1 **Technical note: Ocean Alkalinity Enhancement Pelagic Impact Intercomparison Project**
2 **(OAEPIIP)**

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4 Lennart T. Bach[1]*, Aaron Ferderer[1,2], Julie LaRoche[3], Kai G. Schulz[4]

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6 [1]Institute for Marine and Antarctic Studies, University of Tasmania, TAS 7004, Australia

7 [2]National Collections and Marine Infrastructure, Commonwealth Scientific and Industrial
8 Research Organisation, Hobart, Tasmania, Australia

9 [3]Department of BIology Dalhousie University, Halifax, NS Canada

10 [4]Faculty of Science and Engineering, Southern Cross University, Lismore, NSW, Australia

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12 *corresponding author (lennart.bach@utas.edu.au)

13

14 **Abstract**

15

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

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

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

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

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \underline{\qquad} (1)$$
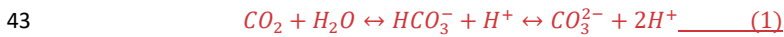
from carbon dioxide (CO_2) on the left to bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}) on the right. The decline in seawater CO_2 concentration lowers the seawater CO_2 partial pressure (pCO_2), thereby enabling an influx of additional atmospheric CO_2, or alternatively, reducing the efflux in cases where the surface ocean is a natural source of CO_2 to the atmosphere. The OAE-induced shift in carbonate chemistry is measurable as an increase in seawater alkalinity – the name-giving feature of OAE. The viability of OAE to serve as a scalable CDR approach critically depends on whether it is environmentally safe. Surface ocean habitats are in focus of the environmental OAE assessment because the surface ocean is where OAE would need to be implemented to enable CO_2 exchange with the atmosphere (Bach et al., 2019).

The environmental OAE assessment is only just starting but seems to be evolving in a similar way as environmental assessments of other drivers of environmental change (e.g. ocean acidification) have been set up in the past: Research funding is provided to individual groups, who will perform individual studies in their local environments, seeking novelty. Each of these studies will be valuable and exceeding previous research is central to scientific progress. However, previous research on environmental drivers has also shown that replication of experiments is perhaps equally important as seeking novelty, since replication allows us to reveal re-occurring response patterns across various scales and environments (Benton et al., 2007; Hamm et al., 2022; Stewart et al., 2013). In ocean acidification research for example, an individual study found that carbon to nitrogen (C/N) stoichiometry of

64 plankton communities is increased under high CO_2 conditions due to CO_2 fertilization of the

65 phytoplankton community (Riebesell et al., 2007). However, replication of the experiment at

66 different locations found that zooplankton communities can strongly modify the response, to

67 the point that the response can be significant in the opposite direction (lower C/N under high

68 CO_2 (Taucher et al., 2021)). Arguably, the crucial progress in this example was understanding

69 of the context-dependency of the C/N response to ocean acidification, which was made

70 possible by replication of a sophisticated experiment across a wide geographical range

71 (Riebesell et al., 2013). Likewise, the intercomparison of climate models via replicated

72 numerical experiments (Dingley et al., 2023) has long been recognised as a cornerstone to the

73 assessment of climate change (Masson-Delmotte et al., 2021), possibly more influential than

74 the output of individual climate models.

75 The Ocean Alkalinity Enhancement Pelagic Impact Intercomparison Project (OAEPIIP) builds

76 upon these insights from previous environmental assessments by establishing a platform that

77 supports replication, while still enabling the pursuit of novelty. In essence, OAEPIIP provides

78 funding for a cost-efficient and standardised OAE experiment, which can be conducted by

79 scientists across the globe (section 2). The experiments will use a microcosm setup to study

80 the response of natural plankton communities to one widely considered two specific OAE

81 implementation strategyscenarios, and they will determine the same set of response

82 variables. Each experiment shall be published on an individual basis in a special issue of a

83 peer-reviewed scientific journal under open access with costs largely covered by OAEPIIP

84 (section 3). Individual publication of OAEPIIP experiments gives room to describe novel

85 observations on how plankton communities respond to OAE. All datasets will be shared and

86 synthesized in a meta-analysis. The standardised experimental design facilitates inclusion of

87 individual datasets into the meta-analysis (Harrison, 2011). Likewise, the collection of all

88 datasets, irrespective of their outcomes, avoids publication bias, which is a known problem

89 of meta-analyses (Field and Gillett, 2010). We expect OAEPIIP to promote consensus among

