Testing mechanistic theories must be based on correct interpretations

Pauly, D.; Müller, J.M.

© 2024. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2024) 227, jeb249637. doi:10.1242/jeb.249637

CORRECTION

Correction: Testing mechanistic theories must be based on correct interpretations

Daniel Pauly and Johannes Müller

There was an error in *J. Exp. Biol.* (2024) **227**, jeb247841 (doi:10.1242/jeb.247841).

In the first sentence of paragraph 10, the following reference was omitted:

Lefevre, S., McKenzie, D. J. and Nilsson, G. E. (2017). Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. *Global Change Biology* **23**, 3449-3459. doi:10.1111/gcb.13652

Instead of citing Lefevre et al. (2017), the authors referred only to Lefevre (2016), which contains the data on which the argument in Lefevre et al. (2017) was based (see the caption to fig. 2 in Lefevre et al., 2017). The text should have read:

'The recent critiques of the GOLT (e.g. Scheuffele et al., 2021; Lefevre et al., 2017, based on data from Lefevre, 2016) on whose assumptions Lonthair et al. (2024) base their argument, do not address these issues.'

Both the online full-text and PDF versions of the paper have been corrected. The authors apologise to the readers for this error.

CORRESPONDENCE

Testing mechanistic theories must be based on correct interpretations

Daniel Pauly[1,*] and Johannes Müller[2]

[1]Sea Around Us, Institute for the Oceans and Fisheries, University of British Columbia, 2022 Main Mall, Vancouver, B.C. V6T 1Z4, Canada
[2]Centre for the Arts in Society, Leiden University, Arsenaalstraat, 1, 2311 Leiden, The Netherlands

*Author for correspondence (d.pauly@oceans.ubc.ca)

D.P., 0000-0003-3756-4793

Journal of Experimental Biology has recently published a number of studies that critically discussed the Gill-Oxygen Limitation Theory (GOLT, see Pauly, 2021), and Lonthair et al. (2024) is the latest contribution to the current debate. We welcome the increasing interest in this theory, especially by physiologists who examine the link between increasing temperatures and the growth of fishes. However, testing a theory must be based on its actual premises and hypotheses, and we have to conclude that the model of Lonthair et al. (2024) does not correspond to any of the GOLT's assumptions and instead, its results are entirely predicted by this theory, as we show here.

As Lonthair et al. (2024) find, higher temperatures reduce the growth of Arctic char (*Salvelinus fontinalis*) while the increase of its gill surface area and metabolic rate scale with similar slopes. The authors interpret this observation as contradictory to the GOLT. However, the similarity in slopes between metabolic rate and gill surface area is actually an inherent component of the GOLT, as explicitly stated (Pauly, 2019, 2021).

Central to the arguments presented by Lonthair et al. (2024) is a parameter introduced by Scheuffele et al. (2021), which these authors believe to be derived from the GOLT: the parameter b_S, i.e. the difference between the scaling exponents of metabolic rate (b_{MR}) and that of gill surface area (b_{GSA}). Thus:

$$b_S = b_{GSA} - b_{MR}. \quad (1)$$

As both Scheuffele et al. (2021) and Lonthair et al. (2024) assert, in order for the GOLT to apply, b_S should be ≤ 0. Therefore, the authors conclude, values of $b_S > 0$ would refute this theory, since the allometric slopes of the GSA would make it increase faster than MR with increasing body size. However, the GOLT always assumed b_{GSA} and b_{MR} to have about the same values (see Pauly, 2019, pp. 36–37), where b_{GSA} and b_{MR} are referred to a 'd_G' and 'd_Q,' respectively, and are used as substitute for one another. Thus, Lonthair et al.'s interpretation overlooks the fundamental assumptions of the GOLT. The theory has always presumed that the scaling exponents of gill surface area and metabolic rate are approximately equal (Pauly, 2019). Random deviations from this balance, whether positive or negative, are not inherently contradictory to the theory. Instead, consistent deviations from this equilibrium would indeed challenge the validity of the GOLT.

Furthermore, the interpretation put forth by Scheuffele et al. (2021) and Lonthair et al. (2024) suggests that the GOLT implies that fish continue to grow until their vital functions are on the verge of collapse due to oxygen limitation, which is biologically implausible. In reality, the theory posits that as fish grow, they gradually allocate less surplus oxygen to growth until, at their maximum size in a given environment, all available oxygen is utilized for non-growth functions. This distinction is crucial; the theory does not suggest that older fish collapse owing to an inability to maintain normal activities, but rather that they reach a point where no surplus oxygen is available to support further growth.

