

# Contributions to the discussion of novel detection of dark oxygen production at the abyssal seafloor

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## Arising from:

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## Statement:

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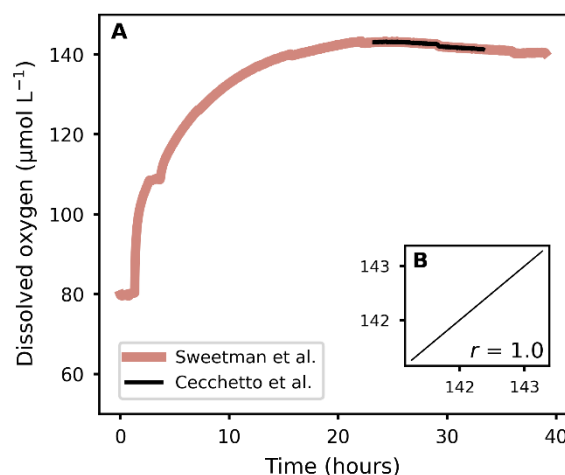
## Main Text

### Introduction

There is an overwhelming consensus between researchers based on a vast body of peer-reviewed literature that deep sea ecosystems constitute an oxygen sink<sup>1-3</sup>. Specific studies on abyssal seafloor regions that contain polymetallic nodules have also confirmed this result<sup>4-6</sup>. In contrast to this well-founded and longstanding paradigm, Sweetman et al.<sup>7</sup> claim to provide evidence to support a hypothesis that polymetallic nodules abiotically produce oxygen in the deep sea. In response, we critique the methodology that the authors outline and present previously non-disclosed data and metadata directly related to the experiments they present. Furthermore, we highlight previously published data that Sweetman et al. include without citation and critical metadata that they omit. The data and metadata that Sweetman et al. omit significantly alters how one interprets the results and directly undermines their claims. Given these revelations, we suggest that the hypothesis that polymetallic nodules produce oxygen can be wholly rejected.

### Publishing duplicate data and omitting key metadata

Sweetman et al. report that they reevaluated in situ O<sub>2</sub> optode data collected from 36-hour benthic chamber experiments in the abyssal eastern and western Clarion-Clipperton Zone (CCZ) in the Pacific Ocean (which they present as Extended Data Figs. 1 and 3). The authors conclude that these data show dark O<sub>2</sub> production (DOP) from multiple locations across the CCZ and claim that these data support their overall conclusions that DOP can be attributed to polymetallic nodules. However, they neglect to cite a previous paper on which Sweetman served as a coauthor—Cecchetto et al.<sup>8</sup>—that presents contradictory results from the same experiments. To wit, both Sweetman et al. (2024) and Cecchetto et al. (2023) report oxygen optode data from one specific chamber experiment (i.e., AKS261-Ch.3) with contradictory conclusions: the former claims O<sub>2</sub> production, while the latter claims O<sub>2</sub> consumption (see Fig. 1). Similarly, even when replicate chamber experiments come from the same benthic lander deployment (as with deployment AKS254), Sweetman et al. report AKS254-Ch.3 as showing production yet omit AKS254-Ch.2 that Cecchetto et al. report as showing oxygen consumption. Finally, and perhaps most importantly, Cecchetto et al. state that: “No nodules were collected [from the chambers] as they were either not present or in the form of manganese oxide granules”. This fact directly contradicts Sweetman et al.’s finding that DOP can be attributed to the presence of nodules. Notwithstanding these issues, we identified at least three published studies that Sweetman et al. do not cite that report in situ chamber incubations with nodules present, and all measured oxygen consumption and not production<sup>4-6</sup>.

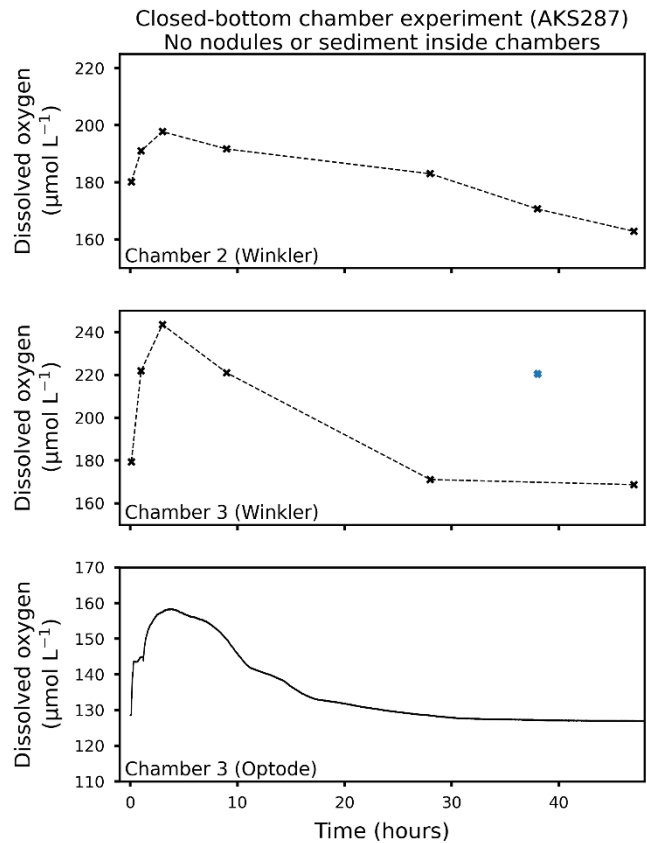


**Fig. 1:** Dissolved oxygen optode data from the chamber lander deployment AKS261-Ch3 as reported in Sweetman et al. (2024) and Cecchetto et al. (2023). Cecchetto et al. report no nodules present in chamber incubations. A) Dissolved oxygen concentration during the chamber incubation as presented in Sweetman et al (2024); the optode data reported in Cecchetto

et al. includes a 23.3-hour time offset. B) The one-to-one relationship between the oxygen optode data reported in Sweetman et al. and the oxygen optode data reported in Cecchetto et al. with the 23.3-hour offset applied, which confirm the oxygen optode measurements are the exact same data. Oxygen optode data from Sweetman et al. comes from <https://www.nature.com/articles/s41561-024-01480-8#MOESM5>. Oxygen optode data from Cecchetto et al. comes from <https://zenodo.org/records/6959870>.

