Influence of Oxygen Minimum Zone on Macrobenthic Community Structure in the Northern Benguela Upwelling System: A Macro-Nematode Perspective Hashim Said Mohamed, Beth Wangui Waweru, Agnes Muthumbi Peer Review – 15/11/2023

Review Summary

This paper provides new data regarding the benthic communities of oxygen minimum zones (in this case, the Northern Benguella Upwelling System), a generally under-studied habitat, and, as such is within the scope of BG. Unlike most macrofauna studies, the macro-nematodes (>0.5 mm) are included in the macrofauna analyses which provides a novel perspective.

The taxonomic discrimination of the general macrofauna was undertaken to 'major taxa' level whilst the macro-nematodes were identified to genus level. This allows for general comparisons with other studies to be undertaken and key patterns in macrofauna and macro-nematode distribution and hypoxia tolerances to be indicated and discussed.

The fact that the samples were sieved on 0.5 mm mesh precludes the entire nematofauna being considered (the majority of which fall within the 'meiofauna' category). This is, however, acknowledged and the term macro-nematodes is used correctly to distinguish these large species from the nematode community as a whole.

The text is, at times, confusing to the reader unfamiliar with the study and study site, and needs revision to enable an easier read. I have listed comments regarding this below along with any formatting errors/queries I have noticed.

The only significant scientific query I have is regarding the nematode feeding type categories applied to the various genera and, therefore, the conclusions drawn from these. This needs clarification and justification with references.

I think this paper should be published following revision and will complement the 'low-oxygen environments and deoxygenation in open and coastal marine waters' BG special issue.

Key Revisions and Clarifications Requested

- The data analyses undertaken to justify the separation of the sites into the microxic, dysoxic and oxic zones (i.e. the SPSS) presents a trip-step in the results section.
 Consideration should be given to stating in the methods that these zone definitions were adopted (following Levin 2003) and removing section 3.2 from the results section. See comment for lines 130-134 and 162-174 below.
- □ Clarification required regarding the Weiser feeding type allocation with justification of the allocation of *Desmolaimus* and *Halanonchus* to Type 2A. If these genera are subsequently considered Type 1B, the results and discussion will need revising accordingly.
- □ Consideration should be given to including a fauna results summary table to include sample/site and community statistics (number of taxa, densities etc) that can be referred to when reading the narrative.
- □ Further comments, queries and suggestions are listed below.

Text and Presentation Comments

Line 34 – Reference required for hypoxia/SDG statement.

More detail on the study location required for reader unfamiliar with the Namibian coast and the BUS:

Line 46 – Location details required. E.g. *The Benguela upwelling system (BUS) is located off the West African coast extending from......*

Line 62 - Location details required. E.g.(namely, Walvis Bay, Namibia (the location of this study), California,.....)

Line 74 – Kunene river location details required. Reference to Figure 1 map with Kunene River labelled?

Lines 85/86 – reference to Figure 1 map required.

Figure 1 – Needs more annotation for readers unfamiliar with the area. Small map needs country boundaries and names. Large map requires additional locations, namely, Luderitz and Kunene River, as these are mentioned in text.

Figure 1 – needs a colour scale bar or key for depth as this is mentioned in the site description (lines 85-89)

Line 94 – clarify whether '*high surface primary production*' is mud surface or ocean surface. Obvious maybe, but avoid ambiguity.

Line 101 – reference to Figure 1 required after at 90 nm.

Line 103 - reference to Figure 1 not required. Reference Table 1 instead??

Line 114-118 – please provide details of the level of taxonomy for non-nematode macrofauna taxa. Later, the term 'macrofauna taxa' is used without the reader knowing the definition of 'taxa'.

Line 119 – Need to state that feeding types were ascribed to genera following Weiser (1953) and insert reference in ref list.

Line 130-144 – As above, it is not clear what level of taxonomic discrimination was used for statistical analyses.

Line 130-134 – This needs clarifying. Something along the lines of.....

Out of the seven sampled stations, only three had replicates. Therefore, direct statistical comparisons between sampling stations was not possible. To overcome this, the sampling stations were grouped into 'habitat types', based on the measured environmental variable that provided the most significant correlation with the various biotic indices. The communities within these key habitat types were then compared using indices and analyses outlined below.

An alternative approach that may be less complicated would be to state up front in this section that, following the approach of Levin (2003), the samples from microxic, dysoxic and oxic habitats will be compared in order to determine the effects of low DO. It could be further stated that this approach was justified by the application of SPSS that indicated DO was a key determinant associated with the microbenthic community structure. The text from lines 169-174 could be modified for this purpose.

