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

2 **(OAEPIIP)**

3

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

5

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

11

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

32   community, will provide a sounder base to facilitate political decision making whether OAE

33   should be upscaled or not.

34

35   **1. Rationale for the Ocean Alkalinity Enhancement Pelagic Impact Intercomparison**

36   **Project (OAEPIIP)**

37

38   Ocean Alkalinity Enhancement (OAE) is an emerging carbon dioxide removal (CDR) approach

39   (Oschlies et al., 2023). OAE drives CDR through the introduction of alkaline substances into

40   seawater which shift the carbonate chemistry equilibrium:

41

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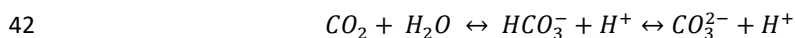
43

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 have been set up in the past:

55   Research funding is provided to individual groups, who will perform individual studies in their

56   local environments, seeking novelty. Each of these studies will be valuable and exceeding

57   previous research is central to scientific progress. However, previous research on

58   environmental drivers has also shown that replication of experiments is perhaps equally

59   important as seeking novelty, since replication allows us to reveal re-occurring response

60   patterns across various scales and environments (Benton et al., 2007; Hamm et al., 2022;

61   Stewart et al., 2013). In ocean acidification research for example, an individual study found

62   that carbon to nitrogen (C/N) stoichiometry of plankton communities is increased under high

63   CO_2 conditions due to CO_2 fertilization of the phytoplankton community (Riebesell et al.,

2

64 2007). However, replication of the experiment at different locations found that zooplankton

65 communities can strongly modify the response, to the point that the response can be

66 significant in the opposite direction (lower C/N under high CO_2 (Taucher et al., 2021)).

67 Arguably, the crucial progress in this example was understanding of the context-dependency

68 of the C/N response to ocean acidification, which was made possible by replication of a

69 sophisticated experiment across a wide geographical range (Riebesell et al., 2013). Likewise,

70 the intercomparison of climate models via replicated numerical experiments (Dingley et al.,

71 2023) has long been recognised as a cornerstone to the assessment of climate change

72 (Masson-Delmotte et al., 2021), possibly more influential than the output of individual

73 climate models.

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

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

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

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

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

79 the response of natural plankton communities to two specific OAE scenarios, and they will

80 determine the same set of response variables. Each experiment shall be published on an

81 individual basis in a special issue of a peer-reviewed scientific journal under open access with

82 costs largely covered by OAEPIIP (section 3). Individual publication of OAEPIIP experiments

83 gives room to describe novel observations on how plankton communities respond to OAE. All

84 datasets will be shared and synthesized in a meta-analysis. The standardised experimental

85 design facilitates inclusion of individual datasets into the meta-analysis (Harrison, 2011).

86 Likewise, the collection of all datasets, irrespective of their outcomes, avoids publication bias,

87 which is a known problem of meta-analyses (Field and Gillett, 2010). We expect OAEPIIP to

88 promote consensus among scientists concerning the potential environmental side effects of

89 OAE on plankton communities, with significant potential for capacity building (section 4). This

90 paper provides a detailed manual for the OAEPIIP experimental setup and describes its
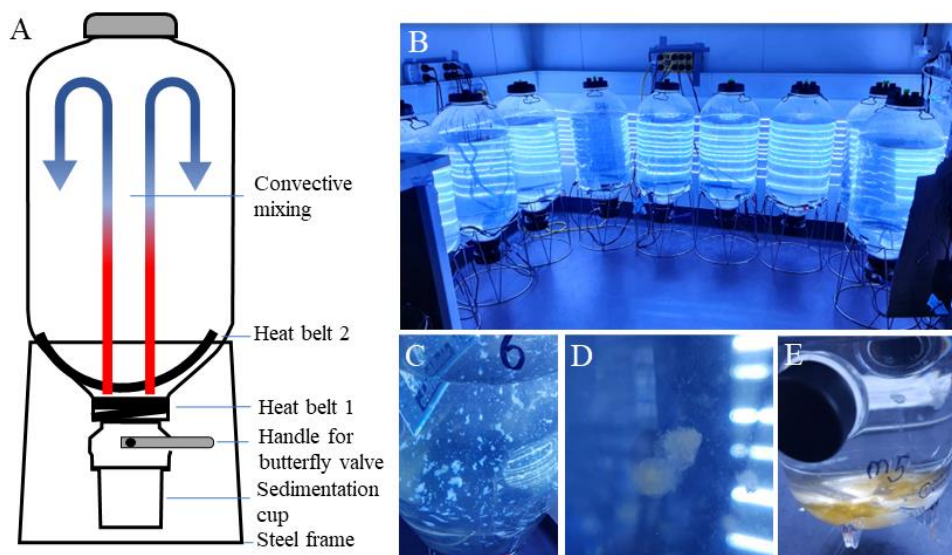
91 benefits.

