We have now addressed the comments and suggestions of the three reviewers. The major changes are: 1) we have explained in more details some of the data, 2) we have added to the paper the oxygen actually experienced by cod during the years; 3) we have deleted the part on otolith analyses and related our findings to those by Limburg and Casini (2019) in the Discussion, 4) we have performed a statistical GAM analyses of the relation between cod condition and oxygen, and 5) we have further improved the Discussion with more discussion about the differences in depth distribution between our study and Orio et al. (2019), the findings of Brander (2020) and the other potential reasons behind cod condition decline, as requested by the reviewers.

Reply to reviewer #1

We thank the reviewer for his thorough comments.

In literature, there have been only two studies investigating the relation between Baltic deoxygenation and cod condition, i.e. Casini et al. (2016) and Limburg & Casini (2019). In the former paper, a strong correlation was found between the extent of hypoxic areas (defined in that paper as km^2 *with oxygen < 2 ml/l) and condition, but the mechanisms potentially explaining the statistical relationships were not investigated but just proposed, i.e. decline in benthic food, changes in cod behavior/distribution, direct physiological stress, or of course a combination of these. In the second paper (Limburg & Casini 2019) it was shown that fish in low condition at capture were exposed during their lives to lower oxygen levels than those in good condition (at least from the mid-1990s), without saying anything about the distribution of the population, and therefore whether or not a large part of the population indeed experienced stressful circumstances. Therefore, the original triggers and the mechanisms relating hypoxia to the average Baltic cod condition in the population were indeed elusive (and we think they still need attention), as we state in the abstract of the new paper (referred to as CHOL, from the initial of the authors names, following the terminology of the Reviewer; we refer here to the Reviewer as KB).*

The CHOL paper takes a further step, showing that the cod population went progressively deeper in autumn and this, concomitant with a shallowing of the low-oxygen layers, increased the spatial overlap between cod distribution and low-oxygen waters, and thus generating stressful circumstances for the cod population (exposure to waters with oxygen < 4 ml/l, detrimental for cod condition as found in experiments by Chabot & Dutil, 1999) (see below about the choice of the oxygen sub-lethal threshold in the CHOL paper). We finally showed that this increased overlap relates statistically to the decline in the mean population condition and to the proportion of fish with very low condition, both for juveniles and large fish. Therefore, the CHOL paper shows the original processes (deepening of the cod population concurrent with the shallowing of low-oxygen layers) creating the stressful circumstances relating to a decline in condition, for both small and large cod. In our opinion, this is a very important step forward in the understanding of the link between low-oxygen and cod condition, and in general for understanding the causes of the declined cod condition. Additionally, it is not so obvious that condition has to be directly linked to a general deoxygenation phenomenon, since mobile fish can change their distribution in response to that, as done by other fish species in other areas. This did not happen for the Baltic cod (conversely it went deeper, in autumn), and we think that finding the answer to why this has happened is one of the next challenges for the scientists. Cod prey should also suffer from deoxygenation, although some are more tolerant to low oxygen; therefore, the question of why cod went deeper is not so trivial in our opinion and should be investigated as we suggested for future studies.

We are therefore totally in line with KB about the fact that "the issues to resolve are firstly whether cod redistribute themselves to remain in areas and depths with sufficient oxygen and if not then *secondly whether the magnitude of ambient oxygen decline that cod experience is sufficient to explain all or only part of the observed change in their condition.” This is exactly what we have done in the paper for both small and large cod in autumn. In addition, we have also investigated the original reasons creating these circumstances (i.e. both deepening of the population and shallowing of the low-oxygen layers), as well as estimated the overlap with the low-oxygen layers, known to affect cod condition, and estimated the relation between this overlap, the mean population condition and the percentage of fish with very low condition, for both small and large cod. This does not mean that direct exposure to low-oxygen is the sole driver of condition (even if oxygen decline is sufficient to explain a large part of the decline in condition), because there can be other contributing drivers and/or drivers that have co-varied with deoxygenation (food availability, parasites, inter- and intra-specific competition, etc…) that could also explain the reduced feeding level (see below). That is why further work is needed here too.*

In the CHOL paper, we used 4 ml/l as sub-lethal oxygen threshold impairing cod condition. As KB correctly stated, 73% oxygen saturation (sub-lethal threshold in Chabot & Dutil (1999)) corresponds to 4.8 ml/l at the experimental conditions, but 65% oxygen saturation is the level from which the decline in condition was significant in Chabot & Dutil (1999) experiment, corresponding to 4.3 ml/l. We therefore used now 4.3 in the revised version of the paper, to improve our analyses.

We agree with KB that the real oxygen levels experienced by cod would be informative, so in the revised paper we showed also the oxygen levels corresponding to the annual depth distribution of cod in autumn, both for small and large cod. However, we think that the information about the shallowing of the 1 ml/l and 4 ml/l (the latter now 4.3 ml/l in the revised paper) depths enriches the story, and together with the deepening of the cod distribution depth, it visually delivers a very clear message. Therefore, we preferred to retain it.

The otolith analysis was already published in Limburg and Casini (2019), but the analysis was re-arranged as a new figure in CHOL. We thought that this was a nice conclusion of the story, but we have now opted to delete it from the paper and to discuss instead the results in Limburg and Casini (2019) in our paper.

Exploring mechanistic relationships would need experimental setups. Using time-series, the statistical relationships have to be interpreted in light of what is known about the biology and ecology of the fish. In our case, we used the experimental results from Chabot & Dutil (1999) to relate the distribution of the cod population with the oxygen levels resulted to affect cod in experimental setups, and in the revised paper we have briefly discussed the information coming from stomach content analysis (see below).

We agree with KB that Neuenfeldt et al. (2020) is an extremely important paper, showing that the lower energy intake observed in cod (using stomach content time-series) would predict a decrease growth in length that could explain the shift in size distribution of cod population towards lower sizes. The lower amount of benthos and pelagic fish in the diet of cod could be due to a decline in their availability (as suggested in Neuenfeldt et al. 2020) but also to a decline in cod appetite due to low-oxygen exposure (Chabot & Dutil 1999, Brander 2020) or other low oxygen-related physiological stress. Food intake can surely be the main driver of growth, but other factors can cause fish to allocate more energy to basic metabolism, reproduction etc… in some circumstances. For example, currently Baltic cod reproduce at a smaller size (around 20 cm) than before (30-35 cm) and this could mean a lower allocation of energy to growth and therefore also explain the growth decline. We agree with KB that such reasoning produces the egg-chicken problem, but it brings us outside the scope of the CHOL paper.

In our analyses we investigate fish condition, not growth in length, and since the two traits are different (fish can grow fast in length, utilizing the stored energy reserves, but this at the detriment of *condition, that is a ratio between weight and length, as shown also in feeding experiments) we do not want to mix them. However, in the revised paper, we have added more discussion about the decline in feeding level found in Neuenfeldt et al. (2020) that could link the increased exposure to low-oxygen levels to declined condition. However, there are some aspects that make this link not as straight forward as it seems. Neuenfeldt et al. (2020) show that feeding level has not declined for large cod, but the observed decline in condition has been more severe for large cod (Casini et al. 2016 and the new CHOL paper), suggesting perhaps that feeding level is not the sole driver of large cod condition and that therefore low oxygen has impacted cod condition also through different mechanisms, other than food intake. For example, large cod could experience shortage of benthic prey and therefore, proportionally, could be forced to eat more pelagic fish that require higher energy to catch. Moreover, cod was not in low-oxygen conditions before the early 1990s (see our CHOL paper), but the feeding level was already low (Neuenfeldt et al. 2020; see also ICES 2016), and so was condition (Casini et al. 2016, new CHOL paper), indicating that direct exposure to hypoxia is not always the driver of feeding level and condition (matching therefore with the results from otolith analyses in Limburg & Casini (2019)). In the revised paper, we have however put our results in relation to Neuenfeldt et al. (2020) findings about feeding levels, to link the increased overlap with low-oxygen waters to feeding level and condition after the early 1990s. We have moreover related more the CHOL paper results with Brander (2020) paper recently published.*

- Minor editorial comments line 23 and 62 -The expansion of hypoxic areas has been quite rapid, but not exponential line 76 – make it clear that this explanation is inference and not based on evidence line 178 “these” presumably refers to “large fish” - better to say so.

We have now edited these specific points.

<u>References</u>

Brander, K (2020). Reduced growth in Baltic Sea cod may be due to mild hypoxia. ICES J. Mar. Sci., doi:10.1093/icesjms/fsaa041.

Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, G., and Hjelm J. 2016. Hypoxic areas, density dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R. Soc. Open Sci., 3, 160416. Doi: 10.1098/rsos.160416.

Chabot, D., and Dutil, J.-D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. J. Fish Biol., 55, 472–491.

ICES (2016). Report of the Workshop on Spatial Analyses for the Baltic Sea (WKSPATIAL), 3-6 November 2015, Rome, Italy. ICES CM 2015/SSGIEA:13. 37 pp.

Limburg, K., and Casini, M. 2019. Otolith chemistry indicates recent worsened Baltic cod condition is linked to hypoxia exposure. Biol. Lett., 15, 20190352.

Neuenfeldt, S., Bartolino, V., Orio, A., Andersen, K. H., Andersen, N. G., Niiranen, S., Bergström, U., Ustups, D. Kallasvuo, M., Kulatska, N., and Casini, M. 2020. Feeding and growth of Atlantic cod (Gadus morhua L.) in the Eastern Baltic Sea under environmental change. ICES J. Mar. Sci., 77: 624–632.

Formatted: English (United States)

<u>Reply to reviewer #2</u>

We thank the reviewer for the helpful comments.

Reply to the general comments:

- The paper is interesting, and the patterns are convincing. Inevitable any conclusions drawn from parallel changes in two or more metrics without a test will be speculative. Nevertheless, I think the authors do a good enough job of highlighting hypoxia as a contributor to decreasing cod condition. However, I think the description on confounding effects and other contribution factors could be improved. For example, although hypoxia may well contribute greatly to low growth of cod in the current system the drivers of a decrease in condition are the triggers of a change in depth distribution and the cause of low oxygen.

We present briefly the potential other factors contributing to the cod condition patterns also in the Introduction to provide some background, specifying that in literature deoxygenation has been advocated as one of the major drivers of the condition decline (e.g. Casini et al. 2016). Our present manuscript is focusing on showing the processes (deepening of cod population and shallowing of low-oxygen layers) explaining the link between the general Baltic deoxygenation and condition (as shown by Casini et al. (2016)) and putting in a population context what found previously in the cod otoliths by Limburg & Casini (2019). We appreciate the reviewer's point, and thus we added some text in the Discussion section about the alternative factors that could contribute to explain the patterns in cod condition. On the other hand, the reasons for the deepening of the cod population have not been investigated, only speculated about in other papers (Orio et al. 2019). These are beyond the scope of our paper, but we have suggested that this is an important question to answer in futures studies.

- Furthermore, there is no description of any statistical analysis. Mostly the patterns are "analyzed" by eye and described in the results chapter (related note referring to figures as you describe results). This approach may occasionally be valid – and the patterns described are convincing enough - but at least some sort of quantification of the size of effects across time should use when describing them (reduced from x to x). A statistic test is used for the otolith data, but this is not included in the methods. The results from the otolith analysis is interesting yet this part of the paper is referred to as an afterthought throughout the paper. I think this analysis warrants increased value, both by adding to the introduction enough background material to allow readers to evaluate the validity of the methods on know of any prior findings and in the a fuller description of methods including how the otoliths were selected.

In the revised manuscript we have been more quantitative, spelling out the most important changes across time. We now also estimated the actual oxygen that the population has been experiencing over time (not only the overlap with low-oxygen levels below a certain threshold) and we perform statistical analysis relating this with fish condition. The otolith analysis has been now deleted from the paper, as suggested by the other reviewers, and we now discuss our results in view of what found by Limburg and Casini 2019.

