Biological Indicators of Oxygen Stress in Marine Water-Breathing Animals

<u>Review #2.</u>

Roman & Levin et al. update the current knowledge of biological effects of ocean deoxygenation and reviews indicators that could be useful if implemented into monitoring programs designed to detect and track ecological shifts as a direct consequence of low oxygen stress.

The authors greatly appreciate the time taken to provide this thoughtful and helpful review and believe this manuscript will be improved by our incorporation of the suggestions made by Dr. Chu. Our responses are indicated in bold after the individual comments.

General comments

There is a great and timely review of the biological response to hypoxia and the large-scale impacts of deoxygenation. Literature examples span multiple ecosystems and are global in representation. The authors target a wide audience and bridges this with examples that span multiple biological levels of organization perspective (and thus interdisciplinary among the biological sciences). As a consequence, they brought to my attention new papers on a topic that I am somewhat familiar with which is an indication that the literature review component alone is immensely valuable. The review furthers the discussion by touching on where deoxygenation may have been overlooked by major research and global science initiatives. This will be a useful guidance document for those who wish to direct resources towards 'oxygen' as a key variable in their monitoring and management efforts, and hopefully cite this review as a result.

The majority of my comments focuses on a meat of the review – the summary of indicators of deoxygenation stress. At the onset, the authors introduce key terms like 'indicator', 'monitoring programs', and cite the Essential Ocean Variables (EOVs) as background context. Readers that already have some buy-in and are tasked with monitoring efforts would predictably ask "what do I measure and how do I measure it?". These are the start-up questions to address when developing indicators for monitoring programs (See Yoccoz et al. 2001, Reynolds et al. 2016 for more). The term 'indicator' could be better defined and applied in the manuscript. In general, ideal indicators have specific criteria when being evalualted for monitoring programs – they should be: quantifiable (measurable with units), sensitive (small change in the system pressure incudes a big change in indicator), responsive (indicators responds in the same time frame as system pressure), specific (not influenced by covariates), and operationally feasible (i.e. costs associated with acquiring enough data points so that a signal can be detected is reasonable)…and be able to detect & track trajectories of change over time (or space) as a consequence of the system pressure (here – deoxygenation).
Excellent suggestion, we have added the following sentence to the paragraph that introduces indicators in the Introduction:" Indicators should have specific criteria when being evaluated for monitoring programs (e.g. Yoccoz et al., 2001; Reynolds et al. 2016). These criteria can include: readily quantifiable, responsive and specific to the stressor of interest, operationally feasible and able to detect response to the stressor over space and time."

Several of the examples as presented are uncoupled from this main manuscript theme which sandwiches the beginning and end of the manuscript. Not all the reviewed biological response examples are presented as indicators in the current text but still exist as a recap of their original citation. Additionally, a science evaluation of the criteria for indicator assessment and associated 'challenges' of using them in monitoring programs could be useful – this is loosely touched upon in Table 1 (e.g. the specific criteria relates to the table's confounding factors), but is inconsistent. Some structured text that considers this for all presented examples could be useful in a minor revision. The translation of the expansive theoretical knowledge into operational guidance is often the missing piece that results in the failure to launch of many monitoring programs.
Our description of indicators of oxygen stress at different levels of biological organization is intended to be a resource for both monitoring programs and research (field programs, laboratory studies and modeling). Some of the indicators we describe are in preliminary stages of understanding which need more research to be useful for monitoring programs. In the revised manuscript we try to make this differentiation. We have modified Table 1 to include a listing of whether the indicator has a "Low, Moderate or High" application to monitoring as described by Jackson et al. 2000. EPA-R-99/005.

Refs:

Yoccoz et al. Monitoring of biological diversity in space and time (2001) Trends in Ecology & Evolution, 16: 446-453.
<u>Added</u>

Reynolds, J.H., Knutson, M.G., Newman, K.B., Silverman, E.D. and Thompson, W.L., (2016). A road map for designing and implementing a biological monitoring program. Environmental Monitoring and Assessment, 188, pp.1-25.
<u>Added</u>

Specific Line comments -

Many of my specific line comments were written before my general comments section; they will mostly focus on whether the biological response examples could meaningfully be used in a monitoring program designed to track deoxygenation impacts.