*Closed-bottom chamber experiment: oxygen production without nodules*

Sweetman et al. also fail to report or discuss that they conducted a benthic chamber lander deployment (AKS287) as an in situ closed-bottom experiment during cruise 5D. In this experiment, the three chambers were maintained above the seafloor without penetrating the sediment. The chamber bottom lids were closed at the start of the experiment, and the incubation was conducted with no nodules or sediment present in the three chambers (see Supplementary Note 1). Importantly, during the incubation period, the oxygen concentration initially increased in two chambers (see Figure 2). The fact that the chambers contained no nodules or sediment contradicts Sweetman et al.'s (2024) main finding that one can attribute oxygen production to the presence of nodules. Furthermore, the initial patterns of oxygen-increase observed during the closed-bottom chamber experiment closely resemble the chamber experiments that Sweetman et al. (2024) report (see Supplementary Figure 1) and, therefore, strongly suggest that oxygen production is an experimental artifact in all their benthic chamber experiments and not attributable to nodules.



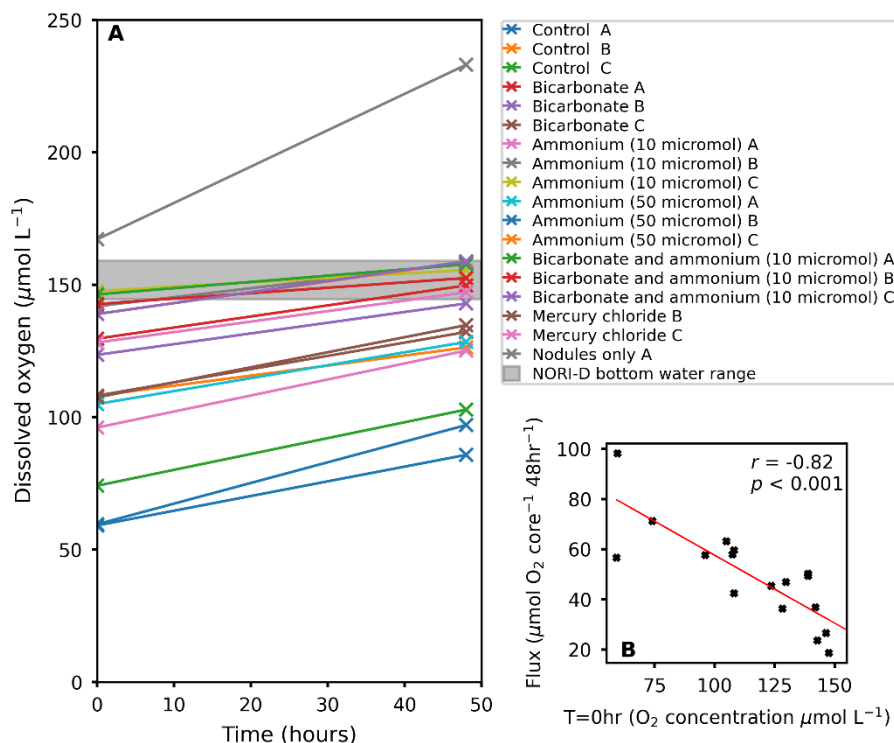
**Figure 2.** Dissolved oxygen concentration in chambers 2 and 3 during the closed-bottom chambers experiment (AKS287) during cruise 5D. Dissolved oxygen was determined using the Winkler titration method; the values represent the mean of duplicate lab measurements. No Winkler oxygen concentration or optode data was available for chamber 1, and no optode data was available for chamber 2. The blue cross indicates an apparent outlier measurement in chamber 3. Optode measurements for chamber 3 were calibrated using pre-cruise saturation calibrations following the procedures outlined in Bittig et al.<sup>9</sup>. Note the varied y-axis between subplots.

### Inaccurate oxygen measurements

Sweetman et al. do not accurately measure the in situ oxygen concentration in NORI-D. Sweetman et al.'s initial in situ concentrations measured inside the chambers vary considerably and consistently exceed the in situ range we measured over multiple years at multiple locations with multiple sensors (see Supplementary Figure 2). An optode outside the chambers also measured oxygen concentrations greater than the in situ range (see Supplementary Figure 2). Sweetman et al. do not address this fundamental discrepancy in the article, which the figures' style (in particular, the thick lines that they use to indicate oxygen concentrations) further masks (see Fig.1 in Sweetman et al.).

### Contaminated ex situ core incubations

Sweetman et al. report ex situ sediment core incubations conducted during cruise 5D as evidence for nodules as an oxygen source (Extended Data Fig. 4 in Sweetman et al.). They state that "opportunistic ex situ experiments were undertaken during the 5D cruise using sediment cores retrieved by a multicorer" and indicate a single deployment on the map. Considering all the 17 sediment core samples were collected within a 1.9 x 1.9 m footprint simultaneously, the initial oxygen concentrations that Sweetman et al. measured vary considerably ( $59.54\text{--}146.31\ \mu\text{mol L}^{-1}$ ), and 15 of the 17 samples contained oxygen concentrations below the in situ bottom water range in NORI-D (see Figure 3A). During the sediment core incubations, the final concentrations never exceeded the maximum in situ range recorded in NORI-D (see Figure 3A). The low initial oxygen concentrations that Sweetman et al. report can in part be attributed to the intrusion of water into the core tube from the oxygen minimum zone in NORI-D during the multicore recovery (see Supplementary Note 2). Additionally, no records in the cruise 5D offshore logs of the nodule-only incubation exist, and Sweetman et al. provide no information about the experimental procedures for this incubation. Thus, we question whether it was conducted as part of the same experiment.