Line 134 – New paragraph starting at The data recorded....

Line 142 – *the various replicates were considered as a single samples*. How was this done? If the replicate data were added together it would constitute 3 times the search effort as that for a single sample, therefore, the number of taxa will likely be elevated. Perhaps a more valid approach would be to randomly select a single replica for use in this analysis, rather than combine the three replicates?

Line 147 – suggest term inverse relationship in preference to opposite trend.

Line 147-150 - not easy to follow. Suggest

Total Organic Matter (%TOM) demonstrated an inverse relationship with depth, with higher organic matter values recorded in the shallower stations. For example, the shallowest stations 20020, 20002, and 23002 had the highest TOM of $38.6\% \pm 2.16$, $27.5\% \pm 1.55$ and 23.1%, respectively, whilst the lowest TOM values were found in the most offshore stations, stations 23070 and 26090, with $9.89\% \pm 1.55$ and 4.4%, respectively (Table 1).

Line 151 - Similarly, dissolved oxygen values had lower values recorded in the shallower onshore station – According to Table 1, this is not correct; needs clarifying or removing. The OFC transect had its highest DO at the shallowest site (33 m) and OWB had lowest DO at middle depth site (128 m), as described in the later text.

Line 158 – New paragraph after (Table 1).

Lines 162-174 (3.2 Biotic Factors) – As discussed for Lines 130-134 above, it is suggested that the use of the approach of considering the sampling stations in terms of their DO zones (following Levin, 2003) be stated up front in the methods section. This section is somewhat of a trip-step in the narrative of the results.

Line 171 – reference error. Should read (Levin, 2003).

Line 162 – Section 3.2. As discussed above for Lines 130-134, it may be easier to follow if this narrative is moved to the data analyses methods section 2.4, to justify using the three DO habitat types.

Line 175 – 3.3 Macrobenthic assemblages. The term taxa is used with no definition (as mentioned for lines 114-118, above). Presumably, this is the taxonomic groups as listed on Figure 3, although the rarer are combined as others. Maybe list the taxa in a table?

Line 176-183 – This paragraph discusses the densities. The references to number of taxa are repeated in the subsequent paragraphs. Suggest the references to number of taxa are removed from this paragraph.

Line 215 – Insert reference to Figure 3, i.e. zones, respectively (see Figure 3).

Line 216-219 – list of data is probably unnecessary as % abundance data already quoted. If a results summary table was included, this could be referred to instead.

Line 222 –their abundance peaking in the Dysoxic station..... Is this relative abundances or total densities. If relative abundance, please state so and reference Figure 6 at the end of sentence.

Line 226 -recorded significant abundance...... If this is not a reference to statistical significance, please use alternative term, e.g. high abundance, to avoid ambiguity. Similarly, in line 232, please use *low abundance* unless statistically insignificant.

Line 230-234 – sentence long and complicated. Need clarifying and/or shortening. Suggest:

For the purposes of graphing the relative abundances, the genera Thoracostomopsis, Anticoma, Cephalanticoma, Trileptium, Mesacanthoides, Terschellingia, and Marylinnia were grouped as 'others' as they were recorded at low abundances (<4%); see Fig. 6.

Line 235-237 – For clarity, please include the feeding type code after the first mention of the feeding type, e.g. *epistratum feeders (type 2A)*.

Line 239 – Clarification and justification is required regarding the allocation of feeding types. Whilst I appreciate that allocation of Weiser's feeding types is based on the morphology of the buccal cavities and is, therefore, partially subjective, most linhomoeid nematodes (with the exception of *Terchellingia*) are considered Type 1B (non-selective detritivore). I would classify *Desmolaimus* as Type 1B due to its unarmed (the cuticular 'arches' are unlikely to act as teeth) and relatively large buccal cavity. Similarly, I have always considered *Halanonchus* a Type 1B nonselective detritivore as, apart from buccal cuticular thickening, there is no dentition and has a large open mouth. Furthermore, these Genera tend to occur in muds. The results and discussion of feeding type guilds may need reviewing.

Line 256 –oxygen correlated significantly with Please state significance of correlation (p value?)

Line 257 – Typographical error. Should readinto three zones based on....

Line 257 – for the benefit of the reader, it would be useful to remind of the three zones. Suggest inserting in parentheses, e.g.oxygen levels recorded (microxic zone (<0.1ml l^{-1}); dysoxic zone (0.1-1.0 ml l^{-1}); oxic zone (>1.0 ml l^{-1})).

