

Exploring key issues of aerobic scope interpretation in ectotherms: absolute versus factorial

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Abstract Aerobic scope represents an animal's capacity to increase its aerobic metabolic rate above maintenance levels (i.e. the difference between standard (SMR) and maximum (MMR) metabolic rates). Aerobic scope data can be presented in absolute or factorial terms (AAS or FAS, respectively). However, the robustness of these calculations to noise or variability in measures of metabolic rate can influence subsequent interpretations of patterns in the data. We explored this issue using simple models and we compared the predictions from these models to experimental data from the literature. First, we investigated the robustness of aerobic scope calculations as a function of varying SMR when MMR is fixed, and vice versa. While FAS is unexpectedly

robust to variability in SMR, even in species with low aerobic scopes, AAS is less sensitive to variation in SMR than is FAS. However, where variation in MMR is the main concern, FAS is more robust than AAS. Our findings highlight the equal importance of minimising variability in MMR, rather than just the variability in SMR, to obtain robust aerobic scope estimates. Second, we analysed metabolic rate accounting for locomotor speed and body mass for swimming fish. The interactions among these factors in relation to AAS and FAS are complex and the appropriate metric is dependent on the specific eco-physiological context of the research question. We conclude with qualified recommendations for using and interpreting AAS and FAS.

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Introduction

In the 1940s, Fred Fry developed the concept of aerobic scope—the range of aerobic metabolic rates available to an animal above maintenance levels (Fry 1947; Fry and Hart 1948). He argued that aerobic scope constrains the physiology and behaviour of animals because it sets limits on the capacity for oxygen delivery to the body's active organs, supporting

processes from digestion to locomotion. The concept has since been applied to a great range of aquatic and terrestrial species (e.g. Auer et al. 2015; Bishop 1999; Moberly 1968; Overgaard et al. 2012; Schippers et al. 2014; Sokolova and Pörtner 2003). On the surface, aerobic scope seems to be a relatively straightforward variable. A more detailed examination, however, immerses us in some unexpected complexities.

For ectotherms in particular, aerobic scope is most commonly represented as the absolute difference between the maximum and standard (resting) rates of aerobic metabolism that an animal can achieve (e.g. Gleeson 1981). In other words, an animal's aerobic scope is the difference between its maximum metabolic rate, MMR, and its standard metabolic rate, SMR (i.e. MMR minus SMR). Hereafter, this is referred to as absolute aerobic scope, AAS. However, an animal's aerobic scope can also be represented as the ratio of its MMR to SMR (i.e. MMR divided by SMR), usually termed the factorial aerobic scope (FAS).

Clearly, calculations of aerobic scope require measurements of SMR and MMR, and both of these typically incorporate error due to factors including equipment noise (e.g. oxygen sensor drift), the behaviour of the subject animal (e.g. restlessness), or poor methodology (see e.g. Clark et al. 2013; Svendsen et al. 2016). A main criticism of FAS as an estimate of aerobic scope is that it may be heavily influenced by relatively minor absolute variations in SMR (the denominator in the derivation of FAS), perhaps to the extent that any observed variation in FAS, within or among individuals, is largely a mathematical artefact. However, there is yet to be an analysis comparing the impact of measurement noise on FAS, or indeed AAS.

Having collected estimates of aerobic scope, those data then require interpretation. The dual definition of aerobic scope (i.e. AAS and FAS) can lead to confusion and contradictory interpretations of the same dataset. For example, changes in aerobic scope with age, and hence size, in developing fish can be positive or negative depending on the aerobic scope definition employed (Clark et al. 2013; Killen et al. 2007). AAS has the advantage of providing a tangible estimate of the absolute capacity for oxygen transport that an animal can achieve above baseline (i.e. above SMR) at any given time, for example to perform physical activity. However, while interpretation of aerobic scope defined as AAS can help us understand how an

animal can function within its environment, it does not account for the fact that different individuals may require a greater proportional investment of oxygen to perform a given physiological task, perhaps most obviously due to size-dependent differences in the relative costs of activity, whereby smaller animals typically (cf. Clark et al. 2012) have a higher relative metabolic rate (Schmidt-Nielsen 1972). Analysis of FAS, on the other hand, evaluates the capacity of the animal's oxygen transport system relative to its own baseline rate of oxygen uptake. The question of under what circumstances each aerobic scope definition may be more appropriate than the other is yet to be addressed. It has, for example, been suggested that animal performance is optimised at temperatures where AAS is maximised (Eliason et al. 2011; Fry 1971; Pörtner and Farrell 2008). However, as highlighted by Clark et al. (2013; their Figure 6), AAS and FAS plotted against temperature can return vastly different, even opposing, conclusions about the relationship between aerobic scope and temperature within a dataset. In fact, for many fish species from temperate to tropical systems, FAS often decreases over the entire temperature range experienced by a species evaluated whereas AAS has been reported to display varying patterns from more or less bell-shaped to continuously increasing or remaining stable (e.g. Clark et al. 2011; Healy and Schulte 2012; Norin et al. 2014; Poletto et al. 2017; Steinhausen et al. 2008; Tirsgaard et al. 2015).