L40 – is 'indicators' an appropriate term here? Biological response seems a better fit.
Changed to "biological response" as suggested.

L82-L85 – Do the authors suggest that 'only' measuring biotic indicators here? Or in companion with existing oxygen monitoring? If the former and with the rest of the manuscript read – it would be challenging to conclude on the causal relationship of any signal in the biotic parameters back to deoxygenation without having the ambient oxygen having been measured in parallel as well.
Sentence changed to: "Oxygen has been proposed as an indicator of ocean health and of large-scale restoration progress; for example, we can use oxygen content to monitor reduced nutrient loading (Grégoire et al., 2021). However, in addition to monitoring oxygen, biotic indicators of low oxygen stress may provide more direct support for environmental managers, fisheries scientists and policy makers in their efforts to better

assess the sensitivity of different ocean species, communities and ecosystems in response to oxygen content."

L85-L89 – it would strengthen the messaging to the intended target audience listed in the previous sentence if a guiding definition of 'indicator' is presented.
Sentence added: "Indicators should have specific criteria when being evaluated for monitoring programs (e.g. Yoccoz et al., 2001; Reynolds et al. 2016). These criteria can include: readily quantifiable, responsive and specific to the stressor of interest, operationally feasible and able to detect response to the stressor over space and time."

L89-L92 – subjective and arguable given the evaluation criteria of what makes a good indicator for monitoring programs hasn't been presented. Changes in animal behavior, unless specific to the focal question/objective of the monitoring program could be considered poor indicators?
Criteria for indicators added (see the above response). In addition, our description of indicators of oxygen stress at different levels of biological organization is intended to be a resource for both monitoring programs and research (field programs, laboratory studies and modeling).

E.g. if the cost of deploying and recovering an oxygen sensor is less than the cost and risks of acquiring and maintaining a population of animals and measuring live animal behavior in a controlled experimental setting…than the latter would not be a good indicator. How would one then apply any lab-derived data back into the natural system state influenced by deoxygenation stress?
Based on laboratory and field results for lethal and sublethal responses of marine animals to low oxygen, countries and states have developed habitat criteria based on oxygen content. Avoidance of bottom waters with specific low oxygen content can thus be used to confirm these ecological predictions.

L98-102 – Just a thought about the idea behind the EOVs and how the relate to this review. Core traits of the EOVs are that they are measurable and inclusive, but also 'non-specific' to just one system pressure/stressor. The challenge with developing deoxygenation indicators is to have them be 'exclusive' – not correlated to other pressures in that they are informative to one very specific stressor.
We agree and have added a cautionary sentence: "Note however that indicators can be misleading if confounding variables are not considered or if the indicator does not have sufficient validation for the level of biological organization considered (e.g. species, population, community or ecosystem)."

L107 – « Garcon et al. 2019 examined… » What did the authors conclude?
Thank you. Sentence changed to: "Garçon et al. (2019) examined the potential application of EOVs to understand biotic responses to oxycline changes within Oxygen Minimum Zones (OMZs) for potential societal benefits such as improved fisheries management."

L124 – The covariate and scaling issue. Given these are established challenges with interpreting the ecological consequences of deoxygenation/hypoxia – can the authors reflect on the presented list of their indicators and suggest which ones are best?

This is addressed in a revised Table 1 and a revised section on "Scaling of Indicators"

L143 – The focus of this review

Reading through – are the levels of biological organization intended to present the 'level' of organization at which (1) the indicator is measured, or (2) the level of organization at which 'the biological response' manifests? The manuscript isn't quite consistent or clear on this.
Sentence changed to: "The focus of this review is on biological indicators of low oxygen stress in water-breathing marine animals. We identify indicators that have been determined for different levels of biological organization, broadly defined: Individuals; Species/Populations; Communities/Ecosystems, (Figure 1).