90 scientists concerning the potential environmental side effects of OAE on plankton

91 communities, with significant potential for capacity building (section 4). This paper provides

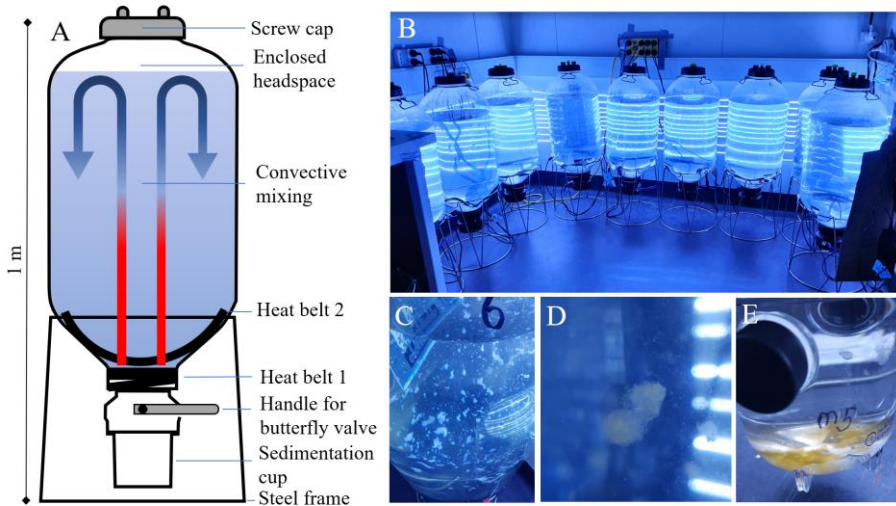92 a detailed manual for the OAEPIIP experimental setup and describes its benefits.

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94 **2. Experimental infrastructure, operation, and design**

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2.1. Microcosm setup

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

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

117 respectively. The bottom opening is equipped with a butterfly valve and a sedimentation cup,
118 used for the collection of settling material. The butterfly valve has a handle so that the
119 sedimentation cup can be isolated from the water column.

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

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

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

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149 volume, temperature, and salinity. Once the weight has been determined, microcosms need

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

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

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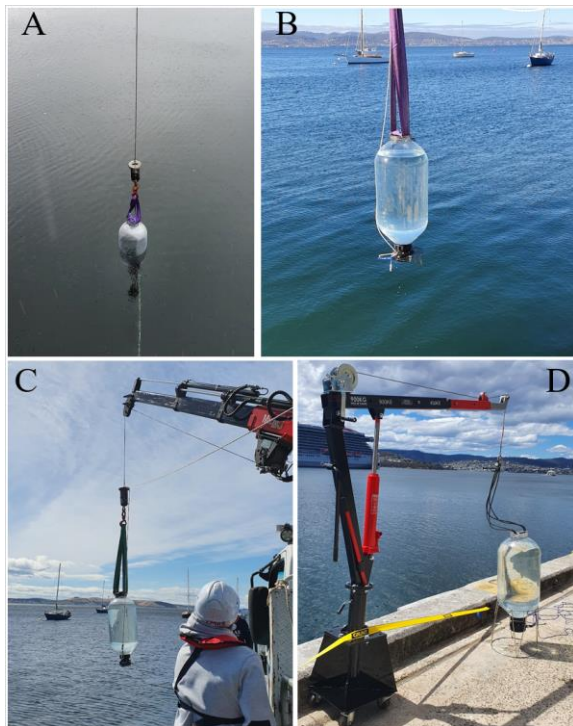
153

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

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

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

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

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

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

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

161

162 **2.2. Mixing, temperature, light, and nutrient conditions in OAEPIIP experiments**

163

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

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

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

167 of the microcosms (Fig. 1, Video S2 (https://doi.org/10.5446/66753)). Based on our

168 experience, these two 30 W heat belts increase the temperature of the enclosed seawater by

169 ~6°C relative to the room temperature, so that room temperature needs to be roughly ~6°C

170 lower than the target temperature in the experiments (please note that testing the

171 temperature difference will be necessary prior to the experiment as temperature offset may

172 differ across temperature-controlled rooms). Once heat belts are attached, microcosms

173 should be placed in front of the light source and heat belts should be plugged in to initiate

174 the convective mixing.

