



Thresholds for Reduction in Fish Growth and Consumption Due to Hypoxia: Implications for Water Quality Guidelines to Protect Aquatic Life

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Abstract

Control of hypoxia is a key element of water quality management, and guidelines are usually based on qualitative reviews of hypoxia impacts. In this study we use segmented regression to identify both thresholds for growth reduction and rate of decline of fish growth and food consumption under hypoxia; and then evaluate whether current freshwater guidelines for dissolved oxygen based on qualitative reviews are consistent with the quantitative analysis of hypoxia thresholds. Segmented regressions were fit to data from published growth-hypoxia studies for freshwater ($N = 17$) and marine fishes ($N = 13$). To understand potential drivers of hypoxia tolerance, we also modelled thresholds as simple functions of environmental and ecological covariates for each species including trophic level, marine vs. freshwater environment, maximum fish length, fish weight, and maximum temperature tolerance. The average threshold for growth reduction (G_{crit} ; $5.1 \text{ mg}\cdot\text{l}^{-1}$ DO) and decreased food consumption ($C_{crit} = 5.6 \text{ mg}\cdot\text{l}^{-1}$ DO) were not significantly different, and did not differ between marine and freshwater taxa. However, salmonids showed a significantly steeper decline in growth with increasing hypoxia relative to other taxa. Growth declined by 22% for every $\text{mg}\cdot\text{l}^{-1}$ reduction in DO below average G_{crit} , and significant regressions indicate that warmwater ($R^2 = 0.25$) and smaller-bodied ($R^2 = 0.44$) species are more likely to be hypoxia tolerant. Observed mean G_{crit} and C_{crit} in the range of $5\text{--}6 \text{ mg}\cdot\text{l}^{-1}$ broadly match minimum water quality guidelines for the protection of aquatic life in freshwater in representative industrialized countries. However, this is much higher than the definition of hypoxia typically used in marine systems ($2\text{--}2.5 \text{ mg}\cdot\text{l}^{-1}$), indicating a need to reconcile definition of hypoxia in the marine environment with empirical data. The principal challenge in freshwater hypoxia management is now translating discretionary guidelines into effective regulatory frameworks to reduce the incidence and severity of hypoxia.

Keywords Hypoxia · Hypoxia thresholds · Fish growth · Food consumption · G_{crit} · C_{crit}

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Introduction

Respiration has been described as the fire of life (Kleiber 1961); most animals rely on oxidative metabolism to carry out their life processes. Oxidative metabolism releases energy by combining reduced organic molecules (e.g., carbohydrates, lipids, proteins) with oxygen, in a process that can be thought of as low-temperature biochemical combustion. Although some heterotrophs are uniquely adapted to living in anaerobic environments, an adequate supply of oxygen is generally essential for short-term survival, and is equally necessary for routine functions like

digestion, tissue maintenance, and somatic growth, which have significant energy demands. Oxygen consumption is directly proportional to metabolic rate and energy expenditures, and chronic hypoxia (reduction of dissolved oxygen below saturation levels) can result in sublethal depression of growth, activity, maintenance metabolism, immune function, and a host of other metabolic processes even if it does not cause direct mortality (Pollock et al. 2007; Vaquer-Sunyer and Duarte 2008; Wu 2002).

Environmental hypoxia occurs when aquatic ecosystem respiration exceeds oxygen supply rate from photosynthesis and diffusion across the water surface. Hypoxia ranges from a marginal reduction of dissolved oxygen below saturation, which is common and generally inconsequential for organism function, to severe hypoxia or complete anoxia (zero dissolved oxygen), as might occur in eutrophic waters with limited circulation and very high respiration rates (Pardo and Garcia 2016; Rabalais et al. 2010; United States Environmental Protection Agency 2003). Some species are robust to hypoxia, having evolved in habitats that are naturally low in dissolved oxygen (Chapman 2015; Currie et al. 2018; Tobler et al. 2009); for instance, tropical wetlands are often characterized by high temperatures and chronic hypoxia, and hypoxia-tolerant cichlids exploit them as refuges from predation (Chapman 2015; Reid et al. 2013). In north temperate latitudes, the profundal zone of lakes is often hypoxic during summer stratification or winter ice-cover, and species like the crucian carp (*Carassius carassius*) or central mudminnow (*Umbra limi*) are well-adapted to surviving and even foraging in toxic hypoxic waters (Nilsson and Renshaw 2004; Rahel and Nutzman 1994). More generally, benthic species might be expected to have greater hypoxia tolerance, because their ecological niche is adjacent to bottom sediments that may generate high respiration rates, and distant from the air-water interface that is the source of dissolved oxygen. Similarly, warmwater species may also be more hypoxia tolerant because solubility of oxygen declines at higher temperatures, increasing their evolutionary exposure to lower oxygen that represents normoxia in warmwater habitats.

Although hypoxia occurs naturally in some lakes, streams, wetlands, and estuaries (Chapman 2015; Rabalais et al. 2010), it has become increasingly widespread in the Anthropocene as a result of nutrient loading and associated eutrophication (Le Moal et al. 2019). Eutrophication elevates oxygen consumption first through the respiratory demand of increased algal biomass, particularly at night, and secondarily through the oxygen demand of bacteria and fungi consuming senescent algae. Elevated temperatures increase heterotroph respiration rates and simultaneously reduce saturation levels of dissolved oxygen, so that climate warming and eutrophication will synergistically increase

the extent and severity of future hypoxia (Niklitschek and Secor 2005; Rabalais et al. 2010). Biological impacts of hypoxia range from loss of sensitive species to large-scale mortality of aquatic organisms, local collapse of commercial fisheries, and de-faunation of profundal dead zones (Breitburg et al. 2018; Stachowitsch 1991; Wu 2002). Although eutrophication of freshwaters in the 1970s was successfully controlled through nutrient removal from municipal wastewaters, eutrophication is now re-emerging in systems where it was considered eradicated (e.g. the Laurentian Great Lakes; Le Moal et al. 2019; Mohamed et al. 2019; Watson et al. 2016).

The expanding footprint of hypoxia elevates the need for dissolved oxygen standards that are protective of aquatic habitats, and many jurisdictions have developed water quality guidelines to protect freshwater habitat and biodiversity. Dissolved oxygen thresholds are generally based on qualitative reviews of hypoxia tolerance studies (e.g., Canadian Council of Ministers of the Environment 1999; Chapman 1986; Franklin 2014) and usually focus on tolerance levels of fish (Saari et al. 2018), presumably because fish are viewed as culturally and economically valued taxa (Franklin 2014). Freshwater guidelines are also usually based on sublethal effects rather than severe impacts like mortality which occur at much lower oxygen levels, presumably because the intent of regulation is to avoid harm to individuals and populations. Generic minimum dissolved oxygen guidelines for the protection of aquatic life in freshwater generally fall in the range of 4–6 mg·l⁻¹ of dissolved oxygen, with short-term (transient) minimums closer to 4 mg·l⁻¹ and longer-term minimums in the range of 5–6 mg·l⁻¹, with lower thresholds for warmwater species (Table 1).

Although fish are frequently the target of studies to identify sublethal thresholds for hypoxic effects, biological response metrics vary widely, and can include altered foraging behaviour, activity levels (Vaquer-Sunyer and Duarte 2008), loss of equilibrium (Wood 2018), physiological diagnostics (e.g. P_{crit} ; Mandic et al. 2009; Speers-Roesch et al. 2013), food consumption, or growth (Herrmann et al. 1962; Hrycik et al. 2017; Wang et al. 2009). Short-term behavioural responses are easily replicated in laboratory trials, but their ecological consequences may be difficult to interpret. P_{crit} is a commonly used index of hypoxia tolerance in fish physiology, and represents the dissolved oxygen concentration below which fish can no longer sustain their maintenance metabolism, and are forced to downregulate their standard metabolic rate and oxygen consumption (Wang et al. 2009). P_{crit} and other physiological metrics are sensitive indicators of organism homeostasis, but their relevance to fitness or population level effects remains unclear (Speers-Roesch et al. 2013; Wood 2018). Studies examining the sublethal effects of hypoxia

