CONCEPTS & SYNTHESIS

EMPHASIZING NEW IDEAS TO STIMULATE RESEARCH IN ECOLOGY

Oxygen supply in aquatic ectotherms: Partial pressure and solubility together explain biodiversity and size patterns

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Abstract. Aquatic ectotherms face the continuous challenge of capturing sufficient oxygen from their environment as the diffusion rate of oxygen in water is 3×10^5 times lower than in air. Despite the recognized importance of oxygen in shaping aquatic communities, consensus on what drives environmental oxygen availability is lacking. Physiologists emphasize oxygen partial pressure, while ecologists emphasize oxygen solubility, traditionally expressing oxygen in terms of concentrations. To resolve the question of whether partial pressure or solubility limits oxygen supply in nature, we return to first principles and derive an index of oxygen supply from Fick's classic first law of diffusion. This oxygen supply index (OSI) incorporates both partial pressure and solubility. Our OSI successfully explains published patterns in body size and species across environmental clines linked to differences in oxygen partial pressure (altitude, organic pollution) or oxygen solubility (temperature and salinity). Moreover, the OSI was more accurately and consistently related to these ecological patterns than other measures of oxygen (oxygen saturation, dissolved oxygen concentration, biochemical oxygen demand concentrations) and similarly outperformed temperature and altitude, which covaried with these environmental clines. Intriguingly, by incorporating gas diffusion rates, it becomes clear that actually more oxygen is available to an organism in warmer habitats where lower oxygen concentrations would suggest the reverse. Under our model, the observed reductions in aerobic performance in warmer habitats do not arise from lower oxygen concentrations, but instead through organismal oxygen demand exceeding supply. This reappraisal of how organismal thermal physiology and oxygen demands together shape aerobic performance in aquatic ectotherms and the new insight of how these components change with temperature have broad implications for predicting the responses of aquatic communities to ongoing global climate shifts.

Key words: altitude; ecology; gigantism; global warming; macroinvertebrate; metabolism; oxygen limitation; physiology; respiratory; salinity; temperature; thermal tolerance.

INTRODUCTION

Aquatic gas exchange is a major challenge as water contains 33 times less oxygen than air, and the diffusion rate of oxygen in water is around 300 000 times lower than in air (Jones 1972; water at 20°C contains 6.3 mL O_2/L, compared with 209.5 mL O_2/L in air, in proportion 1:33, and at 20°C Krogh's oxygen diffusion constant in $cm^2 \cdot atm^{-1} \cdot min^{-1}$ equals 0.000034 in water and 11.0 in air, in proportion 1:323 529). Limitations in environmental oxygen availability explain the wide

range of morphological, physiological, and behavioral adaptations of aquatic ectotherms that are associated with gas exchange (Hynes 1970). These adaptations enable organisms to provide their tissues with sufficient oxygen and balance oxygen demand with environmental supply.

