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1 **Oxygen uptake, heart rate and activities of locomotor muscles during a critical**  
2 **swimming speed protocol in the gilthead sea bream *Sparus aurata***

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13

14 **Abstract**

15 Oxygen uptake, heart rate, and contraction frequencies of slow oxidative (SO) and fast glycolytic  
16 (FG) muscle, were measured simultaneously in gilthead seabream *Sparus aurata* submitted to  
17 stepwise increases in current speed in a swimming respirometer. Variation in oxygen uptake  
18 was closely related to variation in heart rate, over initial steps these rose in concert with an  
19 increase in contraction frequency of SO muscle. There was an asymptote in oxygen uptake and  
20 heart rate at high speeds, that reflected a transition from exclusive use of aerobic SO muscle to a  
21 combination of SO and anaerobic FG muscle, and which preceded fatigue.

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23

24 The critical swimming speed ( $U_{crit}$ ) protocol (Brett, 1964) is a well-established method to  
25 measure exercise performance in fishes (Beamish, 1978; Webb, 1998; McKenzie & Claireaux,  
26 2010). It has been used widely to investigate effects on performance of environmental factors  
27 such as temperature or salinity (Randall & Brauner, 1991; Farrell *et al.*, 2008; McKenzie &  
28 Claireaux, 2010) or as a biomarker of toxicological effects of aquatic pollutants (e.g. Beaumont *et*  
29 *al.*, 1995; McKenzie *et al.*, 2007; Wood *et al.*, 1996), and has had important applications in  
30 research for conservation of valuable migratory species (Farrell *et al.*, 2008; Eliason *et al.*, 2011).  
31 The protocol exposes fish to stepwise increases in speed in a swimming respirometer, until  
32 fatigue. The fish swims against the current by rheotactic reflex, holding position in the swim  
33 channel of the respirometer.

34 In fishes that use body-caudal fin locomotion (Webb, 1998), slow-twitch oxidative (SO)  
35 'red' muscle is used to power swimming at low to intermediate swimming speeds. Strips of  
36 muscle along the flanks beat the tail at steady frequencies directly proportional to swimming  
37 speed and the fish maintains a relatively static position in the respirometer. Contraction of SO  
38 muscle relies on ATP generated aerobically, it is well vascularized and supplied with nutrients  
39 and oxygen in the blood, pumped by the heart. So, increased aerobic swimming speed is linked  
40 to marked increases in oxygen uptake rate ( $M_{O_2}$ ) and heart beat frequency ( $f_H$ ) (Chatelier *et al.*,  
41 2005; McKenzie & Claireaux, 2010). At a certain high current speed in a  $U_{crit}$  test, however, the  
42 fish starts to engage its large myotomal blocks of fast-twitch glycolytic (FG) 'white' muscle, with  
43 irregular powerful tailbeats. These propel the fish forward in the swim channel, after which  
44 they drift back on the current, a 'burst and coast' swimming mode (Webb, 1998). The FG muscle  
45 is poorly vascularised and uses endogenous fuel stores to generate ATP anaerobically so, when it  
46 is recruited at high swim speeds, there can be an asymptote or even slight decline in  $M_{O_2}$  and  $f_H$   
47 (Beamish, 1978; Lee *et al.*, 2003; Chatelier *et al.*, 2005). This may be linked to reduced rates of  
48 SO muscle contraction but this has not been explicitly investigated. Once fishes recruit FG  
49 muscle and engage burst and coast swimming, they typically fatigue quite rapidly.

50 Although patterns of SO and FG muscle recruitment have been studied during forced  
51 swimming in fishes (Roberts & Graham, 1979; Rome *et al.*, 1985; Rome & Alexander, 1990; Geist  
52 *et al.*, 2003) no study has, to the best of our knowledge, simultaneously measured  $M_{O_2}$ ,  $f_H$  and  
53 muscle activity during a  $U_{crit}$  trial. This is interesting better to understand how patterns of  
54 oxygen uptake and heart rate relate to sequential recruitment of aerobic oxidative and then  
55 anaerobic glycolytic muscle. Specifically, to demonstrate that an asymptote in  $f_H$  and  $M_{O_2}$  at high  
56 speeds coincides with recruitment of FG muscle, and to ascertain what SO muscle activity  
57 patterns are at the highest speeds prior to fatigue.

