



HAL
open science

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

Margot Grimmelpont, Thomas Milinkovitch, Emmanuel Dubillot, Christel Lefrançois

► To cite this version:

Margot Grimmelpont, Thomas Milinkovitch, Emmanuel Dubillot, Christel Lefrançois. Individual aerobic performance and anaerobic compensation in a temperate fish during a simulated marine heatwave. *Science of the Total Environment*, 2023, 863, pp.160844. 10.1016/j.scitotenv.2022.160844 . hal-04276226

HAL Id: hal-04276226

<https://hal.science/hal-04276226>

Submitted on 9 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Individual aerobic performance and anaerobic compensation
2 in a temperate fish during a simulated marine heatwave
3

4 Margot Grimmelpont^a, Thomas Milinkovitch^a, Emmanuel Dubillot^a, Christel Lefrançois^a *

5
6 ^a La Rochelle University/CNRS France - UMR 7266 LIENSs, 2 Rue Olympe de Gouges, 17000 La Rochelle

7 * Corresponding author
8

9 **Abstract**

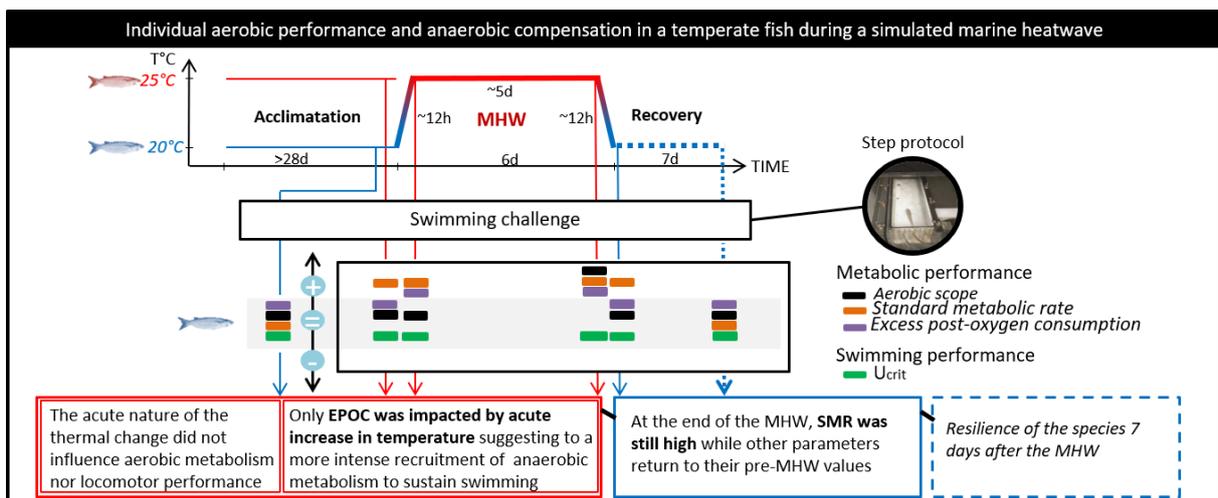
10 Marine heatwaves (MHWs) are becoming more frequent and intense due to anthropogenic climate
11 change and have strong negative effects on ecosystem. For now, few studies have reproduced the
12 complex nature of the temperature changes of a MHW, while it is suggested that ectotherms may be
13 more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

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

25 nature of the MHW had no influence. At the end of the MHW, the SMR remained elevated,
 26 suggesting an additional cost of obligatory activities due to the extreme event. In parallel, U_{crit} did not
 27 vary regardless of the thermal conditions. Concerning EPOC, it significantly increased only when fish
 28 were acutely exposed to 25°C. This strongly suggests that fish may buffer the effects of acute
 29 changes in temperature by shifting to anaerobic metabolism.
 30 Globally, this species appears to be able to cope with this MHW, but that's without taking into
 31 consideration future projections describing an increase in both intensity and frequency of such
 32 events, as well as other stressors like pollution or hypoxia).

33
 34 **Keywords:** MHW, fish, metabolism, swimming performance

35
 36



37
 38
 39
 40
 41
 42

43 1. Introduction

44 Climate change is responsible for a gradual and global increase in temperature but also for the
 45 appearance of extreme climatic events such as marine heatwaves (MHWs), which are predicted to
 46 increase both in frequency and intensity by the end of the century under the actual pathway (IPCC
 47 2021). MHWs have been defined several ways by the scientific community using different metrics
 48 (e.g. Selig et al. 2010; Sorte et al. 2010). In an attempt to facilitate comparisons and understanding of

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

56 MHWs have been shown to have a range of negative effects on ecosystems such as mass mortality
57 events (Garrabou et al. 2009; Hughes et al. 2017; Seuront et al. 2019; Piatt et al. 2020), shifts in
58 community composition, (Wernberg et al. 2013, 2016; Smale and Wernberg 2013), modifications of
59 geographical distributions and decreases in the abundance of species (Sanford et al. 2019). When
60 they are considered in addition to decade-scale changes in temperature, MHWs are expected to
61 contribute to a decline of fishery stocks that are predicted to be decreased by half by 2050 (Cheung
62 and Frölicher 2020). In this regard, Cavole et al. (2016) pointed out that the MHW that persisted from
63 2013 to 2015 in the Northeast Pacific has induced politico-socio-economic problems through
64 fisheries disruptions to species of economic interest (e.g. losses following the closure of the
65 Dungeness crab fishery from Washington to California in 2015-2016 were estimated at nearly \$48
66 million). Considering MHWs when determining the impact of climate change on marine species has
67 therefore become essential. For decades, researchers have investigated the influence of progressive
68 changes in mean temperature on marine organisms, particularly on ectotherms such as fish, whose
69 physiology is temperature-dependent (Burel et al. 1996; Pörtner 2002a; Pörtner and Farrell 2008;
70 Volkoff and Rønnestad 2020). However, as emphasized in Morash et al. (2018), ectotherms might be
71 more sensitive to acute changes in temperature, indicating that the impacts of extreme events such
72 as a MHW should receive increasing attention. Many of the studies on this topic employed the
73 critical thermal maximum (CT_{max}) protocol (i.e a stepwise increase in temperature until the fish loses
74 equilibrium) to assess the tolerance of individuals to an acute increase in temperature (e.g. Kikuchi et

75 al. 2019; Blasco et al. 2020). Recently, in order to specifically simulate temperature variation
76 magnitude during a MHW, other studies exposed organisms to thermal shock following by a
77 stabilization at an elevated temperature (McMahon et al. 2019; Madeira et al. 2020; Johansen et al.
78 2021). One study went even further by considering the capacity of organisms to both acclimate and
79 then recover from a 10-day MHW once the temperature returned to its initial value (Minuti et al.
80 2021). In this study, carried out in the sea urchin *Heliocidaris erythrogramma*, the authors
81 demonstrated an increase in mortality after the end of the MHW, probably a carry-over effect caused
82 by the accumulation of physiological damages they observed during the extreme episode. This
83 highlights the importance of evaluating the effects of these extreme thermal episodes through
84 measurements both during and after their occurrence.

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

101 critical swimming speed (i.e. U_{crit} , the maximum velocity that a fish can attain when exposed to a
102 swimming step protocol). For instance, Mahi-mahi *Coryphaena hippurus* exposed to an increase in
103 acclimation temperature until 32°C showed a decrease in both AS and U_{crit} (Heuer et al. 2021). On the
104 other hand, McMahon et al. (2019) observed a concomitant increase of these two variables when
105 Australasian snapper *Chrysophrys auratus* variables were exposed to an extreme temperature event
106 (i.e. +4°C). It is worth noting that as U_{crit} is approached during a swimming challenge, oxygen supply
107 becomes limiting and swimming effort is progressively sustained by anaerobic metabolism in many
108 species such as sockeye salmon *Oncorhynchus nerka* (Steinhausen et al. 2008), rainbow trout
109 *Oncorhynchus mykiss* (Burgetz et al. 1998) and striped surfperch *Embiotoca lateralis* (Cannas et al.
110 2006), which in some cases may contribute to the decoupling of U_{crit} and AS (e.g. Kunz et al. 2018).

