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Oxygen-dependence of upper thermal limits in fishes

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Key Words

Aerobic scope, Critical oxygen tension (P_{crit}), Critical thermal maximum (CT_{max}), Hypoxia, Oxygen limit for thermal tolerance ($P_{CT_{max}}$), Temperature.

Summary statement

Based on measurements of critical thermal maximum temperatures in marine fishes under hypoxia, this study provides a novel metric ($P_{CT_{max}}$) for assessing the oxygen sensitivity of upper thermal limits in water-breathing ectotherms.

ABSTRACT

Temperature-induced limitations on the capacity of the cardiorespiratory system to transport oxygen from the environment to the tissues, manifested as a reduced aerobic scope (maximum- minus standard metabolic rate), have been proposed as the principal determinant of the upper thermal limits of fishes and other water-breathing ectotherms. Consequently, the upper thermal niche boundaries of these animals are expected to be highly sensitive to aquatic hypoxia and other environmental stressors that constrain their cardiorespiratory performance. However, the generality of this dogma has recently been questioned, as some species have been shown to maintain aerobic scope at thermal extremes. Here, we experimentally tested whether reduced oxygen availability due to aquatic hypoxia would decrease the upper thermal limits (*i.e.*, the critical thermal maximum; CT_{max}) of the estuarine red drum (*Sciaenops ocellatus*) and the marine lumpfish (*Cyclopterus lumpus*). In both species, CT_{max} was independent of oxygen availability over a wide range of oxygen levels despite substantial reductions in aerobic scope (*i.e.*, > 72%). These data show that the upper thermal limits of water-breathing ectotherms are not always linked to the capacity for oxygen transport. Consequently, we propose a novel metric for classifying oxygen-dependence of thermal tolerance; the oxygen limit for thermal tolerance ($P_{CT_{max}}$), which is the water oxygen tension (P_wO_2) where an organism's CT_{max} starts to decline. We suggest that this metric can be used for assessing the oxygen sensitivity of upper thermal limits in water-breathing ectotherms, and the susceptibility of their upper thermal niche boundaries to environmental hypoxia.

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INTRODUCTION

Marine ectotherms largely occupy the extent of latitudes tolerable within their thermal limits (Sunday et al., 2012), and are therefore, expected to shift their latitudinal distribution ranges poleward with climate warming (Pörtner, 2014b; Sunday et al., 2012). The critical thermal maximum (CT_{max}), typically determined as the temperature at which animals exhibit a loss of equilibrium (LOE) (Beitinger et al. 2000), defines the upper limit of a species' fundamental thermal niche and is the temperature where animal function ceases due to the collapse of one or more vital physiological functions (Pörtner, 2010).

Oxygen supply capacity refers to the maximum ability of the cardiorespiratory system to supply oxygen to the tissues by increasing ventilation, cardiac output, and blood oxygen carrying capacity. Over the last two decades, laboratory studies have reported reduced heart and ventilation rates, diminished cardiac output and blood oxygen content, and an accumulation of anaerobic metabolites in water-breathing ectotherms exposed to acute temperature increases that approach their upper thermal limits (Frederich and Pörtner, 2000; Mark et al., 2002; Melzner et al., 2006; Wittmann et al., 2008). This led to the hypothesis that fishes and other water-breathing ectotherms, at temperatures approaching their CT_{max}, are unable to maintain sufficient oxygen supply for basic metabolism due to temperature-induced cardiorespiratory constraints. The temperature where animals become reliant on unsustainable anaerobic metabolism for vital physiological functions is termed the critical temperature (T_{crit}) (Farrell et al., 2009; Pörtner, 2010). Above T_{crit}, extreme hypoxemia develops and the time until terminal ATP-deficiency (*i.e.*, CT_{max}) becomes progressively shorter with increased warming (Pörtner, 2010). In species where the upper thermal limits are determined by insufficient oxygen supply for vital physiological functions, any reduction in oxygen availability should reduce both T_{crit} and CT_{max}. The upper thermal limits of these species can, thus, be described as oxygen-dependent.

In fishes, reduced cardiorespiratory performance and oxygen supply capacity at supra-optimal temperatures have been correlated with reductions in population abundance (Johansen et al., 2015; Pörtner and Knust, 2007) and migration performance (Eliason et al., 2011). It has also been suggested that oxygen-dependent upper thermal limits identified in the laboratory represent those in the field (Giomi et al., 2014; Pörtner and Knust, 2007). However, the link between oxygen supply capacity and upper thermal limits has recently been questioned, as other studies have reported that aerobic scope (AS; the difference between the maximum metabolic rate, MMR, and

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the standard metabolic rate, SMR) and cardiorespiratory performance are maintained in a number of fish and crustacean species experiencing ecologically-relevant thermal extremes (Brijs et al., 2015; Clark et al., 2013a; Ern et al., 2015; Ern et al., 2014 ; Grans et al., 2014; Healy and Schulte, 2012; Jost et al., 2012; Norin et al., 2014). It has, therefore, been suggested that some species possess a more thermally resistant cardiorespiratory system (Ern et al., 2014; Jost et al., 2012), and that insufficient tissue oxygen supply is not the primary determinant of upper thermal limits in all water-breathing ectotherms (Brijs et al., 2015; Wang et al., 2014).

Thermal tolerance can be described as the capacity to maintain the performance of a single physiological function (*e.g.*, heart rate) or a group of related functions (*e.g.*, cardiorespiratory oxygen transport) when temperatures change. Whereas, thermal limits describe the temperatures where the performance of such functions begins to decline, or is reduced to zero. CT_{max} is a widely used metric for evaluating the thermal tolerance and thermal limits of animals (Terblanche et al., 2011 and references within), but involves an acute increase in temperature, representative of relatively few aquatic environments (*e.g.*, shallow tropical estuaries, tide pools, desert streams). Furthermore, because the performance capacity of physiological functions are influenced by both the rate of temperature change and the duration of exposure to new temperatures, the ecological relevance of such measurements must take into account these two parameters (*e.g.*, Somero, 2010; Terblanche et al. 2011; Farrell, 2016); especially if the rates of change greatly exceed those encountered by the species in the wild. Nonetheless, CT_{max} and an organism's thermal breadth ($CT_{max} - CT_{min}$) have been applied widely in both experimental studies and meta-analyses on the impacts of climate warming on aquatic ectotherms, including in predictions of their global (re)distribution (*e.g.*, Sunday et al., 2011; Sunday et al., 2012; Magozzi and Calosi, 2015; Vinagre et al., 2016). Investigating the oxygen-dependence of thermal tolerance using CT_{max} is, therefore, highly relevant for continued research efforts aimed at understanding how climate change will impact ecological physiology and species' distributions. This is especially true as accelerated climate change not only involves increasing (and more extreme) temperatures, but also an increase in the frequency and severity of aquatic hypoxia (Altieri and Gedan, 2015; Diaz and Rosenberg, 2008). This latter condition is likely to play a key role in shaping the distribution of species if their upper thermal limits are oxygen-dependent.

