

CORRESPONDENCE

How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope – remarks on the article by Gräns et al.

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The study by Gräns et al. (Gräns et al., 2014) investigated growth performance and oxygen demand at rest and during recovery from fatiguing exercise in Atlantic halibut (*Hippoglossus hippoglossus*) under simulated scenarios of ocean warming and acidification. The authors claim that their data, when used to evaluate the aerobic scope for exercise, do not explain temperature-dependent growth. They thus question the general use of the concept of ‘oxygen and capacity limited thermal tolerance’ (OCLTT) in explaining the onset of thermal limitation of fishes under field conditions (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). There are important lessons to learn from this study about how, or how not, to investigate the concept of OCLTT and aerobic scope in thermal limitation.

From a conceptual point of view, the term aerobic scope should not be constrained to use only with exercise. In the context of OCLTT, it makes sense to use the term aerobic scope for all routine performances that draw on aerobic energy such as growth, reproduction and steady-state swimming. The question is whether the chasing protocol imposed on a strictly benthic fish such as halibut provide suitable estimates of performance, of aerobic scope as used by growth and of climate sensitivity.

Methodological issues invariably constrain what experimental data can say. In their paper, Gräns et al. routinely measured growth in active fish. In contrast, aerobic scope was determined in fish exercised to exhaustion and from differences between EPOC (excess post-exercise oxygen consumption) and resting metabolism. An over-riding difficulty with tests using exhaustive exercise is that performance itself is not properly quantified. The physiological state of the animals during steady-state growth clearly differs from that during the non-steady recovery state post-exercise. The latter is characterized by exponentially declining oxygen consumption, very low venous P_{O_2} (Farrell and Clutterham, 2003; Lee et al., 2003), release of catecholamines (Reid et al., 1998), acidosis and shifted metabolite concentrations, pathways and ion equilibria, all of which are non-steady state. Furthermore, the data analysis by Gräns et al. does not build on a clear functional background. Linear regressions are used for non-linear data. At normal water pH and the highest temperature, standard metabolic rate and EPOC indicate a clear decline in metabolic scope (their fig. 1), which is not picked up by the selected polynomial fit. Also, the *post hoc* statistics do not support a global effect of CO_2 . For EPOC, statistical significance is reached only at certain temperatures, rather than all test temperatures. Growth was depressed by CO_2 only on the cold side of the studied temperature range, matching predictions of synergistic effects at thermal extremes (Pörtner and Farrell, 2008). Thus, the overall CO_2 effect could be viewed as small and hardly discernible.

In contrast to the authors’ contention, the mechanisms influencing growth have not been investigated. The paper does not provide relevant insight on underlying aerobic scope or the limits important in

the field. However, relevant thermal limitation studied in the laboratory should mimic (be ‘calibrated’ by) field observations. The physiological status of experimental organisms should be similar to that of organisms experiencing thermal limitation in their natural environments. For this, the suitability of non-steady state EPOC measurements after chasing in halibut is not clear. In other cases, calibration by field data exists: in benthic eelpout, limited aerobic scope for growth and associated cardiac limitation parallel a reduction in growth performance and a loss in abundance in extreme summer temperatures in the field (Pörtner and Knust, 2007). In Pacific salmon, limited aerobic scope for steady state swimming and associated cardiocirculatory capacity constrain adult spawners during their upstream migration (Eliason et al., 2011). In both cases, the organisms are in routine steady state, as in the field. This indicates how to investigate OCLTT, performance and relevant aerobic scope more successfully, by preferentially capturing the routine situation on relevant time scales. Fatiguing exercise protocols are poorly qualified to elaborate the subtleties of thermal effects, because of low resolution and rapidly shifting physiological states.