The importance of oxygen in aquatic systems is well recognized, but there is no consensus on what exactly drives oxygen exchange or determines oxygen supply to an organism. Traditionally, ecologists emphasize oxygen solubility, and express environmental oxygen in terms of concentration (e.g., Chapelle and Peck 1999, Allen and Castillo 2007); physiologists emphasize the importance of partial pressure as gases dissolve, diffuse and react according to partial pressure and as it quantifies
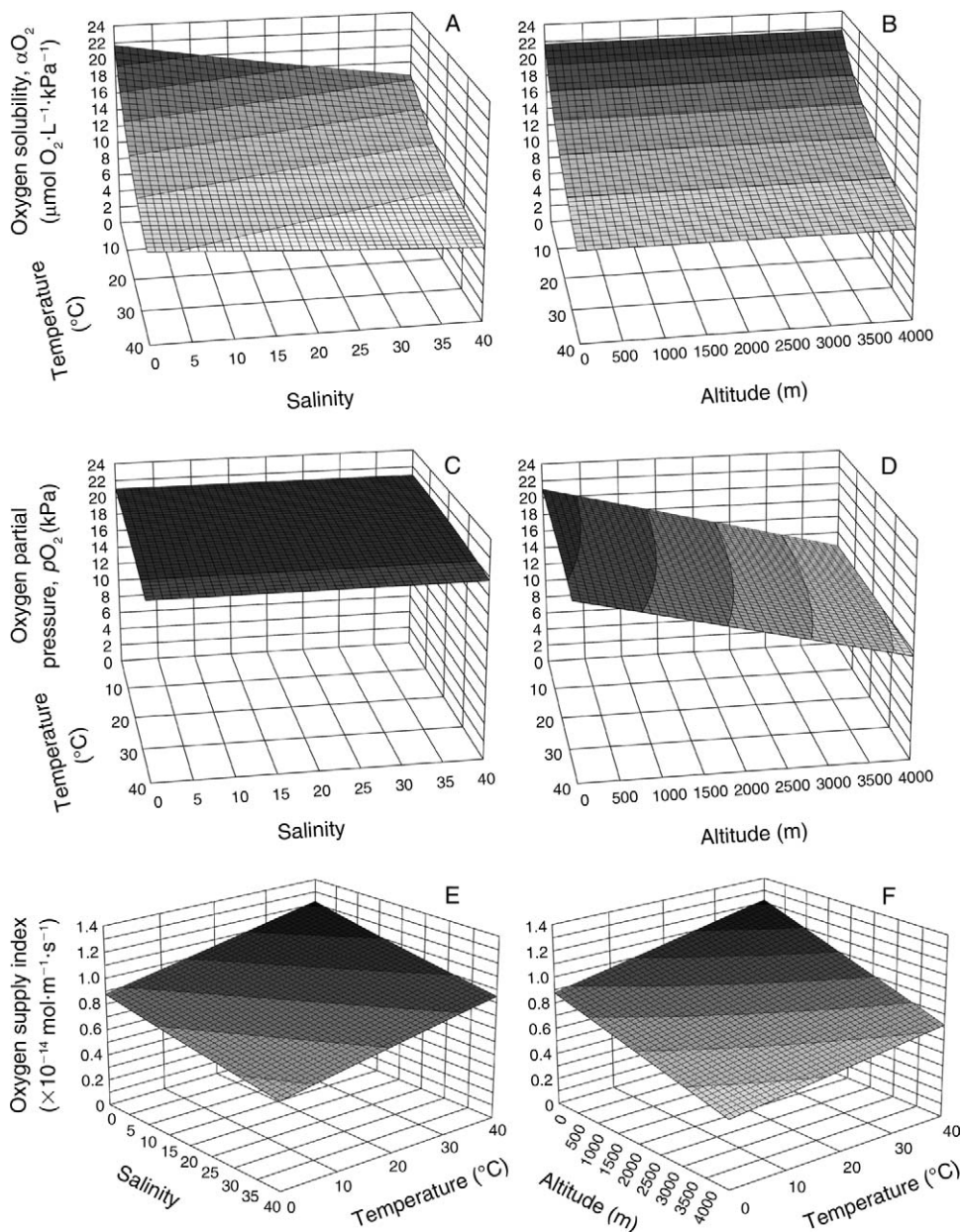
FIG. 1. (A, B) Changes in oxygen solubility (αO_2), (C, D) oxygen partial pressure (pO_2), and (E, F) the oxygen supply index (OSI) plotted over ranges in temperature (0–40°C), salinity (0–40 on the practical salinity scale, PSS) and altitude (0–4000 m above sea level). αO_2 declines with both increasing temperature and salinity (A) but is invariant with altitude (B), whereas pO_2 decreases with increasing altitude (D) but is relatively invariant across both other environmental clines (C). OSI declines with higher salinities and altitude (E, F) but increases with higher temperatures due to higher diffusivity. Isoclines delineate bands that have different shades of gray to facilitate interpretation.

diffusion potential (e.g., Fernández et al. 2003, Willmer et al. 2005). The difference is not merely an issue of semantics: as solubility and partial pressure are not readily interchangeable (Fig. 1), it appears that fundamentally different views on what actually determines oxygen uptake by aquatic ectotherms exist. In ecophysiology, where these two research fields merge, confusion arises from the application of either the physiological or

the ecological approach to explain what limits oxygen supply to aquatic ectotherms in nature. This is illustrated by the unresolved debate on whether oxygen concentrations or partial pressures drive patterns in body size in aquatic ectotherms (Peck and Chapelle 1999, Spicer and Gaston 1999, Jacobsen 2003).

