

23/07/24

### QUOTE

“My co-authors and I stand fully behind the findings in this research paper, which has been published by a highly respected academic journal following a stringent and lengthy peer review process.

“We were the worst critics of this paper for a long time. For eight years I discarded the data showing oxygen production, thinking my sensors were faulty. Once we realised something may be going on, we tried to disprove it, but in the end we simply couldn't.

“We would welcome future peer-reviewed studies that further investigate this phenomenon.

“Following the publication of this paper, I have been approached by other researchers with similar data-sets also showing evidence of dark oxygen production that they discarded thinking equipment was faulty.”

### FULL RESPONSE

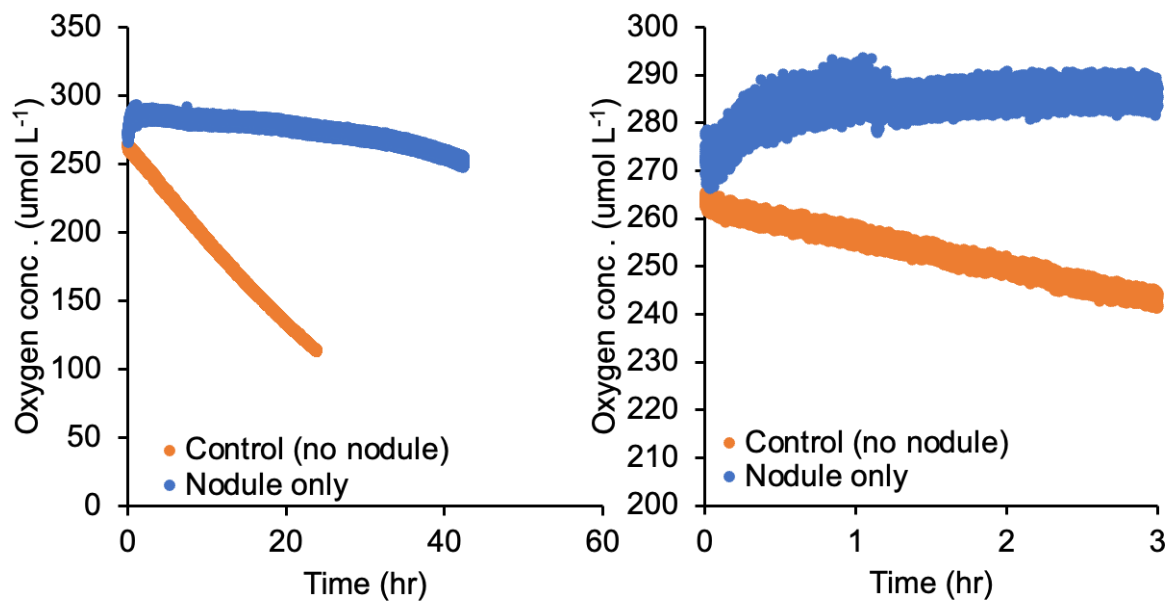
Re. the ex-situ data, we did report in the paper that the starting concentrations were low. For example, in the methods we wrote “*Back in the home laboratory, three of the original core tubes were filled with 40C, 0.2- $\mu$ m-filtered artificial seawater (salinity 35) and sparged with N<sub>2</sub> for 8 min through a filtered pipette tip to achieve an initial dissolved O<sub>2</sub> concentration of ~100  $\mu$ mol l<sup>-1</sup> (**for example, the approximate starting O<sub>2</sub> concentrations for the shipboard experiments**)”.*

We also ran a set of experiments where we tested if O<sub>2</sub> could enter from the outside of the cores if we started at a low O<sub>2</sub> concentration like the concentrations we found in the cores. We found minimal O<sub>2</sub> intrusion from outside. While we did not leave the cores for 24hr before we started the experiments, they were left for a number of hours to settle. There is actually no “golden rule” to leave the cores for 24hr before starting to process cores. Sometimes they are left for 24hrs, sometimes 12 hrs, and sometimes 6hrs. It all depends on the amount of time available to carry out the work and the manpower on board.

If you calculate the amount of oxygen that was produced over 48 hrs in our ex-situ cores, you arrive at a concentration of 50 micromol (measure of concentration) of O<sub>2</sub> being produced per core on average. The minimum and maximum increase we saw was between 20-100 micromol O<sub>2</sub> per core. So, TMC is hypothesising that on average 50 micromol O<sub>2</sub> leaked out of the sediment and into the core. Here is a back of the envelope calculation, and I am assuming an O<sub>2</sub> concentration at the sediment water interface of 150 micromol per litre, which we measured in-situ: To get an increase of 50 micromol O<sub>2</sub>, would mean that 4.4cm of sediment in a 70cm<sup>2</sup> core would need to be completely full of water (i.e., not composed of any sediment, which isn't the case) and each ml of water in that 4.4cm x 70cm<sup>2</sup> slice contain 0.150 micromol per ml (or 150 micromol per litre) as found at the surface. If this was the case, and all the O<sub>2</sub> in that slice diffused out of the sediment in 48hrs, which isn't going to be quick as the diffusion gradient isn't large, you could get a 50 micromol O<sub>2</sub> increase. The problem is that as you enter the sediment, the O<sub>2</sub> decreases exponentially with depth and at 1.5cm depth you're measuring only 50-100 micromol of O<sub>2</sub>. So, if I assume a 1.5cm slice and take into account sediment porosity, and each ml of sediment had 0.150 micromol O<sub>2</sub>, and

all of this O<sub>2</sub> diffused out in 48 hrs, then we would have had an increase of 10-14 micromol, which is below what we were seeing.