Fatigue and recovery involve respiratory (CO_2 accumulation) and non-respiratory (metabolic) changes in acid–base status. These phenomena constrain the validity of the chasing protocol to identify and quantify the effects of ocean acidification (OA) caused by elevated ambient CO_2 levels. Long-term acclimation to OA increases bicarbonate concentrations and associated buffering in blood and tissues and thereby, reduces the acidosis caused by fatiguing exercise. This may protect the EPOC-derived ‘aerobic scope’ from being depressed by more severe acidosis. The data reported by Gräns et al. do not test such a hypothesis. They cannot easily be compared with or be used as evidence against projected effects of OA on thermal limits of performance and fitness under routine aerobic steady-state conditions (Pörtner, 2012).

Whether the thermal windows of exercise and growth are the same is an important question to keep in mind for future studies. The data reported by Gräns et al. do not allow an answer to this question. Both windows may in fact differ if the physiological backgrounds of the fish body differ, for example, as a result of release of catecholamines under exhaustive conditions. As catecholamines push the organism away from resting and activate functional reserves, this blurs the picture with respect to OCLTT at extreme temperatures. Gräns et al. investigated cardiocirculatory scope and limitations after maximum adrenergic stimulation, in an *in situ* perfused heart preparation and during a manipulative increase of input pressure. The resulting mobilisation of cardiac functional reserves and pattern of cardiac limitation would fit the condition of enforced fatiguing exercise more than the subtle thermal limitation seen in growth, at first caused by limited cardiocirculatory capacity and associated cost increments, and the resulting shift in energy

budget (Pörtner and Knust, 2007). The studies on eelpout and halibut are thus not comparable, to say the least, and the results of the halibut study again, cannot easily be interpreted with respect to their relevance in the field.

Accordingly, we need to clearly find out under which conditions a species becomes thermally limited at the ecosystem level and then the respective situation needs to be simulated in experimental work. In most cases, steady-state aerobic functions such as growth and reproduction and their underlying aerobic scope are more universal indicators of subtle thermal constraints. Steady-state aerobic exercise can also become thermally limiting if used during a constraining life phase (e.g. upstream migration of mature salmon). This should not mislead investigators to impose maximum exercise protocols on all animals for assessing aerobic scope, regardless of whether they exercise in nature and reach steady state or not.

Overall, the paper by Gräns et al. is too much about the conceptual debate rather than about how the data might fit OCLTT or what caused apparent differences. There are other comments to make and emphasize the wider context: a recent metaanalysis provided evidence that across organism domains the highest complexity levels and processes coordinating the largest number of body compartments are the first to be thermally constrained (Storch et al., 2014). Heat limits in animals are in fact lower than in most other organism domains and OCLTT provides an explanation because it suggests coordination of the largest number of body compartments across tissues through O₂ supply and demand capacities. OCLTT traces the performance curve of the organism because it primarily focuses on sublethal limits and associated functional constraints, which develop from pejus (mismatch in oxygen supply and demand systems) to critical limits (transition to anaerobic metabolism) and then molecular denaturation, with ecological effects starting beyond pejus limits. Finally, limitations reach lethal values as an end point. These limits, transitions and links need to be further investigated because they are subject to evolutionary adjustments and shifts, for example, during transition to air breathing (Giomi et al., 2014). The evolutionary process and timeline is largely ignored in the current debate but requires consideration. OCLTT is also about linking levels of biological organization, from ecosystem to systemic to molecular, and about oxygen (energy) allocation to specific performances. It thus does not

suffice to study whether oxygen affects lethal limits. A reductionist approach limiting the experimental investigation to one level such as the whole organism, regardless of its natural mode of life, physiological background and ecological context, comes with the risk of misleading results and erroneous conclusions. Last, but not least, testing a conceptual framework such as OCLTT requires considering its most recent definition and underlying theory comprehensively. Also, experimental biologists may need to adjust some of their classical concepts and approaches to better link to other subdisciplines, such as ecology and evolutionary biology.

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Response to ‘How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope – remarks on the article by Gräns et al.’