L154 – Consider "Individual-level indicators"?
Subheading changed to: "Individuals"

Individual-level indicators – these are described more as biological responses – there lacks a discussion on how these can be translated into monitoring programs?
E.g. in research fisheries surveys – individual specimens are brought up in catch. Length (cm), weight (kg), age (otoliths), are measurable, individual-level data. I can envision tissue & blood subsamples being taken if some of the presented indicators can be quantified post-hoc (e.g. protein & enzyme levels present at sampling), but the behavioral ones, at least as presented, would be extremely challenging to implement in a realized monitoring program.
We have a broader focus of the description of indicators than just application to monitoring programs which we have tried to clarify in the revised manuscript and Table.

L163 – The indicator "Amount of HIF-alpha protein"
Changed as suggested.

Indicator sensitivity is species-dependent
L182 – Not clear why HIF may be more difficult to measure? Is it the cost and challenges of the live-animal cultivation requirements to get a data point?
This sentence has been deleted.

L186-L213 – For sensory systems indicators and the suite of variables that require manipulative lab-based experiments to quantify – would these really be 'monitoring indicators'?
They are in the research phase which we now state in Table 1.
Introductory sentence added to this section: "Low oxygen can impair the sensory systems of marine water-breathing animals. Changes in animal vision, olfaction and perception of sound can be sensitive indicators of oxygen stress which impact population and community ecology. While promising, more research is needed to elucidate the primary and secondary effects of sensory impairment by low oxygen stress on different groups of marine animals to be practical for implementation in monitoring programs."

L198 - Would monitoring programs, which infer natural/field systems…be tracking vision loss or sensory loss in situ? How would fisheries actually measure this within their standard equipment? Table 1 suggests 'control' specimens would be required to be brought into the lab,

and manipulated via factorial experiments. Unless these biological responses can be calibrated back to a field-based indicator (can behavioral responses like these be calibrated to a fisheries metric like CPUE?) – these feel a bit uncoupled from being useful monitoring program indicators without additional thought.
Sentence modified to say "possible future monitoring programs": "**Impaired vision could influence their survival (McCormick et al., 2022b) and hence be potentially useful for future management in considering susceptibility to catch and possible fisheries restrictions."**

L186 – Vision – perhaps mention this metric only applies and is limited to mobile animals with eyes. **Changed**

L302-L306 – Pcrit, oxyregulation/oxyconformation and the theory comes from quite a long history of fish physiology research – suggest citing at least one reference here. (e.g. Fry 1933).
Fry reference added as suggested.

L320-L329 – the theory underpinning Pcrit and oxyregulatory ability also relates to ontogeny; this nuance is often lost with the generalization towards 'size'. The ability to oxyregulate is not present throughout developmental life-stages in many marine ecotherms – the ability to oxyregulate manifests in later life history stages (e.g. fish) which also correlates with larger body sizes.
The discussion in L320-329 refers to copepods which do not have gills and obtain oxygen through passive diffusion through their integument. Smaller copepods (same species or different species) have a higher surface/volume ratio and have been shown to survive better at lower oxygen concentrations than larger copepods – the physiological underpinning of which is related to their lower Pcrit. References: Roman, M. R., Brandt, S. B., Houde, E. D., and Pierson, J. J.: Interactive Effects of Hypoxia and Temperature on Coastal Pelagic Zooplankton and Fish, Frontiers in Marine Science, 6, https://doi.org/10.3389/fmars.2019.00139, 2019 and Roman, M. R. and Pierson, J. J.: Interactive Effects of Increasing Temperature and Decreasing Oxygen on Coastal Copepods, The Biological Bulletin, 243, 171–183, https://doi.org/10.1086/722111, 2022.

Pcrit seems out of place here but also is inconsistent with what is / isn't an indicator – which goes back to the comment on what is actually being measured and how it can be measured as part of a monitoring program. Compare this with the units of the other indicators presented which is quantified through a measurement of a biological parameter / response variable.
Pcrit is introduced as a physiological basis for smaller size. Pcrit as part of the Metabolic Index (discussed later in the manuscript) is not an indicator itself but in combination with available oxygen (can be monitored or predicted in climate models) can be an indicator of suitable habitat space as suggested by Deutsch et al. references as well as Cheung and Clarke references provided.