From a physiological perspective, aquatic hypoxia can be defined as any water oxygen tension (P_wO_2) that reduces MMR and AS, with the critical oxygen tension (P_{crit}) characterised as the

P_wO_2 where AS is zero and any further reductions in P_wO_2 result in a proportional decrease in the rate of oxygen consumption below that required to sustain baseline metabolism (*i.e.*, SMR) (Farrell and Richards, 2009). Thus, in species with oxygen-dependent upper thermal limits, the T_{crit} should be reduced at any level of hypoxia that causes a significant reduction in MMR and, thus, AS (Fig. 1A, B). Because upper thermal limits of these species are caused by terminal ATP-deficiency, CT_{max} should also decline accordingly. However, in species where the upper thermal limits in normoxia are not determined by insufficient oxygen supply, a portion of AS would remain at CT_{max} in normoxia (Fig. 1C) and no change in CT_{max} would be expected upon exposure to progressive hypoxia until the P_wO_2 where MMR is reduced down to SMR, and AS reaches zero (Fig. 1D). In such a case, the upper thermal limits in normoxia can be described as oxygen-independent.

To investigate whether acute upper thermal tolerance (*i.e.*, CT_{max}) in fishes is determined by insufficient oxygen supply capacity, we actively constrained AS by lowering water oxygen availability (*i.e.*, induced hypoxia), and then measured CT_{max} at a wide range of water oxygen tensions (P_wO_2), from normoxia to severe hypoxia. This allowed us to investigate if, or when, the fish's upper thermal limit (*i.e.*, CT_{max}) became oxygen-dependent. We used two fish species with different thermal niches and ecologies; the athletic, free-swimming, eurythermal red drum (*Sciaenops ocellatus*; approximate natural temperature range, 15-26°C) and the more sluggish, cold-water lumpfish (*Cyclopterus lumpus*; approximate natural temperature range, 3-11°C) (Aquamaps.org, 2015).

MATERIALS AND METHODS

Animals and maintenance

Red drum (*Sciaenops ocellatus*) were reared at 22±1°C (salinity 35±1 ppt) at the University of Texas at Austin, Marine Science Institute (Port Aransas, Texas, USA), whereas lumpfish (*Cyclopterus lumpus*) were reared at 10±1°C (salinity 32±1 ppt) at the Ocean Sciences Centre, Memorial University of Newfoundland (St. John's, Newfoundland, Canada). Both species were fed with pelleted food every day, but were fasted for 24 h prior to experiments to avoid the metabolic effects of digestion on respirometry measurements. Individual fish were used only once in the experiments described below (*i.e.*, no fish were tested more than once).

Experimental procedures were performed in accordance with policies of the Institutional Animal Care and Use Committee of the University of Texas at Austin (red drum) and the Animal Care Committee of Memorial University of Newfoundland (lumpfish; protocol no. 15-88-KG). Studies on lumpfish also followed the guidelines of the Canadian Council on Animal Care.

Respirometry

Metabolic rates were estimated from rates of oxygen consumption ($\dot{M}_{O_2}$) as measured using fiber-optic oxygen sensors, meters and software (Pyro Science GmbH, Aachen, Germany or Loligo Systems, Tjele, Denmark), and intermittent-flow respirometry (Clark et al., 2013a; Steffensen, 1989). $\dot{M}_{O_2}$ was calculated according to:

$$\dot{M}_{O_2} = \frac{-\delta[O_2] \times (V_{chamber} - V_f)}{BM},$$

where $\dot{M}_{O_2}$ is oxygen consumption rate (mg O_2 h^{-1} kg^{-1}), $\delta[O_2]$ is the slope of the decline in water oxygen concentration (mg L^{-1} h^{-1}) during the closed period of the intermittent respirometry cycle, $V_{chamber}$ is the volume of the respirometer (L), V_f is the volume of the fish (*i.e.*, it was assumed that the fish had a density of 1 L kg^{-1}), and BM is the fish's body mass in kg. Respirometry chamber volumes were 0.40 L for lumpfish and 1.20 L for red drum. Both the standard metabolic rate (SMR) and the maximum metabolic rate (MMR) of lumpfish and red drum were measured, and the aerobic scope (AS) of both species was calculated as MMR − SMR.

Standard metabolic rate (SMR) and critical oxygen tension (P_{crit})

SMR and P_{crit} were measured at 10 and 16°C for lumpfish and at 24 and 30°C for red drum (N = 8 for each temperature and species). For each experimental group, the fish were placed inside respirometry chambers submerged in a tank with a flow-through supply of fully aerated seawater at 10°C for the lumpfish or 24°C for the red drum (the starting temperature for red drum was slightly higher than their acclimation temperature due to limited capacity for cooling in the experimental setup), and allowed 24 hours to settle inside the respirometry chambers. For the 10°C (lumpfish) and 24°C (red drum) groups, $\dot{M}_{O_2}$ recordings were started immediately after the settling period. For the 16°C (lumpfish) and 30°C (red drum) groups, water temperature was gradually increased to these temperatures after the settling period, at a rate of 2°C h^{-1}, upon which $\dot{M}_{O_2}$ recordings were initiated. $\dot{M}_{O_2}$ recordings were then continued for 12-17.5 h, which produced 95-100 (lumpfish) or 160-200 (red drum) individual $\dot{M}_{O_2}$ measurements. For lumpfish the durations of each intermittent measurement cycle (flush/wait/measure) were (in seconds) 300/120/240 and 300/60/180 at 10 and 16°C, respectively, and for red drum were 90/30/120 and 120/30/120 at 24 and 30°C, respectively. Oxygen levels in the respirometry chambers did not fall below 90% air-saturation during $\dot{M}_{O_2}$ measurements. SMR was determined by first taking the mean of the lowest 10% of $\dot{M}_{O_2}$ measurements over the 12-17.5 h respirometry period, excluding outliers that were ±2 s.d. from the mean (no more than two data points were identified as outliers for any fish), and finally calculating the mean of the remaining $\dot{M}_{O_2}$ measurements (Clark et al., 2013a). This method ensured that only $\dot{M}_{O_2}$ measurements recorded when the fish were quiescent were included in the SMR calculations.