111 In the present study, the effects of a MHW were investigated by assessing biological variables in a
112 teleost fish facing temperature variations occurring during such an event and after a thermally stable
113 recovery phase. More specifically, we assessed metabolic performance in the temperate golden gray
114 mullet *Chelon auratus* (Risso 1810), which was chosen because of its ecological relevance as a trophic
115 vector of organic matter from intertidal to subtidal areas (Gautier and Hussenot 2005; Carpentier et
116 al. 2014). - We conducted investigations by measuring aerobic and anaerobic metabolic variables and
117 U_{crit} to gain an integrative view of the possible consequences of a MHW. *Chelon auratus* were
118 exposed to an experimental MHW of a magnitude of 5°C, in agreement with what has been recorded
119 in the Mediterranean basin during the summer extreme episode that occurred in 2003 (Hobday et al.
120 2016). To do so, fish went acutely from acclimation conditions of 20°C to a higher temperature of
121 25°C that was stabilized during five days, before returning to the initial thermal conditions for several
122 days. We hypothesized that the new sudden, high thermal regime may led to an increase of
123 maintenance costs and thereby of SMR. In addition, a limited increase of MMR was expected as this
124 strong and sudden change in temperature could alter optimal functioning at the molecular or
125 membrane level, and/or in organs involved in oxygen transport, such as the heart (e.g. Farrell 2002).
126 Consequences on individual performances were assessed through the determination of U_{crit} and

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

132 **2. Materials and methods**

133 **2.1. Fish husbandry**

134 Juvenile golden gray mullets were captured in salt marshes (L'Houmeau, France 46°12'14"N
135 1°11'42"W) with a fyke net. Fish were held in six different 400L indoor tanks (n = 15–30 individuals
136 per tank) supplied with natural and aerated seawater for nine months. The water quality was
137 maintained with an external biological and mechanical filter (Eheim professional 3 2080, Eheim,
138 Deizisau, Germany) and 30% of the water was renewed each week. During this period, temperature
139 and salinity were recorded three times a week using a WTW Cond 3310 multimeter (Xylem Analytics
140 Germany GmbH) and varied from 18.3 to 20.5°C and from 26.2 to 30, respectively, which is similar to
141 natural conditions encountered during summer in the area where the mullets have been caught.
142 Oxygen level and nitrites were never under 80% of air saturation or above 0.3 mg.L⁻¹, respectively.
143 Animals were fed three times per week in the morning with commercial food pellets (Neo Repro II, Le
144 Gouessant, France) and exposed to a 12h light:12h dark photoperiod cycle. Experiments were carried
145 out respecting regulations of the Animal Care Committee of France (ACCF) (APAFIS#25159-
146 2019102913067306 v7; UMR7266 LIENSs approval number: 173002).

147 **2.2. Temperature treatments**

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

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

155 In the following, exposure to $19.7^{\circ}\text{C} \pm 0.3$ is referred as *20* or *low temperature* conditions; and
 156 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 -
 157 MHW lasting six days, a total of seven treatments were tested, and each fish was exposed to only
 158 one of these seven treatments: three control treatments (i.e. fish acclimated and tested at *low* or
 159 *high* acclimation temperatures), three MHW treatments (i.e. fish tested at different time of the
 160 MHW) and one recovery treatment (i.e. fish tested 7 days after having experienced the whole MHW).
 161 Depending on the temperature treatment they experienced, fish were transferred from their holding
 162 tank to a swim tunnel (a) directly or (b) after having spent time in an intermediary aquaria (table 1).
 163 Before each transfer, fish were starved 48 hours.

164

165 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:
 166 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
 167 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
 168 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
 169 acclimation at 20°C or 25°C . T_{recov} group also spent seven days in the holding tank after experiencing the whole MHW. Three groups ($\text{Control}_{20(1)}$, Control_{25} ,
 170 $T_{20:25(12h)}$) 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
 171 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:
 172 $T_{20:25-25(5d)}$, T_{MHW} and T_{recov} 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
 173 aquaria; it corresponds to the treatment $\text{Control}_{20(2)}$ and is indicated by # in the table. Values outside the brackets are the mean temperature \pm sd. Values in
 174 brackets are the minimum and maximum temperature recorded during the temperature exposition. Values in parenthesis represent the rate of changing
 175 temperature. * and ** indicate the type of experimental set-up used for the temperature changes and/or for the temperature stabilization.

176

177

		Different steps of the simulated MHW lasted ~ 6 days			Recovery 7 days	Swimming challenge ~ 6 hours	
		Acclimation > 28 days	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
	treatment	Control_{25} n = 10	$25.0^{\circ}\text{C} \pm 0.2$ [24.6°C ; 25.4°C]	\emptyset	\emptyset	\emptyset	\emptyset

	<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]

178
179

180

181 2.2.1. Intermediary exposure set-up

182 This set-up consisted of four independent glass-aquaria (13L) that successively hosted fish by groups
183 of two. Each aquarium was connected to a second smaller aquarium (5L) used as a buffer tank to
184 accurately control (a) the water quality using a bacterial and mechanical filter and (b) the
185 temperature, which was set through a submersible heater. The submersible heater associated with
186 the air conditioning system of the experimental room accurately control the water temperature.
187 Temperature was recorded every ten minutes using a 0.001°C HOBO probe (HOBO Tidbit V2 – UTBI-
188 001 – HOBO Onset, Onset Computer Corporation, United States).

189 2.2.2. Protocol of temperature exposure (Table 1)

190 2.2.2.1. Control treatments

- 191 • *Control*₂₀₍₁₎: Fish were directly transferred from their 20°C acclimation tank to the swim tunnel to
192 be individually challenged through a swimming step protocol at 20°C.
- 193 • *Control*₂₀₍₂₎: Fish were transferred in the morning from their 20°C acclimation tank to the
194 intermediary aquaria where they remained for five days under the same 20°C condition. Fish
195 were fed 24 and 72 hours after their transfer. The evening of the fifth day, they were individually
196 placed in the swim tunnel to be challenged later through a swimming step protocol at 20°C.
197 When compared to the *Control*₂₀₍₁₎ group, this treatment allowed us to assess a potential effect
198 of the stress induced by the 5-day period spent in a smaller area (i.e. the 13L aquarium) in
199 comparison to their 400L holding tank.
- 200 • *Control*₂₅: Fish were directly transferred from their 25°C acclimated holding tank to the swim
201 tunnel to be individually tested at this temperature. This treatment permitted us to assess fish
202 metabolic rates while acclimated at the highest assay temperature of 25°C. When compared with
203 the MHW treatments (described below), it allowed us to know if the responses of the fish are a
204 consequence of the thermal acute change experienced during the MHW or only a consequence
205 of exposure to *high temperature* condition.

206 2.2.2.2. MHW treatments

- 207 • *T*_{20:25(12h)}: Fish were individually transferred from their 20°C acclimation tank to the swim tunnel,
208 where they experienced a temperature increase from 20°C to 25°C during a period of ~12 hours
209 (~4°C.h⁻¹). Then, they were individually tested at the *high temperature* condition with a
210 swimming step protocol. When compared with control groups, this treatment evaluated the
211 response of individuals following an initial acute increase in temperature, with a rate and
212 amplitude comparable to what can be observed *in situ* during a MHW (Hobday et al. 2016).
- 213 • *T*_{20:25-25(5d)}: Fish were transferred during the morning from their 20°C acclimation tank to the
214 intermediary aquaria where they experienced a similar temperature increase to the previous
215 group (*T*_{20:25(12h)}) before to remain at 25°C during five days. After this temperature increase, fish

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

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

227 2.2.2.3. Recovery treatment

228
229 T_{recov} : Fish were transferred in the morning from their 20°C acclimation tank to the intermediary
230 aquaria where they experienced similar treatments to the T_{mhw} group. On the evening of the fifth
231 day, they were transferred back to a 20°C acclimated holding tank to permit them to recover. Here,
232 they were fed ~12, ~60 and ~110 hours after their transfer. The evening of the seventh day, fish were
233 moved to the swim tunnel for being individually tested later for a swimming step protocol at the *low*
234 *temperature* condition, i.e. at their initial temperature of acclimation. This treatment allowed us to
235 analyze the recovery capacities or the delayed effects on the fish.

236

237 2.3. Experimental set-up for swimming and metabolic variables

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

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

255

256 2.4. Protocol for swimming and metabolic variables

257

258 Fish were starved 48h before experiencing the swimming challenge. Swimming and metabolic
259 performances were assessed through a critical swimming speed protocol during which the fish was
260 exposed to a stepwise increase of the water velocity (e.g. Brett 1964; Reidy et al. 2000; Vagner et al.
261 2019; Palstra et al. 2020). Fish were transferred into the swim chamber between 5:00 and 6:00 pm.
262 About 30 mins after its transfer, the fish was trained to swim in the swim chamber by progressively
263 and slightly increasing the water velocity over 10 min. Then fish remained for ~12h (i.e. overnight) in
264 the swim chamber at low velocity ($U = 2.75 \text{ BL}\cdot\text{s}^{-1}$). It was during this period that fish of the $T_{20:25(12h)}$
265 group were exposed to the temperature change from 20°C to 25°C, and inversely for those in the
266 T_{mhw} group (Figure 1 and table of the supplementary material). For the $\text{Control}_{20(1)}$, $\text{Control}_{20(2)}$ and
267 T_{recov} groups, temperature was kept stable at 20°C while for the Control_{25} and $T_{20:25-25(5d)}$ groups,
268 temperature was set and kept stable at 25°C.