A Pcrit is just a single environment oxygen level but determined to be a biological relevant threshold using live-animal respirometry experiments. Time-series of the threshold itself is not what would be generated by a monitoring program as it's not the parameter that is responding to the deoxygenation system pressure nor would time-series be generated of Pcrits. A realized

deoxygenation monitoring program that implements Pcrits into its general framework would realistically be generating standard oceanographic oxygen time-series using CTDs with the interpretation/early warning signal being the where and when those oxygen data show environmental oxygen levels to be at Pcrit levels of interest.
Thanks, we agree. There are some monitoring programs in oxygen minimum zones in Eastern Boundary Current Upwelling systems which are trying to monitor oxygen in combination with Pcrits to predict animal distributions. Oxygen is monitored in Chesapeake Bay and we have used laboratory Pcrit determination for the dominant copepod species, *Acartia tonsa,* to predict habitat space in the water column. Field measurements of copepod distributions verified that *A. tonsa* abundance was higher in areas of the water column with a positive predicted MI index (Roman and Pierson, 2019).

L377 – Lethal hypoxia has also been estimated from field measurements with organism presence/absence. I would interpret these as more a sublethal hypoxia threshold being estimated from organism presence/absence for at least mobile species; since most will swim away before at levels higher than the actual lethal levels are reached unless the oxygen loss was rapid and companion data on carcasses was also available.
Sentence modified to: "Field-based estimates of mortality are less certain because of the temporal and spatial variations in oxygen as well as changes in the vertical/horizontal distribution of the organism due to avoidance of the deoxygenated water."

L421 – Metabolic Index –high degree of extrapolation, not only from individual-level lab-based experiments but to entire theoretical niches (based on only a few dimensions…O2 and temperature primarily), but also across entire phyla, coupled with the uncertainties of climate-model forecasts.

The primary discussions coming from this metric have been theoretical hypotheses presented as predictions – as noted, the real-world validation through testing the accuracy of M.I. predictions at the regional scenarios – the extant fisheries perspective remain limited.

When considering the metabolic index under the monitoring / indicator science context – the metabolic index is a derivative of Pcrit theory and so the same comments above apply here as well. The metabolic index in its current formulation doesn't quite seem like an indicator that could be used to track and detect system-state changes.

Thank you for your thoughts. In order to differentiate the Metabolic Index from the other biological indicators of low oxygen stress we have added this sentence in the introductory paragraph of the MI section: "The MI is not an index of biological stress due to low oxygen waters but rather a predictor of the environment that meets the oxygen requirement of a particular species or ecophysiotype."

L489 – Suggest clarifying that indicator species is not a hypoxia-specific idea, and they are bioindicators of a set of environmental conditions or state.
Sentence changed to: "Particular species can be bioindicators of a set of environmental conditions including past and present oxygen state (Schwacke et al., 2013)."

L536 – Indicator species presence may be a straightforward way to detect oxygen changes and is easy to interpret.
Potentially – but cited examples are metazoans only.

The focus of the paper as indicated by the title is on "water-breathing animals" – metazoans only.

Empirical evaluation of how well an organism can act as an indicator species is determined by fidelity and specificity. The hypothetical perfect indicator taxa of deoxygenation waters would have high fidelity (the species is present at all sites with deoxygenated conditions) and highly specific (that species is only present at sites with deoxygenated conditions). Since there are no obligate anaerobic water-breathing animals – using any metazoan as an indicator species of deoxygenation conditions would always require additional context and would not be straight forward. I.e., all metazoans can inhabit normoxic waters…if they are absent, it would be another dimension of their niche that is restricting them from occupying oxygenated waters. Considering the above, consider adding context and specifying obligate anaerobes as potentially more ideal use case for 'indicator taxa' theory.
The focus of the paper as indicated by the title is on "water-breathing animals" – metazoans only which do not include obligate anaerobes but do include taxa which at times can tolerate low oxygen conditions. We added another sentence to this section: "Note that a single indicator taxon niche is rarely unidimensional such that multiple indictors can provide a more robust effect of deoxygenation."