58 Experiments were performed on gilthead seabream *Sparus aurata* L. 1758, a coastal  
59 marine teleost that is highly prized and widely farmed in the Mediterranean. It uses a sub-

60 carangiform swimming mode and, in the wild, adults can migrate over hundreds of kilometres  
61 (Lasserre, 1976). Experimental procedures were approved by the ethics committee for animal  
62 experimentation n° 036 of the French Ministère de l'Enseignement Supérieur, de la Recherche et  
63 de l'Innovation, with reference number APAFIS#10130-201704071516523 v3.

64 Six *S. aurata* with a mean ( $\pm$  SD) mass of  $612 \pm 10$  g and forklength of  $294 \pm 13$  mm were  
65 studied, from a population of animals obtained from Cannes Aquaculture fish farm and  
66 maintained at Ifremer Experimental Aquaculture Station at Palavas-les-Flots, in outdoor 3000 l  
67 tanks provided with a flow of local seawater at prevailing seasonal temperatures and an average  
68 salinity over the study period of 34‰. Fish were held for over 18 months prior to experiments,  
69 fed commercial pellets daily but fasted for 24 h prior to trials. Experiments were in April and  
70 May, when seawater temperature was 15 to 17 °C.

71 Fish were anesthetized by immersion in 0.1 g l<sup>-1</sup> benzocaine in aerated seawater, until  
72 active ventilation ceased, then positioned on an operating table with gills irrigated with aerated  
73 seawater containing 0.05 g l<sup>-1</sup> benzocaine. Insulated stranded stainless steel wire electrodes  
74 (0.05 mm wire diameter, 0.23 mm total diameter; Steel 7 Strand, A-M Systems,  
75 [www.phymep.com](http://www.phymep.com)), 800mm total length, insulated to within 0.5 cm of the tip, were placed to  
76 measure ECG and EMGs. For ECG, two electrodes were inserted on either side of the animal,  
77 under the cleithrum behind the 4<sup>th</sup> gill arch in close proximity to the heart. For EMGs, pairs of  
78 electrodes were inserted on one flank, along the axis of the animal beginning at the insertion of  
79 the dorsal fin and at a distance of 4 cm from each other; for SO muscle superficially just above  
80 midline (avoiding any contact with the lateral line); for FG muscle into epaxial muscle dorsally.  
81 The position of electrodes in SO and FG muscle was confirmed by dissection post mortem. All  
82 wires were held in place with sutures at point of insertion, then gathered and held in place with  
83 a common suture in front of the dorsal fin, so they trailed above fish during swimming trials  
84 (Blasco *et al.*, 2016). After surgery, fish were recovered in a Steffensen-type swim-tunnel  
85 respirometer (vol. 49 l) for at least 24h in aerated seawater at 16°C, swimming in a current  
86 equivalent to 0.5 body lengths per second (BL s<sup>-1</sup>).

87 The respirometer is designed to provide a non-turbulent water flow with a uniform  
88 velocity profile, in which to exercise fish at controlled current speeds while measuring their  
89 rates of oxygen uptake (McKenzie *et al.*, 2001). The anterior portion of the swim section was  
90 shielded with black plastic sheeting to avoid visual disturbance of fish, which spontaneously  
91 occupied this area. After recovery, seabream were exposed to progressive increments of  
92 swimming speed of 0.5 BL s<sup>-1</sup> each 40 min, until fatigue. Fish were considered fatigued when  
93 they rested their caudal fins on the downstream grid for at least 10s. Critical swimming speed  
94 ( $U_{crit}$ , in BL s<sup>-1</sup>) was calculated as described by Brett (1964). The  $M_{O_2}$  was measured in mmol O<sub>2</sub>  
95 kg<sup>-1</sup> h<sup>-1</sup> by respirometry twice at each speed by cyclical “intermittent stopped-flow” (Steffensen,