Reply to the specific comments:

- 26+28: Is "processes" the right word?

We have changed the wording now.

- 100: What is the sample size?

We have added it.

- 101: is this data stable once entered, or is it subject to change? In the last case, a date of retrieval would be handy to include.

We have now added the date of data extraction for the years after 1990, which can undergo slight updates in the ICES DATRAS database. The years before 1990s are from historical databases and therefore not subject to changes.

- 105: there are different ways to measure ‘total length’, maybe explain in more detail how it was done in this study.

Done.

- 107: why is SD26-28 chosen and not for example not 29?

We have now explained the reason.

- 108/109: why is the subdivision of big and small cod made and why those specific lengths? What happens with fish between 29 and 40 cm?

The two length groups for condition were selected to represent small and large fish, as stated in the paper. The small fish can also be seen as juveniles even though the size at maturity has declined with time for this population. The large fish on the other hand can all be considered adults. Currently, there are very few cod above 50 cm and therefore we could not use larger size-classes. We have now been more specific in this part.

- 109: Quarter 4 also includes part of the winter. Why not mentioning the exact months instead of season or quarter 4?

Done

- 117: why are those class divisions different from row 108?

The population distributions, divided in < 30cm and ≥ 30 cm, come from Orio et al. (2019). In the condition estimations, we did not want to use too large ranges of fish sizes in one group because Fulton condition factor (used in the paper we refer to and compare to ours) can be affected by fish size. Moreover, cod start to become piscivorous around 30 cm and therefore fish below 30 cm (but larger than in the plankton- and nektobenthos-feeder phase, around 15 cm) can be considered occupying similar ecological niche. Therefore, the 20-29 and 40-49 size groups were chosen for condition estimation just to represent the small and large sizes with different ecological niches and therefore likely different behavior and food requirements. We have now added some clarification into this part.

- 135: it is later explained, but I would rather put here the <0.8 (Eero et al 2012), explaining the ‘very low’ condition

Done

- 160-191: I see many statements as ‘more’ and ‘lower’ and ‘deeper’, but it is very descriptive, and I miss actual numbers in some places and statistical tests to prove these statements. Also, how many data points were retrieved, how big was the sample size?

We do not think we need statistical tests to explain the long-term patterns, what is important is the overlap between the cod population and low-oxygen layers. However, we tried to add some more quantitative information in the text. We now also estimated the actual oxygen that the population has been experiencing over time (not only the overlap with low-oxygen levels below a certain threshold) and we perform statistical analysis relating this with fish condition. We have also added the samples sizes in the Methods.

- 171: which depth?

Done, we have improved this description.

- 186: The oxygen layers are almost the same, but not totally. I understand this is because they are weighed with the SD-specific distribution of the cod, but I think it makes things clearer if you write somewhere that this means that it differs between the big and the small cod (it took me a while to understand).

Done.

- 267: I miss a note about that it is not 100% sure that the cod are actually in those low oxygen waters, because that was not directly measured. However, the additional otolith results make it very plausible that this is the case.

We agree, fish can move and therefore we cannot be sure that those with very low condition spent most of their time in low-oxygen waters (even if they were caught there) from the time-series, but as the Reviewer #2 also says, this is very plausible also considering the results of the otoliths' analyses in Limburg and Casini (2019).

- 273: Was there a way to directly link otolith chemistry with body condition? (e.g. from the same individual?) Why do you think the overlap between cod and oxygen layers is oscillating? (why is the oxygen stratification oscillating?)

Yes, it is possible analyzing the Mn/Mg elements ratio in the otoliths of individual fish, see Limburg & Casini (2018, 2019).

- 475/476/481/486: you use here the whole word 'subdivision', while in the previous description (472) you al-ready used SD

We have edited this to be more consistent.

- 490 post-2000? This is differently described throughout the text.

We have now deleted the part about otolith analyses, referring instead to Limburg and Casini 2019, as suggested by the other reviewers.

- Figure 3: Is there a possible explanation for the high condition in 1996 in SD25

In general, the mid 1990s are characterized by good oxygen conditions (low extent of hypoxic areas) and a large increase in the sprat stock, probably boosting condition. We feel that going into these details bring us out of the paper's scope and we prefer not to focus on single annual values but on the general patterns.

- Figure 6: 2000 onward is called 'post 2000' in the text. Why are there squares in the boxes

We have now deleted the part about otolith analyses, referring instead to Limburg and Casini 2019, as suggested by the other reviewers.

<u>*References*</u>

Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, G. and Hjelm J. 2016. Hypoxic areas, density dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R. Soc. Open Sci., 3, 160416. Doi: 10.1098/rsos.160416.

Limburg, K.E and Casini, M. 2019. Otolith chemistry indicates recent worsened Baltic cod condition is linked to hypoxia exposure. Biol. Lett., 15, 20190352.

Limburg, K.E. and Casini, M. 2018. Effect of marine hypoxia on Baltic Sea cod Gadus morhua: evidence from otolith chemical proxies. Frontiers in Marine Science, 5: 482.

Orio, A., Bergström, U., Florin, A.-B., Lehmann, A., Šics, I. and Casini, M. 2019. Spatial contraction of demersal fish populations in a large marine ecosystem. Journal of Biogeography, 46: 633-645.

Reply to reviewer #3

We thank the reviewer for the helpful comments.

Reply to the general comments:

- Tendency to oversell the results: this is recurring through the title, abstract and discussion. For example, the study title is not in line with the results. The title implies that the study results alone explain the low condition of Baltic cod, when in reality, the study sheds additional light on one potential mechanism, direct exposure to low oxygen waters, which does not rule out alternative mechanisms (both linked to expanding oxygen minimum zones and to other factors) that have been proposed before. Abstract L27-29: point out more clearly that the study is assessing the role of direct exposure to low oxygen waters, not “the processes”. Discussion L223: should be “one mechanism” not “the mechanisms”. Conclusion L293: should be “shown here one mechanism”, not “the mechanisms”.

In the abstract and discussion, when we speak about processes and mechanisms we refer to the shallowing of low-oxygen layers and deepening of the cod population that create the overlap and therefore the circumstances for a direct exposure effect. However, we have now gone through the manuscript and edited some sentences not to oversell our results.

- Delineation of results from previous work: Exposure to low oxygen water was already previously linked to the Baltic cod condition decline by Limburg and Casini (2019) using otolith microchemistry. This study is cited and referred to by Casini et al., but still, the apparent narrative here is that the exposure to low oxygen waters is shown via the identification of increasing overlap of the depths of low oxygen waters and the cod depth distribution, and that this is then confirmed with otolith microchemistry in this manuscript (e.g., abstract LL 29-34, Introduction LL 89-93, Discussion LL 224-228). This really has it backwards. I suggest to instead clearly lay out key results and conclusions from Limburg and Casini 2019 in the Introduction, and then use this as rationale for the (relevant and interesting) independent confirmation and new insights into the specific patterns of exposure to low oxygen waters in this manuscript.

We have now followed the suggestion from the Reviewer, specifying in the Introduction that in Limburg & Casini (2019) it was shown that fish in low condition at capture were exposed during their lives to lower oxygen levels than those in good condition (at least from the mid-1990s), without saying anything about the distribution of the population, and therefore whether or not a large part of the population indeed experienced stressful circumstances, that could explain the low population condition found in Casini et al. (2016).

- Use and presentation of otolith microchemistry dataset from Limburg and Casini 2019 in this study (connected to previous comment): I would strongly recommend the exclusion of these data from the present manuscript. To me, the analysis and results mirror the previous publication by Limburg and Casini too closely to warrant inclusion here. The authors acknowledge the previous study, but without going into details. However, the dataset, analyses, discussion points (Section4.2) and conclusions are largely the same. Also, the results from otolith microchemistry analyses are not formally correlated to the depth distribution analyses, and appear rather like an “afterthought” in this manuscript. The inclusion in the manuscript thus unnecessarily duplicates previous work. If conclusions from the previous work are instead clearly presented in the Introduction, this will provide the rationale for the real strength and novelty of the present study, the depth distribution analyses. New insights from this independent approach compared to the insights from the original otolith microchemistry approach could then also be discussed more explicitly in the Discussion. Interestingly, all conclusions in the conclusion section of the manuscript (LL293-306) relate to this aspect of the study anyway.

We have now followed the suggestion from the Reviewer, specifying in the Introduction that in Limburg & Casini (2019) it was shown that fish in low condition at capture were exposed during their lives to lower oxygen levels than those in good condition (at least from the mid-1990s), without saying anything about the distribution of the population, and therefore whether or not a large part of the population indeed experienced stressful circumstances, that could explain the low population condition found in Casini et al. (2016).

- Statistical analyses: Right now, the manuscript is lacking in formal statistical assessments. This includes statistical approaches to assess the significance and nature of temporal trends in the depths of low oxygen waters, cod depth distributions and overlap, as well as the formal assessment of the link of overlap and cod condition over time. The Material and Methods should then also include a dedicated section outlining statistical approaches. In this context, looking at Figure 4 of the manuscript, many of the observed temporal changes do not look linear. E.g., for SD26-28, cod mean depth was essentially stable after 1990, and for SD25, neither cod depth distribution nor depth of low oxygen water appears to change significantly between 2008 and 2018. Formal statistical analysis would therefore have the potential to lead to additional insights beyond the points included in the manuscript.

We agree that the trends of the depth patterns are not linear, that is also why standard statistical tests of the temporal patterns would not provide much additional information in our opinion. We now estimated the actual oxygen that the population has been experiencing over time (not only the overlap with low-oxygen levels below a certain threshold) and we perform statistical analysis relating these 2 metrics (overlap and actual oxygen concentration) with fish condition.

Reply to the specific comments:

- Throughout the entire manuscript, I was waiting for an explanation for the discrepancy of the cod depth distribution trends over time between the very similar data sets and analyses in Orio et al. 2019 (showing cod distributions at least for SD26-28 becoming shallower since the 1990s) and this manuscript. This was then given in the second to last sentence of the conclusions:) I suggest to explicitly explain the difference between the datasets (fall versus other seasons) already in the Material and Methods, and then discuss this interesting difference between seasons in the main part of the Discussion, not just in the Conclusion.

We present now a larger discussion of these seasonal differences in the Discussion. We do not think explaining this in the Material and Methods is necessary, since we are very clear that our paper is focusing on Quarter 4.

- L53: Would cite Chabot and Dutil 1999 here already.

Done

- L60: Suggest addition of Reusch et al 2018 as probably best reference for combined strong temporal changes in temperature, eutrophication, oxygen in the Baltic Sea.

Done

- L60-61: to my knowledge, the degradation of benthic communities is NOT well documented in the Baltic Sea, and lack of time series on benthic communities has been one of the issues hampering understanding of consequences of expanding oxygen minimum ones. Rephrase.

Done

- L71: see major comments regarding previous results from Limburg and Casini 2019. Suggest to present in much more depth here and explain that link between low condition and exposure to low oxygen water was established in that study.

Done. We have now specified in the Introduction that in Limburg & Casini (2019) it was shown that fish in low condition at capture were exposed during their lives to lower oxygen levels than those in good condition (at least from the mid-1990s), without saying anything about the distribution of the population, and therefore whether or not a large part of the population indeed experienced stressful circumstances, that could explain the low population condition found in Casini et al. (2016).

- L73: suggest to mention the actual mechanism connected to this, density dependence.

Done, but we also meant change in the habitat occupation, not only contraction, we have now rephrased the sentence.

- L73-75: add mechanism proposed by Brandner 2020, mild hypoxia reducing rate of digestion.