Environmental oxygen concentration has been shown to correlate closely with body size in aquatic amphipods

(Chapelle and Peck 1999, 2004), where oxygen concentrations are assumed to reflect available oxygen through the link with solubility (solubility expresses the amount of oxygen that can dissolve in water at equilibrium for a given partial pressure; hence at a given partial pressure they are equivalent). The problem here is that oxygen diffusion rates (and hence rates of uptake) are governed by gradients in oxygen partial pressure (pO_2), rather than gradients in concentration (Spicer and Gaston 1999, Willmer et al. 2005). Furthermore, the reported sevenfold difference in amphipod body size across clines in salinity and temperature (Chapelle and Peck 1999) is much greater than one would predict from an observed twofold difference in oxygen concentration (Makarieva et al. 2005). Conversely, studies on altitudinal clines, where species richness decreases with decreasing pO_2 at higher altitudes (Jacobsen 2008), support a direct role of pO_2 as a determining factor for oxygen supply. A lower pO_2 at higher altitudes could further explain why highland invertebrates do not attain larger sizes than their lowland counterparts (Jacobsen 2003), an observation that is opposite to what one would predict from greater oxygen solubility at the lower temperatures at high altitude. Yet, if one considers pO_2 as the only determinant of oxygen exchange, problems arise: the rather constant pO_2 of larger water bodies at sea level (Fig. 1C) argues against pO_2 exclusively driving pertinent patterns in size spectra and assemblage diversity (the pO_2 is constant at sea level in an equilibrium situation, whereas a nonequilibrium situation [under- or oversaturation] is unlikely in larger bodies of water). The clear link between environmental oxygen availability and the ecological features mentioned warrants a reappraisal of the relative importance of oxygen concentration and partial pressure in determining gas exchange in aquatic organisms.

Here we propose a measure of oxygen supply, derived from Fick's classic law of diffusion, that combines oxygen solubility and pO_2. This measure unites the former views of ecologists and physiologists (Gaston et al. 2009) and provides a new conceptual framework to explore the effects of oxygen on aquatic ectotherms.

Oxygen Supply Index

All respiratory gas exchange ultimately relies on passive physical diffusion (Jones 1972). An integrated measure of oxygen exchange should therefore incorporate the key features that drive oxygen diffusion from the environment into an organism: diffusion rate, partial pressure, and oxygen solubility. The rate of transfer of an amount of oxygen ($\dot{M}O_2$ in moles/s), can be calculated from a derivation of Fick's equation based on activity or partial pressure gradients (Piiper et al. 1971):

$$\dot{M}O_2 = \alpha O_2 \times DO_2 \times (A \times L^{-1}) \times (\Delta pO_2) \qquad (1)$$

where αO_2 is the solubility of oxygen in water ($mol \cdot m^{-3} \cdot Pa^{-1}$), DO_2 is the diffusivity of oxygen in

water (m^2/s), A is the area of the respiratory surface, and L is the diffusion distance, with ΔpO_2 being the difference in oxygen partial pressure between the hemolymph and the respiratory surface in contact with the environment. Size and shape of an organism will govern A, while L will vary with epithelial and cuticle thickness, as well as the thickness of the boundary layer (see Appendix A for an elaboration). Thus, the physicochemical properties of the respiratory structures and hemolymph pO_2 will largely determine the *absolute* amount of oxygen that can be transferred. Even so, exchange of oxygen will be *proportionally* related to environmental oxygen availability; higher environmental oxygen availability will enable animals to increase their exchange of oxygen, irrespective of their capacities to extract environmental oxygen through differences in surface-area-to-volume ratios, plastron respiration, physical gills, branchial structures and morphological gills, respiratory pigments, ventilatory behavior, or aerial gas exchange. Consequently, all aquatic ectotherms have to deal with the physical availability of oxygen in their aquatic environment, which can be calculated as an oxygen supply index (OSI, in $mol \cdot m^{-1} \cdot s^{-1}$) and incorporates the parameters DO_2, αO_2, and pO_2 as follows:

$$OSI \propto \alpha O_2 \times DO_2 \times pO_2 \qquad (2)$$

(see Appendix B for technical details).

According to this equation, differences in both partial pressure and oxygen solubility affect the rate of oxygen transfer. The solubility of oxygen changes with temperature and salinity (Benson et al. 1979, Benson and Krause 1984). Furthermore, at a given solubility, the amount of dissolved oxygen is directly proportional to atmospheric pO_2. Oxygen concentrations may thus vary even when pO_2 does not change (e.g., across a salinity gradient) or when αO_2 does not change (e.g., across an altitudinal gradient). Consequently, it is not the oxygen concentration that drives oxygen exchange, but instead pO_2 and αO_2 jointly determine both the rate of oxygen transfer and the oxygen concentration.