P_{crit} measurements were made immediately following the determination of SMR, by turning off the flush pumps and allowing the fish to consume the oxygen in the sealed respirometry chambers, thereby gradually exposing them to increasing hypoxia. At a P_wO_2 of ~15 mmHg the flush pumps were turned back on and the fish were returned to a recovery tank. The obtained $\dot{M}_{O_2}$ measurements were then plotted against the declining P_wO_2, and P_{crit} was calculated as the P_wO_2 where $\dot{M}_{O_2}$ first decreased below SMR. It should be noted that the $\dot{M}_{O_2}$ of the fish during the P_{crit} experiment was often elevated above SMR, due to spontaneous movements under hypoxia, and is therefore, designated as routine metabolic rate (RMR) (see Fig. 2).

To account for background microbial respiration, $\dot{M}_{O_2}$ recordings were also made after removal of the fish. $\dot{M}_{O_2}$ from bacterial respiration was not detectable in any of these background measurements, likely due to the thorough cleaning of the respirometry equipment between measurements.

Maximum metabolic rate (MMR) and aerobic scope (AS)

In separate experiments, MMR was measured at 10 and 16°C for lumpfish and 24 and 30°C for red drum (N = 8 for each temperature and species) in both normoxia and in hypoxia (P_wO_2 of 70-76 mmHg; Table 1). For all measurements, the fish were placed in a circular experimental tank (50 L) containing fully aerated seawater at 10°C (lumpfish) or 24°C (red drum) and left overnight. The following morning, water temperature was either maintained at 10°C or 24°C or heated to 16°C (lumpfish) or 30°C (red drum) at a rate of 2°C h^{-1}. The fish were then individually exercised in normoxic water for 2 min (until exhaustion) by manual hand-chasing by the experimenter, and immediately transferred to a respirometry chamber kept at the appropriate temperature and P_wO_2. $\dot{M}_{O_2}$ recordings commenced within 20 s after the cessation of chasing and MMR was determined over a 2-min period during the first $\dot{M}_{O_2}$ recording, which was always the highest. The P_wO_2 (± 2.5 mmHg) in the tank containing the respirometry chambers was regulated using a solenoid system that bubbled air or nitrogen into the water.

To calculate the percentage change in AS with changing P_wO_2, a 3-parameter power function was fitted to MMR in normoxia and in hypoxia, SMR at P_{crit} and RMR below P_{crit}. The reductions in AS (from 0% in normoxia to 100% at P_{crit}) were then calculated as the difference between the corresponding MMR on the regression line and SMR in normoxia (cf. Fig. 2A, B, D, E). Given that MMR was not measured at oxygen levels between ~50% and 100% air-saturation, the calculated change in AS with P_wO_2 does not account for a potential 'zone of hypoxia insensitivity' near 100% air-saturation, where MMR may have been unaffected by mild reductions in dissolved oxygen. Consequently, the values of AS at high P_wO_2 may be subject to a slight underestimate, which diminishes as P_wO_2 approaches P_{crit}.

Critical thermal maximum (CT_{max}) and the oxygen limit for thermal tolerance ($P_{CT_{max}}$)

Finally, in both species, CT_{max} , was measured in normoxia and at multiple levels of hypoxia (see below; N = 8 in all groups). For all measurements, the fish were placed inside respirometry chambers submerged in a 40 L tank containing fully aerated seawater at 10°C (lumpfish) or 24°C (red drum) and left overnight. The following morning, P_wO_2 was either maintained at normoxia or acutely reduced to 100, 80, 67, 60, 40, or 21 mmHg (lumpfish) or 76, 47, 35, 23, or 11 mmHg (red drum) by bubbling the reservoir supplying water to the chambers with nitrogen. The reduction in P_wO_2 took 5-30 min depending on the target P_wO_2, and this level (± 2.5 mmHg) was maintained during the entire CT_{max} measurement by use of the solenoid system. Once the target P_wO_2 was reached, the water temperature was elevated continuously at a rate of 2°C h^{-1} until the fish exhibited loss of equilibrium (LOE). The temperature where LOE occurred was taken as CT_{max}. Due to the morphology of the lumpfish (flattened ventral surface), these fish did not necessarily fall over at the point of LOE, and were therefore, gently prodded with a cotton swab at regular intervals as CT_{max} was approached. The temperature where the fish no longer righted themselves after being gently prodded was taken as CT_{max} for this species. Once CT_{max} was reached for individual fish, they were removed from their chambers and returned to a recovery tank. No fish were used more than once.

Both P_{crit} and the hypoxia induced reduction in AS increased with temperature from 10 to 16°C (lumpfish) (Fig. 2A, B) and from 24 to 30°C (red drum) (Fig. 2D, E). Therefore, the percentage changes in AS at the P_wO_2 levels where CT_{max} was measured (see Fig. 2C, F) were calculated, as described above, from the $\dot{M}_{O_2}$ data at 16°C (lumpfish) or 30°C (red drum). Consequently, the calculated percentage changes in AS are minimal (conservative) estimates (*i.e.*, CT_{max} occurred at temperatures higher than those for which the changes in AS were calculated, meaning that AS would have been reduced even more at those temperatures).

$P_{CT_{max}}$ was determined from the CT_{max} data by fitting a piecewise, two-segmented linear regression through the CT_{max} values not significantly different from CT_{max} in normoxia, and the CT_{max} values significantly different from CT_{max} in normoxia (see Fig. 3).

Statistical analyses

Student's t-tests were used to examine the effect of temperature on MMR, SMR, AS and P_{crit}, as well as the effect of hypoxia on MMR. All *P*-values were corrected for multiple comparisons using

false discovery rate (FDR) correction. FDR cutoff values were 0.0383 and 0.00802 for lumpfish and red drum, respectively. One-way analysis of variance was used to test the effect of P_wO_2 on CT_{max}. All assumptions of these statistical tests were met. Statistical analyses were conducted using SigmaPlot (Systat Software, Inc., Chicago, IL, USA), and the significance level for all tests was $P < 0.05$.

