Meta-analyses

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Sub-lethal effects on fish provide insight into a biologically-relevant threshold of hypoxia

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Hypoxia (low dissolved oxygen) is a mounting concern for aquatic ecosystems as its prevalence increases with rising anthropogenic nutrient inputs. Hypoxia is most commonly defined as 2.0 mg l^{-1} of dissolved oxygen, although this level varies widely across studies and agency regulations. Such definitions may be too conservative, as ecologically-relevant non-lethal effects (e.g. consumption and growth) of hypoxia on important aquatic species, such as fish, often occur at oxygen levels much higher than 2.0 mg l^{-1}. In addition, many mechanisms that regulate hypoxia tolerance in fish have been proposed, including temperature, habitat, location in the water column, and body size, but there is ongoing debate over which mechanisms are most important. Using a structured meta-analysis of published studies, we showed consistent, significant negative effects on fish growth and consumption below 4.5 mg l^{-1}. While the total amount of variation explained was generally low, below 4.5 mg l^{-1} of dissolved oxygen, phylogenetic relationships accounted for most of the explained variation in fish growth. Ecological factors including body size, location in the water column (pelagic, demersal, or benthopelagic), habitat (freshwater, marine, or diadromous), and temperature explained very little of the effect of hypoxia on fish growth and explained only a moderate level of variation in consumption. Our results suggest a dramatically higher threshold for sub-lethal effects of hypoxia on fish than oxygen levels generally set for regulation purposes, and provide little support for accepted ecological mechanisms thought to influence hypoxia tolerance.

Synthesis

Increasing hypoxic conditions in aquatic systems around the globe necessitate not only a clear understanding lethal effects of hypoxia, but also sub-lethal effects on aquatic organisms. We performed a meta-analysis to determine that negative sub-lethal effects (food consumption and growth rate) on fish become significant at approximately 4.5 mg l^{-1} of dissolved oxygen, a much higher threshold than generally assumed for lethal effects. Furthermore, we found little support for several mechanisms thought to influence hypoxia tolerance in fish, including body size, location in the water column (pelagic, demersal, or benthopelagic), habitat (freshwater, marine or diadromous) and temperature.

Hypoxia (low dissolved oxygen) has increased worldwide in aquatic systems in recent decades, and this trend is expected to continue (Diaz 2001, Diaz and Rosenberg 2008, Zhang et al. 2010). While hypoxia is a natural phenomenon in many systems, it can be exacerbated by anthropogenic nutrient loading and climate change (Diaz and Rosenberg 2008, Zhang et al. 2010). Understanding how hypoxia affects important aquatic species, such as fish, becomes more urgent as hypoxia increases in prevalence. A more thorough understanding of the sub-lethal effects of hypoxia in a range of fish species would inform future studies and agency regulations. Existing agency regulations and definitions are inconsistent and primarily based on direct lethal effects, ignoring other sub-lethal factors that are potentially influential on community and population dynamics, such as decreased growth and consumption at low oxygen levels. Furthermore, hypoxia studies point to several different factors that influence hypoxia tolerance in fish, such as temperature, habitat, location in the water column and body size, with little consensus on which factors explain the most variation in hypoxia tolerance among individuals and species. It may be impractical to set separate hypoxia regulations across taxa, and thus elucidating the extent to which hypoxia responses differ across various factors may potentially justify a single, consistent threshold or suggest under which conditions such a threshold may be inadequate.

While direct lethal effects of hypoxia on aquatic organisms are relatively easily observed (Paerl et al. 1998), sub-lethal effects are more difficult to elucidate and quantify. Nonethe-

less, various field and experimental studies have documented behavioral and other non-lethal responses to hypoxia. For example, fish have been shown to alter their distributions to avoid hypoxic waters (Magnuson et al. 1985, Suthers and Gee 1986, Pihl et al. 1991), and the resulting shift may cause changes in predator–prey overlap (Pihl et al. 1992, Kolar and Rahel 1993, Breitburg 1994, Nestlerode and Diaz 1998, Shoji et al. 2005) and lead to altered diets (Aku and Tonn 1999, Ludsin et al. 2009, Roberts et al. 2009, 2012). Moreover, hypoxia-induced distributional shifts may lead to occupation of inferior abiotic (e.g. thermal; Coutant 1985, Price et al. 1985) and biotic (e.g. crowding leading to density-dependent effects; Eby and Crowder 2002) environments. Ultimately, recurring hypoxic conditions may shift aquatic communities toward species compositions favoring more hypoxia-tolerant species (Dauer 1993).

Under many conditions, dissolved oxygen concentrations (hereafter DO) exceeding levels leading to direct mortality may be tolerated by fish even if such levels have meaningful non-lethal effects. That is, habitat selection may require a tradeoff of tolerating certain non-preferred conditions (e.g. low, but not lethal DO) to enable access to other necessary resources such as a specific prey type or a preferred temperature (Aku and Tonn 1999, Roberts et al. 2009, 2012). Studies of various fish species that have exposed individuals to low DO suggest that growth and consumption decrease at levels much higher than oxygen concentrations that cause direct mortality (USEPA 2000; references within the Supplementary material Appendix 1 Table A1). Many aquatic organisms attempt to alleviate the effects of low DO with temporary behavioral or physiological changes, such as increasing ventilation rate or ventilation depth (Marvin and Heath 1968, McNeil and Closs 2007), decreasing activity (Schurmann and Steffensen 1997, Herbert and Steffensen 2005), or changing hemoglobin–oxygen affinity or hemoglobin concentration (Weber and Lykkeboe 1978, Frey et al. 1998). These changes may result in decreased biomass as metabolic activities and consumption decline to reduce oxygen demand (Poon et al. 2002, Wang et al. 2009). Because of the additional physiological stresses associated with hypoxia, fish may experience changes in reproductive success (Wu et al. 2003) or devote more energy to parental care behaviors (Jones and Reynolds 1999) when exposed to hypoxia.