Table 1 A representative selection of water quality guidelines for dissolved oxygen in freshwater and marine habitats from a subset of developed nations; units are $\text{mg}\cdot\text{l}^{-1}$ (parts per million, ppm) of dissolved oxygen

| Habitat | Country | Guideline | | Reference |
|--------------------------------|-------------------|--|------------------------|--|
| FRESHWATER | Canada | Warmwater ^a | Coldwater ^a | Canadian Council of Ministers of the Environment 1999 |
| | | 5.5 ^b | 6.0 ^b | |
| | | 6.0 ^c | 9.5 ^c | |
| | | | | |
| | England and Wales | Cyprinid waters | Salmonid waters | Department for Environment Food and Rural Affairs 2014 |
| | | 4.0–5.0 ^d | 4.5–5.5 ^d | |
| | | | | |
| | New Zealand | Non-salmonid | Salmonid | Franklin 2014 |
| | | 5.0 ^e | 6.0 ^e | |
| | | 3.0 ^f | 5.0 ^f | |
| | United States | Warmwater | Coldwater | United States Environmental Protection Agency 1986 |
| | | 5.5 ^{g,h} | 6.5 ^{g,h} | |
| | | 6.0 ^{g,i} | 9.5 ^{g,i} | |
| 3.0 ^{i,h} | | 4.0 ^{j,h} | | |
| 5.0 ^{i,i} | | 8.0 ^{j,i} | | |
| MARINE (estuarine and coastal) | England and Wales | Condition high | Boundary ^k | Government of the United Kingdom 2015 |
| | | good | 5.7 | |
| | | moderate | 4.0 | |
| | | poor | 3.4 | |
| | United States | Juvenile and adult ^l survival | 2.3 | United States Environmental Protection Agency 2000 |
| | | Growth effects ^l | 4.8 | |

^alowest acceptable concentration^bolder life stages^cearly life stages (eggs and larvae)^dintermittent standards; minimum for 6 hours duration, with a one month to 2 year return period^e7-day mean daily minimum^finstantaneous minimum^g30 day mean^holder life stagesⁱearly life stages (eggs and 30 day post-hatch larvae)^j1 day minimum^k5th percentile value^llimits for constant exposure

on fish growth have the advantage of measuring a more direct correlate of fitness with an unambiguous ecological interpretation (Brett and Blackburn 1981; Hrycik et al. 2017). Growth has the advantage of integrating the effects of relatively mild levels of hypoxia across a period of multiple weeks, increasing sensitivity to detect chronic effects that might be transient over shorter time scales. Growth-hypoxia studies require a more significant time investment than shorter behavioural or physiological assays, but behavioural assays of hypoxia tolerance are rare, while enough laboratory studies of hypoxia effects on fish growth have accumulated so that quantitative analyses of sublethal thresholds are now possible (e.g., Hrycik et al.

2017; Saari et al. 2018; Vaquer-Sunyer and Duarte 2008). In analogy with P_{crit} , we label the threshold levels of dissolved oxygen where growth and food consumption decline as G_{crit} and C_{crit} , respectively.

To define quantitative thresholds of growth impairment as well as the slope of growth reduction under hypoxia, we fit segmented regressions to published growth-hypoxia data for freshwater ($N = 17$) and marine fishes ($N = 13$). To better understand factors that drive variation in hypoxia tolerance among taxa, we also modelled growth thresholds as simple functions of environmental and ecological covariates reported for each species (i.e., marine vs. freshwater; trophic level (omnivores, herbivores, carnivores);

habitat use (benthic, pelagic, bentho-pelagic, demersal, littoral); maximum habitat depth; maximum fish length; study temperature; fish weight; and maximum temperature tolerance). Our objectives were 1) to evaluate the utility of growth and consumption as integrated metrics of hypoxia tolerance, and their relationship with more commonly used physiological metrics like P_{crit} ; 2) to assess whether inter-specific variation in G_{crit} and the slope of growth reduction under hypoxia can be predicted based on ecological or environmental covariates including maximum temperature tolerance, trophic ecology, or habitat use; and 3) to evaluate whether quantitative thresholds for hypoxia impacts on fish growth are consistent with current water quality guidelines for the protection of aquatic life based on qualitative literature reviews. Our general expectations were that G_{crit} and C_{crit} would be highly correlated, and that G_{crit} would be positively related to temperature tolerance and lowest for benthic species.

Methods

Data collection

We used the University of British Columbia Library search engine and Google Scholar to search the web for studies relating growth or food consumption to hypoxia using combinations of “hypoxia”, “fish”, and “growth” using publication dates up to June 2020. We also used citations of published papers to identify additional relevant studies, and used reference lists in older published papers to identify studies that were not available in current searchable databases. Studies or treatments that used supersaturated or cyclic dissolved oxygen (DO) treatments were excluded from analysis, as were studies on larval fish (i.e., <0.05 g; Weber and Kramer 1983) due to concerns that larval fish surface area, diffusion, and metabolic constraints may be qualitatively different than larger life history stages. Because at least three independent treatment levels are required to fit a segmented regression, studies were only included if they reported fish growth at a minimum of 3 dissolved oxygen levels; studies with only 2 dissolved oxygen treatments cannot provide inference concerning breakpoints and were therefore excluded. Although the precision of breakpoint identification might be expected to increase with the number of DO treatment levels, a regression of estimated DO breakpoint values on number of DO treatment levels was not significant ($R^2 = 0.07$, $F_{1,36} = 2.2$, $P < 0.15$; Fig. S1; Table S1, Supporting Information), indicating that the estimated breakpoint is independent of sample size. Two candidate studies with three or more DO treatments were excluded from analysis because the spacing of DO treatment levels could not reasonably be

expected to capture a breakpoint with confidence (Carp: 1, 2, and 7 mg/l, Zhou et al. 2001; Atlantic Halibut 6, 9, 11, 13, and 16 mg/l); Thorarensen et al. 2010). To control for effects of variable ration on oxygen demand, studies or treatments were included only if fish were fed on a satiation ration. Some studies reported growth over multiple time intervals or measured hypoxia effects at different temperatures or across several size classes of fish. To avoid pseudo-replication in the global analysis of hypoxia effects across species, we used growth estimated across the full duration of each experiment, and combined data for both size classes in breakpoint estimation when two sizes of fish were assessed ($n = 5$ studies; exploratory analysis indicated no effect of body size, but the low sample size means limited power to detect any size effect). For studies that reported hypoxia effects at a single temperature, we assumed that researchers chose a temperature that was close to the final temperature preferendum that maximized growth under satiation for the focal species. When growth was reported for multiple temperatures ($n = 5$ studies), we only included data for the temperature that maximized growth in the study, with the rationale that hypoxia effects at maximum growth are most consequential to overall growth and fitness of fish, and to standardize all studies to hypoxia impacts at or near optimal growth. Most studies examined growth of young-of-the-year fish, but large inter-specific variation in size and age at maturity resulted in a wide range of growth rates; consequently, both growth and consumption in each study were re-scaled to a maximum of one to facilitate comparison across studies (i.e., growth rate data were divided by the maximum estimated growth in each experiment as defined by the plateau of the segmented regression fit to the original data).

A total of 38 studies representing 30 species were found that met these criteria for hypoxia effects on growth, and 19 studies representing 16 species for food consumption (Table 2; Tables S2–S5 Supporting Information). For those species that had multiple estimates of hypoxia thresholds from independent published studies we calculated the breakpoint, slope, and intercept for each replicate study separately and then averaged values across replicates to generate a species mean to avoid pseudoreplication. However, all individual studies are included in figures that display raw data.

Potential Predictors of Hypoxia Tolerance

Species-specific ecological attributes that could affect hypoxia tolerance were collated for each species (Table S2, Supporting Information). Attribute values were derived from Fishbase, or from the published and grey literature if data were not reported in Fishbase or suspected to be inaccurate. Variables included marine vs. freshwater

Table 2 Species for which data was available to estimate segmented regression thresholds for growth reduction (G_{crit}) and reduced consumption (C_{crit})

