

## CORRESPONDENCE

# Don't throw the fish out with the respirometry water

Matthew D. Regan<sup>1,\*</sup>, Milica Mandic<sup>2</sup>, Rashpal S. Dhillon<sup>3</sup>, Gigi Y. Lau<sup>4</sup>, Anthony P. Farrell<sup>5,6</sup>, Patricia M. Schulte<sup>5</sup>, Brad A. Seibel<sup>7</sup>, Ben Speers-Roesch<sup>8</sup>, Gordon R. Ultsch<sup>9</sup> and Jeffrey G. Richards<sup>5</sup>

<sup>1</sup>Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI 53706, USA

<sup>2</sup>Department of Biology, University of Ottawa, Ottawa, ON, Canada, K1N 6N5

<sup>3</sup>Department of Biomolecular Chemistry, University of Wisconsin-Madison, Madison, WI 53706, USA

<sup>4</sup>Department of Biosciences, University of Oslo, PO Box 1066, Blindern, 0316 Oslo, Norway

<sup>5</sup>Department of Zoology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

<sup>6</sup>Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

<sup>7</sup>College of Marine Science, University of South Florida, St Petersburg, FL 33701, USA

<sup>8</sup>Department of Biological Sciences, University of New Brunswick-Saint John, PO Box 5050, Saint John, NB, Canada, E2L 4L5

<sup>9</sup>Department of Biology, University of Florida, Gainesville, FL 32611, USA

\*Author for correspondence (mdregan@wisc.edu)

Dr Wood's Commentary (Wood, 2018) provides six reasons to question the usefulness of  $P_{crit}$  and proposes alternative  $\dot{M}_{O_2}$  versus  $P_{O_2}$  analyses as its replacement. While we agree with some of Dr Wood's arguments, we feel that none of them warrant abandoning  $P_{crit}$ , especially in favour of his proposed alternatives, which provide different information than  $P_{crit}$ . A more useful way forward would involve (1) clearly defining  $P_{crit}$  to avoid misinterpretation, and (2) standardizing (or at least clearly describing) the methods used to determine and report  $P_{crit}$ , to optimize its comparative value. This topic demands further discussion because Dr Wood's conclusion could have unwarranted influence on how future hypoxia research is conducted and past hypoxia research is interpreted.

Dr Wood's arguments are either theoretical (reasons 3–6) or methodological (reasons 1, 2). The theoretical arguments, if true, may warrant the abandonment of  $P_{crit}$ . However, contrary to Dr Wood's claims, across species,  $P_{crit}$  is strongly correlated with the environmental  $O_2$  level to which species are exposed and is therefore ecologically relevant (Childress and Seibel, 1998; Mandic et al., 2009; Wishner et al., 2018).  $P_{crit}$  is also correlated with multiple steps of the  $O_2$  transport cascade, from gill surface area through haemoglobin  $P_{50}$  to mitochondrial  $P_{50}$  (Childress and Seibel, 1998; Lau et al., 2017; Mandic et al., 2009), and is a sensitive measure of an animal's overall ability to extract  $O_2$  because altering physiological traits along the cascade can change  $P_{crit}$ . For example, anatomical restructuring of the gill to favour  $O_2$  diffusion [e.g. reducing gill epithelial thickness through seawater acclimation in sculpins (Henriksson et al., 2008); increasing lamellar surface area through hypoxia acclimation in crucian carp (Sollid et al., 2003)] can lower  $P_{crit}$ . These relationships between  $P_{crit}$  and plastic traits along the  $O_2$  transport cascade clearly reflect the physiological relevance of  $P_{crit}$  and indicate that  $P_{crit}$  – especially when it shifts with acclimation – does indeed represent the  $P_{O_2}$  at which  $O_2$  uptake becomes constrained. Therefore, contrary to reason 6,  $P_{crit}$  per se does carry biologically relevant information. Hence,  $P_{crit}$  remains a useful tool for understanding hypoxic performance because it allows for predictive statements.

Much of Dr Wood's reasoning centres on the over-interpretation of  $P_{crit}$ .  $P_{crit}$  does not necessarily quantify an animal's overall hypoxia tolerance (the product of some combination of aerobic metabolism, anaerobic metabolism and metabolic depression; reason 5), reveal what biological processes the  $O_2$  consumed at  $P_{crit}$  is supporting (reason 4), or indicate the onset  $P_{O_2}$  of enhanced glycolytic reliance (reason 4) or metabolic depression (reason 5).