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We appreciate the on-going discussion and healthy evaluation of the hypothesis of oxygen and capacity limitation of thermal tolerance (OCLTT). However, we think it is unfortunate that Pörtner (Pörtner, 2014) sees little value in our study (Gräns et al., 2014), which currently represents the largest long-term experimental test of OCLTT.

The OCLTT hypothesis emphasises the importance of oxygen delivery to aerobic processes as the major evolutionary constraint

shaping organisms, their physiology and ecosystems. However, such a broad view does not easily produce testable predictions, and we argue that the value of a scientific idea lies in its ability to predict future observations. Therefore, we focused on a testable prediction that OCLTT is founded upon; that reduced aerobic scope is the physiological limitation that impairs other organismal performances, such as growth at high temperatures and high P_{CO₂} (Gräns et al.,

2014; Pörtner and Farrell, 2008; Pörtner and Knust, 2007). We found that aerobic scope increased continuously with acclimation temperature, and even more so in CO₂-acidified seawater, whereas growth plateaued at the three intermediate temperatures and declined at the highest acclimation temperatures [fig. 1A and fig. 3 in original article (Gräns et al., 2014)]. This clear mismatch in thermal profiles for aerobic scope and growth indicates that oxygen delivery capacity does not decrease at high temperatures and cannot have limited growth, as OCLTT would have predicted.

Pörtner (Pörtner, 2014) suggests that we should change the definition of aerobic scope to include the scope for all oxygen-requiring performances: ‘use the term aerobic scope for all routine performances that draw on aerobic energy such as growth, reproduction or steady-state swimming’. We, however, use the widely adopted definition of aerobic scope: the difference between standard metabolic rate (SMR) in resting unfed animals, and maximum metabolic rate (MMR) (Fry and Hart, 1948; Pörtner and Farrell, 2008). Growth rate, reproductive output and aerobic scope are all commonly used terms with clear definitions, and we fail to see how redefining and mixing of terminology can improve our understanding of thermal biology. If anything, such a move would risk confusing the debate further with semantic misunderstandings.

Pörtner (Pörtner, 2014) proposes that the thermal mismatch between aerobic scope and growth is due to growth occurring in unstressed fish in ‘steady state’, whereas we measured MMR (and thus aerobic scope) during non-steady state recovery from exhaustive exercise. We suspect the misunderstanding may lie in our differing definitions of aerobic scope. MMR can, in most animals, only be quantified during or immediately after exercise when the fish are using, or recovering from, partly anaerobic white muscle activity. Moreover, Pörtner suggests that oxygen limitation at high temperature can occur for growth at rest, but that oxygen transport capacity can increase greatly during exercise because of catecholamine release and shifts in blood chemistry, which seems unlikely. The stimulatory effects of catecholamines on cardiac performance and oxygen transport also typically decrease with increasing temperature, as a result of blunted β -adrenergic stimulation of the myocardium (Keen et al., 1993). Therefore, our experimental protocol would be expected to have fewer stimulatory effects on aerobic performance at the higher temperatures and yet we still observed the highest aerobic scope at these temperatures.

The positive effect of CO₂ on aerobic scope that we reported (Gräns et al., 2014) is also questioned. Pörtner claims that aerobic scope could be protected by bicarbonate accumulation in fish exposed to high P_{CO_2} , which may be possible. However, this is not a relevant argument against the positive effects of CO₂ on aerobic scope, because this would also occur in nature. Although the effect size on aerobic scope by CO₂ was arguably small, it was nonetheless confirmed by statistical tests across temperatures in the opposite direction to what OCLTT predicts and the increase in aerobic scope from CO₂ was not matched by increased growth.

We feel that Pörtner’s critique of our statistical analysis might be due to misunderstandings. As stated in the original article, the trend lines added to the figures are for visual aid, and not based on the statistical models we used. The experimental design and statistical models were developed together with a mathematical statistician, and we are confident that our statistical analyses are of the highest standard.

