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COMMENTARY

Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology

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ABSTRACT

Observations of climate impacts on ecosystems highlight the need for an understanding of organismal thermal ranges and their implications at the ecosystem level. Where changes in aquatic animal populations have been observed, the integrative concept of oxygen- and capacity-limited thermal tolerance (OCLTT) has successfully characterised the onset of thermal limits to performance and field abundance. The OCLTT concept addresses the molecular to whole-animal mechanisms that define thermal constraints on the capacity for oxygen supply to the organism in relation to oxygen demand. The resulting 'total excess aerobic power budget' supports an animal's performance (e.g. comprising motor activity, reproduction and growth) within an individual's thermal range. The aerobic power budget is often approximated through measurements of aerobic scope for activity (i.e. the maximum difference between resting and the highest exercise-induced rate of oxygen consumption), whereas most animals in the field rely on lower (i.e. routine) modes of activity. At thermal limits, OCLTT also integrates protective mechanisms that extend time-limited tolerance to temperature extremes – mechanisms such as chaperones, anaerobic metabolism and antioxidative defence. Here, we briefly summarise the OCLTT concept and update it by addressing the role of routine metabolism. We highlight potential pitfalls in applying the concept and discuss the variables measured that led to the development of OCLTT. We propose that OCLTT explains why thermal vulnerability is highest at the whole-animal level and lowest at the molecular level. We also discuss how OCLTT captures the thermal constraints on the evolution of aquatic animal life and supports an understanding of the benefits of transitioning from water to land.

KEY WORDS: Organisational complexity, Sublethal thermal limits, Aerobic power budget, Aerobic performance, Oxygen supply, Oxygen demand, Temperature adaptation, Water breather, Air breather

Introduction

Given the impacts of climate warming on ecosystems, it is critical that we increase our understanding of organismal thermal ranges, responses and tolerances. Our understanding has long been insufficient, as studies have often focused on estimates of critical thermal maxima (CT_{max}) or lethal limits (LT_{50}; see Glossary). In fishes, these upper and lower limits and the range between them correlate to varying degrees with latitude, and probably also with latitude-associated temperature regimes (re-assessed by Pörtner and Peck, 2010). However, lethal limits are often more extreme than the temperatures that an animal will experience in its environment.

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Thus, there is a variable 'safety margin' between ambient temperature extremes and lethal temperatures (Sunday et al., 2012, 2014). Negative effects of changing temperature may occur within this margin, impacting ecology and therefore requiring identification. The physiological mechanisms causing heat or chill coma and death have been investigated for more than a century, thanks to the desire to identify the primary mechanism of temperature-associated death, yet a comprehensive mechanism-based understanding of this process has not been established.

Because temperature has a pervasive influence on all levels of biological organisation (Hochachka and Somero, 2002), research should address how mechanisms across these levels combine to shape the thermal limitations of an organism in the context of the ecosystem. The oxygen- and capacity-limited thermal tolerance (OCLTT) concept (Box 1), developed over the last two decades, has been proposed to meet these challenges and to provide a framework explaining how physiological mechanisms co-define an animal's fundamental and realised thermal niches (see Glossary), with a focus on critical life stages (for early summaries of OCLTT, see Pörtner, 2001, 2002; for thermal niches, see Pörtner et al., 2010; Deutsch et al., 2015; Payne et al., 2016). The basic idea underlying the OCLTT is that once temperatures approach limiting values, constraints on the capacity of an animal to supply oxygen to tissues to meet demand cause a progressive decline in performance (e.g. Pörtner and Giomi, 2013; Giomi et al., 2014), with consequences at the ecosystem level (e.g. Del Raye and Weng, 2015; Payne et al., 2016). OCLTT considers that most routine performances are fuelled sustainably by aerobic metabolism in excess of standard metabolic rate (SMR) and largely exclude anaerobic metabolism.

The aim of this Commentary is to summarise (and update) the key elements of the OCLTT concept. We first discuss the use of the OCLTT to understand species' responses to climate change. We then highlight pitfalls that can result from (over-)simplification, from different uses of terms and from combining OCLTT with traditional concepts (e.g. CT_{max}), especially when bypassing the transition from sublethal to lethal thermal limits (see Glossary). We summarise the physiological variables that were measured when developing the OCLTT concept and that should be tested further in order to assess OCLTT, and discuss different understandings of the term 'capacity'. Finally, we consider the evolutionary modulation of the OCLTT.

Using OCLTT to understand species' responses to climate change

The limits of a species' realised niche are thought to determine its large-scale temperature-dependent biogeography, as well as the animals' responses to warming. Combined with consideration of OCLTT, such principles may allow us to explain the currently observed biogeographical shifts of marine animals (Poloczanska et al., 2013). Individuals that undergo biogeographical shifts have experienced non-lethal thermal constraints; however, organisms that stay behind may eventually be lethally affected. Both processes

List of symbols and abbreviations

Ca_{O_2}	arterial oxygen content
Cv_{O_2}	venous oxygen content
CO	cardiac output
CT_{max}	critical thermal maximum
LT_{50}	lethal temperature causing 50% mortality
$\dot{M}_{O_2}$	oxygen consumption rate
OCLTT	oxygen- and capacity-limited thermal tolerance
P_{O_2}	partial pressure of O_2
Pa_{O_2}	arterial partial pressure of O_2
Pv_{O_2}	venous partial pressure of O_2
SMR	standard metabolic rate
T_c	critical temperature
T_d	denaturation temperature
T_{opt}	optimum temperature
T_p	pejus temperature

Glossary

Active thermal tolerance
This occurs in the range of temperatures permanently tolerated. It involves aerobic performance and associated aerobic metabolism fuelling the energy demands of maintenance and additional functions (e.g. growth, reproduction) and behaviours (e.g. roaming, foraging, mating).

Aerobic power budget
The full amount of excess aerobic energy available above maintenance that is recruited from mitochondrial metabolism. It encompasses and is traditionally estimated from aerobic scope for exercise (see below). However, muscles may not be able to fully exploit that aerobic power budget, or may push energy demand beyond routine power budget, through anaerobic contributions and transient mobilisation of functional reserves. Furthermore, trade-offs in energy allocation may occur, affecting the balance between behaviours and exercise, growth of reproductive and somatic tissue and repair processes.

Aerobic scope
The difference between resting and the highest exercise-induced rate of oxygen consumption. In brief, aerobic scope for exercise is a measure of aerobic power budget with the need to consider the complexities and functional constraints discussed under 'aerobic power budget'.

Critical thermal maximum (CT_{max})
The high temperature extreme leading to the onset of spasms (unorganised locomotion), close to the lethal temperature.

Functional capacity
The ability to routinely and permanently maintain a certain rate of functioning, supporting a specific level and kind of performance as needed under routine conditions at the ecosystem level.

Functional reserve
Additional performance capacity activated by hormonal action, e.g. catecholamines (fight and flight response).

Functional scope
The ability to increase the rate of a specific function or set of functions above those at rest, supporting a specific level and kind of performance as needed under routine conditions at the ecosystem level.

Fundamental thermal niche (*sensu* Hutchinson)
The temperature range within which physiological functioning of a species allows tolerance under resting conditions (covering active and passive ranges). Temperature effects may be influenced by specific effects of other environmental factors.

