Research article

Investigating the gill-oxygen limitation hypothesis in fishes: intraspecific scaling relationships of metabolic rate and gill surface area

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Many ectotherms have shown a reduction in maximum body size in the past decades in parallel with climate warming. Indeed, some models forecast a maximum body size decline of 14%–24% by 2050 for numerous fish species. The gill-oxygen limitation (GOL) hypothesis is perhaps the most prominent concept regarding the physiological mechanisms underlying the observed trends, implicating oxygen uptake limitations in driving the decline in fish body size with warming. Current scientific debates, however, demonstrate a clear need for a synthesis of existing empirical evidence to test the fundamental assumptions of the GOL hypothesis. Here, we perform a systematic literature review of the intraspecific allometry of gill surface area (GSA) and metabolic rate. Additionally, we introduce a new parameter, the ratio S, which provides a measure of GSA in relation to the metabolic requirements for maintenance (S_{SMR}) and maximum activity (S_{AMR}). Support for the GOL hypothesis would be evidenced by a universal decline in S with increasing body mass within each species, such that gills become less equipped to supply metabolic requirements as fish grow. In contrast to the predictions of the GOL hypothesis, we show that the scaling exponents for S_{SMR} and S_{AMR} are consistently close to zero, with only a few exceptions where S either increased or decreased. These findings suggest that the GSA of each species is sufficient to meet its oxygen requirements throughout life, and that growth is not universally restricted by oxygen uptake limitations across the gills. We identify the need to investigate hypotheses other than the GOL hypothesis to help explain the observed declines in maximum fish body sizes concurrent with climate warming, in order to facilitate accurate predictions of fish community structure and manage fisheries in the face of climate change.

Introduction

Body size impacts virtually all aspects of an organism's life, including its behaviour, physiology, morphology and ultimately its fitness. A variety of taxa grow larger with increasing distance from the equator and concomitant decreasing environmental temperatures, a principle that has been observed both inter- (Bergmann, 1847, Gilligan, 1991) and intra-specifically (Cappo et al., 2013, Horne et al., 2015, James, 1970, Ricker, 1981, Williams et al., 2003) (Bergmann's rule and James' rule, respectively). Initial reports suggested that these latitudinal trends were due to adaptive changes in body surface-to-volume ratios to optimize physiological thermoregulation in endothermic animals (Bergmann, 1847). However, many ectothermic animals, which lack physiological thermoregulation, also show this latitudinal trend in body size. Additionally, some ectotherms have undergone a reduction in maximum body size over the past decades in parallel with anthropogenically driven climate warming (Baudron et al., 2011, Baudron et al., 2014, Daufresne et al., 2009, Genner et al., 2010, Van Walraven et al., 2010). Some models forecast a 14%–24% decline in maximum body size by 2050 for numerous fish species, with potentially far-reaching ecological and economic impacts (Cheung et al., 2013). The apparently ubiquitous relationship between temperature and body size is often referred to as the 'temperature-size rule'.

The temperature-size rule is reported to be most apparent in aquatic ectotherms (Forster et al., 2012), but disentangling the contribution of temperature from confounding factors can be difficult. Fisheries-induced selection favouring smaller-sized adults (Audzijonyte et al., 2013, Conover and Munch, 2002, Waples and Audzijonyte, 2016), as well as higher fishing mortality of larger-bodied individuals (Sharpe and Hendry, 2009), are proposed mechanisms contributing to the trend of declining body size in fishes. However, the pattern of 'shrinking' is not evident in some commercially fished species (Baudron et al., 2014), and a reduction in body size has also been found in some non-targeted fish and other organisms (Daufresne et al., 2009). This leads to the conclusion that fisheries-induced selection cannot be the sole driver of body size reductions through time, and thus temperature remains implicated.

Studies suggest that climate warming and concomitant heat waves caused by anthropogenic greenhouse gas emissions have played a central role in the decline in organismal body size over the past half-century (Daufresne et al., 2009, Sandström et al., 1995, Sheridan and Bickford, 2011). The effect of temperature may be indirect through changes in ecosystem productivity and food web dynamics (DeLong and Walsh, 2015, Schaum et al., 2018, Schlüter et al., 2012), as well as through changes in population density (Audzijonyte et al., 2019). However, water temperature also directly affects fish, such as through modulating their biological rates. Therefore, environmental temperature influences nearly all physiological processes in fish including metabolism, growth and reproduction (Kingsolver and Huey, 2008).

Perhaps the most influential hypothesis on the mechanisms underlying the temperature-size rule is the gill-oxygen limitation (GOL) hypothesis, sometimes referred to as the gill-oxygen limitation theory (GOLT) (Pauly, 1981, 2019, 2021, Pauly and Cheung, 2018). This hypothesis proposes that there is a mismatch between the body mass scaling exponents of gill surface area (GSA) and aerobic metabolic rate (MR), as the former is considered to be a 2D surface and the latter is considered to be a product of (3D) body volume. According to the GOL hypothesis, the gills become increasingly limited in their capacity to supply oxygen to a growing body, and this issue is amplified by a temperature-induced increase in metabolism. It rests on the fundamental concept of anatomical scaling. The underpinning assumption is that GSA scales with a lower exponent than resting or routine MR, such that a threshold body mass is eventually reached where all available oxygen supplied by the gills is allocated to resting metabolism and thus scope for growth becomes zero (Pauly and Cheung, 2018; Fig. 1A). The increase in maintenance metabolism with temperature, without a proportional increase in GSA, would consequently lead to a decrease in maximum body mass (from $W_{\infty 2}$ to $W_{\infty 1}$ in Fig. 1). GOL is therefore proposed to explain why animals living in warmer waters are not able to reach the same body size as those inhabiting colder environments and why aquatic warming restricts ectotherm body size.