175 While convection provides gentle and non-invasive mixing, there are several trade-offs in

176 regard toregarding temperature control. Firstly, due to the removal of seawater during

177 sampling, the total volume within microcosms declines over the course of the experiment.

178 Since the heat belts cannot be adjusted, there is an increase in heat energy input per liter of

179 enclosed seawater and thus a gradual warming. To mitigate this issue, the external cooling

180 may need to be increased over time by lowering the room temperature. In our experience, a

181 reduction by 1°C for every 5 liters of seawater sampled from the microcosms is sufficient to

182 keep the seawater temperature relatively constant over the course of the study. Secondly,

183 small differences in ventilation at different locations in the temperature-controlled room can

184 lead to seawater temperature differences of around 2°C between microcosms (Ferderer et

185 al., 2022; Guo et al., 2023). To mitigate this experimental constraint, the microcosm

186 placement within the temperature-controlled room must be shuffled on a daily basisdaily.

187 Microcosms can easily be moved when they are being pulled on the steel frame (Fig. 1), but

188 care must be taken to briefly unplug the heat belts and plug them in again after shuffling their

189 position. Furthermore, fans can be utilised to remove heat pockets in the room, although care

190 must be taken as the wind can have a strong cooling effect, resulting in a microcosm that was

191 too warm quickly becoming too cold. Since temperature is a strong driver of physiological

192 processes, it is highly advisable to thoroughly test the setup with all microcosms prior to the

193 experiment (the careful addition of food dye can be used to test advection as explained by

194 Ferderer et al., (2022)). The goal should be to have as little variation in temperatures between

7

195 microcosms as possible, and the seawater temperature should be as the plankton community

196 would have experienced it at the location/season it was collected.

197 Like temperature, light conditions set up for the experiment should reproduce the natural

198 site-specific conditions as much as possible. This applies for the light/dark cycle, the light

199 intensity, and the light spectrum (light spectrum should be between 400 and 750 nm, i.e. cool

200 white light). Since many OAEPIIP participants may not have access to sophisticated computer-

201 controlled light sources, we recommend the ~~delivery~~ application of constant light over a fixed

202 light/dark cycle. In an OAE study in Tasmania, for example, we provided light constantly with

203 200 µmol photons m^{-2} s^{-1} at a 12/12 hours light/dark cycle. These conditions were considered

204 as representative average level for the surface mixed layer at the location/season

205 where/when the natural plankton community were sourced in Tasmania. The light/dark cycle

206 can be achieved by plugging the light source into a timer socket. The microcosms need to be

207 positioned in such a way that light is very similar inside each microcosm. A light meter shall

208 be used to determine light intensity inside the microcosms prior to the experiments and at

209 the end (Table 1). Positioning can be very critical since movement by a few centimetres can

210 often lead to noticeable changes in measured light intensity that are undetectable by the

211 human eye. It is therefore important to adjust light conditions before starting the experiment

212 and marking the spot on the floor where individual microcosms must be placed to ensure

213 replicable light levels. It is also important to have all the other microcosms at their respective

214 locations while doing the adjustments as they might shade each other. The daily shuffling of

215 microcosm positions inside the room, which is essential for the temperature control (see

216 above), will also help to mitigate systematic bias in light regime between microcosms.

217 OAEPIIP experiments shall not add organisms, nutrients, or any substances other than

218 alkalinity/DIC (section 2.4) to the microcosms during or before the experiments.

219

220 **2.3. OAE method in focus of OAEPIIP**

221

222 OAE can be implemented with different approaches, applying different alkaline feedstocks

223 such as solid materials like olivine, calcium/magnesium hydroxides, carbonates, steel slags

224 ~~(Eisaman et al., 2023)~~ or liquid materials like sodium hydroxide (NaOH) dissolved in seawater

225 (Eisaman et al., 2023, NASEM, 2022). ~~, which~~Each alkalinity source ha~~ve~~s different

226 environmental implications as ~~it~~they ~~are~~ is associated with different environmental