92

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

94

95 **2.1. Microcosm setup**

96

97

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, which were originally designed for home brewing (Fig. 1). The tanks are mounted on steel frames and have 120 and 70 mm openings at the top and bottom, respectively. The bottom opening is equipped with a butterfly valve and a sedimentation cup, used for the collection of settling material. The butterfly valve has a handle so that the sedimentation cup can be isolated from the water column.

116   The crucial steps for setting up the microcosms, their filling, and their operation are listed in

117   Table S1, illustrated in Figs. 1 and 2 and Videos S1 (https://doi.org/10.5446/66751) and S2

118   (https://doi.org/10.5446/66753), and briefly described here. OAEPIIP experiments occupy

119   approximately 9 m^2 in a temperature-controlled room with a cooling capacity of roughly 6°C

120   below the temperature aimed for in the microcosm study (e.g., to 14°C if the desired

121   experimental water temperature is 20°C). The microcosms need to be thoroughly cleaned

122   before use (Table S1).

123   Infrastructure needed for filling the microcosms with natural seawater (containing natural

124   plankton communities) depends on the local environment at an OAEPIIP study site. At our

125   site in Tasmania, we fill microcosms from a jetty using a small crane or davit (Fig. 2; Video S1

126   (https://doi.org/10.5446/66751)). Natural seawater with plankton communities shall be

127   collected by opening the top lid and butterfly valve at the bottom and lowering the

128   microcosms slowly into seawater so that each microcosm is filled from bottom to top. Care

129   must be taken to not enclose larger debris, nekton or sediments. Once the microcosm is

130   submersed and only the upper opening is above the sea surface, a rope attached to the

131   handle of the butterfly valve is pulled so that the bottom opening is closed. The microcosm

132   can now be lifted back on shore and put back into its metal frame. Another possibility to fill

133   microcosms is to slowly lower them from a low swimming pontoon or small boat and close

134   the bottom manually. Filling microcosms by slowly lowering them into seawater is a very

135   gentle way to collect plankton communities (Video S1 (https://doi.org/10.5446/66751)),

136   avoiding the physical disturbance to plankton imposed by pumping. Based on our experience

137   it takes roughly 45 minutes to fill 9 microcosms. Longer timescales for the collection (i.e. >>1

138   hour) should be avoided to mitigate the risk of changes in seawater communities over the

139   course of the filling procedure (e.g. through tidal water movement). This potential problem

140   should also be minimised by filling the microcosms in random order. Furthermore, care

141   should be taken to not expose the microcosms to excessive sunlight (or heat) after filling.

142   The weight of the enclosed seawater needs to be determined after the filling procedure as

143   this information is needed for establishing treatments (section 2.5). This could be done using

144   a balance or (if a balance is not available) volumetrically and determining weight with known

