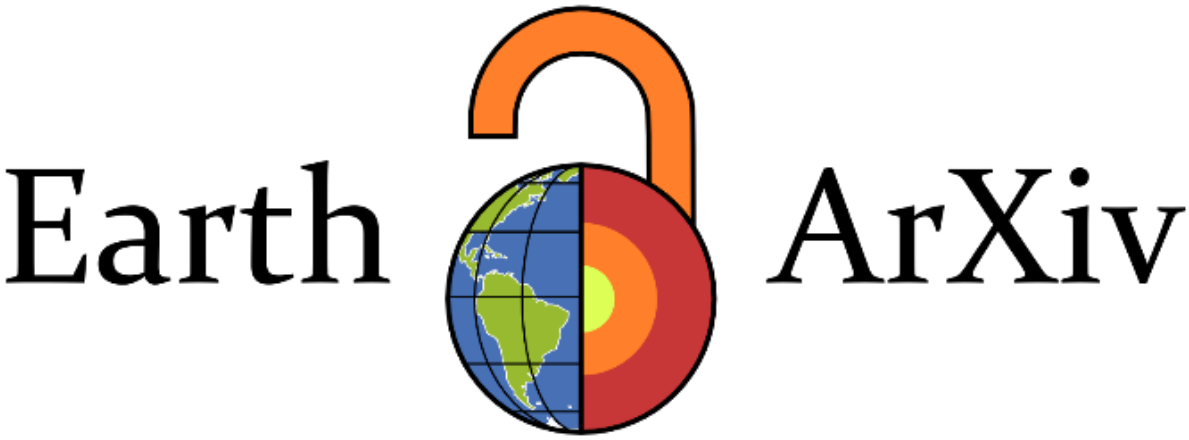

Rebuttal of
Sweetman, A.K., Smith, A.J., de Jonge, D.S.W. et al. Evidence of dark oxygen production at the abyssal seafloor. Nat. Geosci. (2024). https://doi.org/10.1038/s41561-024-01480-8

The paper by Sweetman et al. (2024) is criticized for poor-quality lander incubation experiments, leading to faulty oxygen flux measurements. The authors misinterpret results and make unsupported speculations, raising serious concerns about the methodology, data handling, and overall conclusions of the study.

Main Criticisms:

1. **Data Reuse Without Citation:** The authors reuse data from Cecchetto, Sweetman et al. (2023) without referencing. These earlier deployments were done on sediments without nodules, yet the same patterns of increasing oxygen are presented as nodule incubations, casting serious doubts on the entire experimental approach and on the ethical principles of the authors.
2. **Poor Chamber Ventilation Leading to Inconsistent Oxygen Concentrations inside the incubation chambers:** Initial oxygen concentrations, from sensors and water samples, across different incubations vary widely (ranging from 80-250 µM). Such variability is unrealistic for deep-sea environments (Smith et al 2022 reported a decrease from 145 to 130 µM over a time period of 30 years at station M, in N Pacific, at about 4000 m depth) and points to issues like trapped air bubbles, poor chamber ventilation, or contamination from water layers above. If chambers do not have ambient bottom water background concentrations of oxygen, at the start of incubation, they cannot be well ventilated and the incubations should be discarded since they provide artificial data (Kononets et al. 2021). When re-analyzing the data from these deployments we found that maximum 2 out of 32 incubations from this work might be usable.
3. **Poor Lander Technology and Quality Control:** The authors fail to address well-documented issues with their methodology, ignoring advances in lander incubation technology during the past 20 years. They have not implemented basic quality control measures, such as leakage control, measurement of the incubated water volume (Kononets et al, 2021), oxygen sensors inside all 3 chambers (it seems that they only have 2 sensors available) and outside (Sommer et al. 2008), no measurements of temperature and accidental sediment re-suspension with turbidity sensors (Tengberg et al. 2003), and no control on the correct timing of injections and water sampling, which seems to be wrong (Kononets et al, 2021). Furthermore, several incubations were omitted without explanation.
4. **Artifacts and Questionable Oxygen Fluxes:** Results show highly variable oxygen fluxes across incubations. Results from sensors and water samples analyzed by Winkler titration are very different. In some chambers, there is oxygen consumption and in some oxygen production, that often later turn into consumption. The differences are likely due to artifacts as a result of poorly equilibrated, or ventilated chambers, plastic material (Stevens 1992; Vikstrom et al. 2019), of which the chambers are constructed, leaking oxygen, trapping of air bubbles (se example graphs below) and oxygen-saturated water injections.
5. **Graphical Presentation Issues:** The graphs are very difficult to scrutinize. The use of similar colors and symbols makes it hard to the interpret the results.