| Common Name | Habitat | Species | No. studies | G_{crit} | C_{crit} |
|---------------------|---------|--|-------------|------------------------------|------------------------------|
| Atlantic Cod | M | <i>Gadus morhua</i> | 1 | 6.26 (5.28–7.9) ^a | 8.24 (5.5–11) |
| Atlantic menhaden | M | <i>Brevoortia tyrannus</i> | 1 | 2.24 (1.9–2.6) | – |
| Atlantic sturgeon | FW | <i>Acipenser oxyrinchus oxyrinchus</i> | 1 | 4.06 (3.0–9.8) ^a | 3.65 (3.0–6.1) ^a |
| Beluga sturgeon | FW | <i>Huso huso</i> | 1 | 7.55 (2.5–14.0) ^a | 6.48 (2.5–11.3) ^a |
| Blue Tilapia | FW | <i>Oreochromis aureus</i> | 1 | 3.95 (3.2–4.7) | – |
| Carp | FW | <i>Cyprinus carpio</i> | 1 | 4.29 (4.0–4.6) | – |
| Channel catfish | FW | <i>Ictalurus punctatus</i> | 3 | 6.37 (3.5–9.2) ^b | – |
| Coho Salmon* | FW | <i>Oncorhynchus kisutch</i> | 2 | 4.39 (3.4–6.3) ^c | – |
| Dab | M | <i>Limanda limanda</i> | 1 | 6.83 (4.2–12.1) ^a | – |
| European sea bass | M | <i>Dicentrarchus labrax</i> | 1 | 7.28 (4.5 -) ^a | 6.30 (4.5 -) ^a |
| Mummichog | M | <i>Fundulus heteroclitus</i> | 2 | 2.9 (1.2–6.9) ^c | – |
| Largemouth bass | FW | <i>Micropterus salmoides</i> | 1 | 4.01 (3.4–4.6) | 4.38 (3.9–4.8) |
| Northern pike | FW | <i>Esox lucius</i> | 1 | 3.97 (0.4–7.6) | 4.33 (0.6–8.1) |
| Plaice | M | <i>Pleuronectes platessa</i> | 1 | 5.25 (2.6 -) ^a | – |
| Rainbow trout* | FW | <i>Oncorhynchus mykiss</i> | 1 | 6.6 (6–7.2) | 5.46 (4.9–6.1) |
| Silver bream | M | <i>Rhabdosargus sarba</i> | 1 | 3.68 (3.1–4.2) | 5.77 (4.4–7.2) |
| Southern flounder | M | <i>Paralichthys lethostigma</i> | 2 | 5.7 (2.3–9.0) ^b | – |
| Silver catfish | FW | <i>Rhamdia quelen</i> | 1 | 5.2 (3.1–9.6) ^a | – |
| Sockeye Salmon* | FW | <i>Oncorhynchus nerka</i> | 1 | 3.57 (2.9–4.2) | – |
| Spot | M | <i>Leiostomus xanthurus</i> | 1 | 2.12 (0.23–4) | – |
| Spotted Wolfish | M | <i>Anarhichas minor</i> | 1 | 7.38 (6.0–13.0) ^a | 8.48 (6.0–12.8) ^a |
| Striped Bass | FW | <i>Morone saxatilis</i> | 1 | 6.38 (4.0 -) ^a | 5.63 (4.0 -) ^a |
| Striped Bass hybrid | FW | <i>Morone saxatilis x M. chrysops</i> | 1 | 7 (5.1 -) ^a | 5.69 (3.1 -) ^a |
| Summer flounder | M | <i>Paralichthys dentatus</i> | 1 | 3.91 (3.5–4.7) ^a | – |
| Nile tilapia | FW | <i>Oreochromis niloticus</i> | 3 | 4.62 (0–9.5) ^b | 5.14 (0–9.4) ^b |
| Turbot | M | <i>Scophthalmus maximus</i> | 2 | 7.3 (4.9–9.7) ^b | 6.59 (0–13.2) ^b |
| Winter flounder | M | <i>Pseudopleuronectes americanus</i> | 1 | 6.69 (5.0–27.3) ^a | – |
| White Perch | FW | <i>Morone americana</i> | 1 | 3.07 (1.5 -) ^a | 3.83 (1.5–3.8) ^a |
| Yellow catfish | FW | <i>Pelteobagrus fulvidraco</i> | 1 | 6.39 (4.0–10.7) ^a | 4.86 (4.0–10.8) ^a |
| Yellow perch | FW | <i>Perca flavescens</i> | 1 | 3.65 (2.6–4.7) ^a | – |

The full data set is available as archived data online

M Marine, FW Freshwater (based on the whether the life stage tested for hypoxia tolerance was in fresh or salt water)

*salmonid

^alower 95% confidence interval is based on significant differences in mean growth between DO treatments

^b95% CIs around the average G_{crit} value for all replicate studies of the species, ^c95% CIs are the highest and lowest interval values reported for the 2 studies

occurrence; trophic group (omnivore, herbivore or carnivore); habitat use (benthic, pelagic, benthopelagic, littoral, or demersal); maximum species length (cm); average weight of the fish used in each study (g); average latitude of the species range; maximum depth of habitat use (m); whether the species was warmwater or coolwater; temperature of the growth experiment (°C); and the maximum reported temperature tolerance (°C). Taxa that occurred in both freshwater and marine habitats during different

life-history stages were classified based on the life-history stage where hypoxia was measured, i.e., rainbow trout, sockeye and coho salmon, striped bass, white perch, and Atlantic and Beluga sturgeon were classified as freshwater, despite adult residency in the ocean, because hypoxia tolerance was measured in juveniles in fresh or slightly brackish water. For trophic classification, omnivores ($N = 5$) had broad food preferences that included detrital organic matter, algae, and invertebrates; herbivores

($N = 2$) fed primarily on plant matter; and carnivores ($N = 23$) fed on invertebrates or other fishes (see Table S2, Supporting Information). Fish were considered benthic if they rested on the bottom (e.g., no air bladder), pelagic if they occupied the open water column (e.g., feeding on planktonic or pelagic organisms), benthopelagic if they were generalists occurring both in benthic and open water habitats, demersal if they were closely associated with the bottom but did not rest on it (i.e., occupied the near-benthic water column), and littoral if they were associated with the shoreline and shallower areas of inland waters or the ocean. We also compared hypoxia tolerance of salmonids ($N = 3$) to non-salmonids ($N = 27$), because salmonids are often a priority species for water quality management and thought to have higher sensitivity to hypoxia (Chapman 1986).

The midpoint of the latitudinal geographic range was used as a continuous metric of distribution (e.g., tropical vs. temperate). Species were also classified into two thermal classes: warmwater (average temperature of occurrence or final temperature preferendum greater than 21–22 °C), or coolwater (average temperature of occurrence or final temperature preferendum less than 21–22 °C) after Lyons et al. (2009) and Stuart-Smith et al. (2015). Information on thermal tolerances was derived from published assessments of thermal tolerance for multiple species (e.g., Hasnain et al. 2010; Lyons et al. 2009) or taxon-specific studies. We also included two continuous metrics of thermal preference; first, the reported temperature at which fish were reared in each study (assuming that researchers reared fish at close to optimal temperatures), and the reported thermal maximum or equivalent metric reported in the literature (see Table S2 and the temperature bibliography in Supporting Information). Upper temperature tolerances were inferred based on a variety of reported thermal limit metrics, ranging from temperatures where growth intersects zero to the experimentally derived critical thermal maximum (CT_{max}), or if this information was absent the highest environmental temperature where fish were observed in the wild. We used the maximum temperature that was reported in Fishbase if no other data were available. This necessarily introduced greater variation into our upper thermal tolerance metric, which we assumed to be randomly distributed (i.e., without bias). P_{crit} values for comparison with G_{crit} were extracted from supplementary figures reported in Rogers et al. (2016).

Statistical Analysis

Segmented regression to estimate thresholds of decreased growth and food consumption

Mass-specific growth rate ($\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$) of fish was modelled as a non-linear function of dissolved oxygen ($\text{mg}\cdot\text{l}^{-1}$,

equivalent to parts per million) for each study using segmented regression (package “segmented” in R; Muggeo 2008, 2019). Our application of segmented regression assumed a linear increase in growth (positive slope) up to a threshold dissolved oxygen value beyond which growth plateaus (slope = 0; Chabot and Dutil 1999; Wang et al. 2009). This variant of segmented regression is broadly consistent with the pathways and mechanisms of hypoxia limitation, which generally posit that hypoxia directly limits metabolism up to a dissolved oxygen concentration where supply exceeds demand, beyond which metabolism related to growth becomes limited by factors other than oxygen supply (e.g., gut capacity or digestive physiology; Wang et al. 2009). We also initially fit logarithmic, Michaelis-Menten, and monomolecular asymptotic regressions to growth data sets. However, as expected, segmented regression provided a superior fit in all but 2 studies (sockeye salmon and largemouth bass), where a monomolecular regression provided a somewhat better fit to the data. Therefore, to standardize comparison of slopes and breakpoints, for the final analysis we applied segmented regression to all studies because asymptotic regressions do not estimate thresholds (Toms and Villard 2015). Growth rate data were directly extracted from the text of results, data tables, or digitized from figures of source studies. If studies reported absolute growth rather than specific growth rate, specific growth was calculated from reported data as $\{\log_e(\text{final mass}) - \log_e(\text{initial mass})\} / \text{duration} \times 100$ (Ricker 1975) to ensure consistency in the response variable and data analysis.