While the oxygen diffusivity and the partial pressure gradient drive oxygen across respiratory surfaces, the amount of oxygen delivered at a given pressure differential also depends on solubility. Oxygen exchange thus bears an analogy to transfer of water between two connected vessels, where the amount of water transferred is controlled by gravity (DO_2), height differences in water level (pO_2), and the capacitance of the vessels (αO_2).

Temperature not only affects the purely physical process of oxygen diffusion, but also the organismal oxygen demand as metabolic rate increases with temperature in ectotherms (Gillooly et al. 2001, Jacobsen 2003, Makarieva et al. 2005). Patterns in biological features across habitats differing in temperature may therefore require a control for the effects of temperature on metabolism. Here we use a straightforward correc-
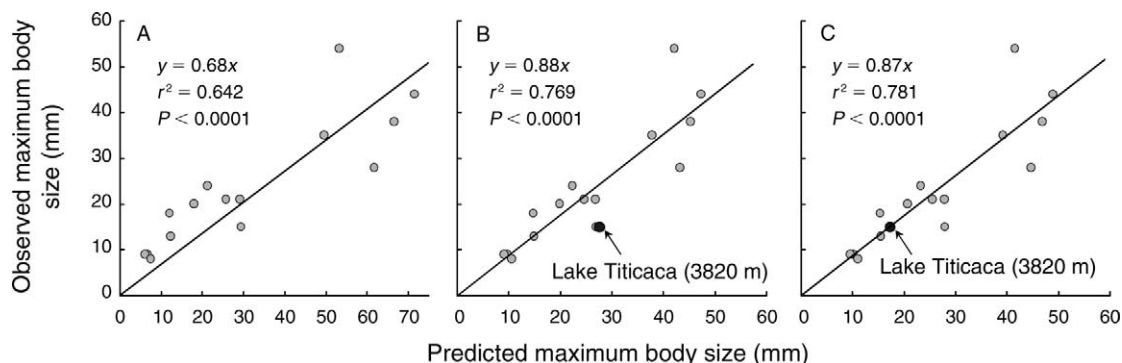
Fig. 2. Relationship between predicted and observed maximum amphipod body sizes (expressed as upper 95th percentiles of the distribution of body sizes in an amphipod assemblage). Predictions are based on the assumption that oxygen acts as a physicochemical ceiling, limiting the maximum size that can be attained by amphipods. As a measure of external oxygen availability, either (A) the oxygen concentration or (B, C) the oxygen supply index (OSI) was taken. For the OSI, plots are shown either assuming (B) no differences in pO_2 or (C) accounting for the lower pO_2 at higher altitudes, which especially affected the predicted body size for the high-altitude Lake Titicaca, at 3820 m above sea level.

tion by calculating oxygen supply relative to oxygen demand (relative oxygen supply index, rOSI):

$$rOSI = OSI/Q_{10}^{\Delta T/10} \qquad (3)$$

where temperature-dependent scaling is commonly expressed in Q_{10} values.

OXYGEN SUPPLY AND ECOLOGICAL PATTERNS

We tested how well our OSI explains patterns in body size and species richness through a reanalysis of published data sets with clines in environmental conditions (altitude, temperature, organic pollution, salinity) purportedly linked with oxygen (Fig. 1). We predicted how maximum body size would differ between sites with different temperatures and salinities, assuming oxygen availability to act as a physicochemical ceiling, limiting the maximum size that can be attained by amphipods (Makarieva et al. 2005; see Appendix C for details regarding the analysis). For species richness, we reanalyzed four data sets on stream invertebrate assemblages across clines in altitude, temperature, and organic pollution (Perry and Schaeffer 1987, Jacobsen 2008, Jacobsen and Marín 2008, Miserendino et al. 2008; see Appendix D for a description). Altitudinal clines have been proposed as the best hope to establish the mechanistic drivers of environmental oxygen supply (Spicer and Gaston 1999), because at higher altitudes pO_2 decreases and oxygen solubility increases as a result of lower temperatures. Consequently such clines allow discrimination between the effects of solubility and partial pressure.