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

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

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

295 each test and before transferring a new fish, the swim respirometer was supplied with new filtered
296 (1 µm) and UV-treated seawater. Also, the swim tunnel was fully cleaned and rinsed with freshwater
297 twice per week. This procedure reduced the bacteria responsible for part of the background oxygen
298 consumption as much as possible.

299 2.5. Calculation of the measured variables

300 2.5.1. Swimming performance

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

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

303 where U_t (BL s^{-1}) is the highest velocity that the fish swam during a complete swimming step, t_1 (min)
304 is the time spent at the fatigue velocity step, t (min) is the time of a complete swimming step (i.e. 15
305 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}).

306 2.5.2. Oxygen consumption (MO_2)

307 MO_{2RAW} (mg O_2 kg^{-1} h^{-1}) was calculated using the following equation including a correction relating to
308 the background respiration (e.g. Lefrançois and Claireaux 2003; Vagner et al. 2008, 2019):

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

309 where $\Delta[O_2]/\Delta t$ (mg O_2 $l^{-1}h^{-1}$) is the oxygen concentration decrease in the swim respirometer over
310 time during each MO_2 measurement period. It is obtained by plotting $[O_2]$ versus time and fitting a
311 linear regression (Graphical Analysis 3.4, Beaverton, OR, USA); only slopes with a regression
312 coefficient above 0.85 were considered for further data analysis. $(\Delta[O_2]/\Delta t)_{bact}$ (mg O_2 $l^{-1}h^{-1}$) is the
313 slope of background respiration over time resulting from the average of two measurements taken
314 before and after the swimming challenge in the empty chamber. V_{resp} is the swim tunnel water
315 volume (10 l) minus the volume of the fish, and m (kg) is the fish body mass. Because fish respiration

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

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

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

324 2.5.3. Metabolic parameters estimation

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

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

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

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

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

334 EPOC was determined ($mg\ O_2\ kg^{-1}$) by subtracting baseline fish MO_2 from the five values of MO_2
335 measured during the post-exercise phases. In order to increase the accuracy of the EPOC assessment,
336 the first two of these five measures were divided in three periods of 5 min each. For each of these
337 periods, MO_2 was assessed as described in equations B and C. Indeed, fish were observed to recover
338 extremely rapidly after the swimming challenge and changes of the slope $\frac{\Delta[O_2]}{\Delta t}$ were usually

339 observed. On the other hand, baseline was defined for each fish as its MO_2 while swimming at 2.75
340 $BL\ s^{-1}$ at the beginning of the swimming challenge. Then, the MO_2 values obtained were plotted
341 *versus* time and the integration of the area bounded between these values and the x-axis (i.e. time)
342 were done using Graphical Analysis vs. 3.4 (Beaverton, OR, USA) (e.g. Lee et al. 2003; Vagner et al.
343 2015).

344 2.6. Statistical analysis

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

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

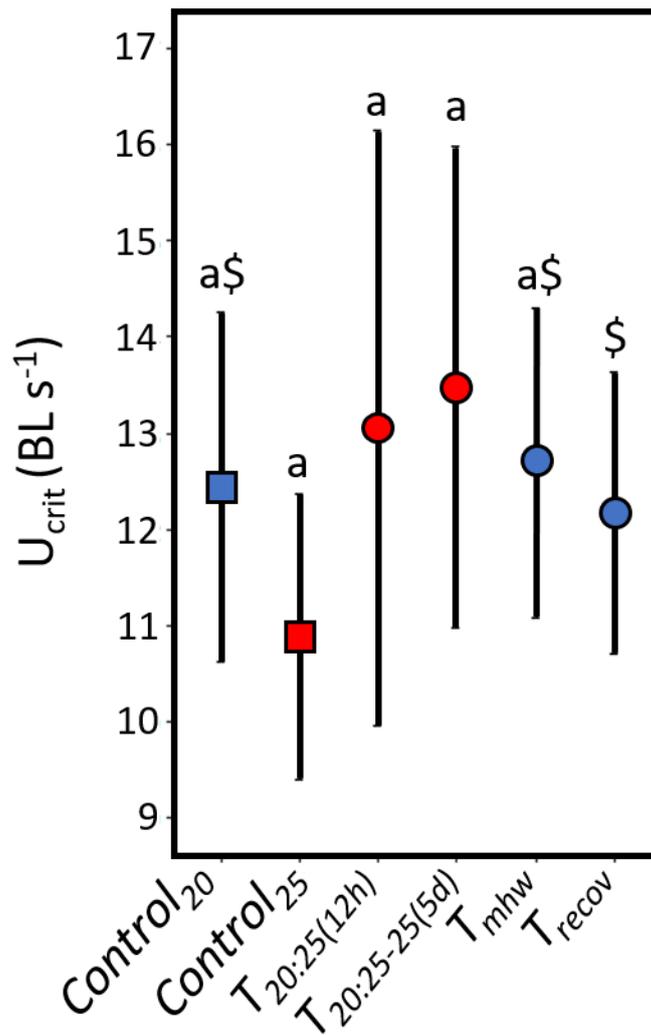
365 performed using the treatments as the fixed factor. If not, a non-parametric test (Kruskal-Wallis test)
366 was used. In cases where the null hypothesis was rejected at a significance level of $P < 0.05$, a post-
367 hoc Tukey HSD after the ANOVA or a pairwise Dunn post-hoc test after the non-parametric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

369 **3. Results**

370 **3.1. Swimming performance**

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

376



377

378
379
380
381
382
383
384
385

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.)

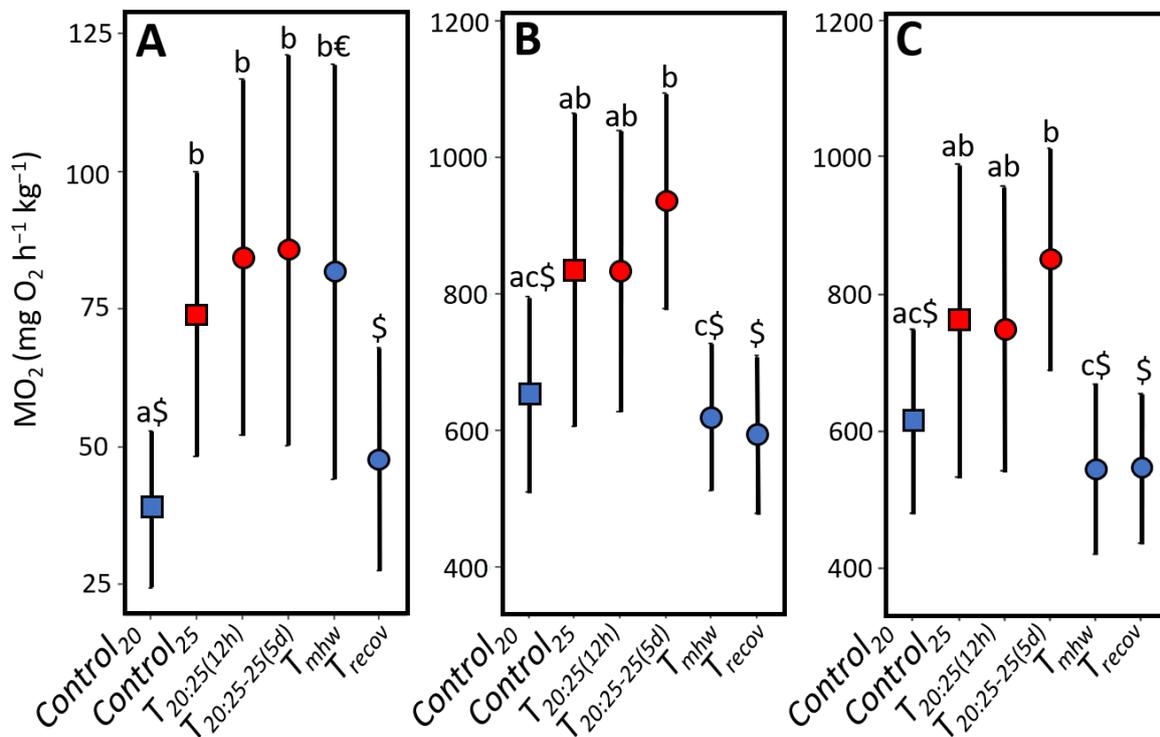
386

387 3.2. SMR, MMR, AMS and EPOC

388 SMR (Figure 2A) showed statistical differences between the two groups of fish *Control*₂₀ and *Control*₂₅
389 ($P < 0.01$). SMR was also significantly different between fish from the *Control*₂₀ group (38.49 ± 14.31
390 mg O₂ h⁻¹ kg⁻¹) and those who only experienced the acute elevation for ~12h (i.e. *T*_{20:25(12h)} : $84.39 \pm$
391 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)} : $85.72 \pm$

392 35.55 mg O₂ h⁻¹ kg⁻¹, P < 0.001), which approximately doubled their SMR. On the other hand, the
 393 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)
 394 or from the SMR of fish from the Control₂₅ group (74.06 ± 25.95 mg O₂ h⁻¹ kg⁻¹, P = 0.25 and P = 0.26,
 395 respectively). Additionally, the SMR of fish whose experienced the whole MHW (i.e. T_{mhw} group) (a)
 396 was not different from fish of the Control₂₅ group (P = 0.33), but (b) was significantly different from
 397 fish of the Control₂₀ group (P < 0.001). This difference disappeared seven days later, as the SMR of
 398 fish from T_{recov} (i.e. 47.70 ± 20.36 mg O₂ h⁻¹ kg⁻¹) and Control₂₀ groups were not statistically different
 399 (P = 0.23).

