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Individual aerobic performance and anaerobic compensation in a temperate fish during a simulated marine heatwave

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Abstract

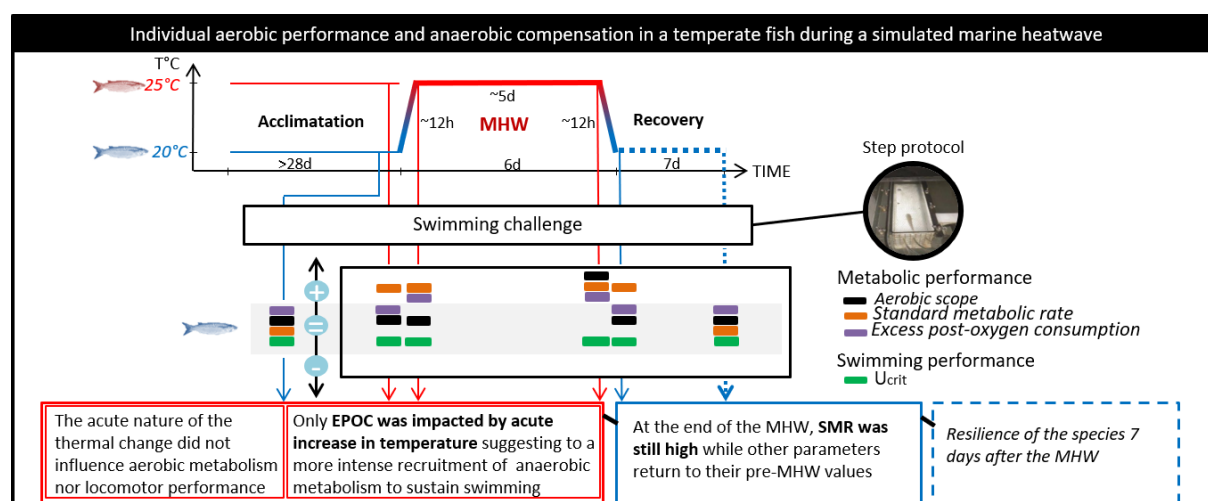
Marine heatwaves (MHWs) are becoming more frequent and intense due to anthropogenic climate change and have strong negative effects on ecosystem. For now, few studies have reproduced the complex nature of the temperature changes of a MHW, while it is suggested that ectotherms may be more vulnerable to rapid changes such as during MHWs. Effects of an experimental MHW were investigated in the golden gray mullet *Chelon auratus*. To do so, juveniles acclimated to 20°C were exposed to a rapid 5°C increase in temperature, followed by a five-day period at 25°C, before quickly returning to their initial temperature of acclimation. Metabolic variables (i.e. SMR-standard, MMR-maximum rate, AS-aerobic scope, EPOC-excess post-oxygen consumption) and critical swimming speed (i.e. U_{crit}) were measured at different phases of this MHW and after a thermally stable recovery phase.

Although the pattern was only significant for the SMR, the aerobic three variables describing aerobic metabolism (i.e. SMR, MMR and AS) immediately increased in fish exposed to the acute elevation of temperature, and remained elevated when fish stayed at 25°C for five days. A similar increase of these metabolic variables was observed for fish that were progressively acclimated to 25°C. This suggests that temperature increases contribute to increases in metabolism; however, the acute

nature of the MHW had no influence. At the end of the MHW, the SMR remained elevated, suggesting an additional cost of obligatory activities due to the extreme event. In parallel, U_{crit} did not vary regardless of the thermal conditions. Concerning EPOC, it significantly increased only when fish were acutely exposed to 25°C. This strongly suggests that fish may buffer the effects of acute changes in temperature by shifting to anaerobic metabolism.

Globally, this species appears to be able to cope with this MHW, but that's without taking into consideration future projections describing an increase in both intensity and frequency of such events, as well as other stressors like pollution or hypoxia).

Keywords: MHW, fish, metabolism, swimming performance



1. Introduction

Climate change is responsible for a gradual and global increase in temperature but also for the appearance of extreme climatic events such as marine heatwaves (MHWs), which are predicted to increase both in frequency and intensity by the end of the century under the actual pathway (IPCC 2021). MHWs have been defined several ways by the scientific community using different metrics (e.g. Selig et al. 2010; Sorte et al. 2010). In an attempt to facilitate comparisons and understanding of

their effects, Hobday et al. (2016) proposed that sea surface temperature anomalies should be defined as a MHW when they exceed the 90th percentile of a 30-year historical period for a given season and location. This consensual definition also includes a spatio-temporal dimension since a MHW must persist for at least five days and spread over thousands of square kilometers to be differentiated from a heat spike (Hobday et al. 2016). In the last decade, all major ocean basins have experienced at least one MHW (Frölicher and Laufkötter 2018), which becomes a complex and increasing subject of concern for ecosystem health (Smale et al. 2019).

MHWs have been shown to have a range of negative effects on ecosystems such as mass mortality events (Garrahou et al. 2009; Hughes et al. 2017; Seuront et al. 2019; Piatt et al. 2020), shifts in community composition, (Wernberg et al. 2013, 2016; Smale and Wernberg 2013), modifications of geographical distributions and decreases in the abundance of species (Sanford et al. 2019). When they are considered in addition to decade-scale changes in temperature, MHWs are expected to contribute to a decline of fishery stocks that are predicted to be decreased by half by 2050 (Cheung and Frölicher 2020). In this regard, Cavole et al. (2016) pointed out that the MHW that persisted from 2013 to 2015 in the Northeast Pacific has induced politico-socio-economic problems through fisheries disruptions to species of economic interest (e.g. losses following the closure of the Dungeness crab fishery from Washington to California in 2015-2016 were estimated at nearly \$48 million). Considering MHWs when determining the impact of climate change on marine species has therefore become essential. For decades, researchers have investigated the influence of progressive changes in mean temperature on marine organisms, particularly on ectotherms such as fish, whose physiology is temperature-dependent (Burel et al. 1996; Pörtner 2002a; Pörtner and Farrell 2008; Volkoff and Rønnestad 2020). However, as emphasized in Morash et al. (2018), ectotherms might be more sensitive to acute changes in temperature, indicating that the impacts of extreme events such as a MHW should receive increasing attention. Many of the studies on this topic employed the critical thermal maximum (CT_{max}) protocol (i.e a stepwise increase in temperature until the fish loses equilibrium) to assess the tolerance of individuals to an acute increase in temperature (e.g. Kikuchi et

al. 2019; Blasco et al. 2020). Recently, in order to specifically simulate temperature variation magnitude during a MHW, other studies exposed organisms to thermal shock following by a stabilization at an elevated temperature (McMahon et al. 2019; Madeira et al. 2020; Johansen et al. 2021). One study went even further by considering the capacity of organisms to both acclimate and then recover from a 10-day MHW once the temperature returned to its initial value (Minuti et al. 2021). In this study, carried out in the sea urchin *Heliocidaris erythrogramma*, the authors demonstrated an increase in mortality after the end of the MHW, probably a carry-over effect caused by the accumulation of physiological damages they observed during the extreme episode. This highlights the importance of evaluating the effects of these extreme thermal episodes through measurements both during and after their occurrence.

As it integrates oxygen supply capacity and energy conversion processes, the metabolic rate of a whole animal is viewed as a relevant parameter for the assessment of its general performance. Particularly, the aerobic scope (AS) represents the oxygen supply the individual may allocate to support energy-demanding activities and is commonly considered to be of ecological significance by the scientific community (e.g. Brett 1964; Fry 1971; Claireaux and Lefrançois 2007; Clark et al. 2013). AS is the difference between the maximum and the standard metabolic rate (MMR and SMR, respectively), which are both under the influence of temperature, notably because of its direct effect on the kinetics of biochemical reactions (Fry 1971; Claireaux and Lagardère 1999; Lefrançois and Claireaux 2003; Clark et al. 2013). SMR represents the costs of maintenance in inactive fish and is expected to exponentially increase with temperature (e.g. Lefrançois and Claireaux 2003, Chabot et al. 2016). On the other hand, MMR is the maximum capacity for oxygen uptake, which is also expected to increase before it levels off as the optimal temperature is approached (e.g. Rummer et al. 2014; Norin and Clark 2016). Variation in SMR and/or MMR may therefore influence the fish's ability to perform essential functions through modifications of the metabolic confines within which it must undertake its aerobic activities (Claireaux and Lefrançois 2007). For instance, several fish species have demonstrated a matching variation of AS and swimming performance assessed through

critical swimming speed (i.e. U_{crit} , the maximum velocity that a fish can attain when exposed to a swimming step protocol). For instance, Mahi-mahi *Coryphaena hippurus* exposed to an increase in acclimation temperature until 32°C showed a decrease in both AS and U_{crit} (Heuer et al. 2021). On the other hand, McMahon et al. (2019) observed a concomitant increase of these two variables when Australasian snapper *Chrysophrys auratus* variables were exposed to an extreme temperature event (i.e. +4°C). It is worth noting that as U_{crit} is approached during a swimming challenge, oxygen supply becomes limiting and swimming effort is progressively sustained by anaerobic metabolism in many species such as sockeye salmon *Oncorhynchus nerka* (Steinhausen et al. 2008), rainbow trout *Oncorhynchus mykiss* (Burgetz et al. 1998) and striped surfperch *Embiotoca lateralis* (Cannas et al. 2006), which in some cases may contribute to the decoupling of U_{crit} and AS (e.g. Kunz et al. 2018).