Lethal temperature limit (LT_{50})
The temperature extreme (cold or warm) causing 50% mortality. It should be noted that the value of LT_{50} found is influenced by the experimental protocol, especially the duration of exposure to step-wise increased or decreased temperature.

Passive thermal tolerance
The range of temperatures sustained passively by an organism through exploitation of residual aerobic and anaerobic metabolism, antioxidative defence, metabolic depression and the heat shock response. As these resources are depleted over time and feeding is constrained, passive tolerance is time limited.

Realised thermal niche (*sensu* Hutchinson)
The range of temperatures within which physiological functioning sustains Darwinian fitness and persistence of a species under routine conditions, including species interactions. Temperature effects may be influenced by specific effects of other environmental factors.

Steady-state routine performance
The rate of performance (feeding, behaviours, reproduction) that an organism displays routinely and permanently to maintain fitness in its natural environment.

Sublethal limits
Constraints in maintaining a functional rate under changing environmental conditions, e.g. warming, with negative implications at the ecosystem level.

Symmorphosis
According to this theory, the components of an organism match its overall functional scope, building on a quantitative match of design and functional parameters. The functional capacity of a complex system such as an animal's body must cope with the highest functional demand (after Weibel et al., 1991).

contribute to local extinction (e.g. Jones et al., 2009). While individuals survive non-lethal thermal constraints, the resulting reductions in available energy will jeopardise reproduction and thus population survival. Furthermore, the borders of the realised niche also depend on species interactions (e.g. changing availability of prey organisms). Current knowledge suggests that the borders of thermal niches can shift as a result of acclimation of individuals between seasons or adaptation over generations; this might allow organisms to tolerate rising temperatures without changes in distribution. However, data reveal ongoing distribution shifts for different species, indicating that the capacities for acclimation and adaptation are limited or that these processes are too slow to prevent biogeographical shifts (evidence reviewed and assessed by IPCC AR5: Poloczanska et al., 2014; Pörtner et al., 2014).

OCLTT has been used to explain the physiology underpinning the climate responses of individual species, as observed in the field, at biogeographical borders of critical life stages or under extreme seasonal conditions. Information on species-specific thermal niches can be derived from data on temperature-dependent steady-state animal performance (see Glossary) under the routine conditions of a species – relevant performances can range from steady-state swimming of salmon during spawning migrations (Eliason et al., 2011), to growth (including feeding) rates of benthic and demersal fish (Pörtner and Knust, 2007), to ventilatory and motor activities as in amphipods (Jakob et al., 2016) – and, more generally, they relate to the scope of routine oxygen demand and associated shifts in species abundance (see Pörtner and Knust, 2007) or biogeographical boundaries (Deutsch et al., 2015). With the possible exception of salmon migrating upstream (Fig. 1), routine performance in the centre of the thermal range usually does not fully exploit the available aerobic power budget (*sensu* Guderley and Pörtner, 2010; see Glossary).

As a result of acclimation or local adaptation and the associated trade-offs, the resulting total thermal performance curves (relating to aerobic power budget and encompassing various performances) differ between species and even between populations of the same species (Box 2, Fig. 1A), leading to different metabolic and performance characteristics across latitudes (Pörtner, 2006; Pörtner et al., 2008; Schröer et al., 2009). As a result of phenotypic diversity and plasticity, a species' biogeographical range is made up of overlapping niches of populations or individuals (Fig. 1A). In addition, differences in thermal curves and optima for individual performances may result from individuals being in different physiological modes when performing (e.g. due to different feeding or hormonal status), such that trade-offs in energy

Box 1. The OCLTT concept

Selected indicators of oxygen- and capacity-limited thermal tolerance (OCLTT) provide a systems view of multiple interlinked parameters characterising the thermal range of an aquatic animal species and its aerobic window (at steady state) (e.g. Pörtner, 2002, 2012; Pörtner et al., 2010, updated according to findings by Deutsch et al., 2015). The example given in the figure is for a warm temperate aquatic animal. The solid line shows the principal pattern of (mixed) body fluid P_{O_2} against temperature in *Maja squinado* (Frederich and Pörtner, 2000). Note that temperature-dependent patterns of body fluid P_{O_2} are not uniform between species and do not closely follow temperature-dependent changes in metabolic rate or performance. The range of active thermal tolerance (see Glossary) is limited on both sides by pejus temperatures (T_p; the box lists processes supporting active tolerance, which become constrained beyond T_p). Towards warm and cold extremes, the transition to passive thermal tolerance (see Glossary) is indicated by a decline in (venous) P_{O_2} (solid black arrows), causing oxidative stress (Heise et al., 2006), heat shock response and, finally, transition to anaerobic metabolism (e.g. Kyprianou et al., 2010; Pörtner and Knust, 2007; dashed black arrows). The model proposes that, in a systemic to molecular hierarchy of thermal tolerance thresholds, these progressive transitions from sublethal to acutely lethal conditions [characterised by critical thermal maximum (CT_{max}), cold shock, denaturation (at denaturation temperature, T_d)] involve feedback between whole-organism and molecular levels. Blue arrows indicate the link between oxidative stress, heat-induced molecular damage and heat shock protein expression (for further details on these interactions, see Kassahn et al., 2009). This whole-organism feedback may narrow molecular thermal windows, such that T_d is reached at lesser extremes of temperature (red arrows shifting upper and lower T_d). The passive tolerance range is a component of the niche used routinely by organisms experiencing extreme temperatures (e.g. in the intertidal zone). Rather than widening the active thermal range at a cost, they minimise metabolic costs and tolerate extremes anaerobically. Extended thermal tolerance is then achieved by protective mechanisms such as metabolic depression, anaerobic metabolism beyond the critical temperature (T_c), antioxidative defence and the use of chaperones such as heat shock proteins (e.g. Tomanek and Somero, 2002).
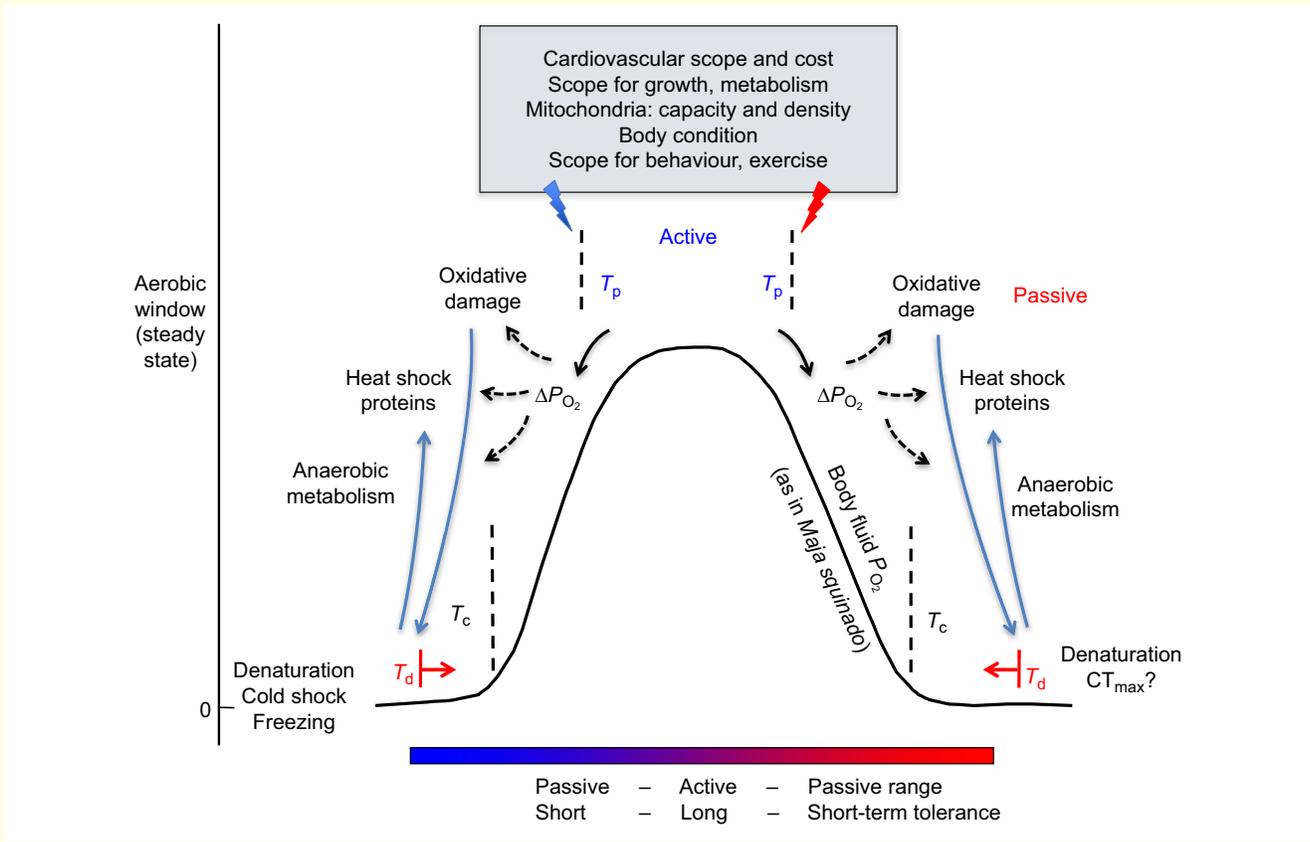
budgets may occur (see Holt and Jorgensen, 2015; for an early example of how starvation modifies thermal performance and optima in salmonids, see Brett, 1971).

