

Thresholds of hypoxia for marine biodiversity

Raquel Vaquer-Sunyer* and Carlos M. Duarte

Department of Global Change Research, Instituto Mediterraneo de Estudios Avanzados (Consejo Superior de Investigaciones Científicas-Universidad de las Islas Baleares), Esporles (Mallorca) 07190, Spain

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Hypoxia is a mounting problem affecting the world's coastal waters, with severe consequences for marine life, including death and catastrophic changes. Hypoxia is forecast to increase owing to the combined effects of the continued spread of coastal eutrophication and global warming. A broad comparative analysis across a range of contrasting marine benthic organisms showed that hypoxia thresholds vary greatly across marine benthic organisms and that the conventional definition of 2 mg O₂/liter to designate waters as hypoxic is below the empirical sublethal and lethal O₂ thresholds for half of the species tested. These results imply that the number and area of coastal ecosystems affected by hypoxia and the future extent of hypoxia impacts on marine life have been generally underestimated.

benthic community | oxygen | coastal ecosystems | eutrophication | impacts

Dissolved oxygen in coastal waters has changed drastically over the past decades, arguably more so than any other ecologically important variable (1, 2), leading to the widespread occurrence of hypoxia. An assessment of the literature shows that the number of coastal sites where hypoxia has been reported has increased with an exponential growth rate of 5.54% year⁻¹ over time [Fig. 1 and supporting information (SI) Table S1]. Although this growth rate can be partially attributed to an increased observational effort, increasing the number of coastal ecosystems monitored and the likelihood of detecting hypoxia therein, this growth also reflects an increase in the prevalence of hypoxia in different types of coastal ecosystems. Multiple reports from careful monitoring time series provide evidence for an unambiguous increase in the number of hypoxic zones and their extension, severity, and duration (3–6). This growth is expected to continue because the prevalence of hypoxia is forecast to increase further owing to the combined effects of eutrophication, leading to the excessive production of organic matter that increases the oxygen demand of coastal ecosystems (7), and the increase in temperature caused by climate change, which enhances the respiratory oxygen demand of the organisms (8), reduces oxygen solubility (9), and reduces the ventilation of coastal waters by affecting stratification patterns (10).

Coastal hypoxia is, thus, emerging as a major threat to coastal ecosystems globally. Hypoxia has been shown to trigger mortality events, resulting in a depletion of metazoans in the ecosystems, resulting in so-called “dead zones” devoid of fisheries resources, such as fish, shrimp, and crabs (11, 12). Hypoxia leads to major loss in biodiversity and impacts the surviving organisms through sublethal stresses, such as reduced growth and reproduction, physiologic stress, forced migration, reduction of suitable habitat, increased vulnerability to predation, and disruption of life cycles (7, 11). Benthic organisms are particularly vulnerable to coastal hypoxia because they live farthest from contact with atmospheric oxygen supply and because coastal sediments tend to be depleted in oxygen relative to the overlying water column.

Assessing the thresholds of oxygen at which lethal and sublethal impacts occur is critical to establish the vulnerability of marine organisms to hypoxia and to set management targets to avoid catastrophic mortality. Hundreds of experiments to determine thresholds of hypoxia for a range of benthic organisms have been conducted. However, the oxygen thresholds for hypoxia proposed

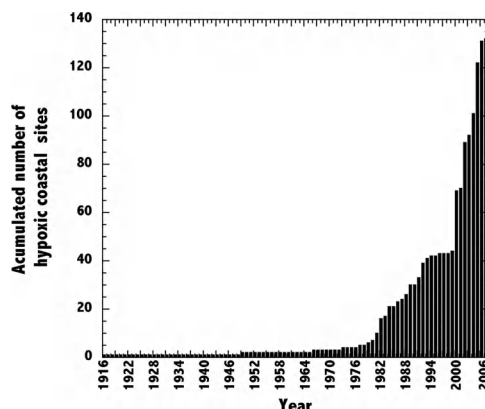


Fig. 1. Accumulated number through time of coastal sites where hypoxia has been reported. Exponential growth rate = 5.54% \pm 0.23% year⁻¹ ($R^2 = 0.86$, $P \leq 0.01$).