In the present study, the effects of a MHW were investigated by assessing biological variables in a teleost fish facing temperature variations occurring during such an event and after a thermally stable recovery phase. More specifically, we assessed metabolic performance in the temperate golden gray mullet *Chelon auratus* (Risso 1810), which was chosen because of its ecological relevance as a trophic vector of organic matter from intertidal to subtidal areas (Gautier and Hussenot 2005; Carpentier et al. 2014). - We conducted investigations by measuring aerobic and anaerobic metabolic variables and U_{crit} to gain an integrative view of the possible consequences of a MHW. *Chelon auratus* were exposed to an experimental MHW of a magnitude of 5°C, in agreement with what has been recorded in the Mediterranean basin during the summer extreme episode that occurred in 2003 (Hobday et al. 2016). To do so, fish went acutely from acclimation conditions of 20°C to a higher temperature of 25°C that was stabilized during five days, before returning to the initial thermal conditions for several days. We hypothesized that the new sudden, high thermal regime may led to an increase of maintenance costs and thereby of SMR. In addition, a limited increase of MMR was expected as this strong and sudden change in temperature could alter optimal functioning at the molecular or membrane level, and/or in organs involved in oxygen transport, such as the heart (e.g. Farrell 2002). Consequences on individual performances were assessed through the determination of U_{crit} and

anaerobic metabolism by measuring excess post oxygen consumption (EPOC, Lee et al. 2003) following the swimming challenge. Overall, this study investigates the biological effects of an experimental MHW and will provide information on the sensitivity of a temperate teleost fish to acute thermal changes, their short-term acclimation capacities, the carry over effects and/or their resilience capacities when faced such extreme events.

2. Materials and methods

2.1. Fish husbandry

Juvenile golden gray mullets were captured in salt marshes (L'Houmeau, France 46°12'14"N 1°11'42"W) with a fyke net. Fish were held in six different 400L indoor tanks (n = 15–30 individuals per tank) supplied with natural and aerated seawater for nine months. The water quality was maintained with an external biological and mechanical filter (Eheim professional 3 2080, Eheim, Deizisau, Germany) and 30% of the water was renewed each week. During this period, temperature and salinity were recorded three times a week using a WTW Cond 3310 multimeter (Xylem Analytics Germany GmbH) and varied from 18.3 to 20.5°C and from 26.2 to 30, respectively, which is similar to natural conditions encountered during summer in the area where the mullets have been caught. Oxygen level and nitrites were never under 80% of air saturation or above 0.3 mg.L⁻¹, respectively. Animals were fed three times per week in the morning with commercial food pellets (Neo Repro II, Le Gouessant, France) and exposed to a 12h light:12h dark photoperiod cycle. Experiments were carried out respecting regulations of the Animal Care Committee of France (ACCF) (APAFIS#25159-2019102913067306 v7; UMR7266 LIENSs approval number: 173002).

2.2. Temperature treatments

After the nine months of acclimatization to the laboratory environment, swimming and metabolic performances were individually assessed for 66 fish [mean weight ± standard error (SE): 13.3 g ± 5.5; mean total length ± SE: 11.4 cm ± 1.2]. To do this, fish were distributed in six holding tanks (n = 15–30 individuals per tank). Five holding tanks were maintained with a recirculating water system at an

acclimation temperature of $19.7^{\circ}\text{C} \pm 0.3$, [min= 18.9°C ; max= 20.5°C] and one tank at $25^{\circ}\text{C} \pm 0.2$, [min= 19.1°C ; max= 20°C]. A period of at least 28 days at these thermal conditions was respected before the beginning of the experiment.

In the following, exposure to $19.7^{\circ}\text{C} \pm 0.3$ is referred as *20* or *low temperature* conditions; and exposure to $25^{\circ}\text{C} \pm 0.2$ as *25* or *high temperature* conditions. In order to assess the effect of a 5°C -MHW lasting six days, a total of seven treatments were tested, and each fish was exposed to only one of these seven treatments: three control treatments (i.e. fish acclimated and tested at *low* or *high* acclimation temperatures), three MHW treatments (i.e. fish tested at different time of the MHW) and one recovery treatment (i.e. fish tested 7 days after having experienced the whole MHW).

Depending on the temperature treatment they experienced, fish were transferred from their holding tank to a swim tunnel (a) directly or (b) after having spent time in an intermediary aquaria (table 1).

Before each transfer, fish were starved 48 hours.

Protocol for temperature exposure of the seven treatments. The grey area symbolizes the whole 5°C - MHW lasting 6 days, which is composed of three phases: acute increase of temperature in ~ 12 h from 20°C to 25°C , 5 days of stabilization at 25°C , acute decrease of temperature in ~ 12 h from 25°C to 20°C , and that can be followed by a 7 days recovery phase at the initial temperature of 20°C . When the fish experienced one of these phases the conditions of temperature are indicated, otherwise \emptyset indicates that the fish was not exposed to the corresponding phase. All fish groups spent at least 28 days in a 400 L-holding tank for acclimation at 20°C or 25°C . *Trecov* group also spent seven days in the holding tank after experiencing the whole MHW. Three groups (*Control*₂₀₍₁₎, *Control*₂₅, *T*_{20:25(12h)}) were directly transferred from the holding tanks to the swim tunnel where the *T*_{20:25(12h)} group endured a 5°C -variation before the swimming challenge. Four groups spent time in an intermediary aquaria before to be tested in the swim tunnel (1) to endure the temperature challenge of 5°C -variation: *T*_{20:25-25(5d)}, *T*_{high} and *Trecov* or (2) to stay at the temperature of acclimation which allowed to test if there is an effect of the 5 days spent in the intermediary aquaria; it corresponds to the treatment *Control*₂₀₍₂₎ and is indicated by # in the table. Values outside the brackets are the mean temperature \pm sd. Values in brackets are the minimum and maximum temperature recorded during the temperature exposition. Values in parenthesis represent the rate of changing temperature. * and ** indicate the type of experimental set-up used for the temperature changes and/or for the temperature stabilization.

| | | Acclimation > 28 days | Different steps of the simulated MHW lasted ~ 6 days | | | Recovery 7 days | Swimming challenge ~ 6 hours |
|---------------------|---------------------------------|-----------------------------------|---|---------------------------|---|--------------------|------------------------------------|
| | | | Acute increase ~ 12 hours | Stabilization ~ 5 days | Acute decrease ~ 12 hours | | |
| Experimental set-up | | Holding tank | In an intermediary aquaria* or in the swim tunnel** | Intermediary aquaria | In an intermediary aquaria* or in the swim tunnel** | Holding tank | Swim tunnel |
| treatme | Control ₂₅ n = 10 | 25.0°C ± 0.2 [24.6°C ; 25.4°C] | ∅ | ∅ | ∅ | ∅ | 25.2°C ± 0.2 [24.7°C ; 25.5°C] |