1989; Svendsen *et al.*, 2016), using the Aquaresp program (University of Copenhagen, [www.aquaresp.com](http://www.aquaresp.com)). Briefly, the cycle alternates two phases; firstly, the swim tunnel receives no water and the fish consumes oxygen within the closed system, measured by an optical sensor (Firesting O2, Pyro-Science, [www.pyro-science.com](http://www.pyro-science.com)) and associated software (Pyro Oxygen Logger), with data taken into Aquaresp. Secondly, Aquaresp starts a pump by USB relay (Cleware, [www.cleware-shop.de](http://www.cleware-shop.de)) that flushes aerated seawater through the tunnel, so renewing oxygen and removing wastes. Each cycle was 20 min, 8 of measurement and 12 of flushing; two cycles were completed at each swimming speed. Standard metabolic rate (SMR; basal metabolic rate at acclimation temperature) was estimated by back-extrapolating the relationship between  $\text{MO}_2$  and speed to a notional speed of zero (Brett, 1964; Chabot *et al.*, 2016). Active Metabolic Rate (AMR; maximal  $\text{MO}_2$  at acclimation temperature) was identified during swimming and usually occurred at speeds approaching  $U_{\text{crit}}$  (McKenzie *et al.*, 2003). Absolute aerobic scope (AAS) was the net difference between AMR and SMR, factorial scope (FAS) was  $\text{AMR}/\text{SMR}$  (Beamish, 1978).

To record  $f_{\text{H}}$ , ( $\text{beats min}^{-1}$ ) and frequencies of SO and FG muscle contractions ( $f_{\text{SO}}$  and  $f_{\text{FG}}$ , respectively, in Hz), ECG and EMG electrodes were connected to a BIOPAC MP36R data acquisition system linked to a computer with BIOPAC Acqknowledge software ([www.biopac.com](http://www.biopac.com)). EMG signals were filtered and recorded using the Acqknowledge 'EMG 30-1000 Hz' acquisition package, which sampled at 2000 Hz with low band pass at 1000 Hz and high band pass at 30 Hz. Signals were displayed on Acqknowledge throughout trials, then recorded twice for 5 min at each swimming speed. The  $f_{\text{H}}$  was calculated based on mean time required for 10 R-R intervals of ECG waveforms. The  $f_{\text{SO}}$  and  $f_{\text{FG}}$  were obtained from the interval between 5 EMG burst onsets, at each speed.

Data were tested for normality using Shapiro-Wilk Test prior to parametric tests. The effects of swimming speed on  $\text{M}_{\text{O}_2}$ ,  $f_{\text{H}}$ ,  $f_{\text{SO}}$  and  $f_{\text{FG}}$  were assessed by one way-analysis of variance (ANOVA) for repeated measures. When significant effects were observed, Bonferroni post-hoc tests were used to compare means. For descriptive purposes of patterns in mean data during the stages of the  $U_{\text{crit}}$  trial, linear or exponential regressions were applied, as detailed below. The relationship of  $f_{\text{H}}$  to  $\text{M}_{\text{O}_2}$  was described by linear regression. All analyses were performed with SigmaStat 4.0 (Systat Software Inc., [www.systatsoftware.com](http://www.systatsoftware.com)).

Mean ( $\pm$  SE)  $U_{\text{crit}}$  was  $3.2 \pm 0.2 \text{ BL s}^{-1}$ , all animals completed at least 20 min swimming at a speed of  $3 \text{ BL s}^{-1}$ , so data were collected for all variables for speeds of 0.5 to  $3 \text{ BL s}^{-1}$  (Fig 1). Mean  $\text{M}_{\text{O}_2}$  increased significantly ( $P < 0.001$  by ANOVA) with swimming speed, steeply until  $2 \text{ BL s}^{-1}$  followed by an asymptote at higher speeds (fig 1A). Application of an exponential relationship to mean data prior to the asymptote, namely  $0.5 \text{ BL s}^{-1}$  to  $2 \text{ BL s}^{-1}$ , revealed a high correlation coefficient ( $R^2 = 0.994$ ). Mean SMR was  $3.37 \pm 0.28 \text{ mmol O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , mean AMR was

