C). The microcosms need to be thoroughly cleaned in
125 a two-step procedure before use as detailed in Table S1.

126 Infrastructure needed for filling the microcosms with natural seawater (containing natural
127 plankton communities) depends on the local environment at an OAEPIIP study site. At our site
128 in Tasmania, we fill microcosms from a jetty using a small crane or davit (Fig. 2; Video S1
129 (https://doi.org/10.5446/66751)). Natural seawater with plankton communities shall be
130 collected by opening the top lid and butterfly valve at the bottom and lowering the
131 microcosms slowly into seawater so that each microcosm is filled from bottom to top. Care
132 must be taken to not enclose larger debris, nekton or sediments. Once the microcosm is
133 submersed and only the upper opening is above the sea surface, a rope attached to the handle
134 of the butterfly valve is pulled so that the bottom opening is closed. The microcosm can now
135 be lifted back on shore and put back into its metal frame. Another possibility to fill microcosms
136 is to slowly lower them from a low swimming pontoon or small boat and close the bottom
137 manually. Filling microcosms by slowly lowering them into seawater is a very gentle way to
138 collect plankton communities (Video S1 (https://doi.org/10.5446/66751)), avoiding the
139 physical disturbance to plankton imposed by pumping. Based on our experience it takes
140 roughly 45 minutes to fill 9 microcosms. Longer timescales for the collection (i.e., >>1 hour)
141 should be avoided to mitigate the risk of changes in seawater communities over the course
142 of the filling procedure (e.g. through tidal water movement). This potential problem should
143 also be minimised by filling the microcosms in random order. Furthermore, care should be
144 taken to not expose the microcosms to excessive sunlight and/or heat after filling.

145 The weight of the enclosed seawater needs to be determined after the filling procedure as
146 this information is needed for establishing treatments (section 2.5). This could be done using
147 a balance or (if a balance is not available) volumetrically and determining weight with known
148 volume, temperature, and salinity. Once the weight has been determined, microcosms need

149 to be transported to the temperature-controlled room where the experiment takes place and

150 light and temperature control needs to be initiated immediately (see following section).

151

152 ***Figure 2***. *Seawater collection for the microcosm experiments. (A) A microcosm slowly lowered*

153 *into seawater to gently collect a plankton community. (B and C) A filled microcosm being*

154 *pulled back on land. Please note that we mostly used a small crane mounted to a truck or a*

155 *davit (as in D) for the seawater collection. However, microcosms filled with seawater only*

156 *weigh about 60 kg, so that lighter gear is probably sufficient for collection. A detailed*

157 *description of seawater collection is provided in Table S1 and Video S1*

158 *(https://doi.org/10.5446/66751).*

159

160 **2.2. Mixing, temperature, light, and nutrient conditions in OAEPIIP experiments**

161

162 OAEPIIP utilises convection to mix the enclosed microcosm volume and keep plankton in

163 suspension (Fig. 1). To establish convective mixing, two 30 Watts heat belts (see Table S1 for

164 where these can be purchased) will be firmly attached to two distinct locations at the bottom

of the microcosms (Fig. 1, Video S2 (https://doi.org/10.5446/66753)). Based on our experience, these two 30 W heat belts increase the temperature of the enclosed seawater by ~6°C relative to the room temperature, so that room temperature needs to be roughly ~6°C lower than the target temperature in the experiments (please note that testing the temperature difference will be necessary prior to the experiment as temperature offset may differ across temperature-controlled rooms). Once heat belts are attached, microcosms should be placed in front of the light source and heat belts should be plugged in to initiate the convective mixing.