227   perturbations (Bach et al., 2019). A widely considered approach is ~~electrodialytical~~ electrochemical OAE, where liquid ~~sodium hydroxide (~~NaOH~~)~~ is the alkalinity source (de Lannoy et al., 2018). ~~electrochemical~~ NaOH-based OAE is in focus of OAEPIIP due to the following reasons. First, ~~electrodialytical~~ 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 already underway. Second, liquid NaOH is suitable as an alkalinity source for applications in pelagic environments as it delivers quasi- instantaneous OAE in seawater. Other methods that involve more slowly dissolving minerals (e.g. olivine) are considered less suitable for pelagic applications as they would partially sink into the deep ocean before dissolving (Köhler et al., 2013; Fakhraee et al., 2023). Third, ~~electrodialytical~~ electrochemical OAE is chemically relatively similar to other OAE methods such as OAE with magnesium hydroxides or ocean liming based on calcium hydroxides. Like NaOH, magnesium and calcium hydroxides dissolve relatively quickly and are comparatively clean sources of alkalinity due to generally less content of bioactive elements like iron or nickel than for example olivine when derived from carbonates (Bach et al., 2019; Renforth et al., 2022), or when magnesium hydroxides are produced chemically (Eisaman et al., 2023). As such, ~~so that~~ their primary potential of these hydroxides to affect pelagic communities is by changing seawater carbonate chemistry. Thus, results from NaOH-based OAE experiments ~~will~~ also have potential to ~~also~~ inform these other approaches. Fourth, NaOH is readily available worldwide, which is logistically beneficial for OAEPIIP.

2.4. Experimental design

250   NaOH-based OAE reduces seawater pCO_2 within seconds (Zeebe and Wolf-Gladrow, 2001), whereas the subsequent equilibration with atmospheric CO_2 takes months to years (Jones et al., 2014; Wang et al., 2023, Mu et al., 2023) or potentially even centuries ~~even longer~~ (He and Tyka, 2023). The carbonate chemistry perturbation is much greater before the equilibration has happened so that more pronounced effects on communities would be expected shortly after alkalinity addition (Bach et al., 2019). As such, an argument can be made to study OAE at ~~in~~ two different ~~scenarios~~timepoints when using rapidly dissolving alkalinity sources like NaOH or other hydroxides. These are the "unequilibrated" timepoint~~scenario~~, simulating the fact that CO_2 influx has not yet happened right after

259 alkalinity addition, and the "equilibrated" timepointscenario, assuming the alkalinity
260 enhanced seawater has already CO_2-equilibrated with the atmosphere. Equilibration could
261 either happen naturally through air-sea CO_2 influx (Ho et al., 2023), or even be enforced within
262 a facility so that alkalinity-enhanced seawater equilibrated with the atmosphere is discharged
263 into the ocean.

264 The nine microcosms available for OAEPIIP experiments will provide triplicate incubations for
265 controls, unequilibrated and equilibrated treatments. An important aspect for OAEPIIP
266 experiments is that the amount of alkalinity added to the treatments is consistent among all
267 studies. Modelling studies suggest that gigatonne-scale OAE sustained for 80 years would
268 increase surface ocean alkalinity by about 100-200 µmol/kg (Burt et al., 2021; Lenton et al.,
269 2018). This seemingly modest perturbation is due to dilution by the huge volume of the ocean
270 (i.e., $9.44*10^{17}$ m^3; Sarmiento and Gruber, 2006). However, the perturbation can be more
271 pronounced at sites where alkalinity is added, before being diluted with unperturbed
272 seawater with a rate that depends on the location (He and Tyka, 2023; Wang et al., 2023). In
273 fast dilution regimes, for example, 1 molar NaOH added at 5 m^3/s from a large ship would
274 initially raise pH to 11 but dilute to pH 8.5 within minutes to hours (He and Tyka, 2023). As
275 such, there is a trade-off for OAE experimentation between the realism and thus the
276 relevance of a simulated OAE perturbation at the timescales (weeks) proposed here (requiring
277 rather low alkalinity perturbation) and the detectability biological effects (facilitated when
278 simulated perturbations are more extreme). Based on thisFor OAEPIIP, we determined an
279 addition alkalinity enhancement of 500 µmol/kg to both the unequilibrated and equilibrated
280 treatments for OAEPIIP. Our rationale for the rather high perturbation is that OAEPIIP has a
281 strong focus on capacity building in OAE research. Setting up clearly distinguishable
282 treatments facilitates data analysis and interpretation, particularly for those entering the
283 field. We emphasize, however, that While a 500 µmol/kg perturbation over the duration of
284 OAEPIIP studies (i.e., weeks; section 2.7) alkalinity increase is on the higher end for what is
285 plausible for OAE (Wang et al., 2023; He and Tyka, 2023), except for perhaps in proximity of
286 a continuous NaOH release site. Thus, the relatively extreme perturbation needs to be taken
287 into account for the eventual interpretation and communication of OAEPIIP studies – since it
288 is likely that a less extreme perturbations would also cause less environmental effect. it
289 seems to be a good compromise between realism and the ability to detect environmental
290 effects on plankton communities (Ferderer et al., 2022).