RESULTS

MMR decreased significantly in both lumpfish and red drum when exposed to hypoxia ($P < 0.001$ for both species and temperatures), and as a result, AS fell with water oxygen tension (P_wO_2), as expected, until the critical oxygen tension (P_{crit}) where AS was zero (Fig. 2A, B, D, E). At the higher temperatures (16 *vs.* 10°C for lumpfish, 30 *vs.* 24°C for red drum), tolerance to hypoxia was reduced (*i.e.*, P_{crit} was significantly increased; $P = 0.038$ for lumpfish and $P = 0.008$ for red drum) and the hypoxia-induced decline in AS was more pronounced (Fig. 2B, E *vs.* Fig. 2A, D). However, CT_{max} did not show a gradual decrease with falling P_wO_2 (Fig. 2C, F). For lumpfish, CT_{max} was maintained at 21.9–22.3°C down to a P_wO_2 of 80 mmHg ($P > 0.05$), at which point AS was reduced by at least 72% (Fig. 2A, B, C). At a P_wO_2 of 67 mmHg, slightly above the P_{crit} at 16°C (63.8 mmHg), CT_{max} was significantly reduced to 20.9°C ($P < 0.05$ compared to CT_{max} in normoxia), and this reduction continued with decreasing P_wO_2 (Fig. 2C). For red drum, a similar pattern was observed, with no significant change in CT_{max} (36.1–36.5 °C) down to a P_wO_2 of 47 mmHg ($P > 0.05$), despite AS being reduced by at least 89% (Fig. 2D, E, F). In red drum, the first sign of reduced CT_{max} occurred at 35 mmHg ($P < 0.05$ compared to CT_{max} in normoxia), slightly below the P_{crit} at 30°C (38 mmHg).

The oxygen limit for thermal tolerance ($P_{CT_{max}}$), as calculated from the CT_{max} data, was 72.2 mmHg for lumpfish (Fig. 3A) and 35.8 mmHg for red drum (Fig. 3B). These values were significantly different ($P < 0.001$).

All results, including fish body masses, are presented as means with associated standard errors in Table 1.

DISCUSSION

This study tested the hypothesis that the upper thermal limits of fishes are determined by insufficient oxygen supply for vital physiological functions and, therefore, affected by oxygen availability. More specifically, we tested the assumption that the fish's critical thermal maximum (CT_{max}) cannot be maintained under aquatic hypoxia where oxygen supply capacity (*i.e.*, AS) is reduced (cf. Fig. 1A, B). Contrary to this assumption, we found that the CT_{max} of both lumpfish and red drum was independent of oxygen availability over a wide range of water oxygen levels. In fact, the AS of lumpfish and red drum could be reduced by more than 72% and 89%, respectively, before CT_{max} was affected (Fig. 2). This shows that the upper thermal limits of water-breathing ectothermic animals are not always determined by oxygen supply capacity (cf. Fig. 1C, D).

In our experimental approach, we initially verified that MMR and AS were constrained in both lumpfish and red drum as water oxygen tension (P_wO_2) was lowered at a constant temperature, both within (10°C for lumpfish, 24°C for red drum) and slightly above (16°C for lumpfish, 30°C for red drum) the species' typical temperature range (Fig. 2A, B, D, E). This was simply a proof of concept, as the constraining effect of hypoxia on AS is a well-known phenomenon in fishes (Claireaux and Chabot, 2016; Claireaux et al., 2000; Farrell and Richards, 2009; Fry, 1971; Lefrancois and Claireaux, 2003). We then defined CT_{max} at water oxygen levels ranging from air-saturation (normoxia) to below the fish's critical oxygen tension (P_{crit}). In determining CT_{max}, we employed a 2°C h^{-1} warming protocol, which approximates the maximum heating rate that fish experience under natural conditions (Fangue et al., 2011; Gamperl et al., 2002; Loong et al., 2005). This rate is also similar to the rate of temperature change (1 to 4°C h^{-1}) used in other key studies investigating the role of oxygen limitations on the thermal tolerance of ectotherms (Eliason et al., 2011; Frederich and Pörtner, 2000; Giomi et al., 2014; Giomi and Pörtner, 2013; Melzner et al., 2006; Wittmann et al., 2008).

In lumpfish, the first sign of a reduced CT_{max} occurred slightly above P_{crit} at 16°C, whereas in red drum, CT_{max} was not reduced until slightly below P_{crit} at 30°C (Fig. 2C, F). Furthermore, the $P_{CT_{max}}$ of lumpfish was closer to normoxia than the $P_{CT_{max}}$ of red drum. Although the two species were acclimated to different temperatures, as befit the species, these data indicate that the lumpfish retained less oxygen supply capacity at CT_{max} in normoxia when compared to red drum. It has been suggested that species occupying a wide thermal range (niche) have been evolutionarily selected for a more thermally resistant cardiorespiratory system (Ern et al., 2014; Jost et al., 2012).

The differences in oxygen supply capacity at CT_{max}, observed here for lumpfish and red drum, may therefore be related to the ecology of the two species. The red drum is an athletic, free-swimming, eurythermal species inhabiting environments such as estuaries and coastal swamps that can fluctuate greatly in temperature over short temporal scales, whereas the lumpfish is a relatively sluggish, cold-water species with a more thermally stable niche (it lives in the coastal zone and in the open ocean). Despite these differences, the CT_{max} of both lumpfish and red drum decreased when P_wO_2 levels fell below 80 and 47 mmHg, respectively, in accordance with the theory that survival below P_{crit} is determined by the capacity for anaerobic metabolism, and is, therefore, time-limited.

Our findings add to a series of recent studies that have suggested that upper thermal limits in a number of water-breathing ectotherms are not determined by insufficient oxygen supply capacity. These studies, performed on a range of species, have found that AS is maintained in normoxia at environmentally-relevant temperature extremes (Ern et al., 2015; Gräns et al., 2014; Norin et al., 2014), or showed that experimentally induced anaemia (internal hypoxia) had very little effect on CT_{max} (Brijs et al., 2015; Wang et al., 2014). The present study bridges these earlier studies, and confirms their findings, by showing an uncoupling of AS and CT_{max} when fish are exposed to environmental (ambient) hypoxia. Our findings are also supported by previous studies which have reported reduced CT_{max} under severe hypoxia (Weatherley, 1970; Rutledge & Beitinger, 1989; Ellis et al., 2013; Healy & Schulte, 2012) and maintained CT_{max} under moderate hypoxia (Weatherley, 1970; Ellis et al., 2013). However, direct comparisons with our results are difficult because these studies either only measured CT_{max} at one (Rutledge & Beitinger, 1989; Healy & Schulte, 2012) or two (Weatherley, 1970; Ellis et al., 2013) levels of hypoxia, applied extremely high rates of temperature increase (18-90°C h^{-1}) (Weatherley, 1970; Rutledge & Beitinger, 1989; Healy & Schulte, 2012), allowed P_wO_2 to drift from normoxia to hypoxia during measurements (Ellis et al., 2013), or were performed on an air-breathing species with surface access (Rutledge & Beitinger, 1989).