Most investigations of the temperature-size rule have been largely theoretical and have selected particular empirical studies to support a narrative, which has led to enthusiastic debate about the GOL hypothesis and the underlying physiological mechanisms of the temperature-size rule (Lefevre et al., 2017, 2018, Pauly, 2021, Pauly and Cheung, 2018). Indeed, forecasts of future fish biomass based on the GOL hypothesis (Cheung et al., 2013) have come under scrutiny (Lefevre et al., 2017), highlighting the need for validated model assumptions in the management and conservation of fish populations.

These recent debates have identified a clear need for synthesis of the scattered empirical evidence to test the fundamental assumptions of the GOL hypothesis. As such, we review the literature and outline the current knowledge on the intraspecific scaling of MR and GSA. Additionally, we introduce a new parameter, the ratio S (GSA:MR), which provides a measure of the available GSA for supplying the metabolic requirements for maintenance (S_{SMR}) and maximum activity (S_{AMR}). We further investigate how this ratio scales with body mass.

Both GSA and MR scale according to power-law relationships:

$$Gill\ surface\ area = a_{GSA} \bullet body\ mass^{b_{GSA}}, \qquad (1)$$

$$Metabolic\ rate = a_{MR} \bullet body\ mass^{b_{MR}}, \qquad (2)$$

where a is the scaling factor (the standardized GSA or MR at 1 g of body mass) and b is the scaling exponent. The ratio S is therefore derived as follows:

$$S = \frac{GSA}{MR} = \frac{a_{GSA}}{a_{MR}} \bullet body\ mass^{b_{GSA} - b_{MR}} \qquad (3)$$

If the predictions of the GOL hypothesis are supported by the literature and GSA indeed scales with a lower exponent than MR, we would expect to find negative scaling exponents for S (as $b_S = b_{GSA} - b_{MR}$; see Fig. 1A and B). If b_S takes on values ≥ 0, however (as in Fig. 1C and D), the fundamental assumption of the GOL hypothesis would not be met. With the ratio S we assess whether respiratory surface area is likely to restrict growth due to an undersupply of oxygen during rest (as framed in the GOL hypothesis and depicted in Fig. 1A and B) or during strenuous exercise (perhaps via a decline in aerobic scope during growth, as suggested by Rubalcaba *et al.*, 2020).

Materials and methods

We conducted a systematic review by screening the literature for publications using the search terms 'gill surface area', 'scaling', 'fish' and 'body weight' or 'body mass' or 'body size' utilizing the search engines Scopus, Web of Science, Science Direct and JSTOR (resulting articles, $N = 215$). In order to supplement the database with additional studies, we also conducted a manual search by scanning the reference lists from studies retrieved through the systematic search ($N = 3$) and a broader investigation with the use of Google Scholar ($N = 2$). The titles and abstracts of the articles found during the database search and during manual search were then screened for suitability for this study (i.e. if the study reported on the GSA scaling of one or more fish species). After this initial elimination, we selected studies that used similar measurements of GSA for different body sizes spanning at least one order of magnitude and conducted full-text assessments of the remaining articles ($N = 22$). Because scaling exponents can be markedly variable when calculated using small mass ranges, we did not use any dataset where the mass range was less than one order of magnitude. Once all suitable publications were identified, the supporting data were extracted using the web application WebPlotDigitizer (Rohatgi, 2020) and stored in a database.

We then searched in the literature for data on the allometry of MR [oxygen uptake rate ($\dot{M}O_2$) used as a proxy for MR] for the same species identified in the GSA investigation using the same search engines as mentioned above. The search terms used for the MR data were the respective species name and a combination of the terms 'body weight' or 'body mass' or 'body size' and 'metabolic rate' or 'oxygen consumption' or 'oxygen uptake' (e.g. '*Anguilla anguilla*' AND 'body weight' OR 'body mass' OR 'body size' AND 'metabolic rate' OR 'oxygen consumption' OR 'oxygen uptake'). Upon finding
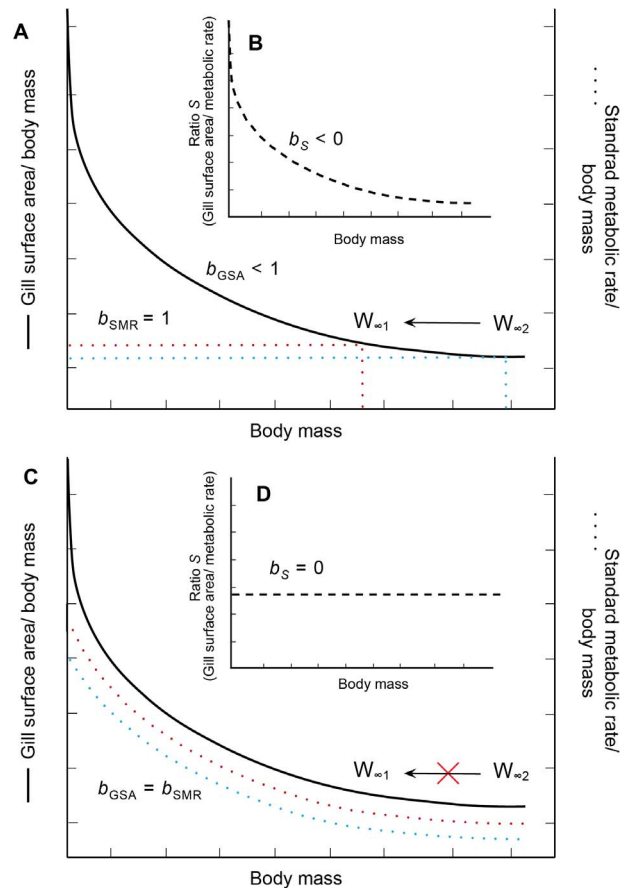