132  $11.45 \pm 0.60 \text{ mmol O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and occurred at  $2.83 \pm 0.14 \text{ BL s}^{-1}$ . The AAS was  $8.07 \pm 0.49 \text{ mmol}$   
133  $\text{O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and FAS  $3.52 \pm 0.29$ . Mean  $f_H$  increased significantly ( $P < 0.001$  by ANOVA) with  
134 swimming speed, this was exponential between 0.5 and  $2 \text{ BL s}^{-1}$  ( $R^2 = 0.996$ ) with evidence of an  
135 asymptote at speeds beyond, coinciding with the asymptote in  $\text{MO}_2$  (fig 1B). Maximum  $f_H$  was  $96$   
136  $\pm 4 \text{ beats min}^{-1}$ , at an average swimming speed of  $2.92 \pm 0.19 \text{ BL s}^{-1}$ . There was a highly  
137 significant linear relationship between  $f_H$  and  $\text{MO}_2$  (fig 2) whereby  $\text{MO}_2 = 0.208(f_H) - 5.648$  ( $R^2 =$   
138  $0.987$ ,  $P < 0.0001$ ). The reciprocal relationship was used to estimate mean  $f_H$  at mean SMR,  
139 being  $50 \text{ beats min}^{-1}$  at  $3.37 \text{ mmol O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , such that average factorial cardiac scope was 1.9  
140 (96/50).

141 Fig. S1 in Supplementary Material shows representative traces of EMG signals for SO and  
142 FG muscle activity, such as were used to calculate their contraction frequency based upon  
143 intervals between burst onsets. There was a significant ( $P < 0.001$  by ANOVA) increase in mean  
144  $f_{SO}$  up to  $2 \text{ BL s}^{-1}$  after which contraction frequency dropped and, at  $3 \text{ BL s}^{-1}$ , it was significantly  
145 lower than at  $2.5 \text{ BL s}^{-1}$  (fig 1C). Among fish, the maximum  $f_{SO}$  of  $4.00 \pm 0.31 \text{ Hz}$  occurred at a  
146 mean speed of  $2.67 \pm 0.2 \text{ BL s}^{-1}$ , providing a mean stridlength (BL swum per SO muscle  
147 contraction) of  $0.69 \pm 0.07 \text{ BL}$ . By contrast, FG muscle had no contractile activity until a speed of  
148  $2 \text{ BL s}^{-1}$ , after which it increased rapidly and significantly ( $P < 0.001$  by ANOVA) to reach a  
149 maximum of  $1.07 \pm 0.30 \text{ Hz}$  at  $3 \text{ BLs}^{-1}$ , the last swimming speed that all fish achieved prior to  
150 fatigue (fig 1D). The engagement of FG muscle was associated with a gait transition to a 'burst  
151 and coast' swimming mode, where sea bream used irregular powerful tailbeats that 'burst' it  
152 forward in the swim channel, after which it 'coasted' back on the current until repeating the  
153 action.

154 The results demonstrate how patterns of  $\text{M}_{O_2}$  and  $f_H$  during a  $U_{crit}$  protocol relate to  
155 sequential recruitment of aerobic oxidative and anaerobic glycolytic muscle in a teleost with  
156 sub-carangiform locomotion. The  $U_{crit}$  values are similar to reports for farmed *S. aurata*  
157 (Basaran et al., 2007; Svendsen et al., 2015), comparisons are confounded by differences in body  
158 size and water temperature, which both affect  $U_{crit}$  performance (Beamish, 1978; McKenzie &  
159 Claireaux, 2010). The changes in  $\text{M}_{O_2}$  with swimming speed, comprising an initial exponential  
160 increase followed by an asymptote, have been reported in various species (Chatelier et al., 2005;  
161 Lee et al., 2003; Tudorache et al., 2015; this study), although others report an exponential  
162 increase up until fatigue (McKenzie et al., 2003; Steinhausen et al., 2005; Methling et al., 2011;  
163 Tudorache et al., 2015). The FAS during  $U_{crit}$ , approximately 3.5, is less than a previous report of  
164 around 5 (Svendsen et al., 2015), which may be due to factors such as fish size and rearing  
165 conditions. The  $f_H$  at low speeds, and as estimated at SMR from the linear relationship between  
166  $f_H$  and  $\text{M}_{O_2}$ , were similar to a previous reports of 'routine'  $f_H$  for *S. aurata* at this temperature  
167 (Aissaoui et al., 2000).

