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In a marine teleost, the significance of oxygen supply for acute thermal tolerance depends upon the context and the endpoint used

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Abstract

Eight juvenile European seabass were exposed to two thermal ramping protocols with different levels of aerobic activity and tolerance endpoint: the critical thermal maximum for swimming (CTS_{max}) while exercising aerobically until fatigue, and the critical thermal maximum (CT_{max}) under static conditions until loss of equilibrium (LOE). In the CTS_{max}, warming caused a profound increase in oxygen uptake rate ($\dot{M}O_2$) culminating in a gait transition, from steady aerobic towards unsteady anaerobic swimming, then fatigue at 30.3 ± 0.4°C (mean ± SE). Gait transition and fatigue presumably indicate an oxygen limitation, an inability to meet the combined demands of swimming plus warming. The CT_{max} also elicited an increase in $\dot{M}O_2$, culminating in LOE at 34.0 ± 0.4°C, significantly warmer than fatigue at CTS_{max}. The maximum $\dot{M}O_2$ achieved in the CT_{max} was, however, less than 30% of that achieved in the CTS_{max}. Therefore, the static CT_{max} did not exploit full cardiorespiratory capacity for oxygen supply, indicating that LOE was not caused by systemic oxygen limitation. Consequently, systemic oxygen supply can be significant for tolerance of acute warming in seabass but this depends upon the physiological context and the endpoint used.

Keywords: critical thermal maximum, fish, oxygen uptake, swimming

Introduction

Global warming will cause a rise in sea surface temperatures of up to 4°C by 2100, accompanied by increased frequency and severity of acute heat waves (Frölicher et al., 2018) . Temperature has direct thermodynamic effects on the physiology of ectotherms, so there has been extensive research to investigate thermal tolerance limits in fishes, and the underlying mechanisms. The Fry paradigm and Oxygen and Capacity Limited Thermal Tolerance hypothesis (Fry-OCLTT) have been proposed as a universal principle underlying thermal tolerance in ectotherms (Fry, 1971; Pörtner, 2010). According to the Fry-OCLTT, oxygen demands of metabolism are accelerated inexorably by warming until they exceed cardiorespiratory capacity for oxygen supply, causing functional collapse (Eliason et al., 2011; Fry, 1971; Pörtner, 2010; Pörtner and Farrell, 2008; Schulte, 2015). Empirical evidence for the Fry-OCLTT paradigm is, however, mixed (Farrell, 2016; Jutfelt et al., 2018; Lefevre, 2016; Lefevre et al., 2021; Pörtner, 2021).

There is particular uncertainty about the role of oxygen limitation in tolerance of acute warming in fishes. Acute thermal tolerance is typically measured with the critical thermal maximum (CT_{max}) protocol, where fish are warmed in steps until loss of equilibrium (LOE) (Lutterschmidt and Hutchison, 1997). The LOE at CT_{max} is an incipient lethal endpoint due to dysfunction of critical organs, brain and heart (Andreassen et al., 2022; Lefevre et al., 2021; Rezende et al., 2011). In some fish species, environmental oxygen availability and/or blood oxygen carrying capacity have little or no effect on CT_{max}, indicating that LOE is not oxygen dependent (Brijs et al., 2015; Ern et al., 2016; McArley et al., 2021). In other species, measurements of rates of oxygen uptake ($\dot{M}O_2$) during a CT_{max} protocol found an initial exponential increase up to a maximum that was comparable to their maximum metabolic rate measured at initial acclimation temperature. This was then followed by a plateau and/or decline, which preceded LOE and which might indicate a limitation in capacity for tissue oxygen supply prior to the endpoint (McArley et al., 2017; Norin et al., 2019). The contrasting evidence indicates that LOE at CT_{max} may have many underlying mechanisms, which may or may not be linked to oxygen limitation, and which differ among species, methodology and context (Andreassen et al., 2022; Ern et al., 2023; Lefevre et al., 2021; Rezende et al., 2014).