**Figure 3.** Ex situ core experiments reported in Sweetman et al. (2024): A) core top water dissolved oxygen concentration at T0 and T48 measured using the Winkler technique (the shaded grey region represents the bottom water dissolved oxygen concentration in NORI-D ( $144.5\text{--}159.0\ \mu\text{mol L}^{-1}$ )); B) the inverse relationship between the core top water oxygen concentration at T0 and the total flux of oxygen measured over the 48-hour incubation period for samples that contained both sediment and nodules (the red line indicates the line of best fit derived from a linear regression). Pearson correlation

coefficient = -0.82,  $R^2 = 0.68$  and  $p < 0.001$ . Data comes from <https://www.nature.com/articles/s41561-024-01480-8#MOESM5>.

The methods that Sweetman et al. used for the ex situ core incubations are not standard practice, and we know of no examples of the same procedures they used in the published literature. Specifically, Sweetman et al. did not follow preincubation protocols that ensure core samples reach a steady state before the incubation starts (see <sup>10-15</sup> for standard preincubation procedures). Consequently, several reasons exist to attribute the DOP to experimental artifacts that Sweetman et al. (2024) do not address (see Supplementary Note 2). For example, we found a significant inverse relationship between the initial (T0) oxygen concentrations and total flux of oxygen during the 48-hour incubations (see Figure 3B), which strongly indicates a diffusion-mediated process for increasing oxygen saturation (see Supplementary Note 2). The figure that Sweetman et al. use (see Extended Data Fig. 4 in Sweetman et al.) to present data from the ex situ core incubations masks the considerable variability in T0 oxygen concentrations and the fact that, at T48, the oxygen concentrations never exceeded bottom water oxygen concentration for NORI-D. Furthermore, Sweetman et al. (2024) do not present the inverse relationship between the initial oxygen concentration of core top water and the total flux of oxygen despite it evidencing a simpler explanation for oxygen increase during incubation (see Figure 3B).

The data that Sweetman et al. collected from the ex situ core incubations clearly indicate that the DOP resulted from experimental artefacts. Standard experimental procedures could have informed the authors as to the oxygen source. For example, had they simply conducted a control experiment with cores from the same multicore deployment but with nodules removed, they could have tested their leading DOP hypothesis and provided evidence to disregard experimental artifacts. This control would have resembled the closed-bottom chamber experiment that showed oxygen increased in the benthic chamber lander incubations in the absence of nodules or sediment (see Figure 2).

### *Summary*

To summarise, Sweetman et al. rely on two main lines of evidence to demonstrate the production of dark oxygen: 1) oxygen production from nodules present in the benthic chamber landers and 2) oxygen production during ex situ core incubations. However, the data that they omit from their study invalidate both lines of evidence. They omit and selectively report pertinent metadata and data from a previously published study and additional experiments conducted as part of this study that show oxygen concentrations rose in chambers that contained no nodules. Furthermore, they omit contemporaneous knowledge of bottom water oxygen concentrations in NORI-D and that T0 oxygen concentrations in ex situ core incubations did not represent this NORI-D bottom water. With these lines of evidence invalidated, the hypothesis that nodules can produce oxygen on the abyssal seafloor is completely unsupported.

## Supplementary Notes

### 1. *Closed-bottom chamber experiment (AKS287)*

Sweetman et al. performed a closed-bottom chamber experiment between 31 May and 2 June 2021 during cruise 5D in NORI-D in the Clarion-Clipperton Zone. To perform this control experiment, they configured the chamber lander system differently compared to deployments used to measure seafloor oxygen fluxes (see Supplementary Tables 1 and 2). First, the weight stacks were adjusted at the base of the lander frame to ensure the chambers were raised above the seafloor to avoid penetration into the sediment (see Supplementary Table 1 and Supplementary Figure 3). Second, the chamber bottom lids were programmed to close as the first step of the procedure at the seafloor and the chambers were not pushed into the sediment (see Supplementary Table 2). Combined, these factors created a mesocosm of bottom water inside the chamber without nodules or sediment. Chamber lander deployment and recovery records and photos confirm the closed-bottom chamber experiment was successful; only seawater was incubated inside the chambers, the chamber doors were closed when the lander was recovered (Supplementary Figure 4), and the water volume inside the chambers was estimated from the chamber dimensions (see Supplementary Table 1). Oxygen optode data was recorded in chamber 3, and Winkler samples were retrieved from chambers 2 and 3; therefore, the oxygen concentration was measured in two of the three chambers. The optode in chamber 1 recorded data; however, data-submission notes state not to use oxygen data from this specific optode because the temperature sensor did not work.

Sweetman et al. estimated the volume of water inside the chambers from the chamber dimensions (see Supplementary Table 2), which indicates that water drained from the chambers during recovery when winched onto the vessel. The lander recovery photos further evidence this water draining (see Supplementary Figure 5). By design, the chamber-bottom lids retain sediment during retrieval and do not completely seal out water. The oxygen increases observed in AKS287 Ch.2 and Ch.3 fall towards the lower end of the DOP rates that Sweetman et al. report because the oxygen source was diluted in a greater water volume because the chambers did not penetrate the sediment. The closed-chamber experiments contained 12.1 L of water compared to ~2–4 L for the chamber experiments with sediment, water, and nodules. After the initial increases in oxygen concentration inside the chambers during the closed-bottom chamber experiments (see Figure 2), the oxygen concentration began to decrease more than the seafloor incubations that contained sediment, water, and nodules because the chamber doors would have leaked slightly and, thus, allowed the oxygen concentration to slowly return to original in situ concentrations.