OCLTT principles are presently integrated into models predicting the effects of climate on species' distributions and the consequences for ecosystems (e.g. Jones and Cheung, 2014; Deutsch et al., 2015). The concept has recently been extended to incorporate the combined effects of various climate change-associated drivers, such as ocean warming combined with acidification and hypoxia (Pörtner, 2010, 2012). It should also be noted that the pattern of acclimatisation to one climate-related driver such as temperature can be modified by the combined effects of multiple drivers (Anttila et al., 2015), indicating that

highly complex mechanisms shape sublethal and lethal thermal constraints.

Complexity shaping thermal limits: pitfalls when addressing OCLTT

Early in the development of the OCLTT concept it was suggested that thermal constraints are first noticeable at the highest level of organisational complexity (i.e. the whole animal), before affecting lower hierarchical levels (e.g. cellular and molecular levels; Pörtner, 2002). Recently, Storch et al. (2014) developed a 'complexity index' to compare the largely different thermal limits found across marine organism domains. They found a relationship between sublethal (and lethal) thermal limits and the number of body and cell
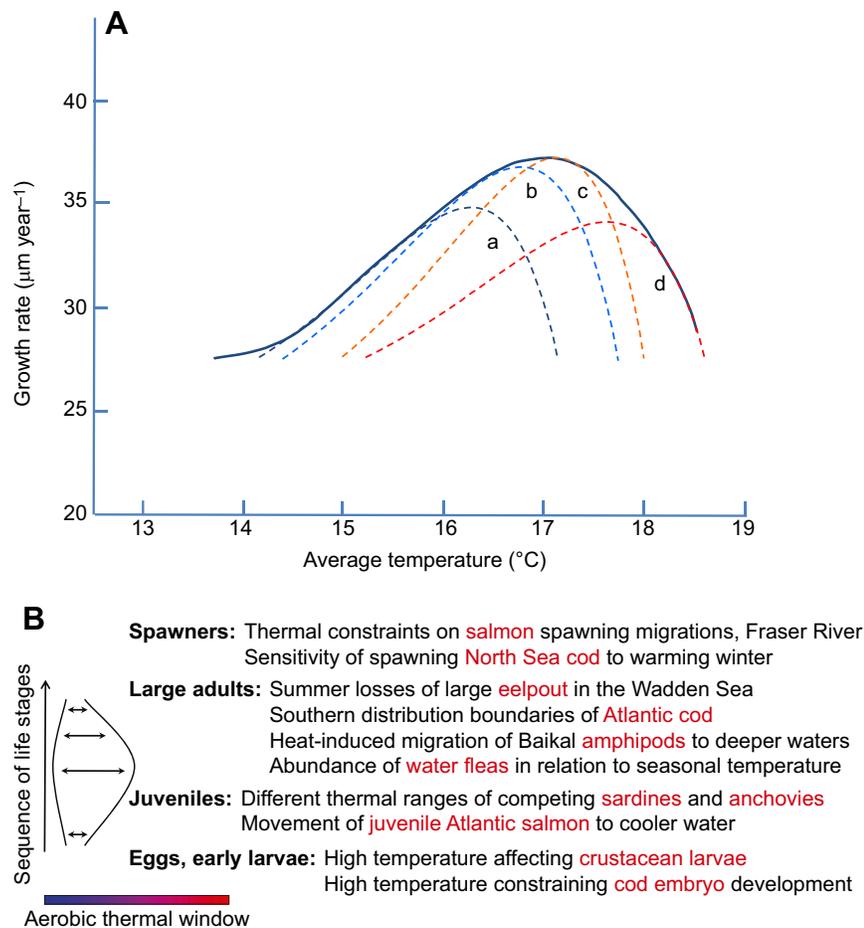
Fig. 1. Dynamics of thermal windows between populations and during ontogeny. (A) Temperature-dependent growth rates (the increment per year) read from otoliths in field populations of banded morwong, *Cheilodactylus spectabilis*, around Australia and New Zealand (after Neuheimer et al., 2011). The overall growth curve may well reflect the combination of different growth curves of individual (hypothetical) populations (a–d), indicating phenotypic diversity of thermal optima and ranges resulting from local acclimatisation or adaptation, as seen in cod, *Gadus morhua.* Trade-offs in energy budget result, and these lead to shifts in growth rate and performance capacities between populations (e.g. Pörtner et al., 2008; see Box 2). (B) Ontogenetic dynamics of thermal windows [curves indicate changes in upper and lower oxygen- and capacity-limited thermal tolerance (OCLTT) limits over time and between life stages; thermal window widths are indicated by arrows; Pörtner and Farrell, 2008] in relation to observed ecosystem/life-history constraints or to field phenomena explained by laboratory evidence for the respective life stage (after Perry et al., 2005; Breau et al., 2011; Eliason et al., 2011, 2013; Deutsch et al., 2015; Dahlke et al., 2016; Jakob et al., 2016; Pörtner and Knust, 2007; Pörtner et al., 2008; Storch et al., 2011; Schwerin et al., 2010; Takasuka et al., 2007).

compartments (separated by structure or function) as a proxy of complexity. The comparatively low thermal limit to animal life, i.e. above about 45°C (or somewhat less in water), would result from functional integration of a large number of compartments at the whole-organism level (Storch et al., 2014). This immediately rules out the possibility that one individual key protein or mechanism has an exclusive role in whole-organism limitation. Instead, constraints felt at lower levels of biological organisation should be embedded into the whole-organism context (see Pörtner, 2012). In heat-tolerant microbes, proteins do function up to 120°C, and there is no reason to assume that animal proteins could not evolve heat limits above the thermal limit of animal life.