Figure 1: Conceptual figure of GSA and SMR divided by body mass and plotted against body mass (**A** and **C**), and the ratio between GSA and SMR (denoted S) plotted against body mass (**B** and **D**). We assume that scaling relationships of both GSA and SMR, as well as the resulting ratio of the two (S), follow power-law functions (GSA, SMR or $S = a \times body\ mass^b$). In (A) (adapted from Pauly and Cheung, 2018, fig. 2 and Pauly, 2021, fig. 4), the 'scope for growth' (the area between SMR and GSA, denoted as the oxygen surplus of gill area after accounting for maintenance metabolism) declines with body mass until it becomes zero and all available oxygen is used for maintenance, limiting maximum body mass to $W_{\infty 2}$. A temperature-induced increase in SMR in (A) (shown as a shift from the dotted blue line to the dotted red line) is proposed to lead to a decline in maximum body mass to $W_{\infty 1}$. In (A) it is assumed that gill area scales with an exponent lower than 1 ($b_{GSA} < 1$), while maintenance metabolism scales with an exponent of 1 ($b_{SMR} = 1$). The ratio S in (B) therefore scales with an exponent lower than 0 ($b_S < 0$, as $b_S = b_{GSA} - b_{SMR}$). A contrasting representation of the scaling relationship of SMR is shown in (C), where b_{SMR} has the same value as b_{GSA} ($b_{GSA} = b_{SMR}$) despite temperature modifying the intercept of the SMR regressions. The resulting 'scope for growth' thus never becomes 0, and the corresponding ratio S in (D) stays constant throughout the entire body mass range ($b_S = 0$). In this latter scenario, maximum body mass declines cannot be explained by GOL.

a relevant publication, we ensured that the data range for body mass was at least one order of magnitude and was overlapping with the body mass range recorded for GSA data.

We then extracted the data as described above and stored the data in our database. Data were pooled for Atlantic bluefin tuna (*Thunnus tynnus*) and Pacific bluefin tuna (*Thunnus orientalis*) to yield a useable dataset for GSA and MR (*N* (GSA) = 1; *N* (MR) = 2). Since the Pacific bluefin tuna has been considered a subspecies of the Atlantic bluefin tuna until recent years (Collette, 1997), we treated this as one single species (labelled bluefin tuna (*Thunnus spp.*) herein). In one study where data points were difficult to manually extract, the raw data were obtained from the original authors. Furthermore, we converted all MR and $\dot{M}O_2$ measurements to the unit $mgO_2 \cdot h^{-1}$ and all GSA data to the unit mm^2. If metabolic data for a particular species were recorded at different temperatures in different studies, we pooled data for that species after standardizing to 20°C using the Q_{10} value available for that species (relevant for two species: Q_{10} of 1.8 for bluefin tuna from Blank *et al.*, 2007; Q_{10} of 1.9 for Atlantic salmon (*Salmo salar*) from Bowden *et al.*, 2018). If any study examined the MR at multiple temperatures, we picked the temperature closest to 20°C [relevant for two species: European eel (*Anguilla anguilla*) and European flounder (*Platichthys flesus*)].

For examining baseline metabolic requirements, we only used MR data from apparently resting animals, so we use the term standard metabolic rate (SMR) henceforth (for bluefin tuna, SMR necessarily includes a low level of swimming activity). For examining maximal metabolic capacity, we used metabolic data from strenuously exercised fish and herein will refer to these data as active metabolic rate (AMR). The total number of publications satisfying our criteria was 23 [6 for GSA only, 14 for MR only (11 for SMR and 3 for SMR and AMR) and 3 for both GSA and SMR]. All references can be found in Table S1 in the supplementary material.

Data describing the relationship between body mass and each of GSA, SMR and AMR were subsequently interrogated by fitting non-linear regressions (power-law relationships) of the form

$$Y = aM^b, \qquad (4)$$

where *Y* is the variable of interest [either GSA, SMR, AMR or *S* (see below)], *a* is the scaling factor (the standardized *Y* variable at 1 g of body mass), *M* is the body mass (in g) and *b* is the scaling exponent. We then checked the data for normality, constant variance and significance of the regressions. The resulting models were consequently used to interpolate and extrapolate GSA, SMR and AMR values for the full mass range available for that species, and a ratio of GSA and MR was calculated according to the formula

$$S = \frac{Gill\ surface\ area}{Metabolic\ rate}. \qquad (5)$$

The calculated variable *S* provides an index of the available GSA (mm^2) per metabolic requirement ($mgO_2 \cdot h^{-1}$) across the body mass range, with higher values meaning more gill surface area per unit of MR. We calculated the *S* index for each of SMR (S_{SMR}) and AMR (S_{AMR}). One study measured GSA and SMR at two temperatures [goldfish (*Carassius auratus*) at 15°C and 25°C], hence for this species we calculated S_{SMR} for each temperature and plotted it against body mass. We then derived standard errors (SEs) for the regression parameters of *S* using the SEs of GSA and MR regression analyses (i.e. the square root of the sum of the squared fractional SEs for *a*, the square root of the sum of the squared SEs for *b*). To provide a quantitative assessment of whether the scaling exponents for S_{SMR} and S_{AMR} were significantly different from zero, we examined whether 95% confidence intervals around *b* encompassed zero (95% confidence intervals calculated as SE × 1.96, as in Fisher, 1925). Regressions and statistics were generated using SigmaPlot version 14.0 (Systat Software, Inc., San Jose California USA, www.systatsoftware.com), and graphs were generated using Rstudio [RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA USA. URL]. Values presented herein are model-derived with ± SE.

