**Muscle bioenergetics of two emblematic Mediterranean fish species: *Sardina pilchardus*
and *Sparus aurata*.**

Loïc Teulier¹, Elisa Thorat¹, Quentin Queiros², David J. McKenzie³, Damien Roussel¹, Gilbert
Dutto⁴, Eric Gasset⁵, Jérôme Bourjea² and Claire Saraux².

¹Université de Lyon, UMR 5023, Écologie des Hydrosystèmes Naturels et Anthropisés,
Université Lyon 1, ENTPE, CNRS, F - 69622 Villeurbanne, France

²MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, Avenue Jean Monnet, 34203
Sète Cedex, France

³MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, Place Eugène Bataillon, 34095
Montpellier, France

⁴Ifremer (Institut Français de Recherche pour l'Exploitation de la MER), Laboratoire SEA,
chemin de Maguelonne, 34250 Palavas-les-Flots, France

⁵MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, chemin de Maguelonne, 34250
Palavas-les-Flots, France

KEY WORDS: red muscle, bioenergetics, marine fishes, lipids

RUNNING TITLE: Cellular fuels in fish muscle

25 SUMMARY

26 We investigated links between swimming behavior and muscle bioenergetics in two
27 emblematic Mediterranean fish species that have very different ecologies and activity levels.
28 European sardines *Sardina pilchardus* are pelagic, they swim aerobically, school constantly
29 and have high muscle fat content. Gilthead seabream *Sparus aurata* are bentho-pelagic, they
30 show discontinuous spontaneous swimming patterns and store less fat in their muscle.
31 Estimating the proportion of red and white muscle phenotypes, sardine exhibited a larger
32 proportion of red muscle (~10% of the body mass) compared to gilthead seabream (~5% of
33 the body mass). We firstly studied red and white muscle fiber bioenergetics, using high-
34 resolution respirometers, showing a 4-fold higher oxidation capacity for red compared to
35 white muscle. Secondly, we aimed to compare the red muscle ability to oxidize either lipids or
36 carbohydrates. Sardine red muscle had a 3-fold higher oxidative capacity than gilthead
37 seabream and a greater capacity to oxidize lipids. This study provides novel insights into
38 physiological mechanisms underlying the different lifestyles of these highly-prized species.

39

40

INTRODUCTION

Fishes have a long history as models for energetics and exercise physiology (Fry, 1957; Brett, 1971). Currently, they are used to investigate muscle metabolism and pathologies at various levels of integration (Li et al., 2017; Bergen et al., 2019; Krishnan and Rohner, 2019). At the *in vivo* level, fish swimming gaits have been well-studied, to define various measures of performance (Brett, 1964; Beamish, 1978; Drucker, 1996; Peake and Farrell, 2004). Gaits comprise steady sustained aerobic swimming, for relatively low speed activities such as migration or holding station, and unsteady burst or sprint anaerobic swimming, for high speed swimming such as predator-prey encounters (Webb, 1984). Aerobic and anaerobic swimming relies on structurally separate muscles in fishes (Bone, 1978; Webb, 1984). Slow-twitch oxidative ‘red’ muscle is used for steady aerobic swimming, it is found in strips along the midline and typically represents about 10% of muscle mass (Bone, 1978; McKenzie, 2011). Fast-twitch glycolytic ‘white’ muscle comprises the large myotomal blocks that form the majority of the muscle mass (Bone, 1978; Johnston, 1983; Jayne and Lauder, 1994). Moreover, red and white muscle are fueled by different energetic substrates and metabolic pathways relying on carbohydrate or lipid fuels are gradually involved in these different locomotion modes among fish species, depending on their swimming needs (Moyes and West, 1995; Weber and Haman, 1996).

The European sardine *Sardina pilchardus* and the gilthead seabream *Sparus aurata* have very different ecologies, in particular in terms of their swimming activity. Sardines are pelagic, they swim constantly in schools, covering great distances (Webb, 1984). Gilthead seabream are coastal benthopelagic, usually foraging in a discontinuous pattern for limited distances, although they perform quite extensive seasonal migrations to and from breeding grounds (Lasserre 1976; McClelland et al., 1995; Grigorakis et al., 2002; Steinhausen et al., 2010; Mercier et al., 2012). Gilthead seabream and sardine also present a different lipid