| | | | | | | | |
|--------------------|---|--|---|-----------------------------------|---|-----------------------------------|-----------------------------------|
| | <i>Control</i> ₂₀₍₁₎ n = 13 | 19.7°C ± 0.3 [18.9°C ; 20.5°C] | ∅ | ∅ | ∅ | ∅ | 19.6°C ± 0.2 [19.3°C ; 19.9°C] |
| | <i>Control</i> ₂₀₍₂₎ n = 12 | 19.7°C ± 0.3 [18.9°C ; 20.5°C] # | ∅ | ∅ | ∅ | ∅ | 19.6°C ± 0.2 [19.2°C ; 19.8°C] |
| MHW treatments | <i>T</i> _{20:25(12h)} n= 12 | 19.7°C ± 0.3 [18.9°C ; 20.5°C] | 20.1°C ± 0.5 to 25.0°C ± 0.3 in 12:25 h ± 1:36 (0.46°C.h ⁻¹) ** | ∅ | ∅ | ∅ | 25.2°C ± 0.2 [24.9°C ; 25.5°C] |
| | <i>T</i> _{20:25-25(5d)} n= 10 | 19.7°C ± 0.3 [18.9°C ; 20.5°C] | 19.7°C ± 0.1 to 24.8°C ± 0.3 in 12:22 h ± 1:05 (0.46°C.h ⁻¹) * | 24.9°C ± 0.2 [24.5°C ; 25.6°C] | ∅ | ∅ | 25.1°C ± 0.2 [24.6°C ; 25.7°C] |
| | <i>T</i> _{mhw} n= 13 | 19.7°C ± 0.3 [18.9°C ; 20.5°C] | 19.6°C ± 0.3 to 25.0°C ± 0.6 in 11:39 h ± 1:07 (0.46°C.h ⁻¹) * | 25.0°C ± 0.4 [24.4°C ; 25.9°C] | 25.0°C ± 0.1 to 20.0°C ± 0.3 in 13:40 h ± 1:07 (0.37°C.h ⁻¹) ** | ∅ | 19.8°C ± 0.1 [19.3°C ; 20.2°C] |
| Recovery treatment | <i>T</i> _{recov} n= 10 | 19.7°C ± 0.3 [18.9°C ; 20.5°C] | 19.4°C ± 0.3 to 24.7°C ± 0.4 in 12:25 h ± 0:58 (0.43°C.h ⁻¹) * | 25.2°C ± 0.4 [24.2°C ; 25.9°C] | 25.2°C ± 0.2 to 19.8°C ± 0.1 in 13:47 h ± 0:51 (0.39°C.h ⁻¹) * | 19.6°C ± 0.2 [18.7°C ; 19.9°C] | 19.5°C ± 0.1 [19.2°C ; 19.8°C] |

2.2.1. Intermediary exposure set-up

This set-up consisted of four independent glass-aquaria (13L) that successively hosted fish by groups of two. Each aquarium was connected to a second smaller aquarium (5L) used as a buffer tank to accurately control (a) the water quality using a bacterial and mechanical filter and (b) the temperature, which was set through a submersible heater. The submersible heater associated with the air conditioning system of the experimental room accurately control the water temperature. Temperature was recorded every ten minutes using a 0.001°C HOBO probe (HOBO Tidbit V2 – UTBI-001 – HOBO Onset, Onset Computer Corporation, United States).

2.2.2. Protocol of temperature exposure (Table 1)

2.2.2.1. Control treatments

• *Control*₂₀₍₁₎: Fish were directly transferred from their 20°C acclimation tank to the swim tunnel to be individually challenged through a swimming step protocol at 20°C.

• *Control*₂₀₍₂₎: Fish were transferred in the morning from their 20°C acclimation tank to the intermediary aquaria where they remained for five days under the same 20°C condition. Fish were fed 24 and 72 hours after their transfer. The evening of the fifth day, they were individually placed in the swim tunnel to be challenged later through a swimming step protocol at 20°C. When compared to the *Control*₂₀₍₁₎ group, this treatment allowed us to assess a potential effect of the stress induced by the 5-day period spent in a smaller area (i.e. the 13L aquarium) in comparison to their 400L holding tank.

• *Control*₂₅: Fish were directly transferred from their 25°C acclimated holding tank to the swim tunnel to be individually tested at this temperature. This treatment permitted us to assess fish metabolic rates while acclimated at the highest assay temperature of 25°C. When compared with the MHW treatments (described below), it allowed us to know if the responses of the fish are a consequence of the thermal acute change experienced during the MHW or only a consequence of exposure to *high temperature* condition.

2.2.2.2. MHW treatments

• *T*_{20:25(12h)}: Fish were individually transferred from their 20°C acclimation tank to the swim tunnel, where they experienced a temperature increase from 20°C to 25°C during a period of ~12 hours (~4°C.h⁻¹). Then, they were individually tested at the *high temperature* condition with a swimming step protocol. When compared with control groups, this treatment evaluated the response of individuals following an initial acute increase in temperature, with a rate and amplitude comparable to what can be observed *in situ* during a MHW (Hobday et al. 2016).

• *T*_{20:25-25(5d)}: Fish were transferred during the morning from their 20°C acclimation tank to the intermediary aquaria where they experienced a similar temperature increase to the previous group (*T*_{20:25(12h)}) before to remain at 25°C during five days. After this temperature increase, fish

remained in the aquaria for five days at 25°C. Here, fish were fed 24 and 72 hours after their transfer. The evening of the fifth day, they were individually placed in the swim tunnel to be tested later through a swimming step protocol at this same temperature. This treatment allowed us to study the short-term acclimation capacities to the new temperature regime.

- T_{mhw} : Fish were transferred in the morning from their 20°C acclimation tank to the intermediary aquaria where they experienced similar treatments to the previous group ($T_{20:25-25_{(5d)}}$). Here, fish were fed 24 and 72 hours after their transfer. On the evening of the fifth day, they were individually placed in the swim tunnel to experience a temperature decrease from 25°C to 20°C in ~12 hours. After this period, they were tested at the *low temperature* condition with a swimming step protocol. This treatment was considered a complete MHW and assessed the potential cumulative effects caused by the temperature variations.

2.2.2.3. Recovery treatment

T_{recov} : Fish were transferred in the morning from their 20°C acclimation tank to the intermediary aquaria where they experienced similar treatments to the T_{mhw} group. On the evening of the fifth day, they were transferred back to a 20°C acclimated holding tank to permit them to recover. Here, they were fed ~12, ~60 and ~110 hours after their transfer. The evening of the seventh day, fish were moved to the swim tunnel for being individually tested later for a swimming step protocol at the *low temperature* condition, i.e. at their initial temperature of acclimation. This treatment allowed us to analyze the recovery capacities or the delayed effects on the fish.

2.3. Experimental set-up for swimming and metabolic variables

To determine the swimming performance and aerobic metabolic rate of each golden gray mullet, two intermittent-flow swim tunnels were used in parallel (Loligo Systems, Tjele, Denmark). We followed the guidelines of Killen et al. (2021) for the reporting of intermittent-flow respirometry method details. The system is fully detailed in Vagner et al. (2014). Briefly, the swim respirometer is

submerged in a buffer tank and composed of a swim chamber (10L, LxHxW= 40x10x10cm) and a hydraulic system, which promotes a laminar flow in combination with a honeycomb structure positioned upstream of the swim chamber. The flow was generated by an external motor equipped with a propeller whose speed is controlled by the experimenter. A flush submersible pump was placed in the buffer tank to ensure a renewal of aerated water in the swim respirometer. The buffer tank also contained a thermo-controlled recirculating system to allow the control of the temperature. To avoid the experimenter visually disturbing the fish and to encourage the fish to swim upstream, a black shelter was positioned in front of the swim chamber. Oxygen level measurements were carried out with an oxygen probe (i.e. optic fiber; Presens, Germany) placed in the swim chamber and connected to an oxymeter (Microx, Presens, Germany), which recorded data every 15s using the software Oxyview (Presens, Germany). The probe was calibrated once at the beginning of the swim test using 0% and 100% air saturation with a controlled temperature at 20°C; no derivation was observed during the duration of the experiment.

2.4. Protocol for swimming and metabolic variables

Fish were starved 48h before experiencing the swimming challenge. Swimming and metabolic performances were assessed through a critical swimming speed protocol during which the fish was exposed to a stepwise increase of the water velocity (e.g. Brett 1964; Reidy et al. 2000; Vagner et al. 2019; Palstra et al. 2020). Fish were transferred into the swim chamber between 5:00 and 6:00 pm. About 30 mins after its transfer, the fish was trained to swim in the swim chamber by progressively and slightly increasing the water velocity over 10 min. Then fish remained for ~12h (i.e. overnight) in the swim chamber at low velocity ($U = 2.75 \text{ BL.s}^{-1}$). It was during this period that fish of the $T_{20:25(12h)}$ group were exposed to the temperature change from 20°C to 25°C, and inversely for those in the T_{mhw} group (Figure 1 and table of the supplementary material). For the $\text{Control}_{20(1)}$, $\text{Control}_{20(2)}$ and T_{recov} groups, temperature was kept stable at 20°C while for the Control_{25} and $T_{20:25-25(5d)}$ groups, temperature was set and kept stable at 25°C.