L577–L624 are behavioral responses described not actually individual-level responses when being measured.

Our interpretation is that the change in depth distribution in the water column or sediment is a biological response to the low oxygen conditions and is thus an indicator of low oxygen stress.

This section repeats the measurable parameter described in the individual-level response associated with sensory systems.

The vertical distribution of organisms can shoal as a result of avoidance of lethal oxygen levels as well as deterioration of vision by non-lethal low oxygen stress such that they shoal for a greater amount of light for visual feeding.

E.g. L589 – this paragraph describes the consequence of low oxygen exposure to individual fish rather than presenting as an actual measurable indicator. For a measurable parameter indicator a population-level indicator (or biological response); the population-level response here when describing the compression into shallow waters (shoaling) is the decrease in the average depth distribution of the population.

Excellent point. We have added this sentence to the introductory paragraph of the section: "Measurable indicators of avoidance of low-oxygen waters can be presence/absence, shallower depths of centers of abundance maxima and reductions of vertical habitat space."

L635 – Population size

As presented – the measurable parameters presented for population size/growth rate/recruitment are standard ones measured by traditional stock assessment monitoring programs.

Can you provide some discussion on whether this would be a 'good' indicator given it relies on having a foundation of another species/individual-specific indicator (lethal O2, L641) and how in reality, if this measurable parameter (standardized counts for a species') is confounded by so many other variables? In general, consider adding some text that clarifies when indicators may or may not be useful in a realized monitoring program.
Thank you for making these points. Fisheries managers worldwide routinely conduct monitoring programs to assess population size/growth rate/recruitment of commercial fish stocks. Increasingly, fisheries managers are employing ecosystem-based fisheries management which considers not only fish stocks but also their predators, prey and water quality (habitat space). Laboratory, field and modeling studies have provided evidence that low oxygen stress (lethal and non-lethal) can directly impact population mortality, growth rate and recruitment. The difficulty of course is trying to isolate the effects of low-oxygen stress from fishing pressure, prey abundance, etc. We try to consider these interactions in the Scaling section and in the revised Table ("Readiness for Monitoring").

L711 - Ecosystem Indicators: L713-L782 – Most of the quantifiable parameters only inform on the community-level of biological organization (e.g. species assemblages metrics); suggest clarifying this in the section subheading.
We agree and will change to Ecosystem to community or Community/Ecosystem. This change will also be made in the Figure and Table.

L752 – there are additional 'diversity indices' not mentioned that integrate some level of functioning into their formulation by including simple traits / functional levels into the calculations. E.g. ITI (infauna trophic index) and the AMBI – AZTI indices are often used in aquaculture-impact assessments marine biotic index that includes simple traits/trophic level categories into the calculations. On the topic of and linking back to anthropogenic over-enrichment; the benthic infauna/macrofauna diversity indices do include ones that integrate a level of functional. While this doesn't explicitly link back to hypoxia sensitivity, they are inversely correlated to hypoxia (as they are correlated to high sulphide levels), and integrate trophic traits into their formulation, thus may be slightly better at representing the ecosystem level of organization than pure species assemblage derived diversity metrics.
We have added a section on these to the trophic section… where they are better discussed.

There are some indices that incorporate elements of diversity and trophic function such as the Infaunal Trophic Index (ITI) (Word 1978), AZTI's Marine Biotic Index (AMBI) Caswell et al. 2019), and the Trophic state Index for Benthic Invertebrates (TSI-BI) (Chalar et al. 2011). These have been applied over a range of sediment ecosystems and over extended periods of time to examine change including in response to oxygen loss.

L799-L814, L838-851 – Abundance and Biomass

As written – this is discussion individual population-level abundances without a quantifiable metric that captures the interspecies component of the community re-organization in mind. E.g. Given hypoxia sensitive differs among species – there can be interspecific shifts in abundance

levels (some increase, some decrease) as some moderate hypoxia thresholds are crossed, but community-wide biomass decreases as oxygen approaches zero
Analogous indices have been developed for infauna to monitor for system degradation as a consequence of anthropogenic enrichment (e.g. polychaete/amphipod ratios).