In a study to investigate effects of acute temperature increases on cardiorespiratory performance of sockeye salmon (*Oncorhynchus nerka*), Steinhausen et al. (2008) warmed fish progressively while they swam aerobically in a swim tunnel. A number of salmon showed a gait-transition to an anaerobic swimming mode and then fatigued at warm temperatures, which the authors attributed to an inability to meet the combined oxygen demands of swimming plus warming (Steinhausen et al., 2008). Blasco et al. (2020) proposed that a similar protocol could be used to evaluate acute thermal tolerance in fishes, warming in steps with fatigue as the endpoint. In two tropical freshwater teleosts exercising aerobically in a swim tunnel, progressive warming caused profound increases in $\dot{M}O_2$, to levels very significantly higher than the individuals' maximum metabolic rate during a critical swimming speed (U_{crit}) test at their acclimation temperature. At a certain warm temperature, however, all individuals transitioned from a steady aerobic to an unsteady anaerobic gait and then fatigued, at temperatures significantly lower than their LOE at CT_{max} (Blasco et al., 2020). Dubbed the critical thermal maximum for swimming (CTS_{max}) by Blasco et al. (2022), the mechanism underlying fatigue would presumably be oxygen supply limitation, an inability to meet the combined oxygen demands of swimming plus warming (Blasco et al., 2020; Blasco et al., 2022; Steinhausen et al., 2008).

Here, we investigated the general hypothesis that capacity for tissue oxygen supply can play a role in acute upper thermal tolerance in fishes, but that this depends upon the prevailing level of aerobic metabolic activity and consequent oxygen demand, and the tolerance endpoint used. We studied the European seabass *Dicentrarchus labrax*, a temperate marine teleost that has been the focus of investigations into the role of oxygen supply in defining CT_{max} (Anttila et al., 2023; Wang et al., 2014). We compared $\dot{M}O_2$ during a CTS_{max} and CT_{max} protocol in the same individuals, in particular the maximum $\dot{M}O_2$ achieved prior to each tolerance endpoint, fatigue or LOE. We reasoned that, if in the CTS_{max} protocol the seabass transitioned to unsteady anaerobic swimming at a certain warm temperature, and then fatigued, then capacity for tissue oxygen supply would underly this thermal tolerance endpoint in this species, as for the species studied by Blasco et al. (2020). We expected that LOE at CT_{max} would occur at a significantly higher temperature than fatigue at CTS_{max}, as observed previously (Blasco et al., 2020; Blasco et al., 2022). We, however, investigated the specific hypothesis that maximum $\dot{M}O_2$ elicited by the CT_{max} would be significantly less than those in the CTS_{max}, indicating that the LOE endpoint was not caused by limitations in capacity for systemic tissue oxygen supply in this species (Wang et al., 2014).

Material and Methods

Juvenile seabass were obtained from a population at the Station Ifremer de Palavas-les-Flots (France), reared in seawater at 18 – 19 °C and tagged (PIT, under benzocaine anaesthesia) for individual identification. Fish were held in a 1 m^3 tank supplied with biofiltered seawater at 18 °C for at least two weeks prior to experimentation, fed a maintenance ration with commercial pellets but fasted for 24 h before experiments. Prior to the main trials, a control was performed at 18 °C on six seabass of mean (± SE) mass 111.4 ± 9.1 g and forklength 202 ± 6 mm, which were swum at the speed of the CTS_{max} (2.5 BL s^{-1}) for 8 h. For the CTS_{max} and CT_{max}, eight seabass were studied, of mean mass 74.2 ± 8.0 and forklength 181 ± 6 mm at the CTS_{max}, then mean mass 75.3 ± 8 g at the CT_{max},.