Despite well-known negative effects of hypoxia on aquatic organisms, definitions of hypoxia vary across studies and agency regulations. Several studies set hypoxia thresholds at 2.0 mg l^{-1} (Diaz and Rosenberg 2008), primarily because significant mortality of various benthic invertebrates occurs at this level (Rosenberg 1980). Agency definitions generally follow the 2.0 mg l^{-1} threshold, including the US Environmental Protection Agency Hypoxia Task Force (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force 2013), US Geological Survey (<http://toxics.usgs.gov/hypoxia/hypoxic_zone.html>, accessed 10 May 2016), Baltic Marine Environment Protection Commission - Helsinki Commission (<http://helcom.fi/baltic-sea-trends/environment-fact-sheets/hydrography/hydrography-and-oxygen-in-the-deep-basins>, accessed 10 May 2016), and the US NOAA Center for Sponsored Coastal Ocean Research (Rabalais et al. 1999). However, DO concentrations that cause sub-lethal effects such as behavioral changes are often drastically different than oxygen concentrations that cause lethal effects (Vaquer-Sunyer and Duarte 2008, Breitburg et al. 2009). Reflecting this discrepancy, other studies have defined hypoxia as 2.8 mg l^{-1} DO (Wu 2002), 4 mg l^{-1} (Paerl et al. 2006), or 3 mg l^{-1} (Howell and Simpson 1994). One USEPA report also proposed hypoxia should be defined as 2.3 mg l^{-1} for aquatic organism survival and 4.8 mg l^{-1} for growth. This recommendation was based on a study of a small number of Atlantic species of fish and invertebrates (USEPA 2000).

Some studies have adopted a broader definition of hypoxia that does not set a specific threshold, or suggests a level of hypoxia that is system-dependent. For example, hypoxia has been defined on an individual level, such as "any level of DO low enough to negatively impact the behavior and (or) physiology of an organism" (Pollock et al. 2007) or "oxygen concentrations that are sufficiently reduced that they affect the growth, reproduction, or survival of exposed animals, or result in avoidance behaviors" (Breitburg et al. 2009). Hypoxia has also been defined on an ecosystem-specific basis with hypoxia defined as the level at which DO drops below the mean DO of the ecosystem (Hughes et al. 2015) or below 50% DO saturation (Breitburg 2002). These abstract definitions are useful in considering how hypoxia influences ecosystems and individuals, but are not sufficient for application to broad-scale agency regulation and tracking of hypoxia.

Examining the constant DO at which fish are significantly, directly affected allows us to make a conservative, general estimate of where we begin to see changes in an aquatic ecosystem due to hypoxia. When DO reaches levels to potentially elicit such negative effects, these waters may be considered impaired habitat regardless of whether fish remain in these waters or actively avoid them. We chose to examine growth and consumption responses of fishes as indicators of hypoxia rather than other metrics or taxonomic groups for several reasons. Fish tend to be more sensitive to low DO than many aquatic invertebrates (Vaquer-Sunyer and Duarte 2008, Ekau et al. 2010), so they are a sufficient indicator of ecosystem health. Because fish tend to be the most commercially and recreationally important species in aquatic ecosystems, many controlled studies on the effects of hypoxia on fish growth and consumption are readily available for incorporation into a standardized meta-analysis. We chose growth and consumption as DO responses because their role as direct, sub-lethal effects makes them relatively easy to observe and quantify in a laboratory setting, often concurrently. Moreover, unlike several behavioral and other non-lethal responses to hypoxia, growth and consumption are ecologically relevant as they are directly related to population production and food web interactions.

Several ecological and phylogenetic factors are thought to influence a fish's tolerance to low DO, and such factors may affect species-specific responses to hypoxia. Body size has often been linked to DO tolerance, with varying interpretations on whether larger body size increases or decreases DO tolerance (reviewed by Nilsson and Östlund-Nilsson 2008). Water temperature may influence a fish's response to hypoxia, with elevated temperatures causing more negative effects of hypoxia (Cech et al. 1984; several references in Supplementary material Appendix 1 Table A1). Physiology specific to certain taxa may play a significant role in DO tolerance in fish and other aquatic organisms. For example, anabantoid fishes can take up oxygen from the air using a specialized labyrinth

organ (Graham 1997). In addition to these ecological factors, phylogenetic relationships should be considered, as similarity between fish species due to sharing common ancestors precludes treatment of related species as truly independent when considering their responses to hypoxia. If relationships between species are not taken into account, the significance of ecological factors may be over-estimated (Felsenstein 1985).

Herein, we employ a structured meta-analysis to assess the effects of low oxygen on consumption and growth across fishes. Previous studies that attempted to set a specific threshold at which hypoxia has significant negative effects have focused on fish species from a single system (USEPA 2000) or were secondary analyses (Vaquer-Sunyer and Duarte 2008). These studies are important to consider, but do not achieve the same generality allowed by a meta-analysis across species from many different ecosystems. Meta-analyses weight variability among studies by calculating standardized effect sizes. Various summaries have demonstrated why such formal standardization across studies is preferred to coarse vote-counting or secondary analyses (Hedges and Olkin 1980, Oliver and Spokane 1983). We developed a large data set of standardized effect sizes of oxygen effects on fish growth and consumption across several fish species. Then, we considered the influence of ecological and phylogenetic factors in a meta-analysis across studies of fish growth and consumption. Specifically, we (a) estimated a general DO level where we observed significant sub-lethal effects of low DO on fish, and (b) explored the extent to which phylogenetic and ecological factors influenced variation surrounding that threshold.