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

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

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

148
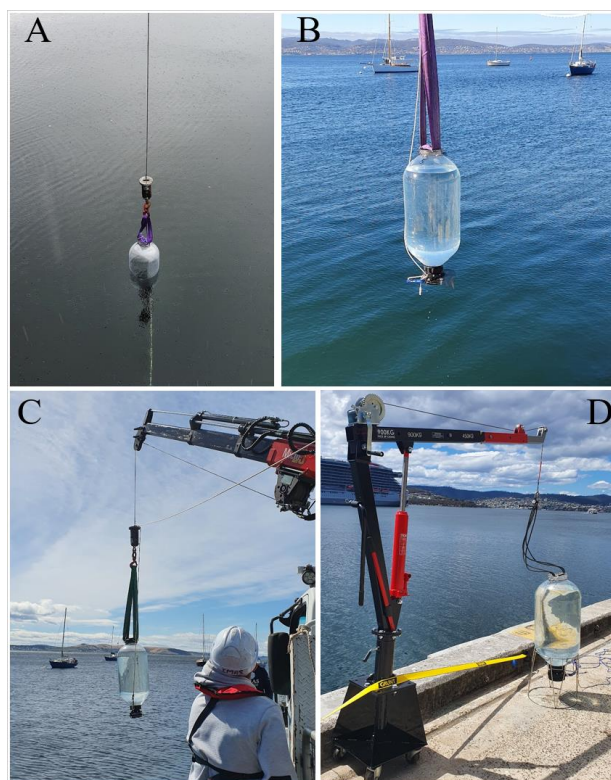
149

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

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

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

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

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

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

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

157

158   **2.2. Mixing, temperature, light, and nutrient conditions in OAEPIIP experiments**

159

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

161   suspension (Fig. 1). To establish convective mixing, two 30 Watts heat belts will be firmly

162   attached to two distinct locations at the bottom of the microcosms (Fig. 1, Video S2

163   (https://doi.org/10.5446/66753)). Based on our experience, these heat belts increase the

6

164   temperature of the enclosed seawater by ~6°C relative to the room temperature, so that

165   room temperature needs to be roughly ~6°C lower than the target temperature in the

166   experiments (please note that testing the temperature difference will be necessary prior to

167   the experiment as temperature offset may differ across temperature-controlled rooms).

168   Once heat belts are attached, microcosms should be placed in front of the light source and

169   heat belts should be plugged in to initiate the convective mixing.

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

171   regard to temperature control. Firstly, due to the removal of seawater during sampling, the

172   total volume within microcosms declines over the course of the experiment. Since the heat

173   belts cannot be adjusted, there is an increase in heat energy input per liter of enclosed

174   seawater and thus a gradual warming. To mitigate this issue, the external cooling may need

175   to be increased over time by lowering the room temperature. In our experience, a reduction

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

177   seawater temperature relatively constant over the course of the study. Secondly, small

178   differences in ventilation at different locations in the temperature-controlled room can lead

179   to seawater temperature differences of around 2°C between microcosms (Ferderer et al.,

180   2022; Guo et al., 2023). To mitigate this experimental constraint, the microcosm placement

181   within the temperature-controlled room must be shuffled on a daily basis. Microcosms can

182   easily be moved when they are being pulled on the steel frame (Fig. 1), but care must be taken

183   to briefly unplug the heat belts and plug them in again after shuffling their position.

184   Furthermore, fans can be utilised to remove heat pockets in the room, although care must be

185   taken as the wind can have a strong cooling effect, resulting in a microcosm that was too

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

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

188   experiment. The goal should be to have as little variation in temperatures between

189   microcosms as possible, and the temperature should be as the plankton community would

190   have experienced it at the location/season it was collected.

191   Like temperature, light conditions reproduce the natural site-specific conditions as much as

192   possible. This applies for the light/dark cycle, the light intensity, and the light spectrum (light

193   spectrum should be between 400 and 750 nm, i.e. cool white light). Since many OAEPIIP

194   participants may not have access to sophisticated computer-controlled light sources, we

195   recommend the delivery of constant light over a fixed light/dark cycle. In an OAE study in

196   Tasmania, for example, we provided light constantly with 200 µmol photons m^{-2} s^{-1} at a 12/12

197   hours light/dark cycle. These conditions were considered as representative average level for

198   the surface mixed layer at the location/season where/when the natural plankton community

199   were sourced in Tasmania. The light/dark cycle can be achieved by plugging the light source

200   into a timer socket. The microcosms need to be positioned in such a way that light is very

201   similar inside each microcosm. A light meter shall be used to determine light intensity inside

202   the microcosms prior to the experiments and at the end (Table 1). Positioning can be very

203   critical since movement by a few centimetres can often lead to noticeable changes in

204   measured light intensity that are undetectable by the human eye. It is therefore important to

205   adjust light conditions before starting the experiment and marking the spot on the floor

206   where individual microcosms must be placed to ensure replicable light levels. It is also

207   important to have all the other microcosms at their respective locations while doing the

208   adjustments as they might shade each other. The daily shuffling of microcosm positions inside

209   the room, which is essential for the temperature control (see above), will also help to mitigate

210   systematic bias in light regime between microcosms.