in the literature (1, 12, 13) are based on limited observations of impacts on organisms (7), and a thorough empirical assessment of the available experimental evidence is still pending. Whereas the thresholds of hypoxia proposed in the literature range broadly from 0.28 mg O₂/liter (14) to 4 mg O₂/liter (15), most reports (55%) refer to a value of 2 mg O₂/l or lower (mean \pm SE of thresholds proposed in the literature: 2.31 \pm 0.10 mg O₂/liter; Table S2) used in most conventional applications (16). This threshold refers to the oxygen level for fisheries collapse (12), but there is ample experimental evidence that a 2-mg O₂/liter threshold may be inadequate to describe the onset of hypoxia impacts for many organisms, which experience hypoxia impacts at higher oxygen concentrations (e.g., 17). Moreover, the diversity of behavioral and physiologic adaptations to hypoxia (18) suggests that different taxa are likely to exhibit different vulnerability to hypoxia and may have, therefore, different oxygen thresholds (19), a possibility that is not addressed by the conventional oxygen thresholds in use (cf. Table S2).

The goal of this article is to examine the variability in oxygen thresholds for hypoxia across benthic organisms and to test for the existence of consistent differences among taxa. We do so on the basis of a comparative analysis of experimentally derived oxygen thresholds for lethal and sublethal responses to hypoxia of benthic organisms. We aim to improve our understanding of the levels of hypoxia that cause significant impacts on marine benthic communities. This understanding will offer a more rigorous basis on which to establish critical thresholds to preserve fishery resources and to effectively conserve coastal

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*To whom correspondence should be addressed. E-mail: raquel.vaquer@uib.es.

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Indeed, we found that the median lethal O_2 thresholds differed significantly with the extent of mobility of the organisms tested (ANOVA, $F = 11.29$, $P < 0.001$). Two-way ANOVA showed that the degree of mobility (F test, $P \leq 0.001$), which accounted for 20% of the variance in median lethal concentrations among experiments, was superior to differences among taxa (F test, $P \geq 0.05$) in accounting for variability across experiments.

Median Sublethal Concentration. Median sublethal concentration (SLC_{50}) thresholds ranged from 10.2 mg O_2 /liter for cod, *Gadus morhua*, which raises its ventilatory water flow below this concentration (21, 22), to 0.085 mg O_2 /liter for the burrowing shrimp *Calocaris macandreae* (Thalassinidea), which switches from aerobic to anaerobic metabolism below this threshold (23). The mean \pm SE SLC_{50} was 2.61 ± 0.17 mg O_2 /liter, the median SLC_{50} was 2.24 ± 0.21 mg O_2 /liter, and the coefficient of variation was 76%, showing important variability in median sublethal thresholds among experiments (Fig. 2B). Ninety percent of the experiments conducted reported median sublethal oxygen concentrations below 5.00 mg O_2 /liter (Fig. 2B).

As for the median lethal O_2 thresholds, some of the variability in median sublethal O_2 thresholds was attributable to differences among taxa, which was stronger for sublethal than for lethal responses (ANOVA, $F = 21.75$, $P < 0.001$; Fig. 3B). Fish had significantly higher oxygen thresholds for sublethal responses (Tukey *post hoc* HSD test, $P < 0.05$; Fig. 3B), which typically involved avoidance of hypoxic waters, depressed activity, shift to oxygen-dependent metabolism, or increased ventilatory water flow. Crustaceans also presented significantly higher oxygen thresholds for sublethal responses than polychaetes, echinoderms, and cnidarians (Tukey *post hoc* HSD test, $P < 0.05$; Fig. 3B), which typically involved avoidance of hypoxic waters, reduced growth, reduced predation rates, lethargy, or decreased activity, among others. Fish and crustaceans, which showed the highest median sublethal O_2 thresholds, are also the taxa with the highest mobility, which confers them some capacity to avoid hypoxic waters. Indeed, two-way ANOVA showed that both differences among taxa and the extent of mobility among organisms were significant (F test, $P \leq 0.001$), accounting for 52% of the variance in median sublethal concentrations among experiments ($F = 11.6$, $P \leq 0.001$).