The following morning, the step-protocol started. At intervals of 15 minutes, the water velocity was increased for three minutes as follows: increments of 0.75 BL s⁻¹ from 2.75 to 6.50 BL s⁻¹ then increments of 1.50 BL s⁻¹. At each swimming step, fish oxygen consumption (MO₂ in mg O₂ kg⁻¹ h⁻¹) was measured by intermittent-flow respirometry. This consists of an alternation between (a) a flushing phase during the increment of water velocity (i.e. 3 min) to restore oxygen saturation using the flush pump (which controls the water flow from the buffer tank to the respirometer) and (b) a MO₂ measurement phase at steady water velocity (i.e. 15 min), during which the respirometer was isolated from the buffer tank by turning off the flush pump. When the fish was exhausted, the water velocity was immediately reduced to recovery speed (i.e. 2.75 BL s⁻¹). The fish was considered exhausted when it was no longer able to swim against the current (i.e. the individual showed a C-shape on the grid located at the back of the swim chamber).

The fish was then allowed to recover for a period of 120 minutes. During this period, MO₂ was recorded to assess excess post-oxygen consumption (EPOC), i.e. the elevation in oxygen consumption due to the solicitation of anaerobic metabolism during the swimming challenge and the subsequent need to restore tissue and cellular oxygen stores (Lee et al. 2003). Five successive MO₂ measurements were recorded. In order to accurately assess the expected rapid decrease in MO₂, the first three oxygen consumption measurements were separated by a short flushing phase of 30s that was enough to reestablish oxygen at its initial concentration. Then, the next two and last MO₂ measurements were separated by 15 minutes of the flushing phase.

At the end of this 120 min-period, fish were removed from the swim chamber and anesthetized (tricaine methane sulphonate MS-222; 0.1 g L⁻¹, Sigma-Aldrich, St Quentin-Fallavier, France). Then, for each individual, biometry (i.e. total length, height, width and weight) was taken. All fish were kept alive at the end of the experimentation and returned to their original holding tanks and maintained at the *low* or *high temperature* condition depending on their acclimation temperature. For each swimming test, the background respiration (e.g. microbial respiration) was measured in the empty tunnel (i.e. without fish) for a minimum of 30 min before and after every swimming challenge. After

each test and before transferring a new fish, the swim respirometer was supplied with new filtered (1 µm) and UV-treated seawater. Also, the swim tunnel was fully cleaned and rinsed with freshwater twice per week. This procedure reduced the bacteria responsible for part of the background oxygen consumption as much as possible.

2.5. Calculation of the measured variables

2.5.1. Swimming performance

U_{crit} (BL s⁻¹) was calculated according the following equation (e.g. Brett 1964; Milinkovitch et al. 2012; McMahon et al. 2019):

$$U_{crit} = U_t + \frac{t_1}{t} \times U_1 \text{equation}(A)$$

where U_t (BL s⁻¹) is the highest velocity that the fish swam during a complete swimming step, t_1 (min) is the time spent at the fatigue velocity step, t (min) is the time of a complete swimming step (i.e. 15 min) and U_1 is the last increase of the velocity before the fish fatigued (i.e. 0.75 BL s⁻¹ or 1.5 BL s⁻¹).

2.5.2. Oxygen consumption (MO₂)

MO_{2RAW} (mg O₂ kg⁻¹ h⁻¹) was calculated using the following equation including a correction relating to the background respiration (e.g. Lefrançois and Claireaux 2003; Vagner et al. 2008, 2019):

$$M_{O_{2RAW}} = \left[\left(\frac{\Delta[O_2]}{\Delta t} \right) - \left(\left(\frac{\Delta[O_2]}{\Delta t} \right)_{bact} \right) \right] \times \left(\frac{V_{resp}}{m} \right) \text{equation}(B)$$

where $\Delta[O_2]/\Delta t$ (mgO₂ l⁻¹h⁻¹) is the oxygen concentration decrease in the swim respirometer over time during each MO₂ measurement period. It is obtained by plotting [O₂] versus time and fitting a linear regression (Graphical Analysis 3.4, Beaverton, OR, USA); only slopes with a regression coefficient above 0.85 were considered for further data analysis. $(\Delta[O_2]/\Delta t)_{bact}$ (mgO₂ l⁻¹h⁻¹) is the slope of background respiration over time resulting from the average of two measurements taken before and after the swimming challenge in the empty chamber. V_{resp} is the swim tunnel water volume (10 l) minus the volume of the fish, and m (kg) is the fish body mass. Because fish respiration

depends on animal body mass, an allometric correction was performed as follow to assess the MO_2 for a 100g-standard fish (e.g. Lefrançois and Claireaux 2003; Vagner et al. 2008, 2019):

$$M_{O_2} = M_{O_{2RAW}} \times \left(\frac{m}{m_{corr}} \right)^{1-A} \text{equation}(C)$$

where MO_2 ($mg\ O_2\ kg^{-1}\ h^{-1}$) is the oxygen consumption for a fish with a standard mass $m_{corr} = 0.1\ kg$, MO_{2RAW} ($mgO_2\ kg^{-1}\ h^{-1}$) is the calculated oxygen consumption with the equation (B) for a fish with mass m (kg) and A is the allometric exponent of the relationship between metabolic rate and fish mass. For this experiment, we used the same value (0.8) already used for *Chelon auratus* in Milinkovitch et al. (2012); Vagner et al. (2014, 2015, 2019) and commonly employed in teleost species (e.g. Wright et al. 2014; Kunz et al. 2018).

2.5.3. Metabolic parameters estimation

Standard metabolic rate (SMR) ($mg\ O_2\ kg^{-1}\ h^{-1}$) was obtained by extrapolating the following relationship to a null swimming velocity (e.g. Brett 1964; Chabot et al. 2016; Vagner et al. 2019; Hachim et al. 2021):

$$MO_2 = SMRe^{bU} \text{equation}(D)$$

where MO_2 ($mg\ O_2\ kg^{-1}\ h^{-1}$) is the oxygen consumption calculated in the equation (C), SMR is the intercept (i.e. the MO_2 at $U=0$), b is a constant and U ($BL\ s^{-1}$) is swimming speed.

The highest MO_2 reached during the U_{crit} test was considered an estimation of MMR, the maximum metabolic rate (in $mg\ O_2\ kg^{-1}\ h^{-1}$, e.g. Norin and Clark 2016).

Aerobic scope AS ($mg\ O_2\ kg^{-1}\ h^{-1}$) was then calculated for each individual by subtracting SMR from MMR (e.g. Brett 1964; Lefrançois and Claireaux 2003).

EPOC was determined ($mg\ O_2\ kg^{-1}$) by subtracting baseline fish MO_2 from the five values of MO_2 measured during the post-exercise phases. In order to increase the accuracy of the EPOC assessment, the first two of these five measures were divided in three periods of 5 min each. For each of these periods, MO_2 was assessed as described in equations B and C. Indeed, fish were observed to recover

extremely rapidly after the swimming challenge and changes of the slope $\frac{\Delta[O_2]}{\Delta t}$ were usually

observed. On the other hand, baseline was defined for each fish as its MO_2 while swimming at 2.75 BL s^{-1} at the beginning of the swimming challenge. Then, the MO_2 values obtained were plotted *versus* time and the integration of the area bounded between these values and the x-axis (i.e. time) were done using Graphical Analysis vs. 3.4 (Beaverton, OR, USA) (e.g. Lee et al. 2003; Vagner et al. 2015).

2.6. Statistical analysis

First, a comparison of the two groups of fish acclimated to 20°C i.e. *Control*₂₀₍₁₎ and *Control*₂₀₍₂₎ was done to assess whether the passage through the intermediary exposure set-up influenced the biological variables subsequently measured in fish. T-tests were used for each variable after performing Shapiro-Wilk and Bartlett tests. Because no significant difference was revealed between *Control*₂₀₍₁₎ and *Control*₂₀₍₂₎ for any variables (i.e. no significant impact of the intermediary exposure aquaria was found), a random sampling of 11 individuals was performed to homogenate the number of fish in regards to the five other treatments and to get an improved balanced plan for the rest of the statistical analyses. The results associated with this new group, called *Control*₂₀, will be the reference for individuals acclimated at the *low temperature* condition and will be used to perform the ANOVA described below.