Results

A total of 22 publications fitted our criteria, yielding data for 9 species (bluefin tuna pooled as 1 species) to be included in our analyses.

Gill surface area

GSA across the 9 species ranged nearly 10 orders of magnitude, from 0.009 mm^2 to 32 819 000 mm^2 across a body mass range of 1.51 mg to 33.14 kg (Fig. 2A). The standardized GSA at 1 g of body mass (scaling factor, *a*) ranged from 352 ± 58 mm^2 (± SE) in the European horse mackerel (*Trachurus trachurus*; temperature not given in study) to 2144 ± 657 mm^2 in the goldfish (at 15°C). Note that the value of *a* was extrapolated to 1 g across a large mass range in several species, so interspecific comparisons should be made with caution. The scaling exponent (*b*) of the relationship between GSA and body mass was steepest for the European horse mackerel (1.23 ± 0.03) and shallowest for the marbled electric ray *Torpedo marmorata* (0.65 ± 0.18). All regression details are given in Table 1.

Standard metabolic rate

SMR data for the 9 species spanned a range of 0.0002 mg $O_2 \cdot h^{-1}$ to 2222 mg $O_2 \cdot h^{-1}$ from fish covering a body mass range of 0.13 mg to 11 kg; Fig. 2B). The scaling factor *a* varied between 0.1 ± 0.04 mg $O_2 \cdot h^{-1}$ in the European horse mackerel at 13°C and 2.61 ± 0.66 mg $O_2 \cdot h^{-1}$ in the bluefin tuna at 20°C. The scaling exponent *b* ranged between 0.64 (± 0.02) in the Nile tilapia (*Oreochromis niloticus*) at 25°C to 1.07 (± 0.02) in Atlantic salmon at 20°C. Regression details are given in Table 1.

Table 1: Allometric regression parameters (a = scaling factor, b = scaling exponent) for GSA, SMR (eight fish species), AMR (three fish species) and the ratios S_{SMR} (GSA/SMR) and S_{AMR} (GSA/AMR).

Species	Body mass range (g)	Overlapping body mass (g)	GSA				SMR				S_{SMR}	
			Temp. (°C)	a (± SE)	b (± SE)	R^2	Temp. (°C)	a (± SE)	b (± SE)	R^2	a (± SE)	b (± SE)
European eel (*A. anguilla*)	10–1160	60–480	9–24	359.6 (± 248.41) *a*	0.9 (± 0.1) ***	0.940	20	0.11 (± 0.06) *a*	0.83 (± 0.09) **	0.989	3260.2 (± 0.87)	0.07 (± 0.14) [−0.19–0.34]
Common carp (*C. carpio*)	0.0015–2210	14.5–225.3	NG	701.59 (± 199.86) **	0.83 (± 0.04) ***	0.989	25	0.26 (± 0.08) ***	0.80 (± 0.06) ***	0.832	2689.1 (± 0.42)	0.03 (± 0.07) [−0.11–0.16]
European flounder (*P. flesus*)	0.002–402.7	4.5–178.5	12–13	740.87 (± 134.84) ***	0.7 (± 0.04) ***	0.963	15	0.14 (± 0.13) *a*	0.77 (± 0.17) ***	0.867	5353.1 (± 0.95)	−0.08 (± 0.17) [−0.41–0.26]
Atlantic salmon (*S. salar*)	0.03–192.5	0.19–1.3	10–22	856.1 (± 15.25) ***	0.97 (± 0.02) ***	0.984	20	0.12 (± 0.01) ***	1.07 (± 0.02) ***	0.935	7418.5 (± 0.1)	−0.09 (± 0.03) [−0.16 − −0.03]
Marbled electric ray (*T. marmorata*)	99.1–3346.7	105.9–3322.2	NG	975.46 (± 1372.91) *a*	0.65 (± 0.18) ***	0.639	16	0.04 (± 0.07) *a*	0.97 (± 0.24) ***	0.742	27 555 (± 2.39)	−0.32 (± 0.30) [−0.92–0.27]
Goldfish (*C. auratus*)	4.37–221.41	4.41–205.4	15	2143.5 (± 657.15) **	0.74 (± 0.06) ***	0.916	15	0.11 (± 0.04) ***	0.87 (± 0.07) ***	0.928	20 089 (± 0.46)	−0.13 (± 0.09) [−0.31–0.05]
Goldfish (*C. auratus*)	4.23–320.1	4.19–226.9	25	1127.8 (± 221.43) ***	0.87 (± 0.04) ***	0.972	25	0.2 (± 0.04) ***	0.83 (± 0.04) ***	0.829	5693.1 (± 0.28)	0.04 (± 0.05) [−0.07–0.15]
European horse mackerel (*T. trachurus*)	1.72–390.1	12–135	NG	352.11 (± 58.22) **	1.23 (± 0.03) ***	0.999	13	0.1 (± 0.04) **	0.89 (± 0.08) ***	0.999	3503.6 (± 0.46)	0.34 (± 0.08) [0.17–0.5]
Bluefin tuna (*Thunnus spp.*)	0.001–33 142	4313–11 000	NG	979.15 (± 834.17) *a*	0.99 (± 0.09) ***	0.919	20	2.61 (± 0.66) ***	0.71 (± 0.03) ***	0.959	375.6 (± 0.89)	0.28 (± 0.09) [0.11–0.46]
Nile tilapia (*O. niloticus*)	0.01–1709.7	18.12–313.79	NG	1253.33 (± 367.9) **	0.67 (± 0.05) ***	0.894	25	0.51 (± 0.07) ***	0.64 (± 0.02) ***	0.900	2447.4 (± 0.33)	0.025 (± 0.05) [−0.08–0.13]

Continued

Table 1: Continued.