Thank you for your thoughts. As you say, we cover this to some extent in the section Taxonomic Shifts section.

L853 – Taxonomic shifts and ratios:

I wrote the above comment before reading this section – which might work better if it shifts before the abundance/biomass paragraphs.

In a system experiencing gradual deoxygenation – the interspecific shift in species abundances usually happens first (among mobile species - sensitive ones leave, tolerant ones invade) before the community-wide decline in abundance occurs. This would be a useful to suggest as the 'early-warning' signal, given the shift in relative abundances among species would theoretically happen first before community-wide abundance decline.

Taxonomic shifts could also go by different names discussed earlier in the paper (i.e., beta diversity), so there is some intellectual overlap here with the earlier section as well.

Thank you for this suggestion. We will move the Taxonomic Shifts section up above abundance and biomass as we agree it fits somewhere between diversity and abundance concepts. We prefer to retain the terminology used, as it is clear what we are discussing and the term Beta diversity does not necessarily invoke the shifts we are discussing. It is a good idea to suggest these as early warning. We have added the sentence: "*Because mobile species can respond quickly to oxygen decline the resultant shifts in taxonomic composition may offer an early warning of hypoxia.*" We have also included a sentence about the Polychaete:Amphipod ratio in this section. "*Among macrofauna, the polychaete to amphipod ratio can reflect changing eutrophication (Dauvin 2018) and water quality (Maximov and Berezina 2023) but does not seem to be a good oxygen indicator.*"

L930-934 – The metric presented seems to be behavioral response and not a measurable indicator of a community-level component. Given this is the ecosystem-level + ecosystem function discussion section, as a reader I would be looking towards quantifiable biological processes capturing the energetics of the system (e.g. biological rates) – this paragraph could use a retool and link the shift in bioturbation behaviour to the measurable ecosystem indicator.

We have added a few sentences to link to metrics and consequences.
"Metrics that reflect these changes include sediment mixed layer depth, burrow size and diversity, and bioturbation rate (Db), although the latter metric is not always positively correlated with oxygen concentration (Smith et al. 2000). Under persistent, stable hypoxia some tolerant species deepen their vertical distributions as long as some oxygen is present (Levin et al., 2009a). In low sedimentation areas hypoxia-induced reduction in sediment mixing can lead to reduced organic matter decomposition and enhanced carbon preservation (Canfield 1994)."

L1020 – Scaling of Indicators

Might be useful to provide an operational definition on what 'scaling' means; the types of 'scaling' aren't clearly defined for the reader. The common theme across the summarized 'scaling' approaches appears to be 'multi-level integration across biological responses to deoxygenation' but not clearly defined in a way that makes the differences obvious. Given 'spatial-temporal' is also discussed (L1023) - I was looking for the traditional concepts/definitions of ecological scale, resolution (grain) and extent, to be presented in a discussion that draws analogies to the biological organization structure theme; wasn't sure if that was the intent. I feel just a slight refocusing of the wording could help with the clarity in this section.

We have edited the Scaling section to improve clarity as suggested:

"Scaling of indicators is often necessary to enable the observed values of the indicator to be interpreted as representing the state of the system and for results to be expressed on spatial and temporal scales that are ecologically or societally meaningful. Consideration of what types and to what extent scaling is needed is important when selecting an indicator, designing a sampling plan and interpreting and communicating the results of an indicator. Scaling often determines what species and life stages to measure, the specific indicator(s) needed and how to allocate effort to sampling locations and frequency of sampling.

Scaling can employ graphical or statistical analyses to extrapolate the measured conditions of individuals to broader areas than those locations sampled (e.g. sub-regions, basin-wide) or to more generalized timescales than those captured by the data (e.g. month, season, years). This scaling employs the statistical concept of looking for patterns in the data collected at different locations and/or over time and subsequently making key assumptions about how these data reflect broader conditions to infer the population of indicator values.