400



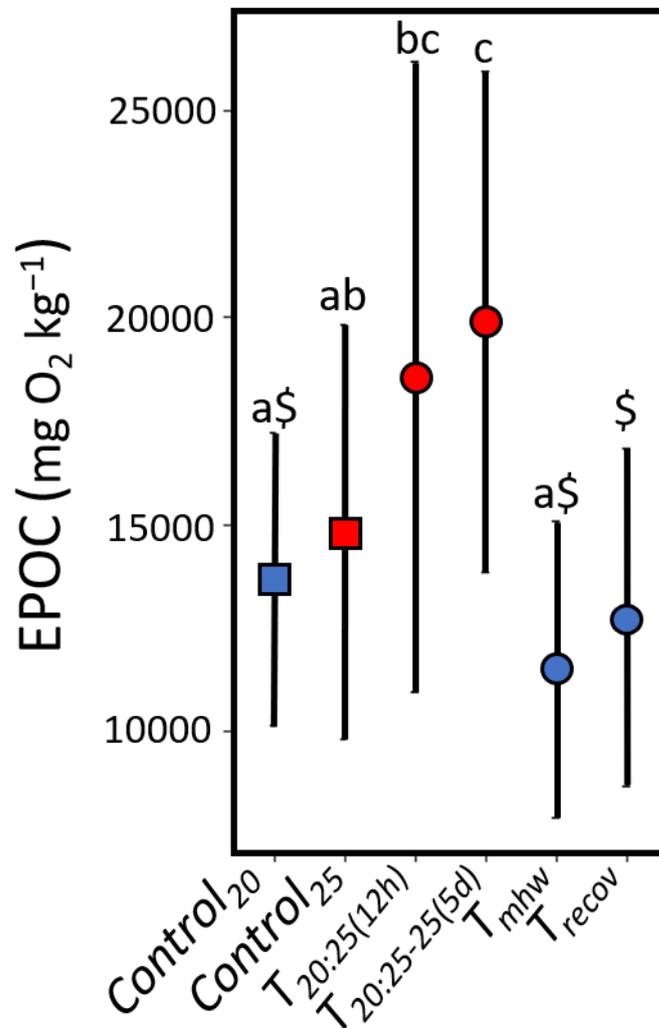
401

402

403 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).
 404 B. Maximum metabolic rate (MMR). C. Aerobic metabolic scope (AS). Color indicates the assay temperature at which fish have been tested in the swimtunnel:
 405 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
 406 experiencing any temperature variation. The round shape indicates the four groups of fish that have endured temperature variation(s). Statistical tests were
 407 performed in two steps for each metabolic variable: letters are used to show if SMR, (Kruskal-Wallis test, Chi₂ = 18.20, P < 0.01), MMR (one-way ANOVA, F =
 408 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
 409 assess if fish recovered 7 days after the MHW (SMR: Kruskal-Wallis, Chi₂ = 10.54, P < 0.01; MMR: one-way ANOVA, F = 0.61, P = 0.55 and AS: one-way
 410 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
 411 in this figure legend, the reader is referred to the web version of this article.)
 412

413

414 The MMR (figure 2B) of both *Control*₂₅ and *T*_{20:25(12h)} showed a trend to be higher than *Control*₂₀, but
 415 were not significantly different ($P = 0.13$ and $P = 0.11$, respectively). However, MMR of fish staying ~5
 416 days at 25°C after experiencing the acute increase in temperature, became significantly higher ($P <$
 417 0.01 , *Control*₂₀ versus *T*_{20:25-25(5d)}). Immediately at the end of the MHW, the MMR returned to a value
 418 comparable to that observed in group *Control*₂₀ ($P = 0.99$) and stayed stable, given that seven days
 419 after,, the MMR showed no difference between the fish of the groups *Control*₂₀, *T*_{mhw} and *T*_{recov} ($P =$
 420 0.55). The AS of fish (Figure 2C) showed a similar pattern to that for MMR was observed under
 421 temperature variations.
 422



423

424

425 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).
426 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
427 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
428 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
429 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
430 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
431 in this figure legend, the reader is referred to the web version of this article.)
432

433 The analysis of EPOC (Figure 3) revealed a different pattern. No statistical difference was found
434 between fish of the groups *Control*₂₀ (13663.18 \pm 3535.41 mg O₂ kg⁻¹) and *Control*₂₅ (14798.90 \pm
435 5006.07 mg O₂ kg⁻¹, $P = 0.29$). On the contrary, acute exposure to 25°C induced a significant increase
436 of EPOC in comparison to 20°C-acclimated fish whether it was assessed immediately (*T*_{20:25(12h)}:
437 18550.25 \pm 7616.51 mg O₂ kg⁻¹, $P = 0.03$) or 5 days after the acute thermal variation (i.e. *T*_{20:25-25(5d)}:
438 19892.90 \pm 6058.98 mg O₂ kg⁻¹, $P < 0.01$). Additionally, EPOC of fish in the *T*_{20:25-25(5d)} group was found
439 statistically different from EPOC of those in the *Control*₂₅ group ($P = 0.03$). Finally, EPOC of fish that
440 experienced the entire MHW (i.e. *T*_{mhw}: 11496.36 \pm 3587.44 mg O₂ kg⁻¹) was not statistically different
441 from fish of the *Control*₂₀ group ($P = 0.19$). Seven days after the MHW, fish showed the same
442 magnitude of EPOC (12759.56 \pm 4076.70 mg O₂ kg⁻¹) as the *Control*₂₀ and *T*_{mhw} groups (one-way
443 ANOVA, $F = 0.94$, $P = 0.40$).

444

445 4. Discussion

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

453 4.1. Effects of the MHW on standard metabolic rate

454 The metabolism of ectotherm species is strongly linked to the temperature which directly affects the
455 kinetics of chemical reactions (e.g. Gillooly et al. 2001; Pörtner 2002b; Brown et al. 2004; Claireaux
456 and Lefrançois 2007; Payne et al. 2016). As described in Fry (1971), SMR was found to exponentially
457 increase with the temperature of acclimation in European sea bass (*Dicentrarchus labrax*, Claireaux
458 and Lagardère 1999; Kir and Demirci 2018), common sole (*Solea solea*, Lefrançois and Claireaux
459 2003), gilthead seabream (*Sparus aurata*, Kir 2020) and salmonids (e.g. sockeye salmon
460 *Oncorhynchus nerka*, Brett 1964; Atlantic salmon *Salmo salar*, Hvas et al. 2017; lake trout or brook
461 trout *Salvelinus namaycush* and *S. fontinalis*, Gibson and Fry 1954), while other kinds of patterns may
462 be observed in barramundi (*Lates calcarifer*, Norin et al. 2014) or in ballan wrasse (*Labrus bergylta*,
463 Yuen et al. 2019).

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

474 In contrast, European perch *Perca fluviatilis* subjected to 18°C and then 23°C show a more intense
475 increase in SMR in acutely warmed individuals than in gradually acclimated fish (Sandblom et al.
476 2016). Kirby et al. (2020) also measured a two-fold higher SMR in minnows *Cyprinodon variegatus*
477 acutely exposed to 32°C (i.e. with an elevation of $2^{\circ}\text{C}\cdot\text{h}^{-1}$) in comparison to acclimated individuals.
478 Elevation in SMR suggests supplementary energy costs to sustain maintenance activities, which could
479 reduce fish fitness and performance (e.g. Fry 1971; Claireaux and Lefrançois 2007; Chabot et al. 2016;

480 Sandblom et al. 2016). Along these lines, Sandblom et al. (2016) and Kirby et al. (2020) suggested
481 that downregulation of SMR during the acclimation at higher temperature could be an energy-saving
482 strategy in eurytherm minnows *Cyprinodon variegatus* and European perch *Perca fluviatilis* to cope
483 with life in highly variable and unpredictable estuarine habitats. On the other hand, the fact that we
484 observed a similar SMR in *C. auratus* independent of the dynamics of thermal change may illustrate
485 an equally adaptation strategy. When living in a highly variable environment, it could be considered
486 crucial to show high plasticity contributing to maintaining the essential costs of life in animals
487 whether undergoing an acute or a progressive change in temperature; especially in future climatic
488 scenarios, where more intense and frequent MHWs are predicted (Darmaraki et al. 2019 ; IPCC
489 2021).