Species	Body mass range (g)	Overlapping body mass (g)	GSA				AMR				S_{AMR}	
			Temp. (°C)	a (± SE)	b (± SE)	R^2	Temp. (°C)	a (± SE)	b (± SE)	R^2	a (± SE)	b (± SE)
Atlantic salmon (S. salar)	0.2–3.44	0.12–1.3	NG	856.1 (± 15.25) ***	0.97 (± 0.02) ***	0.984	20	0.87 (± 0.14) ***	0.75 (± 0.1) ***	0.547	980.8 (± 0.16)	0.23 (± 0.1) [0.03–0.42]
Goldfish (C. auratus)	4.19–323.2	4.5–226.94	25	1127.8 (± 221.43) ***	0.87 (± 0.04) ***	0.972	25	0.55 (± 0.18) **	0.86 (±0.06) ***	0.805	2048.7 (± 0.38)	0.01 (± 0.07) [−0.13–0.16]
Nile tilapia (O. niloticus)	0.01–812.01	20.73–313.79	NG	1253.33 (± 367.9) **	0.67 (± 0.05) ***	0.894	26	1.17 (± 0.53) **	0.87 (±0.08) ***	0.936	1074.3 (± 0.54)	−0.2 (± 0.1) [−0.39 − −0.01]

The body mass range represents the entire range over which the data were collected, while the overlapping body mass range represents the range where data for GSA and MR overlapped.

'Temp.' refers to the temperature (°C) at which the measurements were made (NG, not given; 95% CI, 95% confidence intervals).

Associated references are given in Table S1.

* $P \leq 0.05$.

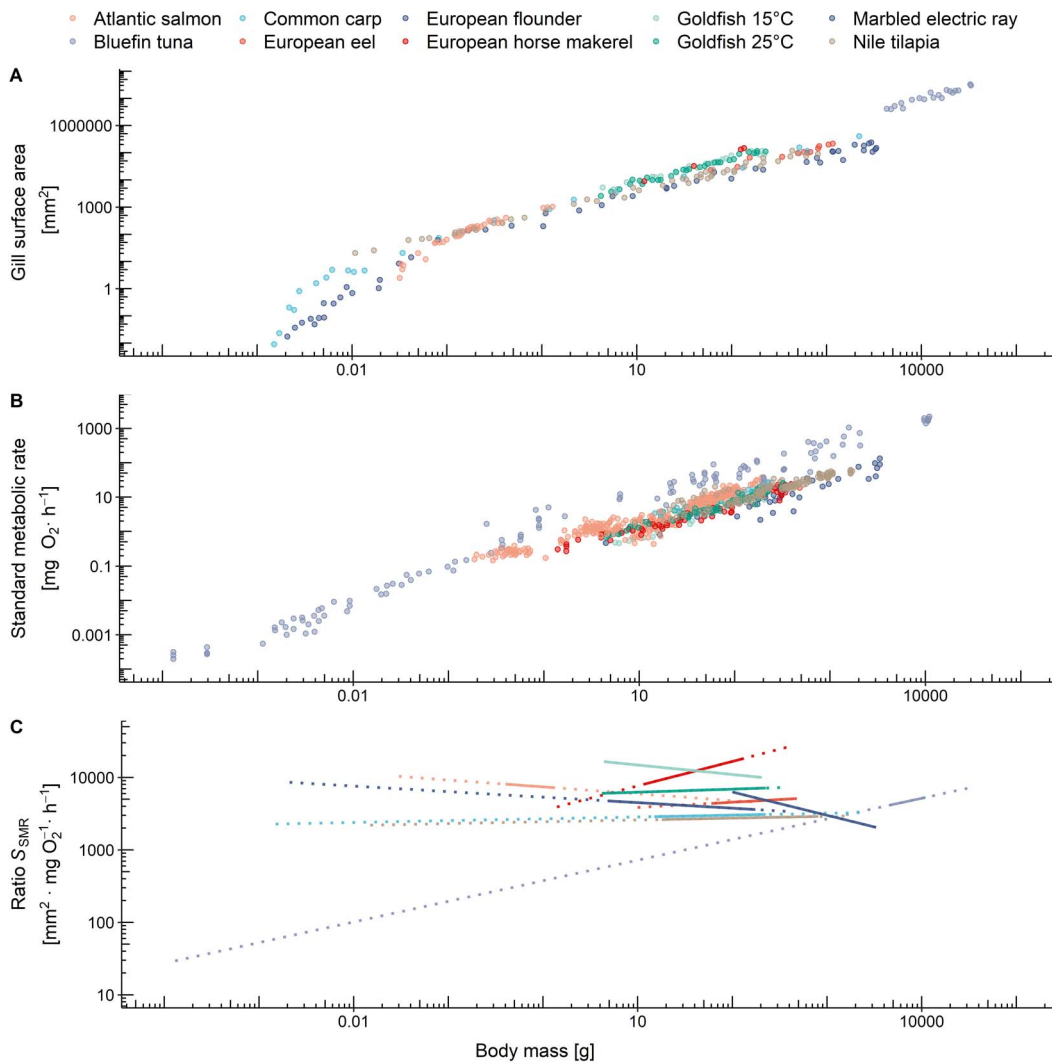** $P \leq 0.01$.