Conclusion: Given scientific ethics, numerous methodological flaws, misinterpretations, and lack of proper quality control, it is strongly recommended that *Nature Geoscience* withdraws this paper.

Sincerely yours,

Associate Prof. Anders Tengberg[1*], Dr. Mikhail Kononets[2] and Prof. Per Hall[2]
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Competing interests: None, the authors are and have never been involved in any investigations related to deep-sea nodules and the companies involved in this work. This rebuttal is not taking any opinion on deep-sea mining. Our input is only a critique of the substandard work presented in Sweetman et al. (2024).

Author contribution statements: All authors contributed to the writing of this document and analyzing of data. Mikhail Kononets created the combined sensors and water sample plots using available data and analyzed the lander functioning in detail.

Our experience: Development, construction and use of multiple autonomous bottom landers comprising 370+ deployments, 1000+ chamber incubations (depth 5-5600 m), 40+ scientific papers (e.g. 1, 6, 11), a lander review (12), papers on lander technology (6,13), quality control (6) and several papers on sensor technology, including on oxygen optode technology (14). The numbers in parenthesis in the text refer to references in the list below.

Extended data:
- Reference list
- Graphs for clarification: We downloaded the available data and made new plots to clarify the issues discussed above. We also included 3 graphs from failed incubations to demonstrate that it is easy to reproduce similar oxygen increases in the chambers if the incubations are not carried out correctly.

Supplementary Information
- A description of how autonomous chamber incubation landers work: *In situ incubations with autonomous benthic chamber landers - for non-experts. Sweetman et al (2024) into perspective*. Numbers in parenthesis in this text refer to references in the list.
- A pdf copy of the Sweetman et al. (2024) paper is included with detailed comments inserted into the relevant places.

Examples of relevant literature

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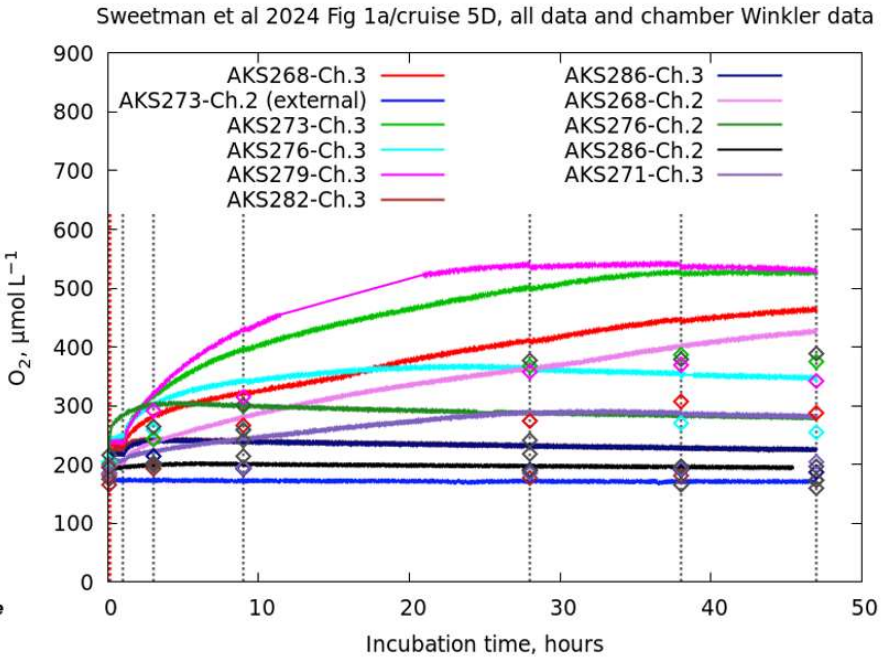
Graphs for clarification, plotted with data from Sweetman et al., 2024. Evidence of Dark Oxygen Production at the Abyssal Seafloor

Sweetman et al 2024
Fig 1a + Winkler data
Grey dotted line: syringe sampling times
Red dotted line: injection time.

Winkler records missing
(only optode data reported)
AKS273-Ch.2 (external)
AKS268-Ch.2
AKS276-Ch.2
AKS286-Ch.2

Optode time series missing (only Winkler data reported)
AKS279-Ch.1
AKS271-Ch.1 No-injection control
AKS271-Ch.2
AKS282-Ch.1 No-injection control

6 incubation datasets missing: 268-Ch.1, 273-Ch.1, 276-Ch.1, 279-Ch.2, 282-Ch.2, 286-Ch.1. Selective data reporting?
Lander not functioning well?