### 2. *Intrusion of oxygen minimum zone water into multicore samples*

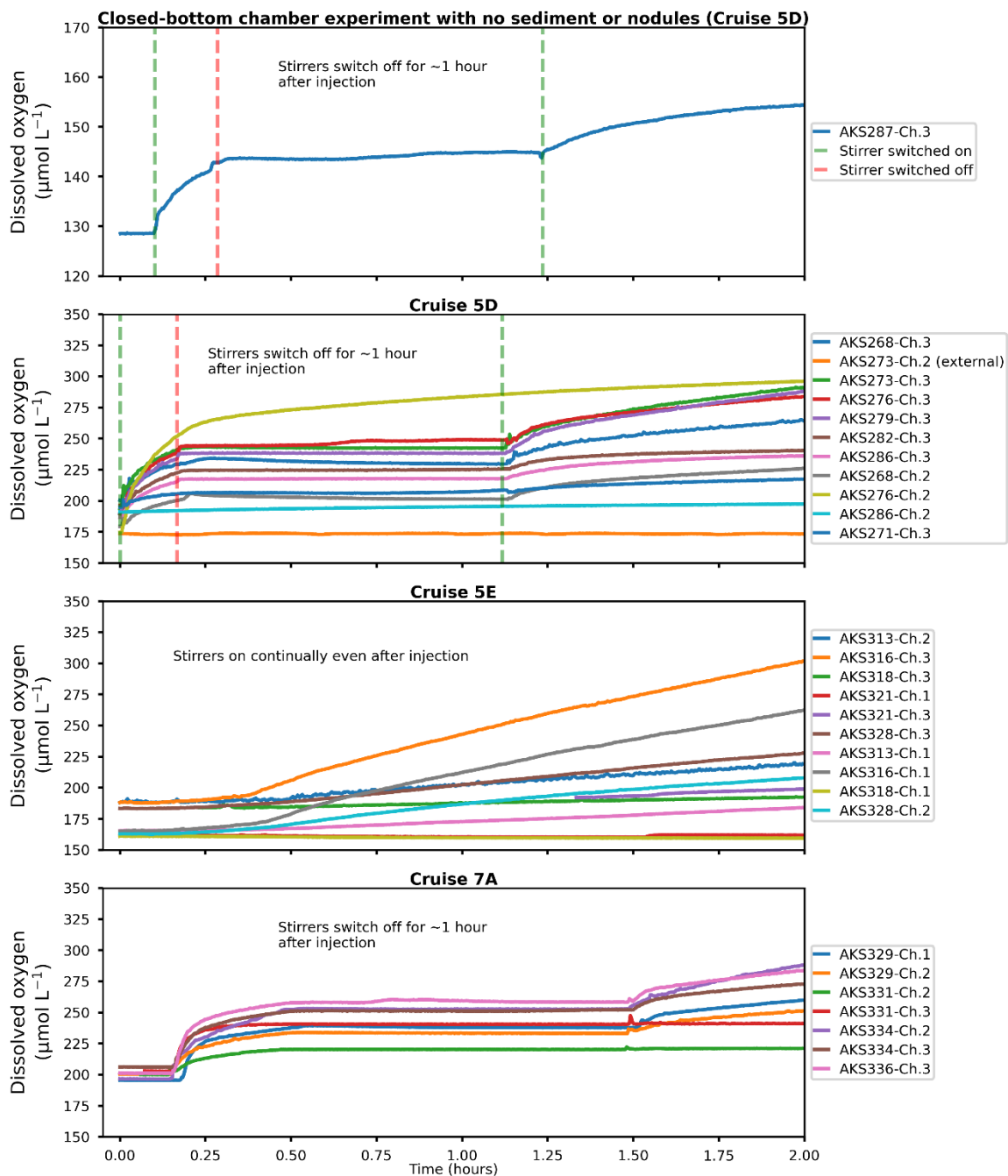
Sweetman et al. performed their ex situ core incubation experiment with 17 core samples collected simultaneously in NORI-D on 26 May 2021. The narrow range in bottom water oxygen concentrations cannot account for the range in oxygen values observed at the start of the core incubation experiments. When one compares the core top clarity and initial (T0) oxygen concentrations that Sweetman et al. measured, one can clearly see a difference in water clarity that correlates with the measured oxygen concentrations. The three core samples with the lowest initial oxygen concentrations (12, 14, and 20) had the clearest core top water (see Supplementary Figure 6). When retrieved from the seafloor to the vessel, core samples pass through the oxygen minimum zone (OMZ) in NORI-D, which extends approximately 80 to 800 m below the ocean surface (see Supplementary Figure 7). Along with extremely low oxygen concentrations, OMZ water is relatively warmer compared to bottom water (see Supplementary Figure 7). OMZ water likely contaminated the core samples during retrieval. We have recognised this artefact on NORI campaigns since cruise 5D, and we now evaluate core top water clarity and temperature as part of the initial core-assessment procedures before allocating cores for laboratory analysis.