For the benefit of optimum functioning, whole-organism and molecular thermal ranges would be interdependent on evolutionary time scales, with molecular thermal ranges being somewhat wider than whole-animal thermal ranges (Pörtner et al., 2012). Lower whole-animal thermal limits would promote the functional optimisation of proteins within the low range of animal body temperatures, resulting in molecular limits beyond but close to whole-organism thermal limits (e.g. Somero, 2010). At the same time, whole-animal constraints can feed back to the protein level, e.g. through oxidative stress (see Kassahn et al., 2009). Consequently, whole-organism limits 'trickle down' to limits at lower organisational levels, such that individual molecular or organellar functions may become limited at less extreme temperatures *in situ* than when extracted from the whole-organism or tissue context (Pörtner et al., 2012; cf. Iftikar and Hickey, 2013; Leo et al., 2017). Characterising the role of aerobic metabolism and underlying mechanisms in thermal limitation (Schulte, 2015) thus requires considering how these mechanisms interact with others

(e.g. antioxidative defence) and whether whole-organism phenomena feed back to these mechanisms (Box 1). This level of complexity may explain why thermal biology has not had a coherent framework and also why experimental work building on reductionist hypotheses (e.g. the idea that thermal damage to one kind of protein causes whole-organism heat death) comes with potential pitfalls. Although experiments must necessarily be reductionist, researchers should strive to embed experimental findings into concepts that capture the full complexity of the mechanisms involved, in an ecological context.

Matches or mismatches in oxygen supply and demand affect all tissues and cells, and thus the largest conceivable number of body compartments in an animal (Storch et al., 2014). Despite the underlying role of functional complexity in OCLTT-induced sublethal thermal constraints, however, some recent studies have exclusively focused on LT_{50} in an attempt to investigate the OCLTT concept; for example, by asking whether oxygen availability can shift LT_{50} or whether a maximally stimulated and exploited cardiovascular system has the capacity to supply oxygen until this point. LT_{50} and CT_{max} are conventional measures of ultimate tolerance limits (e.g. Lutterschmidt and Hutchison, 1997). These limits lie at the edge of or outside the range of aerobic power budget (Zakhartsev et al., 2003; Pörtner and Knust, 2007; Chen et al., 2015), beyond critical limits (T_c, where there is a transition to anaerobic metabolism; Box 1) and close to the co-evolving denaturation temperature (T_d) (see Farrell, 2009, and below). Such testing raises concerns, as it bypasses the sublethal thresholds at the core of OCLTT, such as pejus temperature (T_p, the onset of capacity limitation and hypoxaemia) and T_c, and their ecological relevance (primarily of T_p), which has been demonstrated in field studies

Box 2. Performance and OCLTT

Within the thermal range constrained by the P_{O_2} profile (Box 1), the kinetic stimulation of metabolic processes and energy-dependent functions by warming (and their inhibition upon cooling) leads to an asymmetric whole-organism total performance curve supported by aerobic power budget. The curve has a functional optimum close to the upper T_p. The power budget is shared between growth, immune response, reproduction and exercise during different behaviours (e.g. migration, competition, foraging) at different routine steady-state levels (e.g. sit and wait, roaming). Thermal limitation begins with reduced performance, and tolerance becomes progressively more time limited once functional scope falls below a limiting threshold beyond T_p. At optimum temperature (T_{opt}), maximised steady-state functional scope results from optimum oxygen supply at baseline oxygen demand, which rises exponentially above T_{opt} (dashed exponential curves, see Fig. 2). Capacity constraints lead to a mismatch in oxygen supply and demand beyond T_p. Depending on the level of routine performance and associated energy demand, T_p and T_c are dynamic within species. They are influenced by oxygen demand versus supply and by the temperature dependence of underlying metabolic costs (dashed exponential lines linking upper and lower T_p). The figure illustrates shifts in upper and lower T_p depending on functional rates and associated metabolic demands. Different species use different routine functional rates, and their T_p values are adjusted during their evolution to match ambient conditions. Both ambient hypoxia and elevated carbon dioxide levels may modify thermal windows (Walther et al., 2009; Zittier et al., 2012) and performance optima (green dashed lines). The graph depicts acute performance levels and limitations in response to short-term temperature fluctuations; during acclimatisation or adaptation (double-headed arrows), these may lead to shifts and changing widths of thermal windows (Fig. 1). The exact position of CT_{max} in relation to T_c and T_d is unknown.

Thermal acclimatisation and adaptation shift thermal limits through changing membrane composition or capacities of enzymes and mitochondria, or through mechanisms protecting molecular integrity (influencing T_d; see Pörtner, 2012). For example, in the warmth, reduced oxygen demand and increased T_p and T_c are expected following a decrease in tissue mitochondrial density, capacity and proton leakage costs. The resulting unidirectional shifts of both upper and lower tolerance thresholds, and the changing width of the thermal window involve molecular and cellular adjustments shaping the metabolic capacity of tissues and the functional capacity of relevant energy-consuming processes and their maintenance costs.
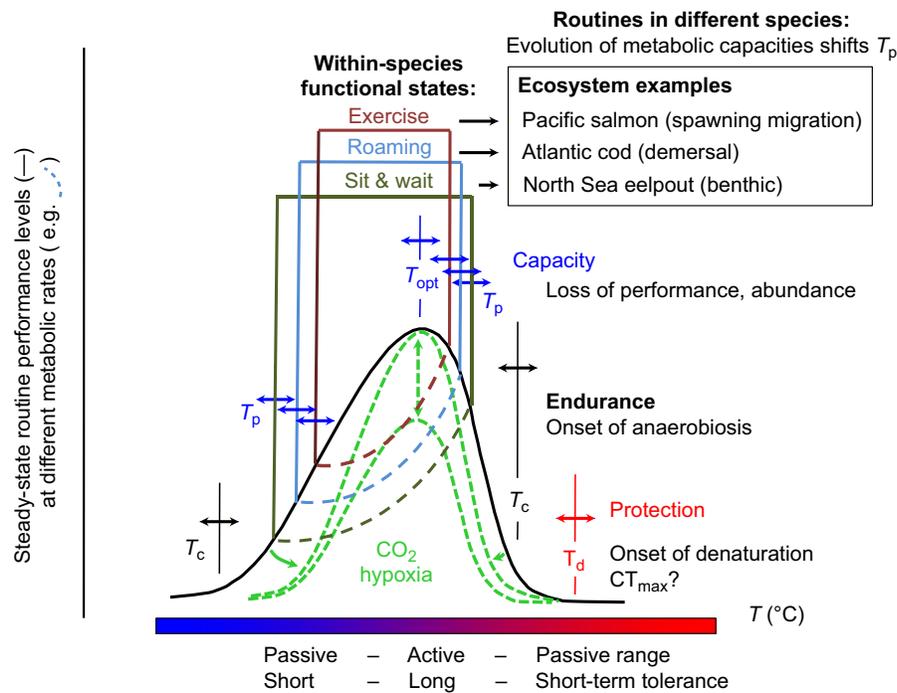
(e.g. Pörtner and Knust, 2007; Eliason et al., 2011; Jakob et al., 2016). By addressing the effect of oxygen on lethality, such studies also overlook the contribution and capacity of mechanisms contributing to passive thermal tolerance (see Glossary; e.g. heat shock response, anaerobic metabolism, metabolic depression and antioxidative defence) as well as its time limitation beyond T_p and T_c, before lethal effects set in at around T_d (Box 1; Pörtner, 2010, 2012; cf. Peck et al., 2009). More generally, passive thermal tolerance starts once hypoxaemia and/or Q_{10}-dependent rate limitations (in the cold) constrain routine performance beyond T_p (Box 1).