Body size spectra

The OSI is a much stronger predictor of amphipod body size than oxygen concentration. The fitted relationship for the OSI ($y = 0.87x$, Fig. 2C, Pearson $r = 0.884$, $P < 0.001$) deviates little from unity and provides a significant improvement (likelihood ratio test,

$P = 0.0052$) compared to the relationship obtained using oxygen concentration as a predictor ($y = 0.68x$; Fig. 2A, Pearson $r = 0.802$, $P < 0.001$). Furthermore, erroneously large maximum body sizes are predicted for amphipods in the high altitude Lake Titicaca when the effect of a lower partial pressure at high altitude is not taken into account (Fig. 2B), and this confirms the importance of pO_2 for environmental oxygen supply, together with oxygen diffusivity and solubility.

Species richness

Patterns in species richness are strongly and significantly related to the OSI (Table 1), which explains an average of 49% of the variation in species richness. Traditional measures of oxygen (oxygen saturation, dissolved oxygen concentration, or BOD_5, the latter being the oxygen demand exerted after five days of a biochemical oxygen demand test) explained only up to 37% of the variation and these were never a better predictor than the OSI; the OSI significantly outperformed BOD_5 in the case of Colorado mountain streams and oxygen concentrations in Patagonian streams, as indicated by likelihood ratio tests (Table 1). Altitude is also significantly related to clines in species richness, but the OSI explains more variation in most cases, especially at higher altitudes (see Appendix E), and significantly outperforms altitude in the relationships with rarefied richness (Table 1). Altitude and oxygen content are closely correlated to two constituents of the index, pO_2 and αO_2. Neither of these alone are consistently powerful predictors. The consistent performance of the OSI demonstrates that both pO_2 and αO_2 are involved in driving environmental oxygen supply. This is illustrated on the Ecuadorian mountain streams (Fig. 3) where the relationship between richness and the OSI is more accurate than with either oxygen concentrations (as a measure of solubility) or oxygen partial pressure.

TABLE 1. Pearson r values for the relationships between species richness and altitude, temperature, and oxygen conditions (oxygen saturation, dissolved oxygen, biochemical oxygen demand [BOD_5]) as well as our oxygen supply index (OSI) and relative oxygen supply index (rOSI).

Biological response parameter	Altitude	Temperature			Oxygen conditions		
		T_{min}†	T_{med}	T_{max}‡	Oxygen§	OSI¶	rOSI#
Higher species richness at high temperatures (low altitude)							
Ecuadorean Andes (Jacobsen 2008; $n = 30$)							
Rarefied richness	−0.456*,bc	**0.406***,c	0.340$^{(*),c}$	−0.019	0.523**,ab	**0.595****,a	0.291
Richness	−0.409*,a	0.237	**0.464****,a	0.375*,a	0.290	0.169	−0.161
Colorado mountains (Perry and Schaeffer 1987; $n = 12$)							
Rarefied richness	−0.435	**0.550**$^{(*),a}$	0.520$^{(*),a}$	0.488	0.021	**0.560**$^{(*),a}$	−0.407
Richness	−0.766**,bc	**0.860****,a	0.830***,ab	0.811**,abc	0.339	0.733**,c	**−0.735****,c
Lower species richness at high temperatures (high pollution)							
Bolivian Altiplano (Jacobsen and Marín 2008; $n = 12$)							
Rarefied richness	N.A.	0.139	**−0.817****,a	−0.243	0.635*,b	0.637*,b	**0.708****,b
Richness	N.A.	0.089	**−0.873****,a	−0.100	0.652*,b	0.669*,b	**0.706****,b
Patagonian streams (Miserendino et al. 2008; $n = 6$)							
Rarefied richness	N.A.	−0.874*,a	−0.853*,a	**−0.877***,ab	0.900*,a	**0.924***,a	0.899*,a
Richness	N.A.	−0.846*,c	−0.897*,bc	**−0.941****,ab	0.907*,bc	**0.964***,a	0.927**,b

Notes: Correlations with the highest R^2 values for significant relationships with temperature and with oxygen are shown in boldface type. "N.A." indicates not applicable. Different superscript letters indicate significant differences between the relationships (tested with pair-wise likelihood ratio tests).

(*) $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Taking the minimum temperatures reported (Jacobsen 2008, Jacobsen and Marín 2008) or the averages minus the square root of the standard deviation (Perry and Schaeffer 1987, Miserendino et al. 2008).

‡ Taking the maximum temperatures reported (Jacobsen 2008, Jacobsen and Marín 2008) or the averages plus the square root of the standard deviation (Perry and Schaeffer 1987, Miserendino et al. 2008).