Core top water undersaturated with oxygen relative to in situ conditions makes ex situ core incubations especially vulnerable to artefacts. Notably, a significant inverse relationship between the core top water T0 oxygen concentrations and the total flux of oxygen calculated over 48 hours (see Figure 3B) strongly implies that an oxygen concentration gradient drives a diffusion-mediated process that causes the oxygen concentration to increase (i.e., according to Fick's first law of diffusion, the diffusive flux is directly proportional to the concentration gradient). This significant inverse relationship evidences an experimental artefact. Notably, because the in situ oxygen concentration exceeded the oxygen concentration in the core top water (see Figure 3A), the sediment porewater would become a transient oxygen source for the core top water. Hence, the flux is greater when the concentration gradient is greater (i.e., when the core top water contains less oxygen, more oxygen diffuses from the sediment porewaters into the core top water). Similar to oxygen that diffused from sediment porewaters into the core top water, microbubbles that initially formed from oxygen out gassing during sample recovery would be an additional oxygen source during the incubations. Warming and depressurisation cause oxygen to out gas from deep sea samples when recovered to the surface as Sweetman et al. note in reference to the chamber lander Winkler samples. A proportion of these microbubbles could feasibly not have escaped the sample and remained trapped on the surface of nodules, sediment, bungs and core tubes before dissolving back into the core top water as the samples cooled during the incubation. While Sweetman et al. conducted control experiments to assess for other sources of oxygen during these incubations, the controls did not represent the experimental conditions. Specifically, shipboard controls were run with Milli-Q water rather than deep-sea bottom water and, therefore, the effect of depressurisation, warming and subsequent cooling, and sediment porewaters remained unaccounted for.

**The authors declare the following competing interests.**

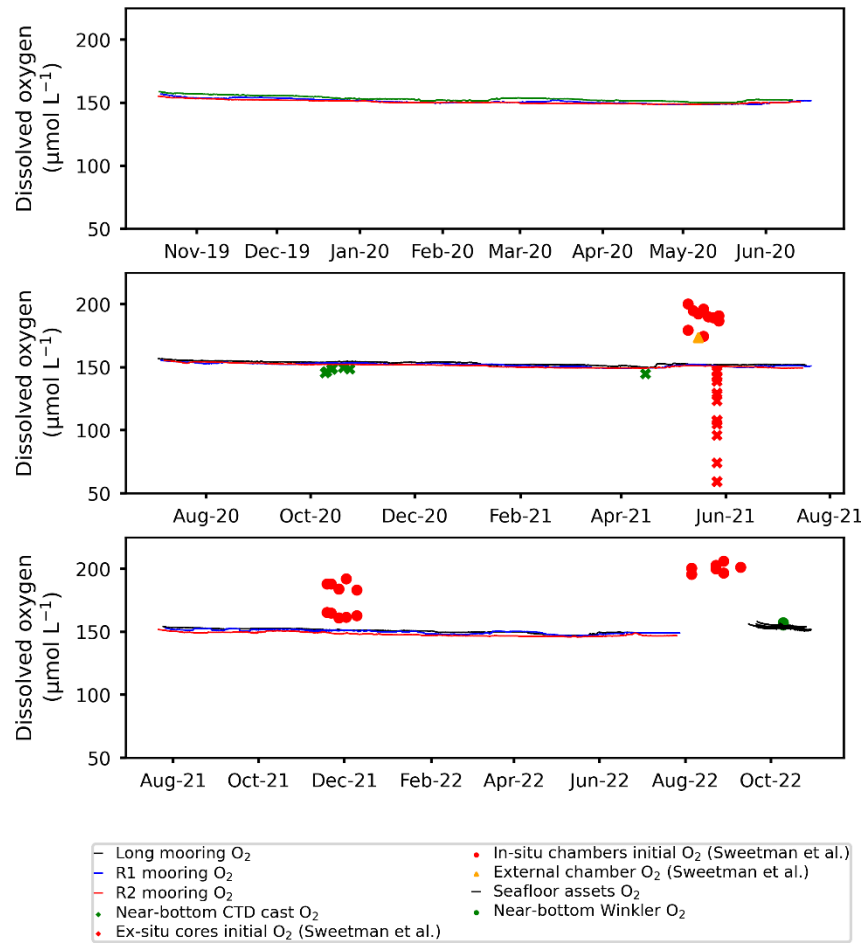
All authors are employed by The Metals Company. The Metals Company partly funded the study reported in Sweetman et al. (2024) through its subsidiary Nauru Ocean Resources Inc. (NORI). NORI holds exploration rights to the NORI-D contract area in the CCZ and is regulated by the International Seabed Authority and sponsored by the Government of Nauru.