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

496 Along these lines, two studies involving the Atlantic salmon parr (*Salmo salar*) showed that for a
497 given tested temperature, the resting metabolic rate of fish differed depending on their thermal
498 history (Beauregard et al. 2013; Oligny-Hébert et al. 2015). SMR of Atlantic salmon parr at 20°C was
499 25%–32% lower when individuals were steadily held at this temperature compared to those
500 maintained under fluctuating thermal regimes (19.8 ± 2.0 °C or 19.5 ± 3.0 °C). The authors proposed
501 that the additional costs of obligatory activities were the result of metabolic reactions to the thermal
502 stress the fish experienced under unsteady thermal conditions. Stress indicators were not assessed in
503 our study. However, based on examples that can be found in the literature, we can assume that at
504 the end of the MHW, *C. auratus* may have to reallocate energy for mechanisms to repair cellular
505 damage. For example, heat shock proteins (HSP) are molecules that can arise in response to acute or

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

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

515 4.2. Effects of the MHW on the maximal metabolic rate and the aerobic scope 516 of *C.auratus*

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

521 In comparison to our study, Vagner et al. (2014) measured a similar value of MMR in swimming *C.*
522 *auratus* acclimated to 20°C (i.e. 590.2 ± 72.0 mg O₂ h⁻¹ kg⁻¹). However, when compared with our
523 work, in a study by Killen et al. (2015) using the same species, MMR was found slightly lower in fish
524 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
525 in the present study fish were exposed to a swimming step challenge, Killen et al. (2015) assessed the
526 metabolic rates of chasing *C. auratus* into a static respirometer, and this difference in protocol may
527 contribute to the discrepancy in the range of values found, as it has been already observed in other
528 species (Reidy et al. 1995). Killen et al. (2015) found no effect of temperature on MMR or on AS in *C.*
529 *auratus* which is consistent with the eurytherm character of this species living in estuaries or
530 lagoons. As suggested by these authors, the absence of significant variations of metabolic rates with
531 temperature may indeed be an adaptation to such environments characterized by large thermal

532 fluctuations.

533 Moreover, the lack of clear temperature influence on MMR and AS observed in Killen et al. (2015),
534 Vagner et al. (2014) and in the present work, may suggest that the temperatures tested surround the
535 optimal temperature of *C. auratus*. Indeed, the thermal performance curve in tolerant and
536 eurythermal species is characterized by the absence of a clear peak at the optimal temperature and a
537 particularly flat shape over a wide thermal breadth (e.g. Fry 1971; Lefrançois and Claireaux 2003;
538 Claireaux and Lefrançois 2007; Farrell 2009; Lefevre 2016). Thus, the variations in metabolic rate are
539 expected to be extremely small around the optimal temperature. Such an explanation is consistent
540 with a study carried out in the flathead grey mullet *Mugil cephalus*, a closely related species,
541 acclimated to five experimental temperatures (i.e. from 10 to 30°C), which showed an optimal
542 temperature of about 25°C (Cucco et al. 2012) and a thermal performance breadth, i.e. the
543 maintenance of 80% of their metabolic performance, between approximately 18 and 30°C (Huey
544 1982).

545 As for the SMR, the rapid temperature change occurring during the MHW did not directly affect
546 either MMR or AS, as acclimated (i.e. *Control*₂₅) and acutely exposed individuals to 25°C (i.e. $T_{20:25(12h)}$)
547 showed a comparable response. While we previously discussed the dissimilarities in the thermal
548 dependence of SMR between *C. auratus* and both sheephead minnow (Kirby et al. 2020) and
549 European perch (Sandblom et al. 2016), the pattern for MMR appears equivalent between these
550 three species. There was indeed no difference in the MMR measured in sheephead minnows that
551 were acutely exposed or acclimated at 10 or 32°C; nor in the MMR of European perch acutely or
552 gradually warmed from 18 to 23°C. In the common killifish (*Fundulus heteroclitus*), Healy and Schulte
553 (2012) also concluded that there was no effect of the type of thermal exposure over the wide range
554 of 5–35°C. On the contrary, the tropical barramundi (*Lates calcarifer*) showed a different pattern,
555 with its MMR varying with the conditions of temperature exposure (Norin et al. 2014). Indeed, the
556 individuals acutely exposed to 38°C from their temperature of acclimation of 29°C (i.e. +2°C h⁻¹) had
557 a higher MMR and AS, compared to those progressively acclimated at 38°C. They interpreted this

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

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

577 4.3. Effects of the MHW on U_{crit} and EPOC

578 Because locomotion during a swimming step protocol partly relies on aerobic slow fibers, U_{crit} is
579 expected to be somehow related to aerobic metabolic performance as shown in various teleost
580 species such as Mahi-Mahi (*Coryphaena hippurus*, Heuer et al. 2021), lumpfish (*Cyclopterus lumpus*,
581 Hvas et al. 2018) and ten damselfish species (Johansen and Jones 2011). Our observations in *C.*
582 *auratus* confirm this expectation as U_{crit} followed the pattern observed in both MMR and AS, i.e. it
583 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; Fanguie 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

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

613 Our results may therefore suggest that fish exposed to acute thermal change showed increased
614 solicitation of anaerobic metabolism, at least when confronted with high energy-demanding
615 activities, as during a swimming effort. It may provide an additional energy source to compensate for
616 a potential deficiency of the aerobic metabolism to sustain swimming effort. This may be the result
617 of prioritization towards energy-demanding compensatory mechanisms induced by temperature
618 changes at the expense of swimming effort and/or a decrease in the efficiency of mitochondria to
619 produce ATP through aerobic pathways (Kunz et al. 2018; Voituron et al. 2022). In European sea bass
620 *Dicentrarchus labrax*, a temperate species with a similar distribution to *C. auratus*, Voituron et al.
621 (2022) recently showed that being acclimated at a high temperature (i.e. 26°C) improved the
622 mitochondrial efficiency (ATP/O, i.e. ratio between ATP synthesis and oxygen consumption). When
623 measured at their acclimation temperature of 26°C, the capacity of mitochondria to anaerobically
624 produce ATP was about 12% higher than when measured at this same temperature, but after an
625 acute thermal change from a lower acclimated temperature (i.e. from 18 to 26°C, or from 22 to
626 26°C).

627 **5. Conclusion**

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

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

650 **References**

- 651 Beaugerard D, Enders E, Boisclair D (2013) Consequences of circadian fluctuations in water
652 temperature on the standard metabolic rate of Atlantic salmon parr (*Salmo salar*). Can J Fish
653 Aquat Sci 70:1072–1081. doi: 10.1139/cjfas-2012-0342
- 654 Blasco FR, Esbaugh AJ, Killen SS, Rantin FT, Taylor EW, McKenzie DJ (2020) Using aerobic exercise to
655 evaluate sub-lethal tolerance of acute warming in fishes. J Exp Biol 223:jeb218602. doi:
656 10.1242/jeb.218602
- 657 Brett JR (1964) The Respiratory Metabolism and Swimming Performance of Young Sockeye Salmon. J
658 Fish Res Board Can 21(5): 1183-1226. doi: 10.1139/f64-103

- 659 Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a Metabolic Theory of Ecology.
660 Ecology 85:1771–1789. doi: 10.1890/03-9000
- 661 Burel C, Ruyet JP-L, Gaumet F, Roux AL, Sévère A, Boeuf G (1996) Effects of temperature on growth
662 and metabolism in juvenile turbot. J Fish Biol 49:678–692. doi: 10.1111/j.1095-
663 8649.1996.tb00064.x
- 664 Burgetz I, Rojas-Vargas A, Hinch S, Randall D (1998) Initial recruitment of anaerobic metabolism
665 during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 201:2711–
666 2721. doi: 10.1242/jeb.201.19.2711
- 667 Cai L, Liu G, Taupier R, Fang M, Johnson D, Tu Z, Huang Y (2014) Effect of temperature on swimming
668 performance of juvenile *Schizothorax prenanti*. Fish Physiol Biochem 40:491–498. doi:
669 10.1007/s10695-013-9860-0
- 670 Cannas M, Schaefer J, Domenici P, Steffensen JF (2006) Gait transition and oxygen consumption in
671 swimming striped surfperch *Embiotoca lateralis Agassiz*. J Fish Biol 69:1612–1625. doi:
672 10.1111/j.1095-8649.2006.01225.x
- 673 Cano-Barbacid C, Radinger J, Argudo M, Rubio-Gracia F, Vila-Gispert A, García-Berthou E (2020) Key
674 factors explaining critical swimming speed in freshwater fish: a review and statistical analysis
675 for Iberian species. Sci Rep 10:18947. doi: 10.1038/s41598-020-75974-x
- 676 Carpentier A, Como S, Dupuy C, Lefrançois C, Feunteun E (2014) Feeding ecology of *Liza spp.* in a tidal
677 flat: Evidence of the importance of primary production (biofilm) and associated meiofauna. J
678 Sea Res 92:86–91. doi: 10.1016/j.seares.2013.10.007
- 679 Cavole L, Diner R, Giddings A, Koester I, Pagniello C, Paulsen M-L, Ramirez-Valdez A, Schwenck S, Yen
680 N, Zill M, Franks P (2016) Biological Impacts of the 2013–2015 Warm-Water Anomaly in the