Methods

Criteria for inclusion of studies

We assembled data for our meta-analysis using the criteria that studies must be controlled lab experiments that test the effects of constant DO on fish growth or consumption (direct, non-lethal effects), with replication and a measure of error reported from that replication. We limited our study to laboratory experiments to control for behavioral avoidance of low oxygen and external ecological factors that may influence growth and consumption, and included experiments with steady temperature and steady DO (rather than fluctuating conditions) to ensure that the effects observed were readily linked to a specific DO level and temperature. To find studies, we first conducted a search in ISI Web of Science on 5 February 2014 with the search terms "effect* hypox*," "consumption," "growth," and "fish" in the field of topic. We retained papers whose titles or abstracts made it apparent that the authors examined the effects of hypoxia on fish consumption and/or growth. Our second method of selecting papers was to use citations from chapter 8, "The effects of hypoxia on growth and digestion" (Wang et al. 2009), in the reference book: 'Fish physiology: hypoxia'. We evaluated the titles and abstracts of papers from Wang et al. (2009) and Web of Science and retained studies that directly assessed hypoxia's effects on fish consumption or growth. We eliminated studies where variance was not reported or was calculated with obvious pseudoreplication (Hurlbert 1984) and lacked sufficient information to re-calculate variance correctly. From the 14 papers obtained through these two methods, we examined backwards citations (papers cited by these studies) and forwards citations (papers that cite these studies) which provided an additional 25 papers for a total of 39 published studies (Supplementary material Appendix 1 Table A1) that experimentally tested fish growth and consumption in response to static treatment and control DO levels.

Table 1. Description of data sets included in analyses.

Data set	No. of articles	No. of effect sizes	No. of species
Conservative consumption	22	22	16
Intermediate consumption	22	47	17
Inclusive consumption	22	63	17
Conservative growth	36	36	27
Intermediate growth	36	91	31
Inclusive growth	36	138	31

In total, 31 different species of fish were included in the 39 experimental studies (Table 1). Of these studies, 17 examined only growth, 3 examined only consumption, and 19 measured both growth and consumption. Different experiments measured growth as instantaneous growth rate, average growth rate, or total growth over the course of the experiment. Consumption responses were reported in terms of consumption rate, total consumption, or egestion rate. For both growth and consumption, we calculated effect sizes (hereafter ES) by comparing control to treatment response for experiments regardless of which metric was used. When DO was reported in a measure other than mg l^{-1} (such as % saturation or torr), we converted to mg l^{-1} using temperatures provided in the paper and assuming pressure at sea level. This assumption regarding pressure had a minimal impact on results because all experiments that reported DO in percent saturation took place in laboratories in low-lying coastal areas (Bergen, Norway; Fiskebäckskil, Sweden; Mont-Joli, Québec, Canada; Sauðárkrókur, Iceland; Solomons, Maryland, USA) or a low-elevation inland location (Stuttgart, Arkansas, USA). Experiments lasted for a duration of 42 h to 225 days and tested temperatures from 8°C to 33°C (Supplementary material Appendix 1 Table A1). We extracted relevant data from figures using ImageJ (Rasband 1997) if data were not reported in text or tables.

Successful meta-analyses rely heavily on the quality of the data collected and its organization. As such, different results may be obtained by more or less conservative methods of data inclusion. If multiple ES are calculated from a particular study, the meta-analysis may be more biased toward studies that tested multiple treatments, but conversely, if only one ES per study is utilized, potentially valuable information is lost. In an effort to take these limitations into account, we analyzed three data sets for both growth and consumption, for a total of six data sets (Table 1). The most 'inclusive' data sets for each growth and consumption included all experimental treatments reported from each study, resulting in 138 ES for growth and 63 ES for consumption. Our 'intermediate' data sets included all dissolved oxygen levels evaluated for a given species in a study, but we limited these data sets to a single temperature per study if more than one temperature was tested. We used only the temperature treatment closest to the experimentally preferred temperature tested for each species according to sources from

the literature in order to eliminate experimental results that may be primarily influenced by extreme temperatures rather than DO treatments. This resulted in 91 ES for growth and 47 for consumption. The 'conservative' data sets consisted of only the preferred temperature for each species and a random DO if more than one level was tested. If more than one species or age class was tested in an article, we randomly chose one to include in the conservative data sets in order to limit these data to a single ES per study. 'Conservative' data sets included 36 ES for growth and 22 for consumption.

Hypoxia threshold

We calculated a Hedge's g ES (Hedges and Olkin 1985) for each treatment–control pairing for both consumption and growth. Hedge's g compares the treatment effect to the control effect by correcting the calculated Cohen's d (Cohen 1977) ES for small sample size. Cohen's d (d) compares the treatment effect to the control effect then scales by standard deviation:

$$\mathrm{DF} = n_{t+}n_c - 2 \tag{1}$$

$$S_{wi} = \sqrt{\frac{(n_t - 1)SD_t^{\,2} + (n_c - 1)SD_c^{\,2}}{\mathrm{DF}}} \tag{2}$$

$$d = \frac{R_t - R_c}{S_{wi}} \tag{3}$$

where DF = degrees of freedom, n_t = number of treatment measurements, n_c = number of control measurements, S_{wi} = within-groups standard deviation, SD_t = standard deviation of treatment responses, SD_c = standard deviation of control responses, R_c = control response, and R_t = treatment response. Control response was assumed to be the response for each experiment at the highest DO level tested, barring hyperoxic (> 100% saturation) treatments. After calculating Cohen's d, we computed Hedge's g (g) to correct for small sample size using the following equations:

