Supplement of Biogeosciences, 21, 3665–3676, 2024
https://doi.org/10.5194/bg-21-3665-2024-supplement


Biogeosciences Open Access EGU

*Supplement of*

# Technical note: Ocean Alkalinity Enhancement Pelagic Impact Intercomparison Project (OAEPIIP)

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**Table S1**. A step-by-step manual on how to set up an OAEPIIP experiments.

| Step | Action |
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| 1 | To establish comparability between OAEPIIP experiments, all participants should use the 55 L brewing tanks "FermZilla" as microcosm incubators. These brewing tanks available worldwide with distributors in China, Australia, USA, and Norway (e.g., https://www.kegland.com.au/products/55l-fermzilla-tri-conical-pressure-brewing-kit?_pos=1&_psq=fermzilla+55&_ss=e&_v=1.0). Both Gen2 and Gen3 FermZilla tanks with sedimentation cups are suitable for OAEPIIP. Ferderer et al. (2022) used Gen2 tanks. |
| 2 | Assemble microcosm tanks as described in the manual. |
| 3 | Fill all microcosms with freshwater to the top and keep water inside for one week to leach out potential contaminants from the microcosm walls and test for leaks. |
| 4 | Empty microcosms and clean 5 times with de-ionised water. |
| 5 | Establish the positions for the microcosms with proper light conditions, i.e. ±5% variability in between them. |
| 6 | Mark the spots on the floor where microcosms were positioned. |
| 7 | Let microcosms dry on the in- and outside. |
| 8 | Weigh all empty microcosms and document their individual masses when empty and dry.* |
| 9 | Transport all microcosms to the site where seawater (with enclosed plankton communities) is collected for the experiment (the filling procedure is also shown in Video S1 https://doi.org/10.5446/66751). |
| 10 | Open butterfly valve at the bottom of the microcosm. |
| 11 | Attach a belt to the handles at the top of the microcosms. Make sure the belt is clean. |
| 12 | Attach the belt to the hook of the crane. |
| 13 | Attach a rope to the handle of the butterfly valve, which is long enough to be pulled from shore. |
| 14 | Fill the microcosm by slowly lowering the microcosm with a crane into the water until the microcosm is almost fully submerged. |
| 15 | Pull the rope attached to the butterfly valve to close the microcosm at the bottom. |
| 16 | Lift the microcosm back on shore and into the metal frame. |
| 17 | Repeat the filling process with all remaining microcosms (ideally < 1 hour for filling all of them). |
| 18 | Transport microcosms into the temperature-controlled room, dry the outside of all microcosms and re-weigh them to be able to calculate the mass of the enclosed seawater by subtraction of the empty weight (see step 8)*. |
| 19 | Attach the first 30W heat belt firmly around the neck just above the butterfly valve at the bottom of the microcosms (Video S2 https://doi.org/10.5446/66753). The 30 W heat belts can be purchased through the same distributor as the FermZilla tanks (e.g., https://www.kegland.com.au/products/30w-heating-belt?_pos=1&_psq=heat+belt&_ss=e&_v=1.0) |
| 20 | Attach the second 30 W heat belt where the steel frame carries the microcosm tank (Video S2 https://doi.org/10.5446/66753). |
| 21 | Put the microcosms in the positions determined by light measurements (see steps 5 and 6). |
| 22 | Plug in 30W heat belts. |
| 23 | Switch on the light source. |
| 24 | Switching on the light source marks the beginning of the experiment (day 0), which should ideally be within hours of seawater collection. After switching on the light, sampling and establishing the treatments will commence. |
| 25 | Shuffle microcosms around the predetermined positions every day to even out potential differences in the amount of light received and potential temperature gradients in the room. |

*microcosm mass is needed for determining the mass of the enclosed seawater by weighing. This step can be ignored if the enclosed seawater mass is determined volumetrically (e.g., by determining microcosm volumes before the experiment and converting enclosed volume to mass by known seawater density).

***Table S2.*** *A list of recommendations for sampling and processing of OAEPIIP core parameters. Recommendations are not comprehensive method descriptions, since methodology will be specific for participating laboratories. Instead, recommendations aim to provide practical advice in microcosm-specific workflows and pitfalls.*