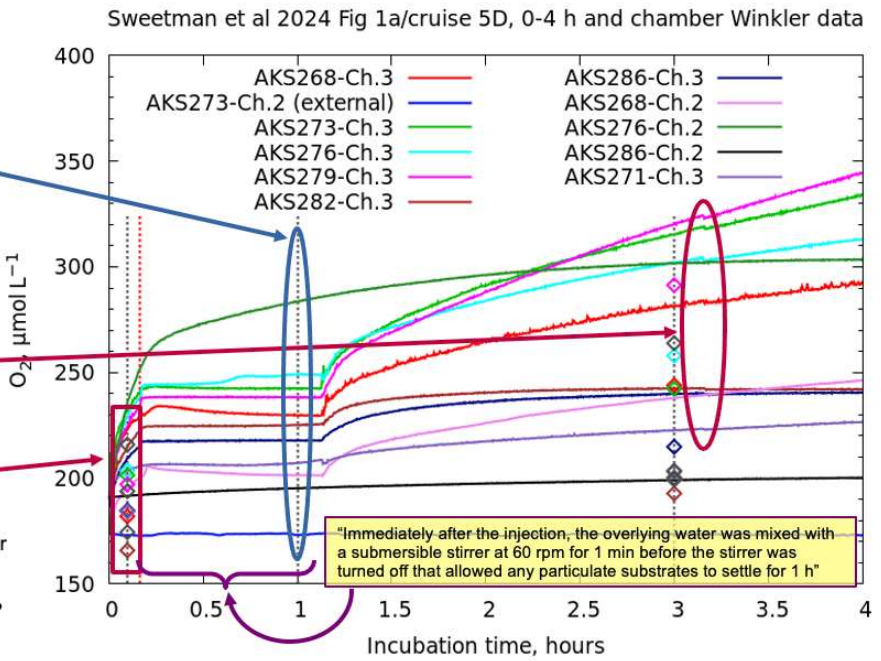


Sweetman et al 2024
Fig 1a + Winkler data
Grey dotted line: syringe sampling times
Red dotted line: injection time.

Reported sampling during the no stirring period (~10 min to ~1h 10 min) does not make sense and likely not done; the sample not present on Winkler data. ***Actual sampling times reported not known? Not accurately reported?***

Dip on the O2 records at ~3.2 h, different than the 3 h timing given in Methods and in the Winkler datafile.
Lander not functioning well?

Winkler data for the first syringe sample, 0.1 h in the incubations showing large span. Should be expected to be close to each other and reflect bottom water Oxygen. Winkler span ~165-215 µM. ***Poor quality Winkler measurements? Poor chamber ventilation?***



Sweetman et al 2024

Fig 1b + Winkler data

Grey dotted line: syringe sampling timings

Red dotted line: injection

Dip on AKS316-Ch.3 O2 time series not matching any sampling time.

Dips on the O2 time series indicating sample timings ~2 h off from the reported timings. ***Reported timings not accurate? Lander not functioning well?***

Winkler records missing (only optode data reported)

AKS313-Ch.2

AKS321-Ch.1

AKS313-Ch.1

AKS318-Ch.1

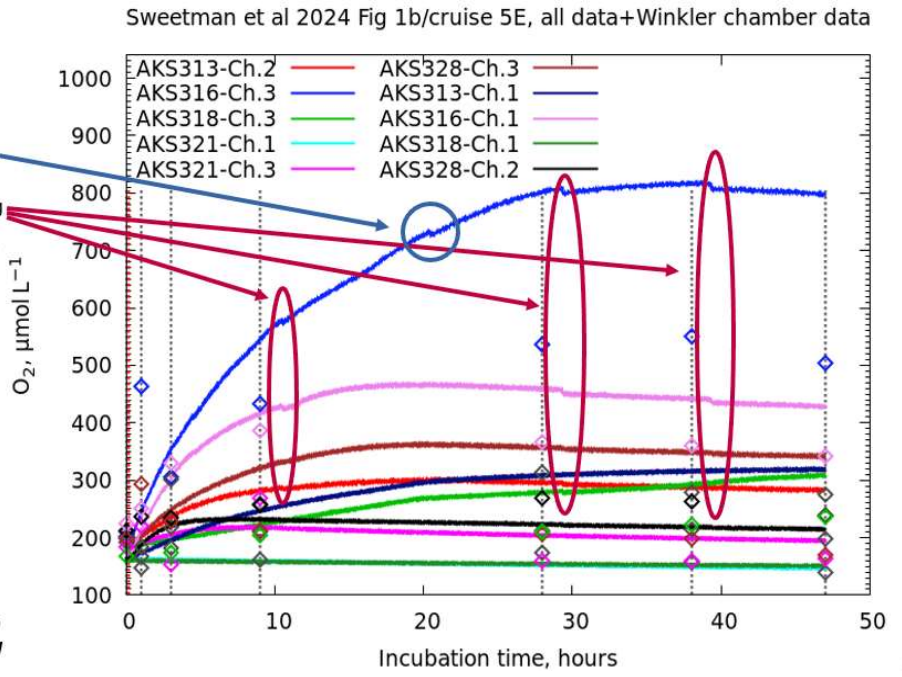
Optode time series missing (only Winkler data reported)