Pörtner (Pörtner, 2014) highlights two examples ‘of how to investigate OCLTT, performance and aerobic scope more successfully’ (Eliason et al., 2011; Pörtner and Knust, 2007). They

are, however, like any study (including ours) not without limitations. In Eliason et al. (Eliason et al., 2011), aerobic and cardiac scope was measured in instrumented sockeye salmon using swim tunnels. Although this is an impressive experimental endeavour, we do not understand why these animals should be considered to be in ‘steady state’. First, SMR was obtained from highly instrumented salmon after an overnight recovery from surgery and during intermittent blood samplings. Second, the thermal challenges were acute for fish at the upper and lower thermal extremes (4°C h⁻¹ and left for 1 h), whereas the intermediate temperatures were tested after short-term thermal acclimation (5°C day⁻¹ and left for 1 day), presumably leaving the fish in different stages of the thermal acclimation process. For MMR measurements, a U_{crit} swim protocol was used with an electric motivator grid. Thus, whereas the MMR measured in Eliason et al. (Eliason et al., 2011), and our study probably consisted of a combination of aerobic and anaerobic metabolism because white muscle is increasingly recruited with increasing swimming speed (Clark et al., 2013a; Jayne and Lauder, 1994), the metabolic measurements in our study were not affected by surgery and variable thermal test protocols.

The other suggested example, Pörtner and Knust (Pörtner and Knust, 2007), combines results from many earlier publications, none of which measured aerobic scope according to conventional definitions. The study reports, in our view, rather weak thermal associations and claims a causal link to oxygen limitation. For example, the authors conclude that oxygen transport limitation was the reason for the drop in field abundance at 19°C, yet in the same paper, growth rate remained high at 20°C (~80% of max) and markers of anaerobic metabolism only became elevated after 72 h at 24°C (in liver, but not heart muscle). In addition, arterial blood flow was reported from only one individual during thermal ramping in a NMR setup with the fish confined in a space half the length of the fish. It is not clear to us how this can represent ‘a similar physiological status as organisms experiencing thermal limitation in their natural environment’.

Curiously, both the suggested examples of how to correctly investigate OCLTT include fish with zero oxygen transport in their datasets, representing either dead fish or measurement errors [no arterial blood flow in Pörtner and Knust, fig. 1C (Pörtner and Knust, 2007); zero MMR in Eliason et al., fig. S2A (Eliason et al., 2011)].

For these reasons, we fail to see how these two studies can be considered to represent aerobic scope measurements under ‘routine steady-state as in the field’, whereas our study is not, as proposed by Pörtner. If these publications represent the best empirical evidence for OCLTT, then the hypothesis is standing on loose ground, and it may not be surprising that a growing number of studies are questioning the generality of OCLTT (Clark et al., 2013a; Clark et al., 2013b; Ern et al., 2014; Norin et al., 2014; Overgaard et al., 2012).

In our paper (Gräns et al. 2014) we aimed to test the core proposition of the OCLTT hypothesis; that tissue oxygen limitation is the mechanism behind reductions in other performances at high temperatures. We demonstrated that the thermal windows for aerobic scope and growth differ, and that reduced aerobic scope was not associated with the decline in growth at high temperatures. This forced us to question the tissue oxygen limitation mechanism that is central to the OCLTT hypothesis. We agree with Pörtner that the ecophysiological community needs to find out at which conditions a species becomes thermally limited. However, we argue that it is unlikely that we will ever identify a single physiological mechanism explaining the complex subject of thermal tolerance and climate change vulnerability in ectothermic animals. Instead, we suggest that

the physiological cause of limitation can vary and will depend on a number of biological and environmental factors including the rate of temperature change, species, lifestyle and physiological state of the organism. We therefore encourage other researchers to look for thermal limitation mechanisms beyond oxygen supply and not to feel obligated to fit their experimental findings into the framework of OCLTT.

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