We consider this mechanism already included in the sentence (stress due to hypoxia exposure). This part of the Introduction has been however changed extensively now.

- L92-96: The otolith works comes in like an afterthought here, since it is not set up in any way in the Introduction section (linked to major comment regarding otolith work)

We have now removed the part about the otolith analysis from the paper, and instead discussed our results in view of Limburg & Casini (2019) in the Discussion.

- Section 2.1: more clearly point out that this (or very similar) cod condition time series were previously published and are here updated to 2018?

Done.

- LL107-109: please explain rationale of using size class 20-29 and 40-49 cm for condition calculations.

Done.

- Section2.2: suggest to point out more clearly the key difference between studies, focus on fall here versus all seasons in Orio et al 2019 (see my previous comment above).

We already stated here that we used the model depth estimates in Quarter 4, consistent with the oxygen used in the study. We prefer to speak about the differences with Orio et al. 2019 in the Discussion.

- LL125-135: I am not a physiologist, but I guess in principle use of oxygen as continuous variable (instead of somewhat arbitrary boundaries) would make sense. I can see that use of specific limits facilitates analysis, but would mention this possible limitation.

The sub-lethal boundary we used (4 ml/l, now more precisely set at 4.3 ml/l following Reviewer #1 comments) is from the experiment by Chabot and Dutil (1999), it is not arbitrary although based on cod from another region. About the boundary 1 ml/l (avoidance), it is a well known boundary for Baltic Sea cod (Schaber et al. 2012). However, we have now also shown, and made analyses with, the actual oxygen experienced by the population.

- L155: Explain the rationale of using a Fulton's k of 0.9. Also give other thresholds(e.g., "very low" used later in L163) here already.

This part has now been deleted and we refer now to the results of Limburg and Casini (2019).

- Section 3.2: in the Discussion section (not here), suggest to discuss the patterns observed for fall here compared to the patterns in Orio et al 2019 reporting cod depth distribution contraction to shallower water for SD26-28 when looking at the entire year.

Done.

- Section 3.2, 3.3, 3.4: would all benefit a lot from formal statistics.

We agree that the trends of the depth patterns are not linear, that is also why standard statistical tests of the temporal patterns would not provide much additional information in our opinion. We now estimated the actual oxygen that the population has been experiencing over time (not only the overlap with low-oxygen levels below a certain threshold) and we performed statistical analysis relating these metrics (overlap and actual oxygen experienced) with fish condition.

- L243: I think the discussion of mechanisms that can explain what drives cod into layers with low oxygen levels is quite central, since it relates to the key novel finding of this manuscript. Suggest to therefore not state that "beyond scope" of manuscript, but rather state that you can only speculate and will discuss possible causes as systematically as possible.

Our paper is focusing on showing the processes explaining the link between the general Baltic deoxygenation and condition (as shown by Casini et al. (2016)) and putting in a population context what found previously in the cod otoliths by Limburg & Casini (2019). Therefore to link the population overlap with low-oxygen waters with fish condition. We really think that explaining the reasons why cod move deeper in autumn deserve a full analysis and this is beyond our scope. We have however provided a potential explanation to the deepening of the distribution in the paper and we say that focused analyses should be done to provide an answer to this interesting question.

- LL244-245: The role of temperature was also the first thing that came to my mind, but I then wondered about actual temperature profiles in fall, and whether they would support these considerations. It would be useful to include information on prevailing temperature depth profiles in fall as background for the discussion.

Since we do not deal with the reasons of the increased depth of the cod population, we prefer not to present temperature (or other information), that would be incomplete to make such analyses on draw conclusions. We think that a focused analysis should be done to answer this question.

- L283: Should read "although we have confirmed here that ..." and refer to Limburg and Casini 2019.

Done.

- LL283-291: Discussion of other factors could be more extensive. Cite Brander et al 2020 here as well.

Done.

- L297: Agree, very interesting future direction, and a question that really results for the first time from the analyses in this manuscript (not possible from Limburg and Casini 2019) – this would be worth pointing out.

We have now deleted the otolith part. We think that the novelty of this new paper (the finding that the population has been progressively more experiencing low-oxygen waters, and that this was due to both a shallowing of low-oxygen layers and deepening of the population) is now clear.

- Figures: I suggest to add a figure to illustrate key findings regarding the correlation of cod condition and the overlap of cod depth distribution and low oxygen waters.

We have now analysed the relation between the actual oxygen experienced and condition that will change somewhat the disposition of the figures.

- Related to the general comment regarding the presentation of otolith microchemistry data in this manuscript, Figure 6 of this manuscript appear to be an alternative view of Figure 2 c in Limburg and Casini 2019, i.e., not adding new information here that could not be provided from that manuscript.

We have now deleted the otolith analysis and referred to, and discussed the results of, Limburg and Casini (2019) instead.

Technical corrections

- LL23-24, L62: wording should be more precise – "exponential increase" not really correct, suggest "strong increase"; "largest marine dead zone", unnecessarily dramatic.

We agree about the first suggestion, but not about the second since the low-oxygen zones are called indeed "dead zones" in literature.

- LL26: "elusive" does not really reflect that specific alternative mechanisms have been proposed.

Here we meant, as stated, that the processes behind the statistical relation between general hypoxia and cod population condition found previously remained elusive.

- LL29-32: rephrase, confusing wording.

Done.

- L59-60: Wording in Breitburg et al 2018 is more scientific ("low O2 areas have become more extensive and severe") – suggest to follow this approach.

Dead-zones is a term used commonly in literature, named also in Breitburg et al. (2018), we prefer to keep this terminology here.

- L81: start new paragraph, focusing on effects and not mechanisms from here on.

Done.

- L82-82: rephrase "lamented"

Done.

- L194: "in a couple..." – word missing?

Correct, done.

- L254: "hostile waters" – suggest to rephrase

We would like to keep this wording, giving a clear idea of the concept.

<u>References</u>

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., Telszewski, M., Yasuhara, M., and Zhang, J. 2018. Declining oxygen in the global ocean and coastal waters. Science, 359, eaam7240. Doi:10.1126/science.aam7240.

Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, G., and Hjelm J. 2016a. Hypoxic areas, density dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R. Soc. Open Sci., 3, 160416.

Chabot, D., and Dutil, J.-D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. J. Fish Biol., 55, 472–491.

Limburg, K., and Casini, M. 2019. Otolith chemistry indicates recent worsened Baltic cod condition is linked to hypoxia exposure. Biol. Lett., 15, 20190352.

Schaber, M., Hinrichsen, H.-H, and Gröger, J. 2012. Seasonal changes in vertical distribution patterns of

Changes in population depth distribution and oxygen stratification explain the current low condition of the Eastern Baltic Sea cod (*Gadus morhua*)

Michele Casini[1,2], Martin Hansson[3], Alessandro Orio[1], Karin Limburg[1,4]

[1] Swedish University of Agricultural Sciences, Department of Aquatic Resources, Institute of Marine Research, Lysekil, Sweden

[2] University of Bologna, Department of Biological, Geological and Environmental Sciences, Bologna, Italy

[3] Swedish Meteorological and Hydrological Institute, Gothenburg, Sweden

[4] State University of New York College of Environmental Science and Forestry, Department of Environmental and Forest Biology, Syracuse, New York, USA

Corresponding author: Michele Casini; e-mail: michele.casini@slu.se

Field Code Changed

Abstract

During the past twenty years, hypoxic areas have expanded ~~exponentially~~ rapidly in the Baltic Sea, which has become one of the largest marine "dead zones" in the world. At the same time, the most important commercial fish population of the region, the Eastern Baltic cod, has experienced a drastic reduction in mean body condition, but the processes behind the relation ~~relating~~ between ~~hypoxia~~ deoxygenation ~~to~~ and condition remain elusive. Here we use extensive long-term monitoring data on cod biology and distribution as well as on hydrological variations, to investigate the processes that relate deoxygenation and cod condition during the autumn season. Our results show that the depth distribution of cod has increased during the past four decades at the same time of the expansion, and shallowing, of waters with oxygen concentrations detrimental ~~of the waters with an oxygen concentration known to be detrimental for~~to cod performance. This has resulted in a progressively increasing spatial overlap between the cod population and low-oxygenated waters after the mid-1990s~~, which relates with the observed decline in cod mean body condition~~. This spatial overlap and the actual oxygen ~~levels~~concentration experienced by cod therein statistically explained the changes in cod condition over the years. These results complement previous ~~Complementary~~ analyses on fish otolith microchemistry that also revealed that since the mid-1990s, cod individuals with low condition were ~~indeed~~ exposed to low-oxygen waters during their life. This study helps to shed light on the processes that have led to a decline of the Eastern Baltic cod body condition, which can aid the management of this population currently in distress. Further studies should focus on understanding why the cod population has moved to deeper waters in autumn and on analysing the overlap with low-oxygen waters in other seasons to quantify the potential effects of the variations in physical properties on cod biology throughout the year.

Keywords: hypoxia, fish body condition, direct exposure, depth distribution, cod *Gadus morhua*

1. Introduction

The oceans and marine coastal areas are experiencing dramatic deoxygenation worldwide (Breitburg et al., 2018). Declining oxygen can have multiple direct and indirect effects on aquatic organisms and entire ecosystems (Breitburg, 2002; Rabalais et al. 2002; Wu, 2002; Diaz and Rosenberg, 2008; Levin et al., 2009). In particular, studies undertaken both in the wild and within experimental set-ups have revealed large effects of hypoxia on basic metabolism, behavior, ecology, distribution and life-history traits of fish (Chabot and Dutil, 1999; Pichavant et al., 2001; Eby et al., 2005; Herbert and Steffensen, 2005; Domenici et al., 2007; Stramma et al., 2012).

The Baltic Sea (Fig. 1) is one of the largest brackish areas in the world where the oxygenated, yet scarce and irregular saline water inflows from the adjacent North Sea, combined with a water residence time of about 25–30 years, make the system particularly prone to hypoxia (Carstensen et al., 2014; Reusch et al., 2018). As a consequence, and in combination with global warming and eutrophication, the Baltic Sea has become one of the largest anthropogenic “dead zones” in the world (Breitburg et al., 2018), with ~~well documented~~ degradation or elimination of benthic communities and disruption of benthic food webs over vast areas (Conley et al., 2009). In particular, since the early 1990s the anoxic and hypoxic areas have increased ~~exponentially~~ rapidly in the southern and central Baltic Sea (Carstensen et al., 2014) (Fig. 2).

In this degraded demersal and benthic environment, ~~the~~ body condition (a morphometric index of fish fatness and well-being) of the dominant demersal fish population, the Eastern Baltic cod *Gadus morhua* (hereafter simply referred to as Baltic cod), has declined since the mid-1990s (Casini et al., 2016a). This decline ~~cod stock~~ was ~~.~~also stressed by the fishery that suffered from an increasingly high proportion of catches of lean cod with low economic value. Low condition has a negative effect on reproductive potential (Mion et al., 2018), mortality (Casini et al., 2016b) and potentially also movements (Mehner and Kasprzak, 2011) with indirect effects on prey and therefore food-web structure and ecosystem functioning as shown in other systems (e.g. Ekau et al., 2010). Therefore, it is very important to understand the ultimate factors leading to low cod condition.