Considering the step-by-step development of OCLTT from upper T_p to T_c – and the increasing involvement of further mechanisms limiting thermal tolerance – leads to the prediction that any effects of experimentally increasing oxygen availability are progressively reduced and may become small at CT_{max} or LT_{50}; such systematic investigation of oxygen effects across thermal thresholds would be

useful. The existence of a whole-organism to molecular hierarchy of thermal sensitivity also suggests that increasing oxygen availability may not cause large shifts in CT_{max} or LT_{50}, as oxygen may not (fully) alleviate the extreme (e.g. denaturation) limits that co-evolved at lower levels of organisation. For example, under ambient hyperoxia, excess oxygen may alleviate thermal stress by reducing the costs of oxygen supply, thereby causing shifts in sublethal but not necessarily in lethal limits (e.g. Mark et al., 2002; Pörtner et al., 2006; Ekström et al., 2016). By contrast, ambient hypoxia or reduced oxygen supply capacity would exacerbate thermal stress and, because of stronger feedback from whole-organism to molecular levels, e.g. through enhanced oxidative stress, may reduce upper CT_{max} or LT_{50} to some extent.

In line with the prediction that reduced oxygen supply capacity may reduce CT_{max}, recent studies in fish have identified a role for haematocrit in thermal tolerance. In support of OCLTT, Beers and

Sidell (2011) found a positive correlation between haematocrit and CT_{max} across Antarctic fish species. A recent study in sea bass manipulated the haematocrit and found a small but significant decline in CT_{max} at low haematocrit (Wang et al., 2014). However, CT_{max} and haematocrit were not correlated, possibly as a result of the effects of reduced sensitivity to oxygen close to CT_{max}, as discussed above, combined with high data variability. The specific mechanisms causing the shift in CT_{max} or the reasons for the high variability in the relationship between CT_{max} and haematocrit remain insufficiently explored (see below). Overall, sublethal thermal constraints are more likely than lethal limits to be responsive to changing oxygen availability, and are likely to be more closely related to tissue functional capacity (see Glossary) and energy budget.

Variables indicative of OCLTT

In light of the above discussion, it seems prudent to avoid focusing exclusively on CT_{max} or LT_{50} in tests of the OCLTT concept. Instead, we should specifically identify sublethal thermal constraints from respiratory and metabolic variables under resting or routine conditions (Table 1). Breakpoints in the temperature dependence of these variables by their nature are 'softer' indicators of thermal limitation than a 'hard' endpoint such as lethal collapse. For example, warming causes SMR to rise exponentially until a breakpoint temperature beyond which SMR no longer increases (indicating the T_c to be surpassed) (e.g. Melzner et al., 2006; Giomi and Pörtner, 2013; see Fig. 2). Increasing ambient oxygen levels can lower the slope of the exponential rise in SMR and cause significantly lowered oxygen consumption rates at high temperatures – as seen in resting fish (Mark et al., 2002) or in amphibious crabs exposed to air (Giomi et al., 2014) – thereby increasing T_c.

Excess oxygen leads to reduced blood flow and thus lowers the cost of cardiovascular activity. This implies that, conversely, lower ambient oxygen levels cause metabolism to rise more strongly with increasing temperature, as a result of increased cardiovascular circulation. Similarly, anaemia can cause an increase in cardiac output (as in anaemic sea bass; see Wang et al., 2014); however, the cost increment in cardiovascular activity may remain small. By reducing viscosity (Farrell, 1991), a lower haematocrit may compensate for the cost increment, balancing the oxygen shortage caused by the reduced haematocrit. Generally, the patterns of heart rate and cardiac output indicate sublethal thermal limitation as they do not increase sufficiently to match the warming-induced rise in O_2 demand and to keep the aerobic power budget large, a lag setting in well below CT_{max} (e.g. Wang et al., 2014). Haematocrit may thus be better correlated with sublethal constraints than with CT_{max} (e.g. Buckley et al., 2014).

So how might variables underlying the OCLTT best be investigated? Analyses of the OCLTT should mimic natural conditions and consider routine activities displayed by the animal in the field, as well as minimising stress phenomena that would transiently mobilise functional reserves (see Glossary), e.g. through release of catecholamines, which stimulate cardiovascular circulation, glycogenolysis or anaerobic metabolism. Such stimulation supports time-limited thermal tolerance but has negative consequences for other components of the energy budget, e.g. growth. It should be noted that not all species display continuous motor activity; thus, measurements of steady-state aerobic scope (see Glossary; Farrell, 2013) for exercise may not always be possible when testing OCLTT. Tissue oxygenation and oxygen supply to sensitive aerobic organs such as the heart (Ekström et al., 2017) or liver may closely trace OCLTT under routine conditions, but such estimates are usually not

Table 1. Variables analysed and interpreted as indicators of OCLTT in animals

Parameter	T_{opt}	T_p	T_c	T_d
Maximum aerobic scope: maximum growth, maximum exercise	✓			
CO_{max} (exercise)	✓			
Reduced performance* (exercise, growth, CO)		✓		
Pv_{O_2}/Cv_{O_2}		✓	✓	
BP Pv_{O_2}/Cv_{O_2}		✓	✓	
BP ventilation		✓	✓	
BP heart rate		✓	✓	
BP stroke volume		✓	✓	
BP $\dot{M}_{O_2}$		✓	✓	
CO_{max} (rest, routine)			✓	
Mitochondrial functioning (permeabilised fibres)		✓?	✓?	
Anaerobic end products (especially succinate)			✓	
Cardiac arrhythmia/bradycardia			✓	
CT_{max}				✓?