291

2.5. Establishing treatments

293

Alkalinity enhancement shall be performed on day 0 of the experiment, shortly after microcosms have been positioned in the temperature-controlled room. Before adding alkalinity, carbonate chemistry samples (i.e., alkalinity and one other carbonate chemistry parameter; section 2.6) should be collected to constrain carbonate chemistry conditions in all microcosms before OAE.

The three control microcosms will not receive any alkalinity addition and remain untreated. The three microcosms of the unequilibrated treatment will receive 500 µmol/kg of NaOH. The simplest way to achieve this is by purchasing and using a 1 molar NaOH solution (ideally in "analytical quality") and adding 500 µL per kg of enclosed seawater. For example, if 54.5 kg of seawater have been enclosed then 54.5 * 500 = 27250 µL of 1 molar NaOH solution needs to be added to the respective microcosm.

The equilibrated treatment is slightly more complicated to establish. Here, most of the alkalinity needs to be added as sodium bicarbonate ($NaHCO_3$) solution and a smaller amount as NaOH solution. We provide an R script based on Seacarb (Gattuso et al., 2021) that can be used to calculate additions of $NaHCO_3$ and NaOH (OAEPIIP, 2024). Furthermore, video tutorials provide detailed instructions on how to use the R script or how to do these calculations with CO2SYS for MSExcel (Pierrot et al., 2021) (Videos S3 and S4; https://doi.org/10.5446/66754, https://doi.org/10.5446/66752). Briefly: In a first step, initial carbonate chemistry conditions need to be calculated for the unperturbed seawater enclosed in the microcosms. For this calculation one needs to assume a current CO_2 partial pressure (e.g., 420 µatm), the target temperature for the experiment, and a salinity and alkalinity estimate based on what the experimentalist expects for their region (or ideally has measured just before collecting the seawater for microcosm experiment). Next, the calculation is repeated for the same conditions except for alkalinity where 500 µmol/kg is added to the assumed value (e.g., 2850 µmol/kg when the assumed value of the unperturbed water was 2350 µmol/kg). The second calculation represents the desired conditions in the equilibrated treatment after the alkalinity enhancement. The calculated dissolved inorganic carbon (DIC) concentrations of the initial carbonate system ($DIC_{initial}$) need to be subtracted from the calculated DIC of the calculated treatment ($DIC_{equilibrated}$):

11

323

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

325

326 where NaHCO$_3$ addition is the amount of NaHCO$_3$ that needs to be added per kg of enclosed

327 seawater (in µmol/kg). The addition of NaHCO$_3$ provides equal amounts of DIC and alkalinity.

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

329 2023; Schulz et al., 2023), so that reaching to +500 µmol/kg requires the addition of slightly

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

331 to be added is calculated as:

332

$$NaOH\ addition = 500 - NaHCO_3 addition \quad (3)$$

334

335 Where 500 is the targeted alkalinity enhancement in µmol/kg. NaHCO$_3$ and NaOH additions

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

337 much NaHCO$_3$ and NaOH need to be added per individual microcosm.

338 It is recommended to use 1 molar stock solutions for both NaHCO$_3$ and NaOH for treatment

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

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

341 µL/kg of NaHCO$_3$ and 80 µL/kg of NaOH (i.e., 22.89 mL/microcosm NaHCO$_3$ and 4.36

342 mL/microcosm NaOH when 54.5 kg of seawater were enclosed). One molar NaHCO$_3$ stock

343 solutions can be prepared by dissolving 8.4 g NaHCO$_3$ powder (dried at 60°C overnight ; note

344 that NaHCO$_3$ decomposes at higher temperatures) in 100 mL deionised water. One molar

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

346 The addition of NaOH to seawater causes precipitation of ~~magnesium hydroxides~~brucite

347 (Mg(OH)$_2$), which appears as white flakes (Fig. 1C). The brucite flakes bind the alkalinity added

348 via NaOH in particulate form and need to be re-dissolved so that dissolved alkalinity is

349 increased by the intended 500 µmol/kg. Furthermore, brucite formation can precipitate