While convection provides gentle and non-invasive mixing, there are several trade-offs regarding temperature control. Firstly, due to the removal of seawater during sampling, the total volume within microcosms declines over the course of the experiment. Since the heat belts cannot be adjusted, there is an increase in heat energy input per liter of enclosed seawater and thus a gradual warming. To mitigate this issue, the external cooling may need to be increased over time by lowering the room temperature. In our experience, a reduction by 1°C for every 5 Liters of seawater sampled from the microcosms is sufficient to keep the seawater temperature relatively constant over the course of the study. Secondly, small differences in ventilation at different locations in the temperature-controlled room can lead to seawater temperature differences of around 2°C between microcosms (Ferderer et al., 2022; Guo et al., 2023). To mitigate this experimental constraint, the microcosm placement within the temperature-controlled room must be shuffled daily. Microcosms can easily be moved when they are being pulled on the steel frame (Fig. 1), but care must be taken to briefly unplug the heat belts and plug them in again after shuffling their position. Furthermore, fans can be utilised to remove heat pockets in the room, although care must be taken as the wind can have a strong cooling effect, resulting in a microcosm that was too warm quickly becoming too cold. Since temperature is a strong driver of physiological processes, it is highly advisable to thoroughly test the setup with all microcosms prior to the experiment (the careful addition of food dye can be used to test advection as explained by Ferderer et al., (2022)). The goal should be to have as little variation in temperatures between microcosms as possible, and the seawater temperature should be as the plankton community would have experienced it at the location/season it was collected.

Like temperature, light conditions set up for the experiment should reproduce the natural site-specific conditions as much as possible. This applies for the light/dark cycle, the light

intensity, and the light spectrum (light spectrum should be between 400 and 750 nm, i.e. cool white light). Since many OAEPIIP participants may not have access to sophisticated computer-controlled light sources, we recommend the application of constant light over a fixed light/dark cycle. In an OAE study in Tasmania, for example, we provided light constantly with 200 µmol photons m^{-2} s^{-1} at a 12/12 hours light/dark cycle. These conditions were considered as representative average level for the surface mixed layer at the location/season where/when the natural plankton community were sourced in Tasmania. The light/dark cycle can be achieved by plugging the light source into a timer socket. The microcosms need to be positioned in such a way that light is very similar inside each microcosm. A light meter shall be used to determine light intensity inside the microcosms prior to the experiments and at the end (Table 1). Positioning can be very critical since movement by a few centimetres can often lead to noticeable changes in measured light intensity that are undetectable by the human eye. It is therefore important to adjust light conditions before starting the experiment and marking the spot on the floor where individual microcosms must be placed to ensure replicable light levels. It is also important to have all the other microcosms at their respective locations while doing the adjustments as they might shade each other. The daily shuffling of microcosm positions inside the room, which is essential for the temperature control (see above), will also help to mitigate systematic bias in light regime between microcosms.

OAEPIIP experiments shall not add organisms, nutrients, or any substances other than alkalinity/DIC (section 2.4) to the microcosms during or before the experiments.

2.3. OAE method in focus of OAEPIIP

OAE can be implemented with different approaches, applying different alkaline feedstocks such as solid materials like olivine, calcium/magnesium hydroxides, carbonates, steel slags or liquid materials like sodium hydroxide (NaOH) dissolved in seawater (Eisaman et al., 2023, NASEM, 2022). Each alkalinity source has different environmental implications as it is associated with different environmental perturbations (Bach et al., 2019). A widely considered approach is electrochemical OAE, where liquid NaOH is the alkalinity source (de Lannoy et al., 2018). NaOH-based OAE is in focus of OAEPIIP due to the following reasons. First, electrochemical OAE was recently evaluated to rank among the highest OAE approaches with regards to their "technological readiness level" (Eisaman et al., 2023), with field trials

229 already underway. Second, liquid NaOH is suitable as an alkalinity source for applications in

230 pelagic environments as it delivers quasi-instantaneous OAE in seawater. Other methods that

231 involve more slowly dissolving minerals (e.g. olivine) are considered less suitable for pelagic

232 applications as they would partially sink into the deep ocean before dissolving (Köhler et al.,

233 2013; Fakhraee et al., 2023). Third, electrochemical OAE is chemically relatively similar to

234 other OAE methods such as OAE with magnesium hydroxides or ocean liming based on

235 calcium hydroxides. Like NaOH, magnesium and calcium hydroxides dissolve relatively quickly

236 and are comparatively clean sources of alkalinity due to generally less content of bioactive

237 elements like iron or nickel than for example olivine when derived from carbonates (Bach et

238 al., 2019; Renforth et al., 2022), or when magnesium hydroxides are produced chemically

239 (Eisaman et al., 2023). As such, the primary potential of these hydroxides to affect pelagic

240 communities is by changing seawater carbonate chemistry. Thus, results from NaOH-based

241 OAE experiments also have potential to inform these other approaches. Fourth, NaOH is

242 readily available worldwide, which is logistically beneficial for OAEPIIP.