§ Minimum oxygen saturation (Jacobsen 2008, Jacobsen and Marín 2008), maximum biological oxygen demand (BOD_5) (Perry and Schaeffer 1987), or average dissolved oxygen minus the square root of the standard deviation (Miserendino et al. 2008).

¶ Calculations based on T_{min}.

Calculations based on T_{max}.

THERMAL RESPONSES: OXYGEN SUPPLY AND DEMAND

Temperature affects both environmental oxygen supply through changes in physical processes (thermally dependent diffusivity and oxygen solubility) and organismal oxygen demand through physiological processes (metabolic rate varies with temperature). The relationships between OSI and the biological features described above do not necessarily reflect a causal link, and temperature could be a strong alternative candidate to explain them. However, the relationship between temperature and amphipod body size is weaker than that for oxygen and the relationships between temperature and species richness are not consistent, with minimum, median, and maximum temperatures alternating as the best regressors and with sign reversals in the relationship (Table 1). Richness and temperature were *positively* correlated for the two data sets with an altitudinal cline, where low species richness corresponds to poor oxygen conditions through a low oxygen supply (at high altitudes and low temperatures, see *Low oxygen supply at low temperatures?*). Richness and temperature were *negatively* correlated for the other two data sets, where low species richness corresponds to poor oxygen conditions through high oxygen consumption (at warm, organically polluted sites, with depleted, under-saturated oxygen conditions). From the consistently positive relationships between the OSI and species richness, we

conclude that oxygen provides the most parsimonious explanation for the observed differences in species richness.

The interplay between oxygen supply and demand is central to understanding patterns in body size and species richness. The 6.75-fold size difference in amphipod maximum body size (Fig. 2C) is correctly predicted only when changes in oxygen supply (1.5-fold gradient) and demand (6.7-fold gradient) are integrated. Expressing oxygen supply relative to demand is also meaningful for the data sets where there is no altitudinal cline. Here the thermal physiologies represented within a species assemblage are similar across sites and the relative OSI, yields better or equally good "oxygen–richness" relationship (Table 1). The constrained physiological diversity arises in the high altitude study on the Bolivian Altiplano because the common species pool is small and uniform, possibly due to harsh environmental conditions of poor oxygen (high altitude) combined with large fluctuations in temperature and oxygen (Jacobsen and Marín 2008). In the Patagonian streams, impacted sites were compared with adjacent upstream reference sites (Miserendino et al. 2008), thus pairs of sites have identical species pools. In contrast, altitudinal shifts in species assemblages have been related to differences in their respiratory physiology (Jacobsen and Brodersen 2008) and such differences invalidate the use of relative
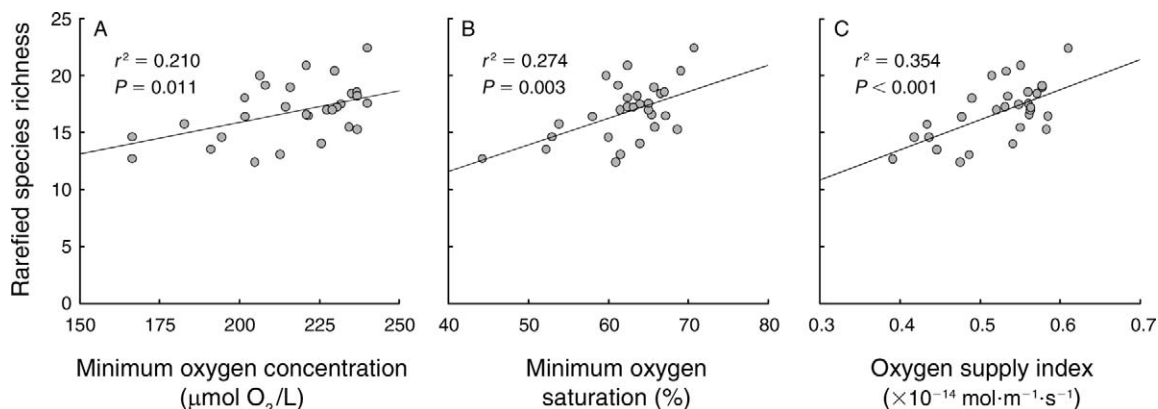
FIG. 3. Relationships between rarefied species richness in Ecuadorean streams and (A) minimum oxygen concentration, (B) minimum oxygen saturation (corresponding to altitudinal partial pressure differences; 100% at sea level, 61% at 3997 m), and (C) the OSI.