The most appropriate index of aerobic capacity may vary depending on context, and in this regard, it is important to note that metabolic rate and aerobic scope in ectotherms are influenced by a number of factors. The most important single factor generating variability in metabolic rate is body size. Both absolute SMR and MMR scale allometrically with body mass, both within- and across-species (Glazier 2005; Killen et al. 2010; Norin and Gamperl 2017). Depending on how aerobic scope is defined (either in terms of AAS or FAS), the scaling of aerobic scope may show very different patterns of allometry (Clark et al. 2013). This is important because it is all but impossible to carry out experiments that do not incur variation in body size among experimental animals. Furthermore, many studies specifically examine changes in physiology or behaviour across life-stages and so a consideration of how to best compare aerobic scope across body sizes is paramount in these cases (Killen et al. 2007; Wilson and Krause 2012). There are also a range of extrinsic

factors that have profound effects on metabolism and aerobic scope in ectotherms. In response to an increase in temperature, for example, SMR and MMR generally increase, with at least some species displaying a decrease in AAS past a species-specific optimum (Farrell 2016; Lefevre 2016). This pattern is often due to a decrease in MMR beyond this point, though not all species display this response and instead reach lethal temperatures before MMR begins to decline (Jutfelt et al. 2018; Lefevre 2016; Nati et al. 2016). Again, however, the exact interpretation of how aerobic scope is affected by temperature is strongly dependent on whether AAS or FAS is used to represent aerobic scope (Clark et al. 2013). Hypoxia can also have a strong effect on aerobic scope by limiting oxygen supply and reducing MMR (Claireaux and Lagardère 1999). Given the importance of understanding how body size and environmental conditions interact to affect responses to climate change (Lefevre et al. 2017; Lindmark et al. 2018; Pauly and Cheung 2018), a consideration of how to best quantify changes in aerobic scope due to these factors is clearly warranted.

In the current article, we empirically investigate two major issues surrounding AAS versus FAS as the derived variable of choice used to underpin studies of aerobic scope. First, we use simple models to examine how variation in estimates of either SMR or MMR can influence the robustness of measurements of AAS and FAS, and we compare the predictions from these models to experimental data from a tropical fish, the barramundi (*Lates calcarifer*). Second, we revisit experimental data in the literature to consider AAS versus FAS when interpreting how the energy costs of transport differ among fish of different sizes and swimming at different speeds. These analyses provide an example for highlighting the varying interpretations of aerobic scope that can be reached depending on which derivation of aerobic scope is used. While the experimental data associated with our analyses are from the fish literature, the models are not fish-specific and should have broad relevance to non-fish taxa.

How does variability in standard and maximum metabolic rate affect measurements of AAS and FAS?

To calculate the aerobic scope of an organism we must measure its SMR and MMR. Both of these

measurements can show variability for many reasons including among- and within-individual biological variation (Burton et al. 2011; Metcalfe et al. 2016; Norin and Malte 2011), experimental error and methodological differences (Clark et al. 2013; Killen et al. 2017; Norin and Clark 2016; Reidy et al. 1995; Roche et al. 2013; Rodgers et al. 2016; Rummer et al. 2016). To interrogate how measurement variability influences calculations of aerobic scope, we generated two simple models that specifically investigated the effects of variability in SMR when MMR is fixed and vice versa. We consider the robustness (i.e. the deviation) of calculations of aerobic scope in the face of this variation.

For the first model, MMR was held constant while SMR was changed, providing a simple simulation of variability in SMR measurements and consequent calculations of aerobic scope. For the second model, SMR was held constant while MMR was changed, providing a simple simulation of variability in MMR measurements along with the consequent aerobic scope calculations. The sizes of the values of SMR and MMR in these models are not important, nor are the sizes of the calculated percentage changes in aerobic scope since these are in part a function of the size of the incremental changes in SMR or MMR within the models. However, the range of values of MMR and SMR in the models were chosen to represent ecologically valid ranges of both FAS (2–25; e.g. Killen et al. 2016b) and AAS (2–50; e.g. Bishop 1999). Readers are directed to focus on qualitative differences in the variation in FAS and AAS as a result of variability in SMR or in MMR.