The events mentioned by TMC also do not explain how O<sub>2</sub> increased when we removed nodules from core tubes, placed them in other core tubes with fresh, cold oxygenated surface seawater and found the O<sub>2</sub> increased in 48hr also. You can see this in image Extended Data Figure 4. We also did other ex-situ experiments in the dark with just nodules incubated with fresh seawater very recently as well as controls (no nodules, just seawater) and found the same DOP process occurring. These experiments were done in the dark and used cold surface seawater chilled to in-situ temperature.



All of this aside, TMC's arguments also still don't explain the O<sub>2</sub> production we found at the seafloor with the lander. The lander technique we used has also been used for decades, is also a "gold standard" and, when used in the deep sea, has only ever shown O<sub>2</sub> consumption. In fact, right after the 2021 experiments we used the same type of instrument in the central Atlantic (seafloor didn't have nodules) and all we found was respiration, no DOP. Moreover, TMC reviewed our proposal when we proposed using the approach we used to do the baseline work and agreed to fund us to do the work with this instrument.

I saw TMC released a statement saying our benthic chambers failed to work and dragged bubbles to the seafloor that then slowly diffused into the water phase above the sediment. We considered this multiple times, but we don't believe it's possible. We were careful to discuss our reasons for ruling this out in the paper, but it's also important to point out the chambers would have had 200 tons of water flushing through them as they sank to the seafloor – more than enough to wash away any bubbles trapped by the chambers, and actually the chambers have multiple valves to release air bubbles as they sink to the seafloor. An important thing to consider is the shape of the profiles are inconsistent with a bubble diffusing into the water phase in the benthic chamber. If the lander was sitting at the seafloor for 6-24 hrs before the experiment started (i.e., the typical times between lander arriving at the seafloor and the start of the experiment), the bubble would have been diffusing all this time, so when the stirrers started, you would have seen a sudden jump in the O<sub>2</sub> from background to very high levels in a matter of seconds as that highly O<sub>2</sub> enriched water started to mix. This is not what we saw; instead we saw a steady rise over many hours that was inconsistent

with the bubble theory. The bubble would have likely also diffused in a matter of seconds at the seafloor anyway (as we write in the paper), but we saw a steady rise over 48hrs. Moreover, some oxygen sensor profiles showed steady O<sub>2</sub> conditions at the seafloor and inside the chamber before the experiment started, also inconsistent with the bubble diffusion theory. If the chambers were flawed, you also have to ask why did some chambers show DOP on some deployments, then “start working again” and not show DOP on the next deployment, and then on the next deployment, suddenly start showing DOP again. At the end of the day, you have to discount a lot of data and theory to adopt the bubble diffusion theory which we weren’t able to do.

We welcome the use of other approaches to look at the process, and we agree that Aquatic Eddy Covariance is another approach to measure O<sub>2</sub> consumption. It is a technique I’m using right now in the NE Atlantic, but it is important to realise that it often is used to measure O<sub>2</sub> exchange over much larger areas, so it will be measuring at “net” flux across a much larger area. Therefore, if you have 2 micromol of O<sub>2</sub> being produced per square metre of seafloor per day and 3 micromol O<sub>2</sub> being consumed you would see a net consumption of 1 micromol O<sub>2</sub>, but miss the 2 micromol of O<sub>2</sub> being produced. As such, DOP could potentially be missed with the AEC approach. Maybe over the scale that the AEC measures you don’t see net production, but that does not mean it is not present and could be being masked by greater respiration rates over larger areas. It also doesn’t mean DOP isn’t important. As you can see in the example I gave above, DOP would be contributing 60% of the O<sub>2</sub> respiration.

Our view is that you need to use both methods concurrently: AEC to see what the wider O<sub>2</sub> cycling is doing and chambers to try and tease out if O<sub>2</sub> is being consumed, or in our cases produced over smaller spatial scales. We have tried to be careful, and we should all be careful about upscaling our results by time and space until the spatial scale and persistence of the process is probed further, which is why we do not and cannot comment on whether DOP is vital to the functioning of the seafloor in NORI-D or elsewhere.

At the end of the day, we were the worst critics of this paper for a long time. For 8 years I discarded the data thinking my sensors were faulty. Once we realised something maybe going on, we tried to disprove it as much as we could, but in the end we simply couldn’t.

Another interesting thing is that researchers are now approaching me with similar data-sets also showing evidence of DOP that they just discarded thinking equipment was faulty.