See Box 1 and 2, and Storch et al. (2014). Indicators are mostly respiratory parameters that have been used to assess different oxygen- and capacity-limited thermal tolerance (OCLTT)-related terms and thresholds (Box 1, Box 2; T_{opt}, T_p, T_c, T_d), as indicated with a tick. A general conclusion from available studies is that the physiological condition of the experimental animals needs to be well defined and to match (long- and short-term) ecosystem conditions. Otherwise, trade-offs in aerobic energy (power) budget may have consequences for individual performances and their thermal constraints. Note that the assessment benefits from an integrative analysis of various processes and taxon- or even species-specific patterns, as not all indicators may display obvious thresholds in all taxa or species. $\dot{M}_{O_2}$, oxygen consumption rate; CO, cardiac output; BP, breakpoint; Pv_{O_2}, venous partial pressure of O_2; Cv_{O_2}, venous oxygen content; ?, proof of concept needed. *Ideally, total aerobic performance (not directly measurable because of trade-offs in energy budget), but often referred to as growth (Fig. 1) or exercise.

available. In fish, measurements of venous rather than arterial P_{O_2} appear appropriate to indicate thermal constraints, because of venous perfusion of the heart in most species (Farrell and Clutterham, 2003; Lannig et al., 2004; Ekström et al., 2016; Farrell, 2009) and the proximity of venous blood to tissues; these analyses would ideally be complemented by those of venous oxygen content (Cv_{O_2}). In crustaceans, measurements of oxygen partial pressure in (mixed
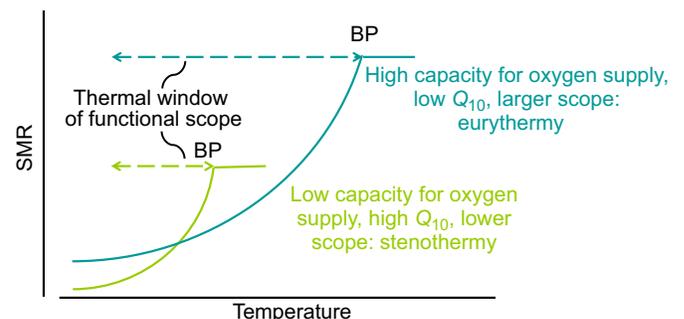

Fig. 2. Conceptual graph illustrating the thermal operating ranges of two hypothetical systems with different temperature-dependent capacities. The schematic diagram shows how low- and high-capacity systems (green and blue curves, respectively) have different baseline costs (standard metabolic rate, SMR) and ranges of operation if exposed to workload (e.g. due to warming) until reaching different capacity limits (under routine conditions, indicated by breakpoints, BP). Through similar principles and differences in thermal responses (Q_{10}), OCLTT distinguishes stenotherms from eurytherms (here exemplified through narrow versus wide temperature ranges and differences in the temperature dependence of the workloads; see Pörtner, 2006). In each case, the thermal window width of functional scope is represented by the length of the dashed arrows.

arterial and venous) haemolymph and of succinate concentrations in tissues can reveal the development of extreme hypoxaemia at thermal extremes (Frederich and Pörtner, 2000). The accumulation of anaerobic metabolites (such as succinate) beyond T_c indicates oxygen-deficient mitochondria. The ecologically important transition phases from earliest sublethal limitation (at T_p) to T_c precede CT_{max} (see Zakhartsev et al., 2003) – but their mechanistic link to CT_{max} clearly deserves further study. In the future, continuous recordings of tissue oxygenation may also support such analyses.

Regulatory responses are likely to involve various molecular factors (Kassahn et al., 2009). For example, hypoxia-inducible factor 1 (HIF-1) contributes to improving anaerobic capacity as well as oxygen supply through erythropoesis and may, thereby, enhance the capacity for heat and cold endurance. Cold adaptation may be supported by thyroid hormones (Little et al., 2013). Depending on the animal phylum, these changes contribute to adjustments of oxygen-transport capacity by both ventilation and circulation (invertebrates) or mainly circulation (fishes), as well as to adjustments in mitochondrial density, capacity, oxygen demand and energy budget (all tissues).

Addressing capacity, performance and energy budget

It should be noted that the term 'capacity' is used in different ways. The original OCLTT literature emphasises the interdependence and trade-offs between the rates and capacities of functions supplying and consuming oxygen and associated energy (e.g. cardiovascular systems, as reflected in cardiac output; or mitochondrial ATP synthesis or transmembrane ion transport in all tissues), their baseline costs (e.g. resting contractile cardiac activity, mitochondrial proton leakage or transmembrane ion leakage) and the resulting performance at various levels of biological organisation, in unstressed animals at rest or during routine activities in variable or stable climates. However, recent studies intending to test OCLTT have focused on the cardiovascular system and pushed it to its limits to determine whether it has the maximum capacity to provide sufficient oxygen to the organism until LT_{50} (Gräns et al., 2014; Wang et al., 2014). This approach probably activates functional reserves (see above), which is possible only transiently. At first sight, both views may appear valid, yet OCLTT emphasises sublethal thermal limitation and whole-organism consequences and trade-offs under resting and routine conditions. The concept addresses the subtle links and constraints in baseline costs, net functional scope (see Glossary) and resulting whole-organism performance capacity (see Fig. 2), as well as any shifts in net energy allocation to routine activities, without transient activation of functional reserves.

Conceptually, functional properties of the fish heart illustrate core aspects of the term 'capacity' and the associated functional and thermal limits, as considered in the OCLTT concept for species with different modes of life and under different temperature regimes. Oxygen consumption of the body ($\dot{M}_{O_2}$, excluding gas exchange via the skin) equals cardiac output (CO) multiplied by the difference in arterial and venous oxygen concentration (Fick's principle):

$$\dot{M}_{O_2} = CO \times (Ca_{O_2} - Cv_{O_2}). \qquad (1)$$

Cardiac output is the product of stroke volume and heart rate, and reflects the 'functional capacity' of the heart. Stroke volume is the blood volume pumped by the fish ventricle during one contraction. Any increase in oxygen demand (e.g. during routine exercise) is reflected in an increase in cardiac output. For most fishes, the contribution of stroke volume to oxygen supply is usually higher

than that of heart rate, e.g. during sustained (routine) swimming (Farrell, 1991).

Functional capacity has three interdependent aspects: (1) how much performance can be achieved, depending on (2) how the respective system is set up and (3) at what cost. A high-capacity cardiovascular system comprises a larger heart with higher resting cardiac output than a low-capacity system (Farrell, 1991). Active fishes usually have a larger relative heart mass (Santer, 1985; Farrell, 1991). Accordingly, exercise training can produce isometric cardiac growth as seen in rainbow trout (Farrell et al., 1990). Cardiac output depends on the volume of the heart and the pressure generated by wall tension. When higher cardiac output is needed (e.g. during warming), heart rate and, possibly, stroke volume increase, at an energetic cost (i.e. increased oxygen consumption). A larger stroke volume entails an increase in ventricle radius and greater filling during diastole.

Morphologically, the fish heart can be simplified as a sphere with defined volume (V) and diameter (D, wall thickness) of the myocardial muscle. The workload (W) of a contracting sphere equals:

$$W = p \times V, \qquad (2)$$

where p is pressure.

Energy requirements (oxygen demand) of the heart are mainly determined by wall tension (T) as defined by Laplace's law:

$$T = p \times r/2D, \qquad (3)$$

where r is the ventricle radius. The equation illustrates that a thicker myocardial muscle produces higher pressure and then output, at the expense of higher baseline costs due to larger, mitochondria-rich tissue mass. The energy turnover during contraction is related to the contractile shortening of heart muscle fibres. In the assumed spherical heart, the length of a circular heart fibre may equal the heart circumference, $L=2\pi r$, with a shrinking radius representing the contractile shortening of the muscle fibre. Because $V=4/3\pi r^3$, the same absolute value of contractile shortening in a large, high-capacity heart (with a large radius) will produce a stroke volume larger than that of a small, low-capacity heart, at rest and during exercise. Similarly, for the same stroke volume, larger hearts need a smaller contraction of the muscle fibre than smaller hearts, resulting in a lower increase in relative cellular oxygen/energy demand for the same increase in performance, and a larger functional and energy reserve in larger hearts to maximise performance. For the same increase in workload, a small-volume heart will thus be stimulated to a greater extent and limited sooner than a large-volume heart (Fig. 2), a conclusion supported by the larger maximum stroke volumes of isolated perfused hearts of active fish (larger hearts) versus sluggish fish (smaller hearts; Farrell, 1991).