*** $P \leq 0.001$.

[a]Not significant.

Figure 2: The influence of body mass on each of (**A**) GSA, (**B**) SMR and (**C**) ratio S_{SMR} (i.e. GSA/SMR) for nine fish species. S_{SMR} represents an index of the available GSA to supply SMR requirements across the body mass range. In (C), dotted lines show S_{SMR} across the full mass range of available data, while solid lines indicate where the mass ranges for GSA and SMR data overlap.

Active metabolic rate

AMR was available for three species and ranged from 2.11 mg $O_2 \cdot h^{-1}$ to 212.91 mg $O_2 \cdot h^{-1}$ measured across a body mass range of 0.3 g to 812 g (Fig. 3B). The scaling factor a varied between 0.55 ($\pm$ 0.18) for the goldfish at 25°C and 1.17 ($\pm$ 0.53) for Nile tilapia at 20°C. The relationship between AMR and body mass scaled steepest in Nile tilapia with a scaling exponent (b) of 0.87 ($\pm$ 0.08) and shallowest in Atlantic salmon with b of 0.75 ($\pm$ 0.1). See Table 1 for regression details.

S_{SMR} index

The index S_{SMR} ranged from 27 to 26 626 mm$^2 \cdot$ mgO$_2^{-1}$ $\cdot$ h^{-1} across nine species with a total body mass range of

0.13 mg to 33.14 kg, yet in the vast majority of species S_{SMR} remained between 1000 and 10 000 mm$^2 \cdot$ mgO$_2^{-1} \cdot$ h^{-1} across the entire mass range (Fig. 2C). The scaling exponent b varied between species from -0.32 ($\pm$ 0.30) in the marbled electric ray to 0.34 ($\pm$ 0.08) in the European horse mackerel, brought about by different scaling exponents for GSA and SMR (e.g. if GSA scales with a larger exponent than MR, S will scale positively). While three species (Atlantic salmon, European flounder and the marbled electric ray) showed a negative exponent in accordance with the GOL hypothesis, five species [the European eel, the common carp (*Cyprinus carpio*), the Nile tilapia, the European horse mackerel and bluefin tuna] showed a positive scaling exponent, representing enhanced capacity of the gills to supply standard metabolic oxygen requirements with increasing body mass. In the case
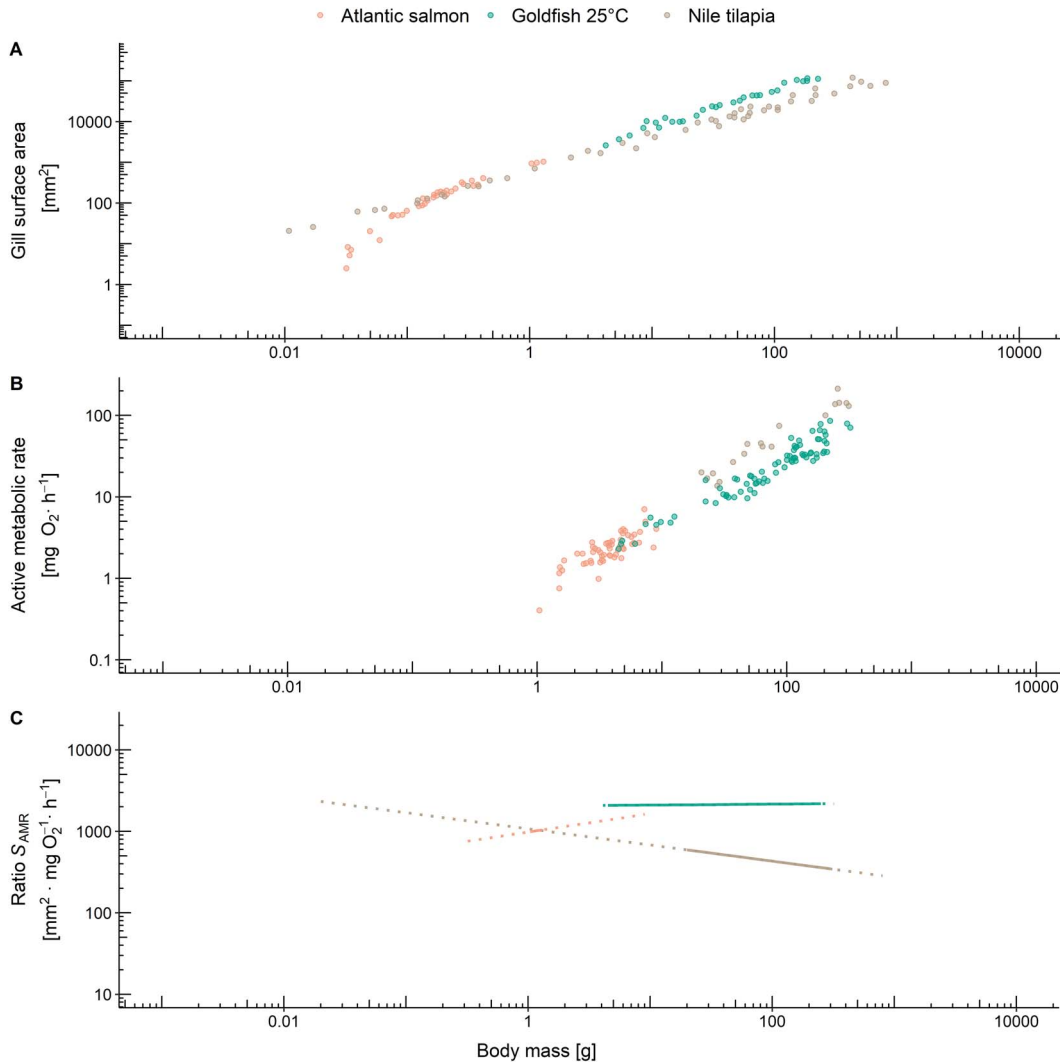
Figure 3: The influence of body mass on each of (**A**) GSA, (**B**) AMR and (**C**) ratio S_{AMR} (i.e. GSA/AMR) for three fish species. S_{AMR} represents an index of the available GSA to supply AMR requirements across the body mass range. In (C), dotted lines show S_{AMR} across the full mass range of available data, while solid lines indicate where the mass ranges for GSA and AMR data overlap.