681 Northeast Pacific: Winners, Losers, and the Future. *Oceanog* 29(2):273-285. doi:
682 10.5670/oceanog.2016.32

683 Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J*
684 *Fish Biol* 88:81–121. doi: <https://doi.org/10.1111/jfb.12845>

685 Cheung WWL, Frölicher TL (2020) Marine heatwaves exacerbate climate change impacts for fisheries
686 in the northeast Pacific. *Sci Rep* 10:1–10. doi: 10.1038/s41598-020-63650-z

687 Claireaux G, Lagardère J-P (1999) Influence of temperature, oxygen and salinity on the metabolism of
688 the European sea bass. *J Sea Res* 42:157–168. doi: 10.1016/S1385-1101(99)00019-2

689 Claireaux G, Lefrançois C (2007) Linking environmental variability and fish performance: integration
690 through the concept of scope for activity. *Philos. Trans. R. Soc. B* 362:2031–2041. doi:
691 10.1098/rstb.2007.2099

692 Claireaux G, Chabot D (2016) Responses by fishes to environmental hypoxia: integration through
693 Fry's concept of aerobic metabolic scope. *J Fish Biol* 88:232–251. doi: 10.1111/jfb.12833

694 Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate
695 change: respirometry, relevance and recommendations. *J Exp Biol* 216:2771–2782. doi:
696 10.1242/jeb.084251

697 Corey E, Linnansaari T, Cunjak RA, Currie S (2017) Physiological effects of environmentally relevant,
698 multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo salar*). *Conserv Physiol* 5(1)
699 doi: 10.1093/conphys/cox014

700 Cucco A, Sinerchia M, Lefrançois C, Magni P, Ghezzi M, Umgieser G, Perilli A, Domenici P (2012) A
701 metabolic scope based model of fish response to environmental changes. *Ecol Mod* 237–
702 238:132–141. doi: 10.1016/j.ecolmodel.2012.04.019

703 Darmaraki S, Somot S, Sevault F, Nabat P, Cabos Narvaez WD, Cavicchia L, Djurdjevic V, Li L, Sannino
704 G, Sein DV (2019) Future evolution of Marine Heatwaves in the Mediterranean Sea. *Clim Dyn*
705 53:1371–1392. doi: 10.1007/s00382-019-04661-z

706 Deutsch C, Ferrel A, Seibel B, Pörtner H-O, Huey RB (2015) Climate change tightens a metabolic
707 constraint on marine habitats. *Science* 348:1132–1135. doi: 10.1126/science.aaa1605

708 Dichiera AM, Khursigara AJ, Esbaugh AJ (2021) The effects of warming on red blood cell carbonic
709 anhydrase activity and respiratory performance in a marine fish. *Comp Biochem Physiol A*
710 *Mol Integr Physiol* 260:111033. doi: 10.1016/j.cbpa.2021.111033

711 Fanguie NA, Mandic M, Richards JG, Schulte PM (2008) Swimming Performance and Energetics as a
712 Function of Temperature in Killifish *Fundulus heteroclitus*. *Physiol Biochem Zool* 81:389–401.
713 doi: 10.1086/589109

714 Farrell AP (2007) Cardiorespiratory performance during prolonged swimming tests with salmonids: a
715 perspective on temperature effects and potential analytical pitfalls. *Philos. Trans. R. Soc. B*
716 362:2017–2030. doi: 10.1098/rstb.2007.2111

717 Farrell AP (2002) Cardiorespiratory performance in salmonids during exercise at high temperature:
718 insights into cardiovascular design limitations in fishes. *Comp Biochem Physiol A Mol Integr*
719 *Physiol* 132:797–810. doi: 10.1016/S1095-6433(02)00049-1

720 Farrell AP (2009) Environment, antecedents and climate change: lessons from the study of
721 temperature physiology and river migration of salmonids. *J Exp Biol* 212:3771–3780. doi:
722 10.1242/jeb.023671

723 Frölicher TL, Laufkötter C (2018) Emerging risks from marine heat waves. *Nat Commun* 9(1):1–4. doi:
724 10.1038/s41467-018-03163-6

725 Fry FEJ (1971) The Effect of Environmental Factors on the Physiology of Fish. *Fish Physiol* 6:1–98.

726 Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonné P, Cigliano M, Diaz D, Harmelin JG, Gambi
727 MC, Kersting DK, Ledoux JB, Lejeusne C, Linares C, Marschal C, Pérez T, Ribes M, Romano JC,
728 Serrano E, Teixido N, Torrents O, Zabala M, Zuberer F, Cerrano C (2009) Mass mortality in
729 Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave.
730 *Glob Chang Biol* 15:1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

731 Gautier D, Hussenot J (2005) *Les Mulets Des Mers D’Europe: Synthèse Des Connaissances Sur Les*
732 *Bases Biologiques Et Les Techniques D’aquaculture*. Editions Quae

733 Gibson ES, Fry FEJ (1954) The performance of the lake trout, *Salvelinus namaycush*, at various levels
734 of temperature and oxygen pressure. *Can J Zool* 32:252–260. doi: 10.1139/z54-025

735 Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on
736 metabolic rate. *Science* 293:2248–2251. doi: 10.1126/science.1061967

737 Hachim M, Rouyer T, Dutto G, Kerzerho V, Bernard S, Bourjea J, McKenzie DJ (2021) Oxygen uptake,
738 heart rate and activities of locomotor muscles during a critical swimming speed protocol in
739 the gilthead sea bream *Sparus aurata*. *J Fish Biol* 98:886–890. doi:
740 <https://doi.org/10.1111/jfb.14621>

741 Healy TM, Schulte PM (2012) Thermal Acclimation Is Not Necessary to Maintain a Wide Thermal
742 Breadth of Aerobic Scope in the Common Killifish (*Fundulus heteroclitus*). *Physiol Biochem*
743 *Zool* 85:107–119. doi: 10.1086/664584

744 Heuer RM, Stieglitz JD, Pasparakis C, Enochs IC, Benetti DD, Grosell M (2021) The effects of
745 temperature acclimation on swimming performance in the pelagic Mahi-mahi (*Coryphaena*
746 *hippurus*). *Front. Mar. Sci.*, 1120. doi: 10.3389/fmars.2021.654276.

747 Hobday AJ, Alexander, Perkins, Smale, Straub (2016) A hierarchical approach to defining marine
748 heatwaves. *Prog Oceanogr* 141:227–238. doi: 10.1016/j.pocean.2015.12.014

749 Huey RB (1982) Temperature, physiology, and the ecology of reptiles. *Biology of the Reptilia* 12:25–
750 74.

751 Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC,
752 Beger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M, Cantin NE, Comeau S,
753 Connolly SR, Cumming GS, Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gilmour JP,
754 Harrison HB, Heron SF, Hoey AS, Hobbs J-PA, Hoogenboom MO, Kennedy EV, Kuo C-Y, Lough
755 JM, Lowe RJ, Liu G, McCulloch MT, Malcolm HA, McWilliam MJ, Pandolfi JM, Pears RJ,
756 Pratchett MS, Schoepf V, Simpson T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis
757 BL, Wilson SK (2017) Global warming and recurrent mass bleaching of corals. *Nature*
758 543:373–377. doi: 10.1038/nature21707

759 Hvas M, Folkedal O, Imsland A, Oppedal F (2017) The effect of thermal acclimation on aerobic scope
760 and critical swimming speed in Atlantic salmon, *Salmo salar*. *J Exp Biol* 220:2757–2764. doi:
761 10.1242/jeb.154021

762 Hvas M, Folkedal O, Imsland A, Oppedal F (2018) Metabolic rates, swimming capabilities, thermal
763 niche and stress response of the lumpfish, *Cyclopterus lumpus*. *Biol Open* 7:bio036079. doi:
764 10.1242/bio.036079

765 IPCC (2021): Summary for Policymakers. *Climate Change 2021: The Physical Science Basis*.
766 Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental
767 Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S.
768 Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R.
769 Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. In Press.