At the core of the GOLT lies the modified Pütter's equation (1920), which describes the growth rate (dW/dt) as a function of anabolic processes (HW^d) and breakdown processes (kW):

$$dW/dt = HW^d - kW. \quad (2)$$

While Pütter (1920) assumed that anabolic processes scale universally with exponents of 2/3, the GOLT suggests a range from 0.6 to 0.9 (and always <1) in adults. This implies that as fish grow larger, less energy (i.e. oxygen) is available to build new body mass, which results in decreasing growth rates.

Lonthair et al. (2024) ignore the literature that documented a problem with the interpretation of measured oxygen uptake data in respirometry experiments and the resulting definition of standard metabolic rate which is understood as reflecting energy consumption without any overhead costs of growth. This view does not reflect current knowledge on this topic and it neglects the fact that juvenile fish continue to allocate energy to growth even after fasting periods of 24–48 h. This leads to an overestimation of their SMR compared with large (old) fish, which are the only ones that do not allocate resources to growth (see e.g. Parry, 1983; Rosenfeld et al., 2015; Chabot et al., 2016).

Like Scheuffele et al. (2021) and Lefevre (2016), Lonthair et al. (2024) assume that fasting fish for 24 h is enough to exclude overhead costs of growth. Rosenfeld et al. (2015), however, who pointed out that fish physiologists use different procedures than colleagues working on endotherms, demonstrated that juvenile fish do not abruptly halt growth after fasting. Instead, the measured values of SMR are heavily influenced by food rations in the days and even weeks before fasting. Animals that were heavily fed before a 35 h fasting period showed overhead costs of growth of up to 65%, suggesting that fasting alone is insufficient to exclude the effects of growth on metabolic rate.

The recent critiques of the GOLT (e.g. Scheuffele et al., 2021; Lefevre et al., 2017, based on data from Lefevre, 2016) on whose assumptions Lonthair et al. (2024) base their argument, do not address these issues. Many studies cited in this literature either do not specify fasting periods or employ short fasting durations that do not effectively exclude the effects of growth on metabolic rate. Without proper consideration of these factors, the conclusions drawn by Lonthair et al. (2024) are not supported by evidence.

In conclusion, while the critique offered by Lonthair et al. (2024) may be valuable as it adds more data that can be related to this

Received 3 June 2024; Accepted 11 June 2024

debate, it is essential to ensure that such critiques are grounded in a thorough understanding of the theories that are examined. Failure to consider the fundamental premises and assumptions of the GOLT, as well as overlooking significant methodological issues in oxygen uptake measurements, will unavoidably lead to misinterpretations and flawed conclusions. What is now required are rigorous approaches to evaluate the validity of the GOLT and its implications for understanding the physiological responses of fish to changing environmental conditions.

References

Chabot, D., Steffensen, J. F. and Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *J. Fish Biol.* **88**, 81-121. doi:10.1111/jfb.12845

Lefevre, S. (2016). Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction. *Conserv. Physiol.* **4**, cow009. doi:10.1093/conphys/cow009

Lefevre, S., McKenzie, D. J. and Nilsson, G. E. (2017). Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. *Glob. Chang. Biol.* **23**, 3449-3459. doi:10.1111/gcb.13652

Lonthair, J. K., Wegner, N. C., Cheng, B. S., Fangue, N. A., O'Donnell, M. J., Regish, A. M., Swenson, J. D., Argueta, E., McCormick, S. D., Letcher, B. H. et al. (2024). Smaller body size under warming is not due to gill-oxygen limitation in a cold-water salmonid. *J. Exp. Biol.* **227**, jeb.246477. doi:10.1242/jeb.246477

Parry, G. D. (1983). The influence of the cost of growth on ectotherm metabolism. *J. Theor. Biol.* **101**, 453-477. doi:10.1016/0022-5193(83)90150-9

Pauly, D. (2019). *Gasping Fish and Panting Squids: Oxygen, Temperature and the Growth of Water-Breathing Animals*, 2nd edn. Oldendorf/Luhe, Germany: International Ecology Institute, 279.