243

244 **2.4. Experimental design**

245

246 NaOH-based OAE reduces seawater pCO_2 within seconds (Zeebe and Wolf-Gladrow, 2001),

247 whereas the subsequent equilibration with atmospheric CO_2 takes months to years (Jones et

248 al., 2014; Wang et al., 2023, Mu et al., 2023) or potentially even centuries (He and Tyka, 2023).

249 The carbonate chemistry perturbation is much greater before the equilibration has happened

250 so that more pronounced effects on communities would be expected shortly after alkalinity

251 addition (Bach et al., 2019). As such, an argument can be made to study OAE at two different

252 timepoints when using rapidly dissolving alkalinity sources like NaOH or other hydroxides.

253 These are the "unequilibrated" timepoint, simulating the fact that CO_2 influx has not yet

254 happened right after alkalinity addition, and the "equilibrated" timepoint, assuming the

255 alkalinity enhanced seawater has already CO_2-equilibrated with the atmosphere.

256 Equilibration could either happen naturally through air-sea CO_2 influx (Ho et al., 2023), or

257 even be enforced within a facility so that alkalinity-enhanced seawater equilibrated with the

258 atmosphere is discharged into the ocean.

259 The nine microcosms available for OAEPIIP experiments will provide triplicate incubations for

260 controls, unequilibrated and equilibrated treatments. An important aspect for OAEPIIP

261 experiments is that the amount of alkalinity added to the treatments is consistent among all

262 studies. Modelling studies suggest that gigatonne-scale OAE sustained for 80 years would

263 increase surface ocean alkalinity by about 100-200 µmol/kg (Burt et al., 2021; Lenton et al.,

264 2018). This seemingly modest perturbation is due to dilution by the huge volume of the ocean

265 (i.e., $9.44*10^{17}$ m^3; Sarmiento and Gruber, 2006). However, the perturbation can be more

266 pronounced at sites where alkalinity is added, before being diluted with unperturbed

267 seawater with a rate that depends on the location (He and Tyka, 2023; Wang et al., 2023). In

268 fast dilution regimes, for example, 1 molar NaOH added at 5 m^3/s from a large ship would

269 initially raise pH to 11 but dilute to pH 8.5 within minutes to hours (He and Tyka, 2023). As

270 such, there is a trade-off for OAE experimentation between the realism and thus the

271 relevance of a simulated OAE perturbation at the timescales (weeks) proposed here (requiring

272 rather low alkalinity perturbation) and the detectability biological effects (facilitated when

273 simulated perturbations are more extreme). For OAEPIIP, we determined an alkalinity

274 enhancement of 500 µmol/kg to both the unequilibrated and equilibrated treatments. Our

275 rationale for the rather high perturbation is that OAEPIIP has a strong focus on capacity

276 building in OAE research. Setting up clearly distinguishable treatments facilitates data analysis

277 and interpretation, particularly for those entering the field. We emphasize, however, that a

278 500 µmol/kg perturbation over the duration of OAEPIIP studies (i.e., weeks; section 2.7) is on

279 the higher end for what is plausible for OAE (Wang et al., 2023; He and Tyka, 2023), except

280 for perhaps in proximity of a continuous NaOH release site. Thus, the relatively extreme

281 perturbation needs to be taken into account for the eventual interpretation and

282 communication of OAEPIIP studies – since it is likely that a less extreme perturbations would

283 also cause less environmental effect.

284

285 **2.5. Establishing treatments**

286

287 Alkalinity enhancement shall be performed on day 0 of the experiment, shortly after

288 microcosms have been positioned in the temperature-controlled room. Before adding

289 alkalinity, carbonate chemistry samples (i.e., alkalinity and one other carbonate chemistry

290 parameter; section 2.6) should be collected to constrain carbonate chemistry conditions in all

291 microcosms before OAE.