~~This~~ ~~In literature, t~~The decline in the Baltic cod condition has been related to a decrease in the main pelagic prey abundance in the main distribution area of cod (Eero et al., 2012; Casini et al., 2016a) and increased parasite infestation (Horbowy et al., 2016), but also to the increased extent of hypoxic and anoxic areas (Casini et al., 2016a). ~~However, the underlying mechanisms of the relationship between cod condition and hypoxia are still elusive (but see Limburg and Casini, 2019).~~ The mechanistic processes linking hypoxia and cod conditions could be various and not mutually exclusive, including stress due to direct hypoxia exposure, suitable habitat contraction and consequent ~~contraction in the spatial distribution of the population~~change in cod spatial distribution, and change in the surrounding biota such as reduction of important benthic prey (Casini et al., 2016a). Limburg and Casini (2019), using otolith microchemistry, showed that fish in low condition at capture were exposed during their lives to lower oxygen levels than those in good condition (at least from the mid-1990s), suggesting that direct exposure to low-oxygen waters could constitute a key factor. However, Limburg and Casini (2019) did not analyse the spatial distribution of the cod population in relation to low-oxygen layers, and therefore whether or not a large part of the population indeed experienced stressful circumstances, which could explain the decline in mean population condition found by Casini et al. (2016a). ~~A recent study pointed out the importance of the decline in the feeding level and energy intake of cod after the mid-1990s, which was explained by the~~ a potential ~~decline in important benthic prey in the environment (Neuenfeldt et al., 2019)~~ ~~or decreased cod appetite due to exposure to low-oxygen waters (Brander, 2020).~~

~~Lately some investigations have also put forward the hypothesis that the observed changes in the distribution of demersal fish species, including cod, were due to the variations in the extent of the hypoxic areas in the Baltic Sea (Orio et al., 2019), although in-depth analyses were not performed to confirm this hypothesis. The low cod condition in recent decades has been stressed also by the fishery that has lamented~~ suffered ~~an increasingly high proportion of catches of lean cod with low economic value. Low condition has a negative effect on reproductive potential (Mion et al., 2018), mortality (Casini et al., 2016b) and potentially also movements (Mehner and Kasprzak, 2011) with indirect effects on prey and therefore food-web structure and ecosystem functioning as shown in other systems (e.g.~~

Ekau et al., 2010). Therefore, it is very important to understand the ultimate factors leading to low cod condition and in particular the processes explaining the correlation between cod condition and deoxygenation of the Baltic Sea water over time.

In this study, we fill this gap and we, we further examine the mechanisms linking deoxygenation to cod condition in the Baltic Sea. We specifically analyse the temporal changes in the depth distribution of cod, from long-term monitoring data, in relation to the actual oxygen levels experienced by the cod population and the oxygen levels acknowledged in literature to affect cod behavior and performance and the actual abiotic conditionsoxygen levels experienced by cod the population. We support these analyses investigating the relation between fish exposure to hypoxia and cod condition using otolith microchemistry. Fish otoliths (ear stones) composed of aragonite accrete continually throughout life and incorporate trace elements, providing a direct, retrospective measure of an individual fish's environmental and physiological history.

2. Materials and methods

2.1 Biological data and estimation of cod condition

Biological data on Eastern Baltic cod individuals (n = 124 165) were collected during the Baltic International Trawl Survey, BITS, between 1991 and 2018 (retrieved from the DATRAS database of the International Council for the Exploration of the Sea, ICES; www.ices.dk; downloaded 28 January 2020) and previous Swedish and Latvian bottom trawl surveys performed in 1979-1990 in the Baltic Sea (Casini et al., 2016a). Cod individual body condition (Fulton's K) was estimated as $K = W/L^3 * 100$, where W is the total weight (g) and L the total length (cm, typically measured from the tip of the snout to the tip of the longer lobe of the caudal fin) of the fish. Mean condition was estimated for ICES Subdivision (SD) 25 (corresponding to the main distribution area for Eastern Baltic cod since the early 1990s, Orio et al., 2017) and SDs 26-28 separately, updating the time-series in Casini et al. (2016a). More northern SDs were not included due to their low and inconsistent survey coverage through the years. Condition was estimated for small fish (represented here by the size-class 20-29 cm) and large

Field Code Changed

Formatted: English (United Kingdom)

fish (represented here by the size-class 40-49 cm) were used in the analyses, as cod change diet during its ontogeny and these two size-classes differ in feeding habits (Neuenfeldt et al., 2020). We could not use smaller and larger size-classes because of their scarcity in the BITS survey catches. The small size-class could also be considered as representing juvenile fish (Eero et al., 2015; ICES, 2017a) although, from around 2005, the mean length at first maturity has decreased below 30 cm (Köster et al., 2017). Years with < 25 observations for the respective length-classes and areas were excluded from the analyses. We focused on the cod condition in autumn (quarter 4 BITS survey, from ~~autumn (~~mid-~~i.e. quarter 4~~October to mid-December), corresponding to the cod main growth season after spawning in spring-summer (Mion et al., 2020). Moreover, for the autumn season, long time-series of oxygen levels and extent of hypoxic areas are also available (Casini et al., 2016a).

2.2 Estimation of cod depth distribution

Indices of cod biomass (calculated as catch-per-unit-effort, CPUE, kg/h, herein referred to as biomass) and depth distribution (i.e. mean depth and interquartile range of predicted depth distribution) from the BITS and historical bottom trawl surveys in SDs 25-28 from 1979 to 2018 were estimated for large (≥ 30 cm) and small cod (~~15-~~< 30 cm) using a modelling procedure similar to the one used in Orio et al. (2019). However, in the current study rather than including environmental variables in the models, quarter was included in interactions with latitude and longitude, and with depth. To estimate the changes in cod depth distribution in SDs 26-28 that account for the changes in the spatial distribution of the cod population, the SD-specific depth distributions were weighted by the annual SD-specific cod CPUEs from the bottom trawl surveys in quarter 4, estimated from the same model, for large and small cod.

2.3 Depth of hypoxic layers

Baltic cod has been shown to avoid oxygen concentrations below 1 ml/l (approximately 1.4 mg/l) (Schaber et al., 2012). Therefore, time-series of the depth at which 1 ml/l oxygen concentration was encountered by SD were obtained from the Swedish Meteorological and Hydrological Institute (SMHI, www.smhi.se).

Field Code Changed

Time-series of depth at which 4.3 ml/l oxygen concentration (approximately 6 mg/l) was encountered by SD were also obtained from SMHI. This oxygen concentration, on average, has been found to affect the performance of fish (Vaquer-Sunyer and Duarte, 2008). Specifically for cod, 4.3 ml/l has been found as threshold ~~under~~ from which an effect on condition and growth starts to be observable (Chabot and Dutil, 1999). Therefore, we expected that the occurrence of cod in areas and depths with an oxygen concentration ≤ 4.3 ml/l would lead to an increase in the proportion of cod individuals with very low condition (K < 0.8; Eero et al., 2012) and a decrease in mean condition in the population. To relate the depths at which 1 ml/l and 4.3 ml/l oxygen concentrations were encountered to cod depth of occurrence ~~and condition~~ in SDs 26-28, the oxygen depths ~~by~~ in each SD were weighted with the annual SD-specific cod CPUEs from the bottom trawl surveys ~~estimated from the same models~~ in quarter 4, for large and small cod. In this way, the oxygen circumstances in the SDs where cod was more abundant were weighted the most.

2.4 Depth overlap between cod and hypoxic layers, and oxygen experienced by cod

We estimated the overlap (% meters) between the cod range of depth distribution and the water layer with oxygen concentration ≤ 4.3 ml/l, as estimated above, in both SD 25 and SDs 26-28. We also reconstructed the time-series of the oxygen concentrations at the mean depth and interquartile range of the predicted cod depth of distribution in each SD (data from SMHI). Also in this case, for SDs 26-28 the oxygen concentrations in each SD were weighted with the annual SD-specific cod CPUEs from the bottom trawl surveys in quarter 4, for large and small cod.

2.5 Modelling of cod condition versus oxygen

To formally analyse the effect of the depth overlap and oxygen concentrations experienced by cod on cod condition, we used generalized additive models (GAMs; Hastie and Tibshirani, 1990). The following additive formulation was used:

Condition ~ s (*Depth overlap*) + s (*Oxygen experienced*) + ε

where *Depth overlap* is the overlap between the cod depth range of distribution and the water layer with oxygen an concentration ≤ 4.3 and *Oxygen experienced* is the actual oxygen level corresponding to the cod depth distribution (we used for this the oxygen corresponding to the deeper interquartile of the cod depth range of distribution). *s* is the thin plate smoothing spline function and *ε* is random error. We limited the maximum degrees of freedom acceptable for each term to *k*=4, which retains model flexibility and allows at the same time an ecological interpretability of the results. A Gamma distribution with an identity function was used. Residuals were inspected for deviation from the assumption of normality and no autocorrelation using graphical methods (Cleveland, 1993). The statistical analyses were performed using the mgcv library of R v. 4.0.2 (www.r-project.org). The significance level was set to α =0.05 for all tests.

2.4 Otolith microchemistry

Otoliths (N = 154) were selected from Baltic cod collected in the study area in the 1980s-2010s from BITS and historical bottom trawl surveys in February (Limburg and Casini, 2019). These were cleaned, transversely sectioned, and analysed by laser ablation inductively coupled plasma spectrometry. A spot of 100-micron diameter was driven at 5 µm/sec, 10 Hz, to create a transect from the otolith core to the outer dorsal edge, collecting a suite of elements (see Limburg and Casini, 2018 for details). For the analysis described here, we took the ratio of manganese to magnesium along this continuous transect. Manganese, although redox sensitive and thus available as dissolved Mn^{2+} and Mn^{3+} at low oxygen concentrations, is also affected by the fish's growth rate (Limburg et al., 2015; suggested by Thomas et al., 2019). Dividing manganese by the corresponding, growth sensitive magnesium (from the same replicate) to some extent corrects for the growth effect (Limburg and Casini 2018, 2019). Our metric for hypoxia exposure is the fraction of an annual growth band wherein this Mn/Mg ratio exceeds an age-based threshold (Limburg and Casini 2018, 2019). We tested this metric as a function of cod condition categorized into "high" (condition ≥ 0.9) and "low" (condition < 0.9) groups, and tested whether this had changed over time (before the year 2000, and from 2000 onward).

3. Results

3.1 Cod condition

Cod condition increased slightly between the mid-1970s and mid-1990s~~,~~ but declined abruptly thereafter. This pattern was similar in SD 25 and SDs 26-28 for both small and large cod (Fig. 3), but after the mid-1990s condition dropped more for large cod (~30% for large cod and 20-25% for small cod). The percentage of large fish with very low condition (< 0.8, see Eero et al., 2012) increased from the end of the 1990s in both SD 25 and SDs 26-28 reaching in recent years 30-40%. The percentage of small fish with low condition also increased, but lagged temporally behind the large cod, and at 10-20% of observations was lower than the high incidences of large cod in poor condition (Fig. 3). In general, in SD 25 condition declined slightly more (and the percentage of fish with very low condition increased more) than in SDs 26-28 after the mid-1990s.

3.2 Cod depth distribution

Large cod in SD 25 were distributed between ~~30~~ 35 and 50 m depth (average of ~~40~~ 43 m depth) at the beginning of the time-series, but have been found in somewhat deeper waters (down to 40-60 m, average 50 m depth) ~~since~~ from the ~~late~~ early ~~1990s~~ 2000s ~~(~~Fig. 4A). In SDs 26-28 large cod were distributed between 35 and 55 m depth (average 45 m) at the beginning of the time-series, ~~while~~ whereas ~~afterwards they moved deeper and since~~ after the mid-1990s they became distributed between 50 and ~~70~~ 75 m depth (average ~~60~~ 62 m) (Fig. 4C). Along with the change in mean depth, large cod in SDs 26-28 have shown a contraction of the range of depth distribution in the past 20 years. Small cod were distributed somewhat shallower than the large fish, but also moved into deeper waters during the time period investigated. In SD 25, ~~these~~ small cod shifted distribution from between 30 and 50 m depth (average 40 m depth) to 45-60 m depth (average 53 m) (Fig. 5A). In SDs 26-28 small cod moved deeper with time as well, from 30-50 m depth (average 40 m) to ~~50~~45-65~~3~~ m depth (average 55 m), and experienced a contraction of the range of depth distribution similar to what occurred for the large fish in this area (Fig. 5C).