AKS313-Ch.3

AKS318-Ch.2

AKS321-Ch.2

2 incubation datasets missing: 316-Ch.2, 328-Ch.1. Selective data reporting? Lander not functioning well?



Sweetman et al 2024

Fig 1c + Winkler data

Grey dotted line: syringe sampling timings

Red dotted line: injection.

Dips on the optode time series indicating sample timings ~40 min off from the reported timings. ***Reported timings not accurate? Lander not functioning well?***

Winkler records missing (only optode data reported)

none

Optode time series missing (only Winkler data reported)

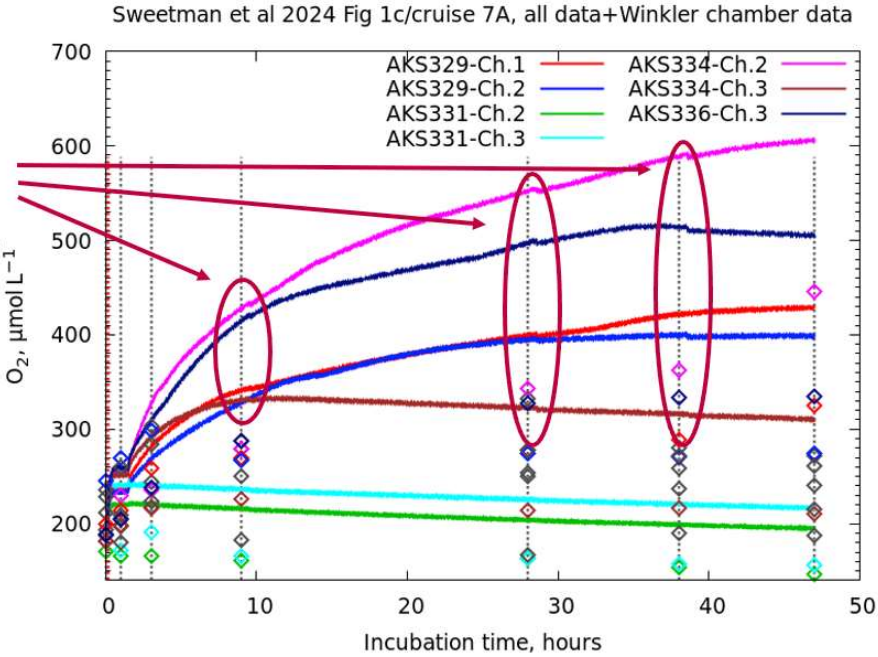
AKS329-Ch.3

AKS334-Ch.1

AKS336-Ch.2

AKS331-Ch.1

AKS336-Ch.1



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In situ incubations with the Gothenburg benthic chamber landers: Applications and quality control

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ABSTRACT

In situ incubations of sediment with overlying water provide valuable and consistent information about benthic fluxes and processes at the sediment-water interface. In this paper, we describe our experiences and a variety of applications from the last 14 years and 305 deployments with the Gothenburg benthic chamber lander systems. We give examples of how we use sensor measurements for chamber leakage control, in situ chamber volume determination, control of syringe sampling times, sediment resuspension and stirring quality. We present examples of incubation data for in situ measurements of benthic fluxes of oxygen, dissolved inorganic carbon, nutrients, metals and gases made with our chamber landers, as well as manipulative injection experiments to study nitrogen cycling (injections of ^{15}N nitrate), phosphate retention (injections of marl suspension) and targeted sediment resuspension. Our main goal is to demonstrate the possibilities that benthic chamber lander systems offer to measure solute fluxes and study processes at the sediment-water interface. Based on our experience, we recommend procedures to be used in order to obtain high quality data with benthic chamber landers.

Published result that gives an example of a faulty incubation. The lid of the lander chamber was closed prematurely which lead to trapping of an air bubble and a poor ventilation, creating similar artefacts as in Sweetman et al. (2024).

