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Effect of temperature on the metabolism, behaviour and oxygen requirements of *Sparus aurata*

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ABSTRACT: We investigated the effect of temperature on the limiting oxygen saturation (LOS) of gilthead sea bream *Sparus aurata*. This threshold was defined as the % O_2 saturation where fish no longer upheld their routine metabolic rate (RMR, the metabolic rate of fed and active fish) during a progressive decline in oxygen saturation. *S. aurata* (398 ± 10 g, mean ± SE) were kept in 3 replicate tanks and subjected to 3 changes in temperature: 16 to 20°C, 20 to 16°C and 16 to 12°C. At each temperature, fish were left to acclimatize for 8 to 10 d, before daily feed intake (DFI), the routine oxygen consumption rate (routine MO_2, mg kg^{-1} min^{-1}) and the LOS were measured. In addition, at 20°C the swimming speed was measured in fish subjected to a decline in O_2 from full air saturation to levels below the LOS (minimum of 8–10 % O_2). For the temperature range tested (12–20°C), DFI, MO_2 and LOS increased exponentially with temperature (7.5-, 3.6- and 2.2-fold, respectively) with mean (± SE) LOS being 17 ± 1, 21 ± 0 and 35 ± 5 % O_2 at 12, 16 and 20°C, respectively. A gradual decline in swimming activity was observed as O_2 declined below the LOS, indicating increasing metabolic stress and/or a 'sit-out' coping strategy which may prolong survival time in severe hypoxia. The results show the importance of temperature as an influential variable over the environmental O_2 requirements of *S. aurata*.

KEY WORDS: Hypoxia · Aquaculture · Metabolism · Behaviour · P_{crit} · S_{crit} · Oxygen threshold · Feeding rate · Temperature

INTRODUCTION

Gilthead sea bream *Sparus aurata* is an important aquaculture species in countries surrounding the Mediterranean Sea, with 154 000 t produced globally in 2011 (FAO 2013). The growth phase is primarily carried out in floating sea cages (Basurco et al. 2011) where fish performance and welfare are closely linked to environmental conditions within the sea cage (Fry 1971, Huntingford & Kadri 2008). The sea cage oxygen (O_2) level is particularly important as it is the main limiting factor of fish aerobic metabolism (Fry 1947, 1971). Studies in Atlantic salmon sea cages have revealed that O_2 may drop to alarmingly low levels, at times down to 30 % O_2. Factors contributing to these low O_2 levels include water temperature, fish stocking density, algal density and water exchange rate (Crampton et al. 2003, Johansson et al. 2006, 2007, Oppedal et al. 2011). There is currently little information on the sea cage O_2 levels in *S. aurata* aquaculture, but variation in the above mentioned factors is expected to cause variable levels of O_2 in *S. aurata* production systems as well. In order to develop production strategies

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that ensure proper physiological function and survival of the fish, it is important to define a limit for acceptable drops in O_2.

When subjected to a gradual decline in O_2, sparid species are able to uphold their oxygen consumption rate (MO_2) over a relatively wide range of O_2 saturations (Cerezo & García García 2004, Valverde et al. 2006). They do this mainly by increasing gill ventilation and perfusion (Perry et al. 2009). At a certain level of O_2, regulatory mechanisms are no longer sufficient to uphold MO_2 and it starts to decrease with further reductions in O_2 (Pörtner & Grieshaber 1993). This threshold is termed the critical oxygen saturation (S_{crit} or P_{crit} if O_2 is presented in units of gas pressure, when determined in resting, fasted fish at a known, stable temperature, i.e. in fish with standard metabolic rates (Schurmann & Steffensen 1997, Claireaux & Lagardère 1999, Behrens & Steffensen 2007). However, when this threshold is determined in fed fish engaged in voluntary swimming activity, i.e. fish with routine metabolic rates (RMRs), this threshold has been termed the limiting oxygen saturation (LOS). The LOS departs from S_{crit} in being a continuum which correlates with RMR at a given temperature (e.g. Claireaux et al. 2000, Claireaux & Lagardère 1999, Remen et al. 2013). The LOS may therefore change according to swimming speed (Steinhausen et al. 2010), specific dynamic action (SDA; the post-prandial increase in metabolism; reviewed by Secor 2009) or other factors that cause variation in RMR. In the present paper, the threshold O_2 level below which MO_2 decreases is presented as LOS, since it is determined in fed *S. aurata* engaged in voluntary swimming activity, and because units of oxygen saturation (% of air saturation) are most commonly used by aquaculturists.