Based on the species-specific dependence of CT_{max} on oxygen availability observed here (*i.e.*, red drum were more resilient to hypoxia than lumpfish), we propose a novel metric for classifying the oxygen-dependent thermal sensitivity of different species; the oxygen limit for thermal tolerance ($P_{CT_{max}}$). By measuring CT_{max} at a range of P_wO_2 levels from normoxia to below P_{crit}, an oxygen-dependent breakpoint can be established where CT_{max} is significantly affected by

hypoxia (Fig. 3A, B). Since $P_{CT_{max}}$ defines the P_wO_2 below which the upper thermal limits of water-breathing ectotherms becomes constrained, the breadth of the thermal niche under hypoxic conditions will increase with the distance between $P_{CT_{max}}$ and the P_wO_2 where AS starts to be reduced by hypoxia. If the latitudinal distribution ranges of marine fishes are shaped by their upper thermal limits (Sunday et al., 2012), then populations of species exhibiting oxygen-independent upper thermal limits at environmentally relevant P_wO_2 levels should be more resilient to the occurrence of environmental hypoxia and oxygen-poor 'dead' zones within their thermal niche. In addition to assessing the oxygen sensitivity of upper thermal limits in water-breathing ectotherms, and the susceptibility of their upper thermal niche boundaries to environmental hypoxia, we also suggest that the $P_{CT_{max}}$ metric can be used to assess the effects of anthropogenic pollutants and other climate change related stressors (*e.g.*, acidification and salinity changes) on the hypoxia sensitivity of upper thermal limits.

In conclusion, the CT_{max} of lumpfish and red drum was unaffected by hypoxia exposure down to P_wO_2 levels of 80 and 47 mmHg, respectively, despite more than 72 and 89% reductions in AS. Thus, constrained oxygen supply capacity did not affect the CT_{max} of these species under conditions of reduced oxygen availability that are routinely encountered by many fishes in the wild. However, increases in the frequency and severity of aquatic hypoxia may cause some species to experience P_wO_2 levels sufficiently low for CT_{max} to be reduced. The proposed $P_{CT_{max}}$ metric can help identify such vulnerable species. In general, our results show that oxygen and upper thermal limits are not intimately linked, thus reinforcing the idea that there are other important physiological constraints that determine the upper thermal limits of fishes.

Abbreviations

$\dot{M}_{O_2}$: Oxygen consumption rate (mg O_2 h^{-1} kg^{-1})

MMR: Maximum metabolic rate (mg O_2 h^{-1} kg^{-1})

SMR: Standard metabolic rate (mg O_2 h^{-1} kg^{-1})

RMR: Routine metabolic rate (mg O_2 h^{-1} kg^{-1})

AS: Aerobic scope (MMR − SMR) (mg O_2 h^{-1} kg^{-1})

CT_{max}: Critical thermal maximum (°C)

CT_{min}: Critical thermal minimum (°C)

T_{crit}: Critical temperature (°C)

P_wO_2: Water oxygen tension (mmHg)

P_{crit}: Critical oxygen tension (mmHg)

$P_{CT_{max}}$: Oxygen limit for thermal tolerance (mmHg)

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Competing interests

The author declares no competing or financial interests.

Author's contributions

R.E., T.N., and A.J.E. conceived the study. All authors participated in aspects of experimental design. R.E. and T.N. performed the experiments and collected the data. R.E., T.N., and A.J.E. analysed and interpreted the data. R.E. and T.N. co-wrote the initial draft, and A.J.E. and A.K.G. provided significant input with regards to revising the paper. All authors gave final approval for publication.