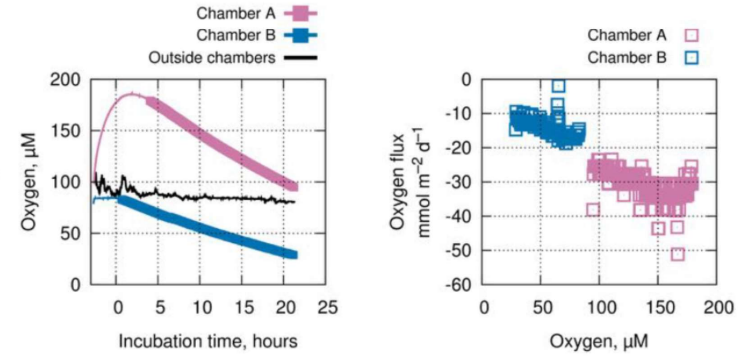


Fig. 6. Air bubble dissolution dynamics. The lid at chamber A (small lander) was prematurely closed so air bubbles were trapped and dissolved. Chamber B carried out a normal incubation (good ventilation, lid closed on time). Left panel: chamber oxygen concentration versus time. The thick line marks data selected for flux calculation. In chamber A, the initial oxygen concentration increase corresponds to the full dissolution of an air bubble of approximately 8 mL volume (normal pressure). Right panel: instantaneous oxygen flux value ($H \times dO_2/dt$, where H is chamber height calculated from chamber volume) versus oxygen concentration. Oxygen dynamics in both chambers appeared to follow the same flux versus oxygen concentration trend.

Un-published result from Gothenburg landers in which we tried to incubate only water to measure pelagic respiration of the bottom water by closing of the contact with the sediment with a plastic film. The lid of the lander chambers were left open for about 4 hours, giving similar O2 concentrations inside the chambers as outside. When lids were closed the oxygen increase in both chambers because the plastic material in the chambers leak out oxygen. In Sweetman et al. 2024 the incubation chambers were never even filled with bottom water.

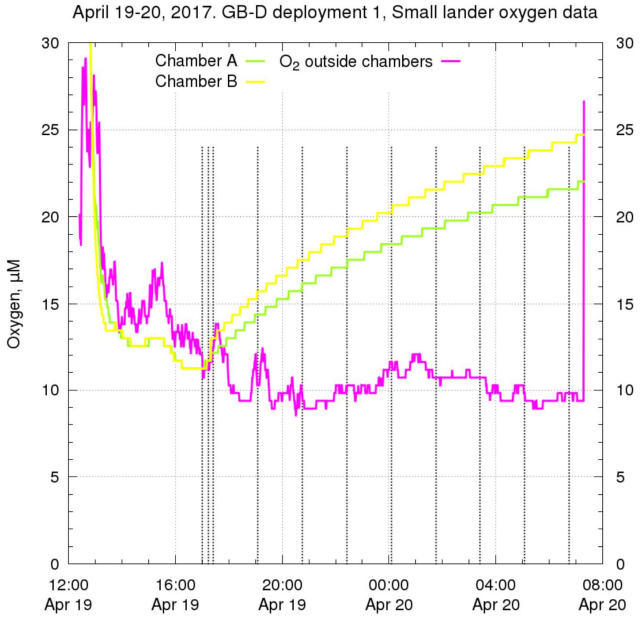
Station GB-D I, 130 m
Small Lander
Incubations
with dummy bottoms
4 h ventilation
14 h real incubation
MQ water injections

Lander started <4 h>
Lid close <15 min>
Injection <10 min>
9 samples
<every 100 min>