When O_2 decreases below the LOS, the fish must increase their anaerobic metabolic rate to compensate for the decreasing ATP production (reviewed by Pörtner & Grieshaber 1993). Anaerobic ATP production is far less efficient than aerobic ATP production, and the duration of survival becomes dependent on the availability of substrates for anaerobiosis (Richards 2009, 2011). Further, a drop in O_2 to levels below the LOS can be expected to induce stress (increased plasma levels of corticosteroids and catecholamines) and lactic acidosis (Van Raaij et al. 1996, Vianen et al. 2001, Petersen & Gamperl 2011, Remen et al. 2012, 2013). The LOS can therefore be implemented in aquaculture as the lower limit for acceptable O_2 levels with regard to the physiological function and welfare of farmed fish (Huntingford & Kadri 2008).

The LOS is expected to increase with any factor that increases the aerobic metabolic rate (Fry 1971, Neill et al. 1994). One of the main factors influencing metabolic rate is temperature (Fry 1947, 1971), which is closely correlated with LOS and S_{crit} (e.g. Schurmann & Steffensen 1997, Claireaux & Lagardère 1999, Barnes et al. 2011, Richards 2011, Mamun et al. 2013, Remen et al. 2013). In order to develop an applicable oxygen threshold for the *S. aurata* industry, it is therefore necessary to investigate the relationship between temperature and LOS for this species. Further, it is important to do so under conditions as similar to production conditions as possible (fed, undisturbed and swimming fish in groups), as RMR, and thus the LOS, can be expected to vary considerably, e.g. with meal size (e.g. Guinea & Fernandez 1997) and swimming speed (e.g. Steinhausen et al. 2010) at a given temperature.

The main aim of this study was to determine the relationship between temperature (12–20°C) and LOS for *S. aurata*. To mimic an aquaculture setting, LOS was determined in fed and undisturbed fish. Further, swimming speed and behaviour of *S. aurata* at O_2 declining from full saturation to levels below the LOS were investigated in order to study the behavioural response of this species to a progressive drop in O_2.

MATERIALS AND METHODS

All experimental work was conducted in accordance with the laws and regulations for experiments and procedures on live animals in Norway, following the Norwegian Regulation on Animal Experimentation 1996. The experiment was approved by Forsøksdyrutvalget (FOTS ID 4580).

Fish material and experimental facilities

Sparus aurata juveniles (5 g) were purchased from Ferme Marine de Douhet hatchery, Ile d'Oléron, France, and transported to the Institute of Aquaculture Torre de la Sal in Spain in July 2011. Fish were fed to satiety (EFICO YM 554, BioMar) and kept in an open flow system (salinity 37.5 ppt) with natural photoperiod and water temperature until they reached 150 g. They were then transported to Matre Research Station, Institute of Marine Research in Norway in January 2012. No fish died during transport. On arrival, a total of 99 fish were distributed into three 500 l squared flow-through experimental tanks with

lids fitted with 18W fluorescent light tubes and automatic feeders (Arvo-Tec T drum 2000, www.arvotec.fi). Feed was provided twice daily during the entire experiment (Amber Neptun 100, 5 mm, Skretting, Norway), aiming for >30% overfeeding. Spill feed was weighed, and feed intake was estimated according to the method of Helland et al. (1996). Calculations for estimation of the tank biomass over time are shown in 'Calculations and statistics' below. Fish were kept on a 12:12 h light:dark h cycle at 34 ppt, 16°C and O_2 saturation > 80% until fish reached a size of 397 ± 10 g (mean ± SE) at the start of the experiment on 13 July 2012 (Table 1).