292 The three control microcosms will not receive any alkalinity addition and remain untreated.

293 The three microcosms of the unequilibrated treatment will receive 500 µmol/kg of NaOH. The

294 simplest way to achieve this is by purchasing and using a 1 molar NaOH solution (ideally in

295 "analytical quality") and adding 500 µL per kg of enclosed seawater. For example, if 54.5 kg

296 of seawater have been enclosed then 54.5 * 500 = 27250 µL of 1 molar NaOH solution needs

297 to be added to the respective microcosm.

298 The equilibrated treatment is slightly more complicated to establish. Here, most of the

299 alkalinity needs to be added as sodium bicarbonate ($NaHCO_3$) solution and a smaller amount

300 as NaOH solution. We provide an R script based on Seacarb (Gattuso et al., 2021) that can be

301 used to calculate additions of $NaHCO_3$ and NaOH (OAEPIIP, 2024). Furthermore, video

302 tutorials provide detailed instructions on how to use the R script or how to do these

303 calculations with CO2SYS for MSExcel (Pierrot et al., 2021) (Videos S3 and S4;

304 https://doi.org/10.5446/66754, https://doi.org/10.5446/66752). Briefly: In a first step, initial

305 carbonate chemistry conditions need to be calculated for the unperturbed seawater enclosed

306 in the microcosms. For this calculation one needs to assume a current CO_2 partial pressure

307 (e.g., 420 µatm), the target temperature for the experiment, and a salinity and alkalinity

308 estimate based on what the experimentalist expects for their region (or ideally has measured

309 just before collecting the seawater for microcosm experiment). Next, the calculation is

310 repeated for the same conditions except for alkalinity where 500 µmol/kg is added to the

311 assumed value (e.g., 2850 µmol/kg when the assumed value of the unperturbed water was

312 2350 µmol/kg). The second calculation represents the desired conditions in the equilibrated

313 treatment after the alkalinity enhancement. The calculated dissolved inorganic carbon (DIC)

314 concentrations of the initial carbonate system ($DIC_{initial}$) need to be subtracted from the

315 calculated DIC of the calculated treatment ($DIC_{equilibrated}$):

316

317 $$NaHCO_3 \ addition = DIC_{equilibrated} - DIC_{initial} \quad (2)$$

318

319 where $NaHCO_3$ addition is the amount of $NaHCO_3$ that needs to be added per kg of enclosed

320 seawater (in µmol/kg). The addition of $NaHCO_3$ provides equal amounts of DIC and alkalinity.

321 However, OAE can only absorb ~0.85 mole of DIC per mole of alkalinity added (He and Tyka,

322 2023; Schulz et al., 2023), so that reaching to +500 µmol/kg requires the addition of slightly

323 more alkalinity without DIC. NaOH is used for this purpose and the exact amount that needs

324 to be added is calculated as:

325

326 $$NaOH\ addition = 500 - NaHCO_3 addition \qquad (3)$$

327

328 Where 500 is the targeted alkalinity enhancement in µmol/kg. $NaHCO_3$ and NaOH additions

329 need to be multiplied with the weight of the enclosed microcosm seawater to calculate how

330 much $NaHCO_3$ and NaOH need to be added per individual microcosm.

331 It is recommended to use 1 molar stock solutions for both $NaHCO_3$ and NaOH for treatment

332 manipulations because in that case required additions in µmol/microcosm are equivalent to

333 µL/microcosm. For example, in the equilibrated treatment a typical addition would be 420

334 µL/kg of $NaHCO_3$ and 80 µL/kg of NaOH (i.e., 22.89 mL/microcosm $NaHCO_3$ and 4.36

335 mL/microcosm NaOH when 54.5 kg of seawater were enclosed). One molar $NaHCO_3$ stock

336 solutions can be prepared by dissolving 8.4 g $NaHCO_3$ powder (dried at 60°C overnight; note

337 that $NaHCO_3$ decomposes at higher temperatures) in 100 mL deionised water. One molar

338 NaOH (ideally in "analytical quality") should be purchased as such.

339 The addition of NaOH to seawater causes precipitation of brucite ($Mg(OH)_2$), which appears

340 as white flakes (Fig. 1C). The brucite flakes bind the alkalinity added via NaOH in particulate

341 form and need to be re-dissolved so that dissolved alkalinity is increased by the intended 500

342 µmol/kg. Furthermore, brucite formation can precipitate phosphates (Karl and Tien, 1992).