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

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

213

214   **2.3. OAE method in focus of OAEPIIP**

215

216   OAE can be implemented with different approaches (Eisaman et al., 2023), which have

217   different environmental implications as they are associated with different environmental

218   perturbations (Bach et al., 2019). A widely considered approach is electrodialytical OAE,

219   where liquid sodium hydroxide (NaOH) is the alkalinity source (de Lannoy et al., 2018). NaOH-

220   based OAE is in focus of OAEPIIP due to the following reasons. First, electrodialytical OAE was

221   recently evaluated to rank among the highest OAE approaches with regards to their

222   "technological readiness level" (Eisaman et al., 2023), with field trials already underway.

223   Second, liquid NaOH is suitable as an alkalinity source for applications in pelagic environments

224   as it delivers quasi instantaneous OAE in seawater. Other methods that involve more slowly

225   dissolving minerals (e.g. olivine) are considered less suitable for pelagic applications as they

226   would partially sink into the deep ocean before dissolving (Köhler et al., 2013; Fakhraee et al.,

227   2023). Third, electrodialytical OAE is chemically relatively similar to other OAE methods such

228   as OAE with magnesium hydroxides or ocean liming based on calcium hydroxides. Like NaOH,

229   magnesium and calcium hydroxides dissolve relatively quickly and are comparatively clean

230   sources of alkalinity so that their primary potential to affect pelagic communities is by

231   changing seawater carbonate chemistry. Thus, results from NaOH-based OAE experiments

232   will also inform these other approaches. Fourth, NaOH is readily available worldwide, which

233   is logistically beneficial for OAEPIIP.

234

235   **2.4. Experimental design**

236

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

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

239   al., 2014) or even longer (He and Tyka, 2023). The carbonate chemistry perturbation is much

240   greater before the equilibration has happened so that more pronounced effects on

241   communities would be expected shortly after alkalinity addition (Bach et al., 2019). As such,

242   an argument can be made to study OAE in two different scenarios when using rapidly

243   dissolving alkalinity sources like NaOH or other hydroxides. These are the "unequilibrated"

244   scenario, simulating the fact that CO_2 influx has not yet happened right after alkalinity

245   addition, and the "equilibrated" scenario, assuming the alkalinity enhanced seawater has

246   already CO_2-equilibrated with the atmosphere.

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

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

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

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

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

252   2018). This seemingly modest perturbation is due to dilution by the huge volume of the ocean

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

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

255   seawater (He and Tyka, 2023). Based on this, we determined an addition of 500 µmol/kg to

256   both the unequilibrated and equilibrated treatments for OAEPIIP. While a 500 µmol/kg

257   alkalinity increase is on the higher end for what is plausible for OAE, it seems to be a good

258   compromise between realism and the ability to detect environmental effects on plankton

259   communities (Ferderer et al., 2022).

260

261   **2.5. Establishing treatments**

262

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

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

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

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

267   microcosms before OAE.

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

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

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

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

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

273   to be added to the respective microcosm.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

288   2350 µmol/kg). The second calculation represents the desired conditions in the equilibrated

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

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

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

292

$$NaHCO_3 \; addition = \; DIC_{equilibrated} - DIC_{initial}$$

293
294

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

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

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

298 2023; Schulz et al., 2023), so that reaching to +500 µmol/kg requires the addition of slightly

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

300 to be added is calculated as:

301

$$NaOH \; addition = 500 - \; NaHCO_3 \; addition$$

302
303

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

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

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

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

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

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

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

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

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

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

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

315 The addition of NaOH to seawater causes precipitation of magnesium hydroxides, which

316 appear as white flakes (Fig. 1C). Therefore, microcosms should be stirred with a clean plastic