Temperature and oxygen were continuously monitored using Oxyguard 420 probes mounted inside the tanks (accuracy of ±0.2% O_2, Oxyguard International), and logged every 31 s using the Commander software (OxyGuard). In each tank, a camera (VN-SVUC-MINI, Seavision, SubSea) was attached to the tank wall approx. 20 cm below the water surface, directed outwards into the tank. Daily inspections of the tanks and recordings of fish behaviour were managed using Hikvision capture card and DVRserver V6.67 software (Hikvision).

Experimental set-up

All fish were anaesthetized (MS-222, 150 mg l^{-1}, Fluca Analytical, Sigma-Aldrich), weighed (to the nearest g) and measured (cm fork length) at the start of the experimental period (Day 0, Table 1). During the experimental period, fish in all 3 tanks were subjected to 3 changes in temperature: 16 to 20°C (Days 1–2), 20 to 16°C (Days 12–13) and 16 to 12°C (Days 20–21). This regime was chosen in order to minimize the change in temperature at each temperature adjustment, and the temperature range (12–20°C) was used because 12°C is a minimum temperature for growth (Mingarro et al. 2002, Hernández et al. 2003) and because 20°C was the maximum temperature possible in the laboratory. The change in temperature was changed in a stepwise manner at a rate of 2°C d^{-1}. At each new temperature (20, 16, 12°C) the fish were left to acclimatize for 8 to 10 d before measurements of MO_2 and LOS (Days 12, 20 and 28, respectively). After the final LOS measurement on Day 28, all fish were anaesthetized (MS-222, 150 mg l^{-1}) and weights and lengths measured. They were then returned to the tanks and kept for another 12 d at 20°C before the final study of swimming speed during a decline in O_2 at 20°C. This temperature was chosen because the activity level in terms of feeding was highest (see below for details on swimming speed measurements). After this, anaesthetized fish were killed by a blow to the head and the weights and lengths of all fish were recorded (Table 1).

Table 1. Overall mean (± SE) gilthead sea bream *Sparus aurata* weights (*W*, g), lengths (*L*, cm fork length), condition factor (*K*, calculated as 100 W/L^3) and total number of fish (N) in the 3 replicate tanks at the start of the experiment (Day 0), on Day 28 and on Day 45. Two fish from 1 of the replicate tanks were taken out on Day 28 due to their low weights (approximately 100 g), and 5 fish from each tank were taken during blood sampling on Day 35

Day	*W* (g)	*L* (cm)	*K*	N
0	397 ± 10	25.0 ± 0.2	2.3 ± 0.0	82
28	421 ± 15	26.4 ± 0.2	2.3 ± 0.0	82
45	485 ± 8	27.2 ± 0.2	2.3 ± 0.0	65

Measurements of routine MO_2 and LOS

The tank water level was recorded 1 d before measurements started. Fish were fed as usual in the morning, and MO_2 measurements were initiated 1.5 h later in order to achieve a measure of routine MO_2. The water O_2 level within the tank was raised to 120–140% O_2 by adding oxygen-supersaturated water (~400% O_2), the flow in and out of the tanks was stopped, and the O_2 level was allowed to decline as a result of fish respiring within the tank. This continued until the rate of O_2 decline was visibly lowered, suggesting that the MO_2 of the fish was depressed and the LOS had been surpassed (a more accurate estimation of LOS was performed later on, see below). The duration of this period ranged from 2 h at 20°C to 5 h at 12°C, and O_2 saturation was between 7 and 10% at the end. Water in- and outflow was then restored. During the period with no water exchange, there is an inevitable build-up of wastes (CO_2, ammonia etc.), which occurs at a rate that corresponds to the MO_2 of the fish. As this is also the case in an aquaculture situation where a drop in O_2 is caused by fish/algae/bacteria consumption, no attempt was made to correct for a possible effect of such wastes on fish MO_2.