Swimming trials were performed in a plexiglass Steffensen-type swim tunnel (volume 30 l) provided with biofiltered aerated seawater at 18 °C. Fish were measured for mass, width, height, and fork length, to calculate relative swimming speed in body length per second (BL s^{-1}) corrected for solid blocking effect (Bell and Terhune, 1970), then acclimated overnight at a low swimming speed of 1 BL s^{-1}. The next day, current speed was increased by 0.25 BL s^{-1} every 2 min until 2.5 BL s^{-1}. In the control trial, the fish then swam for 8h. In the CTS_{max}, after 30 min at 18 °C the temperature was increased by 1°C each 30 min until fish fatigued, resting their tails for at least 10 s against the rear grid. Fish were immediately removed from the tunnel into a tub of aerated seawater at 18°C for 30 min, then returned to their holding tank. CTS_{max} was calculated as the last temperature step fully completed plus the proportion of the ultimate step prior to fatigue (Blasco et al., 2020). The experiments were performed over three weeks, with the last individual studied 24h before measurement of its CT_{max} (below).

The CT_{max} was performed in eight individual respirometry chambers (volume 3 l) that were supplied with aerated seawater at 19 °C, due to an overnight increase in the surface temperature of the local seawater supplying the facilities. Fish were acclimated to the chambers overnight then, the next morning, temperature was increased by 1°C every 30 min until LOE (McArley et al., 2017; Penney et al., 2014). Chambers were screened from view with an opaque plastic sheet containing a small observation hole, to monitor fish throughout. As soon as fish lost dorso-ventral equilibrium, they were removed and placed in aerated water at 19 °C for 30 min, then returned to their holding tank. CT_{max} was calculated as for CTS_{max} but using LOE as endpoint (Blasco et al., 2020).

The $\dot{M}O_2$ was measured in mgO_2 kg^{-1} h^{-1} over the last 10 min of each temperature increment, in both protocols, by intermittent-flow respirometry (Blasco et al., 2020; Steffensen, 1989). $\dot{M}O_2$ was corrected for body mass with a scaling coefficient of b = 0.8 for metabolic rate in European seabass (Rodde et al., 2021). The maximum $\dot{M}O_2$ achieved in each protocol was identified for each individual. Absolute thermal scope for CT_{max} and CTS_{max} was calculated as maximum $\dot{M}O_2$ minus the relevant $\dot{M}O_2$ at 19°C. The proportional change in $\dot{M}O_2$ was calculated as a Q_{10} value over 19 – 29°C.

A two-way ANOVA with repeated measures was used to evaluate effects of temperature on $\dot{M}O_2$, with one factor protocol (CTS_{max} versus CT_{max}), repeated factor the temperature increments, each fish as an individual, and an interaction term between protocol and temperature steps. Data were considered for a range of 19 to 30°C, which was measured on all individuals in both protocols. An error function was included to correct for repeated tests on the same individual. Tukey post hoc tests, with Holm correction for multiple comparisons, were used to identify the temperature at which $\dot{M}O_2$ first increased significantly from the value at 19 °C, and to compare $\dot{M}O_2$ between protocols at each temperature. Single variables, such as CTS_{max} and CT_{max}, maximum $\dot{M}O_2$, thermal scope, and Q_{10} were compared by pairwise Welch t-tests. All statistical analyses were performed in R 4.0.2 within the lsmeans package (version 2.27-61), with significance accepted at $p < 0.05$.

Results

At a speed of 2.5 BL s^{-1}, the seabass swam with a sustained aerobic body-caudal swimming gait. In the control trial the seabass swam for 8h with no sign whatsoever of fatigue. During CTS_{max}, all individuals eventually transitioned in swimming gait, from an entirely steady aerobic gait with rhythmic beating of the tail, to increasing engagement of unsteady anaerobic 'burst-and-coast' episodes. This led to fatigue within less than one temperature increment (30 min). No fish lost equilibrium in the swimming trials. During CT_{max}, the fish exhibited erratic behaviour, rolling sideways, then complete loss of dorsoventral orientation. The mean CTS_{max} was 30.3 ± 0.4°C, significantly and almost 4°C lower than mean CT_{max} at 34.0 ± 0.4°C (Figure 1A).