350 phosphates (Karl and Tien, 1992). This must be avoided as the loss of phosphate from the

351 dissolved phase in the treatments would be a problematic confounding factor. The formation

352 of brucite~~is problem~~ will be particularly pronounced in the unequilibrated treatment where

353 all alkalinity is added as NaOH. ~~Therefore~~To dissolve all brucite, microcosms should be gently

354 stirred with a clean plastic paddle during and after NaOH additions until all white flakes

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355 disappear. Our previous experiments resembling the OAEPIIP approach (Ferderer et al., 2022)
356 revealed that dissolution of all brucite by gentle stirring leads to the desired outcome, i.e.,
357 alkalinity was increased by 500 µmol/kg and no phosphate was lost. ~~This problem will be~~
358 ~~particularly pronounced in the unequilibrated treatment where all alkalinity is added as~~
359 ~~NaOH.~~ For consistency, control and equilibrated microcosms should be stirred as much as
360 the unequilibrated microcosms. If OAEPIIP participants do not have prior practical experience
361 with seawater carbonate chemistry manipulation, it is advised to test the above-mentioned
362 procedures (including the measurement of resulting carbonate chemistry parameter changes
363 such as in TA and DIC) before commencing the main OAEPIIP experiment.

364

365 **2.6. Essential parameters to be measured in OAEPIIP experiments**

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367 Next to an identical experimental design and setup, the same parameters need to be
368 measured in individual OAEPIIP experiments to make them comparable (Iglesias-Rodríguez et
369 al., 2023). A list of "core" parameters with justifications for their choice is provided in Table
370 1, and additional recommendations on how to sample and process these is provided in Table
371 S2. The core parameters (Table 1) should provide a relatively comprehensive, yet cost-
372 efficient insight into processes within the plankton community. Although all core parameters
373 need to be measured in all participating OAEPIIP studies, there may be unsurmountable
374 logistical constraints which prohibit a participant from determining a core parameter. Such
375 cases should be mentioned upon application for OAEPIIP participation so that mitigation
376 pathways can be explored and that potential participants with less infrastructure capacity still
377 have the opportunity to participate if possible (see also section 4).
378 If they wish to do so, OAEPIIP participants can also measure additional parameters to
379 maximise their individual experimental outcomes. However, the following issues should be
380 considered:

381

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

387   2) Any type of contamination (particulate or dissolved organic or inorganic) must be kept

388   at a minimum.

389   3) It is possible to sample mesozooplankton with a customized net (Guo et al., 2023), but

390   sampling should be restricted to 3 occasions during the experiment (e.g., beginning,

391   middle, end) to avoid overfishing.

392   4) Aggregation and sedimentation are often observed in these microcosm studies and it

393   is encouraged to sample settling~~sedimenting~~ materials from the sediment trap

394   (Ferderer et al., 2022). However, care must be taken to not remove significant

395   volumes of seawater.

396

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

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

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

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

401 *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 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
Microscopy		
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

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

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

404 *establishment of the treatments.*

405

406 **2.7. Duration of experiment**

407

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

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

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

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

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

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

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

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

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

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

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

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

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

421

422 $$Duration = \frac{0.5611}{\left(0.5611 \times 1.47^{\frac{T_{exp}-15}{10}}\right)} \times 20 \qquad (4)$$

423

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

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

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

427

428   **2.8. Sampling operations and logistics**

429

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

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

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

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

434   of the experiment due to the withdrawal of sampl~~ing volume~~es. While an increasing

435   headspace will lead to some ~~limited~~ CO_2 exchange between the atmosphere and the enclosed

436   volume, previous studies with the same setting found that this has no ~~e~~affect on the OAE

437   treatments established in the experiments (Guo et al., 2023; Ferderer et al., 2022).

438   The convective system mixes the water column so that no manual mixing is needed prior to

439   sampling. A peristaltic pump is recommended to withdraw the seawater samples from the

440   microcosms.

441   The total number of samplings for specific parameters is listed in Table 1 (for example, POC

442   and PON need to be sampled 11 times in total). The frequency of sampling needs to be

443   adjusted based on the temperature-dependent duration of the experiment (section 2.7.).