Pauly, D. (2021). The gill-oxygen limitation theory (GOLT) and its critics. *Sci. Adv.* **7**, eabc6050. doi:10.1126/sciadv.abc6050

Pütter, A. (1920). Studien über physiologische Ähnlichkeit VI. Wachstumsähnlichkeiten. *Pflüg. Arch. Ges. Phys.* **180**, 298-340. doi:10.1007/BF01755094

Rosenfeld, J., Van Leeuwen, T., Richards, J. and Allen, D. (2015). Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life–history adaptation in salmonids. *J. Animal Ecology,* **84**, 4-20. doi:10.1111/1365-2656.12260

Scheuffele, H., Jutfelt, F. and Clark, T. D. (2021). Investigating the gill-oxygen limitation hypothesis in fishes: intraspecific scaling relationships of metabolic rate and gill surface area. *Conserv. Physiol.* **9**, coab040. doi:10.1093/conphys/coab040

doi:10.1242/jeb.247841

The gill oxygen limitation hypothesis remains unsupported by experimental evidence. Response to 'Testing mechanistic theories must be based on correct interpretations'

Joshua K. Lonthair[1,2], Nicholas C. Wegner[3], Brian S. Cheng[1], Nann A. Fangue[4], Matthew J. O'Donnell[5], Amy M. Regish[5], John D. Swenson[1], Estefany Argueta[1], Stephen D. McCormick[5,1], Benjamin H. Letcher[1] and Lisa M. Komoroske[1,*]

[1]Department of Environmental Conservation, University of Massachusetts Amherst, Amherst, MA 01003-9285, USA
[2]National Research Council under contract to Fisheries Resources Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, CA 92037-1508, USA
[3]Fisheries Resources Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla 92037-1508, CA, USA
[4]Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, Davis, CA 95616, USA
[5]US Geological Survey, Eastern Ecological Science Center at the S. O. Conte Research Laboratory, Turners Falls, MA 01376-1000, USA

*Author for correspondence (lmkomoroske@umass.edu)

J.K.L., 0000-0002-0400-3458; N.C.W., 0000-0002-8447-1488; B.S.C., 0000-0003-1679-8398; N.A.F., 0000-0001-5419-0282; M.J.O., 0000-0002-9089-2377; A.M.R., 0000-0003-4747-4265; J.D.S., 0000-0002-3740-3984; E.A., 0009-0002-0790-3256; S.D.M., 0000-0003-0621-6200; B.H.L., 0000-0003-0191-5678; L.M.K., 0000-0003-0676-7053

Lonthair et al. (2024) present data from an experiment designed to test the gill oxygen limitation (GOL) hypothesis by coupling measurements of gill surface area (GSA) and metabolic rate (MR) scaling with fish growth. We found that brook trout (*Salvelinus fontinalis*) reared under warmer temperatures grew slower than those under cold temperatures and concluded that there was no evidence for oxygen limitation at the gills as GSA grew faster (scaled higher) than MR. In contrast, the correspondence article of Pauly and Müller (2024) asserts that our results are not in conflict with GOL. However, after careful review of the prior literature on the topic as well as our data, we maintain the validity of our original data interpretations.

To understand our results, we must first understand the assumptions and predictions of GOL as proposed by Pauly and colleagues. Pauly (1981, 2021) argues that GSA scales with body mass with an allometric slope (b) less than 1.0 because GSA is two-dimensional, while MR should ideally scale with a greater b around 1.0 because it is associated with three-dimensional body volume. Support for the GOL hypothesis has been viewed as either GSA suppressing metabolic scaling (i.e. scenario 1: GSA and MR scale similarly and less than 1.0) or as GSA scaling lower than MR creating a mismatch in oxygen supply and demand with growth (e.g. scenario 2: see Fig. 1, adapted from Lonthair et al., 2024). The physiology community generally agrees that most GSA and MR scaling relationships manifest as scenario 1, but view MR scaling as the driver (Lefevre et al., 2017; Scheuffele et al., 2021) with the gills typically growing to match oxygen demand [rather than the gills constraining metabolism as proposed in Pauly (1981, 2021)]. Thus, the scaling of GSA and MR at similar rates and less than 1.0 are consistent with both classical physiology views and the GOL hypothesis as present in Scenario 1, and this correlation cannot be used to prove either, since correlation does not inform the underlying mechanism. For this reason, examining deviations from the typical pattern can be informative, and as Pauly (2021) asserts, showing that GSA can scale at a rate that does not constrain metabolism (i.e. at or greater than b=1.0) would 'eviscerate' the GOL hypothesis. Interestingly, there are several robust datasets in the literature of GSA scaling close to or even above b=1.0, which