## Supplementary Figures

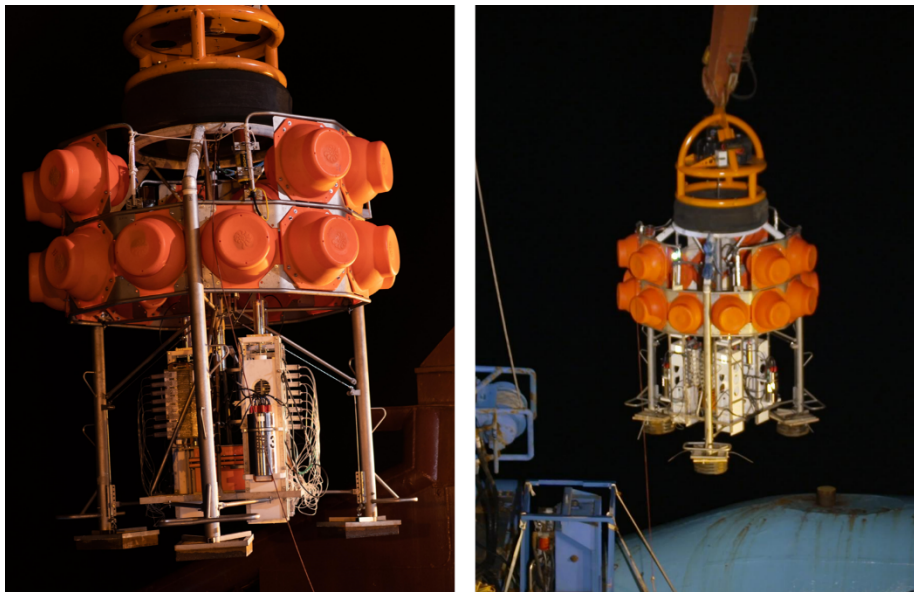


**Supplementary Fig. 1:** DOP's dependence on the chamber stirrer during the closed-bottom chamber experiment and chamber experiments reported in Sweetman et al. (2024). Heriot-Watt University supplied oxygen optode data from AKS287 chamber 3 to The Metals Company; all other optode data comes from <https://www.nature.com/articles/s41561-024-01480-8#MOESM5>. Oxygen optode data for AKS287 was calibrated using the using pre-cruise saturation calibrations, and procedure outlined in Bittig et al.<sup>7</sup>. The stirrer on and off times for panel "Cruise 5D" are specific for AKS276-Ch.3 and approximate for other chambers; for panel "Cruise 7A", no stirrer data was available. Note that the y-axis varies between subplots.





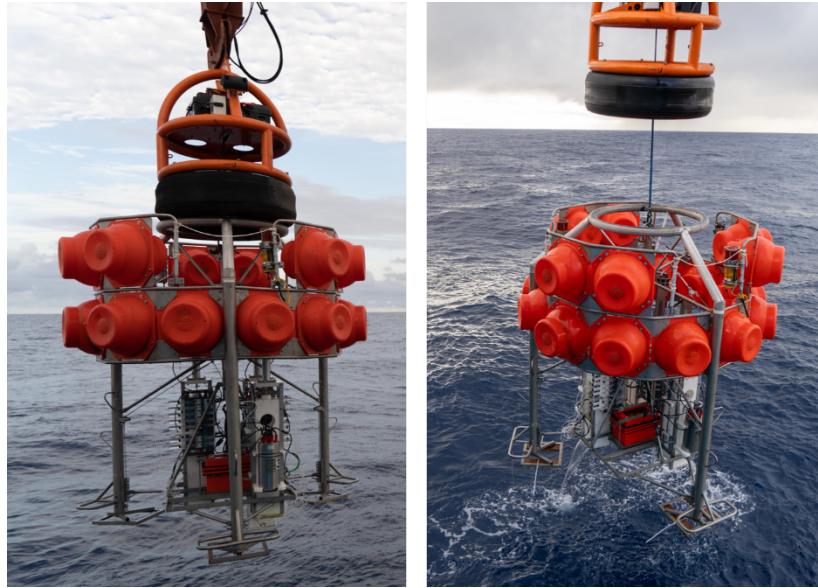
**Supplementary Fig. 2.** Comparison of bottom water oxygen measurements in NORI-D. Oxygen measurements from NORI cruises includes oxygen optode measurements from three oceanographic moorings (red, blue, and green lines), nine seafloor assets (black lines), seven near bottom CTD casts (green crosses), and three near-bottom Winkler measurements measured on water samples collected 50 m, 17 m, and 1 m above the seafloor (green circles). Mooring, seafloor asset, and CTD locations appear in Supplementary Figure 8. The red circles, orange triangle, and red crosses represent initial oxygen concentrations from the chamber incubations, external chamber optode, and ex situ core incubations, respectively, that Sweetman et al. report.



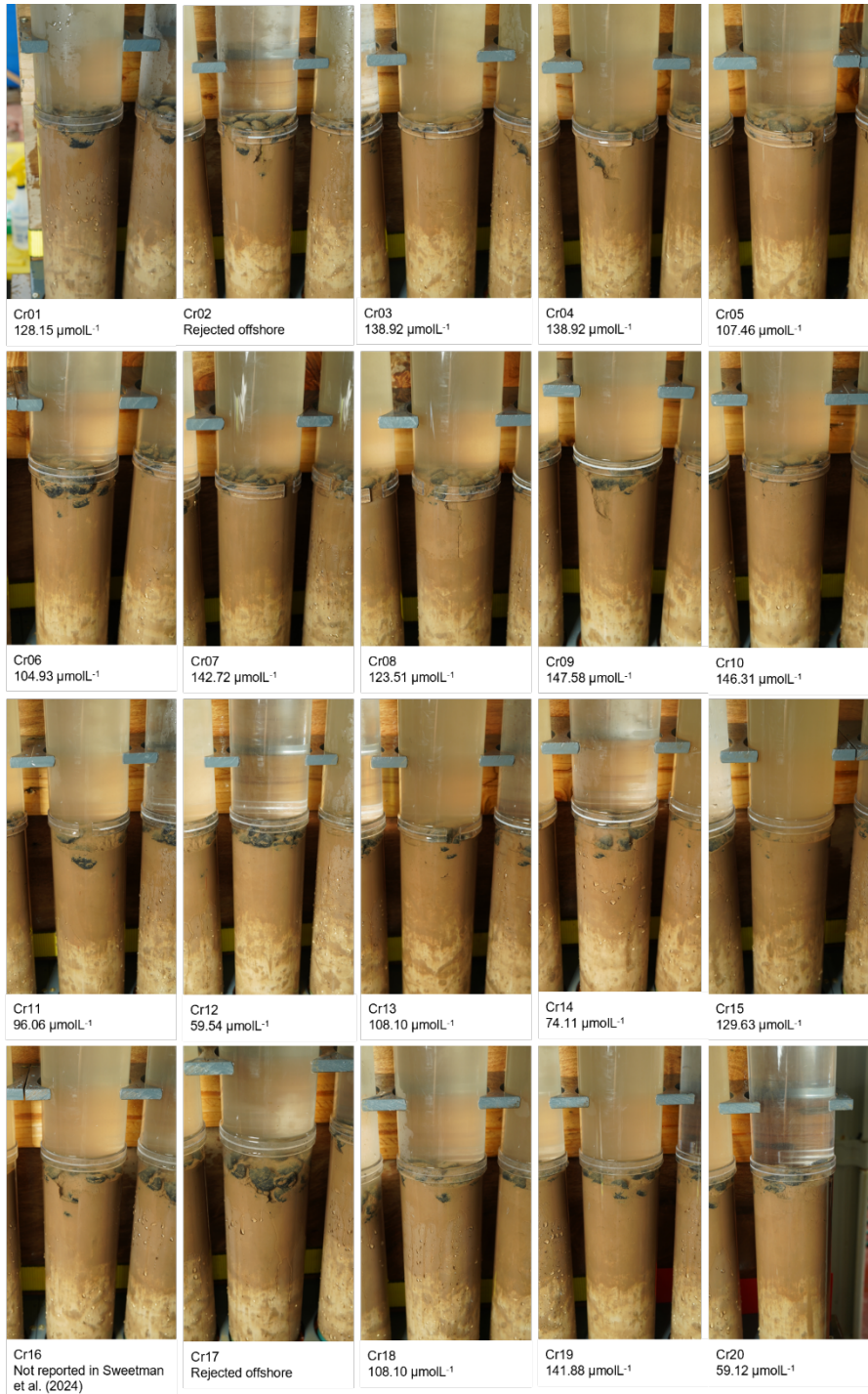
**Supplementary Fig 3:** Benthic chamber lander deployment images during cruise 5D. Left image: a standard seafloor oxygen flux deployment configuration (AKS286). Right image: closed-bottom chamber experiment deployment (AKS287)—note the raised weight stacks to ensure chambers do not penetrate the sediment. Right image is a still obtained from a video recording; both original images have been cropped.