of the goldfish, GSA and SMR were recorded at two different temperatures, with a negative scaling exponent for S_{SMR} at 15°C [$b = -0.13$ ($\pm$ 0.09)] and a positive exponent at the warmer temperature of 25°C [$b = 0.04$ ($\pm$ 0.05)]. This was driven by a higher b for GSA and a lower b for SMR at 25°C, which contradicts the GOL hypothesis. Despite the above-mentioned trends, when the exponents for S_{SMR} were examined with consideration of the uncertainty around the estimates, 6 of the 9 species had S_{SMR} exponents that were not different from zero (i.e. 95% confidence intervals for b bounded zero) (Table 1). The exceptions were Atlantic salmon with a clearly negative exponent (95% confidence bands: −0.16 to −0.03) and two species with a clearly positive exponent (European horse mackerel: 0.17 to 0.50; bluefin tuna: 0.11 to 0.46). For visual comparison with the schematic

diagram presented in Fig. 1, we have plotted the data for three representative scaling relationships [tilapia, goldfish (15°C), and goldfish (25°C)] in the same format in the supplementary material (Fig. S1–S3).

S_{AMR} index

The ratio S_{AMR} for three species ranged from 285 to 2334 mm² · mgO₂⁻¹ · h⁻¹ with a body mass range of 0.32–812 g (see Fig. 3C). The scaling exponent for S_{AMR} (b) again varied between species, ranging from −0.2 ($\pm$ 0.1) in the Nile tilapia to 0.23 ($\pm$ 0.1) in Atlantic salmon. Two of the three species (goldfish and Atlantic salmon) showed a positive scaling exponent for S_{AMR} across body mass, while the scaling exponent for Nile tilapia declined with body mass.

When examining these relationships in light of the level of uncertainty around the calculated exponents, the positive scaling exponent remained for one of the species (Atlantic salmon 95% confidence bands: 0.03–0.42) and the negative scaling exponent remained for Nile tilapia (−0.39 to −0.01). Regression parameters for all fish species are given in Table 1.

Discussion

Evidence for/against the GOL hypothesis

The GOL hypothesis predicts that as fish grow, the maintenance oxygen demand of the body progressively approaches the oxygen supply capacity of the gills. When fish approach this intersection point, the growth rate declines and their size asymptotes. If this hypothesis were true, the ratio S_{SMR} (GSA divided by SMR) would universally scale negatively with body mass, i.e. with negative exponent b (as depicted in Fig. 1B). Furthermore, if increased temperature exacerbates this issue and results in the oxygen supply/demand mismatch occurring at a smaller body mass, as proposed by the GOL hypothesis (Pauly, 1981, 2019), then S_{SMR} would scale even more negatively in warmer environments and/or reach a critically low threshold at a smaller body mass in warmer environments.

The data presented here provide the first broadscale assessment of the assumptions of the GOL hypothesis in fishes. Only three species (Atlantic salmon, European flounder and marbled electric ray) provided some support for the GOL hypothesis by exhibiting a negative scaling exponent for the ratio S_{SMR}. However, for the majority of species (European eel, common carp, Nile tilapia, European horse mackerel and bluefin tuna), S_{SMR} showed a positive scaling trend with body mass, meaning gill-oxygen supply in fact increased with body mass relative to maintenance metabolic demand. This suggests that most of the current empirical evidence does not support the GOL hypothesis.

It is important to keep in mind that our S ratios come with some level of uncertainty around the calculated values. When taking this uncertainty into account, six species showed S_{SMR} scaling exponents encompassing zero (similar scaling relationships as shown in Fig. 1D). Only three species had scaling exponents that were significantly different from zero (Atlantic salmon had negative b_S, while European horse mackerel and bluefin tuna had positive b_S) (see Table 1).

We only found one study that measured GSA and MR in fish acclimated to different temperatures (goldfish in Li *et al*., 2018), emphasizing the need for further empirical studies in this area. In that study, the scaling exponent for S_{SMR} was positive at the cooler temperature (15°C) and negative at the warmer temperature (25°C), although neither trend was significantly different from zero when considering 95% confidence intervals. This suggests that there is negligible change in the capacity of the gills to supply oxygen to the body as goldfish grow, independent of temperature. Temperature-

dependent scaling of GSA in the study by Li et al. (2018) indicates that this trait is not inflexible, but rather indicates that the goldfish can adjust its respiratory morphology according to its metabolic needs (Table 1). Increased metabolic demands at higher temperatures, reflected in a higher scaling factor a at 25°C (0.2 ± 0.04) compared with 15°C (0.11 ± 0.04), can be met by physiological processes increasing oxygen supply to the tissues, such as changes in cardiac output, blood oxygen-carrying capacity and tissue oxygen extraction (Audzijonyte *et al*., 2019, Clark *et al*., 2008). Furthermore, some fish, including the genus *Carassius*, can rapidly remodel their GSA depending on abiotic factors, including changing environmental oxygen levels (Sollid *et al*., 2003) and temperature (Sollid *et al*., 2005). Gill remodelling is thought to be a mechanism to optimize the GSA in a varying environment and is considered to be a trade-off between maximizing oxygen supply, while minimizing osmoregulatory costs, the uptake of toxic substances and the risk of gill infection (Nilsson *et al*., 2012, Perry and Gilmour, 2010).