For the first model we set MMR at 50. To account for the fact that constant incremental changes in SMR across its range will have a varying effect on the change in FAS depending upon the magnitude of SMR, SMR values were calculated by varying FAS (between 25 and 2), at constant FAS increments (arbitrarily, 0.1). Next, we calculated AAS for all values of SMR and presented these, alongside the generated values of FAS (Fig. 1a). When MMR is fixed, clearly both AAS and FAS decrease as SMR increases; AAS linearly and FAS curvilinearly (Fig. 1a). We then compared these modelled data with experimental data from barramundi (Norin et al. 2016). We chose the barramundi dataset because both SMR and MMR of the same 60 fish were measured under different environmental conditions, which

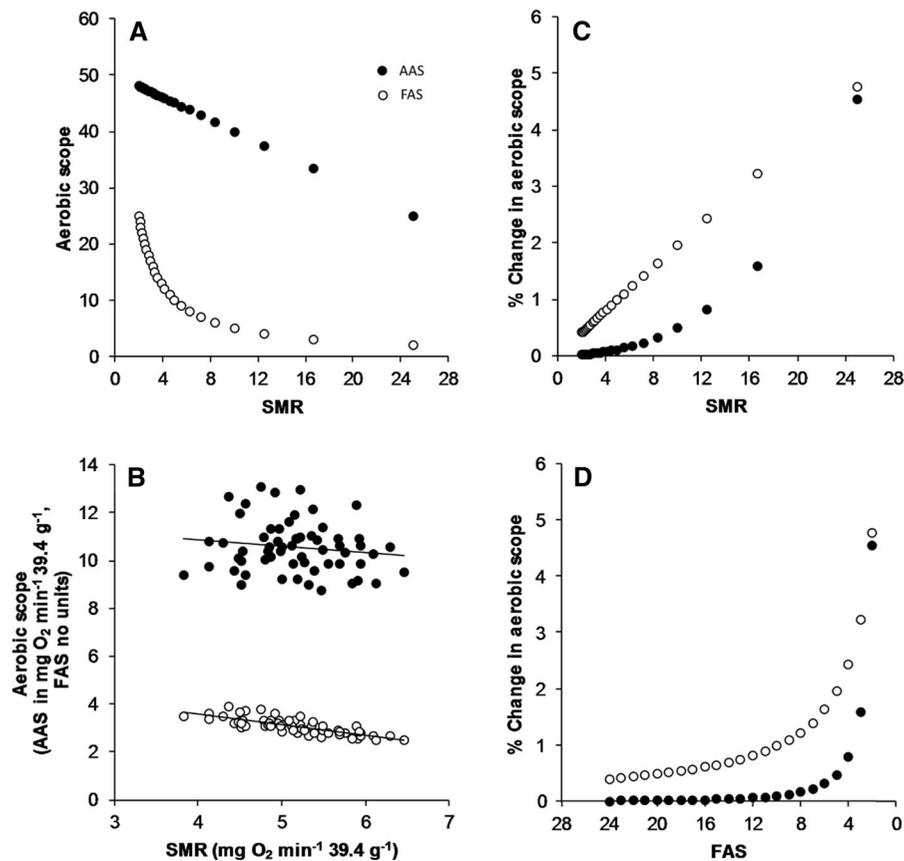


Fig. 1 The effects on the estimate of absolute aerobic scope (AAS) and factorial aerobic scope (FAS) due to variability in standard metabolic rate (SMR). (AAS: closed circles; FAS: open circles). **a** Modelled variation in calculations of aerobic scope due to variation in SMR (where MMR is fixed, at 50). **b** Experimental data from barramundi exposed to hypoxia (which constrained MMR more than SMR) for comparison with the model data in panel **a**. Note that these data, which are from Norin et al. (2016), are presented body-mass-adjusted whereas the statistical analyses detailed in the main text were performed on the raw data with body mass as a covariate. Linear regressions for the presented data are: $AAS = -0.259 SMR + 11.874$ ($r^2 = 0.0197$) and $FAS = -0.446 SMR + 5.371$

constrained either SMR or MMR to different degrees. We are not aware of any other datasets where either SMR or MMR is constrained to different degrees in a way that would allow comparisons with our modelled data. A particular strength of the barramundi data set is that the same fish were measured under the different experimental conditions, meaning that we can exclude variation between fish stocks, labs, experimenters and protocols as a cause for the observed patterns in the data. When exposed to hypoxia (45% air saturation at