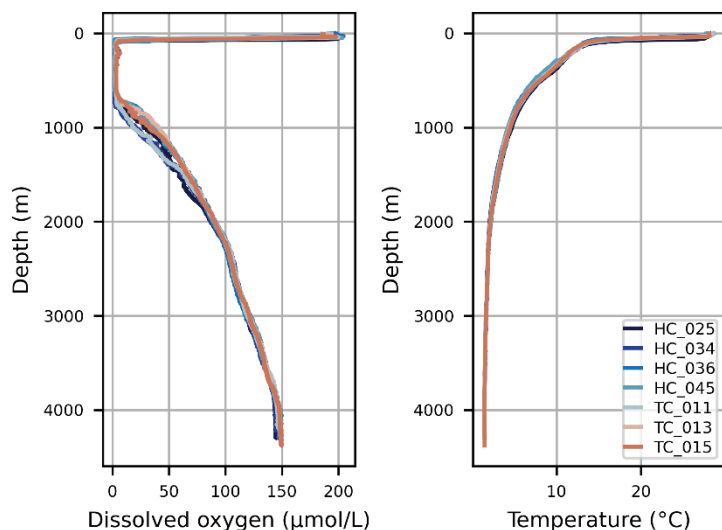
**Supplementary Fig 4:** Image of the benthic chamber lander recovery after the closed-bottom chamber experiment (AKS287). Note that all three chamber bottom lids (doors) are closed. The original image has been cropped.



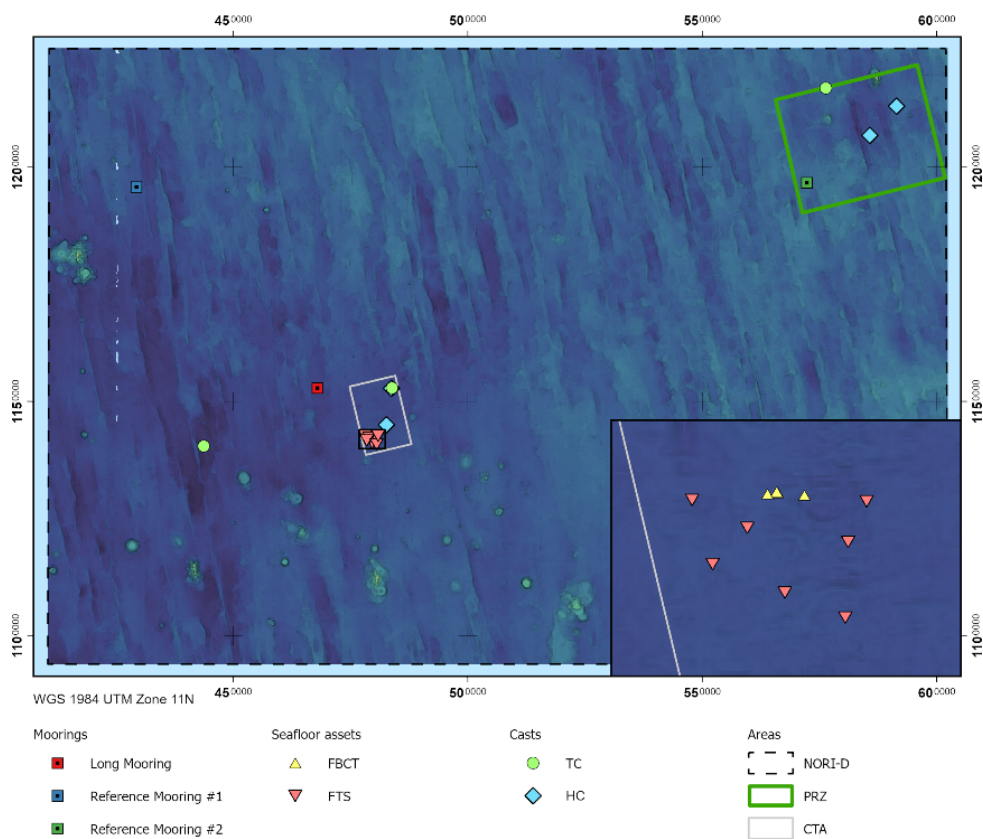
**Supplementary Fig 5:** Benthic chamber lander recovery images during cruise 5D. Left image: a standard seafloor oxygen flux recovery (AKS282). Right image: closed-bottom chamber experiment recovery (AKS287)—note water leaking from chambers. Both original images have been cropped.