Confidence intervals (CIs) on thresholds for individual species were calculated using the delta method in R segmented. Although it is possible to fit a segmented regression with only 3 data points, estimating CIs on the breakpoint requires at least 4 observations (i.e., dissolved oxygen treatments), and the CIs tend to be large at low sample size. However, even with only 3 dissolved oxygen treatment levels a conservative lower 95% confidence limit can be inferred if growth is significantly reduced at the lower treatment levels, i.e., the breakpoint will be somewhere between the lower and upper dissolved oxygen treatments that are significantly different. We used the level of significance between hypoxia (DO) treatments, as reported in the original study at $\alpha = 0.05$, to infer a lower 95% CI on the growth or consumption breakpoint when this information was reported. This allowed us to infer lower 95% CIs on G_{crit} for 11 of 12 studies with only 3 oxygen treatment classes; however, the same logic could not be applied to estimating upper CIs, which are absent for these studies. Fortunately, certainty in the lower 95% CI is most consequential for minimum water quality guidelines. Applying this method of inference to studies with more than 3 oxygen treatments also reduced the size

of the lower 95% CI in several cases, as noted in results. Nevertheless, confidence intervals around G_{crit} and C_{crit} estimates tended to be wide for many species, indicating comparatively large uncertainty in estimates (Table 2, Figs. S2 and S4) particularly for hypoxia experiments with a low number of dissolved oxygen levels. While this should not bias the overall mean threshold averaged across multiple species, it would tend to diminish power to detect relationships between thresholds and ecological covariates across species.

Comparison of segmented regressions and parameter values among species was facilitated by standardizing regressions to a maximum of 1. First, a segmented regression was fit to the reported specific growth rate data from each study (range of maximum reported daily specific growth among species 0.3–6.5%). The original growth data was then divided by the asymptote or plateau of the segmented regression (i.e., the fitted maximum observed growth) to re-scale the regression to a maximum predicted value of 1 for all species. This approach makes growth reduction under hypoxia scale-independent, and the predicted response can then be interpreted as a proportion of maximum growth at satiation. Food consumption data were extracted from studies in the same fashion as growth data, and generally expressed as grams consumed·gram body weight⁻¹·day⁻¹ (dry mass food:wet mass fish), and standardized and modelled as described for growth. Some studies reported reduced food consumption in units other than mass-specific consumption, but this should not affect the identification of the threshold or slope of standardized data.

We used two approaches to estimate the average threshold for initiation of hypoxic effects on fish. First, we combined data from all studies (total $n = 300$ observations for growth, $n = 189$ for food consumption) and fit a global segmented regression to estimate average G_{crit} and C_{crit} as described above. This explicitly weighted the contribution of different studies to parameter estimates based on study sample sizes. However, it could also be argued that slopes and breakpoints for each species should be treated as individual replicates and weighted equally (i.e., assuming that all studies were of equal value rather than weighting them by sample size); to this end we also calculated mean G_{crit} and C_{crit} by averaging the breakpoints calculated independently for each species. We also calculated the mean slope for all species (relative decline in growth per mg·l⁻¹ dissolved oxygen) to allow interpretation of the magnitude of average sublethal effects on growth below the breakpoint. Because the species average breakpoint, slope, and intercept were estimated with error, the species average intercept was fit based on the average slope and breakpoint for this second estimation method.

Although most studies were conducted on fish from a relatively narrow size range, five studies explicitly tested for differences in hypoxia tolerance between two discrete juvenile size classes (Table S3). For the purposes of calculating G_{crit} and C_{crit} for each species, we standardized observed growth and food consumption separately by dividing growth by the maximum observed value in each respective size class, before combining standardized data from the 2 size classes in a single segmented regression. The potential for size effects to influence hypoxia tolerance thresholds within a species was tested in a separate analysis, where G_{crit} was modelled as a function of weight while controlling for study effects (i.e., ANCOVA with weight as the covariate).

Relationship between thresholds and ecological and environmental covariates

We performed a series of analyses to test potential ecological drivers of variation in hypoxia tolerance. First, we used t-tests and ANOVA to assess the effects of individual class variables on G_{crit} and the slope of segmented regressions (t-tests: marine vs. freshwater species, warmwater vs. coolwater species; ANOVA: foraging mode, trophic group). The relationships between G_{crit} and continuous covariates (maximum temperature, experimental temperature, log of weight, log of maximum length, depth, average latitude) were then assessed using univariate regressions. We also assessed the degree of correlation among independent continuous variables using Pearson's R correlation coefficient. Residuals were tested for normality and homogeneity using Shapiro-Wilks W and a plot of residuals against predicted values. Weight had to be log-transformed to meet assumptions of normality. Slopes of segmented regressions could not be normalized, and were analyzed using a Wilcoxon 2-sample test to compare classes. Post-hoc comparisons of classes in ANOVA were assessed using a Tukey test.

After testing a priori hypotheses related to individual factors, the relative importance of covariates in predicting G_{crit} was evaluated using model selection with the Akaike Information Criteria for small sample sizes (AICc; Burnham and Anderson 2002). We first fit a global linear model using all covariates with the `lm` function in the `lme4` package of R. Models were then ranked using the `dredge` function in the `Mumin` package of R (Bartoń 2009) and considered plausible if they were within 7 delta AIC of the most likely model with the lowest AIC without model averaging (Burnham et al. 2011), and). The most parsimonious model was selected based on the lowest AICc. All analyses other than segmented regression and model selection were performed using SAS (SAS version 14.1; SAS Institute 2015).

Results

When parameter estimates for species were weighted equally, the average threshold for growth reduction (G_{crit} , $N = 30$ species) was $5.1 \text{ mg}\cdot\text{l}^{-1}$ dissolved oxygen (95% confidence intervals (CI) $4.5\text{--}5.7 \text{ mg}\cdot\text{l}^{-1}$). The average relative decrease in growth per $\text{mg}\cdot\text{l}^{-1}$ reduction in dissolved oxygen was 22%, with the declining leg of the segmented regression defined by $\text{SGR} = (0.22 \cdot \text{DO}) - 0.205$ (Fig. 1a). There was considerable variation in both slope and G_{crit} among species (Fig. 1b, Fig. S2; Table 2), with no significant difference between marine and freshwater taxa for either G_{crit} ($t_{28} = -0.30$, $P < 0.76$) or slope ($t_{28} = 0.19$, $P < 0.85$). Although the breakpoint of the segmented regression did not differ between salmonids and all other taxa combined ($t_{28} = 0.25$, $P < 0.80$), the regression slope was significantly steeper for salmonids (Wilcoxon 2-sample test, $N = 30$, $z = 2.35$, $P < 0.03$) and the intercept was significantly lower (Wilcoxon 2-sample test, $N = 30$, $z = 2.49$, $P < 0.02$; Fig. 1b), indicating greater sensitivity to hypoxia in salmonids. P_{crit} values reported in the literature were not significantly related to G_{crit} ($R^2 = 0.11$, $F_{1,8} = 1.01$, $P < 0.35$; Fig. S3) for the limited number of species where both were available ($N = 10$). There was no significant effect of fish size on G_{crit} for the five studies that measured growth in two juvenile size classes ($F_{1,8} = 1.41$, $P < 0.27$). When studies were weighted by sample size with all data combined in a single segmented regression, parameter estimates for G_{crit} , ($5.6 \text{ mg}\cdot\text{l}^{-1}$, 95% CI $5.0\text{--}6.2$) and the decrease in growth per $\text{mg}\cdot\text{l}^{-1}$ DO reduction (15%) were similar to weighting species equally.

When species were weighted equally, the average threshold for reduced food consumption (C_{crit} , $N = 16$) was $5.6 \text{ mg}\cdot\text{l}^{-1}$ dissolved oxygen (95% CI $4.9\text{--}6.4 \text{ mg}\cdot\text{l}^{-1}$; Fig. 2a, Fig. S4), which was not significantly different from the G_{crit} value of $5.1 \text{ mg}\cdot\text{l}^{-1}$. This was similar to C_{crit} , when studies were weighted by sample size with all data combined in a single segmented regression ($5.9 \text{ mg}\cdot\text{l}^{-1}$, 95% CI $5.3\text{--}6.5$). Marine and freshwater species did not differ in their breakpoints or slopes for segmented regressions of food consumption against dissolved oxygen, nor did salmonids compared to all other species (Fig. 2b). Consistent with growth, salmonids exhibited a very steep decline in food consumption at reduced dissolved oxygen that was only exceeded by one other non-salmonid species (Fig. 2b, Fig. S4), but the difference was not significant for this smaller data set (Wilcoxon 2-sample test, $N = 16$, $z = 1.83$, $P < 0.09$). Thresholds for reduction in growth and food consumption were significantly correlated ($R^2 = 0.50$, $F_{1,14} = 13.8$, $P < 0.002$), and the slope of the relationship (0.79) was not significantly different from one (Fig. 3), which is the expectation if reduced consumption is the mechanism limiting growth.