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Figures

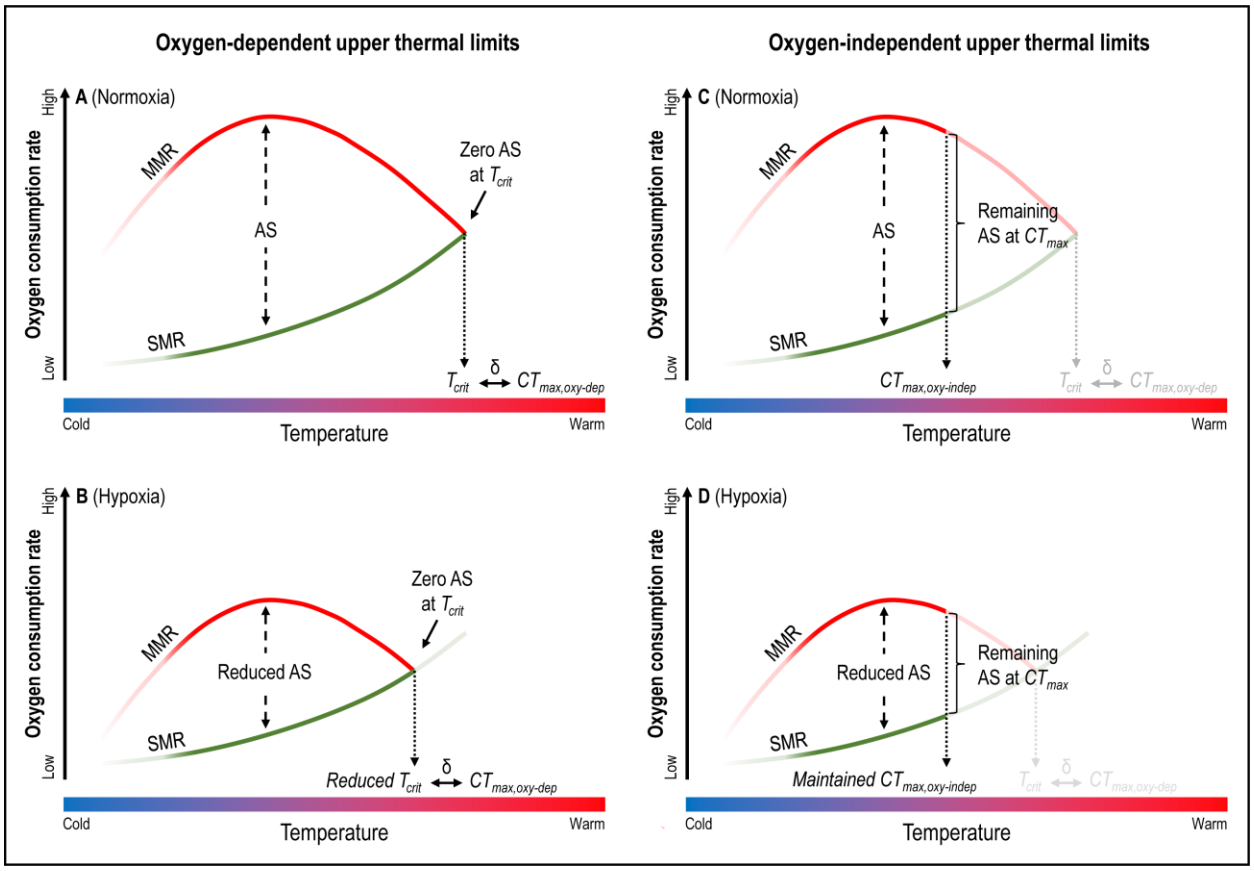

Fig. 1. Theoretical representation of the effects of temperature and hypoxia on maximum metabolic rate (MMR), standard metabolic rate (SMR), aerobic scope (AS) (*i.e.*, MMR – SMR) and critical temperature (T_{crit}) in (A, B) species with oxygen-dependent upper thermal limits ($CT_{max,oxy-dep}$) and (C, D) species with oxygen-independent upper thermal limits ($CT_{max,oxy-indep}$). In species with oxygen-dependent upper thermal limits, the exponential increase in SMR surpasses the temperature constrained MMR at T_{crit}. Above T_{crit}, aerobic SMR cannot be maintained and $CT_{max,oxy-dep}$ defines the temperature where ATP-deficiency becomes terminal (A). Because survival above T_{crit} is determined by the capacity to support energy demands through anaerobic metabolism, the distance from CT_{max} to T_{crit} (δ) should decrease with increasing temperature and increase with increasing temperature raping rate. Furthermore, any hypoxia-induced reduction in MMR will lower the temperature where MMR is surpassed by SMR,

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resulting in a reduced T_{crit} and $CT_{max,oxy-dep}$. Therefore, $CT_{max,oxy-dep}$ should decline with water oxygen tension (P_wO_2) upon exposure to any level of hypoxia sufficient to reduce MMR (B). In species with oxygen-independent upper thermal limits, the critical thermal maximum ($CT_{max,oxy-indep}$) is determined by the collapse of physiological functions not directly related to oxygen supply. Consequently, MMR is not surpassed by SMR at CT_{max} and a portion of AS remains at $CT_{max,oxy-indep}$ (C). If a hypoxia-induced reduction in MMR is not sufficient for MMR to be surpassed by SMR, then $CT_{max,oxy-indep}$ will remain unchanged in such species (D). Thus, in species with oxygen-independent upper thermal limits, $CT_{max,oxy-indep}$ should not decline with P_wO_2 upon exposure to hypoxia above the level required to reduce AS at $CT_{max,oxy-indep}$ in normoxia to zero.