| Parameter | Recommendation |
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| Total Alkalinity | Total Alkalinity should be sampled directly from the microcosm. We recommend filtration of the sample for reasons discussed in Schulz et al. (2023). Filtration can be achieved quite effortlessly if the sample is withdrawn from a microcosm and pumped through a syringe filter directly into the storage container. Alkalinity sample storage and measurement should follow best practices described by Dickson et al. (2007) and Schulz et al. (2023). Alkalinity should be reported in µmol/kg. |
| Second carbonate chemistry parameter (e.g., pH or DIC) | Sampling, sample storage, and measurement of the second carbonate system parameter has to follow best practices described by Dickson et al. (2007) and should consider OAE-specific recommendations by Schulz et al. (2023). OAEPIIP participants can decide which second carbonate chemistry parameter they prefer to measure but must keep in mind that daily measurements are needed, regardless of the duration of the experiment. Data should generally be reported in units recommended by Dickson et al. (2007) and pH specifically on the total pH scale. |
| Salinity | Salinity should be measured after water collection inside each microcosm. A second measurement should be made in all microcosms at the end of the experiment. Measurements are ideally via conductivity and temperature and reported on the practical salinity scale (PSS-78) (Lewis and Perkin, 1981). |
| Light | Light should be measured before the experiment starts to establish that all microcosms are receiving the same amount of light. Light measurements can be tricky and time-consuming as small changes in the position of the light meter often lead to rather pronounced changes in light intensity. Aiming for consistent light at all locations where microcosms will be positioned in the room is very important to minimize variability in the dataset due to unequal light supply. We urge OAEPIIP participants to take great care when setting up the light environment as any deviations can have a large influence on the data quality. Light measurements should be repeated at the end of the experiment to reaffirm light conditions. Light should be reported in µmol/$m^2$/s, meaning that rather photosynthetically active radiation (PAR) is measured than luminous (Lux) or radiant flux (W). |
| Temperature | Temperature needs to be measured daily in all microcosms, irrespective of the duration of the experiment. We recommend measurements before shuffling microcosm positions with a probe at ~0.1 m depth. Temperature should be reported in °C. |

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| Nutrients ($NO_x^-$, $PO_4^{3-}$, $Si(OH)_4$) | We recommend to sample nutrients like alkalinity (i.e., filter the seawater upon collection directly into the clean storage container). Samples should be analysed quickly (e.g. within 3 hours) after collection or stored appropriately so that nutrient concentrations do not change during storage. Nutrient concentrations should be analysed with seawater-compatible protocols, for example following Hansen and Koroleff (1999). If possible, we recommend the use of certified reference material for accuracy-control (e.g. https://www.jamstec.go.jp/scor/property.html). Nutrient concentrations should be reported in µmol/L. |
| Chlorophyll a (Chla) | Chla is sampled by filtering an appropriate amount of seawater onto glass fibre filters (GF/F). The filtration should be performed quickly after sampling the seawater and the sample should not be exposed to excessive light. We recommend collecting filtration volume for Chla and the other particulate matter samples that require filtration (i.e., POC/PON and BSi, see below) in the same container. However, care must be taken to gently mix the volume before sub-sampling for particulate matter samples to avoid sedimentation bias. Chla on GF/F filters should be stored dark in liquid nitrogen or at -80°C until analysis. Storage at -20°C may also be ok if storage is short (few days). Storage can be avoided if Chla is immediately extracted, e.g. with acetone. A measurement technique appropriate for seawater Chla analysis must be used, e.g., following Welschmeyer (1994). Chla probes can be acceptable under certain circumstances, for example due to logistical constraints of the participant or limitations in sampling volume. However, the necessity for using probes should be discussed with the OAEPIIP administration before deciding on their use. Chla should be reported in µg/L. |
| Particulate organic carbon and nitrogen (POC and PON) | POC/PON is sampled by filtering an appropriate amount of seawater onto glass fibre filters (e.g., GF/F or QMA). We recommend storage of filters in combusted glassware to minimize potential contamination with C or N. POC and PON should be reported in µmol/L. |
| Biogenic silica (BSi) | BSi is sampled by filtering an appropriate amount of seawater onto polycarbonate filters (pore size = 0.8 µm). The samples should be stored at -20°C in plasticware, not glassware, to avoid Si contamination. Analysis of BSi should follow protocols appropriate for seawater, e.g., (Hansen and Koroleff, 1999). BSi should be reported in µmol/L. |
| Flow cytometry (FC) | Approximately 1 mL of sample should be collected from each microcosm. The volume can be collected from the same volume that has already been collected for particulate matter samples (see above). However, care must be taken to gently mix the sample before sub-sampling for flow cytometry to avoid any sedimentation bias. Appropriate sample fixation needs to be applied before sample storage (Veldhuis et al., 2001; Marie et al., 2014). Samples should be flash-frozen in liquid $N_2$ directly after their fixation and then stored at -80°C. For the analysis, investigators should separate the community into at least: picoeukaryotes (0.2-2 µm), nanoeukaryotes (2-20 µm), microeukaryotes (>20 µm), and Synechococcus-like cells (if present) (See e.g., Ferderer et al., (2022)). The determination of size classes can be achieved by size calibrations with |