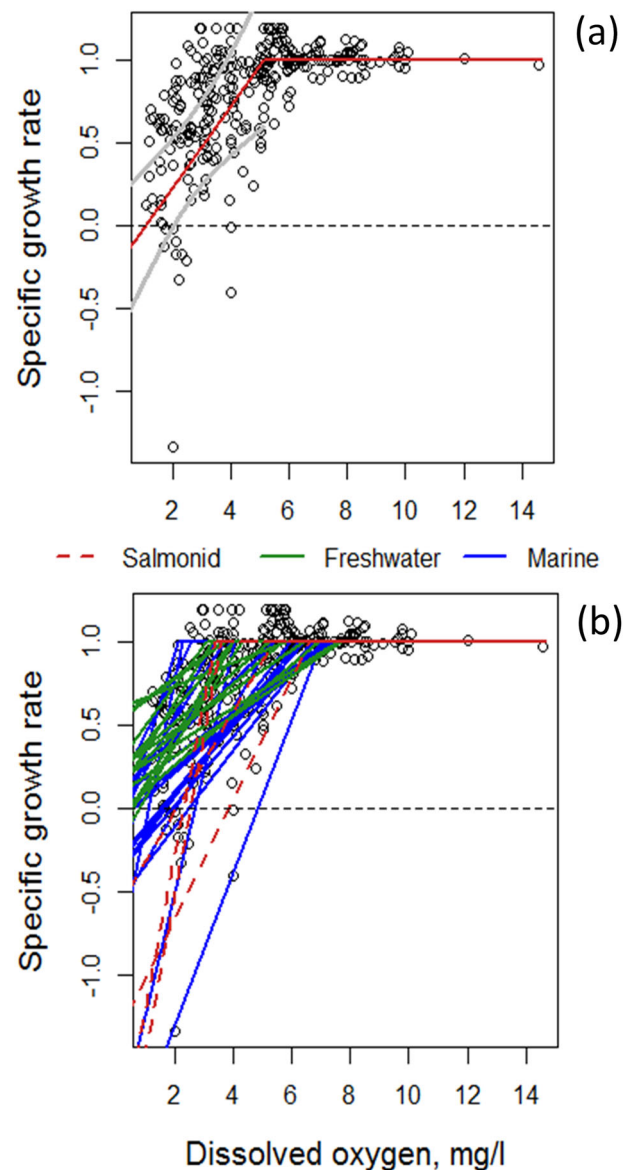


Fig. 1 **a** Segmented regression of specific growth rate (standardized to a maximum of 1) as a function of dissolved oxygen, averaged across 30 species. The average breakpoint value (G_{crit}) is 5.11 mg/l dissolved oxygen ($4.5\text{--}5.7 \text{ mg/l}$ 95% confidence interval). Open circles represent the mean standardized growth for individual oxygen treatments, grey lines are 95% confidence intervals. **b** Segmented regressions of specific growth rate on dissolved oxygen for 38 studies; green lines represent freshwater fish species, blue are marine taxa, and broken lines are salmonids

G_{crit} was significantly related to ecological and environmental covariates, but the variance explained was generally low. G_{crit} varied significantly with habitat use based on the significance of the overall ANOVA ($R^2 = 0.32$, $F_{4,25} = 2.9$, $P < 0.042$), but no habitat use pairs (e.g., benthic vs. pelagic) were significantly different with an a posteriori Tukey test, making the results difficult to interpret; and contrary to expectation benthic species had the highest G_{crit} ($6.1 \text{ mg}\cdot\text{l}^{-1}$) rather than the lowest. G_{crit} varied significantly

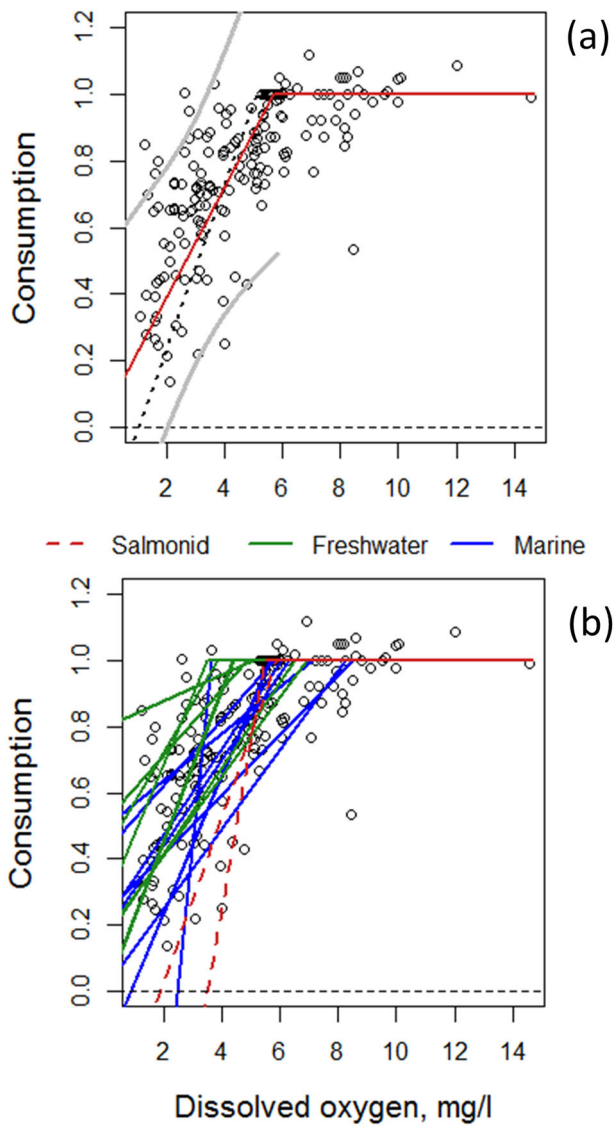


Fig. 2 **a** Segmented regression of food consumption (standardized to a maximum of 1) as a function of dissolved oxygen averaged across 16 species. The average breakpoint value (C_{crit}) is 5.67 mg/l dissolved oxygen (4.9–6.4 mg/l 95% confidence interval). Open circles represent the mean standardized consumption for individual oxygen treatments, grey lines are 95% confidence intervals, and the black broken line represents the growth vs. dissolved oxygen regression from Fig. 1a. **b** Segmented regressions of food consumption for 19 studies; green lines represent freshwater fish species, blue are marine taxa, and broken lines are salmonids

with trophic guild ($R^2 = 0.26$, $F_{2,27} = 4.85$, $P < 0.016$), and was lowest for omnivores ($N = 5$, $G_{crit} = 3.4 \text{ mg}\cdot\text{l}^{-1}$), intermediate for herbivores ($N = 2$, $G_{crit} = 4.3 \text{ mg}\cdot\text{l}^{-1}$), and highest for carnivores ($N = 23$, $G_{crit} = 5.5 \text{ mg}\cdot\text{l}^{-1}$). G_{crit} also increased with the reported maximum depth of habitat used by each species ($R^2 = 0.19$, $F_{1,25} = 5.73$, $P < 0.025$), the log of species maximum body length ($R^2 = 0.21$, $F_{1,28} = 7.59$, $P < 0.012$), and the log of average weight of

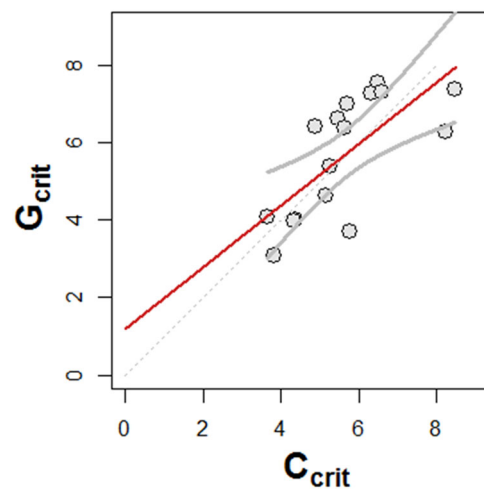


Fig. 3 Positive relationship ($R^2 = 0.50$, $P = 0.002$) between the breakpoint for growth (G_{crit}) and consumption (C_{crit}). Dotted line indicates a 1:1 relationship

fish used in each study ($R^2 = 0.44$, $F_{1,27} = 20.8$, $P < 0.0001$; $G_{crit} = [1.62 \cdot \log_{10}(\text{weight}_{\text{expt}})] + 3.06$; Fig. 4).

Consistent with expectation, coolwater species had a higher threshold for growth reduction ($N = 12$, $G_{crit} = 5.8 \text{ mg}\cdot\text{l}^{-1}$) relative to warmwater species ($N = 18$, $G_{crit} = 4.6 \text{ mg}\cdot\text{l}^{-1}$), although the difference was marginally non-significant ($t_{28} = 1.99$, $P < 0.056$). However, when temperature was expressed as a continuous variable G_{crit} was significantly correlated with maximum reported temperature tolerance for a species (Fig. 4a; $R^2 = 0.25$, $F_{1,26} = 8.72$, $P < 0.007$; $G_{crit} = [-0.127 \cdot T_{\text{max}}] + 9.23$; Fig. 4a) as well as the temperature of the growth experiment ($R^2 = 0.18$, $F_{1,28} = 6.17$, $P < 0.019$; $G_{crit} = [-0.126 \cdot T_{\text{study}}] + 7.75$), which was assumed to be near the optimal temperature for growth. Following a similar trend, G_{crit} also increased with latitude although the relationship was relatively weak and not statistically significant ($R^2 = 0.11$, $F_{1,28} = 3.37$, $P < 0.08$; $G_{crit} = [0.037 \cdot \text{average latitude}] + 3.61$).