343 This must be avoided as the loss of phosphate from the dissolved phase in the treatments

344 would be a problematic confounding factor. The formation of brucite will be particularly

345 pronounced in the unequilibrated treatment where all alkalinity is added as NaOH. To dissolve

346 all brucite, microcosms should be gently stirred with a clean plastic paddle during and after

347 NaOH additions until all white flakes disappear. Our previous experiments resembling the

348 OAEPIIP approach (Ferderer et al., 2022) revealed that dissolution of all brucite by gentle

349 stirring leads to the desired outcome, i.e., alkalinity was increased by 500 µmol/kg and no

350 phosphate was lost. For consistency, control and equilibrated microcosms should be stirred

351 as much as the unequilibrated microcosms. If OAEPIIP participants do not have prior practical

352 experience with seawater carbonate chemistry manipulation, it is advised to test the above-

353 mentioned procedures (including the measurement of resulting carbonate chemistry

354 parameter changes such as in TA and DIC) before commencing the main OAEPIIP experiment.

2.6. Essential parameters to be measured in OAEPIIP experiments

Next to an identical experimental design and setup, the same parameters need to be measured in individual OAEPIIP experiments to make them comparable (Iglesias-Rodríguez et al., 2023). A list of "core" parameters with justifications for their choice is provided in Table 1, and additional recommendations on how to sample and process these is provided in Table S2. The core parameters (Table 1) should provide a relatively comprehensive, yet cost-efficient insight into processes within the plankton community. Although all core parameters need to be measured in all participating OAEPIIP studies, there may be unsurmountable logistical constraints which prohibit a participant from determining a core parameter. Such cases should be mentioned upon application for OAEPIIP participation so that mitigation pathways can be explored and that potential participants with less infrastructure capacity still have the opportunity to participate if possible (see also section 4).

If they wish to do so, OAEPIIP participants can also measure additional parameters to maximise their individual experimental outcomes. However, the following issues should be considered:

1) Not more than approximately 1/3 of the microcosm volume should be sampled over the course of the study (a) to limit the build-up of a headspace and (b) to avoid too much heat input per Liter of enclosed volume via the heat belts (the room temperature might need lowering to compensate for reducing volume throughout the experiment; section 2.2).

2) Any type of contamination (particulate or dissolved organic or inorganic) must be kept at a minimum.

3) It is possible to sample mesozooplankton with a customized net (Guo et al., 2023), but sampling should be restricted to 3 occasions during the experiment (e.g., beginning, middle, end) to avoid overfishing.

4) Aggregation and sedimentation are often observed in these microcosm studies and it is encouraged to sample settling materials from the sediment trap (Ferderer et al., 2022). However, care must be taken to not remove significant volumes of seawater.

387 *Table 1. List of core parameters that essentially need to be measured in all individual OAEPIIP*

388 *studies. The "Samplings" column indicates how often all 9 microcosms need to be sampled for*

389 *a specific parameter during the study. "Daily" means that this parameter needs to be*

390 *measured every day, irrespective of the temperature-dependent duration of the study. "b/e"*

391 *means that samples need to be taken at the beginning and the end of the experiment.*