According to OCLTT, the capacity of the heart plays a role in determining thermal tolerance and associated energy turnover. As outlined above, excess oxygen can cause reduced blood flow and visibly lower whole-organism oxygen demand in the warmth, possibly by reducing the rising cost of cardiovascular activity. Increasing temperature induces increased cardiac output (e.g. Lannig et al., 2004; Farrell, 2009; Franklin et al., 2013), building on different baseline costs of maintenance and with different exponential slopes in hearts of different sizes according to a species' active or passive lifestyle (Fig. 2).

Even within-species variability as seen in European sea bass indicates that larger hearts in fish with lower SMR support higher temperature tolerance and faster recovery from exercise than smaller hearts in fish with higher SMR (Ozolina et al., 2016). This

emphasises that there is poorly understood variability in the patterns depicted in Fig. 2. The following hypothetical picture emerges: the comparison of sluggish versus active fish appears analogous to that of cold stenothermal versus cold eurythermal fish (or ectotherms in general). To meet the same absolute or relative increase in oxygen demand, a low-capacity, low-cost system as in a sluggish fish (or a cold-adapted stenotherm) would experience a stronger stimulus than a higher-capacity system as in an active fish (or in a temperate eurytherm), causing a greater percentage cost increment in the sluggish fish/stenotherm and thus contributing to a higher Q_{10} and earlier thermal limitation under routine conditions. This pattern is also mirrored in the low-capacity, low-cost mitochondria seen in cold-adapted stenotherms versus high-capacity, high-cost mitochondria as in cold-adapted eurytherms (Pörtner, 2006; Fig. 2). In line with these findings, more active, mobile Antarctic stenotherms are indeed more heat tolerant than sessile sluggish ones (Peck et al., 2009; for the role of cold adaptation and eurythermy in the evolution of high-energy turnover endotherms, see Pörtner, 2004; Clarke and Pörtner, 2010). Further observations are also in line with these emerging principles. Population-specific adaptation to various temperature regimes in salmon involves different heart sizes and adrenoceptor densities (Eliason et al., 2011, 2013). Acclimation of individual fish to temperature also involves changes in cardiac performance of fish. Acclimation to warming in salmon causes an increase in maximum heart rate, meeting the rising baseline cost (Anttila et al., 2014).

The mechanical picture drawn from this simplified approach will thus be modified by potential cellular or morphological differences, such as in the oxidative capacity of mitochondria (see Pörtner, 2006), pacemaker activity, and size and capillarisation of the heart, or blood viscosity. For example, a low contribution of blood oxygen transport to aerobic scope is compensated for to some extent by the evolution of relatively large hearts, as in Antarctic icefishes (Farrell, 1991). The interplay of all of these factors will shape the contribution of the cardiovascular system to the species-specific oxygen and capacity limitation of the whole organism.

In general, maintenance costs (measured as SMR) are relatively low within the optimal thermal range but rise exponentially towards the upper limit of thermal tolerance, constraining functional (aerobic) scope. Although for some performances (like growth or reproduction) or routine activities (roaming and feeding) aerobic scope is not fully exploited, rising maintenance costs will still introduce constraints on aerobic power budget. Thus, functional scope, e.g. of the heart, is highest at the thermal optimum (T_{opt}) when maintenance costs are still relatively low. At temperatures below T_{opt}, functional scope is depressed by cooling more than maintenance costs are, finally leading to the failure of oxygen supply to meet demand as seen in warm temperate animals at critically low temperatures (Frederich and Pörtner, 2000). High-capacity systems (e.g. tuna), while having a higher baseline cost (Fig. 2), come with the benefit of easily buffering demand under routine conditions, e.g. during warming or exercise or both, with a smaller percentage increment in cost and limitation setting in at higher temperatures than for the same condition in a low-capacity system (as in hagfish or in Antarctic icefish, considering the loss of haemoglobin in the latter). Here, baseline costs are lower but percentage increments are higher, and the system runs into capacity limitations at lesser extremes. Because of the interdependence of capacity and cost, the percentage increment of cost per degree of warming is thus highest in energy-saving, low-capacity systems, such as polar or winter stenotherms (e.g. Pörtner, 2006; Wittmann et al., 2008; Pörtner et al., 2013), emphasising a link between energy

turnover, mode of life and the level of eurythermy (see Pörtner, 2004; Peck et al., 2009; Clarke and Pörtner, 2010). It should be noted that temperate-zone animals may be able to exploit the energetic benefits of being either winter stenotherms or spring and summer eurytherms through seasonal acclimatisation (e.g. Wittmann et al., 2008).

Looking at capacity just in terms of its maximum exploitability thus misses the role of underlying design and its plasticity under routine conditions, as well as the subtleties in the functional transitions and limitations. In a living animal, an early subtle indication of capacity limitation can be the presence of a breakpoint temperature (Fig. 2). This more complex approach to capacity captures the progressive development of thermal limitation from the earliest constraints to lethal temperatures (Box 1), as well as the difference between stenotherms and eurytherms (Pörtner, 2006).

In this context, measurements of aerobic scope for exercise as an estimate of aerobic power budget have to be interpreted very carefully, as analyses of aerobic scope using critical swimming speed (U_{crit}) protocols in fish can include exploitation of non-sustainable short-term functional reserves that rely on hormonal (adrenergic) stimulation or anaerobic processes, beyond the onset of kick-and-glide swimming (Lurman et al., 2007). As the degree of mobilisation of anaerobic reserves can have a strong behavioural component (Peake and Farrell, 2006), the use of fatigue-based exercise protocols may overestimate aerobic capacity, thereby again missing earliest functional constraints at pejus limits (Table 1).

It is also important to note that T_p and T_c may shift depending on the routine performances used and their steady-state energy demand at the ecosystem level (Box 1). For some species in a specific life phase (e.g. spawning migrations in salmon), T_p and T_c are best determined during full exploitation of aerobic scope for exercise. For other species and life phases (and more widely), this should be during their lower levels of routine activities (e.g. Atlantic cod; Deutsch et al., 2015). In species regularly experiencing temperature extremes (such as at low tide in the intertidal zone), their capacity to exploit the passive tolerance range may become important in shaping fitness. Ideally, for assessing ecologically relevant T_p and T_c values, mode of life and associated energy demand, life phase and habitat challenges require consideration.

Evolutionary modulation of OCLTT

The OCLTT framework has identified phenomena of thermal limitation in various animal phyla against ecological and evolutionary backgrounds (e.g. Pörtner et al., 2005; Knoll et al., 2007). It has been suggested to be an early evolutionary principle in animals that has been modified according to life stage (see Pörtner and Farrell, 2008) or climate zone (Pörtner, 2006; Beers and Sidell, 2011; Pörtner et al., 2013), or during the evolution of air breathing (Giomi et al., 2014).