$$J = 1 - \frac{3}{4DF - 1} \tag{4}$$

$$g = J \times d \tag{5}$$

where J = correction factor for small sample sizes. To determine a threshold level, we defined the DO threshold where sub-lethal effects end as the point where the 95% confidence interval around the mean ES overlapped zero or was positive, i.e. the level at which we no longer observed a significantly negative effect of DO. ES values were not distributed normally or uniformly across the entire range of DO and could not be successfully transformed to improve normality. In addition, confidence intervals around most standard regressions would be challenging to interpret due to difficulty in incorporating error both within and across studies. As a result, we split individual ES into smaller increments of 1 mg l^{-1} DO of the treatment group. We calculated means and 95% confidence intervals within each 1 mg l^{-1} interval using the following equations from Gurevitch et al. (2001):

$$\overline{E} = \frac{\sum_{i=1}^{n} w_i E_i}{\sum_{i=1}^{n} w_i} \tag{6}$$

$$s^2_{\overline{E}} = \frac{1}{\sum_{i=1}^{n} w_i} \tag{7}$$

$$CI = \overline{E} \pm t_{\alpha/2[n-1]}(s_{\overline{E}}) \tag{8}$$

where $\overline{E}$ = grand mean, n = number of data points within a bin, E_i = ES for the ith data point, and $w_i = 1/v_i$ where v_i = sampling variance, $S^2_{\overline{E}}$ = the variance of $\overline{E}$, CI = 95% confidence interval of $\overline{E}$, and t is the two-tailed critical value from the Student's t-distribution at the critical level of α (here, 0.05) at n–1 degrees of freedom. This analysis allowed us to calculate a reliable confidence interval using an accepted meta-analytical technique for weighted means. We repeated this process for each of our six data sets for 1.0 mg l^{-1} bins starting at 0.0 mg l^{-1} DO (bins = 0.0–1.0 mg l^{-1}, 1.0–2.0 mg l^{-1}, etc.) then for 1.0 mg l^{-1} bins starting at 0.25, 0.5 and 0.75 mg l^{-1} (in other words, bins starting at 0.25–1.25, 0.5–1.5 mg l^{-1}, 0.75–0.75 mg l^{-1}) to account for differences in structuring intervals on the results of our analyses. We considered the intervals where the 95% confidence interval either overlapped zero or was positive to be the level where DO no longer influences consumption or growth. All analyses were carried out in the statistical software R ver. 3.1.2 (<www.r-project.org>).

Contributing factors

We performed phylogenetic variance partitioning (Desdevises et al. 2003) to obtain estimates of variation in DO response due to phylogenetic relatedness, ecological variables, and the overlap between the two (niche conservatism). This analysis included only ES that tested DO levels below the threshold we found in the previous analysis (4.5 mg l^{-1}) in order to limit the factors that influence growth and consumption to those that operate under oxygen deprivation. When oxygen levels are high, other contributing factors not included in this analysis may play a role and therefore were not included in regression analyses. We eliminated the conservative consumption data sets for growth and consumption from regression analyses due to low sample size, leaving four datasets for this analysis. Phylogenetic variance partitioning is based primarily on phylogenetic eigenvector regression, which is robust for groups of species where autocorrelation is low or sample size is small (Diniz-Filho et al. 1998). Our ecological variables included temperature, habitat (freshwater, marine or anadromous), location in the water column (demersal, pelagic or benthopelagic), and log-transformed body mass at the beginning of each experiment. We classified habitat and location in the water column using FishBase (<www.fishbase.org>), and used species-specific length–weight regressions from FishBase if starting length was given in original papers rather than starting mass. For regression analyses, we scaled ES by the minimum in each data set to avoid negative values, then log + 1 transformed the data, and weighted the ES by variance by dividing by the standard error.

For phylogenetic relationships and variance partitioning, we followed methods described in Gonçalves-Souza et al. (2014), which adapts a procedure for variance partitioning in Desdevises et al. (2003). This procedure allowed us to determine variance in ES that can be explained by a) ecological variables, b) niche conservatism, c) phylogenetic relationships, and d) unexplained variance. We removed hybrid species from one study (Green and Rawles 2011) due to difficulty in understanding phylogenetic relationships for those individuals. We

performed all phylogenetic variance partitioning analyses in the statistical software R using packages 'ape' (Paradis et al. 2004), 'MASS' (Venables and Ripley 2002), and 'PVR' (Santos et al. 2012) following the procedure summarized below.

For each of the four datasets, we recreated a phylogenetic tree of our species using taxonomic classifications and assuming a branch length of one, then we performed a principal coordinates analysis (PCoA) using a Cailliez correction (Cailliez 1983) to determine the relative relatedness between species. We regressed all axes against our transformed ES and kept only axes that explained a significant amount of the variation in ES. Our methods differed from Gonçalves-Souza et al. (2014) and Desdevises et al. (2003) only in that we used a forward and backward selection procedure using AIC to determine significant axes rather than only forward selection because we found that forward selection was not sufficient to eliminate axes that described very little variation in our effect size. Using only the significant axes, we performed a multiple linear regression on our transformed ES and retained the proportion of variance explained by phylogeny (R^2_{phylo}).

Next, we determined the variance explained by ecological variables using a multiple linear regression on our transformed ES with temperature, habitat, location in the water column, and log-transformed body size. We did not include DO as a predictor variable because we had already accounted for its effects by estimating a DO threshold in the previous analyses. Temperature and body mass were modeled as continuous variables, while habitat (marine, freshwater or anadromous) and location in the water column (pelagic, demersal or benthopelagic) were treated as categorical with anadromous and benthopelagic conditions set as baseline levels. Selection of baselines do not influence significance levels, only interpretation of relative coefficients for each level of categorical factors. From this regression, we retained the variance explained by the ecological variables (R^2_{eco}).