168 The exponential increase in  $M_{O_2}$  and  $f_H$  during initial stages of the  $U_{crit}$  was clearly a  
169 response to increasing oxygen and nutrient demands of mitochondria in working SO muscle  
170 (Teulier *et al.*, 2019), required to contract the muscle and beat the tail faster as speed steps were  
171 imposed. It is well-established that  $M_{O_2}$  rises exponentially with speed of aerobic swimming in  
172 fishes (Beamish, 1978; Webb, 1998). The estimated stridlength of SO muscle, approximately  
173 0.7 BL per tailbeat, is typical for teleosts with sub-carangiform locomotion (Beamish, 1978). The  
174 asymptote in  $M_{O_2}$  and  $f_H$  at 2 BL  $s^{-1}$  coincided with  $f_{SO}$  also reaching an asymptote coupled with  
175 engagement of FG muscle. The fact that SO muscle continued to contract at relatively high  
176 frequencies, alongside recruitment of the large, powerful FG muscle blocks, may explain why  
177  $M_{O_2}$  and  $f_H$  did not decline but essentially remained stable up until fatigue at  $U_{crit}$ . Although FG  
178 muscle is poorly vascularised, it does receive some blood flow and is a very large organ,  
179 representing up to 70% of the mass of the seabream (Teulier *et al.*, 2019). The very close  
180 coupling of  $M_{O_2}$  to  $f_H$ , throughout the  $U_{crit}$  protocol, was confirmed by the fact that variation in  $f_H$   
181 explained almost 99% of variation in  $M_{O_2}$ .

182 The  $f_{FG}$  was much lower than  $f_{SO}$ , with much more variation around the mean because  
183 contractions were aperiodic. It has been suggested that fatigue in a  $U_{crit}$  swim test is in fact a  
184 behavioural response, that occurs when a fish cannot engage the full power of FG muscle in the  
185 limited confines of the swim tunnel, so 'chooses' to fall back against the rear screen (Peake &  
186 Farrell, 2006; Tudorache *et al.*, 2007).

187 In conclusion, the data provide the first simultaneous measure of  $M_{O_2}$ ,  $f_H$  and contraction  
188 frequencies of SO and FG muscle in a fish during a  $U_{crit}$  protocol. The results demonstrate  
189 unequivocally that variation in  $M_{O_2}$  is closely related to variation in  $f_H$  and that an asymptote in  
190  $M_{O_2}$  and  $f_H$ , at high speeds, reflects a transition from exclusive use of aerobic SO muscle to a  
191 combination of SO and anaerobic FG muscle.

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196

## 197 **References**

- 198 Aissaoui, A., Tort, L., & Altimiras, J. (2000). Circadian Heart Rate Changes and Light-Dependence  
199 in the Mediterranean Seabream Sparus Aurata. *Fish Physiology and Biochemistry*, 22, 89–94.
- 200 Basaran, F., Ozbilgin, H., & Ozbilgin, Y. D. (2007). Comparison of the Swimming Performance of  
201 Farmed and Wild Gilthead Sea Bream, Sparus Aurata. *Aquaculture Research*, 38, 452–456.
- 202 Beamish, F. W. H. (1978). Swimming Capacity. In *Fish Physiology Volume 7* (Hoar, W. S., Randall,  
203 D. J., eds), pp. 101–187 New York: Academic Press.

204 Beaumont, M., Butler, P., & Taylor, E. (1995). Plasma Ammonia Concentration in Brown Trout in  
205 Soft Acidic Water and Its Relationship to Decreased Swimming Performance. *The Journal of*  
206 *experimental biology*, *198*, 2213–2220.

207 Blasco, F. R., McKenzie, D. J., Taylor, E. W., & Rantin, F. T. (2016). The Role of the Autonomic  
208 Nervous System in Control of Cardiac and Air-Breathing Responses to Sustained Aerobic  
209 Exercise in the African Sharptooth Catfish *Clarias Gariepinus*. *Comparative biochemistry and*  
210 *physiology. Part A, Molecular & integrative physiology*, *203*, 273–280.

211 Brett, J. R. (1964). The Respiratory Metabolism and Swimming Performance of Young Sockeye  
212 Salmon. *Journal of the Fisheries Research Board of Canada*, *21*, 1183–1226.

213 Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The Determination of Standard Metabolic Rate  
214 in Fishes. *Journal of Fish Biology*, *88*, 81–121.

215 Chatelier, A., McKenzie, D. J., & Claireaux, G. (2005). Effects of Changes in Water Salinity upon  
216 Exercise and Cardiac Performance in the European Seabass (*Dicentrarchus Labrax*). *Marine*  
217 *Biology*, *147*, 855–862.