The log of fish weight in each study and habitat guild were the most consistent predictors of G_{crit} , and were included in all models (Table 3). Trophic guild was the only other covariate retained within 2 deltaAIC of the top model. Covariates retained in models with some support (2–7 deltaAIC) included maximum temperature tolerance, the log of adult body length, maximum depth of habitat use, the midpoint of the latitudinal distribution, and whether a species occurred in marine or freshwater (Table 3). However, interpreting the relative significance of different continuous covariates as potential drivers of G_{crit} is complicated by the high correlations among most of the independent ecological or environmental drivers (Table 4).

Fig. 4 a Scatterplots and regressions showing a reduction in G_{crit} with increasing upper temperature tolerance (T_{max}) for 28 species of fish; and an increase in G_{crit} with greater maximum observed depth of occurrence of a species (b), greater maximum body length (c), or greater weight of fish used in the study (d). Error bars represent 95% confidence intervals around point estimates

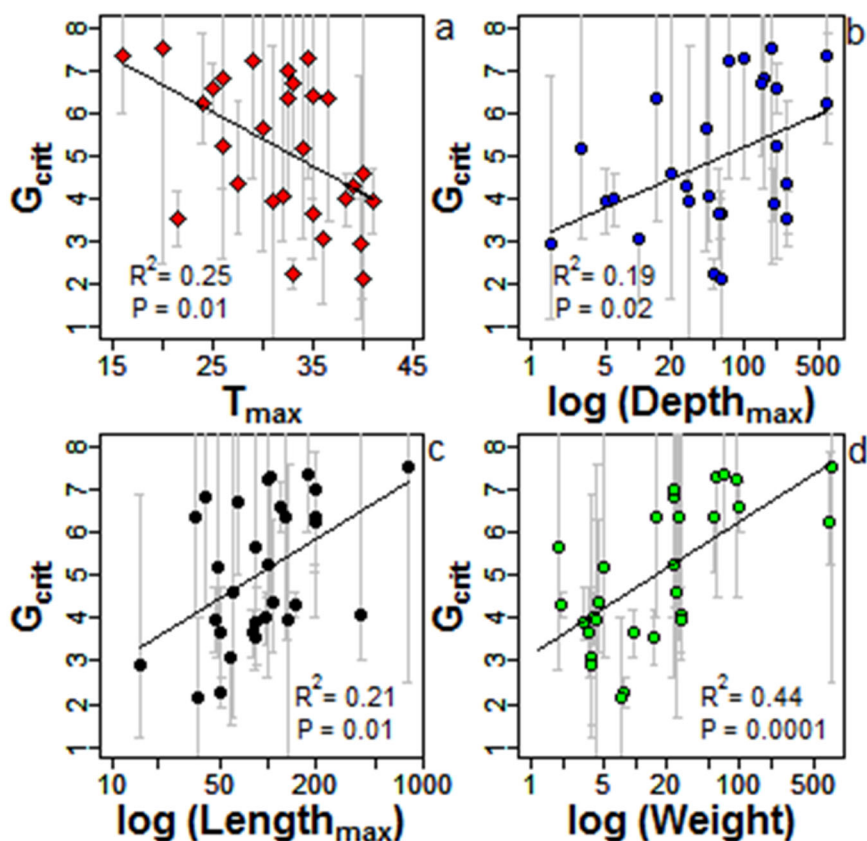


Table 3 Top ranked models and variables for predicting G_{crit}

| Model | df | Log likelihood | AIC _c | ΔAIC | weight | R ² |
|--|----|----------------|------------------|------|--------|----------------|
| Habitat + Log(wt) | 7 | -28.41 | 77.82 | 0 | 0.37 | 0.58 |
| Habitat + Log(wt) + TrophicGp | 9 | -24.38 | 79.63 | 1.81 | 0.15 | 0.67 |
| Habitat + Log(wt) + T _{MAX} | 8 | -24.49 | 80.59 | 2.77 | 0.09 | 0.64 |
| Habitat + Log(wt) + Log(length) | 8 | -27.84 | 81.28 | 3.46 | 0.07 | 0.78 |
| Habitat + Log(wt) + Max _{DEPTH} | 8 | -28.31 | 82.23 | 4.41 | 0.04 | 0.77 |
| Habitat + Log(wt) + AvgLat | 8 | -28.33 | 82.26 | 4.44 | 0.04 | 0.77 |
| Habitat + Log(wt) + MF | 8 | -28.41 | 82.42 | 4.60 | 0.04 | 0.76 |
| Habitat + Log(wt) + AvgLat + TrophicGp | 10 | -23.19 | 83.31 | 5.49 | 0.02 | 0.85 |

Habitat represents the dominant habitat classification for a species (benthic, pelagic, benthopelagic, demersal, littoral), wt represents the average weight of fish used in the experiment, TrophicGp represents trophic group (omnivore, herbivore, carnivore), T_{MAX} represents the maximum temperature tolerance reported in the literature, length is the maximum reported species length, AvgLat represents the average latitudinal distribution of a species, and MF represents marine or freshwater residency of the life stage tested

Discussion

Although quantitative assessments of hypoxia tolerance are limited, the mean threshold G_{crit} ($5.1 \text{ mg}\cdot\text{l}^{-1}$) and C_{crit} ($5.6 \text{ mg}\cdot\text{l}^{-1}$) values we derived are similar to but slightly higher than those reported in earlier reviews (e.g., Hryciuk et al. 2017: $G_{crit} = 4.5$, $C_{crit} = 4.5 \text{ mg}\cdot\text{l}^{-1}$). Using a more traditional dose-response toxicological approach with data from 11 fish species, Saari et al. (2018) reported $5.0 \text{ mg}\cdot\text{l}^{-1}$

as the threshold for a 10% reduction in growth (EC_{10}) for warmwater fishes and $5.2 \text{ mg}\cdot\text{l}^{-1}$ as the EC_{10} for coldwater fishes. Our values are also somewhat higher than the median threshold for sublethal effects of hypoxia on fish reported by Vaquer-Sunyer and Duarte (2008; $4.4 \text{ mg}\cdot\text{l}^{-1}$), although most of the sublethal effects included in their meta-analysis included performance metrics other than fish growth. Vaquer-Sunyer and Duarte (2008) also reported that 90% of published studies on hypoxia tolerance had

Table 4 Correlations among continuous habitat and species covariates used to predict G_{crit}

| | T_{MAX} | T_{STUDY} | Avg. Lat. | $Depth_{MAX}$ | $Length_{MAX}$ | Weight | |
|---|-----------|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------|
| | | 0.79 ^{***} | 0.68 ^{***} | -0.83 ^{***} | -0.52 ^{**} | -0.54 ^{**} | T_{MAX} |
| – | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.005 | 0.003 | |
| | 28 | 28 | 25 | 28 | 27 | 27 | |
| | | | 0.70 ^{***} | 0.76 ^{***} | -0.35 | -0.52 ^{**} | T_{STUDY} |
| | – | <0.0001 | <0.0001 | <0.0001 | 0.06 | 0.004 | |
| | | 30 | 27 | 30 | 29 | 29 | |
| | | | 0.55 ^{**} | 0.21 | 0.29 | 0.29 | Avg. Lat. |
| | | – | 0.003 | 0.27 | 0.13 | 0.13 | |
| | | | 27 | 30 | 29 | 29 | |
| | | | | 0.45 [*] | 0.49 [*] | 0.49 [*] | $Depth_{MAX}$ |
| | | | – | 0.02 | 0.01 | 0.01 | |
| | | | | 27 | 26 | 26 | |
| | | | | | 0.54 ^{**} | 0.54 ^{**} | $Length_{MAX}$ |
| | | | | – | 0.002 | 0.002 | |
| | | | | | 29 | 29 | |
| | | | | | – | – | Weight |

The first row is the Pearson correlation coefficient (r), followed by the probability and the sample size. Depth, length, and weight are log-transformed values. Bolded correlation coefficients are statistically significant at ^{*} $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$

thresholds for sublethal effects below $5.0 \text{ mg}\cdot\text{l}^{-1}$, and that 90% of lethal hypoxia thresholds were below $4.6 \text{ mg}\cdot\text{l}^{-1}$. This indicates that using average thresholds for sublethal effects on fish growth as regulatory criteria would be protective to most other aquatic taxa (i.e., fish can act as an umbrella taxon to protect 90% of other species; but see Saari et al. (2018) for a more detailed consideration of freshwater invertebrate hypoxia sensitivity).

