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EDITED BY
Iris Eline Hendriks,
Spanish National Research Council (CSIC), Spain

REVIEWED BY
Sam Dupont,
University of Gothenburg, Sweden
Jinlin Liu,
Tongji University, China
Emily Rivest,
College of William & Mary, United States

*CORRESPONDENCE
Paul McElhany
paul.mcelhany@noaa.gov

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Ocean acidification thresholds for decapods are unresolved

Paul McElhany* and D. Shallin Busch

National Oceanic and Atmospheric Administration, Northwest Fisheries Science Center, Conservation Biology Division, Seattle, WA, United States

A published analysis of ocean acidification thresholds for decapod crustaceans highlights data showing the negative effects of low pH on many species. However, the methods used in the paper have substantial flaws that call into question the proposed thresholds. The quantitative metrics calculated for the meta-analysis are uninformative with respect to pH sensitivity, which raises concerns about the validity of the thresholds developed by the expert opinion process. We recommend against using the published thresholds and for a reanalysis of the data to identify new thresholds.

KEYWORDS
decapod, ocean acidification, thresholds, meta-analysis, ocean change

Introduction

Identifying thresholds of biological response to environmental stressors can provide valuable information for marine resource managers (Heinze et al., 2021). In the field of ocean acidification (OA), meta-analysis of species pH exposure experiments have been used to develop thresholds or relative risk metrics for multiple taxa (Bednaršek et al., 2019; Bednaršek et al., 2021b; Hancock et al., 2020; Cornwall et al., 2022). Decapod crustaceans are an important ecological and economic component of marine ecosystems, and species in this group are potentially vulnerable to OA. Using quantitative analysis based on published data from species pH exposure experiments and expert opinion, Bednaršek et al. (2021a) developed pH exposure thresholds for a number of biological responses of decapods. The Bednaršek et al. (2021a) thresholds have been used in other analyses to characterize potential risk of decapod species to OA (Alin et al. 2023; Alin et al., 2024; Hamilton et al., 2023; Zeldis et al., 2022; Siegel et al., 2022). Although there is no doubt that many decapod species are sensitive to elevated CO_2 and exhibit thresholds in their sensitivity, the quantitative analysis in Bednaršek et al. (2021a), which informs the expert opinion, has several methodological issues which raise questions about the utility of the recommended thresholds. This brief research report describes the methodological issues and discusses the implications for our current understanding of pH thresholds for decapods. It does not recommend a specific pathway for re-evaluation of the data, but does provide some relevant references that could guide a re-evaluation.

Methods/results

We evaluated the methods used to derive the two quantitative metrics presented in Bednaršek et al. (2021a) for threshold development (least squares regression and piecewise regression). We then compared their quantitative values to the final threshold recommendations to better understand how much the quantitative metric values likely influenced the expert opinion based results.

Least squares regression

The LSR threshold is defined by Bednaršek et al. (2021a) as:

> "…the data were fitted to least squares regression (LSR). When the LSR was significant (p value < 0.05), a threshold value was calculated as pH at which the response variable had decreased by 25% of the difference between the highest and lowest pH treatment in the dataset. The significance of this 25% decline is to determine in a systematic fashion where the response parameter substantially differed from the control value."

This metric is fundamentally flawed as a method for establishing OA sensitivity thresholds. The metric has no relationship to pH sensitivity – it is purely a function of the range of pH values used in the experiments that are part of the meta-data set. Because the model is a linear relationship, a 25% decline in the range of the fitted response corresponds to a 25% decline in the range of experimental pH. A metric based only on experimental pH range was calculated as:

$$pH_{75} = pH_{min} + 0.75 * (pH_{max} - pH_{min})$$

Where pH_{75} is 75% of the way between the minimum and maximum experimental pH values from all of the studies used for a particular response analysis. As an example, the Bednaršek et al. (2021a) Supplementary Figure S2 shows the data that contributed to the LSR threshold for decapod hatching success. The lowest pH tested (pH_{min}) was 7.18 in an experiment from Miller et al. (2016) and the highest pH tested (pH_{max}) was 8.03 in an experiment from Swiney et al. (2016). The pH_{75} based on these pH_{min} and pH_{max} values is 7.82, which is the same as the LSR value for hatching success reported in Bednaršek et al. (2021a; Table 1). The range of values in the literature that researchers have used for experiments is not a useful metric for setting response thresholds as it has no inherent relationship to the sensitivity of organisms to pH.