Core parameter	Rationale	Samplings
Alkalinity	The treatment-defining parameter of the study.	7*
Second carbonate chemistry parameter (e.g., pH or DIC)	Required to constrain the carbonate system. Also provides insights for net autotrophy/heterotrophy.	Daily*
Salinity	Required to define the marine system under investigation.	b/e
Light	To constrain physical conditions for growth.	b/e
Temperature	To monitor its influence on metabolic rates and assess temperature stability due to convective mixing.	daily
Nutrients (NO_x^-, PO_4^{3-}, $Si(OH)_4$)	Nitrate+Nitrite (NO_x^-) and phosphate (PO_4^{3-}) availability largely determines the productivity of the plankton community. Availability of $Si(OH)_4$ provides insights if productivity will likely be driven by diatoms.	11
Chlorophyll a (chla)	Chla is a widely used proxy for phytoplankton biomass	11
Particulate organic carbon and nitrogen (POC and PON)	POC and PON dynamics are related to the increase and decline of biomass. Their ratio (POC/PON) is an important metric in biogeochemical element cycling.	11
Biogenic silica (BSi)	BSi is a widely used proxy for diatom biomass	11
Flow cytometry (FC)	FC is a cost-efficient tool that reveals shifts in phytoplankton size classes and specific groups with distinguishable fluorescence/scatter characteristics. FC is particularly good for enumeration of small phytoplankton and heterotrophic bacteria.	11
Microscopy	Microscopy is a widely available tool to assess dynamics in phytoplankton and microzooplankton communities. It is complementary to FC as it is better suited for larger phytoplankton/microzooplankton.	7
Nucleic acid sample	Nucleic acid samples (DNA and possibly RNA) will provide a detailed assessment of microbial diversity. Basic requirements for this parameter will be metabarcoding for 16S rRNA genes (variable region of V4-V5). Further analysis for metagenomics and metatranscriptomics will be possible depending on the timing of sample collection but are not essential for the participation.	b/e

392 **These parameters must be sampled directly before and after establishment of the OAE*

393 *treatments in all 9 microcosms. All other parameters must be sampled for the first time after*

394 *establishment of the treatments.*

395

2.7. Duration of experiment

396

397

398 To the best of our knowledge, there is no general rule for the ideal duration of microcosm

399 experiments. Experiments that are too short may miss important responses of plankton

400 communities while long experiments may exacerbate so-called "bottle effects", non-specific

401 effects from confinement rather than the experimental perturbation itself (Pernthaler and

402 Amann, 2005). For example, 3 days of experiment may be too short to observe a

403 differentiation of plankton species composition between treatments, while a community

404 contained for 2 months could be dominated by those that best survive in a laboratory

405 environment. Based on experiments with the OAEPIIP setup in Tasmania we consider 20 days

406 as a good compromise for an experiment at 15°C. However, metabolic rates increase with

407 temperature so that experimental duration needs to be adjusted based on respective

408 locations. Informed by Q10 temperature dependencies (Sherman et al., 2016), we

409 recommend the following framework: 20 days is the reference duration at 15°C. The duration

410 (in days) increases/decreases from this reference point using Q10 kinetics:

411

412
$$Duration = \frac{0.5611}{\left(0.5611 \times 1.47^{\frac{T_{exp}-15}{10}}\right)} \times 20 \qquad (4)$$

413

414 where 0.5611 is the reference growth rate at 15°C, T_{exp} is the anticipated temperature in the

415 OAEPIIP experiment, and 1.47 is the Q_{10} factor derived by (Sherman et al., 2016). For example,

416 an experiment at 25°C should last for 14 days and an experiment at 5°C for 29 days.

417

2.8. Sampling operations and logistics

418

419

420 All microcosm incubators shall be closed after the filling procedure with the black screw cap

421 (Fig. 1) and kept closed over the course of the experiment except during the establishment of

422 treatments (section 2.5) and sampling. The enclosed headspace (Fig. 1) may vary slightly in

423 between microcosms after the filling procedure (section 2.1) and will increase over the course

424 of the experiment due to the withdrawal of samples. While an increasing headspace will lead

425 to some CO_2 exchange between the atmosphere and the enclosed volume, previous studies

with the same setting found that this has no effect on the OAE treatments established in the experiments (Guo et al., 2023; Ferderer et al., 2022). The convective system mixes the water column so that no manual mixing is needed prior to sampling. A peristaltic pump is recommended to withdraw the seawater samples from the microcosms.

The total number of samplings for specific parameters is listed in Table 1 (for example, POC and PON need to be sampled 11 times in total). The frequency of sampling needs to be adjusted based on the temperature-dependent duration of the experiment (section 2.7.). OAEPIIP experiments at higher temperatures require higher sampling frequency because metabolic processes are faster. Table 1 lists the minimum number of days a parameter should be sampled. This number is to guarantee that there will be enough comparable data points across OAEPIIP experiments. For example, nutrient samples should be taken at least 11 times in each microcosm during the experiment. For an experiment at 15°C (20 days), this could mean a sampling on day 0 (directly after establishing treatments) and then days 2, 4, 6,…,20. However it may also be reasonable to increase frequency during periods of phytoplankton blooms (e.g., daily) and then reduce the frequency (e.g. every 4 days) when nutrients are depleted. In general, OAEPIIP experimentalists can best decide on an individual basis what sampling schedule is most appropriate for their experiment, but the total number of samplings must be at least as defined in Table 1 for each of the listed parameters.