770

771 Islam MJ, Kunzmann A, Slater MJ (2021) Responses of aquaculture fish to climate change-induced
772 extreme temperatures: A review. *J World Aquac Soc* 53(2):314-366. doi: 10.1111/jwas.12853

773 Johansen JL, Jones GP (2011) Increasing ocean temperature reduces the metabolic performance and
774 swimming ability of coral reef damselfishes. *Glob Chang Biol* 17:2971–2979. doi:
775 10.1111/j.1365-2486.2011.02436.x

776 Johansen JL, Nadler LE, Habary A, Bowden AJ, Rummer J (2021) Thermal acclimation of tropical coral
777 reef fishes to global heat waves. *eLife* 10:e59162. doi: 10.7554/eLife.59162

778 Katopodis C, Gervais R (2012) Ecohydraulic Analysis of Fish Fatigue Data. *River Res Appl* 28:444–456.
779 doi: 10.1002/rra.1566

780 Kikuchi E, Grande FRD, Duarte RM, Vaske-Júnior T (2019) Thermal response of demersal and pelagic
781 juvenile fishes from the surf zone during a heat-wave simulation. *J Appl Ichthyol* 35:1209–
782 1217. doi: 10.1111/jai.13970

783 Killen SS, Reid D, Marras S, Domenici P (2015) The interplay between aerobic metabolism and
784 antipredator performance: vigilance is related to recovery rate after exercise. *Front Physiol*
785 6:111. doi: 10.3389/fphys.2015.00111

786 Killen SS, Christensen EAF, Cortese D, Závorka L, Norin T, Cotgrove L, Crespel A, Munson A, Nati JJH,
787 Papatheodoulou M, McKenzie DJ (2021) Guidelines for reporting methods to estimate
788 metabolic rates by aquatic intermittent-flow respirometry. *J Exp Biol* 224:jeb242522. doi:
789 10.1242/jeb.242522

790 Kirby AR, Crossley DA, Mager EM (2020) The metabolism and swimming performance of sheephead
791 minnows (*Cyprinodon variegatus*) following thermal acclimation or acute thermal exposure. *J*
792 *Comp Physiol B* 190:557–568. doi: 10.1007/s00360-020-01293-2

793 Kir M (2020) Thermal tolerance and standard metabolic rate of juvenile gilthead seabream (*Sparus*
794 *aurata*) acclimated to four temperatures. J Therm Biol 93:102739. doi:
795 10.1016/j.jtherbio.2020.102739

796 Kir M, Demirci Ö (2018) Thermal tolerance and standard metabolic rate of juvenile European sea bass
797 (*Dicentrarchus labrax*, Linnaeus, 1758) acclimated to four temperatures. J Therm Biol
798 78:209–213. doi: 10.1016/j.jtherbio.2018.10.008

799 Kunz KL, Claireaux G, Pörtner H-O, Knust R, Mark FC (2018) Aerobic capacities and swimming
800 performance of polar cod (*Boreogadus saida*) under ocean acidification and warming
801 conditions. J Exp Biol 221(21), jeb184473. doi: 10.1242/jeb.184473

802 Labille J, Slomberg D, Catalano R, Robert S, Apers-Tremelo M-L, Boudenne J-L, Manasfi T, Radakovitch
803 O (2020) Assessing UV filter inputs into beach waters during recreational activity: A field
804 study of three French Mediterranean beaches from consumer survey to water analysis. Sci.
805 Total Environ 706:136010. doi: 10.1016/j.scitotenv.2019.136010

806 Lee CG, Farrell AP, Lotto A, Hinch SG, Healey MC (2003) Excess post-exercise oxygen consumption in
807 adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed
808 swimming. J Exp Biol 206:3253–3260. doi: 10.1242/jeb.00548

809 Lefevre S (2016) Are global warming and ocean acidification conspiring against marine ectotherms? A
810 meta-analysis of the respiratory effects of elevated temperature, high CO₂ and their
811 interaction. Conserv Physiol 4:cow009. doi: 10.1093/conphys/cow009

812 Lefrançois C, Claireaux G (2003) Influence of ambient oxygenation and temperature on metabolic
813 scope and scope for heart rate in the common sole *Solea solea*. Mar Ecol Prog Ser 259:273–
814 284. doi: 10.3354/meps259273

815 Madeira D, Narciso L, Cabral HN, Vinagre C, Diniz MS (2013) Influence of temperature in thermal and
816 oxidative stress responses in estuarine fish. *Comp Biochem Physiol, Part A Mol Integr Physiol*
817 166:237–243. doi: 10.1016/j.cbpa.2013.06.008

818 Madeira D, Madeira C, Costa PM, Vinagre C, Pörtner H-O, Diniz MS (2020) Different sensitivity to
819 heatwaves across the life cycle of fish reflects phenotypic adaptation to environmental niche.
820 *Mar Environ Res* 162:105192. doi: 10.1016/j.marenvres.2020.105192

821 McCabe RM, Hickey BM, Kudela RM, Lefebvre KA, Adams NG, Bill BD, Gulland FMD, Thomson RE,
822 Cochlan WP, Trainer VL (2016) An unprecedented coastwide toxic algal bloom linked to
823 anomalous ocean conditions. *Geophys Res Lett* 43:10366-10376. doi:
824 10.1002/2016GL070023

825 McMahon SJ, Parsons DM, Donelson JM, Pether SMJ, Munday PL (2019) Elevated CO₂ and heatwave
826 conditions affect the aerobic and swimming performance of juvenile Australasian snapper.
827 *Mar Biol* 167:6. doi: 10.1007/s00227-019-3614-1

828 Milinkovitch T, Lucas J, Le Floch S, Thomas-Guyon H, Lefrançois C (2012) Effect of dispersed crude oil
829 exposure upon the aerobic metabolic scope in juvenile golden grey mullet (*Liza aurata*). *Mar*
830 *Pollut Bull* 64:865–871. doi: 10.1016/j.marpolbul.2012.01.023

831 Mills KE, Pershing AJ, Brown CJ, Chen Y, Chiang F-S, Holland DS, Lehuta S, Nye JA, Sun JC, Thomas AC,
832 Wahle RA (2013) Fisheries Management in a Changing Climate: Lessons from the 2012 Ocean
833 Heat Wave in the Northwest Atlantic. *Oceanography* 26(2):191–195. doi:
834 <http://dx.doi.org/10.5670/oceanog.2013.27>

835 Minuti JJ, Byrne M, Hemraj DA, Russell BD (2021) Capacity of an ecologically key urchin to recover
836 from extreme events: Physiological impacts of heatwaves and the road to recovery. *Sci Total*
837 *Environ* 785:147281. doi: 10.1016/j.scitotenv.2021.147281

838 Morash AJ, Neufeld C, MacCormack TJ, Currie S (2018) The importance of incorporating natural
839 thermal variation when evaluating physiological performance in wild species. *J Exp Biol*
840 221:jeb164673. doi: 10.1242/jeb.164673

841 Norin T, Clark TD (2016) Measurement and relevance of maximum metabolic rate in fishes. *J Fish Biol*
842 88:122–151. doi: 10.1111/jfb.12796

843 Norin T, Malte H, Clark TD (2014) Aerobic scope does not predict the performance of a tropical
844 eurythermal fish at elevated temperatures. *J Exp Biol* 217:244–251. doi: 10.1242/jeb.089755

845 Oligny-Hébert H, Senay C, Enders EC, Boisclair D (2015) Effects of diel temperature fluctuation on the
846 standard metabolic rate of juvenile Atlantic salmon (*Salmo salar*): influence of acclimation
847 temperature and provenience. *Can J Fish Aquat Sci* 72:1306–1315. doi: 10.1139/cjfas-2014-
848 0345

849 Palstra AP, Kals J, Böhm T, Bastiaansen JWM, Komen H (2020) Swimming Performance and Oxygen
850 Consumption as Non-lethal Indicators of Production Traits in Atlantic Salmon and Gilthead
851 Seabream. *Front Physiol* 11:759. doi: 10.3389/fphys.2020.00759

852 Payne NL, Smith JA, van der Meulen DE, Taylor MD, Watanabe YY, Takahashi A, Marzullo TA, Gray CA,
853 Cadiou G, Suthers IM (2016) Temperature dependence of fish performance in the wild: links
854 with species biogeography and physiological thermal tolerance. *Funct Ecol* 30:903–912. doi:
855 10.1111/1365-2435.12618

856 Peake SJ, Farrell AP (2006) Fatigue is a behavioural response in respirometer-confined smallmouth
857 bass. *J Fish Biol* 68:1742–1755. doi: 10.1111/j.0022-1112.2006.01052.x

858 Piatt JF, Parrish JK, Renner HM, Schoen SK, Jones TT, Arimitsu ML, Kuletz KJ, Bodenstein B, García-
859 Reyes M, Duerr RS, Corcoran RM, Kaler RSA, McChesney GJ, Golightly RT, Coletti HA, Suryan
860 RM, Burgess HK, Lindsey J, Lindquist K, Warzybok PM, Jahncke J, Roletto J, Sydeman WJ