3.3 Depth of hypoxic layers

The depth at which 1 ml/l was encountered remained fairly constant at around 70 m in SD 25, while in SDs 26-28, it decreased from below 1~~0~~20 m to ~~70~~ around 80 m over the past 20 years ~~while in SDs 26-28 it became shallower from being deeper than 100 m before the early 1990s to 70-80 m in the past twenty years~~ (Fig. 4A,C and 5A,C). ~~The~~ Over this same time period, the depth at which 4.3 ml/l was encountered diminished in SD 25 from ~ 60~~-65~~ m ~~at the beginning of the time period~~ to ~ 50~~-55~~ m ~~during the past twenty years~~, ~~while~~ and in SDs 26-28 ~~it became shallower from being~~ the 4.3 ml/l threshold shifted from ~~70-~~ ~ 80 m ~~before the early 1990s~~ to ~~55-~~ ~ 60 m ~~in the past fifteen years~~since the early 1990s (Fig. 4A,C and 5A,C). The oxygen depths in SDs 26-28, accounting for the SD-specific distribution of the cod, did not differ much between large and small cod (note the slightly different patterns in the oxygen depths between Fig. 4C and Fig. 5C, which is due to the different distribution of small and large cod among these three SDs).

3.4 Depth overlap between cod and hypoxic layers, and oxygen experienced by cod

L~~In SD 25, l~~arge cod depth distribution never overlapped with the depth ~~with~~ of oxygen ≤ 1 ml/l along the time period analysed except in the very last year in SD 25 ~~, while in SDs 26-28 there was an overlap in a couple of years toward the end of the time series~~ (Fig. 4A,~~-~~C). On the other hand, large cod distribution heavily overlapped with the depth with oxygen ≤ 4.3 ml/l since the mid-1990s (Fig. 4A,C) and the overlap, although oscillating, increased in the past twenty years reaching values above ~~50~~90% in SD 25 and ~~up to~~above ~~100~~80% in SDs 26-28 (Fig. 4B,~~-~~D).

Also s~~S~~mall cod distribution never overlapped with depth with oxygen ≤ 1 ml/l along the time period analysed, ~~neither in SD 25 nor SDs 26-28~~ except in the very last year in SD 25 (Fig. 5A,C). On the other hand, small cod distribution overlapped with the depth with oxygen ≤ 4.3 ml/l since ~~mid~~early-~~1990s~~ 2000s (Fig. 5A,C) and the overlap, although oscillating, increased in the past fifteen years reaching values higher than ~~60~~90% ~~both~~ in SD 25 and up to 50% in SDs 26-28 (Fig. 5B,D).

The actual oxygen concentrations experienced by cod changed extensively during the study period. Large cod in SD 25 experienced oxygen concentrations of 5-7 ml/l (average ~ 6.5 ml/l) during the late 1970s and early 1980s, while especially from the mid-1990s a decline, paralleled by a widening of the experienced oxygen range, occurred until reaching values of 2.5-5.5 ml/l (average ~ 4 ml/l). Similar patterns occurred also in SDs 26-28 although the oxygen at the lower interquartile range of the predicted cod depth distribution declined further down to be close to 1 ml/l.

Small cod in SD 25 experienced oxygen concentrations of 6-7.5 ml/l (average ~ 7 ml/l) during the late 1970s and early 1980s, while especially from the late-1990s a decline, paralleled by a widening of the experienced oxygen range, occurred until reaching values of 3-6 ml/l (average ~ 4 ml/l). Similar patterns occurred also in SDs 26-28 although the oxygen experienced in the latest years was relatively better than in SD 25, being 3.5-6.5 ml/l (average ~ 5 ml/l).

There was a strong positive correlation between the percentage of the cod population in waters ≤ 4.3 ml/l and the percentage of cod individuals with very low condition (for large cod, r = 0.71 and 0.74 in SD 25 and SDs 26-28, respectively; for small cod, r = 0.58 and 0.59 in SD 25 and SDs 26-28, respectively). There was also a strong negative correlation between the percentage of the cod population in waters ≤ 4 ml/l and mean cod condition (for large cod, r = –0.77 and –0.76 in SD 25 and SDs 26-28, respectively; for small cod, r = –0.60 and –0.54 in SD 25 and SDs 26-28, respectively).

3.5 Modelling of cod condition versus oxygen

The GAMs explained 68.3 % and 61.8 % of the total deviance, for large and small cod, respectively (see the caption of Fig. 6 for more statistics). For both models, *Oxygen experienced* by cod was the most important predictor of condition, while *Depth overlap* explained a minor part of the model deviance. For large cod, the effect of *Oxygen experienced* was positive and seemed to reach an asymptote at around 5 ml/l (Fig. 6A), while for the small cod this was not the case with a positive effect over the whole range of the experienced oxygen (Fig. 6B). *Depth overlap* was negatively correlated to condition, although this effect was much stronger for the large cod (Fig. 6A,B). The residuals of the models did not strongly violate the normality and homogeneity assumptions and were not autocorrelated (Fig. S1). The use of GAMs with an interactive formulation (i.e. *Depth overlap* and *Oxygen experienced* used in interaction) explained a similar amount of deviance (69.4 % and 62.1 %, for large and small cod, respectively).

~~**3.5 Otolith microchemistry**~~

~~Fish exposed to hypoxia as measured by otolith chemistry showed different responses as a function of their condition at time of capture and the time period (pre- or post 2000; Fig. 6). Prior to 2000, the annual duration of hypoxia exposure was relatively low (35.4%); for the years 2000 and onward, the percent duration rose to 51.8%. More strikingly, when divided further into groups by fish condition, pre-2000 fish were not significantly different with respect to hypoxia exposure regardless of condition. After 2000, fish with condition < 0.9 had been exposed considerably longer to hypoxia (62.7% ± 3.6) than fish with condition ≥ 0.9 (40.9% ± 5.1; Fig. 6). The effect sizes of interaction of time period and condition were large and highly significant ($F_{1,746}$ = 23.287, p = 2 x 10^{-6}).~~

4. Discussion

In this paper, we analysed the potential mechanisms relating Baltic Sea deoxygenation with changes in Eastern Baltic cod body condition during the past four decades. To this end, we investigated the changes in depth distribution of the cod population and the vertical changes in oxygen gradients based on long-term biological and hydrological monitoring data. Moreover, we ~~supplemented~~ related the results of these analyses with proxies for hypoxia exposure from individual fish otolith microchemistry recently published in literature.

4.1 Cod depth ~~of~~ distribution and overlap with hypoxic ~~areas~~layers

Our analyses show an increase in the areas with an oxygen level below cod tolerance (i.e. oxygen ≤ 1 ml/l; Schaber et al., 2012). Moreover, this oxygen threshold has also shifted with time towards shallower depths, determining an overall contraction of the potentially suitable habitat for cod (Casini et al., 2016a). Declines in oxygen concentrations have caused a contraction of the habitat and the distribution of fish in other systems (Eby and Crowder, 2002; Stramma et al., 2012; Breitburg et al., 2018) with measurable effects on, for example, individual growth (e.g. Campbell and Rice, 2014). In the Baltic Sea, however, this change seems not to have affected the cod depth of distribution in autumn, since the latter has been always above 70-75 m, a depth only almost never ~~in few years~~ reached by the waters with 1 ml/l. On the other hand, it could be hypothesized that during the latest decade the cod population was unable to occupy even deeper habitats because of the vertical rise of this oxygen layer. This hypothesis seems to be supported by the decline in the range of depth distribution (i.e. a squeeze of the cod habitat occupation) shown by both large and small cod in SDs 26-28 during the past twenty years. Explaining the temporal changes in the depth distribution of cod is beyond the scope of this paper, but a potential reason could be that cod seek deeper layers to avoid too warm waters, which could be detrimental when resources are scarce. In fact, pelagic prey have declined after the mid-1990s in the southern and central Baltic Sea (Casini et al., 2016a) and therefore cod might go deeper to optimize metabolism. Small cod, moreover, could seek deeper waters to escape from the predation of the increased seals and aquatic birds (Orio et al., 2019). The temporal patterns revealed by our study for quarter 4 generally conform to what found by Orio et al. (2019) for quarter 1. However, the increased depth distribution of large cod in SDs 26-28 found in our study in quarter 4 contrasts with ~~what found~~findings in the same areas in quarter 1, where a shallowing has instead been observed since the mid-1990s after an initial deepening (Orio et al., 2019). In quarter 1 cod is in general distributed deeper than in quarter 4 (Orio et al., 2019 and this study, respectively) and it could be that in quarter 1, when the water is also less stratified, large cod were able to dwell in exceptionally deep layers in the mid-1990s because of the good oxygen circumstances during that period (Carstensen et al. 2014; this study). A different requirements that the large mature fish have for their maturation and successful reproduction when they approach spawning in late spring (Røjbek et al., 2012) could potentially also contribute to the difference in the temporal patterns of distribution between the quarters.

The depth where dissolved oxygen falls to ≤ 4.3 ml/l (“sub-lethal” level, i.e. level that has been shown in previous studies to affect cod performance; Chabot and Dutil, 1999; Vaquer-Sunyer and Duarte, 2008) has shallowed during the past four decades, as a consequence of deoxygenation. Our analysis revealed that this vertical rise, together with the deepening of the cod depth distribution, has resulted in that cod has started to dwell more and more in these hostile low-oxygen waters. ~~This is consistent with observations of hypoxia exposure proxied by otolith chemistry (Limburg and Casini, 2018 and 2019; this study, see below).~~ The depths at which cod has been dwelling during the past two decades correspond to the depths of the Baltic Sea permanent stratification where the oxygen drops quickly, explaining the wider range of oxygen concentrations that cod has experienced during this period. Moreover, our analysis reveals that the oxygen concentrations that the cod population indeed experienced has progressively decreased until approaching the 1 ml/l at the lowest boundary of its distribution (tolerance level, i.e. level that has been shown in previous studies to be avoided by cod; Schaber et al., 2012). The overlap between cod depth distribution and “sub-lethal” oxygen layers occurred ~~and reinforced only~~mainly after the mid-1990s, concomitant with the ~~decline~~ drop in cod condition, while in earlier years the cod population was occurring mostly above those layers. ~~Therefore, according to our expectations and hypothesis, the negative effects of hypoxia on cod condition could only arise after the mid-1990s. This is also in accordance with our otolith microchemistry analysis (Limburg and Casini (2019), see section 4.2 below) and previous investigations that suggested that in the earlier years (before the mid-1990s) cod condition was regulated by other factors, such as pelagic prey biomass and density dependence (Casini et al., 2016a, Limburg and Casini, 2019).~~ The progressively higher proportion of the cod population in “sub-lethal” oxygen layers after the mid-1990s, as revealed by our study, conforms also to the increasingly higher proportion of individuals in extremely low condition (< 0.8 Fulton’s K), which include starving fish and fish close to the condition mortality threshold (Eero et al., 2012; Casini et al., 2016b).