317 paddle during and after NaOH additions until all white flakes disappear. This problem will be

318 particularly pronounced in the unequilibrated treatment where all alkalinity is added as

319 NaOH. For consistency, control and equilibrated microcosms should be stirred as much as the

320 unequilibrated microcosms. If OAEPIIP participants do not have prior practical experience

321 with seawater carbonate chemistry manipulation, it is advised to test the above mentioned

322 procedures (including the measurement of resulting carbonate chemistry parameter changes

323 such as in TA and DIC) before commencing the main OAEPIIP experiment.

324

2.6. Essential parameters to be measured in OAEPIIP experiments

326

Next to an identical experimental design and setup, the same parameters need to be measured in individual OAEPIIP experiments to make them comparable. 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:

341

1) Not more than approximately 1/3 of the microcosm volume should be sampled over the course of the study 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 sedimenting materials from the sediment trap (Ferderer et al., 2022). However, care must be taken to not remove significant volumes of seawater.

355

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

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

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

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

360 *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

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

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

363 *establishment of the treatments.*

364

365   **2.7. Duration of experiment**

366

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

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

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

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

371   Amann, 2005). Based on experiments with the OAEPIIP setup in Tasmania we consider 20

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

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

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

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

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

377

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

379

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

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

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

383

384   **2.8. Sampling operations and logistics**

385

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

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

388   microcosms.

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

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

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

392   OAEPIIP experiments at higher temperatures require higher sampling frequency because

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

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

395 across OAEPIIP experiments. For example, nutrient samples should be taken at least 11 times
396 in each microcosm during the experiment. For an experiment at 15°C (20 days), this could
397 mean a sampling on day 0 (directly after establishing treatments) and then days 2, 4, 6,…,20.
398 However it may also be reasonable to increase frequency during periods of phytoplankton
399 blooms (e.g., daily) and then reduce the frequency (e.g. every 4 days) when nutrients are
400 depleted. In general, OAEPIIP experimentalists can best decide on an individual basis what
401 sampling schedule is most appropriate for their experiment, but the total number of
402 samplings must be at least as defined in Table 1 for each of the listed parameters.

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

407

408 **2.9. Statistical analyses**

409

410 Microcosm data contains complex ecological data which require specific (often complicated)
411 statistical tools for their analysis. A common issue is the presence of non linear relationships,
412 which without gross transformation of the variables prevents the fitting of data to linear
413 models. Furthermore, OAEPIIP microcosms will be sampled several times over an extended
414 period. This sampling strategy results in temporal-pseudoreplication, where observations are
415 not independent of each other and therefore violate the assumption of independence
416 required for simple linear models and Generalised additive models (GAMs). The expansion of
417 GAMs to Generalized Additive Mixed Models (GAMMs) allows for correlations between
418 observations and the modelling of data structures which are nested as well as for non-linear
419 relationships between the response and explanatory variables.

420 To facilitate and standardize statistical analyses of individual datasets we provide an R-based
421 pipeline (OAEPIIP, 2024). This pipeline is tailored towards the evaluation of individual OAEPIIP
422 data sets using GAMMs. The files contain a workflow which demonstrates the use of GAMMs
423 and facilitates the seamless integration of individual datasets gathered during OAEPIIP
424 experiments into the workflow. Theoretical background, knowledge and details on how to fit
425 such models can be found in the textbooks by Zuur et al. (2009) and Wood (2017).

426

427   **3. Logistics and administration**

428

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

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

431   intercomparison-project/).

432

433   **3.1. Eligibility and funding**

434

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

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

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

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

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

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

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

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

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

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

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

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

447   applications in close proximity.

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

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

450   rates). Standardized components like the microcosms will be supplied and fees for the

451   publication of individual studies in an OAEPIIP special issue will be covered (see section 3.2).

452   The remaining funding will be transferred via invoicing. Thus, participants must have a bank

453   account associated with their affiliation to which funding can be transferred from Australia.