Finally, we regressed our transformed ES against all predictor variables (significant PCoA axes and ecological variables) to obtain the total variance explained by the model (R^2_{total}). We determined the amount of variance explained by a) ecological variables, b) niche conservatism, c) phylogenetic relationships, and d) unexplained variance with the following equations:

$$a = R^2_{total} - R^2_{phylo} \quad (9)$$

$$b = R^2_{eco} - a \quad (10)$$

$$c = R^2_{total} - R^2_{eco} \quad (11)$$

$$d = 1 - (a + b + c) \quad (12)$$

Publication bias

Meta-analyses may be susceptible to publication bias, or the idea that published studies do not reflect all completed studies (Rothstein et al. 2006). For instance, it is plausible that studies with significant results are more likely to be published than studies with insignificant results. To assess the possibility of publication bias, we calculated Rosenthal's fail safe number (Rosenthal 1979) and both of Rosenberg's fail safe numbers (Rosenberg 2005) for each of our six data sets including all DO levels tested. Fail safe numbers address the question of whether publication bias influences the observed effect in our meta-analysis. Rosenthal's fail safe number has been used extensively in meta-analysis literature and represents the number of studies with an ES of zero that would be needed to make the overall effect of the meta-analysis insignificant ($\alpha = 0.05$). The Rosenberg method reports two fail safe numbers. One, N_+, is interpreted in a similar manner to Rosenthal's fail safe number, but it weights studies based on their variance (Rosenberg 2005). The other Rosenberg fail safe number, N_1, is interpreted as the relative sample size of one study with no effect that would nullify the significance of the overall analysis (Rosenberg 2005). When analyzed for a random-effects model, N_+ often collapses to its fixed-effects value. Therefore, we calculated both N_+ and N_1 to allow for easier interpretation from N_+ but also to provide a random-effects model value that better accounts for variability between studies. A fail safe number greater than $5n + 10$, where n is the number of studies, suggests a robust fail safe number for Rosenthal's and Rosenberg's N_+ fail safe numbers (Rosenberg 2005).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.td771> (Hrycik et al. 2016).

Results

Hypoxia threshold

Across the studies considered, effects of oxygen concentrations on consumption and growth were most consistently evaluated in the interval 1.0 mg l^{-1} to 6.0 mg l^{-1}. Although the size of each data set varied, across all data sets of growth and consumption ES values were generally low at low DO levels and approached zero as DO increased (Fig. 1). Within 1.0 mg l^{-1} intervals, 95% confidence intervals for most weighted means were negative and did not overlap zero below concentrations ~4.5 mg l^{-1} dissolved oxygen, although the exact value of this threshold depended on where intervals started (Fig. 2, Supplementary material Appendix 2–4). A discrepancy with this pattern was demonstrated in consumption data sets where 95% confidence intervals overlapped zero or were positive at 0.25–1.25 mg l^{-1} due to very high variance in the 0.25–1.25 mg l^{-1} interval (Supplementary material Appendix 1 Fig. A1). This discrepancy was not visible when data were grouped differently. Weighted means of growth data sets and larger data sets had smaller confidence intervals due to their higher sample sizes (Table 1, Fig. 2, Supplementary material Appendix 2–4). In addition, ES at very low or very high DO levels had large confidence intervals around their weighted means, again due to small sample sizes in these intervals.

Contributing factors

Our data set included 29 species with varying degrees of relatedness within the class Actinopterygii (Fig. 3). Ecological variables (habitat, temperature, location in the water column, and body mass) explained little variation in each data set (1.9–20.7%; Table 2). Phylogenetic relationships explained a large portion of variation in growth (31.3–38.4%), but very little in consumption (7.7–13.5%). The effect of niche