4.2 Relation to ~~O~~otolith microchemistry from literature

Limburg and Casini (2019) using otolith microchemistry recently found that Baltic cod with low condition at capture experienced during their lives lower oxygen levels than cod with high condition. However, Limburg and Casini (2019) did not analyse to what extent the cod population indeed experienced low-oxygen levels, and therefore whether the exposure to low-oxygen waters could explain the decline in the mean condition of the cod population. Our study did this, showing that a large part of the population ~~have~~has dwelled in sub-lethal low-oxygen levels after the mid-1990s in quarter 4. Together, the individual-based study by Limburg and Casini (2019) and the population-level present study provide consistent and robust indications that the decline in mean cod condition of the population from the mid-1990s is due to an increased overlap with low-oxygen layers. This suggests that currently~~,~~ condition may carry over from chronic exposure to low oxygen concentrations, which weakens fish and produces a cascade of effects, from reduced metabolic scope leading to lower activity and slower digestion (Claireaux and Chabot, 2016), to greater susceptibility to disease and parasites (e.g., Sokolova et al., 2018). Both Limburg and Casini (2019) and the present study also revealed that the exposure to low-oxygen ~~layers~~concentrations was lower in the period before the mid-1990s, and was unrelated to cod condition confirming that, before the mid-1990s, factors other than direct low-oxygen exposure played a greater role in shaping cod condition as concluded also by Casini et al. (2016a).

4.3 Mechanisms shaping cod condition~~The complementary analyses performed on fish otolith microchemistry confirmed that since the mid-1990s, cod individuals with low condition were indeed exposed to low-oxygen waters during their life. Duration of hypoxia exposure as measured in Baltic cod otoliths has increased markedly since mid-1990s (Limburg and Casini, 2018) and was found in our study to be significantly greater in fish in poor condition at time of capture. This is a remarkable finding, given that condition is measured only once during life (at capture), and the observations of hypoxia exposure are taken throughout life. This suggests that currently, condition may carry over from chronic exposure to low oxygen, which weakens fish and produces a cascade of effects, from reduced metabolic scope leading to lower activity and slower digestion (Claireaux and Chabot, 2016), to greater susceptibility to disease and parasites (e.g., Sokolova et al., 2018). In contrast, in fish captured prior to 2000 the overall exposure to hypoxia was lower and showed no relationship with condition. Thus the otolith microchemistry analysis confirmed the that, pre-2000, factors other than hypoxia played a greater role in shaping cod condition as concluded also by Casini et al. (2016a).~~

W~~Although w~~e have ~~shown~~ confirmed here, using population-level monitoring data, that direct oxygen exposure is likely a key factor shaping cod condition after the mid-1990s (Limburg and Casini, 2019).

Low-oxygen exposure ha~~ve~~s been shown in laboratory experiments to reduce cod appetite with consequent significant decline in body condition and growth (Chabot and Dutil, 1999). This seems to conform to the observation of a decline in Eastern Baltic cod feeding level from stomach content analyses (Neuenfeldt et al., 2020) that has been put earlier in relation with cod growth by Brander (2000). Therefore, a lower appetite due to an increased direct exposure to low-oxygen waters seems to be a sound explanation to both the decline in growth (Brander 2000) and condition (this study). In our estimations of cod overlap with sub-lethal waters we considered the oxygen thresholds affecting cod performance as found in laboratory experiments performed on relatively large fish (~ 45 cm, Chabot and Dutil, 1999). Interestingly, in our statistical analysis the inflection of the curve relating the actual oxygen experienced and condition for large cod (40-49 cm in our study) started to occur at ~ 4.5-5 ml/l, corresponding well to the threshold found experimentally in Chabot and Dutil (1999). The threshold for small fish could be however higher, although a size-dependent hypoxia tolerance in fish is still debated (Vaquer-Sunyer and Duarte, 2008; Nilsson and Östlund-Nilsson, 2008). This could however explain why in our statistical analysis the effect of oxygen experienced on small cod condition was linear without reaching an asymptote at high oxygen concentrations. In this case our assumption of a 4.3 ml/l sub-lethal threshold for small cod could be considered very conservative.

Beside direct exposure to sub-lethal oxygen levels, other factors, not mutually exclusive, ~~–~~might contribute to explain the decline in condition as well (Casini et al., 2016a). For example deoxygenation, by deteriorating the benthic communities, has likely affected ~~negatively~~ important benthic prey for cod in negative ways, and therefore also influenced ~~also~~ indirectly cod condition and growth (Neuenfeldt et al., 2020). Moreover, ~~T~~the more severe decline in condition in SD 25 compared to SDs 26-28, for example, could be due to the higher density of cod in the southern Baltic Sea during the past twenty years (Orio et al., 2017) leading to density-dependent effects, and the lower abundance of sprat, the main pelagic fish prey for cod, in this area (Casini et al., 2014). ~~Moreover, deoxygenation, by deteriorating the benthic communities, has likely affected negatively important benthic prey for cod and therefore influenced also indirectly cod condition and growth (Neuenfeldt et al., 2019).~~~~An~~ Aadditional potential reasons of the decline in cod condition after the early 1990s ~~is~~are constituted by the increased biomass of flounder that could have deprived cod of important benthic food resources (Haase et al., 2020) and increased parasite infestation (Horbowy et al., 2016). All these factors could have acted, singularly or in combination, on cod together with direct low-oxygen exposure shaping the decline in its condition observed in the past three decades. Moreover, cod condition was relatively low also in the 1970s-1980s (although not showing individuals with very low condition, Fig. 2), when the cod population did not seem to ~~dwell~~spend time in low-oxygen waters, confirming that the main drivers of mean condition can vary in time.

5. Conclusions

We have shown here the potential mechanisms linking deoxygenation to cod condition in the Baltic Sea. A combination of increased depth of distribution of the cod population and a vertical rise of the "sub-lethal" oxygen layers has led cod dwelling progressively more in hostile low-oxygen waters, contributing to explain the reduction in cod condition in the past two decades. Further analyses should focus on revealing the reasons of the shift of cod distribution to deeper and less-oxygenated waters. We stress that our depth analyses were focused on the autumn season, when cod growth is maximised for the accumulation of energy reserves to be utilized for spawning the following spring-summer (Mion et al., 2020). The changes in cod depth of distribution are different in other seasons, especially those before and during spawning (Orio et al., 2019), when cod could have different environment requirements for reproduction. Therefore, further analyses should be performed to investigate the changes in cod population depth distribution in relation to oxygen stratification in other seasons to better understand the biotic and abiotic spatio-temporal dynamics, and their effects on cod performance, over the entire year.

Data availability

Time-series used in this study are available upon request to the corresponding author.

Author contribution

MC designed and coordinated the study. MC, MH, AO and KL prepared the raw data. MC estimated cod condition, MH performed the hydrographic modelling, and AO performed the cod distribution modelling. MC prepared the first draft of the manuscript and all authors contributed to the final version.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

We thank all the personnel involved in the long-term fish and hydrological monitoring programmes and data collection at the SLU's Department of Aquatic Resources (and former Swedish National Board of Fisheries) and at the Swedish Meteorological and Hydrological Institute. We also thank the Institute of Food safety, Animal Health and Environment "BIOR", Latvia, for the historical Latvian data on cod condition and survey catches. We are grateful to Keith Brander, Jan Dierking and one anonymous reviewer for their constructive suggestions that improved the manuscript.

Financial support

This study was funded by the Swedish Research Council Formas (grant no. 2018-00775 to Michele Casini: "Fish interactions in the marine benthic habitat: a knowledge gap in Baltic Sea fish ecology and multispecies fisheries management") and the US National Science Foundation (project OCE-1923965 to Karin Limburg: "Shifting the hypoxia paradigm – new directions to explore the spread and impacts of ocean/Great Lakes deoxygenation").

References

Brander, K.: Reduced growth in Baltic Sea cod may be due to mild hypoxia, ICES Journal of Marine Science, https://doi.org/10.1093/icesjms/fsaa041, 2020.

Breitburg, D.: Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries, Estuaries, 25, 767–781, https://doi.org/10.1007/BF02804904, 2002.

Formatted: English (United Kingdom)
Formatted: English (United Kingdom)
Formatted: English (United Kingdom)
Formatted: English (United Kingdom)
Field Code Changed
Formatted: English (United Kingdom)
Formatted: English (United Kingdom)

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., Telszewski, M., Yasuhara, M., and Zhang, J.: Declining oxygen in the global ocean and coastal waters, Science, 359, eaam7240, https://doi.org/10.1126/science.aam7240, 2018.

Campbell, L. A., and Rice, J. A.: Effects of hypoxia-induced habitat compression on growth of juvenile fish in the Neuse River Estuary, North Carolina, USA. Mar. Ecol. Prog. Ser., 497, 199–213, https://doi.org/10.3354/meps10607, 2014.

Carstensen, J., Andersen, J. H., Gustafsson, B. G., and Conley, D. J.: Deoxygenation of the Baltic Sea during the last century, Proc. Natl Acad. Sci. USA, 111, 5628–5633, https://doi.org/10.1073/pnas.1323156111, 2014.

Casini, M., Rouyer, T., Bartolino V., Larson N., and Grygiel, W.: Density dependence in space and time: opposite synchronous variations in population distribution and body condition in the Baltic Sea sprat (*Sprattus sprattus*) over three decades, PLoS ONE, 9, e92278, https://doi.org/10.1371/journal.pone.0092278, 2014.

Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, G., and Hjelm J.: Hypoxic areas, density dependence and food limitation drive the body condition of a heavily exploited marine fish predator, R. Soc. Open Sci., 3, 160416, https://doi.org/10.1098/rsos.160416, 2016a.

Casini, M., Eero, M., Carlshamre, S., and Lövgren, J.: Using alternative biological information in stock assessment: condition-corrected natural mortality of Eastern Baltic cod, ICES J. Mar. Sci., 73, 2625-2631, https://doi.org/10.1093/icesjms/fsw117, 2016b.

Chabot, D., and Dutil, J.-D.: Reduced growth of Atlantic cod in non-lethal hypoxic conditions, J. Fish Biol., 55, 472–491, https://doi.org/10.1111/j.1095-8649.1999.tb00693.x, 1999.

Claireaux, G., and Chabot, D.: Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope, J. Fish Biol., 88, 232-251, https://doi.org/10.1111/jfb.12833 , 2016.

Cleveland, W. S.: Visualizing data. Summit, NJ: Hobart Press, 1993.

Conley, D. G. Björck, S., Bonsdorff, E., Carstensen, J., Destouni, G., Gustafsson, B. G., Hietanen, S., Kortekaas, M., Kuosa, H., Meier, H. E. M., Müller-Karulis, B., Nordberg, K., Norkko, A., Nürnberg, G., Pitkänen, H., Rabalais, N. N., Rosenberg, R., Savchuk, O. P., Slomp, C. P., Voss, M., Wulff, F., and Zillén L.: Hypoxia-related processes in the Baltic Sea, Environ. Sci. Technol., 43, 3412–3420, https://doi.org/10.1021/es802762a, 2009.

Diaz, R. J., and Rosenberg, R.: Spreading dead zones and consequences for marine ecosystems, Science, 321, 926–929, https://doi.org/10.1126/science.1156401, 2008.

Domenici, P., Lefrançois, C., and Shingles, A.: Hypoxia and the antipredator behaviours of fishes, Phil. Trans. R. Soc. B, 362, 2015–2121, https://doi.org/10.1098/rstb.2007.2103, 2007.

Eby, L.A., and Crowder, L. B.: Hypoxia-based habitat compression in the Neuse River Estuary: context-dependent shifts in behavioral avoidance thresholds, Can. J. Fish. Aquat. Sci., 59, 952–965, https://doi.org/10.1139/f02-067, 2002.

Eby, L. A., Crowder, L. B., McClellan, C. M., Peterson, C. H., Powers, and M. J.: Habitat degradation from intermittent hypoxia: impacts on demersal fishes, Mar. Ecol. Prog. Ser., 291, 249–261, https://doi.org/10.3354/meps291249, 2005.

Eero, M., Vinther, M., Haslob, H., Huwer, B., Casini, M., Storr-Paulsen, M., and Köster, F. W.: Spatial management of marine resources can enhance the recovery of predators and avoid local depletion of forage fish, Cons. Lett., 5, 486–492, https://doi.org/10.1111/j.1755-263X.2012.00266.x, 2012.