In the control swim, $\dot{M}O_2$ remained stable for 8h (Figure S1, supplementary materials). caused There were marked increases in $\dot{M}O_2$ in both thermal ramping protocols (Figure 2), that became significant from 19 °C at 25°C in both cases. There was, however, a significant interaction between protocol and temperature (Two-way ANOVA, $F_{11,74}=13.71$, $p < 0.001$). All measures of $\dot{M}O_2$ in CTS_{max}, irrespective of temperature, were higher than any measure of $\dot{M}O_2$ in the CT_{max} (Two-way ANOVA, Holm $p < 0.001$, Table S1, supplementary materials). Consequently, the maximum $\dot{M}O_2$ achieved and absolute thermal scope were significantly higher in the CTS_{max} (Figure 1). Despite these differences in $\dot{M}O_2$ and absolute thermal scope, Q_{10} did not in fact differ between protocols (Figure 1).

Discussion

The results demonstrate that capacity for tissue oxygen supply can be significant for upper thermal tolerance in this teleost species, but this depends upon the physiological context and the tolerance endpoint used. When warmed while exercising aerobically, all individuals eventually transitioned to unsteady anaerobic swimming, evidence of limitations in capacity for tissue oxygen supply (Steinhausen et al., 2008), leading to fatigue at CTS_{max}. When warmed under static conditions, the animals eventually lost equilibrium at a CT_{max} that was significantly higher than their CTS_{max} but, in line with our specific hypothesis, maximum $\dot{M}O_2$ in the CT_{max} was very significantly less than achieved in the CTS_{max}. Therefore, the full cardiorespiratory capacity for oxygen supply was not exploited during thermal ramping in the static CT_{max}, indicating that LOE is not linked to limitations in systemic oxygen delivery in this species (Wang et al., 2014).

Fatigue in the CTS_{max} was not due to limited aerobic endurance because, at their acclimation temperature, seabass swam at 2.5 BL s^{-1} for longer than the duration of the warming challenge.

Similar aerobic endurance was observed in Nile tilapia *Oreochromis niloticus* and pacu *Piaractus mesopotamicus* (Blasco et al., 2020). This confirms that the fatigue endpoint in the CTS_{max} is due to the progressive warming. The swimming responses to the CTS_{max} protocol, gait transition followed by fatigue, were also very similar in tilapia and pacu (Blasco et al., 2020). The gait transition is qualitatively indistinguishable to that observed prior to fatigue in a critical swimming speed (U_{crit}) protocol, where it is stimulated by increments in current velocity rather than temperature. This indicates that the transition occurs due to a similar mechanism. In the U_{crit}, it is typically assumed that gait transition and then fatigue occur because a fish has reached its maximum cardiorespiratory capacity for oxygen supply (Brett, 1964; Claireaux et al., 2005; Farrell, 2007; Webb, 1998). Although it is not known what causes the gait transition in a U_{crit} test, one plausible theory focuses on the heart. An aerobic organ, the teleost heart receives much of its oxygen supply from venous return in the single circulation, after the blood has unloaded oxygen to respiring tissues (Farrell and Jones, 1992; Jones and Randall, 1978). It has been suggested that during an incremental swim challenge, fishes engage the gait transition towards anaerobic swimming when tissue oxygen extraction outstrips capacity for supply, and oxygen levels in venous blood returning to the heart drop below a critical level. By engaging the white muscle, the fish reduces rates of oxygen extraction by the working red muscle and so venous oxygen supply to the heart is assured (Farrell and Clutterham, 2003; McKenzie and Claireaux, 2010). The gait transition preceding fatigue in the CTS_{max} may reflect a similar decline in venous oxygen levels, that risked compromising oxygen supply to the working heart (Blasco et al., 2020).

Steinhausen et al., (2008) found that fatigue from exercise during acute warming in the sockeye salmon coincided with cardiac pumping capacity reaching its maximum. Cardiac performance plays a major role in defining acute thermal tolerance in many fish species (Ekström et al., 2014; Ekström et al., 2016; Farrell, 2007; Gilbert et al., 2019), so represents a focus for future studies regarding the mechanisms for the CTS_{max} endpoint. Other mechanisms may also contribute, progressive warming can impair locomotor coordination in fishes, possibly as a consequence of limitation in oxygen supply to the brain (Andreassen et al., 2022). Further research is therefore required to understand what causes gait transition and fatigue in a CTS_{max} but it is interesting that the test solicits similar physiological responses in multiple species (Blasco et al., 2020).