$r^2 = 0.609$). **c** Percentage change in modelled aerobic scope per single incremental increase in SMR, providing a measure of robustness of aerobic scope to variation in SMR. The incremental increases in SMR can be seen to increase in magnitude as SMR becomes larger such that the resultant decrease in FAS per increment is always constant. **d** Percentage change in modelled aerobic scope per unit increment increase in FAS due to a decrease in SMR (as per panel **c**). Note that the x axis values are presented in reverse order for ease of comparison with panels **a**, **c**, since increases in SMR result in decreases in FAS. All modelled data points presented in **a**, **c** and **d** are a subsample to limit the number of plotted data points, for visual clarity

29 °C), MMR of the barramundi was constrained more than SMR (1.38- vs. 1.69-fold variation among individuals in MMR and SMR, respectively), which produced a biological example similar to the modelled data where MMR was fixed. For the barramundi data, we compared the change in AAS and FAS with SMR (or MMR for the second model below) using a linear regression with \log_{10} —aerobic scope as the response variable and \log_{10} —body mass, \log_{10} —SMR (or \log_{10} —MMR), and aerobic scope category (i.e. FAS

or AAS) as the predictor variables. Although the observed decrease in AAS with SMR did not reach significance ($P = 0.236$), the decrease in FAS did ($P < 0.001$; Fig. 1b). As for the modelled data, this decrease in FAS was faster than that for AAS (interaction between SMR and aerobic scope category; $P < 0.001$). The lack of a significant decrease for AAS in the barramundi data could be due to the much narrower range of SMR values among individual fish, as compared to the modelled data. Nonetheless, with respect to the differences in AAS and FAS the barramundi data qualitatively agreed with the modelled data within the same range of SMR values; as SMR increases, the calculation of FAS decreases faster than does the calculation of AAS, although the rate of change for the modelled data becomes similar at high SMR values. To quantify the ‘robustness’ of calculations of AAS and FAS to variability in SMR, we then calculated the absolute percentage change in modelled AAS and FAS for each increment in SMR (Fig. 1c). Finally, we calculated those percentage changes in terms of each 0.1 increment in FAS (Fig. 1d) to illustrate the relationship between the SMR values and (consistent) increments in FAS. Variations in SMR will have a greater influence on the calculated value of aerobic scope when aerobic scope is small, and Fig. 1d enables interpretation of the difference in robustness to SMR variation in AAS and FAS in instances of low aerobic scope, or indeed higher aerobic scope.

When MMR is constant, a change in SMR (Fig. 1c; creating a single unit change in FAS, Fig. 1d) has a varying effect on the percentage change in calculations of both AAS and FAS. When SMR is high, values of FAS are therefore lower (Fig. 1a); at higher SMR values an incremental change in SMR and hence FAS results in a larger percentage change in aerobic scope than occurs at lower values of SMR (where FAS is therefore high) (Fig. 1c, d). For AAS, the percentage change in aerobic scope with a change in SMR tends towards a constant at low values of SMR (high values of FAS; Fig. 1c). Across the range of FAS values modelled, the percentage change in FAS is always greater than the percentage change in AAS (Fig. 1c, d). However, the difference is relatively small at the lowest values of FAS, where SMR is high, and diminishes at the highest values of FAS where SMR is low. The main message arising from this simple model is that calculations of AAS change less than do

calculations of FAS as a result of variability in SMR, indicating that AAS is more robust to variability in SMR than is FAS.

For the second model we set SMR at 2 and induced MMR to vary between 4 and 50. This time we plotted AAS and FAS against MMR (Fig. 2a), and plotted percentage changes in AAS and FAS for each unit increase in MMR (Fig. 2c) and in FAS (Fig. 2d).

When SMR is constant, variation in MMR of course results in changes in both AAS and FAS. The absolute change in AAS for a given change in MMR is greater than that for FAS for all but the very lowest range of modelled MMR values (Fig. 2a). This pattern is also seen in the experimental data for barramundi (Fig. 2b) when the fish were measured under their acclimation conditions (normoxia and 29 °C), where SMR varied less than MMR (1.71- vs. 2.27-fold among individuals, respectively). For these data, both AAS and FAS increase significantly with MMR ($P = 0.003$ and $P < 0.001$, respectively), and the slope for AAS is much greater than that for FAS across a comparable range of MMR values (interaction between MMR and aerobic scope category; $P < 0.001$). Despite differences in absolute changes in AAS and FAS due to variation in MMR, the percentage changes in AAS and FAS are often very similar (Fig. 2c). However, at low values of MMR (and hence low values of FAS), the percentage change in AAS is greater; at this point, AAS shows less robustness to changes in MMR than does FAS (Fig. 2c, d).