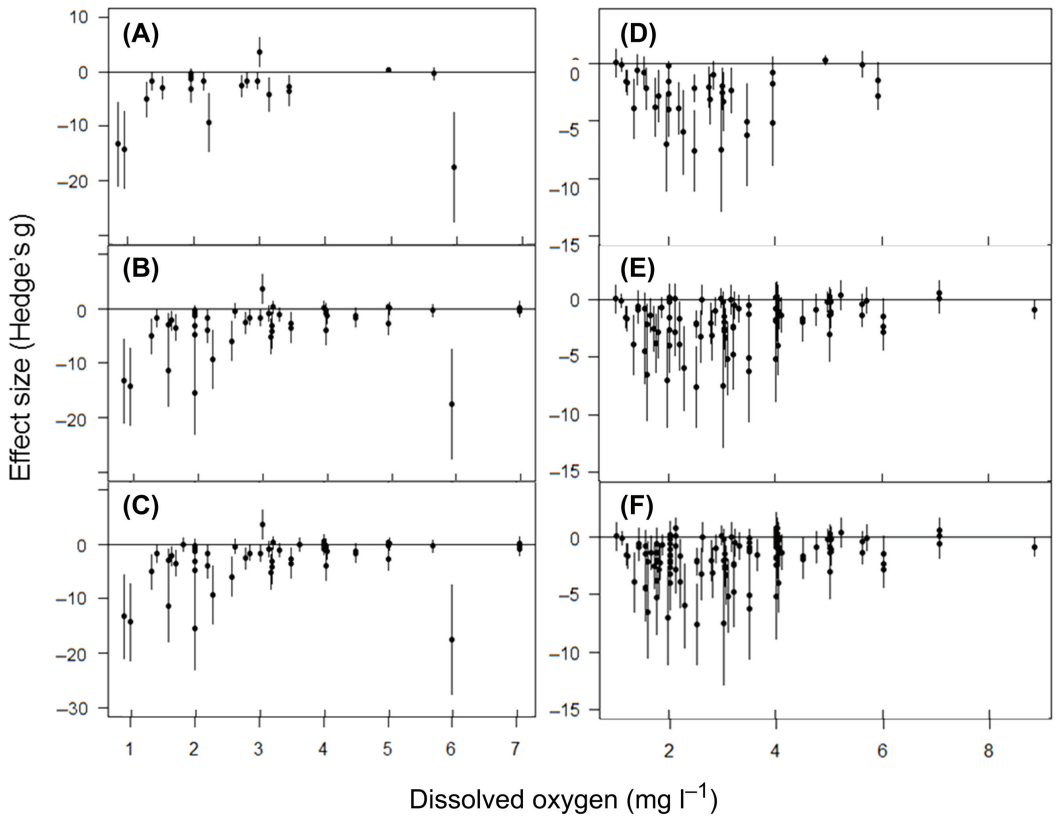

Figure 1. Hedge's g effect sizes for all data points included in this study. Error bars represent one standard error. Data sets are conservative consumption (A), intermediate consumption (B), inclusive consumption (C), conservative growth (D), intermediate growth (E), and inclusive growth (F).

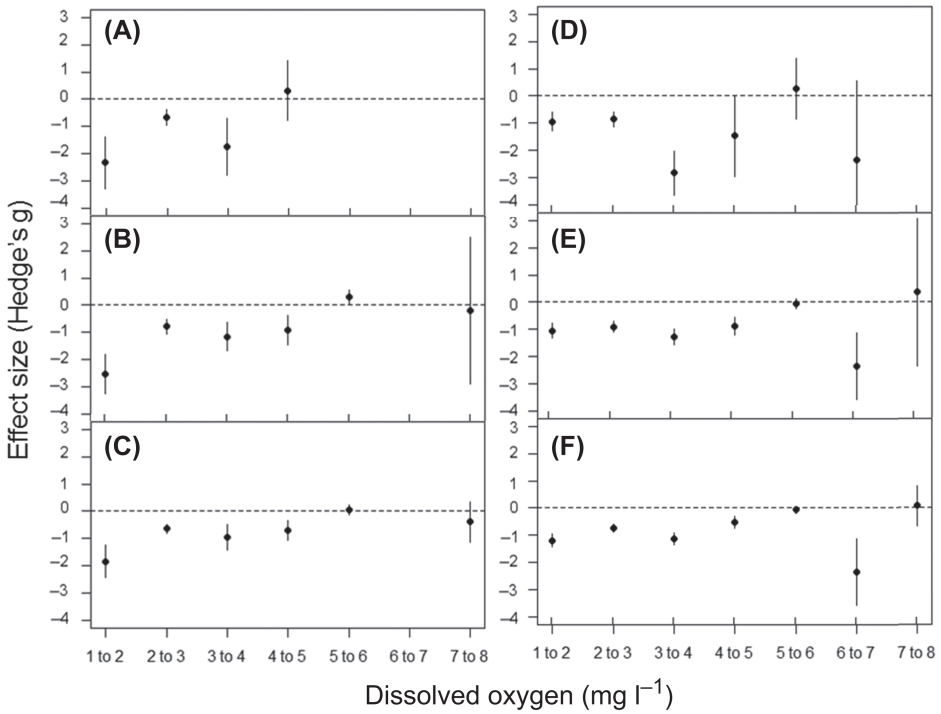

Figure 2. Weighted means for 1.0 mg l^{-1} bins for data sets conservative consumption (A), intermediate consumption (B), inclusive consumption (C), conservative growth (D), intermediate growth (E), and inclusive growth (F). Error bars represent 95% confidence intervals, and the dotted line represents a null effect size.

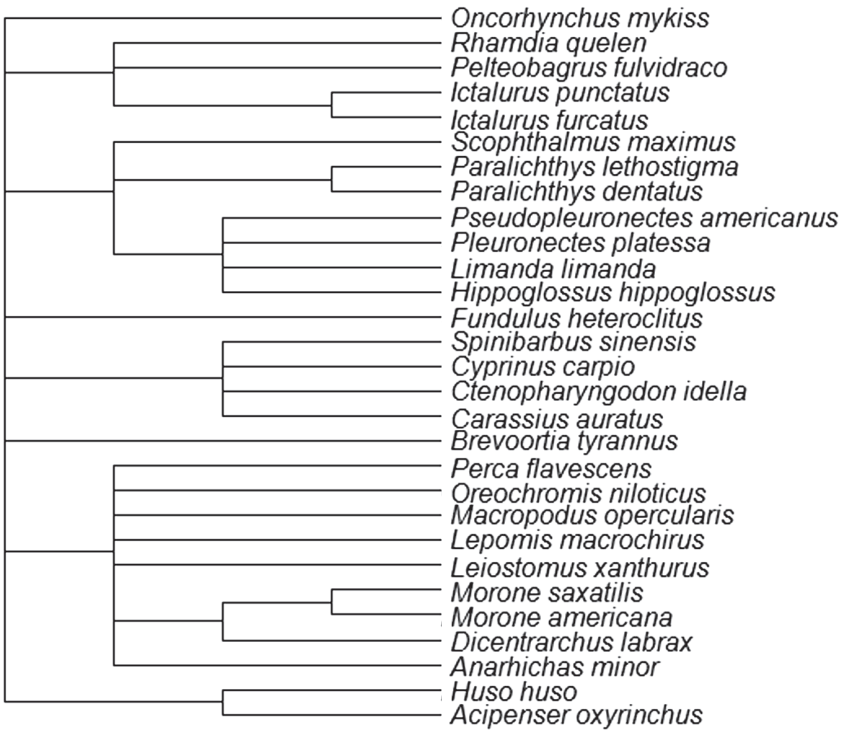

Figure 3. All species included in phylogenetic variance partitioning, assuming a branch length of one between each species. The species displayed were included in the 'inclusive' data sets.

conservatism was very small across data sets (1.4–11.1%). Ultimately, a large portion (45.5–67.6%) of the variation in ES remained unexplained after accounting for phylogenetic factors, ecological factors and niche conservatism (Table 2).