OSI. The higher diversity in thermal physiologies along an altitudinal cline may also be the cause for the higher degree of scatter in the OSI–richness relationships for the Ecuadorean and Colorado mountain streams (Table 1) compared to the OSI–richness relationships for the Bolivian Altiplano and the Patagonian streams.

Given the effects of temperature on oxygen supply and demand, it follows that many thermal responses may originate through organismal oxygen demand exceeding supply. With increasing ambient temperature, organismal oxygen demand increases exponentially, while ambient oxygen supply in water bodies effectively increases linearly (Figs. 1 and 4A). Each species will have an optimal temperature where relative oxygen supply as a measure of aerobic scope is maximal, with the optimum being governed by its thermal physiology and respiratory adaptations (Pörtner 2006). Aerobic

scope will decrease at both higher and lower temperatures (Fig. 4A), which is consistent with ideas of a temperature-defined window for aerobic scope (Pörtner 2006), and a temperature window for growth (Vannote and Sweeney 1980). Lower limits have been suggested to arise from limitations in ventilatory and circulatory capacity (Pörtner 2006). Upper limits have been previously suggested to arise from the combined effect of decreased oxygen supply (lower solubility) and increased metabolic rate, constituting a double challenge. Instead we show here that oxygen supply increases with increasing temperatures, but not sufficiently to match the exponential rise in metabolic rate. In this new perspective, the point at which oxygen supply no longer matches oxygen demand is much more variable. Responses to global warming will be very different for species or individuals with contrasting
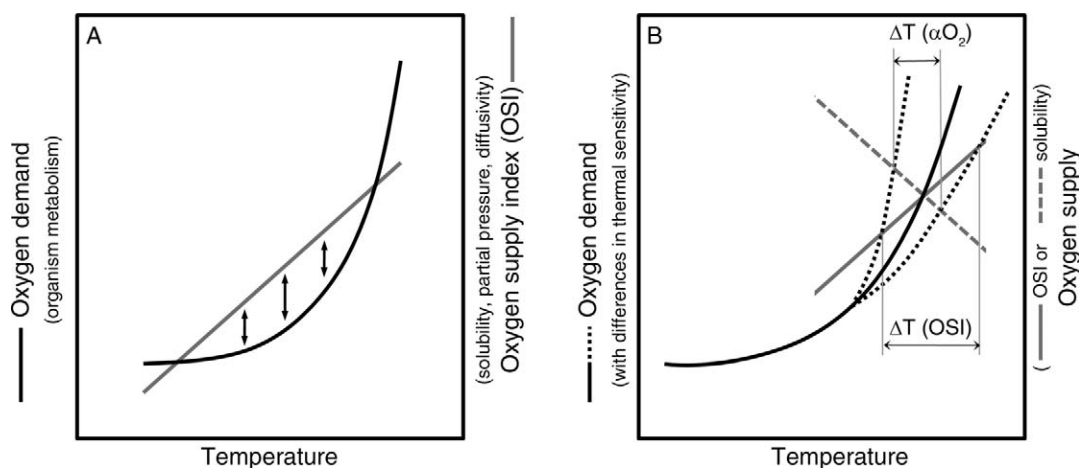

FIG. 4. (A) Conceptual model of an oxygen defined window for aerobic scope (lines with double arrowhead) determined by temperature-related changes in environmental oxygen supply (gray) and organismal oxygen demand (black), showing (B) how physiological differences related to the thermal sensitivity of oxygen demand (dotted black lines) will shift the critical temperature at which oxygen supply no longer meets demand. However, there is a larger scope for physiological variation in setting upper thermal tolerance when oxygen supply increases with temperature as predicted by the OSI (gray line), compared to the widely held notion of a lower oxygen supply at high temperatures owing to decreased solubility (dashed gray line).

thermal sensitivity of their respiratory physiology (Fig. 4B). The new insights gained from our OSI show that upper limits may mainly arise from physiological effects increasing metabolic rate under warm conditions, whereas physical thermal effects lowering oxygen supply under cold conditions (see *Low oxygen supply at low temperatures?*) provide an additional mechanism for oxygen to set lower limits.

Low Oxygen Supply at Low Temperatures?