Median Lethal Time. The median lethal time (LT_{50}) upon exposure to acute hypoxia ranged greatly across organisms tested (Fig. 2C), from only 23 min for the flounder *Platichthys flesus* (19, 24), to more than 32 weeks for the bivalve *Astarte borealis* at temperatures below 20°C (25). The mean (\pm SE) LT_{50} was 267.9 ± 22.0 h, the median was 116.7 ± 27.67 h, and the coefficient of variation was 178% across experiments, indicative of considerable variability in these thresholds across organisms (Fig. 2C). Ten percent of the organisms showed LT_{50} upon exposure to acute hypoxia of less than 6.8 h (Fig. 2C). There were significant differences in LT_{50} under hypoxia among taxa (ANOVA, $F = 11.12$, $P < 0.001$; Fig. 3C). In particular, Priapulida, the most tolerant group, had significantly longer LT_{50} under hypoxia than other taxonomic groups, and molluscs, the second most tolerant group, also showed significantly longer LT_{50} under hypoxia than annelids, fish, and crustaceans, which were the most sensitive groups (Fig. 3C). Sessile organisms also had longer LT_{50} than mobile organisms did (F test, $P \leq 0.01$). However, two-way ANOVA showed that differences among taxa (F test, $P \leq 0.001$), which explained 17% of the variance in LT_{50} , were superior to differences in mobility (F test, $P \geq 0.05$), to account for variability among experiments.

We also found significant ontogenic shifts in survival time, with early stages having survival times, on average, $64\% \pm 7\%$ (H_0 linear regression slope = 1, t test, $P < 0.05$) of those of more

developed stages for any one species, whereas similar effects were not found for lethal or sublethal concentrations.

Discussion

The results presented here provide evidence of the broad, order-of-magnitude variability in the thresholds of oxygen concentrations for hypoxia among benthic marine organisms, which cannot be adequately captured by a single, universal threshold. This variability partially derived from significant differences in oxygen thresholds across taxa. The most sensitive organisms were crustaceans, which showed the highest LC_{50} and the shortest LT_{50} , whereas fish exhibit sublethal responses at the highest O_2 concentration (Fig. 3). On the other hand, molluscs, with the lowest LC_{50} , are the organisms most tolerant to hypoxia, together with cnidarians, which showed the lowest LC_{50} for sublethal threshold, and priapulids, which showed the longest LT_{50} .

The differences in oxygen thresholds for hypoxia across taxa probably reflect the broad differences in adaptations to cope with low oxygen conditions among benthic organisms, which span a broad range of behavioral and metabolic changes. Mobile organisms have the capacity to avoid hypoxic waters and thus tend to show comparably high oxygen thresholds. Benthic fish have been reported to move to near-surface waters to breathe when bottom waters become hypoxic (26), and crustaceans move to shallower areas (27), where these organisms are more vulnerable to predation. Yet fast-moving organisms (e.g., fish) do not necessarily show higher lethal thresholds than those with more restrictive mobility (e.g., crustaceans), pointing to differences among taxa independent of their relative mobility.

Many benthic organisms (polychaetes, annelids, crustaceans, bivalves, priapulids, and anemones) leave their burrows or tubes to move to the sediment surface or reduce their burial depth (28, 29) in the presence of hypoxia. Some bivalves stretch their siphons upward into the water column to reach waters with higher oxygen concentrations (30). Some echinoderms stand immobile on their arm tips with the central disk elevated to avoid the hypoxic bottom water (31), and some gastropods climb structures to reach waters with higher oxygen concentration. Metabolic adaptations to cope with hypoxia include depression of activity in the presence of hypoxia, as reported for echinoderms (32); reduced feeding activity (e.g., some crustaceans, molluscs, and polychaetes; refs. 33–35); reduced metabolic rates (e.g., cnidarians; ref. 36) and heartbeat rate (some crustaceans; ref. 37); and shift to anaerobic metabolism over time scales of hours to days, an adaptation widespread among bivalves (38, 39), polychaetes (40), oligochaetes (41), echinoderms (42), and the mud-shrimp *Calocaris macandreae* (23), among others.