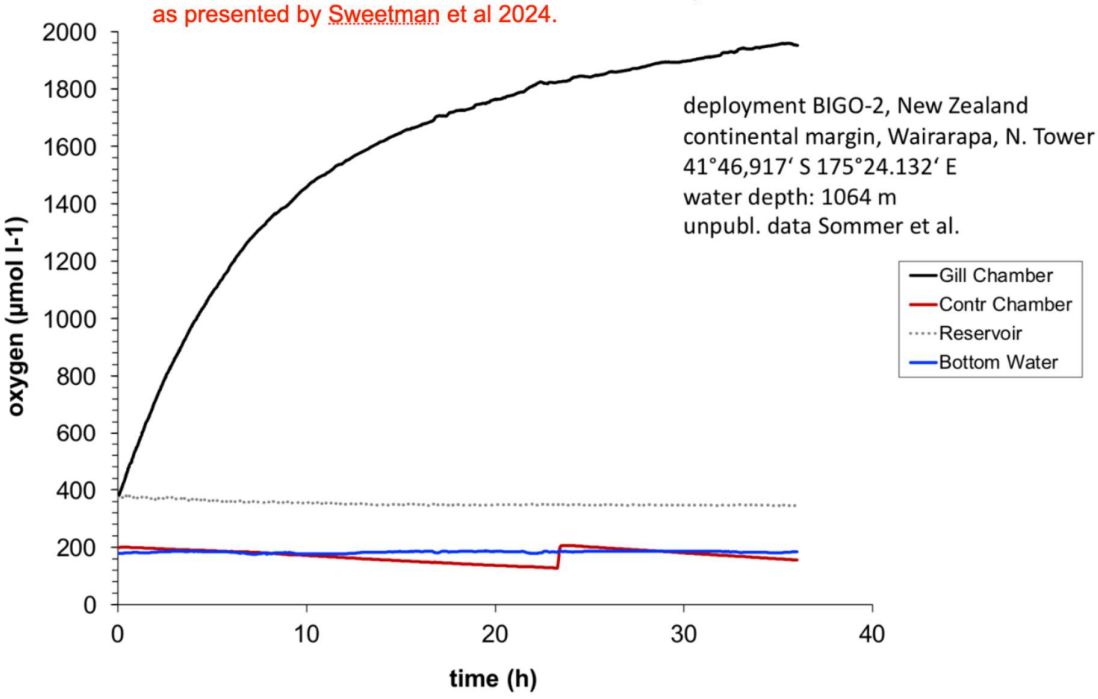
Incubation with dummy bottoms
Chamber height 35 cm

BW O2 was around 10 µM

Chamber A (green-yellow) – premature injection



Un-published result from one of the Geomar landers. During deployment the ventilation valve in the lid of one chamber was not opened. As a result an air bubble was included in the flux chamber which dissolved and showed a similar shape of the O2 concentration curve with time as presented by Sweetman et al 2024.

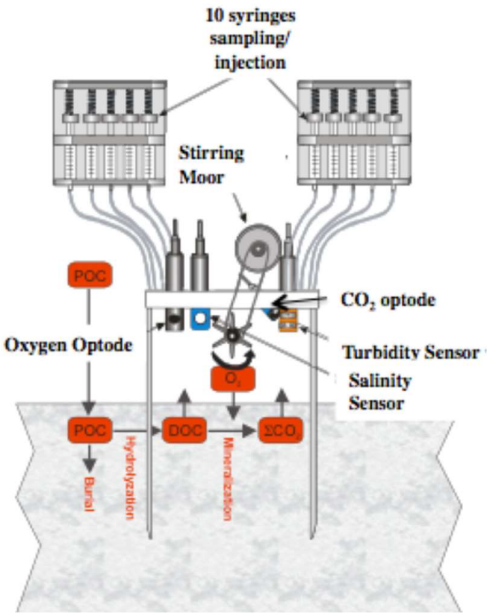


Supplementary Information

In situ incubations with autonomous benthic chamber landers - for non-experts. Sweetman et al (2024) into perspective

(Numbers in parenthesis refer to references in the list above)

Biological, chemical and physical processes at the seabed play an important role in the environment. Sediment-water incubation measurements with autonomous benthic landers, directly on the seabed, provide valuable information on what is leaking out and being taken up by the seafloor (e.g. 2,6,7,8, 9,12). Lander measurements on deep-sea plains (4000-5000 m) have often been focused on understanding the role such sediments play in the global carbon and nutrient cycles. For example, in a climate context, it is important to know how much carbon is stored in the deep-sea sediments that cover about 60% of the Earth's surface (8). In addition to studies of natural processes, the benthic landers also make it possible to carry out controlled experiments to simulate different types of events and study mechanisms of chemical processes in sediment (e.g. 6,11). For example, injecting ^{15}N labeled nitrate makes it possible to study in detail processes of N cycle (2). The landers have also been used in several projects that deliberately seek to study the effects of sediment stirred up by natural processes such as strong currents and waves, or by human activities such as trawling and dredging. To simulate natural processes, the trapped sediment was stirred up and the effects of this were studied (e.g. 1,6,11).



Conducting measurements and experiments directly at the seabed provides advantage of avoiding the sources of error created by taking sediment out of its natural environment to a laboratory at the surface. In addition to mechanical disturbance during sampling and recovery, other factors such as pressure, temperature, light, oxygen, animals and bacteria are affected (e.g. 4,5). However, in situ incubation experiments with landers, while providing this significant advantage, are neither easy nor simple. It is quite a complex experimental method and there is no standardized equipment. Use of the method does not automatically guarantee that results are good, because a lot of things can go wrong in situ, on the sea floor. The experiments are run fully autonomously, without any supervision, so use of sensors has become of extreme importance as there is no other way of making sure that incubation experiments were successful down there on the sea floor.