454   This criterion therefore excludes laboratories in countries under relevant sanctions from

455   Australia to receive funding, although they are still welcome to be part of the OAEPIIP

456   community. Practically, participants will send two invoices to the University of Tasmania, one

457   at the beginning of the experiment to support purchasing of materials (e.g. the microcosms)

458   and the second one towards the end when the data is available and has been submitted.

459   OAEPIIP cannot provide funding for salaries. Therefore, the experiment was designed to be

460   suitable for a Master thesis or a chapter of a PhD thesis.

461

462   **3.2. Data management and publication**

463

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

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

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

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

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

469   individual basis in an OAEPIIP special issue (publication fees of up to 1600 US$ are part of the

470   ~12,000 US$ funding provided by OAEPIIP). Individual publication will enable identification of

471   novel observations on how plankton communities respond to OAE. If participants prefer not

472   to publish their data they still need to submit their data to OAEPIIP so that it can be included

473   in the OAEPIIP synthesis. This is critically important because the synthesis must avoid

474   publication bias.

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

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

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

478

479   **4. Capacity building and inclusivity**

480

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

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

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

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

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

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

487   worldwide is what we aim for since the OAE assessment requires the inclusion of the global

488   community. Indeed, participation in the process of assessing marine CDR methods (such as

489   OAE), rather than being on the receiving end of information only, has been expressed as an

490   important aspect by stakeholders from developing countries.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

506 guarantee geographical diversity).

507 Furthermore, potential participants that simply have no chance to measure one (or more)

508 core parameters due to unsurmountable logistical constraints can still hand in an application,

509 if they indicate which parameters they are unable to deliver on their application form

510 (available on the OAEPIIP website). The OAEPIIP administration will then evaluate such

511 applications on a case-by-case basis and explore if there is a way for participation despite this

512 limitation. This pathway is set in place specifically for potential participants with less

513 developed infrastructure and less capacity for collaboration with an experienced (e.g., due to

514 geographic isolation).

515 Altogether, we hope the cost-efficient design of OAEPIIP, its eligibility criteria that refrain

516 from classic measures of scientific success, and potential support via an evolving OAEPIIP

517 community could promote an inclusive assessment of OAE. One primary goal of OAEPIIP is

518 capacity building to provide more informed decisions concerning OAE that encompass data

519 from a geographically diverse range of plankton ecosystems.

520

521 **Competing interests**

522

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

524

525   **Acknowledgements**

526

529

530   **Data availability**

531

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

533   2024).

534

535   **References**

536

537   Bach, L. T., Gill, S. J., Rickaby, R. E. M., Gore, S., and Renforth, P.: CO2 Removal With
538   Enhanced Weathering and Ocean Alkalinity Enhancement: Potential Risks and Co-benefits
539   for Marine Pelagic Ecosystems, Frontiers in Climate, 1, 2019.

540

541   Benton, T. G., Solan, M., Travis, J. M. J., and Sait, S. M.: Microcosm experiments can inform
542   global ecological problems, Trends in Ecology & Evolution, 22, 516–521,
543   https://doi.org/10.1016/j.tree.2007.08.003, 2007.

544

545   Burt, D. J., Fröb, F., and Ilyina, T.: The Sensitivity of the Marine Carbonate System to
546   Regional Ocean Alkalinity Enhancement, Frontiers in Climate, 3, 2021.

547

548   Dingley, B., O'Rourke, E., Turner, B., CMIP Panel Members, WGCM Infrastructure Panel
549   Members, and CMIP7 Task Team Members: CMIP Annual Report 2022-2023,
550   https://doi.org/10.5281/zenodo.8101810, 2023.

551

552   Eisaman, M. D., Geilert, S., Renforth, P., Bastianini, L., Campbell, J., Dale, A. W., Foteinis, S.,
553   Grasse, P., Hawrot, O., Löscher, C. R., Rau, G. H., and Rønning, J.: Assessing the technical
554   aspects of ocean-alkalinity-enhancement approaches, State of the Planet, 2-oae2023, 1–29,
555   https://doi.org/10.5194/sp-2-oae2023-3-2023, 2023.