While ecological factors explained little variation in general, some ecological variables were significant (Supplementary material Appendix 1 Table A2). Within the growth data sets, there were no significant factors in the inclusive data set according to regressions only including ecological factors, but location in the water column was significant in the intermediate data set with pelagic fish being the most sensitive to low DO. In the intermediate consumption data set, both habitat and body weight were marginally significant ($p < 0.10$; Supplementary material Appendix 1 Table A2) with larger fish and freshwater fish being most sensitive to low DO. For the inclusive consumption data, habitat was the only significant ($p < 0.05$) factor suggesting that freshwater fish were the most sensitive to low DO.

Publication bias

Failsafe numbers indicate little evidence of publication bias. Our sample sizes were considered robust for all data except the conservative consumption data set (Table 3). Robustness is generally not calculated for Rosenberg's N_1 fixed-effects and Rosenberg's N_1 random-effects failsafe numbers, but their large quantities indicate that a single experiment would need a very large ES to produce a null mean effect size. Rosenberg's N_+ random-effects model collapsed to its fixed-effects counterpart for all data sets except the smallest consumption data set.

Discussion

Our results from a combination of 39 studies demonstrate consistent, negative effects of DO on fish growth and consumption at concentrations up to 4.5 mg l^{-1}. This level is much higher than most current agency regulations of hypoxia which commonly use a 2.0 mg l^{-1} threshold that considers the level at which high mortality rates occur. This considerable disparity between regulatory DO levels and levels where organisms are sub-lethally affected has implications for classifying habitat suitability and impairment. For example, Lake Erie, North America, is a large, commercially- and recreationally-important ecosystem (Hushak

Table 2. Proportion of variance explained by ecological variables, niche conservatism, and phylogenetic relationships as well as proportion of unexplained variance for each data set. The conservative consumption and growth data sets were not included in these analyses due to low sample size.

	Intermediate consumption	Inclusive consumption	Intermediate growth	Inclusive growth
Ecological (a)	0.207	0.114	0.050	0.019
Niche conservatism (b)	0.040	0.097	0.111	0.014
Phylogenetic (c)	0.077	0.135	0.384	0.313
Unexplained (d)	0.676	0.655	0.455	0.654

Table 3. Rosenthal's and Rosenberg's fail safe numbers for each full data set. Rosenthal's, Rosenberg's, and Rosenberg's N_+ are interpreted as the number of studies with an effect size of zero that would result in a mean effect size of zero. These are considered robust if they are greater than 5n + 1 (marked with an asterisk). Rosenberg's N_1 is interpreted as the effect size of a single study needed to produce a null mean effect. Rosenberg's N_1 and Rosenberg's N+ can be run as both fixed-effects models and random-effects models, with some random-effects models collapsing to the fixed-effects model.

Data set	5n + 10	Rosenthal	Rosenberg	Rosenberg N_1	Rosenberg N_+	Rosenberg N_1 (random)	Rosenberg N_+ (random)
Conservative consumption	120	706*	101	55	62	15	35
Intermediate consumption	245	2,747*	404*	255	268*	110	collapse
Inclusive consumption	325	4,105*	1,071*	705	733*	176	collapse
Conservative growth	190	2,782*	901*	582	621*	111	collapse
Intermediate growth	465	10,532*	4,399*	2,989	3,070*	650	collapse
Inclusive growth	700	19,879*	9,102*	6,258	6,369*	1,359	collapse

et al. 1986) that has experienced hypolimnetic hypoxia during most summers in recent decades (Hawley et al. 2006). Using data from a one-dimensional limnological model for the Central Basin of Lake Erie (Rucinski et al. 2014) averaged over 1987–2005, the number of days that experience hypoxia would be 50 using the criteria of 2.0 mg l^{-1}, but that number increases to 93 hypoxic days when we set the threshold of hypoxia at 4.5 mg l^{-1}. Furthermore, some fish species demonstrate mortality at DO levels higher than 2 mg l^{-1} (Landman et al. 2005, Vaquer-Sunyer and Duarte 2008), and our proposed 4.5 mg l^{-1} level would encapsulate not only ecologically-relevant non-lethal effects, but also direct mortality of these hypoxia-sensitive species.

Our phylogenetic variance partitioning results revealed an influence of phylogenetic relationships on fish growth responses to hypoxia, indicating that phylogenetically similar species tended to react comparably to low DO levels. In general, Fundulidae, Centrarchidae, Percidae and Sciaenidae were more tolerant to low DO and Osphronemidae, Anarhichadidae, Bagridae and Heptapteridae were more sensitive. This is likely due to physiological adaptations to hypoxia specific to these families. For example, some Fundulidae have been shown to perform aerial respiration (Halpin and Martin 1999) and many members of the families Centrarchidae and Percidae exhibit aquatic surface respiration, moving close to the surface of the water where DO is higher (Chapman and Mckenzie 2009). However, there was little influence of phylogeny on DO sensitivity when measured using consumption, suggesting that there may be some behaviors in fish that influence their consumption but are not specific to certain clades. Searching for, digesting and assimilating food is energetically costly for fish, as it may account for up to 60% of energy expenditures (Van Dam and Pauly 1995). Decreased consumption is thought to be a mechanism to decrease oxygen consumption (Pichavant 2001, Gan et al. 2013), which may be beneficial across clades as a response to short term low DO exposure.