Line 262-264 – It is essential to note.....their relative abundance. Does this mean polychaetes were recorded at their highest density at the microxic sites or they were the most abundant taxa in this zone? I assume the former. Needs clarification to avoid ambiguity.

Line 265 – Please define the *core OMZ*. OMZ spatial centre? Area of lowest DO? Maybe in parentheses.

Line 273-275 – At the microxic zone.....in relative abundance. Meaning unclear. Does it mean, at the microxic sites, the polychaetes were the most abundant taxa in terms of total density, but the average relative abundance was lower than that of cumaceans? (How was the relative abundance of multiple sites calculated?)

Line 276 - Were there only 4 specimens found at 23002, i.e. 3 cumaceans (75%) and 1 other taxa (25%)? If so, the 75% relative abundance, whilst correct, gives a misleading impression of the community and, when averaged with the other microxic station could be misinterpreted.

- This paragraph needs reviewing as it is not clear what point is being made and it read more like the results narrative than discussion.

Lines 269, 274, 275 – The term cumacean should not be capitalised whereas Cumacea should. Please use terms consistently, either cumaceans or Cumacea wherever possible. Similarly, polychaetes or Polychaeta; nematodes or Nematoda etc. My preference would be polychaetes/nematodes etc when discussing the results, unless referring specifically to the phylum or class as a whole, in which case 'the Nematoda' or 'the Polychaeta' is good. Line 279 – please use the term macro-Nematoda or macro-nematodes, throughout. The Nematoda includes the meiofaunal size category whereas, this study, considers only those retained on a 500 μ m mesh, i.e. the macrofauna size class.

Line 286 – Should this read Apart from the increase in nematode size....

Line 289 – suggestcoupled with a reduction in predation by larger fauna that are affected adversely by reduced oxygen concentrations.....

Line 296 - reference should read Gutierrez et al. (2008)

Line 301 - Once the DO levels to dusoxic levels..... This implies a temporal data set that documented an increase in DO. Suggest At dysoxic sites (DO $0.1 - 1 \text{ ml } l^{1-}$), other taxa.....

Line 304 – as above. Suggest At the oxic sites, where DO levels were above 1.0 ml l¹, more taxa.....

Line 308 – Lowest densities, diversity and species of macrofauna taxa or macro-nematodes or both? Please clarify.

Line 310 – As 301 and 304 above. Suggest At sites with DO above 1 ml l⁻¹.....

- Does this refer to your study or Zettler's? Avoid ambiguity, suggest *During the present study, at sites with....*

Line 314-316 – Most of tolerance/intolerance to hypoxia. Meaning unclear. Suggest: Of these fauna, crustaceans were most abundant. This conforms to the observations of Soto et al. (2017) at oxic sites in an upwelling system in Chile. Conversely, Zettler et al. (2009) recorded amphipod species in low oxygen areas. These contradictory results indicate that, at least amongst the Amphipoda, tolerance/intolerance to hypoxia is species specific.

Line 357 – Suggest: Tolerance to hypoxia is indicated by both the presence and absence of taxa......

Line 361 – insert Wieser reference

Line 362-363 – Need consistence of terminology for feeding types in text and Figure 7. Standard definitions are:

Туре	Buccal Cavity	Feeding Method
1A	Small, unarmed	Selective deposit feeders and bacteriovores
1B	Large, unarmed	Non-selective deposit feeders
2A	Small, armed	Selective epigrowth feeders and herbivores
2B	Large, armed	Carnivores and omnivores

Line 363 - Typographical errors – I think it should read(2A), with the former dominating the oxic zone.

Line 367 – suggest These observations appear to be.....

Line 375-376 - The areas around the OMZ have also been observed to contain a high level of diatomaceous mud, which forms a significant component of epistratum feeders' diet, whose abundance was also high in the dysoxic areas.

Is it true that epistratum feeders (2A species) feed on diatomaceous mud? Need reference. 2A species feed on live diatoms whereas diatomaceous mud comprised dead, settled diatoms. Does the surface of the mud support epibenthic diatoms? The shallowest site was 33 m (oxic) whilst the rest of the oxic and dysoxic sites were in excess of 100 m where primary production would be very limited.

Line 377 – Below the OMZ,.... Does this mean offshore from the OMZ, in deeper water?

Line 385 – should this read Ostracoda and Bivalvia observed in limited numbers in the oxic zone....? (not anoxic?)

Line 380-391 (5.0 Conclusion) – the conclusion does not mention any thoughts on the macronematodes despite the title stating "a macro-nematode perspective".