218 Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., ... Farrell, A. P.  
219 (2011). Differences in Thermal Tolerance among Sockeye Salmon Populations. *Science*  
220 *(New York, N.Y.)*, *332*, 109–112.

221 Farrell, A. P., Hinch, S. G., Cooke, S. J., Patterson, D. A., Crossin, G. T., Lapointe, M., & Mathes, M. T.  
222 (2008). Pacific Salmon in Hot Water: Applying Aerobic Scope Models and Biotelemetry to  
223 Predict the Success of Spawning Migrations. *Physiological and Biochemical Zoology*, *81*,  
224 697–708.

225 Geist, D. R., Brown, R. S., Cullinan, V. I., Mesa, M. G., Vanderkooi, S. P., & McKinstry, C. A. (2003).  
226 Relationships between Metabolic Rate, Muscle Electromyograms and Swim Performance of  
227 Adult Chinook Salmon. *Journal of Fish Biology*, *63*, 970–989.

228 Lasserre, G. (1976). Dynamique Des Populations Ichtyologiques Lagunaires - Application a  
229 *Sparus aurata* L., Université des Sciences et Techniques du Languedoc, Montpellier.

230 Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G., & Healey, M. C. (2003). The Effect of  
231 Temperature on Swimming Performance and Oxygen Consumption in Adult Sockeye  
232 (*Oncorhynchus Nerka*) and Coho (*O. Kisutch*) Salmon Stocks. *Journal of Experimental*  
233 *Biology*, *206*, 3239–3251.

234 McKenzie, D. J., Cataldi, E., Romano, P., Owen, S. F., Taylor, E. W., & Bronzi, P. (2001). Effects of  
235 Acclimation to Brackish Water on the Growth, Respiratory Metabolism, and Swimming  
236 Performance of Young-of-the-Year Adriatic Sturgeon (*Acipenser Naccarii*). *Canadian*  
237 *Journal of Fisheries and Aquatic Sciences*, *58*, 1104–1112.

238 McKenzie, D. J., & Claireaux, G. (2010). The Effects of Environmental Factors on the Physiology of  
239 Aerobic Exercise. In *Fish Locomotion: An Eco-ethological Perspective* (Domenici, P., Kapoor,

240 B. G., eds), pp. 308–344 Boca Raton: CRC Press.

241 McKenzie, D. J., Martinez, R., Morales, a., Acosta, J., Morales, R., Taylor, E. W., ... Estrada, M. P.  
242 (2003). Effects of Growth Hormone Transgenesis on Metabolic Rate, Exercise Performance  
243 and Hypoxia Tolerance in Tilapia Hybrids. *Journal of Fish Biology*, *63*, 398–409.

244 McKenzie, D. J., Garofalo, E., Winter, M. J., Ceradini, S., Verweij, F., Day, N., ... Taylor, E. W. (2007).  
245 Complex Physiological Traits as Biomarkers of the Sub-Lethal Toxicological Effects of  
246 Pollutant Exposure in Fishes. *Philosophical transactions of the Royal Society of London.*  
247 *Series B, Biological sciences*, *362*, 2043–2059.

248 Methling, C., Tudorache, C., Skov, P. V., & Steffensen, J. F. (2011). Pop up Satellite Tags Impair  
249 Swimming Performance and Energetics of the European Eel (*Anguilla Anguilla*). *PLoS ONE*,  
250 6.

251 Peake, S. J., & Farrell, A. P. (2006). Fatigue Is a Behavioural Response in Respirometer-Confined  
252 Smallmouth Bass. *Journal of fish biology*, *68*, 1742–1755.

253 Randall, D. J., & Brauner, C. J. (1991). Effects of Environmental Factors on Exercise in Fish.  
254 *Journal of Experimental Biology*, *126*, 113–126.

255 Roberts, J. L., & Graham, J. B. (1979). Effect of Swimming Speed on the Excess Temperatures and  
256 Activities of Heart and Red and White Muscles in the Mackerel, *Scomber Japonicus*. *Fishery*  
257 *Bulletin*, *76*, 861–867.

258 Rome, L. C., & Alexander, R. M. (1990). The Influence of Temperature on Muscle Velocity and  
259 Sustained Performance in Swimming Carp. *Journal of experimental biology*, *154*, 163–178.