444   OAEPIIP experiments at higher temperatures require higher sampling frequency because

445   metabolic processes are faster. Table 1 lists the minimum number of days a parameter should

446   be sampled. This number is to guarantee that there will be enough comparable data points

447   across OAEPIIP experiments. For example, nutrient samples should be taken at least 11 times

448   in each microcosm during the experiment. For an experiment at 15°C (20 days), this could

449   mean a sampling on day 0 (directly after establishing treatments) and then days 2, 4, 6,…,20.

450   However it may also be reasonable to increase frequency during periods of phytoplankton

451   blooms (e.g., daily) and then reduce the frequency (e.g. every 4 days) when nutrients are

452   depleted. In general, OAEPIIP experimentalists can best decide on an individual basis what

453   sampling schedule is most appropriate for their experiment, but the total number of

454   samplings must be at least as defined in Table 1 for each of the listed parameters.

455 Sampling for all OAEPIIP experiments should begin two hours after the onset of the light
456 period on a sampling day. This coordination of initial sampling ensures that the plankton
457 community is in a similar diurnal growth state. Hence, sampling of all 9 microcosms should
458 ideally not last longer than 3 hours.
459

460 **2.9. Statistical analyses**

461

462 Microcosm data contains complex ecological data which require specific (often complicated)
463 statistical tools for their analysis. A common issue is the presence of non linear relationships,
464 which without gross transformation of the variables prevents the fitting of data to linear
465 models. Furthermore, OAEPIIP microcosms will be sampled several times over an extended
466 period. This sampling strategy results in temporal-pseudoreplication, where observations are
467 not independent of each other and therefore violate the assumption of independence
468 required for simple linear models and Generalised additive models (GAMs) (Zuur et al., 2009;
469 Wood, 2017). The expansion of GAMs to Generalized Additive Mixed Models (GAMMs) allows
470 for correlations between observations and the modelling of data structures which are nested
471 as well as for non-linear relationships between the response and explanatory variables.
472 To facilitate and standardize statistical analyses of individual datasets we provide an R-based
473 pipeline (OAEPIIP, 2024). This pipeline is tailored towards the evaluation of individual OAEPIIP
474 data sets using GAMMs. The files contain a workflow which demonstrates the use of GAMMs
475 and facilitates the seamless integration of individual datasets gathered during OAEPIIP
476 experiments into the workflow. Theoretical background, knowledge and details on how to fit
477 such models can be found in the textbooks by Zuur et al. (2009) and Wood (2017).
478

479 **3. Logistics and administration**

480

481 Basic instructions and updates on OAEPIIP will be provided on the OAEPIIP website
482 (https://appliedbgc.imas.utas.edu.au/ocean-alkalinity-enhancement-pelagic-impact-
483 intercomparison-project/).
484

485 **3.1. Eligibility and funding**

486

487 To join OAEPIIP, participants need to be capable of performing an OAEPIIP study and provide
488 all data by December 2025. This capacity shall be confirmed on a simple 1 page form (available
489 on the OAEPIIP website) that potential participants need to fill in and send to the email
490 provided on the form. Career stage, publication record, or other parameters of a scientist's
491 curriculum vitae have no relevance for OAEPIIP. As such, application success is determined by
492 logistical and infrastructure-related aspects, for example whether a participant has access to
493 a temperature-controlled room and can provide the various data in the given timeframe (but
494 see also section 4 on suggestions on how to mitigate individual limitations to infrastructure).
495 Ultimately, participation is restricted by total funding available to OAEPIIP. Should there be
496 more applications than there is funding, participants will be selected based on two criteria:
497 First, we will consider the locations of the experiments to obtain the best possible geographic
498 spread. Second, participants will be selected by chance should there be clusters of
499 applications in close proximity.
500 OAEPIIP provides a maximum of around 12,000 US$ per study in materials and funding for
501 analytical costs and publication fees (the exact amount is slightly variable due to exchange
502 rates). All materials and Sstandardized components like the microcosms will be suppliedshall
503 be purchased by the individual participants with ~10,400 US$ made available to them for the
504 experiments. The OAEPIIP administration will provide all necessary information for the
505 purchase of standardized components so that all experiments are conducted in the same type
506 of incubators. The remaining ~1,600 US$ will be retained by the OAEPIIP administration and
507 made available to support the publication of individual OAEPIIP studies and fees for the
508 publication of individual studies in an OAEPIIP special issue will be covered (see section 3.2).
509 The remaining funds for materials and standardised components ing will be transferred via
510 invoicing. Thus, participants must have a bank account associated with their affiliation to
511 which funding can be transferred from Australia. This criterion therefore excludes
512 laboratories in countries under relevant sanctions from Australia to receive funding, although
513 they are still welcome to be part of the OAEPIIP community. PracticallyIn practice, participants
514 will send two invoices to the University of Tasmania, one at the beginning of the experiment
515 to support purchasing of materials (e.g. the microcosms)and standardized components and
516 the second one towards the end when the data is available and has been submitted. OAEPIIP
517 cannot provide funding for salaries. Therefore, the experiment was designed to be suitable
518 for a Master thesis or a chapter of a PhD thesis.