556

557   Fakhraee, M., Li, Z., Planavsky, N. J., and Reinhard, C. T.: A biogeochemical model of
558   mineral-based ocean alkalinity enhancement: impacts on the biological pump and ocean
559   carbon uptake, Environ. Res. Lett., 18, 044047, https://doi.org/10.1088/1748-9326/acc9d4,
560   2023.

561

562   Ferderer, A., Chase, Z., Kennedy, F., Schulz, K. G., and Bach, L. T.: Assessing the influence of

563   ocean alkalinity enhancement on a coastal phytoplankton community, Biogeosciences, 19,
564   5375–5399, https://doi.org/10.5194/bg-19-5375-2022, 2022.

565

566   Field, A. P. and Gillett, R.: How to do a meta-analysis, British Journal of Mathematical and
567   Statistical Psychology, 63, 665–694, https://doi.org/10.1348/000711010X502733, 2010.

568

569   Gattuso, J.-P., Epitalon, J.-M., Lavigne, H., and Orr, J. C.: Seacarb: seawater carbonate
570   chemistry. R package version 3.3.0., , CRAN, 2021.

571

572   Guo, J. A., Strzepek, R. F., Swadling, K. M., Townsend, A. T., and Bach, L. T.: Influence of
573   Ocean Alkalinity Enhancement with Olivine or Steel Slag on a Coastal Plankton Community
574   in Tasmania, EGUsphere, 1–27, https://doi.org/10.5194/egusphere-2023-2120, 2023.

575

576   Hamm, T., Barkhau, J., Gabriel, A.-L., Gottschalck, L. L., Greulich, M., Houiller, D., Kawata, U.,
577   Tump, L. N., Leon, A. S., Vasconcelos, P., Yap, V., Almeida, C., Chase, Z., Hurd, C. L., Lavers, J.
578   L., Nakaoka, M., Rilov, G., Thiel, M., Wright, J. T., and Lenz, M.: Plastic and natural inorganic
579   microparticles do not differ in their effects on adult mussels (Mytilidae) from different
580   geographic regions, Science of The Total Environment, 811, 151740,
581   https://doi.org/10.1016/j.scitotenv.2021.151740, 2022.

582

583   Harrison, F.: Getting started with meta-analysis, Methods in Ecology and Evolution, 2, 1–10,
584   https://doi.org/10.1111/j.2041-210X.2010.00056.x, 2011.

585

586   He, J. and Tyka, M. D.: Limits and CO_2 equilibration of near-coast alkalinity enhancement,
587   Biogeosciences, 20, 27–43, https://doi.org/10.5194/bg-20-27-2023, 2023.

588

589   Jones, D. C., Ito, T., Takano, Y., and Hsu, W.-C.: Spatial and seasonal variability of the air-sea
590   equilibration timescale of carbon dioxide, Global Biogeochemical Cycles, 28, 1163–1178,
591   https://doi.org/10.1002/2014GB004813, 2014.

592

593   Köhler, P., Abrams, J. F., Völker, C., Hauck, J., and Wolf-Gladrow, D. A.: Geoengineering
594   impact of open ocean dissolution of olivine on atmospheric CO2, surface ocean pH and
595   marine biology, Environ. Res. Lett., 8, 014009, https://doi.org/10.1088/1748-
596   9326/8/1/014009, 2013.

597

598   de Lannoy, C.-F., Eisaman, M. D., Jose, A., Karnitz, S. D., DeVaul, R. W., Hannun, K., and
599   Rivest, J. L. B.: Indirect ocean capture of atmospheric CO2: Part I. Prototype of a negative
600   emissions technology, International Journal of Greenhouse Gas Control, 70, 243–253,
601   https://doi.org/10.1016/j.ijggc.2017.10.007, 2018.

602

603   Lenton, A., Matear, R. J., Keller, D. P., Scott, V., and Vaughan, N. E.: Assessing carbon dioxide
604   removal through global and regional ocean alkalinization under high and low emission
605   pathways, Earth System Dynamics, 9, 339–357, https://doi.org/10.5194/esd-9-339-2018,
606   2018.