Despite the concern that calculations of FAS could be strongly influenced by variation in the ratio’s denominator, SMR, our simple models indicate that when FAS is low, overall, calculations of AAS are as variable as calculations of FAS when SMR varies. Further, while there is a focus on SMR as a source of variation in FAS, our simple models support experimental evidence in showing that variation in MMR (which could be due either to biological variation or experimental noise) is also an important consideration, particularly when aerobic scope is low and defined as AAS. The fact that substantial variation in the calculation of MMR can indeed result from *bona fide* experimental factors is evident from studies that have specifically tested how different techniques and protocols to exhaust fish can produce significantly different estimates of MMR (Norin and Clark 2016; Reidy et al. 1995; Roche et al. 2013; Rummer et al. 2016; Soofiani and Priede 1985; Killen et al. 2017).

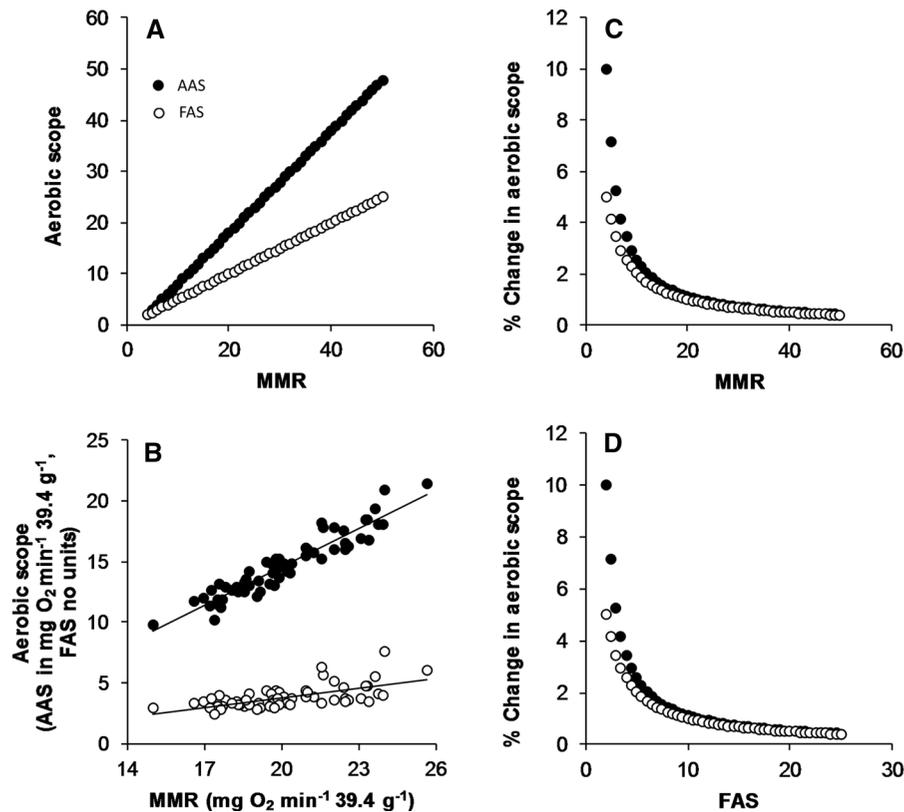


Fig. 2 The effects on the estimate of absolute aerobic scope (AAS) and factorial aerobic scope (FAS) due to variability in maximal metabolic rate (MMR). (AAS: closed circles; FAS: open circles). **a** Modelled variation in calculations of aerobic scope due to variation in MMR (where SMR is fixed, at 2). **b** Experimental data from barramundi under their acclimation conditions (where SMR varied less than MMR) for comparison with the model data in panel **a**. Note that these data, which are from Norin et al. (2016), are presented body-mass-adjusted whereas the statistical analyses detailed in the main text were performed on the raw data with body mass as a covariate. Linear regressions for the presented data are: $AAS = 1.062 MMR -$

6.689 ($r^2 = 0.893$) and $FAS = 0.266 MMR + 1.523$ ($r^2 = 0.414$). **c** Percentage change in aerobic scope per single incremental increase in MMR, providing a measure of robustness of aerobic scope to variation in MMR. The incremental increases in MMR can be seen to decrease in magnitude as MMR becomes larger such that the resultant decrease in FAS per increment is always constant. **d** Percentage change in aerobic scope per unit increment increase in FAS due to an increase in MMR (as per panel **c**). All modelled data points presented in **a**, **c** and **d** are a subsample to limit the number of plotted data points for visual clarity

Among fish species, MMR has also been noted as having a much stronger effect than SMR on calculations of both AAS and FAS (Killen et al. 2016b).