Received 16 July 2024; Accepted 18 July 2024

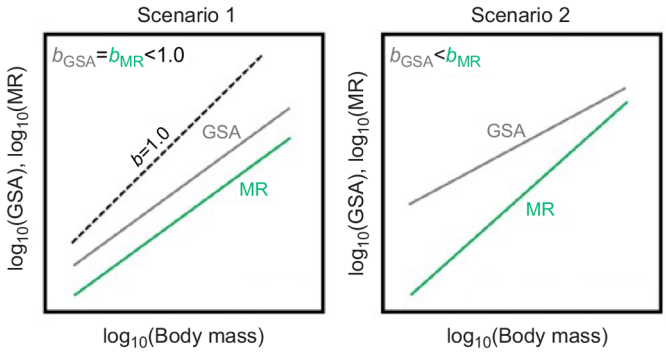

Fig. 1. Conceptual diagram depicting two scenarios where hypothesized allometric slopes (*b*) for gill surface area (GSA) and metabolic rate (MR) could align with expectations of gill oxygen limitation. In scenario 1, as conceptualized by Pauly (2021), geometric constraints on GSA (i.e. surface area to volume relationships) limit the allometric slope of GSA to less than b<1.0, resulting in a similarly constrained allometric slope for MR at b<1.0. Importantly, data that conform to the pattern in scenario 1 could also be driven by the reverse relationship (i.e. MR scales at b<1.0 and GSA scales similarly to meet oxygen demand, see extended discussion in the main text). Under scenario 2, geometric constraints result in GSA scaling less than that of MR, ultimately resulting in a mismatch between oxygen supply and demand. Results from Lonthair et al. (2024) do not support the gill oxygen limitation hypothesis under either scenario. Image reproduced from Lonthair et al. (2024).

would seem to negate the GOL hypothesis through Pauly's (2021) own admission (e.g. Holeton, 1976; Prinzing et al., 2023; see Wegner and Farrell, 2024 for review). The strength of Lonthair et al. (2024) is that we provide more than an isolated dataset on gill scaling; we show that brook trout GSA scaling is not significantly different from 1.0, while also showing that brook trout growth slows at warmer temperatures, suggesting that GSA scaling is not the primary factor limiting metabolism and growth.

Because the respiratory physiology community generally agrees that GSA can scale at or close to 1.0 if needed to support oxygen demands, others have also searched for a proposed 'mismatch' in GSA and metabolic scaling that could indicate a constraint on oxygen supply in relation to oxygen demand as fish grow. This is the basis of scenario 2 in Lonthair et al. (2024) as originally tested in Scheuffele et al. (2021) and subsequently in other studies (e.g. Somo et al., 2023; Prinzing et al., 2023; Skeeles and Clark, 2024). To examine potential evidence for a scaling mismatch between GSA and MR in the literature, Scheuffele et al. (2021) developed the S metric or difference in the allometric scaling of GSA and MR (b_S),

$$b_S = b_{GSA} - b_{MR},$$

in which b_S>0 would indicate that more GSA is available per unit of metabolic demand as a fish grows, while b_S<0 would support the GOL hypothesis by indicating that less GSA is available per unit of metabolism as a fish grows [Fig. 1, scenario 2 adapted from Lonthair et al. (2024)]. Here again, we see that our results argue against the concept of gill oxygen limitation as b_S is positive in all instances for brook trout (whether measuring resting metabolic rate or maximum metabolic rate). Thus, in our experiment, the amount of GSA per unit of metabolism actually increased as the fish grew, even though the fish at warm temperatures grew more slowly. Despite this clear evidence that GSA is not constraining metabolism and growth, Pauly and Müller (2024) argue that the slopes in our data between GSA and MR are 'similar' and the differences are 'random deviations', and align with GOL expectations. This is simply not accurate: the confidence intervals for GSA and resting metabolic rate (RMR) do not overlap for either temperature.

Finally, in response to Pauly and Müller's (2024) concern on the potential impact of growth on metabolic rate, we must first clarify that Lonthair et al. (2024) measured both RMR and maximum metabolic rate (MMR), not standard metabolic rate (SMR) as stated by Pauly and Müller (2024). We thus report the full range of typical oxygen uptake (aerobic scope) used by brook trout over the size range examined. MMR and aerobic scope were reported in this study to emphasize the 'excess' capacity available above baseline (resting) costs, which would include oxygen uptake available for growth. Whether examining RMR or MMR our results do not support gill oxygen limitation as defined above, nor do the vast majority of other paired datasets directly comparing GSA and MR scaling (e.g. Scheuffele et al., 2021; Prinzing et al., 2023; Skeeles and Clark, 2024).