Ekau, W., Auel, H., Pörtner, H.-O., and Gilbert, D.: Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish), Biogeosciences, 7, 1669–1699, https://doi.org/~~doi:~~10.5194/bg-7-1669-201, 2010.

Nilsson, G. E., and Östlund-Nilsson, S.: Does size matter for hypoxia tolerance in fish? Biol. Rev., 83, 173–189, https://doi.org/10.1111/j.1469-185X.2008.00038.x, 2008.

Haase, K., Orio, A., Pawlak, J., Pachur, M., and Casini, M.: Diet of dominant demersal fish species in the Baltic Sea: is flounder steeling benthic food from cod? Mar. Ecol. Progr. Ser., 645, 159-170, https://doi.org/10.3354/meps13360, 2020.

Hastie T. J., Tibshirani R. J.: Generalized additive models. London, UK: Chapman and Hall/CRC, 1990.

Herbert, N. A., and Steffensen, J. F.: The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress, Mar. Biol., 147, 1403–1412, https://doi.org/10.1007/s00227-005-0003-8, 2005.

Horbowy, J., Podolska, M., and Nadolna-Ałtyn, K.: Increasing occurrence of anisakid nematodes in the liver of cod (*Gadus morhua*) from the Baltic Sea: Does infection affect the condition and mortality of fish? Fish. Res., 179, 99-103, http://dx.doi.org/10.1016/j.fishres.2016.02.011, 2016.

Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., Neira, C., Rabalais, N. N., and Zhang, J.: Effects of natural and human-induced hypoxia on coastal benthos, Biogeosciences, 6, 2063–2098, https://doi.org/10.5194/bg-6-2063-2009, 2009.

Limburg, K. E., Walther, B. D., Lu, Z. G. Jackman, Mohan, J., Walther, Y., Nissling, A., Weber, P. K., and Schmitt, A. K.: In search of the dead zone: use of otoliths for tracking fish exposure to hypoxia, J. Mar. Syst., 141, 167-178, https://doi.org/10.1016/j.jmarsys.2014.02.014, 2015.

Limburg, K. E., and Casini, M.: Effect of marine hypoxia on Baltic Sea cod *Gadus morhua*: evidence from otolith chemical proxies, Front. Mar. Sci., 5, 482, https://doi.org/10.3389/fmars.2018.00482, 2018.

Limburg, K., and Casini, M.: Otolith chemistry indicates recent worsened Baltic cod condition is linked to hypoxia exposure, Biol. Lett., 15, 20190352, https://doi.org/10.1098/rsbl.2019.0352, 2019.

Mehner, T., and Kasprzak, P.: Partial diel vertical migrations in pelagic fish, J. Anim. Ecol., 80, 761–770, https://doi.org/10.1111/j.1365-2656.2011.01823.x, 2011.

Mion, M., Thorsen, A., Dierking, J., Herrmann, J.-P-, Huwer, B., Vitale, F., von Dewitz, B., and Casini, M.: Effect of fish length and nutritional condition on the fecundity of distressed Atlantic cod *Gadus morhua* from the Baltic Sea, J. Fish Biol., 92, 1016-1034, https://doi.org/10.1111/jfb.13563, 2018.

Mion, M., Hilvarsson, A., Hüssy, K., Krumme, U., Krüger-Johnsen, M., McQueen, K., Mohamed, E., Motyka, R., Orio, A., Plikšs, M., Radtke, K., and Casini, M.: Historical growth of Eastern Baltic cod (*Gadus morhua*): setting a baseline with international tagging data, Fish. Res., 223, 105442, https://doi.org/10.1016/j.fishres.2019.105442, 2020.

Neuenfeldt, S., Bartolino, V., Orio, A., Andersen, K. H., Andersen, N. G., Niiranen, S., Bergström, U., Ustups, D. Kallasvuo, M., Kulatska, N., and Casini, M.: Feeding and growth of Atlantic cod (*Gadus morhua* L.) in the Eastern Baltic Sea under environmental change, ICES J. Mar. Sci., 77, 624–632, https://doi.org/10.1093/icesjms/fsz224, 2020~~19~~.

Orio, A., Florin, A.-B., Bergström, U., Šics, I., Baranova, T., and Casini, M.: Modelling indices of abundance and size-based indicators of cod and flounder stocks in the Baltic Sea using newly standardized trawl survey data, ICES J. Mar. Sci., 74, 1322-1333, https://doi.org/10.1093/icesjms/fsx005, 2017.

Orio, A., Bergström, U., Florin, A.-B., Lehmann, A., Šics, I., and Casini, M.: Spatial contraction of demersal fish populations in a large marine ecosystem, J. Biogeogr., 46, 633-645, https://doi.org/10.1111/jbi.13510, 2019.

Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A., and Boeuf, G.: Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass, J. Fish Biol. 59, 875–883, https://doi.org/10.1111/j.1095-8649.2001.tb00158.x, 2001.

Rabalais, N. N, Turner, R. E, and Wiseman Jr, W. J.: Gulf of Mexico hypoxia, A.K.A 'The dead zone', Annu. Rev. Ecol. Syst., 33, 235–263, https://doi.org/10.1146/annurev.ecolsys.33.010802.150513, 2002.

Reusch, T. B. H., Dierking, J., Andersson, H., Bonsdorff, E., Carstensen, J., Casini, M., Czajkowski, M., Hasler, B., Hinsby, K., Hyytiäinen, K., Johannesson, K., Jomaa, S., Jormalainen, V., Kuosa, H., Kurland, S., Laikre, L., MacKenzie, B. R., Margonski, P., Melzner, F., Oesterwind, D., Ojaveer, H., Refsgaard, J. C., Sandström, A., Schwarz, G., Tonderski, K., Winder, M., and Zandersen, M.: The Baltic Sea as a time machine for the future coastal ocean. Sci. Adv., 4, eaar8195, https://doi.org/10.1126/sciadv.aar8195, 2018.

Røjbek, M. C., Jacobsen, C., Tomkiewicz, J., Støttrup, J. G.: Linking lipid dynamics with the reproductive cycle in Baltic cod *Gadus morhua*. Mar. Ecol. Progr. Ser., 471, 215-234, https://doi: 10.3354/meps10012, 2012.

Schaber, M., Hinrichsen, H.-H, and Gröger, J.: Seasonal changes in vertical distribution patterns of cod (*Gadus morhua*) in the Bornholm Basin, Central Baltic Sea, Fish. Oceanogr., 21, 33–43, https://doi.org/10.1111/j.1365-2419.2011.00607.x, 2012.

Sokolova, M., Buchmann, K., Huwer, B., Kania, P. W., Krumme, U., Galatius, A., Hemmer-Hansen, J., and Behrens, J. W.: Spatial patterns in infection of cod *Gadus morhua* with the seal-associated liver worm *Contracaecum osculatum* from the Skagerrak to the central Baltic Sea, Mar. Ecol. Progr. Ser., 606, 105-118, https://doi.org/10.3354/meps12773, 2018.

Stramma, L., Prince E. D., Schmidtko, S., Luo, J., Hoolihan, J. P., Visbeck, M, Wallace, D. W. R., Brandt, P., and Körtzinger, A.: Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes, Nat. Clim. Change, 2, 33–37, https://doi.org/10.1038/nclimate1304, 2012.

Thomas, O. R., Swearer, S. E., Kapp, E. A., Peng, P., Tonkin-Hill, G. Q., Papenfuss, A., Roberts, A., Bernard, P., and Roberts, B. R.: The inner ear proteome of fish, The FEBS journal, 286, 66-81, https://doi.org/10.1111/febs.14715, 2019.

Vaquer-Sunyer, R., and Duarte, C. M.: Thresholds of hypoxia for marine biodiversity, Proc. Natl Acad. Sci. USA, 105, 15452–15457, https://doi.org/10.1073/pnas.0803833105, 2008.

Formatted: English (United States)

Formatted: English (United Kingdom)

Formatted: English (United Kingdom)

Formatted: English (United Kingdom)

Field Code Changed

Formatted: English (United States)

Formatted: English (United Kingdom)

Formatted: English (United States)

Wu, R. S. S.: Hypoxia: from molecular responses to ecosystem responses, Mar. Pollut. Bull. 45, 35–45, https://doi.org/10.1016/S0025-326X(02)00061-9, 2002.

Formatted: English (United Kingdom)

Formatted: English (United Kingdom)

Formatted: English (United States)

Formatted: English (United Kingdom)

Formatted: English (United Kingdom)

Figure captions

Fig. 1. Bathymetric Map of the Baltic Sea divided into ICES Subdivisions ~~(SDs)~~. The study area includes the SDs 25–28 (i.e. the Central Baltic Sea).

Fig. 2. Maps of the Baltic Sea with superimposed the areas with oxygen concentration ≤ 1 ml/l (black, avoided by cod) and ≤ 4.3 ml/l (grey, sub-lethal level, producing negative effects on cod performance)

in 1990 (panel A) and 2018 (panel B). Time-series of the total area (km^2) with oxygen concentration ≤ 1 ml/l and ≤ 4.3 ml/l in the Subdivision~~D~~s 25-28 (panel C). Data were from the Swedish Meteorological and Hydrological Institute (SMHI, www.smhi.se) (see also Casini et al., 2016a).

Fig. 3. Temporal developments of mean cod condition (± 1 s.d.) in Subdivision (SD) 25 and ~~Subdivisions~~ SDs 26-28 for small cod (20-29 cm) and large cod (40–49 cm). Superimposed (grey bars) the temporal developments of the percentage of cod with very low condition (< 0.8) for the respective areas and length classes.

Fig. 4. Time-series of large cod (≥ 30 cm) depth distribution (mean and interquartile range of each predicted depth distribution; see Orio et al., 2019) as well as depths of oxygen concentration 1 ml/l and 4.3 ml/l, for Subdivision (SD) 25 (panel A) and ~~Subdivisions~~ SDs 26-28 (panel C). Panels B and D, time-series of the percent of large cod in waters with oxygen concentration ≤ 4.3 ml/l (grey bars), and oxygen at the mean depth and interquartile range of cod distribution (solid line and dotted lines), in ~~Subdivision~~ SDs 25 and ~~Subdivisions~~ SDs 26-28.

Fig. 5. Time-series of small cod (~~15-~~ < 30 cm) depth distribution (mean and interquartile range of each predicted depth distribution; see Orio et al., 2019) as well as depth of oxygen concentration 1 ml/l and 4.3 ml/l, for Subdivision (SD) 25 (panel A) and ~~Subdivisions~~ SDs 26-28 (panel C). Panels B and D, time-series of the percent of small cod in waters with oxygen concentration ≤ 4.3 ml/l (grey bars), and oxygen at the mean depth and interquartile range of cod distribution (solid line and dotted lines), in ~~Subdivision~~ SD 25 and ~~Subdivisions~~ SDs 26-28.

Fig. 6. Results of the General Additive Models (GAMs). The plots show the partial effects of the depth overlap (i.e. overlap between cod depth range of distribution and the depth of the water layer with oxygen concentration ≤ 4.3 ml/l) and of the actual oxygen experienced (at the lowest interquartile of the cod depth of distribution) on cod condition, for large (A) and small (B) cod. Blue and red dots represent SD 25 and SDs 26-28, respectively. Statistics for large cod (A): *Depth overlap* (edf = 1.00; F = 6.46; p = 0.01), *Oxygen experienced* (edf = 2.86; F = 10.88; p < 0.00001). Statistics for small cod (B):

Field Code Changed

Formatted: Font: Italic

Formatted: Font: Italic

Depth overlap ($edf = 1.30$; $F = 0.82$; $p = 0.55$), *Oxygen experienced* ($edf = 1.51$; $F = 17.60$; $p < 0.00001$). See tables S1 and S2 for the analysis of the residuals.