The effect of temperature treatments on biological variables (i.e. SMR, MMR, AS, EPOC, U_{crit}) was analyzed in two steps. At first the response of fish during and directly after the extreme episode was investigated through a comparison between the *Control*₂₀, *Control*₂₅, *T*_{20:25(12h)}, *T*_{20:25-25(5d)}, and *T*_{mhw} groups. In a second step, the carry over effect in fish that underwent the MHW was explored considering only the *Control*₂₀, *T*_{mhw} and *T*_{recov} groups. In the results, the statistical differences between the temperature treatments during and directly after the extreme event (*Control*₂₀, *Control*₂₅, *T*_{20:25(12h)}, *T*_{20:25-25(5d)} and *T*_{mhw}) are illustrated on the figures by letters, while the differences between *Control*₂₀, *T*_{mhw} and *T*_{recov} (i.e. assessing the carry over effect) are illustrated by symbols. In both cases, application conditions were assessed for each variable using a Shapiro-Wilk test for normality and Bartlett test for homoscedasticity. If the conditions were met, a one-way ANOVA was

performed using the treatments as the fixed factor. If not, a non-parametric test (Kruskal-Wallis test) was used. In cases where the null hypothesis was rejected at a significance level of $P < 0.05$, a post-hoc Tukey HSD after the ANOVA or a pairwise Dunn post-hoc test after the non-parametric test was performed. The values were expressed as mean \pm standard error (SD) of the mean.

3. Results

3.1. Swimming performance

Average U_{crit} tended to be lower for the *Control*₂₅ group (10.88 ± 1.48 BL s^{-1}) than for the other treatments where it ranged between 12.44 ± 1.81 BL s^{-1} and 13.48 ± 2.50 BL s^{-1} . However, no statistical difference was revealed (Kruskal-Wallis test, $Chi^2 = 6.41$, $P = 0.17$). Furthermore, U_{crit} was not significantly different between the groups where the fish were tested at 20°C (i.e. *Control*₂₀, *T_{mhw}* and *T_{recov}*; one-way ANOVA, $F = 0.23$, $P = 0.80$, Figure 1).

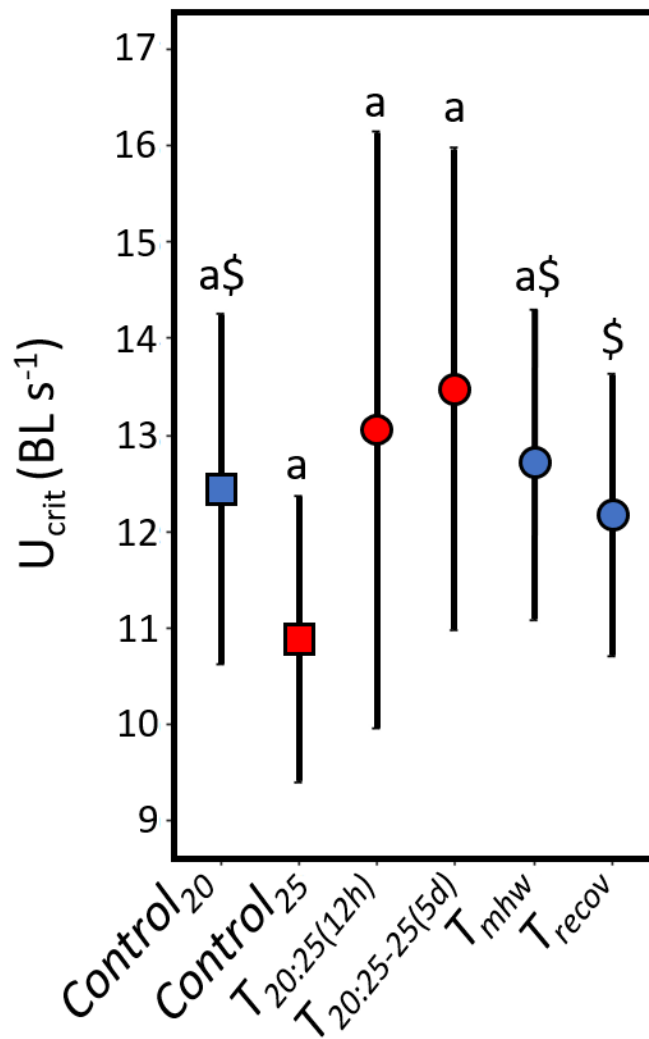


Fig. 1. Effect of MHW and recovery period on U_{crit} in *Chelon auratus* (mean \pm sd; BL s⁻¹). Color indicates the assay temperature at which fish have been tested in the swim tunnel: blue is for 20 °C and red is for 25 °C. The square shape indicates the two control groups of fish, i.e. tested at their low or high acclimation temperature without experiencing any temperature variation. The round shape indicates the four groups of fish that have endured temperature variation(s). Statistical tests were performed in two steps: letters are used to show if U_{crit} was significantly impacted during or directly after the MHW (Kruskal-Wallis test, $\chi^2=6.41$, $P = 0.17$), and symbols are used to assess if fish recovered 7 days after the MHW (One-way ANOVA, $F = 0.23$, $P = 0.80$). Different letters or symbols indicate that treatments were significantly different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. SMR, MMR, AMS and EPOC

SMR (Figure 2A) showed statistical differences between the two groups of fish *Control*₂₀ and *Control*₂₅ ($P < 0.01$). SMR was also significantly different between fish from the *Control*₂₀ group (38.49 ± 14.31 mg O₂ h⁻¹ kg⁻¹) and those who only experienced the acute elevation for ~12h (i.e. *T*_{20:25(12h)} : 84.39 ± 32.35 mg O₂ h⁻¹ kg⁻¹, $P < 0.001$) or spent five days at the *high temperature* (i.e. *T*_{20:25-25(5d)} : $85.72 \pm$

35.55 mg O₂ h⁻¹ kg⁻¹, $P < 0.001$), which approximately doubled their SMR. On the other hand, the SMR measured in fish of the $T_{20:25(12h)}$ and $T_{20:25-25(5d)}$ groups did not differ from each other ($P = 0.50$) or from the SMR of fish from the $Control_{25}$ group (74.06 ± 25.95 mg O₂ h⁻¹ kg⁻¹, $P = 0.25$ and $P = 0.26$, respectively). Additionally, the SMR of fish whose experienced the whole MHW (i.e. T_{mhw} group) (a) was not different from fish of the $Control_{25}$ group ($P = 0.33$), but (b) was significantly different from fish of the $Control_{20}$ group ($P < 0.001$). This difference disappeared seven days later, as the SMR of fish from T_{recov} (i.e. 47.70 ± 20.36 mg O₂ h⁻¹ kg⁻¹) and $Control_{20}$ groups were not statistically different ($P = 0.23$).