Comparing aerobic scope across body masses: an example investigating locomotion costs

Subsequent to taking metabolic rate measurements in order to calculate aerobic scope, those aerobic scope values must be interpreted. An example of when the choice of FAS or AAS may affect interpretation of results is when aerobic scope is compared across a

range of body sizes and life stages (Bishop 1999; Gillooly and Allen 2007; Glazier 2009; Weibel and Hoppeler 2005). Relatively few studies have quantified changes in both SMR and MMR over orders of magnitude of body sizes for a single species (Brett 1965; Clark et al. 2012; Killen et al. 2007). In each of these three studies, the authors presented changes in aerobic scope with mass in terms of FAS, or presented AAS on a log scale. A stated reason for reporting FAS in this context is that the proportion of aerobic scope that is occupied during active behaviours, such as locomotion, will vary for animals of different sizes. For example, the cost of transport per unit distance is

known to be relatively higher in smaller animals, meaning they must spend more energy than larger animals per unit of body mass to move a given absolute distance (Schmidt-Nielsen 1972). Therefore, a smaller animal could have a large total AAS but in theory use a substantial proportion of that to move at the same absolute speed as a larger animal. However, the justification for the use of FAS when comparing aerobic scope between different body masses has not been thoroughly explored.

We investigated this issue using an extensive dataset for sockeye salmon from Brett (1965), which represents masses over three orders of magnitude and to our knowledge is the only dataset that considers changes in metabolic rate in response to both swimming speed and large changes in body mass. A re-examination of these data first shows that both AAS and FAS increase with body mass when considered on a whole-animal basis (Fig. 3a, b). For sockeye salmon, FAS differs by almost fourfold between an animal that is 3 g and one that is 1500 g. AAS increases in almost direct proportion to body mass, indicating that the mass-scaling exponent for AAS is close to 1.0 in this dataset.

Second, we examined how the proportion of aerobic scope occupied during locomotion varies with body size. Both small and large fish are able to swim at speeds that are slow relative to their own body length ($1\text{--}2\text{ BL s}^{-1}$) without using a large proportion of their aerobic scope (Fig. 3c, e). For large fish, however, as they swim progressively faster the proportion of AAS remaining decreases dramatically (Fig. 3e). For example, a 500 g sockeye salmon would not be able to swim faster than 3 BL s^{-1} entirely aerobically. Indeed, at faster swim speeds relative to body length, smaller individuals use a much lower proportion of their AAS than do larger fish. At any given speed relative to body length, however, larger fish cover a greater absolute distance and it is therefore not surprising that they spend a greater amount of energy. When considering absolute swim speeds (e.g. cm s^{-1}), the effects of mass on locomotor costs are reversed—larger fish are able to swim much faster in absolute terms while still having a large proportion of AAS available for other oxygen-demanding tasks (Fig. 3d, f). The relationships between the proportion of AAS remaining against body mass for different swim speeds, both absolute and relative to body length, are the same

whether considering mass-specific values or whole animals values (Figure S1).

These calculations suggest that there is no ‘correct’ way to assign relevance of locomotor costs within the context of aerobic scope when comparing the constraints imposed by aerobic scope across body masses. Instead, the choice of whether to consider AAS or FAS as a more important parameter will depend on biological context. For example, studies concerned with dispersal or migration may focus on absolute distances and speeds. In this case FAS may be the most useful index of aerobic scope, particularly when there is variation in body size, because animals of differing body sizes will face vastly different locomotor costs per unit of absolute distance. Smaller individuals need to move faster to cover a specific absolute distance within a finite time. An increase in absolute speed has a disproportionate effect on smaller animals, quickly reducing their remaining AAS. In contrast, larger individuals can move the same absolute distances quickly and can do so while occupying less of their aerobic scope. This potentially makes AAS a poor basis for comparison in datasets where absolute movement speeds and distances are of interest and there is large variation in body size among animals. In contrast, routine locomotor costs associated with daily activities (e.g. within a home range) are probably more related to relative speeds and distances because individuals will display levels of locomotion that are relative to their body size. In this case, AAS may be more valid for comparisons, even across large mass ranges, because relative movement speeds will generally be towards the lower end of the spectrum where the effect of body size on locomotor costs is minimal.