Partitioning variance due to phylogenetic factors and niche conservatism allowed us to better understand which ecological factors independent of phylogenetic relatedness play a role in regulating hypoxia tolerance. Surprisingly, ecological factors (temperature, fish body size, location in the water column and habitat) and niche conservatism across related species generally had little influence on growth and consumption. Some factors were statistically significant, but contributed very little to the overall variation in response to hypoxia, indicating that they are not of high ecological importance despite significance. In particular, larger, pelagic and freshwater fish tended to be most sensitive to low DO, although these trends were weak. Pelagic fish would likely be exposed to low DO less frequently than demersal or benthopelagic fish, and therefore would be less likely to have evolved adaptations to hypoxia. This trend occurred in both phylogenetic and ecological components, despite low niche conservatism, suggesting that both species-specific traits and homologous traits across larger clades play a role in determining hypoxia tolerance. The small amount of variation explained by phylogenetic and ecological factors warrants further study to evaluate factors that influence hypoxia tolerance, but was not possible here due to limited information reported in the studies used in our meta-analysis. Nonetheless, the study findings that combined phylogenetic factors, niche conservatism and ecological factors explained a relatively small portion of the overall variation in DO sensitivity in fish, providing support for the utility of a broad threshold value.

Failsafe numbers indicated robustness against publication bias for all datasets except the conservative consumption data set. Trends in ES were consistent across data sets, suggesting little bias in overall conclusions in harmful DO levels across methods of selecting ES. However, calculations of ES and publication bias may not be interpreted entirely analogously as in other studies due to the range of oxygen concentrations considered. ES calculation depends partially on 'control' DO levels, which varied by experiment and depended on factors such as temperature and logistical constraints in laboratory settings. In this meta-analysis, we accepted the original authors' determination of a control DO level as sufficient. Methods for calculating standard metrics of publication bias such as failsafe numbers (Rosenthal 1979, Rosenberg 2005), funnel plots (Light and Pillemer 1984), or trim and fill methods (Duval and Tweedie 2000) may not be straightforward because they are designed to test binary predictor variables, rather than continuous gradients. Thus, our failsafe numbers should be interpreted over the range of all DO levels tested. The similar conclusions and trends in data from a single ES per study (conservative data sets) versus multiple ES per study (inclusive data sets) also supports the notion that our results were not an artifact of publication bias.

Similar to our findings, some previous studies have suggested higher thresholds for hypoxia than the widely accepted 2.0 mg l^{-1}. Many of these were based on specific species or ecosystems (USEPA 2000, Breitburg 2002, Paerl

et al. 2006). However, Vaquer-Sunyer and Duarte (2008) synthesized results from numerous studies in a secondary analysis to establish a broader sublethal threshold among a variety of aquatic taxa, including fish. These authors reported a mean sub-lethal threshold across taxa of 2.24 mg l^{-1} and a fish-specific mean sub-lethal threshold of 4.41 mg l^{-1}. The latter value was based on 34 studies which examined a variety of behavioral and physiological non-lethal responses. Our estimated threshold value is generally consistent with that reported by Vaquer-Sunyer and Duarte (2008) for fish. In addition, our study adds further support to this threshold using a different set of reference studies limited to ecologically-relevant responses (growth and consumption), and furthermore, accounts for magnitude and variability of effect within each study used by performing a structured meta-analysis.

Several studies have found a more nuanced, non-linear relationship between low DO and fish response than is acknowledged by a threshold DO value. Effects of DO on mortality rates (Plante et al. 1998, Shimps et al. 2005, Small et al. 2014) and various sub-lethal responses (Stierhoff et al. 2006, Flint et al. 2015) may not be abrupt and instead display continuous, non-linear relationships. However, other studies have used breakpoint analyses or similar threshold-dependent methods to determine critical values at which low DO effects fish (Chabot and Dutil 1999, Yang et al. 2013), similar to finding a threshold as we did in this study. A single value, although simplified, is useful in several contexts, such as policy decision-making and ecological modeling that relates to fish taxa that have not been specifically examined for response to low oxygen.

Our meta-analysis tested direct, sub-lethal effects of consistent levels of low DO in controlled laboratory settings. Fish may react differently when exposed to fluctuating treatments (Carlson et al. 1980, Bejda et al. 1992) that simulate natural diurnal fluctuations or move out of hypoxic areas when possible (Aku and Tonn 1999, Ludsin et al. 2009, Roberts et al. 2009, 2012). Our results represent a DO level where we expect to see sub-lethal effects in aquatic organisms that may encompass a variety of responses, including behavioral changes that may lead to avoidance of hypoxic waters or decreases in growth and subsequently biomass if individuals remain within that habitat. We suggest that waters are impaired if DO levels are low enough to negatively affect fish consumption, growth, and, ultimately, population biomass. The threshold effect level of 4.5 mg l^{-1} was found among many freshwater and marine species from a variety of habitats. Moreover, since ecological factors do not appear to strongly modify responses to low DO, the threshold level represents a general DO level where hypoxia would be expected to significantly influence aquatic ecosystems.

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Supplementary material (available online as Appendix oik-03678 at <www.oikosjournal.org/appendix/oik-03678>) Appendix 1–4.