**Supplementary Fig. 6:** Images of multicore samples used in Sweetman et al. (2024) for ex situ core incubations. Initial T0 oxygen concentrations appear beneath each image. Oxygen concentration data comes from <https://www.nature.com/articles/s41561-024-01480-8#MOESM5>.



**Supplementary Fig. 7:** Dissolved oxygen and temperature profiles in NORI-D measured using CTD rosette casts during cruises 5B and 5C. The figure shows data from casts that achieved a maximum depth of within 20 m of the seafloor.



**Supplementary Fig. 8:** Locations of CTD casts and oxygen optodes on moorings and seafloor assets used to determine the in situ bottom water oxygen concentration in NORI-D. The oxygen optodes on the three oceanographic moorings were situated within 3 m of the seafloor. The optodes on the seafloor assets were within 1 m of the seafloor. (Legend: PRZ: Preservation Reference Zone, CTA: collector test area, HC: hydrographic CTD cast, TC: trace metal CTD cast, FBCT: fixed-bottom current and turbidity station, FTS: fixed turbidity station). Black rectangle indicates inset map area. Image background shows seafloor bathymetry.

**Supplementary Table 1:** Meta data recorded for the closed-bottom chamber experiment (AKS287) that Sweetman et al. conducted during cruise 5D. Positional data, water depths, and deployment recovery times come from The Metals Company’s offshore logs. Heriot-Watt University supplied preparation and recovery comments related to AKS287 to The Metals Company.

Easting (EPSG:32611 UTM zone 11)	478210.49
Northing (EPSG:32611 UTM zone 11)	1140725.73
Water depth (m)	4276.53
Time off deck (UTC)	2021-05-31 04:20:00
Time on deck (UTC)	2021-06-02 15:25:00
Preparation comments	“Injecting cold, filtered SW [sea water] in all 3 chambers (Bromide forgotten). Weight stacks raising lander to point where adequate sediment penetration depth not possible (no algae injection needed). All chambers closing with no sediment penetration: TUNA action 'Push chambers down' closes the chamber door because we switched the drive motor cables. Cables clear of chamber side holes. 96-98 kg weight on each leg (x3 in total).”
Recovery comments	“No sediment samples. Injector and syringe samplers didn’t work [for chamber 1 only]. Optode 9 did not record data [chamber 2]. Chamber doors did close because they were still closed when aboard the ship. Water volume assumed from chamber dimensions 22 x 22 x 25 cm.

**Supplementary Table 2:** Comparison of the chamber lander order of events at the seafloor during the closed-bottom chamber experiment (AKS287) and a typical deployment (AKS279) conducted on cruise 5D. Sweetman et al. used the manufacturers software “TUNA” to programme the chamber landers.

AKS287 chamber 2 and 3 (closed-bottom chamber experiment with no nodules or sediment)		AKS279 chamber 3 (chamber experiment with sediment penetration)	
Event	Time (UTC)	Event	Time (UTC)
Close chamber lid	2021-05-31 10:00:00	Chambers pushed down (takes 1 hour and 20 minutes)	2021-05-22 10:00:00
Stirrers on	2021-05-31 11:21:00	Stirrers on	2021-05-22 11:21:00
Syringe 1: Control	2021-05-31 11:21:00	Syringe 1: Control	2021-05-22 11:21:00
Optode power on*	2021-05-31 11:22:00	Optode power on*	2021-05-22 11:22:00
Injector: Incubation start	2021-05-31 11:31:00	Injector: Incubation start	2021-05-22 11:31:00
Stirrer stop, wait 1 h for algae to sink**	2021-05-31 11:32:00	Stirrer stop, wait 1 h for algae to sink	2021-05-22 11:32:00
Start stirrers after injection	2021-05-31 12:29:00	Start stirrers after injection	2021-05-22 12:29:00
Syringe 2	2021-05-31 12:30:00	Syringe 2	2021-05-22 12:30:00
Syringe 3	2021-05-31 14:30:00	Syringe 3	2021-05-22 14:30:00
Syringe 4	2021-05-31 20:30:00	Syringe 4	2021-05-22 20:30:00
Syringe 5	2021-06-01 15:20:00	Syringe 5	2021-05-23 15:20:00
Syringe 6	2021-06-01 01:20:00	Syringe 6	2021-05-24 01:20:00
Syringe 7	2021-06-02 10:20:00	Syringe 7	2021-05-24 10:20:00
Optode power off*	2021-06-02 11:17:00	Chambers closing (takes 1 hour)	2021-05-24 10:21:00
Push chamber down minimally	2021-06-07 10:40:00	Optode power off*	2021-05-24 11:17:00
Stirrers stop	2021-06-07 11:18:00	Stirrers stop	2021-05-24 11:18:00
Chamber comes up	2021-06-07 11:20:00	Chamber comes up	2021-05-24 11:20:00

\*Raw optode data shows that the optodes recorded data throughout the deployments and were not turned on or off. We copy the TUNA programme table verbatim from the metadata that Heriot-Watt University supplied.

\*\*No algae was injected into chambers during the closed-bottom chamber incubations instead, cold filtered seawater was injected.

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