Although the CTS_{max} results provide evidence that tissue oxygen supply can play a role in thermal tolerance in swimming seabass when fatigue is the endpoint, the CT_{max} data support the hypothesis that LOE is not due to limitations in systemic oxygen supply in this species (Wang et al., 2014). The mean CT_{max} of 34.0 °C in our juvenile seabass reared at 18° C, was comparable to previous reports of 34.7 and 35.6 °C for juveniles acclimated for 30 days to 15 or 20 °C, respectively (Kır and

Demirci, 2018). The slightly higher CT_{max} reported by Kir and Demirci (2018) may also be linked to a more rapid thermal ramping of 0.3 °C min^{-1} compared to 0.033 °C min^{-1} in this study, as more rapid ramping is known to lead to higher CT_{max} values in fishes (Desforges et al., 2023; Ern et al., 2023). We ramped at 1°C every 30 min to ensure that fish core temperature was equilibrated with the water at each step, and that we could measure M_{O2} over the last 10 min. While MO_2 increased markedly with warming in the static CT_{max}, as observed in other studies (McArley et al., 2017; Norin et al., 2019), and the Q_{10} was similar in both protocols, the actual rates were always significantly lower than at any temperature in the CTS_{max}. That is, LOE occurred at MO_2 levels lower than those of the same individuals exercising at 2.5 BL s^{-1} at 19 °C. Static respirometry does not constrain gas exchange in seabass, juveniles achieved similar high maximum rates of oxygen uptake when either swum in a U_{crit} test or chased to exhaustion and then placed in a static chamber (Killen et al., 2017). Therefore, warming did not challenge capacity for oxygen supply in stationary fish, it caused LOE by an oxygen-independent mechanism. There are a variety of possible contenders, particularly direct temperature-induced effects on critical organs such as heart and brain, which cause their dysfunction. There is evidence of this in fishes, including that such effects can interact with oxygen limitation within these organs (Andreassen et al., 2022; Friedlander et al., 1976; Vornanen, 2020). Overall, the consensus is that LOE can have multiple causes and that systemic oxygen limitation is not a universal mechanism across fish species (Andreassen et al., 2022; Ern et al., 2023; McArley et al., 2021).

The Fry-OCLTT argues that capacity for oxygen supply is a mechanism underlying thermal tolerance in fishes (Fry, 1971; Pörtner, 2010), the CTSmax test indicates that it can be in multiple species, if fatigue from aerobic activity is the tolerance endpoint. Here we show that this is true for a species where LOE in a CT_{max} protocol appears to occur by an oxygen-independent mechanism. The CT_{max} is easy and rapid to perform but the endpoint has a complex multitude of potential mechanisms. The CTS_{max} is more technically demanding but the mechanism underlying tolerance may be similar across fish species.

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Ethics.

Experimental procedures were approved by the ethics committee for animal experimentation n° 036 of the French Ministère de l’Enseignement Superieur, de la Recherche et de l’Innovation, with reference number APAFIS#19567-2018100910598940 v2.

Data accessibility. Data are available in the electronic supplementary material

Author contribution. All authors contributed to study conception, design, securing funding, animal husbandry. J.J.H.N., F.R.B. and C.R. carried out the experiments and completed the data analyses. J.J.H.N and D.J.M. drafted the initial manuscript; all authors then reviewed and revised the manuscript.