The broad variability in oxygen thresholds shown here is in contrast with the widespread use of uniform thresholds for hypoxia in the literature (Table S2). The vast majority of studies and reports continue to use the 2-mg O_2 /liter convention, originally derived as the oxygen threshold for fisheries collapse (12). A total of 43% and 21.5% of the published reports used the 2-mg O_2 /liter and the 2-ml/liter (i.e., 2.85 mg O_2 /liter) threshold, respectively, and a single study (15) used a threshold of 4 mg O_2 /liter (Table S2). A seminal review by Gray *et al.* (19), which included experimental studies reporting mortality thresholds well above 2 mg O_2 /liter, concluded that “mortality occurs where concentrations are below 2.0 to 0.5 mg O_2 /liter”; and the U.S. Environmental Protection Agency recommends a threshold of 2.3 mg O_2 /liter for juvenile and adult aquatic organism survival (43). In a recent review, Díaz and Rosenberg (54) state that “hypoxia occurs when DO falls below 2 ml of O_2 /liter ... culminating in mass mortality when DO declines below 0.5 ml of O_2 /liter.” The results presented here show that the conventionally accepted level of 2 mg O_2 /liter falls well below the oxygen thresholds for the more sensitive taxa.

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Mean \pm SE	1.54 \pm 0.07	4.41 \pm 0.39	59.9 \pm 12.3
90th percentile	2.51	8.09	0.9
<i>n</i>	77	34	39

Crustaceans			
Mean \pm SE	2.45 \pm 0.14	3.21 \pm 0.28	55.5 \pm 12.4
90th percentile	5.72	5.0	1.0
\underline{n}	168	30	102
Gastropods			
Mean \pm SE	0.89 \pm 0.11		
90th percentile	1.62		
\underline{n}	12		
Bivalves	1.42 \pm 0.14		
Mean \pm SE			
90th percentile	3.43		
\underline{n}	19		
Molluscs			
Mean \pm SE		1.99 \pm 0.16	412.9 \pm 37.3
90th percentile		2.83	55.4
\underline{n}		28	239
Annelids			
Mean \pm SE		1.20 \pm 0.25	132.2 \pm 18.7
90th percentile		1.37	37.8
\underline{n}		10	43
Echinoderms			
Mean \pm SE		1.22 \pm 0.22	201.1 \pm 44.8
90th percentile		2.12	33.6
\underline{n}		8	23
Cnidarians			
Mean \pm SE		0.69 \pm 0.11	232.5 \pm 114.4
90th percentile		1.43	24
\underline{n}		19	8
Priapulids			
Mean \pm SE			1512.0 \pm 684.0
90th percentile			820.8
\underline{n}			3

Shown are mean \pm SE, 90th percentile (10th percentile for lethal times), and number of observations of LC₅₀, SLC₅₀, and LT₅₀ for the various groups.

ratory experiments). Hence, the results derived from laboratory experiments should be considered conservative.

Consideration of the different thresholds of hypoxia among taxa derived here (Fig. 3 and Table 1) predicts that the sequence of losses of benthic fauna during hypoxic events should be initiated by the loss of fish, followed by crustaceans, then worms, echinoderms, and molluscs as oxygen declines. This prediction is consistent with the observed sequence of losses of benthic fauna during hypoxic events, as reported in the Danish fjords (30) and the Baltic Sea (49). The agreement between the sequences of losses of various taxa with hypoxia predicted from laboratory experiments and those observed in coastal areas impacted by hypoxia provides additional confidence in the relevance of

laboratory experiments. The pattern of recolonization of benthic fauna lost to hypoxia upon subsequent improvement of the oxygen conditions differs, however, from the pattern of loss, because recolonization patterns, which are initiated by polychaetes (50, 51), are determined by life-history and dispersal properties of the organisms and not their resistance to hypoxia.