An in-situ incubation experiment involves enclosing a small part of the seabed with overlying water using a benthic chamber and to measure what is released or taken up by the sediments. Benthic chamber is usually a piece of a few decimeters wide cylindrical or rectangular “up-side down bucket” used to isolate sediment with bottom water. Benthic chambers are equipped with many special devices (stirring system (13), solute injection and sampling system, ventilation lids, mechanism to retain incubated sediment) and sensors necessary for performing the measurements and sampling during the incubation experiments. Benthic lander is thus a platform that is specially

designed for delivering benthic chambers to the seafloor and back, and for carrying out the incubation experiments (e.g. 6,7,8,12).

After preparing the landers on deck, they are sent to the bottom all by themselves by hanging weights under a buoyancy platform. After landing on the bottom, the incubation chambers are left open to ventilate for several hours. After venting, the chambers are gently pushed into the sediment to about half their height.

On most landers, incubation measurements start when the chamber lids are automatically closed by a minicomputer. During the incubation experiments, a stirring motor stirs the water inside the chamber (13), sensors continuously measure inside and outside the chambers and automatic syringes, controlled by a minicomputer, take time series of water samples from the enclosed water. Depending on how biologically/chemically active the sediments are, the experiments last between 6 hours, in organically rich sediments, to 48-72 hours in low-active sediments, such as those found in the deep sea.

Once the measurements are complete, the incubated sediment is often collected and the weights holding the lander to the bottom are released, using an acoustic remote control, so that it rises to the surface. After the lander is lifted on board, the sensor data is downloaded and checked to determine if incubations have gone according to plan. Water samples from the syringes are emptied and analyzed, either on board or after the expedition, for the parameters and processes being studied. If sediments are collected these can be analyzed for fauna, grain size, organic content and mineral composition.

It is crucial that the chambers are clean and contain 100% ambient bottom water at the beginning of incubations, otherwise the measurement results will be wrong. Especially when measuring oxygen in environments with low oxygen levels, such as in deeper parts of the Pacific Ocean, care must be taken to avoid artefacts. Plastic materials often used for the chambers absorbs oxygen and other gases when they are on deck, which will leak out if the equipment is not sufficiently ventilated at the bottom before incubations start (e.g. 10,15). Another problem is trapping of air bubbles inside the chamber that dissolve when you start your measurements and/or injecting water that contains more oxygen than the chamber water (6). Some landers (for example that of Sweetman et al., 2024) have valves to release air and water trapped on the way down. If these valves do not work, you get the wrong measurement results because chamber oxygen levels are affected by the bubbles and the incubated water is not 100% ambient bottom water. Air bubbles trapped inside the chamber water sampling system and tubing represent risk of oxygen contamination of incubation water samples used for Winkler analysis. Most common method of mitigating this, namely, pre-filling all the dead spaces with e.g. MilliQ water does not really eliminate the contamination as it is not possible to ideally mimic bottom water oxygen levels in the water used for the pre-filling.

Modern well working landers are equipped with water quality sensors, inside and outside the chambers, which both measure ongoing processes, such as oxygen consumption, and are used for quality control. The sensors used inside the incubation chambers measure salinity, temperature, pressure, turbidity, oxygen (14), pH and sometimes carbon dioxide (6). The salinity measurement is used to determine the volume of water being incubated by the chamber and to detect if any of the incubators are leaking. This is done by injecting the lander with a known volume of distilled water, which lowers the salinity slightly. Simple dilution calculations give the volume, stability of chamber water salinity after the injections gives indication that chambers are firmly inserted in sediment and do not leak. The pressure sensor provides feedback that all mechanical functions are working and are done at the right time. These include closing the lid, injecting and sampling water with syringes and taking the sediment. Measuring turbidity, i.e. how much sediment particles are in

the water, serves to check when chambers are inserted into sediment and whether sediment is inadvertently stirred up from the bottom during the lander deployment and the measurements (1,11). Sensors outside the chambers measure the same parameters as inside, this helps to make sure chamber water is actually 100% ambient bottom water at the beginning of incubations and that chambers are not leaking. In addition, acoustic Doppler current sensors are commonly used to measure currents at and above the bottom. These sensors also have built-in inclinometers (accelerometers) and compasses, which are useful for checking whether the lander was correctly deployed, standing upright, and check for lander tilt and movements during deployment and measurements.