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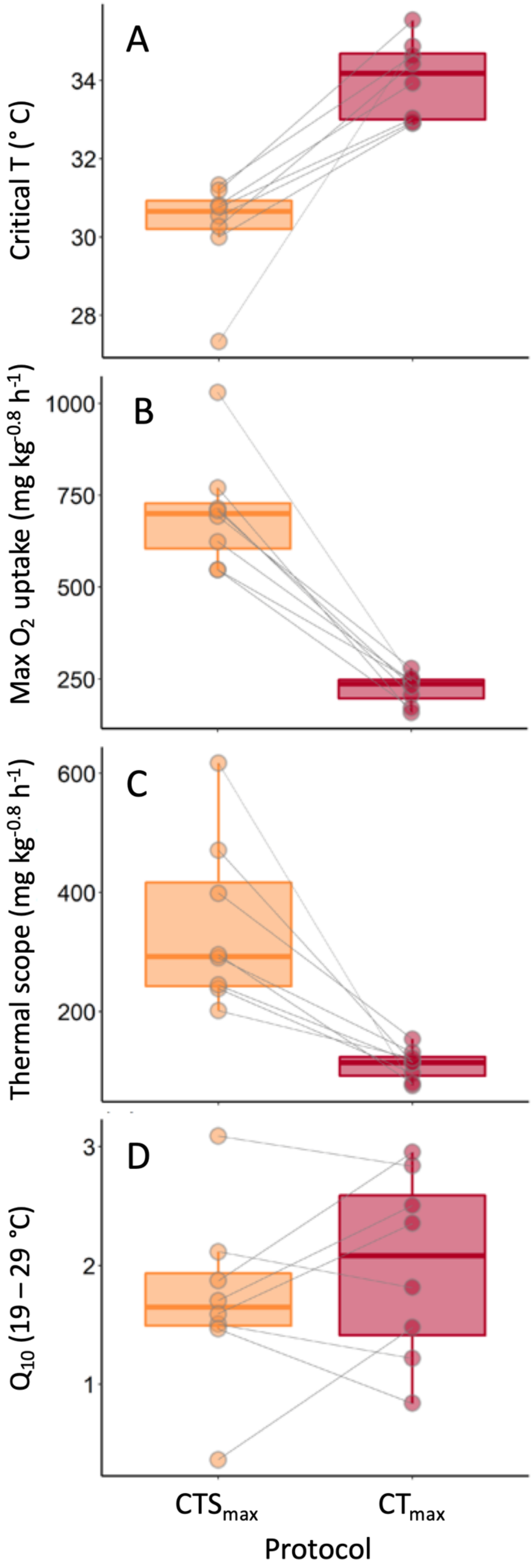
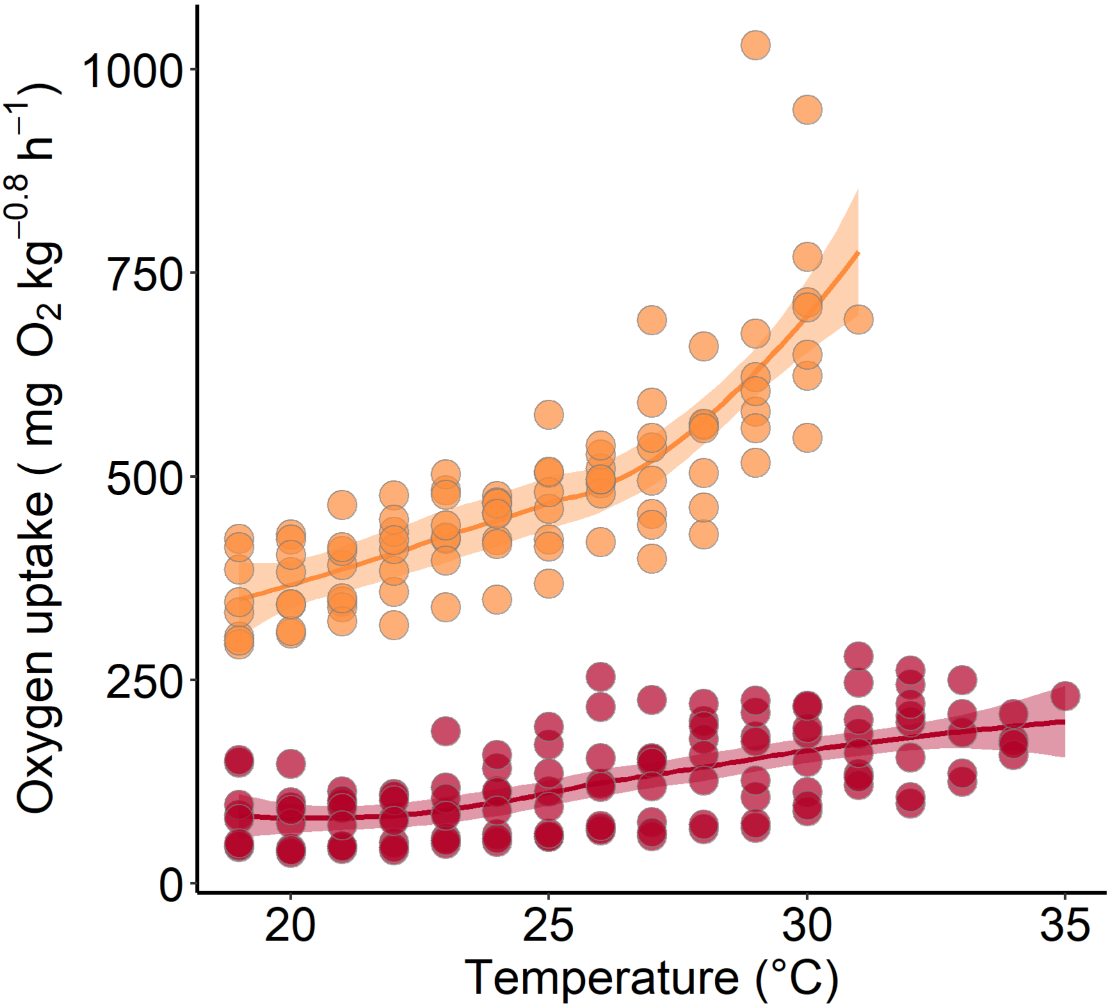
Figure legends