The oxygen influx over the water surface during the progressive decline in oxygen was examined by measuring the O_2 change over time in a tank without fish with 16°C seawater that had been oxygen-stripped using N_2 gas (O_2; 11% of air saturation). In a similar situation to the experiment, there was no

water entering or leaving the tank, and the circulation of water caused by fish movements was mimicked using a recirculation pump connected to the inlet (EHEIM Universal pump 1250, 20 l min^{-1}). The influx of O_2 to the water volume through diffusion (O_2 influx, mg O_2 $l^{-1}min^{-1}$) was modelled as:

$$O_2 \text{ influx} = k\,(\alpha - C_t) \quad (1)$$

where k is the diffusion constant, α is oxygen solubility (mg l^{-1}) at the prevailing temperature and salinity, and C_t is the oxygen concentration (mg l^{-1}) within the tank at time t. The diffusion constant, k, was estimated to be 0.0013275, by finding the value of k that maximized the correlation between the observed and modelled increase in oxygen saturation after oxygen-stripping (R^2 = 0.99). It should be noted that the O_2 influx depends on the circulation of water within the tank. With no circulation, the contribution of O_2 influx on the change in tank C_t was negligible, but a significant contribution was found when introducing the recirculation pump. The pump created a circulation considered to be noticeably higher than what can be achieved by a group of fish swimming in the tank (water current speed ranging between 7 and 23 cm s^{-1} depending on the distance from the tank centre), and the real value of k can therefore be expected to be somewhere in the range of 0 to 0.0013275. The estimated LOS did not change as a result of k varying in the range of 0 to 0.0013275, but MO_2 did. The effect of k on MO_2 increased with the duration of no inflow (from 2 h at 20°C to 5 h at 12°C), resulting in an increase in normoxic MO_2 (O_2 between LOS and 100% O_2) equivalent of 4 ± 1, 10 ± 1 and 18 ± 1% at 20, 16 and 12°C, respectively, using a k = 0.0013275 compared to using a k = 0. This corresponds to an absolute change in MO_2 equivalent to 0.15–0.18 mg O_2 kg^{-1} min^{-1}, a possible overestimation that was considered within acceptable limits for a measure of routine MO_2. The routine MO_2 (mg O_2 kg^{-1} min^{-1}) was calculated as

$$MO_2 = V\,(C_{t-1} - C_t + O_2 \text{ influx}) / BM \quad (2)$$

where V is the tank volume (~460 l), C_t is the oxygen concentration (mg l^{-1}) at time t, C_{t-1} is the oxygen saturation 1 min earlier, and BM is the biomass (kg).

In order to find the LOS and the normoxic MO_2, 5 min (20°C) or 10 min (12 and 16°C) averages of MO_2 were plotted against the average O_2 saturations measured during the time intervals. A broken-line regression model was fitted to the data, and the curve break-point, representing the LOS, was established using the 'segmented' package in R 2.14.0 (The R Foundation for Statistical Computing © 2011, www.r-project.org). This method simultaneously estimates slope parameters and the turning point(s) within a standard linear model framework (Muggeo 2008). Only MO_2 values for $O_2 \leq 100$% of air saturation were used, and the normoxic MO_2 was determined by averaging MO_2 at O_2 above the LOS. A Davies test was used to test for a difference in slopes, and break-points were considered valid when the p-value returned by the test was ≤0.05 (Muggeo 2008).

Swimming speed measurement

During the decline in O_2 at 20°C (Day 45), swimming speeds were recorded every 10 min using cameras submerged halfway in the water column and placed in 1 corner of the tank, pointing towards the opposite corner of the squared, 500 l tank. The inlet pipe in the opposite corner was used as a reference point for measuring the swimming speed (body lengths per second, BL s^{-1}; Fig. 1). This was done by measuring the time it took for a fish to pass the pipe (snout to tailfin), and was recorded for 10 fish at the start of every 10 min period during the decline in O_2 (down to 8–10% O_2). Only fish swimming close to the pipe were chosen in order to avoid an effect of the distance between the camera and the fish. All measurements were performed within the first 2 min of the 10 min period. The swimming speed mea-