The conclusion that oxygen depletion induces significant mortality at critical oxygen thresholds exceeding by 2.3 times the 2-mg O₂/liter threshold generally used in the literature implies that the present inventory of the number and extent of hypoxic areas in the coastal zone, which uses the occurrence of oxygen levels ≤ 2 mg O₂/liter (Fig. 1), represents an underestimate of the coastal areas experiencing mortality of benthic organisms attributable to hypoxia. Hence, benthic organisms may be suffering substantial mortality in areas not presently designated as hypoxic. The conventional 2-mg O₂/liter limit serves to separate “dead zones,” depleted of most of the commercially harvested species, from waters supporting significant benthic animal communities. However, it fails to reflect the oxygen threshold at which these communities experience hypoxia-derived mortality. The pace of growth of hypoxia as a major threat to coastal biodiversity and associated living resources may be, therefore, greater than hitherto considered. Moreover, there is ample evidence that the oxygen requirements of marine animals are even higher in the presence of concurrent stresses, such as high temperature (52) or sulfide concentrations (53), suggesting that areas under stress are particularly prone to experience hypoxia-derived catastrophic mortality. These interactions are not considered in present assessments and classifications but are likely to play a more prominent role in the future as global warming and other mounting stresses in the coastal ocean increase the sensitivity of benthic organisms to oxygen depletion. Indeed, a recent assessment concluded that the area of hypoxia (defined as ≤ 2 mg O₂/liter) in Danish coastal waters, one of the countries most severely affected by this problem, will more than double under the projected temperature increase over the 21st century (6), an estimate that needs to be revised upwards in light of the higher oxygen thresholds for hypoxia proposed here.

The analysis presented here demonstrates that hypoxia impacts occur at a broad range of oxygen concentrations, including oxygen concentrations well above the oxygen thresholds generally used to diagnose hypoxia at present. The vulnerability of coastal ecosystems to hypoxia is, thus, greater than currently recognized, with fish and crustaceans being the most vulnerable faunal components. The number and extent of the coastal zones

affected by hypoxia is, thus, likely to be greater than hitherto realized, and the prospects for future expansion of these areas more disturbing than currently forecasted. Coastal hypoxia is, thus, emerging as a major threat to coastal ecosystems globally. The revised thresholds of hypoxia provided here will help better protect these ecosystems, conserve their biodiversity, and set successful management targets to avoid hypoxia-derived biodiversity losses in coastal waters.

Methods

We searched the literature for reports of hypoxia on the Web of Science and Scholar Google using the keywords “hypoxia,” “marine,” “benthic,” and “sea” and their combinations to guide the search. This search delivered more than 6000 published reports of responses of benthic marine organisms to hypoxia, which were then examined further for the availability of experimental assessments of responses to reduced oxygen content. This search delivered a total of 872 experimental assessments examining the distribution of oxygen thresholds, involving 206 different species of marine benthic organisms.

The outcome of experimental assessments, which follow standard toxicity tests, was summarized using the following indicators of oxygen thresholds: LC₅₀ and SLC₅₀, representing the statistically derived O₂ concentration at which 50% of the organisms in a given population die or exhibit sublethal responses, respectively, and LT₅₀, representing the statistically derived time interval at which 50% of a given population dies after exposure to low O₂ levels. The vast majority (99.1%) of the experiments designed to assess LT₅₀ chose ≤ 2 mg O₂/liter as experimental conditions, consistent with the widespread acceptance of 2 mg O₂/liter as the threshold for hypoxia in the literature (Table S2). Yet this choice indicates that the lethal times reported represent lethal times under acute hypoxia. Only a few experiments testing species particularly sensitive to hypoxia (0.87%) used higher experimental O₂ conditions. We analyzed these indicators to extract oxygen thresholds conducive to the effective conservation of marine biodiversity.

ANOVA was used to test for differences in oxygen thresholds among taxonomic groups. ANOVA analysis was conducted after checking for normality using the Shapiro-Wilk test and homogeneity of variance using the Levene test. The Tukey *post hoc* HSD test was used to determine differences between mean threshold values among taxa ($\alpha = 0.05$). We also classified the species tested according to their mobility as “fast moving” (fish and a few mollusks, such as octopus), “highly mobile” (most crustaceans), “reduced mobility” (some crustaceans, gastropods, polychaetes, echinoderms, jellyfish, comb fish (ctenophora), priapulids, flatworms, and sipunculida), and “sessile” (anemones, bryozoans, and bivalves). ANOVA was used to test for differences in thresholds with mobility, according to the procedures outlined above. Two-way ANOVA was used to test for the combined effect of taxonomic membership and the extent of mobility of the organisms tested on the experimentally derived thresholds.

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