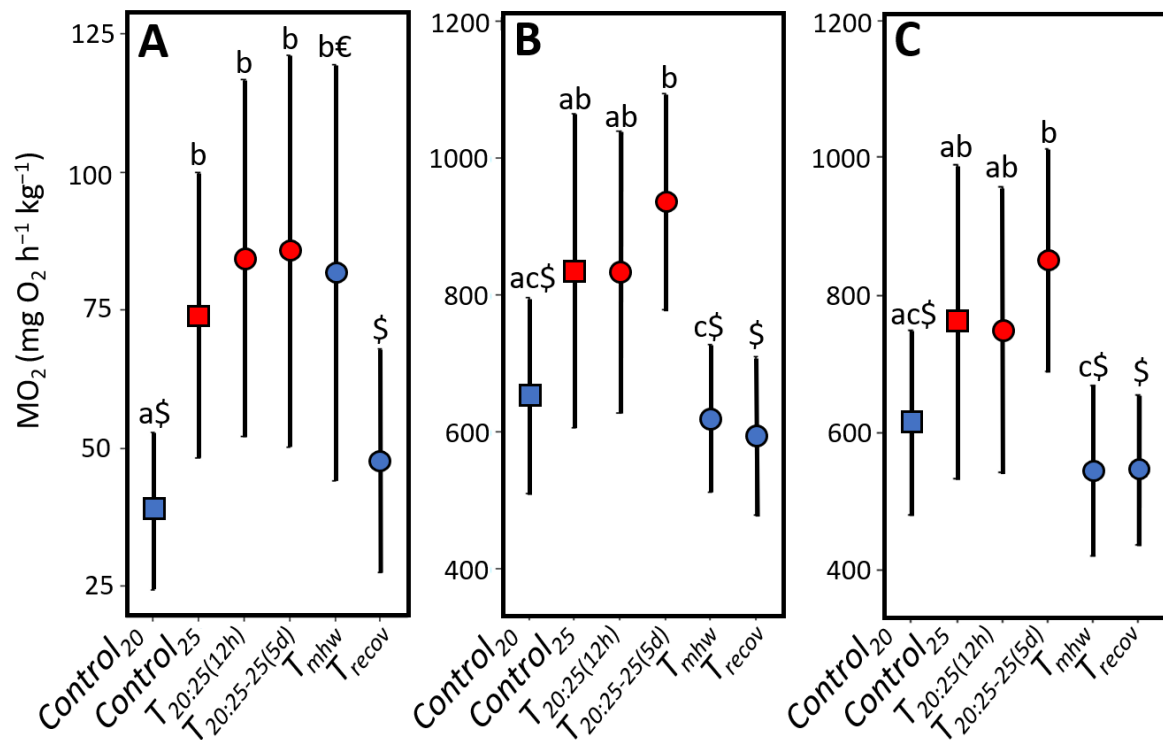


Fig. 2. Effect of the MHW and recovery period on the metabolic performance of *Chelon auratus* (mean ± SD; mg O₂ h⁻¹ kg⁻¹). A. Standard metabolic rate (SMR). B. Maximum metabolic rate (MMR). C. Aerobic metabolic scope (AS). Color indicates the assay temperature at which fish have been tested in the swimtunnel: blue is for 20 °C and red is for 25 °C. The square shape indicates the two control groups of fish, i.e. tested at their low or high acclimation temperature without experiencing any temperature variation. The round shape indicates the four groups of fish that have endured temperature variation(s). Statistical tests were performed in two steps for each metabolic variable: letters are used to show if SMR, (Kruskal-Wallis test, $\chi^2 = 18.20$, $P < 0.01$), MMR (one-way ANOVA, $F = 6.60$, $P < 0.001$) and AS (one-way ANOVA, $F = 5.45$, $P < 0.01$) were significantly impacted during and directly after the MHW whereas symbols are used to assess if fish recovered 7 days after the MHW (SMR: Kruskal-Wallis, $\chi^2 = 10.54$, $P < 0.01$; MMR: one-way ANOVA, $F = 0.61$, $P = 0.55$ and AS: one-way ANOVA, $F = 1.16$, $P = 0.33$). Different letters or symbols indicate that treatments were found significantly different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The MMR (figure 2B) of both *Control*₂₅ and *T*_{20:25(12h)} showed a trend to be higher than *Control*₂₀, but were not significantly different (*P* = 0.13 and *P* = 0.11, respectively). However, MMR of fish staying ~5 days at 25°C after experiencing the acute increase in temperature, became significantly higher (*P* < 0.01, *Control*₂₀ versus *T*_{20:25-25(5d)}). Immediately at the end of the MHW, the MMR returned to a value comparable to that observed in group *Control*₂₀ (*P* = 0.99) and stayed stable, given that seven days after,, the MMR showed no difference between the fish of the groups *Control*₂₀, *T*_{mhw} and *T*_{recov} (*P* = 0.55). The AS of fish (Figure 2C) showed a similar pattern to that for MMR was observed under temperature variations.

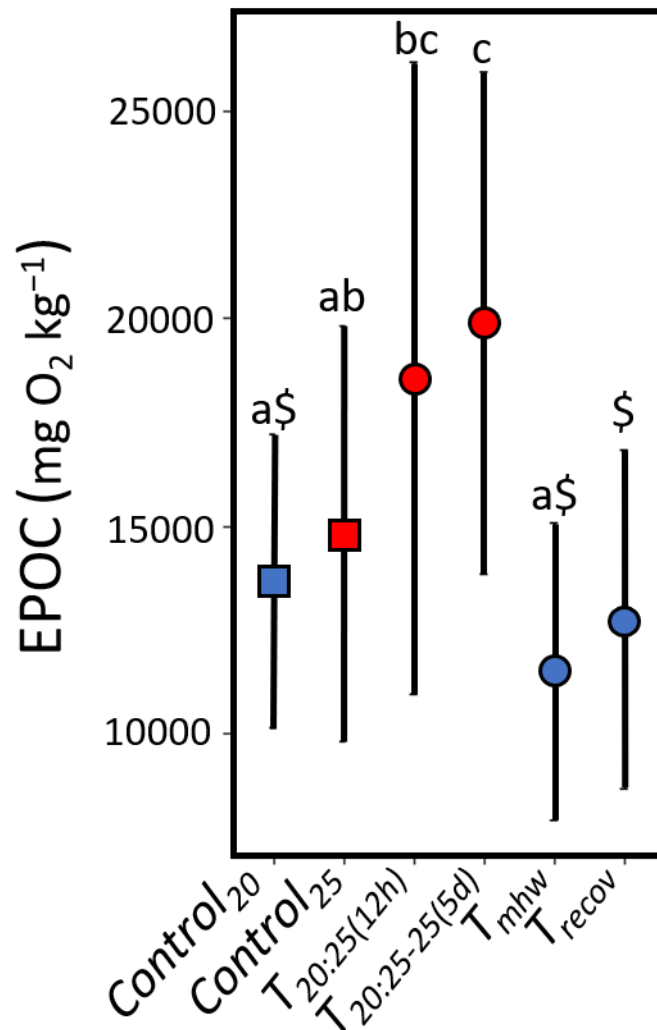


Fig. 3. Effect of the MHW and recovery period on *Chelon auratus* excess post-oxygen consumption after the swimming challenge (EPOC mean \pm sd; mg O₂ kg⁻¹). Color indicates the assay temperature at which fish have been tested in the swim tunnel: blue is for 20 °C and red is for 25 °C. The square shape indicates the two control groups of fish, i.e. tested at their low or high acclimation temperature without experiencing any temperature variation. The round shape indicates the four groups of fish that have endured temperature variation(s). Statistical tests were performed in two steps: letters are used to show if EPOC was modulated during and directly after the MHW (Kruskal-Wallis test, $\chi^2 = 14.9$, $P < 0.01$), whereas symbols are used to assess if fish recovered 7 days after the MHW (One-way ANOVA, $F = 0.94$, $P = 0.40$). Different letters or symbols indicate that treatments were found significantly different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The analysis of EPOC (Figure 3) revealed a different pattern. No statistical difference was found between fish of the groups *Control*₂₀ (13663.18 ± 3535.41 mg O₂ kg⁻¹) and *Control*₂₅ (14798.90 ± 5006.07 mg O₂ kg⁻¹, $P = 0.29$). On the contrary, acute exposure to 25°C induced a significant increase of EPOC in comparison to 20°C-acclimated fish whether it was assessed immediately (*T*_{20:25(12h)}: 18550.25 ± 7616.51 mg O₂ kg⁻¹, $P = 0.03$) or 5 days after the acute thermal variation (i.e. *T*_{20:25-25(5d)}: 19892.90 ± 6058.98 mg O₂ kg⁻¹, $P < 0.01$). Additionally, EPOC of fish in the *T*_{20:25-25(5d)} group was found statistically different from EPOC of those in the *Control*₂₅ group ($P = 0.03$). Finally, EPOC of fish that experienced the entire MHW (i.e. *T*_{mhw}: 11496.36 ± 3587.44 mg O₂ kg⁻¹) was not statistically different from fish of the *Control*₂₀ group ($P = 0.19$). Seven days after the MHW, fish showed the same magnitude of EPOC (12759.56 ± 4076.70 mg O₂ kg⁻¹) as the *Control*₂₀ and *T*_{mhw} groups (one-way ANOVA, $F = 0.94$, $P = 0.40$).

4. Discussion

The aim of the present study was to assess the physiological responses of a teleost fish facing different temperature regimes representative of a MHW and their ability to recover after it. To reach these objectives, aerobic and anaerobic metabolic variables were assessed, as well as swimming performance in *C. auratus*. To disentangle the contribution of the different phases of temperature change to the observed impacts, a succession of measurements has been done during a six-days MHW of a magnitude of 5°C. Our results suggest that *C. auratus* may shift from aerobic to anaerobic metabolism in response to acute changes in temperature during MHW.