Piecewise regression

A second metric used to develop the thresholds was a piecewise regression of biological response vs. pH that was fit with the R package "segmented" using fitting methods described in Muggeo (2017). Conceptually, the fitted breakpoint in a linear segmented model could provide threshold information as it could indicate a value where biological response to pH changes abruptly. However, as implemented in Bednaršek et al. (2021a), the analysis is uninformative. The regression fit was based on pooling all data points from all the studies, and treating each point as an independent value, thus implicitly giving more weight to studies that have larger sample sizes. In a meta-analysis, studies are usually weighted by the inverse of the study variance estimate (Viechtbauer, 2010; Cuijpers, 2016). By treating each of the data points as independent values, the studies were given essentially arbitrary weights based on the number of pH levels or biological response levels selected by the experimenter. Treating all the data for each study as independent also inflates the sample size, making it more likely to incorrectly claim statistical significance. This issue was particularly acute for the survival analyses. For some studies, the survival response used in the model was a single point for each pH value – the fraction of individuals surviving at the end of the experiment. For other studies, a data point was included for every time step in a survival curve at each pH value. For example, in the analysis of juvenile survival, the Agnalt et al. (2013) study on European lobster is represented by three data points on end-point survival while the Long et al. (2013) study on red king crab is represented by over 300 data points from the daily time step survival curves (see Bednaršek et al. (2021a) Figure 4). The Long et al. (2013) study does not contain 100 times as much information on pH thresholds as the Agnalt et al. (2013) study – the skewed weighting is an artifact of inappropriately treating the data.

Model class

The response families used for the quantitative metrics in the Bednaršek et al. (2021a) study are inappropriate for some datasets because the models are not well suited to the data being examined. As examples, Bednaršek et al. (2021a) fit piecewise regression models to hatching success (Supplementary Figure S2) and survival (Supplementary Figures S7-S12). In both cases, probability is treated as a response, with a normal (Gaussian) error distribution assumed. An entire class of models (logistic regression, a binomial response with a logit link) has been developed for modeling probabilities; using these approaches would have been the best treatment of the data (Viechtbauer, 2010). Assuming a normal response, as Bednaršek et al. (2021a) did, is especially problematic at low or high probability values, where standard errors may be less than zero or greater than one.

Expert opinion

The final thresholds presented in Bednaršek et al. (2021a) are the result of an expert opinion process in which a group of scientists combined a consideration of the quantitative analysis described above, their own expertise in some aspect of decapod OA sensitivity and open discussion to reach consensus threshold values. With this process, it is not possible to know exactly how much the flawed quantitative analyses influenced the final thresholds. However, it is possible to get some idea of their contribution by comparing the final thresholds to the quantitative analysis results (Table 2) and by reviewing the discussion text in the paper. The expert threshold is

TABLE 1 Table comparing pH_{75} and LSR values for each of the response metrics assessed in Bednaršek et al. (2021a).

Figure number in Bednaršek et al.	Response metric	pH_{min}	pH_{max}	pH_{75}	LSR Thresholds in Bednaršek et al.
Main Text #4	Juvenile survival (all)	6.77	8.06	7.74	7.74**
Supplement #2	Hatching success	7.18	8.03	7.82	7.82
Supplement #3	Adult respiration	6.57	8.17	7.77	7.77
Supplement #4	Adult hemolymph pH	6.11	8.10	7.60	7.6
Supplement #5	Adult feeding rate	6.94	8.12	7.82	7.82
Supplement #6	Adult search time	6.70	8.11	7.75	7.77
Supplement #7	Larval survival (<7d)	7.16	8.07	7.84	7.84
Supplement #8	Larval survival (8-30d)	7.16	8.20	7.94	N.S.
Supplement #9	Juvenile survival (8-30d)	7.44	8.06	7.90	7.91
Supplement #10	Juvenile survival (30-180d)	6.77	8.20	7.84	7.84
Supplement #11	Adult survival (0-30d)	6.11	8.17	7.65	7.66
Supplement #12	Adult survival (30-207d)	7.17	8.17	7.92	7.92

The minimum and maximum pH values were extracted using the metaDigitise R package Pick et al., 2019 from each of the Response vs pH plots in Bednaršek et al. (2021a). The pH_{75} and Bednaršek et al. (2021a) thresholds are essentially the same, though there is some noise because of small errors in digital data extraction of the Bednaršek et al. (2021a) graphs. In supplemental Figure #8, Bednaršek et al. (2021a) did not find a significant slope so the LSR threshold is reported as N.S. **Note that there appears to be a typo in the legend of Bednaršek et al. (2021a) Figure 4. In the figure legend, LSR is reported as 7.84, but digitizing the LSR indicator line on the graph shows the value to be 7.74.