861 (2020) Extreme mortality and reproductive failure of common murres resulting from the
862 northeast Pacific marine heatwave of 2014-2016. PLOS ONE 15:e0226087. doi:
863 10.1371/journal.pone.0226087

864 Pörtner HO (2002a) Climate variations and the physiological basis of temperature dependent
865 biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp
866 Biochem Physiol A Mol Integr Physiol 132:739–761. doi: 10.1016/S1095-6433(02)00045-4

867 Pörtner HO (2002b) Physiological basis of temperature-dependent biogeography: trade-offs in
868 muscle design and performance in polar ectotherms. J Exp Biol 205:2217–2230. doi:
869 10.1242/jeb.205.15.2217

870 Pörtner HO, Farrell AP (2008) Physiology and Climate Change. Science 322:690–692. doi:
871 10.1126/science.1163156

872 Pörtner H-O, Bock C, Mark FC (2017) Oxygen- and capacity-limited thermal tolerance: bridging
873 ecology and physiology. J Exp Biol 220:2685–2696. doi: 10.1242/jeb.134585

874 Reidy SP, Nelson JA, Tang Y, Kerr SR (1995) Post-exercise metabolic rate in Atlantic cod and its
875 dependence upon the method of exhaustion. J Fish Biol 47:377–386. doi: 10.1111/j.1095-
876 8649.1995.tb01907.x

877 Reidy SP, Kerr SR, Nelson JA (2000) Aerobic and anaerobic swimming performance of individual
878 Atlantic cod. J Exp Biol 203:347–357. doi: 10.1242/jeb.203.2.347

879 Rummer JL, Couturier CS, Stecyk JAW, Gardiner NM, Kinch JP, Nilsson GE, Munday PL (2014) Life on
880 the edge: thermal optima for aerobic scope of equatorial reef fishes are close to current day
881 temperatures. Glob Chang Biol 20:1055–1066. doi: 10.1111/gcb.12455

882 Sandblom E, Clark TD, Gräns A, Ekström A, Brijs J, Sundström LF, Odelström A, Adill A, Aho T, Jutfelt F
883 (2016) Physiological constraints to climate warming in fish follow principles of plastic floors
884 and concrete ceilings. Nat Commun 7:11447. doi: 10.1038/ncomms11447

885 Sanford E, Sones JL, García-Reyes M, Goddard JHR, Largier JL (2019) Widespread shifts in the coastal
886 biota of northern California during the 2014–2016 marine heatwaves. Sci Rep 9:1–14. doi:
887 10.1038/s41598-019-40784-3

888 Selig ER, Casey KS, Bruno JF (2010) New insights into global patterns of ocean temperature
889 anomalies: implications for coral reef health and management. Glob Ecol Biogeogr 19:397–
890 411. doi: 10.1111/j.1466-8238.2009.00522.x

891 Seuront L, Nicastrò KR, Zardi GI, Goberville E (2019) Decreased thermal tolerance under recurrent
892 heat stress conditions explains summer mass mortality of the blue mussel *Mytilus edulis*.
893 Science Rep 9:1–14. doi: 10.1038/s41598-019-53580-w

894 Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming
895 species. Proc R Soc Lond B Biol Sci 280:20122829. doi: 10.1098/rspb.2012.2829

896 Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander LV,
897 Benthuyzen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell
898 HA, Gupta AS, Payne BL, Moore PJ (2019) Marine heatwaves threaten global biodiversity and
899 the provision of ecosystem services. Nat Clim Chang 9:306–312. doi: 10.1038/s41558-019-
900 0412-1

901 Sokolova I (2021) Bioenergetics in environmental adaptation and stress tolerance of aquatic
902 ectotherms: linking physiology and ecology in a multi-stressor landscape. J Exp Biol
903 224:jeb236802. doi: 10.1242/jeb.236802

904 Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an
905 integrative tool for assessing limits of environmental stress tolerance in aquatic
906 invertebrates. *Mar Environ Res* 79:1–15. doi: 10.1016/j.marenvres.2012.04.003

907 Sorte CJB, Fuller A, Bracken MES (2010) Impacts of a simulated heat wave on composition of a
908 marine
909 community. *Oikos* 119:1909–1918. doi: 10.1111/j.1600-0706.2010.18663.x

910 Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, Farrell AP (2008) The effect of acute temperature
911 increases on the cardiorespiratory performance of resting and swimming sockeye salmon
912 (*Oncorhynchus nerka*). *J Exp Biol* 211:3915–3926. doi: 10.1242/jeb.019281

913 Svendsen JC, Tudorache C, Jordan AD, Steffensen JF, Aarestrup K, Domenici P (2010) Partition of
914 aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. *J*
915 *Exp Biol* 213:2177–2183. doi: 10.1242/jeb.041368

916 Vagner M, Lefrançois C, Ferrari RS, Satta A, Domenici P (2008) The effect of acute hypoxia on
917 swimming stamina at optimal swimming speed in flathead grey mullet *Mugil cephalus*. *Mar*
918 *Biol* 155:183–190. doi: 10.1007/s00227-008-1016-x

919 Vagner M, Zambonino-Infante J-L, Mazurais D, Imbert-Auvray N, Ouillon N, Dubillot E, Le Delliou H,
920 Akbar D, Lefrançois C (2014) Reduced n-3 highly unsaturated fatty acids dietary content
921 expected with global change reduces the metabolic capacity of the golden grey mullet. *Mar*
922 *Biol* 161:2547–2562. doi: 10.1007/s00227-014-2526-3

923 Vagner M, Lacoue-Labarthe T, Infante J-LZ, Mazurais D, Dubillot E, Delliou HL, Quazuguel P,
924 Lefrançois C (2015) Depletion of Essential Fatty Acids in the Food Source Affects Aerobic
925 Capacities of the Golden Grey Mullet *Liza aurata* in a Warming Seawater Context. *PLOS ONE*
926 10:e0126489. doi: 10.1371/journal.pone.0126489

- 927 Vagner M, Pante E, Viricel A, Lacoue-Labarthe T, Zambonino-Infante J-L, Quazuguel P, Dubillot E,
928 Huet V, Delliou HL, Lefrançois C, Imbert-Auvray N (2019) Ocean warming combined with
929 lower omega-3 nutritional availability impairs the cardio-respiratory function of a marine
930 fish. *J Exp Biol* 222(8), jeb187179. doi: 10.1242/jeb.187179
- 931 Voituron Y, Roussel D, Teulier L, Vagner M, Ternon Q, Romestaing C, Dubillot E, Lefrancois C (2022)
932 Warm Acclimation Increases Mitochondrial Efficiency in Fish: A Compensatory Mechanism to
933 Reduce the Demand for Oxygen. *Physiol Biochem Zool* 95:15–21. doi: 10.1086/716904
- 934 Volkoff H, Rønnestad I (2020) Effects of temperature on feeding and digestive processes in fish.
935 *Temperature* 7:307–320. doi: 10.1080/23328940.2020.1765950
- 936 Wernberg T, Smale D, Tuya F, Thomsen M, Langlois T, Bettignies TD, Bennett S, Rousseaux CS (2013)
937 An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot.
938 *Nat Clim Change*. doi: 10.1038/NCLIMATE1627
- 939 Wernberg T, Bennett S, Babcock RC, Bettignies T de, Cure K, Depczynski M, Dufois F, Fromont J,
940 Fulton CJ, Hovey RK, Harvey ES, Holmes TH, Kendrick GA, Radford B, Santana-Garcon J,
941 Saunders BJ, Smale DA, Thomsen MS, Tuckett CA, Tuya F, Vanderklift MA, Wilson S (2016)
942 Climate-driven regime shift of a temperate marine ecosystem. *Science* 353:169–172. doi:
943 10.1126/science.aad8745
- 944 Wood CM (1991) Acid-Base and Ion Balance, Metabolism, and their Interactions, after Exhaustive
945 Exercise in Fish. *J Exp Biol* 160:285–308. doi: 10.1242/jeb.160.1.285
- 946 Wright S, Metcalfe JD, Hetherington S, Wilson R (2014) Estimating activity-specific energy
947 expenditure in a teleost fish, using accelerometer loggers. *Mar Ecol Prog Ser* 496:19–32. doi:
948 10.3354/meps10528

949 Yuen JW, Dempster T, Oppedal F, Hvas M (2019) Physiological performance of ballan wrasse (*Labrus*
950 *bergylta*) at different temperatures and its implication for cleaner fish usage in salmon
951 aquaculture. *Biol Control* 135:117–123. doi: 10.1016/j.biocontrol.2019.05.007

952 Zeng L-Q, Zhang Y-G, Cao Z-D, Fu S-J (2010) Effect of temperature on excess post-exercise oxygen
953 consumption in juvenile southern catfish (*Silurus meridionalis* Chen) following exhaustive
954 exercise. *Fish Physiol Biochem* 36:1243–1252. doi: 10.1007/s10695-010-9404-9

955

956

957