4.1. Effects of the MHW on standard metabolic rate

The metabolism of ectotherm species is strongly linked to the temperature which directly affects the kinetics of chemical reactions (e.g. Gillooly et al. 2001; Pörtner 2002b; Brown et al. 2004; Claireaux and Lefrançois 2007; Payne et al. 2016). As described in Fry (1971), SMR was found to exponentially increase with the temperature of acclimation in European sea bass (*Dicentrarchus labrax*, Claireaux and Lagardère 1999; Kir and Demirci 2018), common sole (*Solea solea*, Lefrançois and Claireaux 2003), gilthead seabream (*Sparus aurata*, Kir 2020) and salmonids (e.g. sockeye salmon *Oncorhynchus nerka*, Brett 1964; Atlantic salmon *Salmo salar*, Hvas et al. 2017; lake trout or brook trout *Salvelinus namaycush* and *S. fontinalis*, Gibson and Fry 1954), while other kinds of patterns may be observed in barramundi (*Lates calcarifer*, Norin et al. 2014) or in ballan wrasse (*Labrus bergylta*, Yuen et al. 2019).

In the present study, the SMR assessed in *C. auratus* was in accordance with the range observed on the same species by Vagner et al. (2014) or Killen et al. (2015), and was found to double in individuals acclimated at 25°C compared to those acclimated at 20°C (i.e. $Control_{20}$ versus $Control_{25}$). Interestingly, exposure of *C. auratus* to 25°C, but in an acute way (i.e. group $T_{20:25(12h)}$), also led to a doubling of the SMR. At 25°C, the SMR therefore increased similarly regardless of the dynamics of the thermal change *C. auratus* experienced. This suggests that although an elevation of temperature contributes to an increase in metabolism, the acute character of the thermal challenge has no specific influence upon the basal oxygen requirements of *C. auratus*. It is worth noting that further maintenance at 25°C for five days (i.e. $Control_{20}$ versus $T_{20:25-25(5d)}$) results in no additional change in SMR.

In contrast, European perch *Perca fluviatilis* subjected to 18°C and then 23°C show a more intense increase in SMR in acutely warmed individuals than in gradually acclimated fish (Sandblom et al. 2016). Kirby et al. (2020) also measured a two-fold higher SMR in minnows *Cyprinodon variegatus* acutely exposed to 32°C (i.e. with an elevation of $2^{\circ}\text{C}\cdot\text{h}^{-1}$) in comparison to acclimated individuals. Elevation in SMR suggests supplementary energy costs to sustain maintenance activities, which could reduce fish fitness and performance (e.g. Fry 1971; Claireaux and Lefrançois 2007; Chabot et al. 2016;

Sandblom et al. 2016). Along these lines, Sandblom et al. (2016) and Kirby et al. (2020) suggested that downregulation of SMR during the acclimation at higher temperature could be an energy-saving strategy in eurytherm minnows *Cyprinodon variegatus* and European perch *Perca fluviatilis* to cope with life in highly variable and unpredictable estuarine habitats. On the other hand, the fact that we observed a similar SMR in *C. auratus* independent of the dynamics of thermal change may illustrate an equally adaptation strategy. When living in a highly variable environment, it could be considered crucial to show high plasticity contributing to maintaining the essential costs of life in animals whether undergoing an acute or a progressive change in temperature; especially in future climatic scenarios, where more intense and frequent MHWs are predicted (Darmaraki et al. 2019 ; IPCC 2021).

Effects were also observed at the end of the experimental MHW (i.e. $Control_{20}$ versus T_{mhw}), suggesting that individuals demonstrated a limited capacity to adjust their SMR when returned to their acclimation temperature of 20°C. To our knowledge, such persistence of elevated SMR has never been measured, possibly because other studies have rarely investigated aerobic metabolic rate in resting individuals facing a succession of different thermal regimes, combining acute and/or progressive changes.

Along these lines, two studies involving the Atlantic salmon parr (*Salmo salar*) showed that for a given tested temperature, the resting metabolic rate of fish differed depending on their thermal history (Beauregard et al. 2013; Oligny-Hébert et al. 2015). SMR of Atlantic salmon parr at 20°C was 25%–32% lower when individuals were steadily held at this temperature compared to those maintained under fluctuating thermal regimes (19.8 ± 2.0 °C or 19.5 ± 3.0 °C). The authors proposed that the additional costs of obligatory activities were the result of metabolic reactions to the thermal stress the fish experienced under unsteady thermal conditions. Stress indicators were not assessed in our study. However, based on examples that can be found in the literature, we can assume that at the end of the MHW, *C. auratus* may have to reallocate energy for mechanisms to repair cellular damage. For example, heat shock proteins (HSP) are molecules that can arise in response to acute or

chronic heat stress to prevent structural damage to cells and tissues (Islam et al. 2021). Yet, an up-regulation of heat shock protein 70 (HSP70) was observed in both *Chelon ramada* and in *Salmo salar* exposed to temperature variations (i.e. from 24°C to CT_{max} in Madeira et al. 2013 and to multiple daily Δ5°C-Δ9°C fluctuations in Corey et al. 2017, respectively).

Whatever the mechanisms involved in the increase in SMR, seven days were sufficient for *C. auratus* to fully recover from the metabolic disturbance they experienced (i.e. T_{mhw} versus T_{recov}). Living in variable areas such as estuarine habitats (Gautier and Hussenot 2005) may contribute to this resilience. However, the recovery dynamics should be investigated more in details in further studies by measuring SMR, as well as stress indicators, during a shorter recovery period.

4.2. Effects of the MHW on the maximal metabolic rate and the aerobic scope of *C. auratus*

Compared to the marked effects of temperature on its SMR, the thermo-dependence of MMR of *C. auratus* is less clear. Despite an increase of at least 28% in fish acutely exposed or acclimated to 25°C, MMR was indeed not significantly impacted by the 5°C temperature change except in the individuals exposed at 25°C for 5 consecutive days ($T_{20:25(5d)}$).

In comparison to our study, Vagner et al. (2014) measured a similar value of MMR in swimming *C. auratus* acclimated to 20°C (i.e. 590.2 ± 72.0 mg O₂ h⁻¹ kg⁻¹). However, when compared with our work, in a study by Killen et al. (2015) using the same species, MMR was found slightly lower in fish acclimated at 18, 22 and 26°C (i.e. respectively around 359.2, 404.1 and 381.7 mg O₂ h⁻¹ kg⁻¹). While in the present study fish were exposed to a swimming step challenge, Killen et al. (2015) assessed the metabolic rates of chasing *C. auratus* into a static respirometer, and this difference in protocol may contribute to the discrepancy in the range of values found, as it has been already observed in other species (Reidy et al. 1995). Killen et al. (2015) found no effect of temperature on MMR or on AS in *C. auratus* which is consistent with the eurytherm character of this species living in estuaries or lagoons. As suggested by these authors, the absence of significant variations of metabolic rates with temperature may indeed be an adaptation to such environments characterized by large thermal

fluctuations.

Moreover, the lack of clear temperature influence on MMR and AS observed in Killen et al. (2015), Vagner et al. (2014) and in the present work, may suggest that the temperatures tested surround the optimal temperature of *C. auratus*. Indeed, the thermal performance curve in tolerant and eurythermal species is characterized by the absence of a clear peak at the optimal temperature and a particularly flat shape over a wide thermal breadth (e.g. Fry 1971; Lefrançois and Claireaux 2003; Claireaux and Lefrançois 2007; Farrell 2009; Lefevre 2016). Thus, the variations in metabolic rate are expected to be extremely small around the optimal temperature. Such an explanation is consistent with a study carried out in the flathead grey mullet *Mugil cephalus*, a closely related species, acclimated to five experimental temperatures (i.e. from 10 to 30°C), which showed an optimal temperature of about 25°C (Cucco et al. 2012) and a thermal performance breadth, i.e. the maintenance of 80% of their metabolic performance, between approximately 18 and 30°C (Huey 1982).