These ideas have long been excluded from the definition of  $P_{crit}$ . Simply,  $P_{crit}$  defines the lowest water  $P_{O_2}$  at which the animal can maintain some benchmark  $\dot{M}_{O_2}$  state (e.g.  $\dot{M}_{O_2, std}$ , the  $\dot{M}_{O_2}$  of an inactive and post-absorptive ectotherm, is a useful benchmark for evaluating  $O_2$  supply capacity at low  $P_{O_2}$ ), and this has physiological and ecological relevance.

Dr Wood's methodological arguments mention that  $P_{crit}$  calculation requires the portion of the  $\dot{M}_{O_2}$  curve at  $P_{O_2}$  above  $P_{crit}$  to have a slope of zero (Box 1A in Wood, 2018), a response that not all animals display. However, this is not required with the above definition, and indeed widely used calculation methods (e.g. Yeager and Ultsch, 1989) can effectively assign  $P_{crit}$  when the slope of this portion of the curve is positive or negative (Box 1B,D in Wood, 2018). In the case of true oxy-conforming species (Box 1C in Wood, 2018),  $P_{crit}$  may be impossible to calculate (though some species once thought to be oxy-conformers are actually oxy-regulators when measured with modern respirometric techniques). However, this does not negate the usefulness of  $P_{crit}$  for oxy-regulating species, which comprise the vast majority of animals measured to date (Steffensen, 2006).

As Dr Wood states, cross-study comparisons can become muddled in the absence of standardized methods for  $P_{crit}$  calculation and experimentation [though the two-segmented lines method (Yeager and Ultsch, 1989) has been used >250 times]. However, this criticism is hardly unique to  $P_{crit}$  and does not necessarily justify abandoning  $P_{crit}$  or any other non-standardized measurement. Methods can be standardized, or at least carefully described and presented with all relevant data (e.g.  $\dot{M}_{O_2}$  versus  $P_{O_2}$  traces; reason 6) to optimize comparative value. But even if they cannot,  $P_{crit}$  would remain a useful comparator within studies exploring differences in environmental condition, treatment, genotype, strain, population and/or species.

Two important points must be made regarding Dr Wood's proposed alternatives. First, we welcome such measurements and others like them (e.g. Claireaux and Chabot, 2016; Cobbs and Alexander, 2018), as they add valuable physiological information on animal performance in hypoxia. However, caution is needed, as some measures may be less relevant to hypoxia tolerance per se than  $P_{crit}$  (e.g. regulation index, which simply quantifies an animal's oxy-regulatory ability) and others unsuitable for a complex system like the  $O_2$  cascade (e.g. Michaelis–Menten approach, which is based on simple enzyme kinetics). Second, the methods for experimentation and calculation of the proposed alternatives have

not been rigorously standardized, and so Dr Wood's critique of  $P_{crit}$  applies to his alternatives as well. For example, to determine regulation index, how many and what types of curves are needed to best fit the data (reason 2)? And, if there is variation in  $\dot{M}_{O_2, std}$ , which point is used to anchor the diagonal line of oxy-conformation (reason 1)?

To summarize,  $P_{crit}$  should not be abandoned, but it should be carefully defined to avoid misinterpreting what it represents. The theoretical argument against  $P_{crit}$  is incorrect, and the methodological argument against  $P_{crit}$  can be rectified through standardization and/or clearly defining and reporting the conditions and methods of measurement. What will aid in this are published guidelines that the community accepts and uses for applicable species. Importantly,  $P_{crit}$  defines the lower bound of the  $P_{O_2}$  spectrum over which an animal supports its metabolic rate predominantly using aerobic metabolism, albeit with a diminishing aerobic scope for activity as  $P_{O_2}$  approaches  $P_{crit}$ . On its own, this information has important ecological relevance and serves as an effective comparator within and among species. When paired with other hypoxia-related physiological measurements such as lactate accumulation and calorimetry-based measurements of metabolic depression (e.g. Regan et al., 2017),  $P_{crit}$  contributes to a more complete picture of an animal's total hypoxic response by capturing the suite of aerobic contributions to hypoxic survival in a single value. In other words, when properly measured and interpreted,  $P_{crit}$  is useful.

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