Sampling for all OAEPIIP experiments should begin two hours after the onset of the light period on a sampling day. This coordination of initial sampling ensures that the plankton community is in a similar diurnal growth state. Hence, sampling of all 9 microcosms should ideally not last longer than 3 hours.

2.9. Statistical analyses

Microcosm data contains complex ecological data which require specific (often complicated) statistical tools for their analysis. A common issue is the presence of non linear relationships, which without gross transformation of the variables prevents the fitting of data to linear models. Furthermore, OAEPIIP microcosms will be sampled several times over an extended period. This sampling strategy results in temporal-pseudoreplication, where observations are not independent of each other and therefore violate the assumption of independence required for simple linear models and Generalised additive models (GAMs) (Zuur et al., 2009;

458   Wood, 2017). The expansion of GAMs to Generalized Additive Mixed Models (GAMMs) allows

459   for correlations between observations and the modelling of data structures which are nested

460   as well as for non-linear relationships between the response and explanatory variables.

461   To facilitate and standardize statistical analyses of individual datasets we provide an R-based

462   pipeline (OAEPIIP, 2024). This pipeline is tailored towards the evaluation of individual OAEPIIP

463   data sets using GAMMs. The files contain a workflow which demonstrates the use of GAMMs

464   and facilitates the seamless integration of individual datasets gathered during OAEPIIP

465   experiments into the workflow. Theoretical background, knowledge and details on how to fit

466   such models can be found in the textbooks by Zuur et al. (2009) and Wood (2017).

467

468   **3. Logistics and administration**

469

470   Basic instructions and updates on OAEPIIP will be provided on the OAEPIIP website

471   (https://appliedbgc.imas.utas.edu.au/ocean-alkalinity-enhancement-pelagic-impact-

472   intercomparison-project/).

473

474   **3.1. Eligibility and funding**

475

476   To join OAEPIIP, participants need to be capable of performing an OAEPIIP study and provide

477   all data by December 2025. This capacity shall be confirmed on a simple 1 page form (available

478   on the OAEPIIP website) that potential participants need to fill in and send to the email

479   provided on the form. Career stage, publication record, or other parameters of a scientist's

480   curriculum vitae have no relevance for OAEPIIP. As such, application success is determined by

481   logistical and infrastructure-related aspects, for example whether a participant has access to

482   a temperature-controlled room and can provide the various data in the given timeframe (but

483   see also section 4 on suggestions on how to mitigate individual limitations to infrastructure).

484   Ultimately, participation is restricted by total funding available to OAEPIIP. Should there be

485   more applications than there is funding, participants will be selected based on two criteria:

486   First, we will consider the locations of the experiments to obtain the best possible geographic

487   spread. Second, participants will be selected by chance should there be clusters of

488   applications in close proximity.

489   OAEPIIP provides a maximum of around 12,000 US$ per study in materials and funding for

490   analytical costs and publication fees (the exact amount is slightly variable due to exchange

491   rates). All materials and standardized components like the microcosms shall be purchased by

492   the individual participants with ~10,400 US$ made available to them for the experiments. The

493   OAEPIIP administration will provide all necessary information for the purchase of

494   standardized components so that all experiments are conducted in the same type of

495   incubators. The remaining ~1,600 US$ will be retained by the OAEPIIP administration and

496   made available to support the publication of individual OAEPIIP studies(see section 3.2).

497   The funds for materials and standardised components will be transferred via invoicing. Thus,

498   participants must have a bank account associated with their affiliation to which funding can

499   be transferred from Australia. This criterion therefore excludes laboratories in countries

500   under relevant sanctions from Australia to receive funding, although they are still welcome

501   to be part of the OAEPIIP community. In practice, participants will send two invoices to the

502   University of Tasmania, one at the beginning of the experiment to support purchasing of

503   materials and standardized components and the second one towards the end when the data

504   is available and has been submitted. OAEPIIP cannot provide funding for salaries. Therefore,

505   the experiment was designed to be suitable for a Master thesis or a chapter of a PhD thesis.