519

3.2. Data management and publication

521

Datasets of individual OAEPIIP studies should be formatted using a standardised template available on the OAEPIIP homepage (section 3) and submitted to OAEPIIP as soon as they are available. All data must be uploaded and made available under open access. Participants will be listed on the OAEPIIP homepage and their individual datasets will be linked to their names and affiliations as soon as it is made available. OAEPIIP experiments shall be published on an individual basis in an OAEPIIP special issue (publication fees of up to 1600 US$ are ~~part of the ≃12,000 US$ funding~~ provided by OAEPIIP). Individual publication will enable identification of novel observations on how plankton communities respond to OAE. If participants prefer not to publish their data, they still need to submit their data to OAEPIIP so that it can be included in the OAEPIIP synthesis. This is critically important because the synthesis must avoid publication bias.

The OAEPIIP synthesis will be prepared once all datasets have been delivered. First and last authors of individual studies will automatically be co-authors on the synthesis publication(s) at the end of the project, unless they prefer not to be.

536

4. Capacity building and inclusivity

538

OAEPIIP has potential benefits that go beyond scientific knowledge gain. The community effort helps to build a network of OAE scientists and provides an incentive and access to those who have not yet engaged with OAE research. Indeed, growing the OAE research community is essential to accelerate the OAE assessment and make it more comprehensive. Providing the same amount of funding, regardless of the location, may increase the attractiveness of OAEPIIP studies to those that currently have less funding. Participation of scientists worldwide is what we aim for since the OAE assessment requires the inclusion of the global community. Indeed, participation in the process of assessing marine CDR methods (such as OAE), rather than being on the receiving end of information only, has been expressed as an important aspect by stakeholders from developing countries.

We are aware that the infrastructure demands for OAEPIIP (section 2), still put barriers on participation. To mitigate those barriers, potential participants from more experienced

551 laboratories can offer to serve as a partner for a less experienced laboratory. Likewise,
552 potential participants from less experienced laboratories can indicate if they essentially need
553 support from an experienced laboratory. This information shall be disclosed on the
554 application form (available on the OAEPIIP website) so that OAEPIIP can establish
555 partnerships between participants. Partners can support each other through knowledge
556 exchange but also more practically by analysing samples for each other. For example, if an
557 interested participant has no capacity to measure alkalinity or flow cytometry samples, it may
558 partner with another participant to share analytical duties. The distribution of funding for
559 analytical costs via invoicing allows for such flexibility as it provides an opportunity to easily
560 re-distribute funding between project participants when this is communicated with the
561 OAEPIIP administration. For example, when two laboratories partner, they together have
562 access to twice the funding (~24,000 US$), which they share among them for the two
563 experiments they would have to do (the two experiments must be at different locations to
564 guarantee geographical diversity).
565 Furthermore, potential participants that simply have no chance to measure one (or more)
566 core parameters due to unsurmountable logistical constraints can still hand in an application,
567 if they indicate which parameters they are unable to deliver on their application form
568 (available on the OAEPIIP website). The OAEPIIP administration will then evaluate such
569 applications on a case-by-case basis and explore if there is a way for participation despite this
570 limitation. This pathway is set in place specifically for potential participants with less
571 developed infrastructure and less capacity for collaboration with an experienced (e.g., due to
572 geographic isolation).
573 Altogether, we hope the cost-efficient design of OAEPIIP, its eligibility criteria that refrain
574 from classic measures of scientific success, and potential support via an evolving OAEPIIP
575 community could promote an inclusive assessment of OAE. One primary goal of OAEPIIP is
576 capacity building to provide more informed decisions concerning OAE that encompass data
577 from a geographically diverse range of plankton ecosystems.
578
579 **Competing interests**
580
581 The contact author has declared that none of the authors has any competing interests.
582

587

588   **Data availability**

589

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

591   2024).

592

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