It should be noted that these patterns have been derived from a single dataset on sockeye salmon (Brett 1965). To date, this remains the only fish dataset which provides measures of SMR, MMR, and activity costs at several swimming speeds across a large range of body masses. While this dataset is useful for illustrating general interactions among the factors of interest, we encourage further experimental studies to understand whether these relationships among body mass, locomotion speed and aerobic metabolic parameters are consistent across taxa.

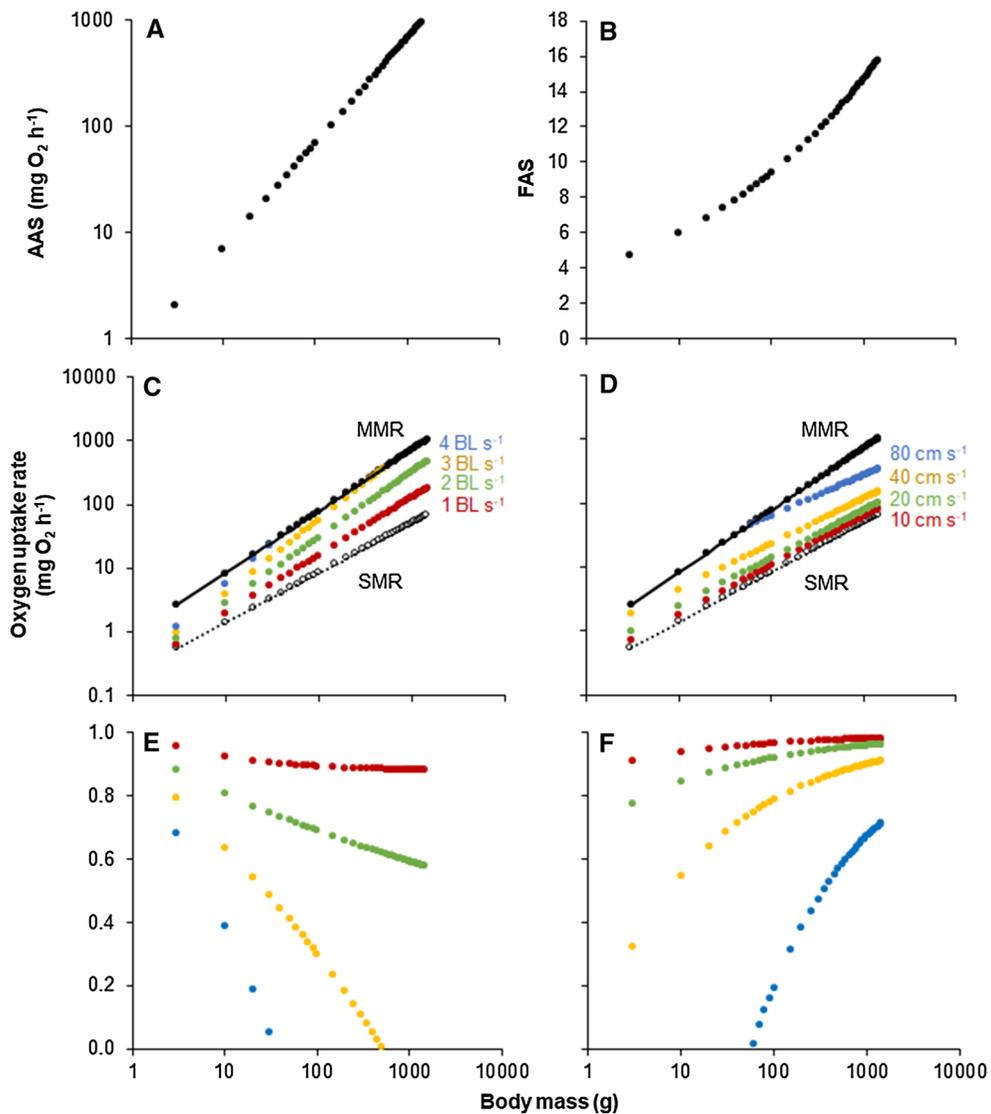


Fig. 3 Effects of swimming energy costs on the available aerobic scope for sockeye salmon *Oncorhynchus nerka* of various sizes. Curves are produced from data and equations provided by Brett (1965). Panels **a**, **b** show changes in AAS (**a**) and FAS (**b**) with body mass. Panels **c**, **d** show the interactive effects of body mass and relative swim speed on rate of oxygen uptake (indicative of metabolic rate). The black points represent MMR and the white points represent SMR. The coloured points represent oxygen uptake with increasing swim speeds (speeds denoted in each panel). In panel **c**, speeds are relative to the size of the fish; in panel **d** speeds are absolute values. Panels **e**, **f** show the proportion of total AAS that remains after accounting for the