Best practices have been developed and published (6) based on our multiyear experience and more than 1000 individual chamber incubations using sensors and our methods of data analysis using simultaneous sensor measurements inside and outside chambers. These have been used for routinely identifying and discarding bad incubation datasets, enabling constant improvements of the in-situ incubation method thus making possible the high-quality incubation experiments with an overall success rate over 90%. To make this possible, the following set of sensors can be recommended as absolute minimum: salinity, temperature, oxygen, and turbidity in each incubation chamber plus salinity, temperature and oxygen outside. Measurements of O2, T and Salinity inside and outside are critical as these enable control of leakage and make sure chambers are well ventilated and filled with 100% ambient bottom water at the beginning of incubations.

Benthic lander used by Sweetman et al was obviously equipped with only 2 (two) oxygen optodes per their 3 chambers and for measurements outside. Use of other sensors was not reported, so probably there wasn't any. Most of their deployments Sweetman et al made with no measurements of any parameter outside chambers, as there was only one single optode dataset reported as external per their 16 reported lander deployments. By using only two oxygen optodes in their chambers and choosing not to measure other parameters either inside or outside chambers, Sweetman et al practically disabled any possibility of water quality control needed to prevent and avoid contamination due to air bubbles, surface water trapped inside the chambers on the way to the sea floor, leakage from plastics of the chambers. This means Sweetman et al had no experimental measurements confirming their lander actually got rid of the oxygen artifacts using the valve system on the way down and over the following ventilation period and make sure that their chambers incubated ambient bottom water with ambient bottom water oxygen levels. After their 16 (sixteen) lander deployments Sweetman et al could not even report dissolved oxygen levels in bottom water for their study sites. Our analysis of their oxygen data shows that they possibly have 2 good quality incubations (AKS318-Ch.1, AKS321-Ch.1) out of 32 possible (2 optodes*16 deployments). Even this is not 100% sure as Sweetman et al could not measure temperature, salinity and oxygen both inside and outside those chambers to confirm the 2 incubations were not contaminated with oxygen. All their other oxygen incubation datasets showed signs of strong contamination. This indicates highest possible overall success rate of max 4% for their incubations (2 incubations with reasonable oxygen levels and respiration rates, and chamber volumes available out of 16 deployments*3 chambers). Making any conclusions or statements other than that Sweetman et al incubation oxygen data is of poor quality appears impossible and not acceptable.

Honest and serious critical assessment of such a low success rate of in situ oxygen uptake flux measurements would necessarily lead to the question whether the incubation chambers did function properly and to the need of critical evaluation of chamber design with regard to ensuring best possible quality of incubations as explained above: making sure chamber ventilation works as it should and providing experimental evidence indicating presence and magnitude of known

contamination factors affecting dissolved oxygen levels. When identified, the effect can be mitigated to diminish it as much as possible, for example, as needed for deployments on the seafloor with low oxygen levels and very low respiration rates. This is of course first and foremost the responsibility of Sweetman et al. who operated the lander and designed the incubation experiments. It is our opinion that experienced and serious researchers are expected to identify and deal with problems with their experimental equipment rather than trying to publish a large number of suspicious, almost obviously faulty experimental datasets in a journal high ranked like Nature Geoscience providing unlikely explanations on the basis of extremely weak experimental data.

Sweetman et al. actually appear to have a well- functioning lander platform. Their publication record suggests many successful deployments meaning the lander platform has been deployed and retrieved on many occasions at different seafloor depths. Successful deployment and recovery of a deep-sea lander is never a luck. It is a result of thorough lander design consideration and long preparations. Deep-sea environment is not forgiving to mistakes, a wrongly designed or operated lander risks failing and staying on the sea floor forever.

In this light it is a real pity to see such a low rate of successful measurements coming out of a good lander platform and miserable incubation data suggesting presence of well-known contamination factors affecting oxygen optode incubation records and Winkler samples. This cries for improvement, and the means of improving this is well within the reach. A lot of expertise is available, including published papers. There are colleague researchers operating similar incubation chamber systems. They were faced with these problems, identified causes and implemented modifications needed to reduce negative effects to a minimum. See for example the figure by Sommer et al.

From our side the recommendation to Sweetman et al. is to equip their lander with sensors. We consider measurements of temperature, salinity and oxygen inside each incubation chamber and outside chambers (in the ambient bottom water) as a recommended minimum for quality control and respiration measurements. Additional sensors (Turbidity, Pressure and others) will provide further means of deployment and incubation quality control (6). The use of modern water quality sensors has been an important key to the success of many (>90%) of our own incubation experiments.