Another type of scaling analysis is used with indicators to derive a mechanistic understanding of how the indicator logically and causally relates to higher levels of biological organization (population, community or ecosystem). For example, low-DO impaired vision affects detecting prey that determines feeding and growth that affects vulnerability to predator (mortality) and fecundity, which affect population abundance. This integration and scaling across levels of biological organization from the organismal to ecosystem level can be represented in a conceptual diagram (Altieri and Witman, 2006), where low-oxygen stress reduced survivorship and growth of individual mussels and impacted the density and spatial extent of mussel populations. Individuals of a single species could be used to infer the state of the population while observations on multiple species can be leveraged to community (e.g. diversity) and food web levels (e.g. energy pathways). The condition of individuals as indicated by lipid content (e.g. Herbinger et al.,1991) suggests sufficient exposure to low-DO can elicit a response of the bioenergetics and physiology of the individual. Reduced animal condition can be related to the oxygen state of the system and can lead to higher mortality, lowered fecundity and other responses that can be directly related to population, community or food web levels. While values of indicators on subsets of individuals can stand alone to show exposure and responses of individuals, scaling translates indicator observations into potentially more-relevant levels of biological organization and scales of time and space. This mechanistic scaling approach was used by Rose et al. (2018a, b) to examine how reduced growth, increased mortality, and reduced fecundity due to low-DO exposure affected croaker (*Micropogonias undulatus*)

population dynamics in the Gulf of Mexico. By using an agent-based model with a 2-D grid that included dynamic DO field, the time-dependent exposures of individuals were simulated and avoidance behavior was projected.

Scaling of indicators can include numerical models which provide a quantitative translation of the indicator into variables that are more relevant to management and society. Common situations requiring such modeling are when multiple stressors covary and DO effects need to be isolated or when expressing indicators in units explicitly chosen to inform policy (e.g. economic impacts of reduced biodiversity) and management decisions (e.g. fisheries yield). For instance, Franco et al. (2022) scaled low-DO effects to habitat changes of Pacific halibut (*Hippoglossus stenolepis*) in the Northeastern Pacific. They used fisheries-independent data and model predictions from ROMS-BEC of oxygen and a metabolic index was used to map suitable aerobic habitat."

L1085 – "oxygen-stress", perhaps reword to deoxygenation stress. Review doesn't discuss hyperoxia effects (oxygen being too high).
Good point, thank you. Text changed to: "low oxygen stress"

L1186-L1188 - Many of the biological indicators of oxygen stress described in this
1187 paper, if tied to specific DO response thresholds,

From the perspective of, "what is needed in the practical launch and implementation of a realized deoxygenation monitoring program" – this might be the key statement to emphasize. Implementation of any of the parameters to produce longitudinal data (time-series) to track biological responses to deoxygenation will require the natural oxygen levels to be monitored in tandem.
Thank you, good point, we have added text to say that oxygen needs to be monitored and also refer to Table 1 which categorizes the application potential of the indicators of low oxygen stress we describe: "Many of the biological indicators of oxygen stress described in this paper, if tied to specific DO response thresholds and *in situ* oxygen concentrations, can be used in monitoring and applied to management of water quality, biodiversity and fisheries."

Technical corrections:

L433 – Howard et al. (2020) missing from References
Reference added: "Howard EM, Penn JL, Frenzel H, Seibel BA, Bianchi D, Renault L, Kessouri F, Sutula MA, McWilliams JC, Deutsch C. Climate-driven aerobic habitat loss in the California Current System. Science advances. 2020 May 15;6(20): eaay3188."

L1079 – ROMS-BEC – could you define this acronym?
Full name added: "Regional Ocean Modeling System- Biogeochemical Elemental Cycling (ROMS-BEC)"

L1087 – Table 1. Difficult to read with some text cut off within the excel table format.

Table has been edited and now has an additional column "Readiness for Monitoring" with ratings for the indicators: "Low, Medium or High" to indicate their practicality for monitoring implementation at this time.

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