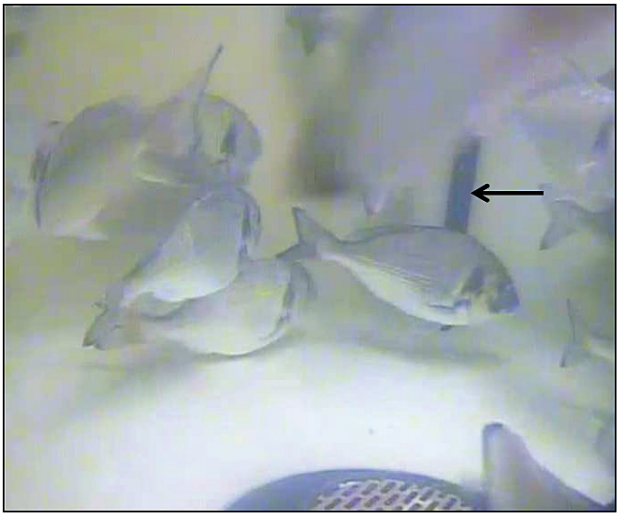

Fig. 1. Measurements of gilthead sea bream *Sparus aurata* swimming speed during declining O_2 were performed by analysing videos from cameras submerged in the tanks. The time it took for a fish to pass (snout to tail fin) the right side of the inlet pipe (arrow) was noted and swimming speed was recalculated to body lengths per second

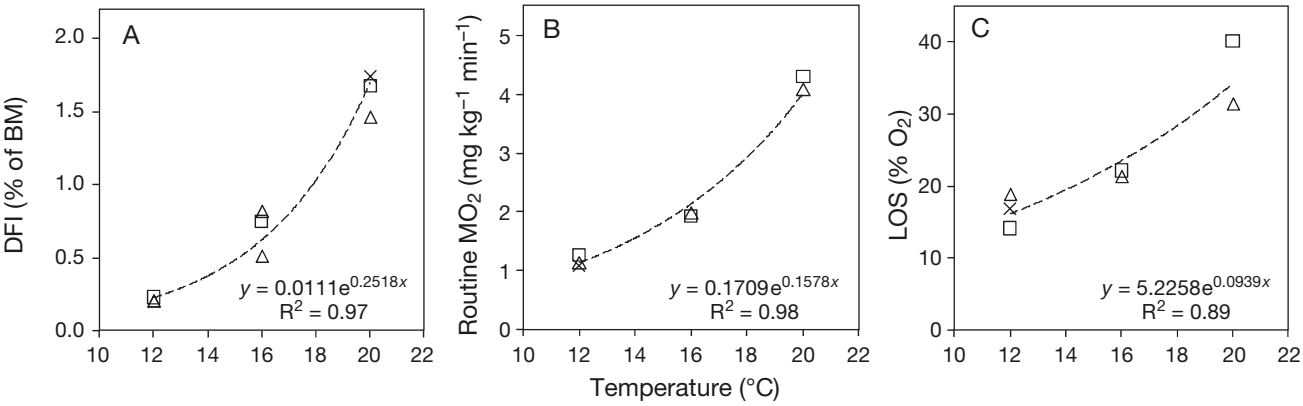

Fig. 2. Relationship between temperature and (A) the daily feed intake (DFI, % of biomass, BM, measured on the day before limiting oxygen saturation, LOS, measurement), (B) the routine oxygen consumption rate (routine MO_2, mg O_2 kg^{-1} min^{-1}) and (C) the LOS (% of air saturation) in 3 replicate tanks with groups of undisturbed, fed and freely swimming adult gilthead sea bream *Sparus aurata*. Each replicate tank is given a separate symbol in the graphs (note that one replicate is missing for MO_2 and LOS measurements at 16 and 20°C). Measurements at 20, 16 and 12°C were performed on Days 12, 20 and 28, respectively, after 8 d of temperature acclimation. In all panels, exponential trend lines are fitted to the data, and the resulting equations are shown

surement was performed in 2 tanks only, as water leakage in the third tank led to reduced tank water level.

Calculations and statistics

The feed conversion ratio (FCR) was calculated as:

$$FCR = FI / (BM_{end} - BM_{start}) \quad (3)$$

where FI represents the total feed intake (g) during the period in question, BM_{end} is the biomass (g) at the end of this period, and BM_{start} is the biomass (g) at the start of the period. The total feed intake and the tank biomass measured on Days 0, 28 and 45 were used to calculate FCR for Days 0 to 28 and Days 29 to 45.