607

608   Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen,
609   Y., Goldfarb, L., Gomis, M. I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J. B. R., Maycock,

610 T. K., Waterfield, T., Yelekçi, Ö., Yu, R., and Zhou, B. (Eds.): Climate Change 2021: The
611 Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of
612 the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge,
613 United Kingdom and New York, NY, USA, https://doi.org/10.1017/9781009157896, 2021.

615 OAEPIIP: Additional resources on carbonate chemistry calculation and statistical evaluations
616 of OAEPIIP, 2024.

618 Oschlies, A., Bach, L. T., Rickaby, R. E. M., Satterfield, T., Webb, R., and Gattuso, J.-P.:
619 Climate targets, carbon dioxide removal, and the potential role of ocean alkalinity
620 enhancement, State of the Planet, 2-oae2023, 1–9, https://doi.org/10.5194/sp-2-oae2023-
621 1-2023, 2023.

623 Pernthaler, J. and Amann, R.: Fate of Heterotrophic Microbes in Pelagic Habitats: Focus on
624 Populations, Microbiology and Molecular Biology Reviews, 69, 440–461,
625 https://doi.org/10.1128/mmbr.69.3.440-461.2005, 2005.

627 Pierrot, D., Epitalon, J.-M., Orr, J. C., Lewis, E., and Wallace, D. W. R.: MS Excel program
628 developed for CO2 system calculations – version 3.0, , GitHub repository, 2021.

630 Riebesell, U., Schulz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhöfer, M., Neill,
631 C., Nondal, G., Oschlies, A., Wohlers, J., and Zöllner, E.: Enhanced biological carbon
632 consumption in a high CO2 ocean, Nature, 450, 545–548,
633 https://doi.org/10.1038/nature06267, 2007.

635 Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M.,
636 Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R., and Schulz, K. G.:
637 Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean
638 change research, Biogeosciences, 10, 1835–1847, https://doi.org/10.5194/bg-10-1835-
639 2013, 2013.

641 Schulz, K. G., Bach, L. T., and Dickson, A. G.: Seawater carbonate chemistry considerations
642 for ocean alkalinity enhancement research: theory, measurements, and calculations, State
643 of the Planet, 2-oae2023, 1–14, https://doi.org/10.5194/sp-2-oae2023-2-2023, 2023.

645 Sherman, E., Moore, J. K., Primeau, F., and Tanouye, D.: Temperature influence on
646 phytoplankton community growth rates, Global Biogeochemical Cycles, 30, 550–559,
647 https://doi.org/10.1002/2015GB005272, 2016.

649 Stewart, R. I. A., Dossena, M., Bohan, D. A., Jeppesen, E., Kordas, R. L., Ledger, M. E.,
650 Meerhoff, M., Moss, B., Mulder, C., Shurin, J. B., Suttle, B., Thompson, R., Trimmer, M., and
651 Woodward, G.: Chapter Two - Mesocosm Experiments as a Tool for Ecological Climate-
652 Change Research, in: Advances in Ecological Research, vol. 48, edited by: Woodward, G. and
653 O'Gorman, E. J., Academic Press, 71–181, https://doi.org/10.1016/B978-0-12-417199-
654 2.00002-1, 2013.

656 Taucher, J., Boxhammer, T., Bach, L. T., Paul, A. J., Schartau, M., Stange, P., and Riebesell, U.:

657 Changing carbon-to-nitrogen ratios of organic-matter export under ocean acidification, Nat.
658 Clim. Chang., 11, 52–57, https://doi.org/10.1038/s41558-020-00915-5, 2021.
659
660 Wood, S. N.: Generalized Additive Models: An Introduction with R, Second Edition, 2nd ed.,
661 Chapman and Hall/CRC, Boca Raton, 496 pp., https://doi.org/10.1201/9781315370279,
662 2017.
663
664 Zeebe, R. E. and Wolf-Gladrow, D.: CO2 in Seawater: Equilibrium, Kinetics, Isotopes, Gulf
665 Professional Publishing, 382 pp., 2001.
666
667 Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M.: Mixed effects models
668 and extensions in ecology with R, Springer, New York, NY, https://doi.org/10.1007/978-0-
669 387-87458-6, 2009.