In closing, we contend that understanding the mechanisms driving relationships between fish body size and temperature is critical from both fundamental and applied perspectives. This requires examination of hypotheses proposed in the literature and assessing the weight of evidence in support of each one. To accomplish this, it is imperative that the assumptions and predictions of candidate hypotheses are clear, and that they can be rigorously tested. Hypotheses that rely solely on correlations that can be explained by multiple, contrasting mechanisms not only cast doubt on the likelihood of a unifying theory, but more importantly are essentially untestable, and by extension unfalsifiable. Claiming support for a hypothesis under these circumstances is a false equivalency. Pauly and Müller (2024) contend that the results of Lonthair et al. (2024) do not provide sufficient evidence against GOL due to their design and data interpretations. While we respectfully disagree, if there are alternate or more rigorous experimental designs that would clearly test the validity of the GOL hypothesis, we strongly encourage their elaboration and testing in order to advance our understanding of body size–temperature relationships.

Acknowledgements

We are thankful for constructive feedback from Timothy Clark, Jill Leonard, and Ken Jeffries on a prior draft of this article. Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Holeton, G. F. (1976). Respiratory morphometrics of white and red blooded Antarctic fish. *Comp. Biochem. Physiol.* **54A**, 215-219. doi:10.1016/S0300-9629(76)80100-4

Lefevre, S., McKenzie, D. J. and Nilsson, G. E. (2017). Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. *Glob. Chang. Biol.* **23**, 3449-3459. doi:10.1111/gcb.13652

Lonthair, J. K., Wegner, N. C., Cheng, B. S., Fangue, N. A., O'Donnell, M. J., Regish, A. M., Swenson, J. D., Argueta, E., McCormick, S. D., Letcher, B. H. et al. (2024). Smaller body size under warming is not due to gill-oxygen limitation in a cold-water salmonid. *J. Exp. Biol.* **227**, jeb246477. doi:10.1242/jeb.246477

Pauly, D. (1981). The relationships between gill surface area and growth performance in fish: a generalization of von Bertalanffy's theory of growth. *Berichte der Deutschen Wissenchaftlichen Kommission für Meeresforschung* **28**, 251-282.

Pauly, D. (2021). The gill-oxygen limitation theory (GOLT) and its critics. *Sci. Adv.* **7**, eabc6050. doi:10.1126/sciadv.abc6050

Pauly, D. and Müller, J. (2024). Testing mechanistic theories must be based on correct interpretations. *J. Exp. Biol.* 227, jeb247831. doi:10.1242/jeb.247841

Prinzing, T. S., Bigman, J. S., Skelton, Z. R., Dulvy, N. K. and Wegner, N. C. (2023). The allometric scaling of oxygen supply and demand in the California Horn Shark, *Heterodontus francisci*. *J. Exp. Biol.* **226**, jeb246054. doi:10.1242/jeb.246054

Scheuffele, H., Jutfelt, F. and Clark, T. D. (2021). Investigating the gill-oxygen limitation hypothesis in fishes: intraspecific scaling relationships of metabolic rate and gill surface area. *Conserv. Physiol.* **9**, coab040. doi:10.1093/conphys/coab040

Skeeles, M. R. and Clark, T. D. (2024). Fish gill surface area can keep pace with metabolic oxygen requirements across body mass and temperature. *Funct. Ecol.* **38**, 755-764. doi:10.1111/1365-2435.14507

Somo, D. A., Chu, K. and Richards, J. G. (2023). Gill surface area allometry does not constrain the body mass scaling of maximum oxygen uptake rate in the

tidepool sculpin, *Oligocottus maculosus*. *J. Comp. Physiol. B* **193**, 425-438. doi:10.1007/s00360-023-01490-9

Wegner, N. C. and Farrell, A. P. (2024). Plasticity in gill morphology and function. In *Encyclopedia of Fish Physiology: From Mechanism to Application*, 2nd edn, Vol. 1 (ed. S.L. Alderman and T. E. Gillis), pp. 762-779. Elsevier: Academic Press. doi:10.1016/B978-0-323-90801-6.00028-8

doi:10.1242/jeb.248173