As for the SMR, the rapid temperature change occurring during the MHW did not directly affect either MMR or AS, as acclimated (i.e. *Control*₂₅) and acutely exposed individuals to 25°C (i.e. *T*_{20:25(12h)}) showed a comparable response. While we previously discussed the dissimilarities in the thermal dependence of SMR between *C. auratus* and both sheephead minnow (Kirby et al. 2020) and European perch (Sandblom et al. 2016), the pattern for MMR appears equivalent between these three species. There was indeed no difference in the MMR measured in sheephead minnows that were acutely exposed or acclimated at 10 or 32°C; nor in the MMR of European perch acutely or gradually warmed from 18 to 23°C. In the common killifish (*Fundulus heteroclitus*), Healy and Schulte (2012) also concluded that there was no effect of the type of thermal exposure over the wide range of 5–35°C. On the contrary, the tropical barramundi (*Lates calcarifer*) showed a different pattern, with its MMR varying with the conditions of temperature exposure (Norin et al. 2014). Indeed, the individuals acutely exposed to 38°C from their temperature of acclimation of 29°C (i.e. +2°C h⁻¹) had a higher MMR and AS, compared to those progressively acclimated at 38°C. They interpreted this

temperature-dependent pattern by concluding that the lower MMR observed in acclimated individuals is likely to be a strategy for not maintaining high performance when it isn't required to meet the daily needs of the species.

According to Healy and Schulte (2012), in addition to appearing to be species-specific, the responses of aerobic metabolic rate in fish to acute conditions is likely to be influenced by their initial temperature of acclimation. Together with the variation in thermal tolerance of the tested species, it participates in limiting both the interpretation and comparison of studies carried out under different experimental conditions. Despite this, the variability of responses reported in the literature certainly illustrates the complexity and diversity of physiological and behavioral responses to perform and survive in thermally variable environments. Among these, and as observed in *C. auratus*, a strategy that allows aerobic capacity to be minimally affected by an acute change in temperature would probably be beneficial for maintaining performance in variable thermal environments. Additionally, such a lack of change in MMR is reminiscent, at least partially, with the concept of plastic floors and concrete ceilings. Sandblom et al. (2016) proposed that resting requirements (e.g. standard metabolic rate, as well as associated cardiorespiratory requirements) are plastic in warming conditions, while maximum capacities are much less flexible. Observations they made in European perch validated this concept, while our observations in *C. auratus* only illustrate the 'concrete ceiling' with stable values of MMR measured immediately or a few days after the acute increase in temperature.

4.3. Effects of the MHW on U_{crit} and EPOC

Because locomotion during a swimming step protocol partly relies on aerobic slow fibers, U_{crit} is expected to be somehow related to aerobic metabolic performance as shown in various teleost species such as Mahi-Mahi (*Coryphaena hippurus*, Heuer et al. 2021), lumpfish (*Cyclopterus lumpus*, Hvas et al. 2018) and ten damselfish species (Johansen and Jones 2011). Our observations in *C. auratus* confirm this expectation as U_{crit} followed the pattern observed in both MMR and AS, i.e. it did not significantly change with increasing temperature, regardless of whether we considered

584 acclimation or acute changes in temperature occurring in few hours. Furthermore, as in MMR and
 585 AS, no significant change was observed either immediately or seven days after the completion of the
 586 MHW. An analogy can be made with observations on Australian snapper larvae (*Chrysophrys auratus*)
 587 whose MMR and U_{crit} showed a similar response three weeks after an acute elevation of temperature
 588 (i.e. between 18 and 22°C, McMahon et al. 2019). On the contrary, Kunz et al. (2018) found that the
 589 MMR of polar cod *Boreogadus saida* increased with temperature of acclimation without inducing any
 590 modulation of U_{crit} . In *Cyprinodon variegatus*, the higher AS observed in fish acclimated to 32°C
 591 compared to acutely exposed individuals also did not translate into improved swimming
 592 performance (Kirby et al. 2020). These few examples illustrate the wide range of trends reported in
 593 the literature. It is suggested that this may arise from potential differences in thermal plasticity
 594 between aerobic performance and skeletal muscle properties (e.g. nerve stimulation, contractile
 595 kinetics, glycogen store; Fangue et al. 2008, Kirby et al. 2020), the animal's behaviour (e.g. Katopodis
 596 and Gervais 2012; Peake and Farrell 2006), as well as the level of recruitment of anaerobic fast
 597 fibers during swimming effort (Wood 1991).

598 None of these variables was measured in the present study, but excess post-exercise oxygen
 599 consumption was. Swimming until U_{crit} is known to be partly anaerobically fueled, which involves an
 600 oxygen debt that has to be repaid after the exercise (e.g. Brett 1964; Lee et al. 2003; Farrell 2007;
 601 Svendsen et al. 2010; Zeng et al. 2010; Cai et al. 2014; Vagner et al. 2014; Dichiera et al. 2021). EPOC,
 602 associated with aerobic and swimming performances, participates in a holistic view of the *C. auratus*
 603 strategy to sustain such a high-energy demanding activity. Interestingly, although the elevation in
 604 acclimation temperature provoked no change in EPOC (i.e. *Control*₂₀ versus *Control*₂₅), it increased
 605 significantly in fish acutely exposed to 25°C ($T_{20:25-25(12h)}$) suggesting that *C. auratus* use glycolytic-
 606 based locomotion more intensively to sustain its swimming performance under such conditions. In
 607 addition, although a few days at 25°C should have been beneficial to achieve homeostasis, the
 608 difference in EPOC was instead accentuated when compared to fish held in acclimated conditions
 609 (i.e. *Control*₂₀ or *Control*₂₅ versus $T_{20:25-25(5d)}$). This therefore suggests an even higher cost to sustain

swimming effort; especially when it is accumulated to the immediate oxygen requirements illustrated by the concomitant elevation (even if not significant) of MMR or AS observed in the same individuals.

Our results may therefore suggest that fish exposed to acute thermal change showed increased solicitation of anaerobic metabolism, at least when confronted with high energy-demanding activities, as during a swimming effort. It may provide an additional energy source to compensate for a potential deficiency of the aerobic metabolism to sustain swimming effort. This may be the result of prioritization towards energy-demanding compensatory mechanisms induced by temperature changes at the expense of swimming effort and/or a decrease in the efficiency of mitochondria to produce ATP through aerobic pathways (Kunz et al. 2018; Voituron et al. 2022). In European sea bass *Dicentrarchus labrax*, a temperate species with a similar distribution to *C. auratus*, Voituron et al. (2022) recently showed that being acclimated at a high temperature (i.e. 26°C) improved the mitochondrial efficiency (ATP/O, i.e. ratio between ATP synthesis and oxygen consumption). When measured at their acclimation temperature of 26°C, the capacity of mitochondria to anaerobically produce ATP was about 12% higher than when measured at this same temperature, but after an acute thermal change from a lower acclimated temperature (i.e. from 18 to 26°C, or from 22 to 26°C).

5. Conclusion

Considering only the variables related to aerobic metabolism (SMR, MMR, AS), *C. auratus* appears to be highly tolerant to acute temperature changes that occurring during a MHW. However, if we broaden the perspective, maintenance of energy-demanding activities (such as swimming effort) appears to have relied on an increasing proportion of the anaerobic pathway. This pattern suggests that in order to determine the ability of an individual to cope with such temperature changes, it seems of great importance to understand the relationships between thermal conditions and reliance on aerobic, but also anaerobic metabolism.

Furthermore, the shift to white anaerobic muscle fibers during these acute temperature phases generated an oxygen debt, and it cannot be excluded that this contributed to the increase in SMR observed at the end of the MHW, i.e. when the fish returned to their initial acclimation temperature. The additional oxygen requirements to process the accumulated lactic acid in the blood and muscle tissue could indeed have induced delayed effects relative to the triggering event. This pattern disappears very quickly, however, so it is very unlikely that the increased allocation to maintenance will impact the individual's growth or other activities (e.g. Claireaux and Lefrançois 2007; Sokolova et al. 2012; Sokolova 2021; Pörtner et al. 2017). Nevertheless, given the predicted increase in the frequency of MHWs in the coming decades (Frölicher et al. 2018), it can be hypothesized that the succession of such events could more profoundly impact aquatic organisms. Combined with other environmental changes that may coincide with MHW events, such as oxygen depletion (IPCC 2021; Deutsch et al. 2015; Claireaux and Chabot 2016), toxic algal blooms (McCabe et al. 2016), as well as inputs of pollutants (e.g. UV filters in sunscreen products, Labille et al. 2020), the effects could be even more severe, and further investigations in this direction appear essential to better assess the organism's response to such environmental stressors.

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