identical to one of the quantitative estimates for four of the response metrics (adult respiration, adult search time, long-term juvenile survival and long-term adult survival) and the expert threshold appears to be a small rounding adjustment of one of the quantitative measures for three of the thresholds (hatching, short-term larval survival, and medium-term adult survival). Thus, half of the expert threshold values appear to match one of the quantitative estimates. The range of pH thresholds in Bednaršek et al. (2021a) that match a quantitative metric is 7.4 - 7.8 and the range that do not match a metric is 7.52 - 7.75. In several places in the text, it is explicitly stated that a threshold was based on one of the quantitative metrics, for example, with regard to hatching success: "Considering the two findings where significant effects were observed, the expert panel unanimously set the threshold for hatching success at pH 7.82, based largely on an LSR of 7.82" and with regard to respiration: "This observation was supported by an LSR- derived threshold of 7.77 ($p < 0.0001$, $R^2 = 0.17$)." However, in some cases (adult and juvenile growth), it was explicitly stated that the quantitative analyses were ignored, for example: "The final adult and juvenile threshold identified was unanimously set at 7.75 for 105 days (Table 2), based on significant experimental effects in individual studies rather than statistical threshold analyses."

TABLE 2 Comparison of the quantitative metrics and expert pH thresholds for decapods.

Response metric	Regression breakpoint	LSR metric	Expert threshold
Hatching success	—	7.82	7.80
Juvenile and adult growth rate	—	—	7.75
Adult respiration rate	—	7.74	7.74
Adult hemolymph pH	—	7.56	7.70
Adult feeding rate	7.94	7.83	7.69
Adult search time	—	7.76	7.76
Larvae survival (<7d)	7.44	NA	7.40
Larvae survival (3-8d)	—	7.19	7.52
Juvenile survival (8-30d)	7.83	7.91	7.60
Juvenile survival (31-180d)	7.75	7.84	7.75
Adult survival (0-30d)	7.60	7.66	7.52
Adult survival (31-180d)	7.68	7.92	7.65
Adult survival (181-365d)	7.80	—	7.80

Data from Bednaršek et al. (2021a) Table 2. The orange cells of the table indicate cases where the expert threshold exactly matched one of the quantitative metrics and the pink cells indicate cases where the expert threshold was within a few hundredths of one of the quantitative metrics.

Discussion

The development of quantitative metrics combining the results from multiple studies should rely on standard meta-analysis techniques that use estimates of effect size to provide a valid unit of comparison Kroeker et al., 2010. The quantitative measures in Bednaršek et al. (2021a) are uninformative with regard to the question they attempt to answer. Although the final thresholds were established by expert consensus, the process was confounded by the fact that the information provided to the expert panel was not an accurate synthesis of the available literature. We suggest that further work is needed to identify OA thresholds for decapods. As such, we recommend that the thresholds presented in Bednaršek et al. (2021a) not be used in studies evaluating OA risks or for establishing management goals for decapods, and suggest re-evaluation of the published works based on them. Any reconsideration of decapod sensitivity to OA should apply appropriate methods tailored to the available data as described in the established statistical literature on meta-analysis (e.g., Borenstein et al., 2021; Harrer et al., 2021).

This critique has focused on specific methodological issues in Bednaršek et al. (2021a) and not on broader questions about OA thresholds, which are key to address when presenting thresholds so that they are used appropriately. For example, presentation of a generic global decapod threshold created by pooling across all species and locations would likely require nuanced discussion to guide readers away from making mistakes in its application. Given the general variability in pH sensitivity at the species and/or population level, the range in local baseline pH conditions, the importance of variability in pH on species response, and the importance of co-stressors like temperature or food availability (Kroeker et al., 2013; Vargas et al., 2017, 2022), we believe that a generic, global-level threshold may not be particularly predictive of OA risk to a focal species of interest or of the vulnerability of biota in a particular region. Although generic thresholds are a useful means of summarizing the literature and provide some information when comparing high-level taxonomic groups, it is important to be explicit about their limitations, especially when thresholds may be considered in a management or regulatory context.

Data availability statement

Publicly available datasets were analyzed in this study. The data are available in the published paper: Bednarsek, Nina, Richard Ambrose, Piero Calosi, Richard K. Childers, Richard A. Feely, Steven Y. Litvin, W. Christopher Long, et al. "Synthesis of Thresholds of Ocean Acidification Impacts on Decapods." Frontiers in Marine Science 8 (2021): 1542. https://doi.org/10.3389/fmars.2021.651102.

Author contributions

PM: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. DB: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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