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| | standardized beads or by filtrations of some phytoplankton samples with filters or meshes (here, 0.2, 2, and 20 µm filters/meshes; Veldhuis and Kraay, 2000). Identifying more than these four groups for the evaluation of the individual OAEPIIP studies is encouraged but for the synthesis data provided to the OAEPIIP database, these four groups must be included. Importantly, the gates which are set to distinguish these four groups must be identical in all 9 microcosms in an individual OAEPIIP experiment. If the gates vary for example between the control and equilibrated treatment, then the populations enclosed within these gates could not be compared (it would be an apples and oranges comparison (Bach et al., 2017)). Abundances of picoeukaryotes, nanoeukaryotes, microeukaryotes, and Synechococcus-like cells should be reported in counts/mL. |
| Microscopy | Between 10-100 mL of sample should be collected for microscopic quantification of microphytoplankton (>20 µm) and microzooplankton (>20 µm). Samples should be fixed with Lugol solution and stored in a dark and cool place until analysis. Whether acidic or basic Lugol solution is chosen for the fixation depends on the experimentalist's focus on the phytoplankton community. Acidic Lugol is appropriate for preserving diatoms while it will dissolve coccolithophorid $CaCO_3$, making them unrecognisable. Conversely, basic Lugol is suitable to preserve coccolithophores but inappropriate to preserve diatoms as BSi will dissolve. Microphyto- and zooplankton abundances should be determined with the Utermoehl method. If possible, biovolume of the counted species should be determined following (Hillebrand et al., 1999). The identified species should be determined to the highest taxonomic level possible. Abundance should be reported in cells/mL and biovolume in µL/mL. |
| Metagenomics | This sample collection protocol is modified from an standard operating procedure developed for Ocean Sampling day (https://www.vliz.be/projects/assembleplus/file/3495 ) and has been used from 2014 until now. It is based on (Gilbert et al., 2010). The metagenomics component of OAEPIIP is coordinated by Prof. Julie LaRoche at Dalhousie University (Canada) who can provide logistical support on sample analyses.<br><br>Materials and supplies needed for sampling comprise: Surgical gloves; Nine (i.e., one for each microcosm) 60 ml sterile disposable Luer-lock plastic syringes (Can be reused if carefully acid washed, rinsed with double de-ionised water (Milli-Q) and dried); Sterivex cartridge filters (https://www.sigmaaldrich.com/AU/en/product/mm/svgp010); blu tack for sealing the sterivex cartridge filters after sampling, transparent tape, clean sampling container (acid washed, Milli-Q rinsed and dried).<br><br>Samples should be collected at the beginning of the experiment and at the end. The following describes how to take a sample from one microcosm. This procedure has to be repeated for all other microcosms. Wear surgical gloves at all times during the water filtration and filter processing.<br><br>Fill a sampling container (acid washed, rinsed with double de-ionised water (Milli-Q) and dried) with approximately 2 L water from a microcosm. Fill a sterile disposable Luer-lock plastic syringe (Can be reused if carefully acid washed, rinsed with Milli-Q and dried) the syringe with water from the sampling |

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| | container. Attach a 0.22 µm Sterivex cartridge filter (https://www.sigmaaldrich.com/AU/en/product/mm/svgp010) to the end of the syringe and push the volume through the filter. Repeat this procedure until you have filtered the 2 L or when no water can be pushed through the cartridge anymore. Note that the sterivex will have to be disconnected and reconnected each time you filter through a new 50 mL of sample. Record the volume that has been filtered in your laboratory book. Then fill your syringe with air and push the air through the cartridge to remove the residual water. Plug the ends with blu tack or something equivalent (and appropriate) available to you. Please note that it is also possible to use a peristaltic pump instead of the syringe for filtering samples. However, if you use this alternate way of collecting your samples, it should be noted in your laboratory notebook (including filtered volumes) and be communicated to OAEPIIP upon data delivery for the synthesis.<br><br>Label the Sterivex cartridge with either cryolabels or using a permanent marker that does not decay at -80°C. If you are using the permanent marker, it is advisable to cover the label with transparent tape that does not fall off at -80°C. Please make sure that the label does not get lost under any circumstances.<br><br>For storage, put sample cartridges into cold-resistant plastic bags. You can also use knee length nylon stockings as bags as those are resistant even down to -80°C. The best DNA conservation method is storing at -80°C until shipping for analysis but if that is not available the filters can be stored at -20°C for a short time. Samples must be frozen immediately after collection and labelling.<br><br>The 18 cartridges collected for the experiment (9 at the beginning and 9 at the end of the study) should be sequenced for the 16s RNA by a professional service laboratory. If necessary, support can also be provided by Prof. Julie LaRoche's laboratory, which should be directly communicated with her team. For shipping samples should ideally be transported on dry ice if possible or with dry shippers (liquid nitrogen). |

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