In contrast to commonly held views, environmental oxygen availability at the respiratory surface (expressed by the OSI) *decreases* with decreasing temperature. This results principally from the fact that the strong decrease in oxygen diffusivity at lower temperatures is only partly compensated by a concomitant increase in oxygen solubility; something that was indeed suggested earlier (Spicer and Gaston 1999). This notion has far-reaching implications for our understanding of the ecological and evolutionary importance of oxygen supply in aquatic habitats. It provides new insights in oxygen-driven limits to body size and the way in which oxygen sets thermal limits (see *Thermal responses: oxygen supply and demand*).

Larger body sizes at low temperatures cannot be due to greater oxygen supply (via higher solubility) as previously suggested, since oxygen supply decreases with decreasing temperatures. Instead, lower temperatures may increase *relative* oxygen supply, primarily through reduced metabolism (Fig. 4). The combined importance of oxygen supply and demand explains why increased body sizes are associated with low temperatures at high latitudes (Vamosi et al. 2007), but not at high altitudes (Jacobsen 2003). At high altitudes, the combined effect of a lower pO_2 and lower diffusivity decrease oxygen supply sufficiently (Fig. 1F) to offset any benefit from a lower oxygen demand in the cold (Table 1), while at high latitudes, oxygen demand decreases relative to oxygen supply, and thus relative oxygen supply increases. A similar rationale explains why oxygen concentrations have been relatively successful in explaining ecological patterns; although concentration overestimates supply, this holds only for low temperature situations, when relative oxygen supply is indeed likely to be higher.

Conclusion: Oxygen Forges a Link between Ecology and Physiology

The OSI, derived from first principles of gas diffusion, brings together the different viewpoints that fuelled the debate on the importance of either αO_2 or pO_2 to explain patterns in body size. We show here the power of an integrated approach including effects of αO_2, pO_2, and diffusivity to consistently and accurately explain patterns in species richness and body size, covering a broad species spectrum from both marine and freshwater ecosystems. Systematically incorporating environmental oxygen supply relative to organismal oxygen demand in

data analyses will reveal a more pervasive influence of oxygen on ecological patterns. A far-reaching, perhaps counterintuitive, implication of the OSI is the appreciation of reduced environmental oxygen supply in cooler habitats. This notion warrants a reappraisal of the role of oxygen in setting limits to maximum body size and thermal tolerance.

The link between oxygen supply and demand epitomizes the need to (re-)unite the related, but often separate fields of physiology and ecology (Gaston et al. 2009) into ecophysiology. An analysis informed by both physiology (differences between species' oxygen consumption and respiratory capabilities) *and* ecology (differences in species assemblages and oxygen availability across habitats) would enable a better understanding of how oxygen supply matches a species' capabilities and constraints. Their integration at the large-scale level is not only logical, but also essential if we are to tackle key scientific questions and global environmental challenges (Chown and Gaston 2008, Wang and Overgaard 2007).

Methods

For body size spectra, we took upper 95th percentiles of body sizes previously reported in aquatic amphipod assemblages along clines in temperature, salinity and altitude (see Appendix C). For species richness, we reanalyzed two data sets on stream invertebrate assemblages across clines in altitude and temperature (see Appendix D). Here confounding environmental changes with altitude were minimal (e.g., stream width, substrate composition, human impact). In both data sets, species richness decreased with altitude and increased with temperature. Since our index should consistently explain richness patterns independent of whether species richness is positively or negatively related to temperature, we also included two data sets (see Appendix D) where oxygen is (periodically) lower due to organic pollution, and where low oxygen coincided with higher temperatures. The oxygen supply index was calculated for each site from reported environmental conditions and related to the reported species richness using linear regressions.

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APPENDIX A

Boundary layer effect on the relationship between OSI and species richness (*Ecological Archives* E092-131-A1).

APPENDIX B

Derivation of the OSI from first principles on gas diffusion (*Ecological Archives* E092-131-A2).

APPENDIX C

Description of the data sets on body size and data analysis (*Ecological Archives* E092-131-A3).

APPENDIX D

Description of the data sets on species richness and data analysis (*Ecological Archives* E092-131-A4).

APPENDIX E

Additional analysis, showing oxygen to be of greater importance at higher altitudes (*Ecological Archives* E092-131-A5).

SUPPLEMENT

Data and formulas used in the analyses on body size and richness (*Ecological Archives* E092-131-S1).