Daily feed intake (DFI, % of BM) was calculated according to the method described by Helland et al. (1996). The daily biomass, $BM_{day\ n}$ (g), was approximated using the FCR and the DFI (g) according to the following formula:

$$BM_{day\ n} = (BM_{day\ n-1} + DFI_{day\ n-1}) / FCR \quad (4)$$

R (2.14.0 The R Foundation for Statistical Computing © 2011) was used to test for a difference in slopes (Davies test), for the 2 lines representing MO_2 at O_2 above and below the LOS (Muggeo 2008). All further statistical tests were performed using Statistica© (StatSoft). Fixed non-linear regression ($y = ae^{bx}$) was used to test the non-linear relationships between temperature and the parameters DFI, MO_2 and LOS. A significance level of 5% was used for all tests.

RESULTS

Effect of temperature on DFI, routine MO_2 and LOS

DFI and routine MO_2 increased exponentially with temperature (fixed non-linear regression, $p < 0.02$, $R^2 > 0.97$, Fig. 2A,B). Mean DFI (± SE) was 0.22 ± 0.01, 0.70 ± 0.09 and 1.63 ± 0.08% of BM, and the mean routine MO_2 was 1.2 ± 0.1, 1.6 ± 0.4 and 3.4 ± 0.8 mg O_2 kg^{-1} min^{-1} at 12, 16 and 20°C, respectively.

Broken-line regression models were significant for all plots of routine MO_2 against ambient O_2 for the 3 temperatures tested (Davies test, $p < 0.001$, $R^2 > 0.93$). LOS increased exponentially with temperature (fixed non-linear regression, $p < 0.01$, $R^2 = 0.89$), being 17 ± 1, 22 ± 0 and 36 ± 4% O_2 at 12, 16 and 20°C, respectively (Fig. 2C). One replicate is missing from both 16 and 20°C measurements, due to a small water leak from the tank during measurements.

Behaviour during declining O_2

The swimming speed of *Sparus aurata* during a progressive decline in O_2 at 20°C is shown in Fig. 3A,B. There was no change in swimming speed during the period of time when ambient O_2 dropped towards the LOS. However, a steady decline in swimming speed was observed as O_2 continued to decline below this threshold. No fish rested on the tank bottom, tried to escape, performed air-surface respiration or lost equilibrium during the decline in O_2 to levels below