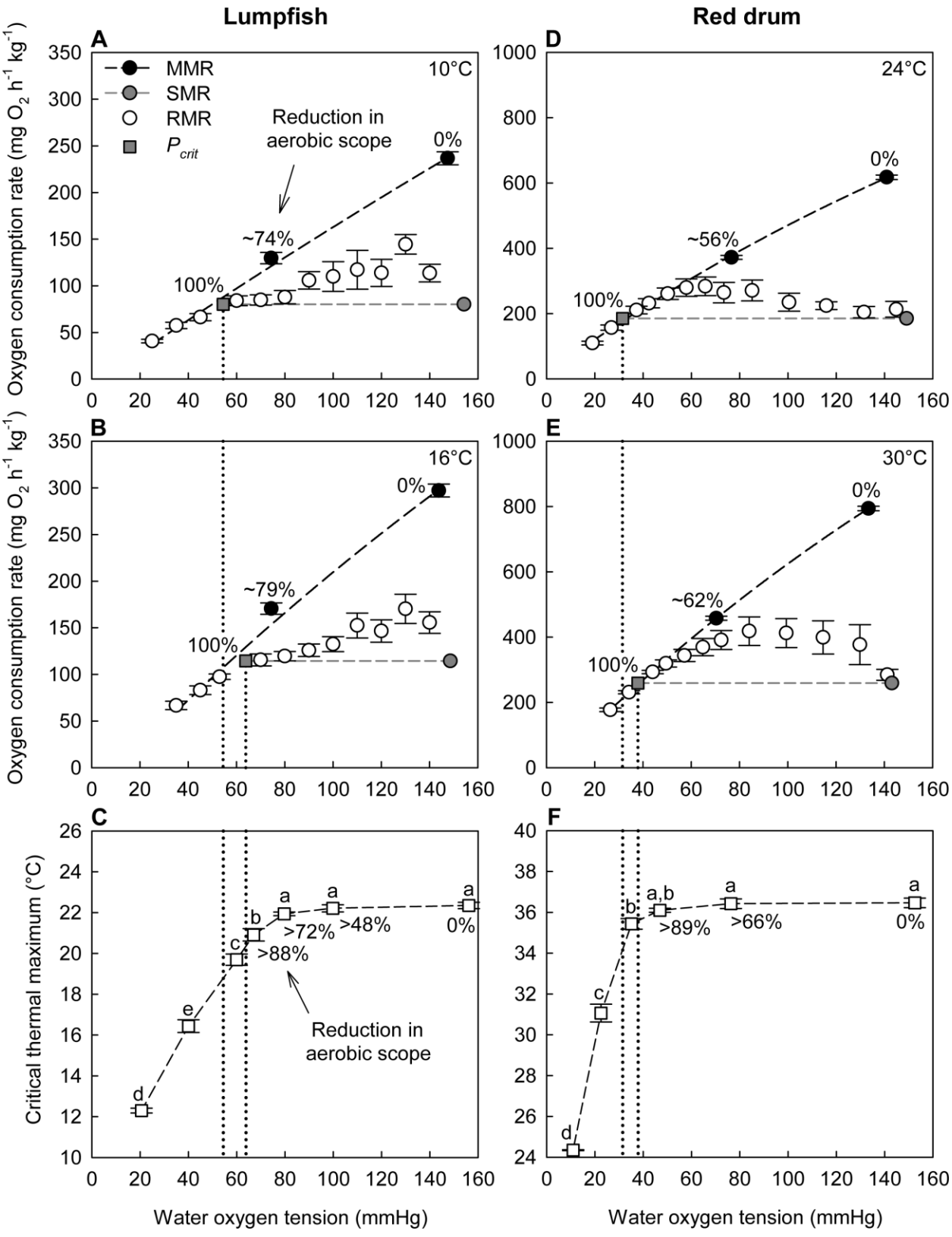

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Fig. 2. Effects of hypoxia on oxygen consumption rate ($\dot{M}_{O_2}$) and critical thermal maximum (CT_{max}) in (A, B, C) lumpfish (*Cyclopterus lumpus*) and (D, E, F) red drum (*Sciaenops ocellatus*). Data showing the depressing effect of hypoxia (reduced water oxygen tension, P_wO_2) on aerobic scope (AS) (maximum- minus standard metabolic rate; MMR − SMR) of lumpfish (*Cyclopterus lumpus*) (A, B) and red drum (*Sciaenops ocellatus*) (D, E) at two temperatures. Also shown is the critical oxygen tension (P_{crit}) of the two species, which is where AS is zero. Note that, at P_wO_2 levels above P_{crit}, routine metabolic rate (RMR) was greater than SMR in both species due to spontaneous activity. Despite the large reductions in AS, the CT_{max} of both species was maintained over a wide range of P_wO_2 values (C, F). In lumpfish acclimated to 10±1°C, CT_{max} at 80 mmHg was not significantly different from CT_{max} in normoxia (P < 0.001) despite a > 72% reduction in AS (C). In red drum acclimated to 22±1°C, CT_{max} at 47 mmHg was not significantly different from CT_{max} in normoxia (P < 0.001) despite a > 89% reduction in AS (F). Upper thermal limits were, therefore, independent of oxygen availability over this very broad P_wO_2 range. This means that the thermal sensitivity of physiological functions other than those governing oxygen supply capacity were primarily responsible for CT_{max} of both lumpfish and red drum until close to these species' P_{crit} (*i.e.*, these species follow the oxygen-independent model shown in Fig. 1C, D). Different lower-case letters indicate significant differences in CT_{max} between P_wO_2 levels (one-way ANOVA, P < 0.05). Values are means ± 1 s.e.m. (N = 8 in all groups; Table 1).