260 Rome, L. C., Loughna, P. T., Goldspink, G., & others. (1985). Temperature Acclimation: Improved  
261 Sustained Swimming Performance in Carp at Low Temperatures. *Science (New York, NY)*,  
262 *228*, 194.

263 Steffensen, J. F. (1989). Some Errors in Respirometry of Water Breathers: How to Avoid and  
264 Correct for Them. *Fish Physiology and Biochemistry*, *6*, 49–59.

265 Steinhausen, M. F., Steffensen, J. F., & Andersen, N. G. (2005). Tail Beat Frequency as a Predictor  
266 of Swimming Speed and Oxygen Consumption of Saithe (*Pollachius Virens*) and Whiting  
267 (*Merlangius Merlangus*) during Forced Swimming. *Marine Biology*, *148*, 197–204.

268 Svendsen, J. C., Tirsgaard, B., Cordero, G. A., & Steffensen, J. F. (2015). Intraspecific Variation in  
269 Aerobic and Anaerobic Locomotion: Gilthead Sea Bream (*Sparus Aurata*) and Trinidadian  
270 Guppy (*Poecilia Reticulata*) Do Not Exhibit a Trade-off between Maximum Sustained  
271 Swimming Speed and Minimum Cost of Transport. *Frontiers in Physiology*, *6*, 43.

272 Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and Setup of Intermittent-  
273 Flow Respirometry System for Aquatic Organisms. *Journal of Fish Biology*, *88*, 26–50.

274 Teulier, L., Thorat, E., Queiros, Q., McKenzie, D. J., Roussel, D., Dutto, G., ... Saraux, C. (2019).  
275 Muscle Bioenergetics of Two Emblematic Mediterranean Fish Species: *Sardina Pilchardus*

276 and Sparus Aurata. *Comparative Biochemistry and Physiology Part A: Molecular &*  
277 *Integrative Physiology*, 235, 174–179.

278 Tudorache, C., Viaenen, P., Blust, R., & De Boeck, G. (2007). Longer Flumes Increase Critical  
279 Swimming Speeds by Increasing Burst-Glide Swimming Duration in Carp *Cyprinus Carpio*,  
280 L. *Journal of Fish Biology*, 71, 1630–1638.

281 Tudorache, C., Burgerhout, E., Brittijin, S., & van den Thillart, G. (2015). Comparison of Swimming  
282 Capacity and Energetics of Migratory European Eel (*Anguilla Anguilla*) and New Zealand  
283 Short-Finned Eel (*A. Australis*). *Frontiers in Physiology*, 6, 256.

284 Webb, P. W. (1998). Swimming. In *The Physiology of Fishes* (Evans, D. D., ed), pp. 1–38 Boca  
285 Raton: CRC Press.

286 Wood, a W., Johnston, B. D., Farrell, a P., & Kennedy, C. J. (1996). Effects of  
287 Didecyldimethylammonium Chloride (DDAC) on the Swimming Performance, Gill  
288 Morphology, Disease Resistance, and Biochemistry of Rainbow Trout (*Oncorhynchus*  
289 *Mykiss* ). *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 2424–2432.