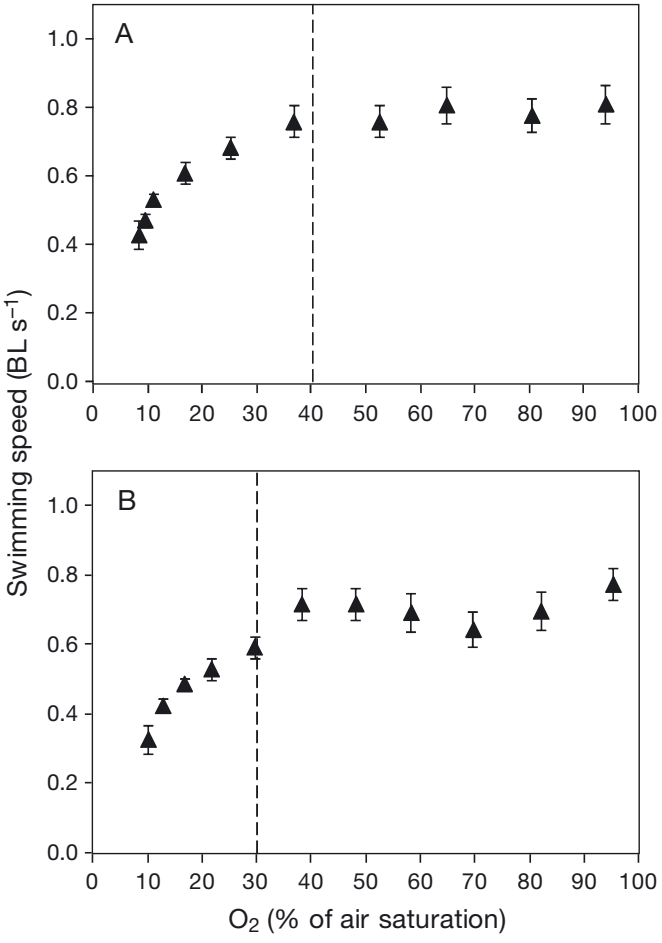

Fig. 3. Mean (± SE, n = 10) swimming speeds (body lengths, BL, s^{-1}) of gilthead sea bream *Sparus aurata* during a progressive decline in O_2 in 2 replicate tanks (A and B) at 20°C. The limiting oxygen saturation levels found for these 2 replicate tanks are represented as vertical broken lines. Swimming speed was measured every 10 min during the decline in O_2

the LOS. Furthermore, the fish did not show any startle response when the tank lid was lifted after the end of the trial, compared to overt responses during normoxic conditions.

DISCUSSION

This study is the first to present the relationship between temperature (12–20°C) and the RMR of *Sparus aurata* around harvest size (~400 g), and the resulting increase in the minimum O_2 required to uphold their RMR (the LOS; 17–36% O_2). When O_2 declined below the LOS, the swimming speed of the fish started to decrease.

Effect of temperature on RMR and LOS

As expected, MO_2 increased with temperature (Guinea & Fernandez 1997, Ibarz et al. 2003, Libralato & Solidoro 2008). As temperature increases, biochemical reactions proceed faster (Angilletta et al. 2002), and the metabolic cost of maintenance increases (Fry 1947, 1971). The capacity for feeding and growth increases with temperature until the thermal optimum is reached, and decreases thereafter (reviewed by Fry 1947, 1971, Farrell 2009, Pörtner 2010). The low feed intake and RMR observed at 12°C is in line with suggestions by Ibarz et al. (2003) that this temperature represents a lower limit for suitable temperatures in *S. aurata* aquaculture. The highest temperature used in the present study (20°C) can be expected to be close to the optimal temperature for growth. Although higher temperatures of 24–26°C were found to maximize growth in the *S. aurata* growth model presented by Hernández et al. (2003), the same range (24–26°C) was considered lethal by Feidantsis et al. (2009). Temperatures up to 26°C may occur in the surface water of the Mediterranean during summer (Damianidis & Chintiroglou 2000), and further studies are therefore warranted in order to determine the relationship between temperature, RMR and LOS for the entire temperature range relevant for *S. aurata* aquaculture.

In line with previous observations, we found that the increase in RMR with temperature led to an elevation of the LOS (Schurmann & Steffensen 1997, Claireaux & Lagardère 1999, Valverde et al. 2006, Barnes et al. 2011, Richards 2011, Svendsen et al. 2012, Mamun et al. 2013, Remen et al. 2013). This confirms that temperature is an important factor to consider when determining oxygen thresholds. The LOS can be expected to increase with any factor that influences the aerobic metabolic rate of a fish (Fry 1971, Neill et al. 1994), and since the level of RMR is highly dependent on the feeding status of the fish (Guinea & Fernández 1997), both temperature and feed intake are likely to have influenced the LOS (Thuy et al. 2010). The contribution of SDA to the value of RMR, and thus the LOS, in the present study is not clear, since none of these parameters were measured in fasted fish. It may, however, be considered small at 12°C, due to the low feed intake (DFI = 0.21% of BM), and considerable at 20°C (DFI = 1.67% of BM). For the practical use of LOS in aquaculture, it is important to point out that LOS may be lower in fasted fish, particularly at the upper end of the temperature range tested.