An implicit assumption of our analysis is that the suite of studies available in the literature reflect a representative subset of fish species. However, species studied for hypoxia tolerance are almost certainly non-randomly selected; some target species were chosen because they are important to sport or commercial fisheries (e.g. largemouth bass, turbot), others because they are keystone species (e.g. cod), or because they are known to be hypoxia tolerant or model species in physiology (e.g., *Fundulus*). The comparatively large sample size (30 species) covering a diversity of ecological niches suggests that the central tendency of the data is likely representative for bony fishes. However, given that carnivores ($N = 23$) and temperate fishes ($N = 23$) are generally better represented compared to tropical species ($N = 7$), inferences are likely most robust for north temperate fish communities.

Direct mortality or population collapse is the most severe biological outcome of hypoxia, and may be used as a default benchmark for hypoxia classification (e.g., Rabalais et al. 2010). However, negative impacts of hypoxia can occur at DO levels well above thresholds that induce mortality (Vaquer-Sunyer and Duarte 2008; Wu 2002), and

DO guidelines for the protection of aquatic life in freshwater are generally based on incipient sub-lethal effects rather than lethal ones (e.g., Franklin 2014; Chapman 1986). Growth reduction represents a credible and meaningful index of negative impact, since it integrates behavioural, physiological, and energetic responses into a single ecological metric that has well-understood fitness consequences. While measurement of growth is a time-consuming assay compared to more short-term behavioural metrics (e.g., loss of equilibrium; Wood 2018), it requires a relatively low level of technology and generates unambiguous results. Fitting segmented regression to growth data also allows interpretation not only of the breakpoint where growth declines, but also the incremental decline in growth with increasing hypoxia (i.e. slope and intercept of the declining segment). These additional metrics allow finer resolution of hypoxia sensitivity than the breakpoint alone, as illustrated by the significantly steeper decline in growth observed for salmonids relative to other taxa, despite similar G_{crit} .

The strong correlation that we observed between G_{crit} and C_{crit} indicates that the threshold for decreased food consumption can be used as a credible alternative to G_{crit} . C_{crit} has the advantage of being a much more rapid assay than a 2–4 week growth experiment, which makes it an appealing metric of hypoxia tolerance. Measuring food consumption is also a simple enough assay that it can probably be performed in the field for endangered species when transfer of wild fish to laboratory facilities could compromise their subsequent re-introduction to the wild

because of disease concerns. Coherence between G_{crit} and C_{crit} also indicates that the primary mechanism whereby hypoxia affects growth is through a reduced ability to consume food, or to convert it into somatic tissue (e.g., Zambonino-Infante et al. 2017), compounded by diversion of energy away from somatic growth to stressor costs associated with hypoxia. Digestion and protein synthesis (e.g. specific dynamic action) have very high energy demands (up to 70% of the maximum metabolic rate for fish; Alsop and Wood 1997), which means that hypoxia will directly limit consumption and growth at dissolved oxygen thresholds well above those required for maintenance metabolism (Wang et al. 2009). Although less well-studied, hypoxia likely has similar effects on invertebrate food consumption; for instance Low and Micheli (2018) observed a 39–47% decrease in sea urchin grazing at 5.5 mg·l⁻¹ dissolved oxygen.

P_{crit} is a commonly used index of hypoxia tolerance, but has recently been criticized as a potentially ambiguous metric for methodological and conceptual reasons (Speers-Roesch et al. 2013; Wood 2018; but see Ultsch and Regan 2019 and Seibel et al. 2021 for alternative perspectives). The weak relationship we observed between P_{crit} and G_{crit} supports the view that it is a poor index of longer-term tolerance to sub-lethal effects of hypoxia, although this needs to be tempered by our comparatively low sample size of paired G_{crit} and P_{crit} values ($N = 10$). While measuring P_{crit} remains a valuable assay for understanding the immediate homeostatic physiological response to hypoxia, if the intent is to inform regulatory thresholds we would advocate for the use of G_{crit} or C_{crit} as more directly interpretable metrics of chronic hypoxia tolerance.

Univariate regressions identified several factors that were correlated with hypoxia tolerance, although none explained a very large proportion of the observed variation among species. The relatively weak relationships between G_{crit} and covariates are likely due in part to the wide confidence intervals around breakpoints for many species (Figs. S2 and S4, Table 2), which would reduce power to detect underlying trait associations. Some relationships with covariates were consistent with expectation: G_{crit} decreased with maximum temperature tolerance, and detritivores and herbivores were more hypoxia tolerant than carnivores, as might be expected based on their habitat associations and the aerobic demands of their lifestyles; for instance, Munn et al. (2020) found that the relative abundance of detritivorous fish species increased at lower minimum dissolved oxygen values. Other covariate associations were less clear; for instance, the weight of fish used in experiments explained the largest variation in G_{crit} ($R^2 = 0.44$). Since most growth experiments were conducted on juveniles, this may be due to the greater respiratory demand associated with the growth of juveniles of species that are larger at

maturity, and therefore at an earlier stage in their growth trajectory for a given body size (Monnet et al. 2020; Sibly et al. 2015). Alternatively, it may simply be an artefact of the correlation between the weight of experimental fish and adult size, since larger species had a higher G_{crit} (Fig. 4d).

Model ranking identified habitat use (lifestyle) as the most significant factor explaining variation in G_{crit} , but interpretation of habitat use effects was unclear; benthic fish had the highest G_{crit} , rather than being the most hypoxia tolerant as predicted. However, the high degree of correlation among covariates makes it difficult to infer causation; for instance, 62% of the variation in maximum temperature tolerance is explained by the variables “habitat use” and “trophic group” ($P < 0.0012$). Hypoxia tolerance is likely affected by context-specific selection on habitat use, trophic level, and life history as well as environmental tolerances and phylogenetic constraints, so it is probably unsurprising that most inter-specific variation remains unaccounted for. Intrinsic differences in activity levels among species may also affect oxygen consumption and hypoxia tolerance, and this variation may be poorly captured by habitat associations (i.e., a benthic guild might include both active and sedentary species). Hrycik et al. (2017) similarly concluded that most inter-specific variation in G_{crit} was associated with phylogeny rather than ecological covariates. The overall pattern that emerges is one where smaller, warmwater fishes in shallow waters are more likely to be hypoxia tolerant than larger coolwater species capable of using deeper habitats; however, these habitat relationships are likely too weak to be considered highly predictive for data deficient species.

Although regulatory criteria for dissolved oxygen are based exclusively on oxygen concentration (mg·l⁻¹ or ppm), a case can be made that concentration is not the best metric of oxygen supply to aquatic life (Deutsch et al. 2015; Verberk et al. 2011). Oxygen supply to the gills is influenced by both dissolved oxygen concentration, which decreases with temperature, and partial pressure of oxygen, which increases with temperature. The increase in partial pressure of oxygen with temperature tends to stabilize oxygen supply and may partially or entirely compensate for lower dissolved oxygen concentrations at elevated temperatures (Deutsch et al. 2015). The lower G_{crit} observed for warmwater species in our analysis may therefore partly reflect the stabilizing effect of partial pressure on oxygen supply at higher temperatures, rather than actual adaptations to hypoxia.

Implications for Water Quality Guidelines for the Protection of Aquatic Life

Average thresholds for initiation of reduced growth and food consumption in our analysis are broadly consistent

with established freshwater minimum water quality guidelines for dissolved oxygen in multiple jurisdictions, including Canada, New Zealand, the United States, and Great Britain (Table 1). Most guidelines explicitly identify higher hypoxia thresholds for coldwater streams, which is consistent with our observation of higher G_{crit} for coolwater species; they also generally recognize the greater sensitivity of egg and larval life stages, which was not part of our analysis. These guidelines are based on qualitative literature reviews considering a variety of biological responses focused on fish, and represent a consensus opinion across multiple agencies. Our analysis indicates a sound quantitative foundation for these guidelines in terms of thresholds of growth impairment for fish, as do earlier quantitative reviews of hypoxia impairment (e.g., Hrycik et al. 2017; Saari et al. 2018; Vaquer-Sunyer and Duarte 2008). Convergence of quantitative analyses and earlier qualitative weight-of-evidence assessments increases confidence that current water quality guidelines are well supported.

All of the studies that we included in analysis manipulated dissolved oxygen as a single independent stressor. However, hypoxia is often associated with additional water quality issues that further elevate organism stress (Breitburg et al. 2018; Niklitschek and Secor 2005). Release of hydrogen sulphide from benthic sediments often accompanies hypoxia, causing toxicity by binding to cytochrome c oxidase and blocking cellular respiration; survival time under hypoxia declines by an average of 30% in the presence of hydrogen sulphide (Vaquer-Sunyer and Duarte 2010). Similarly, elevated temperature increases respiratory demand, resulting in an average $0.7 \text{ mg}\cdot\text{l}^{-1}$ increase in the threshold for hypoxia-driven mortality for every 10°C rise (Vaquer-Sunyer and Duarte 2011). Consequently, our estimates of G_{crit} and incremental growth reduction (22% drop per $\text{mg}\cdot\text{l}^{-1}$ decrease in dissolved oxygen) are likely to underestimate the realized effect of hypoxia when it occurs with other stressors in the natural environment.