Formatted: English (United States)

Formatted: English (United States)

Fig. 6. Differences in otolith chemistry as related to hypoxia and fish condition for pre-2000 and 2000 onwards. Within-year hypoxia exposure duration is proxied by the fraction of each annual growth band in which the otolith Mn/Mg ratio exceeds age-specific thresholds. These are categorized by condition factor (high condition is 0.9 or greater) measured at time of capture (see Limburg and Casini, 2019).

Figure 1

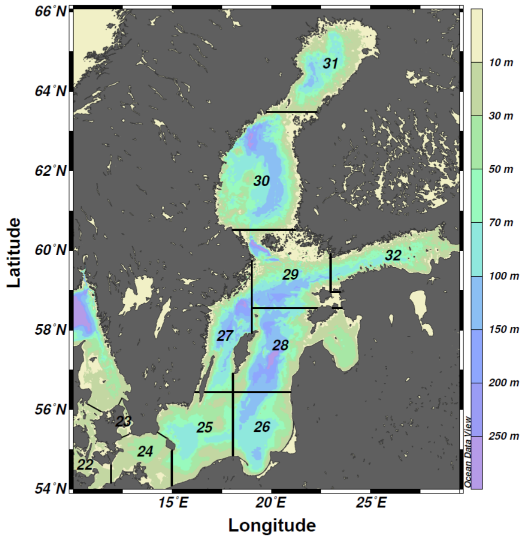

66°N
64°N
62°N
60°N
58°N
56°N
54°N
Latitude
15°E
20°E
25°E
Longitude
31
30
32
29
27
28
25
26
23
24
22
10 m
30 m
50 m
70 m
100 m
150 m
200 m
250 m
Ocean Data View

Figure 2

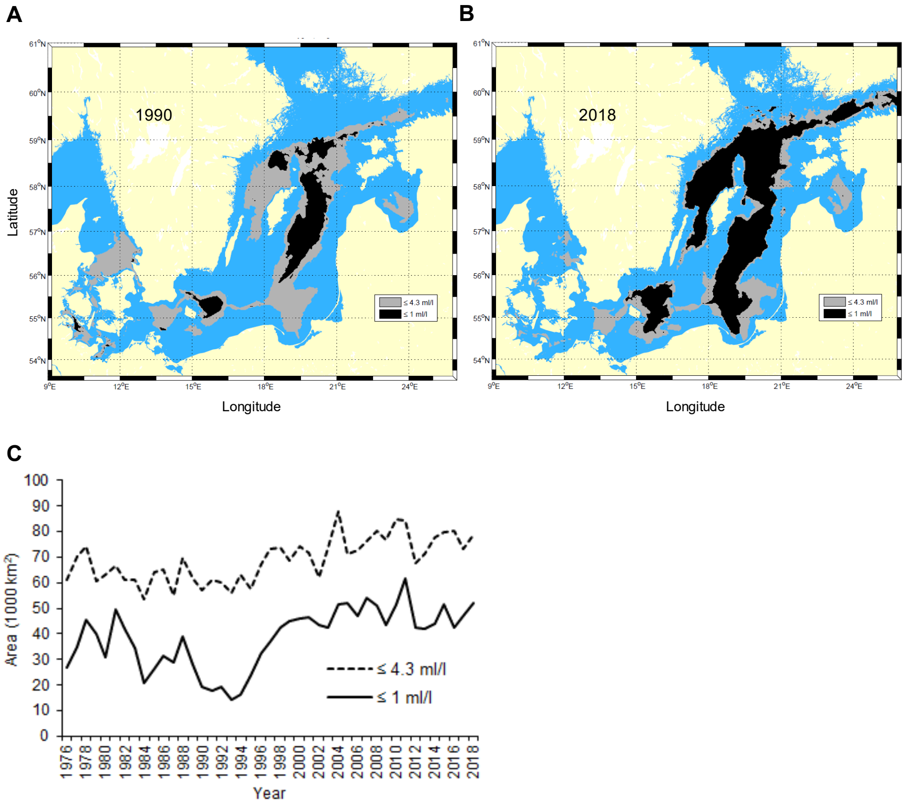
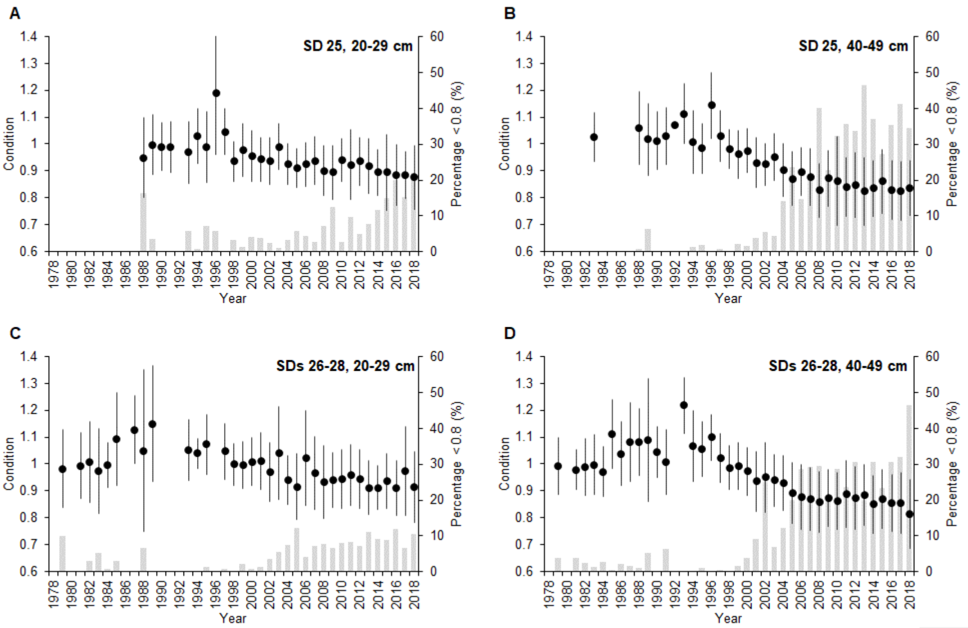

A
B
1990
2018
Latitude
Longitude
Longitude
≤ 4.3 ml/l
≤ 1 ml/l
C
Area (1000 km²)
Year
100
90
80
70
60
50
40
30
20
10
0
1976
1978
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
≤ 4.3 ml/l
≤ 1 ml/l

Figure 3



A
SD 25, 20-29 cm
B
SD 25, 40-49 cm
C
SDs 26-28, 20-29 cm
D
SDs 26-28, 40-49 cm
Condition
Percentage <0.8 (%)
Year

Figure 4

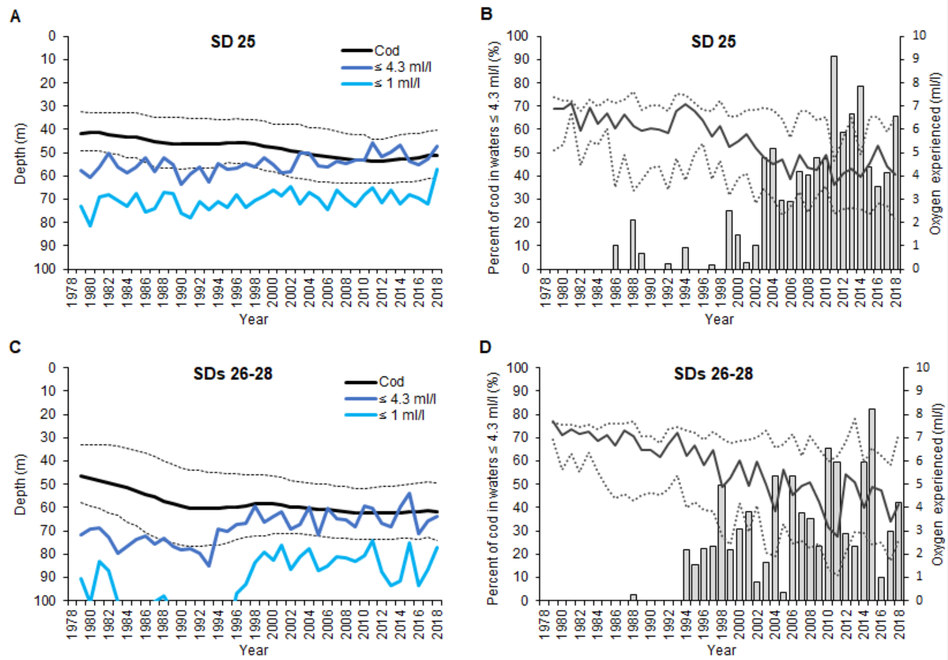

A
SD 25
Cod
≤ 4.3 ml/l
≤ 1 ml/l
Depth (m)
Year
B
SD 25
Percent of cod in waters ≤ 4.3 ml/l (%)
Oxygen experienced (ml/l)
Year
C
SDs 26-28
Cod
≤ 4.3 ml/l
≤ 1 ml/l
Depth (m)
Year
D
SDs 26-28
Percent of cod in waters ≤ 4.3 ml/l (%)
Oxygen experienced (ml/l)
Year

Figure 5

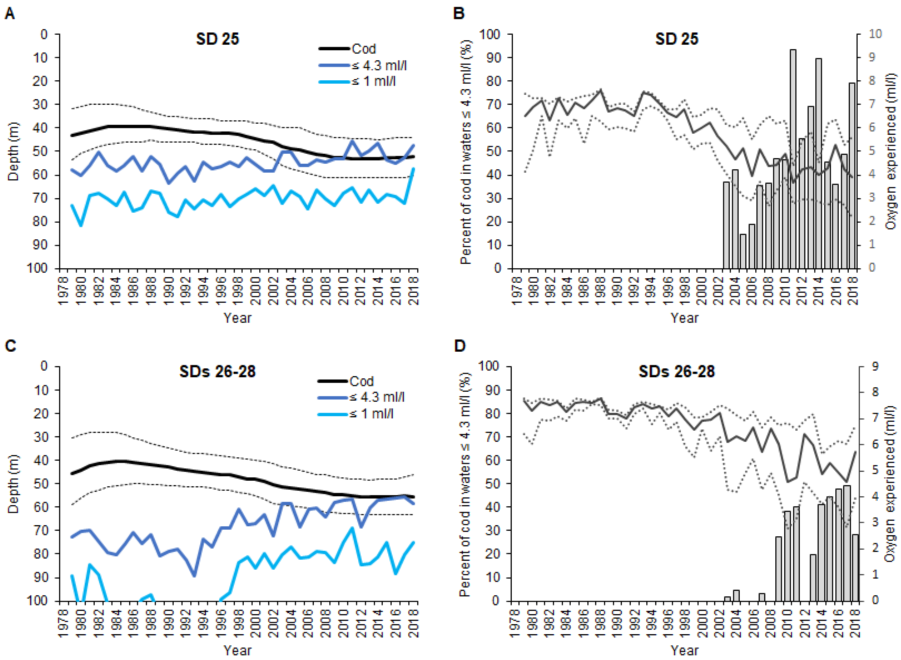

A
SD 25
Cod
≤ 4.3 ml/l
≤ 1 ml/l
Depth (m)
Year
B
SD 25
Percent of cod in waters ≤ 4.3 ml/l (%)
Oxygen experienced (ml/l)
Year
C
SDs 26-28
Cod
≤ 4.3 ml/l
≤ 1 ml/l
Depth (m)
Year
D
SDs 26-28
Percent of cod in waters ≤ 4.3 ml/l (%)
Oxygen experienced (ml/l)
Year

Figure 6