Practical use of LOS as a limit for unacceptable drops in O_2

The LOS measured in the present study represents a minimum level of O_2 required to uphold RMR, maintain physiological integrity and ensure survival (Neill et al. 1994). No fish died during or after the 1 h progressive drop in O_2 below LOS in our study, suggesting that such a short-term exposure to O_2 levels below this threshold is not a threat to survival. It may be expected that survival is threatened during longer exposure, as a result of the rapid consumption of fuels, and the accumulation of anaerobic end-products (reviewed by Richards 2009, 2011). Further, a drop in O_2 below the LOS is expected to represent a stressor for the fish, resulting in elevated production of stress hormones (Van Raaij et al. 1996, Vianen et al. 2001, Petersen & Gamperl 2011, Remen et al. 2012, 2013). Unpublished results from the present study confirm that plasma lactate and cortisol increased in *S. aurata* after a progressive drop in O_2, declining below the LOS for the last hour. It is therefore suggested that LOS is a suitable limit for acceptable drops in O_2 in *S. aurata* aquaculture, with respect to the physiological integrity and welfare of the fish (Huntingford & Kadri 2008). It should be emphasized that higher levels of O_2 are expected to obtain fast growth and efficient production, as appetite is generally reduced at higher levels of O_2 than the LOS. For example, the appetite of post-smolt Atlantic salmon was found to be reduced at 70% O_2 at 16°C, while the LOS was found to be ~52% O_2 at the same temperature (Remen et al. 2012, 2013). In a general recommendation on suitable O_2 levels in sparid aquaculture, 80–85% O_2 was suggested as a minimum for proper feeding and growth (Mozes et al. 2011), but further studies are required in order to confirm this recommendation and to determine the possible effect of temperature on the O_2 required for maximal feeding and growth.

Effect of O_2 on swimming speed

The swimming activity of *S. aurata* gradually decreased as the O_2 level declined below the LOS, and no signs of agitation were observed. According to the species and the context, hypoxia may result in increased swimming activity (attempt to flee), a reduction ('sit out') in swimming activity (reviewed by Chapman & McKenzie 2009) or no change (Metcalfe & Butler 1984, van Raaij et al. 1996). The response of *S. aurata* to a progressive decline in O_2 suggests that this species copes with hypoxia by reducing its activity level, a response that may conserve energy and prolong survival time when hypoxia cannot be avoided (reviewed by Chapman & McKenzie 2009). A similar response has been seen in crucian carp *Carassius carassius*, eelpout *Zoarces viviparus*, white sturgeon *Acipenser transmontanus* and common sole *Solea solea* (Fischer et al. 1992, Nilsson et al. 1993, Dalla Via et al. 1998, Cech & Crocker 2002). In species such as Atlantic herring *Clupea harengus*, rainbow trout *Oncorhynchus mykiss* and red hake *Urophycis chuss* (Bejda et al. 1987, van Raaij et al. 1996, Domenici et al. 2000), attempts to flee from the situation seem to be more prevalent. However, since the reduction of swimming speed was only observed at O_2 below the LOS, it cannot be excluded that the reduction in swimming speed was a direct consequence of the increasing energy deficit experienced at these levels of O_2. Further experiments are therefore required to identify whether the observed lack of response to visual stimuli in swimming fish after 1 h below LOS is a 'sit-out' strategy or a sign of pronounced energy deficit and severe metabolic stress.

Conclusion and future perspectives

We investigated the effect of temperature (12–20°C) on feed intake and the RMR of *S. aurata* kept under conditions mimicking an aquaculture setting, and the resulting change in LOS. All measured parameters increased exponentially within the temperature range tested, with LOS being 17% O_2 at 12°C and 36% O_2 at 20°C. The LOS can be implemented in aquaculture as a lower limit for acceptable drops in O_2 with respect to physiological function and welfare of farmed *S. aurata*. Higher levels of O_2 are required for efficient production. During a progressive decline in O_2 below the LOS, *S. aurata* gradually reduced their swimming speed, indicative of increasing metabolic stress and/or a 'sit-out' coping strategy which may prolong survival time in severe hypoxia. The feed intake at 12–20°C suggests that growth is minimal at the lowest temperature, and that high feeding rates and fast growth can be expected at temperatures ≥20°C. Further studies are required to determine the relationship between temperature, MO_2 and LOS for the entire temperature range experienced by *S. aurata* in sea cages, i.e. up to 26°C.

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