Figure 1. Boxplots of data for critical temperature of endpoint (A); maximum rate of oxygen uptake achieved (B); thermal scope, calculated as maximum rate of oxygen uptake minus rate at 19 °C (C), and Q10 for effects of temperature on oxygen uptake between 19 °C and 29 °C (D). Two thermal ramping protocols are compared in n = 8 European seabass, either critical thermal maximum for swimming (CTS_{max}) exercising aerobic with fatigue as endpoint, or critical thermal maximum (CT_{max}) under static conditions with loss of equilibrium as endpoint. Each point is an individual, the lines connecting the two protocols show how that individual responded to each. Boxplot lower and upper hinges represent the 25th and 75th percentiles. The horizontal line within the box is the median. The length of whiskers represents the range of data points between each hinge and 1.5× the difference between the 25th and 75th percentiles. Data beyond these limits are outliers.

Figure 2. Rate of oxygen uptake in n = 8 European seabass during exposure to two thermal ramping protocols, either critical thermal maximum for swimming (CTS_{max}) exercising aerobic with fatigue as endpoint (19 °C to 31 °C, orange symbols and curve), or critical thermal maximum (CT_{max}) under static conditions with loss of equilibrium as endpoint (19 to 35°C, red symbols and curve). Each point represents an individual, the temperature ranges are those for which oxygen uptake data was collected on all individuals, the shaded area shows the 95% confidence interval.


A
Critical T (° C)
28
30
32
34
B
Max O_2 uptake (mg $kg^{-0.8}$ h^{-1})
250
500
750
1000
C
Thermal scope (mg $kg^{-0.8}$ h^{-1})
200
400
600
D
Q_{10} (19 – 29 °C)
1
2
3
CTS_{max}
CT_{max}
Protocol

393


Oxygen uptake (mg O_2 $kg^{-0.8}$ h^{-1})
1000
750
500
250
0
20
25
30
35
Temperature (°C)

394