Minimum dissolved oxygen guidelines for freshwater life (i.e., $5\text{--}6 \text{ mg}\cdot\text{l}^{-1}$; Table 1) contrast sharply with commonly used definitions of marine hypoxia in the primary literature, which generally center around $2\text{--}2.5 \text{ mg}\cdot\text{l}^{-1}$ (Vaquer-Sunyer and Duarte 2008; Rabalais et al. 2010), although recent studies have argued for revising these upwards based on meta-analyses demonstrating negative effects of hypoxia at thresholds well above $2.5 \text{ mg}\cdot\text{l}^{-1}$ (Steckbauer et al. 2011). Nevertheless, the apparent difference in recognized hypoxia thresholds in freshwater and the conventional use of hypoxia with reference to marine habitats is striking. Its origin may reflect implicit assumptions concerning what constitutes normoxia in freshwater vs. marine environments, or it may simply reflect a *de facto* labeling convention that has perpetuated itself in the marine literature (Rabalais et al. 2010; Steckbauer et al. 2011).

Higher thresholds in freshwater may also reflect implicit recognition of the greater vulnerability of confined inland waters to hypoxia relative to larger-volume marine environments (Jenny et al. 2016a), or perceptions concerning the greater ability to manage nutrient inputs and water quality in confined inland waters vs. the marine environment (Geist and Hawkins 2016). However, regulatory guidelines for dissolved oxygen in marine habitats, where available, are often only slightly lower than those for freshwater (Table 1; United States Environmental Protection Agency 1986, 2003), suggesting that regulators are basing guidelines on observed thresholds of impacts rather than labeling conventions.

Operationally, freshwater guidelines in the range of $5\text{--}6 \text{ mg}\cdot\text{l}^{-1}$ are intended as both management triggers and minimum water quality targets. However, it is less clear whether $2\text{--}2.5 \text{ mg}\cdot\text{l}^{-1}$ is viewed as a management target in marine environments (e.g., Government of the United Kingdom 2015), or simply a threshold for defining hypoxia. The main functional difference between freshwater and *de facto* marine hypoxia definitions is the diagnostic level of biological response. Freshwater criteria are based on incipient sublethal effects on individuals (e.g. growth reduction), while marine hypoxia in the primary literature is more conventionally defined by population collapse or catastrophic species loss (Vaquer-Sunyer and Duarte 2008). To some extent guidelines based on impacts to individuals can be viewed as more precautionary; however, thresholds set near a presumed level of population collapse (e.g. $2\text{--}2.5 \text{ mg}\cdot\text{l}^{-1}$) are problematic for four reasons. First, it is difficult to precisely manage oxygen to low thresholds, particularly in confined inland or estuarine waters that are subject to stochastic interannual or diel variation in flows or temperature that can trigger a rapid increase in respiration and hypoxia (e.g., Niklitschek and Secor 2005; Pardo and García 2016; Dubuc et al. 2017; Zinn et al. 2021). Stochasticity inevitably increases the margin of error required to ensure that thresholds are not exceeded, necessarily elevating thresholds that are intended as management triggers (Larned and Schallenberg 2019; Scholes and Kruger 2011). Second, as noted earlier hypoxia frequently co-occurs with other stressors (e.g. sulphide toxicity, elevated temperatures) that can elevate a management threshold higher than expected from hypoxia alone. Third, nutrient inputs tend to enhance fish community production, which will initially delay or mask the negative impacts of hypoxia (Breitburg et al. 2018), despite an impending threshold. Fourth, once a hypoxia threshold is crossed, system recovery may be difficult due to positive feedbacks (e.g., release of P from hypoxic sediments; Conley et al. 2009; Watson et al. 2017), which greatly increases the likelihood that the hypoxic state will self-perpetuate; these

feedbacks generate resistance to a return to normoxic conditions, meaning that recovery may be multidecadal, partial, or even non-existent (Duarte et al. 2009; Steckbauer et al. 2011; Jenny et al. 2016b). Thus regulatory thresholds that are set too low (i.e., 2–2.5 mg·l⁻¹) run the risk of perpetuating hypoxic states, rather than preventing them. Finally, legacy effects in nutrient dynamics create significant temporal lags in ecosystem response to management interventions; this means that thresholds set at the cusp of population collapse may trigger management actions too late to be effective (Scholes and Kruger 2011). Given these considerations, the generally higher thresholds in freshwater that are calibrated to incipient growth reduction or other sublethal effects should be viewed not as precautionary, but as necessarily protective (Larned and Schallenberg 2019); this is explicitly recognized in regulatory guidelines for some marine habitats (e.g., United States Environmental Protection Agency 2003).

This review was partially motivated by an assessment of recovery needs for Salish sucker, a federally threatened species in Canada experiencing widespread hypoxia throughout its designated critical habitat (Pearson 2015; Rosenfeld et al. 2021). Uncertainty around the benefits and necessity of targeted experiments on hypoxia tolerance for this species complemented conservation concerns over small population size that precluded collection of wild fish for laboratory experiments. However, the broader science and conservation issue is whether adequate information already exists to guide species recovery without the need for taxon-specific hypoxia studies.

In practical terms, regulatory guidelines for dissolved oxygen can be established based on species-specific tolerances, or a reference condition approach based on what is considered normoxic for a given ecosystem or habitat and associated fish community (Rabalais et al. 2010). For example, normoxia in most north temperate waterbodies is at or near oxygen saturation, barring habitats that might naturally tend towards hypoxia, e.g. wetlands or marshes with low circulation and relatively high respiratory demand, or the hypolimnion of stratified or winterkill lakes. As a generic standard for north temperate water bodies, a G_{crit} in the range of 5.1 mg·l⁻¹ is appropriate. In contrast, applying saturation as the normoxic reference condition to naturally oxygen depleted sulfide springs and the sulfur-tolerant poeciliids that are endemic to them would be non-sensical (Tobler et al. 2009). It is also important to note that hypoxia tolerance often varies with life stage, but the growth and food consumption data we present are almost exclusively derived from juveniles, presumably because rapid juvenile growth increases power to detect treatment effects over a short time interval. Although eggs (lacking mobility) have relatively low oxygen tolerance, the hypoxia tolerance of larger adult fish may be related more to species-specific

lifestyle adaptations than allometry of body size (Nilsson and Östlund-Nilsson 2008), and may deviate from the average thresholds for juveniles observed in our analysis.

With reference to threatened species like Salish sucker, which co-occur in mixed communities with salmonids and other native coolwater species, taxon-specific studies of hypoxia tolerance are likely unwarranted for practical management. Even for taxa tolerant of hypoxia (i.e., with a low G_{crit}), adoption of lower dissolved oxygen guidelines should be approached with caution because hypoxia management involves managing ecosystems, rather than individual species. As noted above, managing dissolved oxygen to low thresholds is complicated by unanticipated non-linearities (Larned and Schallenberg 2019; Rosenfeld 2017); in systems prone to hypoxia, stochastic temperature increases or flow reductions (e.g., random heat waves or drought; Niklitschek and Secor 2005) can rapidly drop water quality below target levels, and this risk is greatest at lower thresholds where system stability is often compromised. Exceptions are highly regulated systems, where discharge and dissolved oxygen can be managed with precision by directly manipulating river flows (Graeber et al. 2013). More detailed research to refine criteria beyond generic guidelines may be warranted for ecosystems that are the nexus of multi-billion dollar socio-economic systems (Kauffman 2019; United States Environmental Protection Agency 2003), or when endangered species are suspected of unusually high sensitivity to hypoxia (United States Environmental Protection Agency 2000). However, in most cases dealing effectively with hypoxia will mean managing ecosystems with a wider margin of error (Larned and Schallenberg 2019; Scholes and Kruger 2011). It is also important to note that the freshwater DO thresholds listed in Table 1 are currently non-binding guidelines (rather than management triggers). Unlike regulatory requirements, guidelines are generally discretionary and are not always effective because they are easily ignored (e.g., Boesch 2019; Elliott and Rossio 2003; Rosenfeld et al. 2021). Operationally, guidelines need to be translated into enforceable regulations if water quality for species and ecosystems at risk is to be managed effectively (Boesch 2019).

Data availability

All data are available in the Supporting Information and the Figshare online repository <https://figshare.com/s/164a76a8aeadea807233>, or by contacting the corresponding author.

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Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests.

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