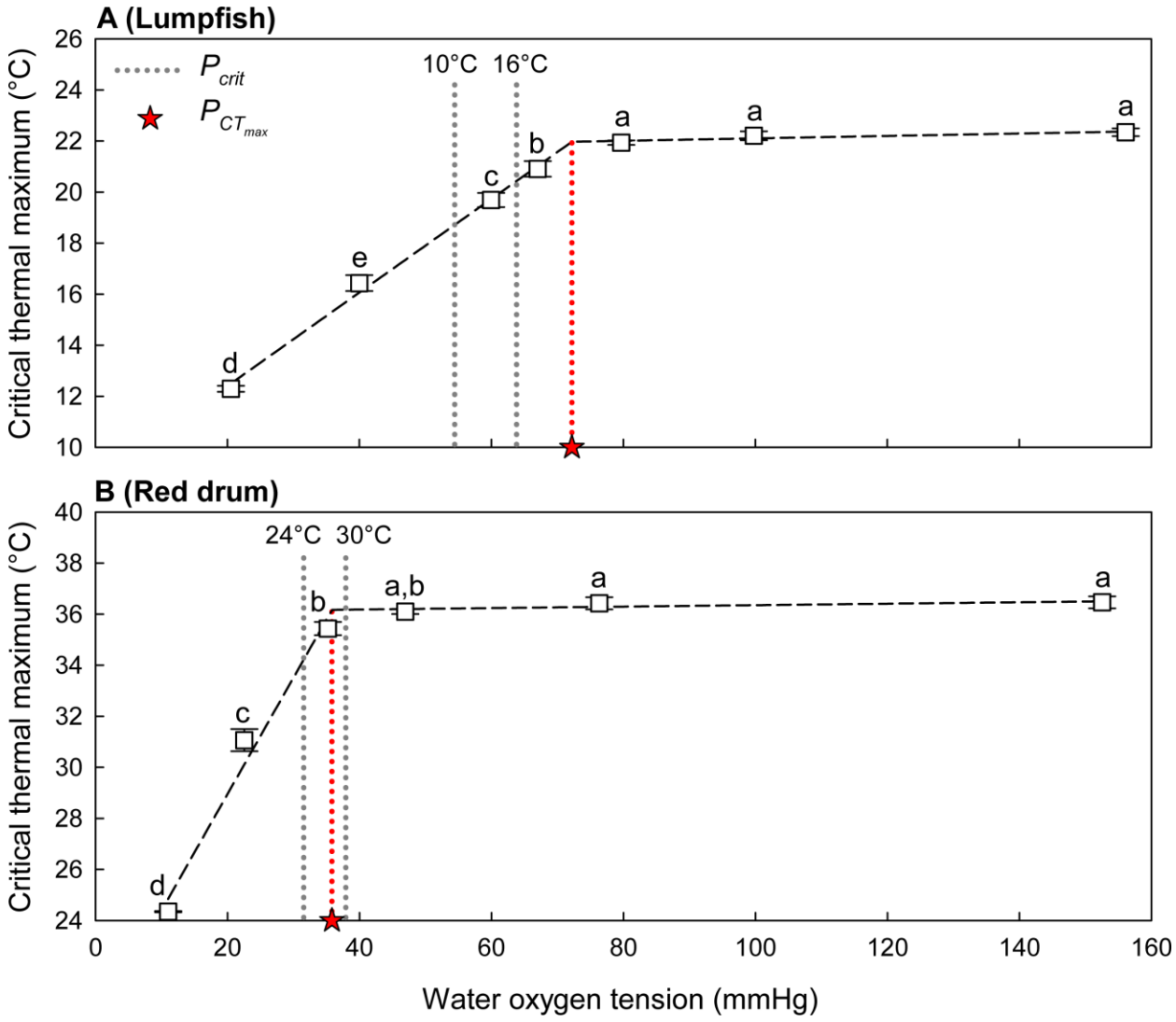

Fig. 3. The oxygen limit for thermal tolerance ($P_{CT_{max}}$) in (A) lumpfish (*Cyclopterus lumpus*) and (B) red drum (*Sciaenops ocellatus*) acclimated to 10±1ºC and 22±1ºC, respectively. $P_{CT_{max}}$ was determined by fitting a piecewise two-segmented linear regression through the critical thermal maximum (CT_{max}) values that were not significantly different from CT_{max} in normoxia, and the CT_{max} values that were significantly different from CT_{max} in normoxia. In both lumpfish and red drum, $P_{CT_{max}}$ values (72.2 ± 1.8 mmHg and 35.8 ± 2.4 mmHg, respectively) lie well below the water oxygen tension (P_wO_2) associated with large decreases in the fish's aerobic scope (Fig. 2A, B, D, E). The upper thermal limits of these two species, therefore, are oxygen-independent (as

illustrated in Fig. 1C, D) across a wide range of environmental hypoxia. Different letters indicate significant differences between P_wO_2 levels (one-way ANOVA, $P < 0.05$). Values are means ± 1 s.e.m. (N = 8 in all groups; Table 1). The critical oxygen tensions (P_{crit}) of lumpfish and red drum (cf. Fig. 2) are included for reference.

Table

Table 1. Maximum metabolic rate (MMR), standard metabolic rate (SMR), aerobic scope (AS), critical oxygen tension (P_{crit}), critical thermal maximum (CT_{max}), and the oxygen limit for thermal tolerance ($P_{CT_{max}}$) of lumpfish (*Cyclopterus lumpus*) and red drum (*Sciaenops ocellatus*). Included are the corresponding water temperatures (°C), water oxygen tensions (mmHg), and fish body masses (g). Different lower-case superscript letters indicate significant differences. Values are means ± 1 s.e.m. (N = 8 in all groups). Note: there are no body masses listed for AS and $P_{CT_{max}}$ as these parameters were calculated from the other data. Fixed experimental conditions are shown in regular font and measured parameters are presented in italics.

Lumpfish (*Cyclopterus lumpus*)	**Water temperature (°C)**	**Water oxygen tension (mmHg)**	**Oxygen consumption rate (mg O_2 h^{-1} kg^{-1})**	**Body mass (g)**
MMR	10 ± 0.1	147.4 ± 1.2	*236.7 ± 6.9*^{a}	23.0 ± 1.3
		74.3 ± 0.5	*129.6 ± 6.1*^{b}	22.7 ± 1.1
	16 ± 0.1	143.8 ± 1.0	*297.2 ± 11.4*^{a}	23.0 ± 1.2
		74.4 ± 0.5	*170.7 ± 8.6*^{b}	22.3 ± 1.1
SMR	10 ± 0.1	>148	*80.2 ± 3.7*^{a}	23.0 ± 1.5
	16 ± 0.1		*114.4 ± 5.2*^{b}	22.5 ± 1.7
AS	10 ± 0.1	147.4 ± 1.2	*156.6 ± 10.6*^{a}	-
		74.4 ± 0.5	*49.5 ± 9.8*^{b}	
	16 ± 0.1	143.8 ± 1.0	*182.9 ± 16.6*^{a}	
		74.4 ± 0.5	*56.3 ± 13.9*^{b}	
P_{crit}	10 ± 0.1	*54.4 ± 5.4*^{a}	-	23.0 ± 1.5
	16 ± 0.1	*63.8 ± 5.5*^{b}		22.5 ± 1.7
CT_{max}	*22.3 ± 0.2*^{a}	156.1 ± 2.5	-	22.7 ± 0.9
	22.2 ± 0.2^a	99.8 ± 2.5		21.4 ± 0.6
	21.9 ± 0.1^a	79.7 ± 2.5		23.2 ± 1.1
	20.9 ± 0.3^b	67.0 ± 2.5		22.9 ± 1.4
	19.7 ± 0.3^c	59.9 ± 2.5		21.6 ± 1.0
	16.4 ± 0.3^d	40.0 ± 2.5		23.6 ± 0.6
	12.3 ± 0.1^e	20.5 ± 2.5		22.1 ± 0.8
$P_{CT_{max}}$	-	*72.2 ± 1.8*	-	-

Red drum (*Sciaenops ocellatus*)	**Water temperature (°C)**	**Water oxygen tension (mmHg)**	**Oxygen consumption rate (mg O_2 h^{-1} kg^{-1})**	**Body mass (g)**
MMR	24 ± 0.1	140.9 ± 0.8	*617.8 ± 18.2*^{a}	46.2 ± 3.8
		76.6 ± 1.0	*372.4 ± 14.6*^{b}	45.9 ± 3.7
	30 ± 0.1	133.4 ± 0.6	*794.2 ± 43.7*^{a}	42.4 ± 2.4

		70.3 ± 1.0	*458.1 ± 14.0*[b]	42.2 ± 2.3
SMR	24 ± 0.1	>145	*185.1 ± 13.2*[a]	35.7 ± 1.2
	30 ± 0.1		*259.1 ± 10.3*[b]	40.0 ± 2.0
AS	24 ± 0.1	140.9 ± 0.8	*432.8 ± 31.3*[a]	-
		76.6 ± 1.0	*187.3 ± 27.7*[b]	
	30 ± 0.1	133.4 ± 0.6	*535.1 ± 54.0*[a]	
		70.3 ± 1.0	*199.0 ± 24.3*[b]	
P_{crit}	24 ± 0.1	*31.5 ± 1.7*[a]	-	35.7 ± 1.2
	30 ± 0.1	*37.9 ± 1.2*[b]		40.0 ± 2.0
CT_{max}	*36.5 ± 0.2*[a]	152.5 ± 2.5	-	41.5 ± 2.1
	36.4 ± 0.2[a]	76.3 ± 2.5		40.1 ± 1.7
	36.1 ± 0.1[a,b]	46.9 ± 2.5		38.5 ± 1.8
	35.4 ± 0.3[b]	35.2 ± 2.5		44.4 ± 3.1
	31.1 ± 0.4[c]	22.5 ± 2.5		39.1 ± 2.3
	24.4 ± 0.1[d]	11.0 ± 2.5		42.6 ± 3.9
$P_{CT_{max}}$	-	*35.8 ± 2.4*	-	-