506

507   **3.2. Data management and publication**

508

509   Datasets of individual OAEPIIP studies should be formatted using a standardised template

510   available on the OAEPIIP homepage (section 3) and submitted to OAEPIIP as soon as they are

511   available. All data must be uploaded and made available under open access. Participants will

512   be listed on the OAEPIIP homepage and their individual datasets will be linked to their names

513   and affiliations as soon as it is made available. OAEPIIP experiments shall be published on an

514   individual basis in an OAEPIIP special issue (publication fees of up to 1600 US$ are provided

515   by OAEPIIP). Individual publication will enable identification of novel observations on how

516   plankton communities respond to OAE. If participants prefer not to publish their data, they

517   still need to submit their data to OAEPIIP so that it can be included in the OAEPIIP synthesis.

518   This is critically important because the synthesis must avoid publication bias.

519 The OAEPIIP synthesis will be prepared once all datasets have been delivered. First and last

520 authors of individual studies will automatically be co-authors on the synthesis publication(s)

521 at the end of the project, unless they prefer not to be.

522

523 **4. Capacity building and inclusivity**

524

525 OAEPIIP has potential benefits that go beyond scientific knowledge gain. The community

526 effort helps to build a network of OAE scientists and provides an incentive and access to those

527 who have not yet engaged with OAE research. Indeed, growing the OAE research community

528 is essential to accelerate the OAE assessment and make it more comprehensive. Providing the

529 same amount of funding, regardless of the location, may increase the attractiveness of

530 OAEPIIP studies to those that currently have less funding. Participation of scientists worldwide

531 is what we aim for since the OAE assessment requires the inclusion of the global community.

532 Indeed, participation in the process of assessing marine CDR methods (such as OAE), rather

533 than being on the receiving end of information only, has been expressed as an important

534 aspect by stakeholders from developing countries.

535 We are aware that the infrastructure demands for OAEPIIP (section 2), still put barriers on

536 participation. To mitigate those barriers, potential participants from more experienced

537 laboratories can offer to serve as a partner for a less experienced laboratory. Likewise,

538 potential participants from less experienced laboratories can indicate if they essentially need

539 support from an experienced laboratory. This information shall be disclosed on the

540 application form (available on the OAEPIIP website) so that OAEPIIP can establish

541 partnerships between participants. Partners can support each other through knowledge

542 exchange but also more practically by analysing samples for each other. For example, if an

543 interested participant has no capacity to measure alkalinity or flow cytometry samples, it may

544 partner with another participant to share analytical duties. The distribution of funding for

545 analytical costs via invoicing allows for such flexibility as it provides an opportunity to easily

546 re-distribute funding between project participants when this is communicated with the

547 OAEPIIP administration. For example, when two laboratories partner, they together have

548 access to twice the funding (~24,000 US$), which they share among them for the two

549 experiments they would have to do (the two experiments must be at different locations to

550 guarantee geographical diversity).

Furthermore, potential participants that simply have no chance to measure one (or more) core parameters due to unsurmountable logistical constraints can still hand in an application, if they indicate which parameters they are unable to deliver on their application form (available on the OAEPIIP website). The OAEPIIP administration will then evaluate such applications on a case-by-case basis and explore if there is a way for participation despite this limitation. This pathway is set in place specifically for potential participants with less developed infrastructure and less capacity for collaboration with an experienced (e.g., due to geographic isolation).

Altogether, we hope the cost-efficient design of OAEPIIP, its eligibility criteria that refrain from classic measures of scientific success, and potential support via an evolving OAEPIIP community could promote an inclusive assessment of OAE. One primary goal of OAEPIIP is capacity building to provide more informed decisions concerning OAE that encompass data from a geographically diverse range of plankton ecosystems.

Competing interests

The contact author has declared that none of the authors has any competing interests.

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Data availability

All code provided for experimental design and statistical analysis can be found here: (OAEPIIP, 2024).

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