energy costs of swimming, for sockeye salmon of a given size while swimming at a given speed. Each colour corresponds to the speed in the panel directly above. All modelled data points are a subsample to limit the number of plotted data points for visual clarity. Relationships for mass-specific rates of oxygen uptake (i.e. $\text{mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$) are presented in Fig. S1 in the online supplementary material. Notably, trends with regards to how speed and body mass constrain available AAS (panels **e**, **f**) are identical regardless of whether absolute or mass-specific data are used, despite mass-specific SMR, MMR, and activity costs decreasing (instead of increasing) with body mass

Conclusions

Variability in metabolic rate values affecting estimates of aerobic scope

The simple models presented in the current article should be interpreted in terms of the general trends rather than the quantified outputs; these trends are robust to variation in the input parameters. In turn, we offer qualified, not quantified, conclusions and recommendations based on the model outputs. There are two main conclusions arising from these models. First, FAS is unexpectedly robust to variation in measurements of metabolic rate. Although in general estimates of AAS are more robust to variability in measurements of SMR than are estimates of FAS, this difference is fairly small, particularly when FAS is large. Furthermore, variation in MMR can cause variation in estimates of AAS that are proportionally just as great as, or even greater than, the variation in estimates of FAS. Second, and arising from the first conclusion, variation in measurements of MMR can be at least as influential on calculations of aerobic scope as is the more commonly considered variability in SMR.

Consequently, we offer two specific suggestions to those scientists recording metabolic values with a focus on estimating aerobic scope in species with low aerobic scopes and hence where measurement variability has a greater effect: (1) if variability in SMR is a predominating concern, perhaps because the subject animals display chronic restlessness, AAS will provide the more robust estimate of aerobic scope; (2) if variability in MMR is the main concern, perhaps because the animals are averse to performing activity in a laboratory setting, FAS tends to be the more stable estimate of aerobic scope.

As a caveat to these recommendations, we caution that the mathematical derivations of aerobic scope will not compensate for poorly collected data and care should be placed on using best practice respirometry techniques and obtaining robust datasets to be used for aerobic scope calculations (Chabot et al. 2016; Clark et al. 2013; Steffensen 1989; Svendsen et al. 2016). This is true in all cases but is particularly important when the goal is to quantify variation among individuals or treatments.

Aerobic scope evaluated within a biological context

As well as consideration of how best to deal with biological and measurement variation, biological context is also crucial in determining whether AAS and/or FAS should be used to represent aerobic scope, as demonstrated by our analyses of aerobic scope within the eco-physiological context of body mass and locomotion speed. In turn, we urge those that use estimates of aerobic scope to carefully consider which form of the estimate they employ, based on their specific research questions, rather than to ensure conformity with past literature. For example, there has recently been increased effort to understand how individual variation in behaviour or stress responsiveness can reduce the amount of aerobic scope available for other physiological functions by increasing oxygen uptake above that required for maintenance alone. A major advantage of AAS over FAS in this regard is that the physiological components that occupy space within an individual's aerobic scope can be quantified and subtracted from the total AAS, to quantify the proportion of AAS remaining (Killen et al. 2014, 2016a). Partitioning aerobic scope in this way is difficult and complex to interpret in terms of FAS. In contrast, for biological factors which limit MMR (e.g. exposure to hypoxia), FAS after accounting for this lowered metabolic "ceiling" may also be a useful index to calculate because it reveals how the factor of interest constrains MMR in relation to the animal's own baseline requirements. Studies that measure how energetic costs change with body size may also benefit from assessing FAS due to the exaggerated effects of scaling on the calculation of AAS. Often, the most reasonable choice may be to show both derivations of aerobic scope (e.g. Norin et al. 2014). Even if one derivation is deemed most relevant to the research question in hand and is thus the focus of the analysis, the other might also be presented in the manuscript unobtrusively, to the advantage of other researchers who may wish to interpret the aerobic scope data differently.

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