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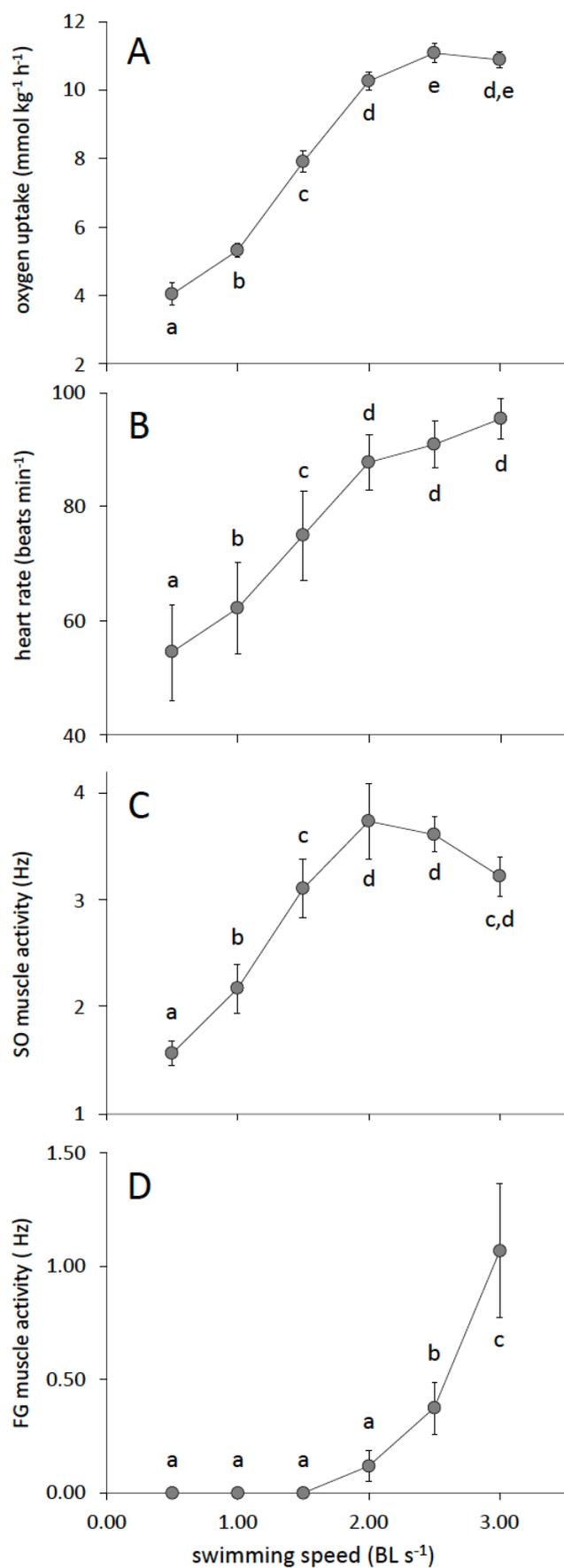
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292 **Figure legends**

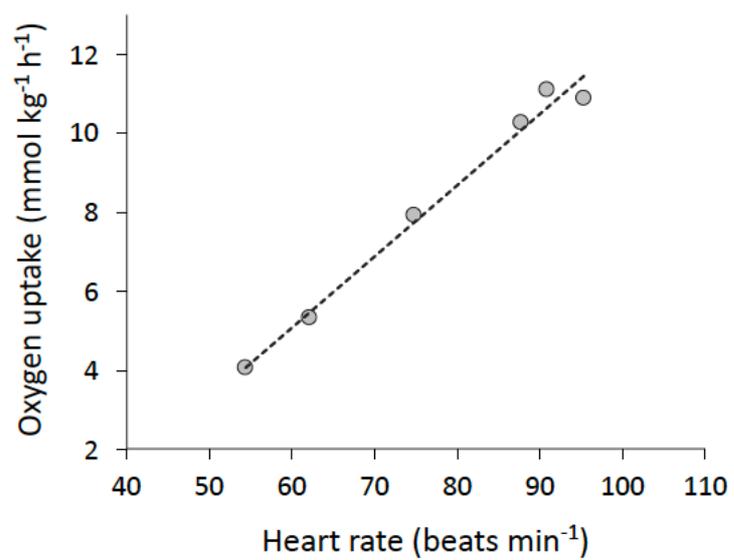
293 **Figure 1.** Effects of swimming speed, in bodylengths  $s^{-1}$ , on mean ( $\pm$  SEM) oxygen uptake rate  
294 (A); heart rate (B), and the contraction frequencies of slow-twitch oxidative (SO) and fast-twitch  
295 glycolytic (FG) muscles (C and D, respectively), in  $n = 6$  *Sparus aurata* submitted to a critical  
296 swimming speed protocol. On each panel, similar letters indicate no significant difference in the  
297 mean, by 1-way ANOVA for repeated samples and Bonferroni post-hoc test ( $P > 0.05$ ).

298 **Figure 2.** Least squares linear relationship between mean heart beat frequency ( $f_H$ ) and mean  
299 oxygen uptake rate ( $M_{O_2}$ ) in  $n = 6$  *Sparus aurata* submitted to a critical swimming speed protocol.  
300 The line is described by the equation  $M_{O_2} = 0.180(f_H) - 5.738$  ( $R^2 = 0.987$ ).

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