Notably, we targeted studies that investigated GSA and MRs covering body masses of at least one order of magnitude, as scaling relationships can be particularly unreliable when examining small mass ranges. While there are numerous other studies that have measured GSA and/or MRs over smaller mass ranges, we are confident that our findings are broadly applicable. For example, Luo *et al*. (2020) measured SMR and GSA of 6 cyprinid species across a mass range between 0.48 and 0.87 orders of magnitude, and the scaling exponents for S_{SMR} (calculated by us) decreased slightly for three species and increased slightly for the other three species, resulting in an average b_S of −0.01. Moreover, a meta-analysis of a broad range of animals by Gillooly *et al*. (2016) found similar scaling exponents for respiratory surface area and metabolism as the present study [i.e. a b_{GSA} of 0.85 (amphibians excluded) and a b_{SMR} of 0.84 in Gillooly *et al*., 2016 versus an average b_{GSA} of 0.86 and a b_{SMR} of 0.84 in the present study]. The resulting b_S becomes slightly positive at 0.01 when calculated for the dataset in Gillooly *et al*. (2016). This supports the general applicability of our findings and further questions the universality of the GOL hypothesis.

Comparative physiologists have questioned one of the fundamental assumptions of the GOL hypothesis by highlighting that gills are folded organs, not ellipses as proposed by Cheung *et al*. (2013), and their surface area can therefore increase proportionally with body mass (Lefevre *et al*., 2017). Lamellae are not suggested to be inefficiently stacked behind one another (as proposed by Pauly and Cheung (2018) and Pauly (2021)), but can rather grow hyper-allometrically, with a concomitant increase in buccal water volume as highlighted in Lefevre *et al*. (2018). Empirical data (Oikawa and Itazawa, 1985) support the claim that fish will not 'run out of space' for gills when growing, as the gills take up a decreasing proportion of the head, but maintain ample surface area, with increasing body mass (Lefevre *et al*., 2018). It is rather suggested that a given gill size reflects the adaptation of

that species to its metabolic requirements and not the other way around (but see alternative suggestions in Gillooly *et al.*, 2016). Given the above-mentioned mechanisms for enhancing oxygen supply and transport, the risk of oxygen undersupply causing a limitation in growth seems unlikely.

Maintenance vs. active metabolism

A notable observation in the GOL hypothesis is that the predictions are based upon maintenance metabolism (i.e. SMR), whereby growth is proposed to cease once gill-oxygen supply intersects maintenance oxygen demand (Pauly, 1981, 2019). This makes little biological sense for two reasons. Firstly, if fish could reach a size where the gills could only serve the oxygen requirements for maintenance without surplus, then they would not have the aerobic capacity to undertake critical activities such as foraging, digestion, predator avoidance and reproduction. Secondly, structural measurements such as GSA can only provide reasonable insight into maximal capacities because it is rarely possible to determine how much of the structure might be used in any particular circumstance (e.g. fish do not perfuse all available gill lamellae while at rest; Perry and Gilmour, 2010). As such, it may be more appropriate to examine GSA alongside active (maximal) MR, rather than maintenance MR, when investigating the presence of GOL, as this will indicate whether fish may become compromised in their capacity to undertake simultaneous oxygen-demanding activities as they grow.

In this context, we found sufficient data for three species to compare the scaling relationships of GSA and AMR. While the S_{AMR} for Nile tilapia decreased with increasing body mass due to a higher scaling exponent for AMR compared to GSA, two of the three species (Atlantic salmon and goldfish) showed a positive scaling exponent for S_{AMR} (although the 95% confidence intervals for the goldfish encompassed zero; see Table 1). The latter indicates that the oxygen supply capacity of the gills increases proportionally more than the maximal metabolic requirements of these species as they grow, which provides strong evidence against the hypothesis that fish gills cannot even keep pace with maintenance metabolic requirements as fish increase in body mass.

Conclusions

The lack of support found in this study for the GOL hypothesis suggests that the GSA of a species is adapted to the specific oxygen requirements of its life history, and not vice versa. There does not appear to be any evidence of a universal limitation in the gills to supply oxygen for growth in fishes (in contrast to the suggestions of Pauly, 2021), and hence there is a need to investigate other mechanisms that may underlie the temperature-size rule.

Another revelation from our analysis is that data on the body mass scaling of GSA and MR are rather scarce, particularly when examining for body mass ranges that cover an order of magnitude. This field of study would benefit from more research on the scaling of GSA and MR (standard and active), especially of large-growing species and individuals at their maximum attainable body sizes. Furthermore, as seen in the goldfish, the scaling exponent of S can be temperature sensitive, so we encourage scaling studies to encompass full thermal windows of the focal species.

It seems clear that many aquatic ectotherms will become smaller as their environment continues to warm (Daufresne *et al.*, 2009, Sandström *et al.*, 1995). The mechanisms underpinning this phenomenon cannot be explained by the GOL hypothesis, and therefore greater research attention is needed to better understand the physiological basis of the temperature-size rule and accurately parametrize models to predict fish size in the face of ongoing climate change.

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Supplementary material

Supplementary material accompany the online version of this article. Additionally, raw data are available at: https://figshare.com/s/dd8bae70d044849a61e3.

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