OCLTT varies with ontogeny, the associated development of organ functioning, metabolic plasticity and the organ's resulting capacity and body size. Thermal windows are typically narrow during early life and adult spawning stages and wider during juvenile and young adult stages (Pörtner and Farrell, 2008; Poletto et al., 2017; Fig. 1B). Such bottlenecks constrain where early and spawning life stages can live, and expose them to strong evolutionary pressures, leading to adaptive changes with functional consequences for the next life stage. These are virtually unexplored. Knowing the life history of a species in the context of habitat fluctuations is thus relevant to fully identify evolutionary bottlenecks and their consequences for physiology and biogeography.

In permanently oxygen-rich polar waters, adaptation may have alleviated thermal constraints on the cold side of the thermal window (Wittmann et al., 2012; Pörtner et al., 2013), leaving residual cold limitation to kinetic constraints on functional capacity. Improved cold tolerance was facilitated by excess oxygen dissolved in cold water, which supported low metabolic rates by allowing a lowered oxygen supply capacity and cost. However, this would come at the price of enhanced heat intolerance (Pörtner et al., 2013). In addition, excess oxygen supply at stable low temperatures may have enabled the loss of myoglobin and haemoglobin functions in Antarctic icefish, which lowered oxygen supply capacity and increased heat intolerance even further (Beers and Sidell, 2011).

The situation is less clear for the evolutionary adaptation to breathing air, which has 30-fold higher oxygen levels than water. The transition to terrestrial life often required the evolution of completely new gas-exchange systems (lungs, trachea), while convective 'blood'-bound oxygen supply to tissues persisted in most taxa (except for some adult insects). The symmorphosis principle (see Glossary; Weibel et al., 1991) suggests that these new convective oxygen-supply systems also evolved with their capacity limits set to cope with temperature extremes. In lower vertebrates, some evidence in fact indicates a progressively limited scope for oxygen uptake by the lungs towards higher temperatures (see fig. 6 in Pörtner, 2002; Jackson, 2007). In contrast, crustaceans still use their gills when in air and are therefore suitable models to investigate the potential benefits of air breathing for thermal tolerance. In fact, findings in amphibious crabs corroborate that oxygen supply costs are reduced in air and that this causes enhanced heat tolerance (Giomi et al., 2014). Insects may also have exploited this route; while aquatic larvae are subject to OCLTT principles (Verberk and Calosi, 2012), the tracheal oxygen supply to tissues in terrestrial adults supports elevated heat tolerance at minimised oxygen-supply costs.

Recent studies of thermal tolerance in air breathers may not have considered this diverse evolutionary background, or the pitfalls mentioned above. A study of the tropical toad *Rhinella marina* reported evidence for thermal acclimation at two temperatures (Seebacher and Franklin, 2011) but did not explore the related changes in steady-state cardiovascular functions or in tissue oxygenation (or venous oxygen tensions as determined by Pörtner et al., 1991). Such evidence is required when investigating a role for oxygen in thermal limitation. A later study of the same species by Overgaard et al. (2012) found a typical exponential increase in resting oxygen consumption, which entered the steep phase beyond 30°C, in line with a transition to pejus range (see Fig. 2). However, activity was enforced and non-steady state; and data variability was high and may not have provided sufficient resolution to determine limiting thresholds. A later study on python (Fobian et al., 2014) drew conclusions from measurements of temperature-dependent arterial oxygen tensions, which remained high even at high temperatures. It needs to be considered that arterial oxygen values are often not suitable to investigate OCLTT, as also seen in fish, for example, where arterial P_{O_2} (Pa_{O_2}) remained high when venous P_{O_2} (Pv_{O_2}) fell with rising temperatures (see Sartoris et al., 2003). In line with OCLTT, however, the python developed a warming-induced reduction in aerobic scope, which was determined as the difference between metabolic rates of fasting and digesting snakes. The drop in scope was paralleled by a levelling off in temperature-dependent arterial P_{O_2} and oxygen consumption in digesting snakes (see fig.1 of Fobian et al., 2014). As a corollary, future studies addressing sublethal constraints in oxygen supply towards extreme temperatures in air require careful consideration of best practices for studying OCLTT (Pörtner, 2014).

Further study of the role of OCLTT in animal evolution and ontogeny is needed. For example, the mechanisms of thermal adaptation and limitation have been poorly investigated across all life stages of a species or in both aquatic and terrestrial animals from the sub-tropics and the tropics. These organisms are probably adapted to ambient temperatures closer to metazoan heat limits of about 45°C, suggesting a steep transition from optimum temperatures via sublethal to lethal limits. This makes it more difficult to accurately identify sublethal thresholds, analogous to the study of cold limitation in (sub-)polar organisms. Thermal adaptation to the warmth would cause a down-regulation of metabolic rate at high oxygen diffusivity, which might alleviate oxygen-dependent constraints. Nonetheless, some data in tropical fish indicate constraints on aerobic scope for exercise at high temperatures (Munday et al., 2009). That said, the available data are presently too limited to clearly identify typical patterns of complexity limits in both tropical and subtropical aquatic and terrestrial ectotherms.

Conclusions

The ad hoc mixing of OCLTT with classical concepts in thermal biology such as CT_{max} requires care, as such studies tend to overlook the subtle limits to aerobic metabolism and performance, and the systemic to molecular hierarchy of thermal tolerance.

For understanding ecological patterns, the functional rates of organisms need to be explained. Darwinian fitness does not depend on one performance trait only, but various traits come together in their additive energy cost. Fitness is thus related to routine metabolism, reflecting how aerobic power budget is used by different performances simultaneously and at the required minimum level and above. Therefore, OCLTT should not be simplified to comprise aerobic scope for exercise only. OCLTT is about a cause and effect understanding for different performances and their role in fitness and share in energy budget of a specific life stage. Such cause and effect understanding is at the core of physiological studies in an ecological context. This principal understanding is also an asset when making predictions on the fate of populations in a distant future.

The OCLTT concept explains the first line of thermal limitation at the whole-organism level in animals, and may represent an evolutionary constraint that was modified depending on life stage and climate, and during transition to life in air. Neglecting to consider the links between levels of biological organisation will lead to insufficient explanations of thermal limits that fall behind what the OCLTT concept has already achieved. It is possible that the combination of OCLTT and molecular limits shape lethal limits and their response to oxygen availability. As experiments can rarely resolve all facets of complex phenomena, addressing such complexity requires the parallel development of theoretical background and experimental approaches at multiple levels; current theories of evolutionary biology illustrate these requirements (e.g. Angilletta, 2009). Thermal physiology should strive to interpret experimental results in light of an organism's ontogeny and ecology, using comprehensive, ecologically relevant concepts (Bozinovic and Pörtner, 2015). Conversely, reductionist lines of interpretation should remain coherent with the widest possible conceptual framework.

Sublethal thermal limitation according to OCLTT can also vary depending on activity level (e.g. resting, roaming or high rates of steady-state energy use); thus, it is important to consider which situation is typical for a species and its critical life stage(s) in the wild. Whether limitations to routine metabolic scope set

biogeographical borders and, thus, limits to the realised niche needs to be investigated more widely. In the context of thermal biology and climate change, we require integrative, ecosystem-oriented and evolutionary modes of interpretation, ideally combining field and experimental data. With this aim, the OCLTT concept has connected ecological and physiological findings in animals, and we hope that these connections will be developed further in the future.

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