storage capacity, which can be related to their lifestyle and locomotor behavior. The sardine stores lipids in its muscle, as the primary site of rapidly available substrate (Venugopal and Shahidi, 1996). By contrast, gilthead seabream mainly stores lipids in the liver (McClelland et al., 1995). Beyond the fundamental ecophysiological interest of comparing these two species, our study has broader ecological and socio-economical implications. *Sparus aurata* is highly prized in inshore fisheries, representing the second most captured species (Weiss et al., 2018, <https://archimer.ifremer.fr/doc/00478/58970/>) and is also the main aquaculture species in the Mediterranean Sea, accounting for 44% of the farmed fish production. *Sardinus pilchardus* is the second most fished species in the Mediterranean as a whole, representing 17% of landings (FAO 2018. Global Capture Production 1950-2016 (online query), www.fao.org). Further, the sardine stock in the Gulf of Lions is currently considered to be ecologically unbalanced, with a major decline in final adult size and body condition (GFCM 2018), possibly mediated by differences in prey composition and feeding behaviour (Saraux et al., 2019; Queiros et al., submitted). While significant information is available on the seabream, due to its status as a farmed species (Pavlidis and Mylonas, 2011), much less is known about the physiology of sardines, due to the difficulties of maintaining them in captivity.

The aim of this study was to characterize aerobic (slow-twitch oxidative) muscle bioenergetics in the sardine and gilthead seabream. We hypothesized that the lifestyle of the sardine would be associated with adaptations for constant aerobic exercise, notably a high capacity for lipid-oxidative metabolism in their muscles compared with those of the more sedentary gilthead seabream.

MATERIAL AND METHODS

Fishes

The *S. aurata* were bought in April 2016 from Cannes Aquaculture (Cannes, France), and transported to the Ifremer research station at Palavas-les-Flots (France). They were then held at a density of 4 kg.m⁻³ in outdoor 4 m³ tanks supplied with well-aerated seawater at prevailing seasonal temperatures (8–25°C). Fish were fed once a day in the morning with 1% body weight of commercial feed. The *S. pilchardus* were captured in March 2016 off Sète (South of France) by a commercial purse-seiner adapted for this purpose. They were transported to the same site as seabream and held in quarantine tanks for health assessment.

After confirmation of an absence of pathogens, sardines were moved into indoor 3 m³ tanks supplied with water pumped directly from the sea (more details in Queiros et al., submitted). The photoperiod was adjusted each week to follow the natural cycle and sea water temperature was not controlled (averaging 12°C at this period of time) except to maintain a minimum of 10°C or a maximum of 25°C. Sardines were fed every day with 0.6% body mass of aquaculture pellets. Experiments were performed in February 2017 and approved by the Ministère Français de l'Enseignement Supérieur, de la Recherche et de l'Innovation (APAFIS # 4000-2016020415387815 v.3; APAFIS# 7097-2016093008412692).

Tissue sampling and Fiber bioenergetics

Fishes were fasted for one day before being euthanized with an overdose of benzocaine (1000 ppm) and small muscle samples were immediately withdrawn and stored at 4°C in MIR05 buffer containing 0.5 mM EGTA, 3 mM MgCl₂, 60 mM K-lactobionate, 20 mM Taurine, 10 mM KH₂PO₄, 20 mM HEPES, 110 mM sucrose, 1g/L free-fatty-acid bovine serum albumin, pH = 7.1 (Kuznetsov et al., 1998). Thereafter, the entire red and white muscles, liver and visceral tissues were dissected and weighed.

Respiration rates of both red and white muscle fibers were measured using high resolution respirometers (O2K, Oroboros Instruments; www.oroobros.at) in 2 ml of MIR05 solution at

12°C, following a protocol of sequential injections of substrates and inhibitors of the mitochondrial electron transport system (ETS) adapted from a previous study on *Danio rerio* (Teulier et al., 2018). According to this protocol, instead of using chemical permeabilization, fibers were gently mechanically disrupted to measure the cellular respiration rates. Basal non-phosphorylating respiration rate was obtained by addition of either a mixture of respiratory substrates (5 mM pyruvate/2.5 mM malate/5 mM succinate; PMS), which allow a full activation of the ETS by generating a convergent electron flow at the coenzyme-Q junction, or a lipid-derived substrate (40 μ M palmitoyl-carnitine/2.5 mM malate; PCM). The phosphorylating rate of respiration was initiated by the injection of ADP (1 mM). The integrity of mitochondrial membranes was systematically checked by adding cytochrome c (10 μ M). Maximal respiration rate was obtained using carbonyl cyanide-p-trifluoromethoxyphenyl hydrazine (FCCP; 1 μ M). Finally, the complex III (ubiquinol-cytochrome c oxydoreductase) of the ETS was inhibited by an injection of antimycin A (2.5 μ M). The factorial cellular aerobic scope refers to the ratio of FCCP-induced maximal uncoupling respiration rate to basal non-phosphorylating respiration rate (Roussel et al., 2015).

Statistical analysis

All data are expressed as means $\pm$ SEM. Species (sardine vs. seabream) and substrate (PMS vs PCM) effects on bioenergetics parameters were tested using 2-way ANOVA, or non-parametric 2-way ANOVA on ranks, depending on normality (Shapiro-Wilk test) and homoscedasticity (Levene test). When apposite, pairwise comparisons with adjustments for multiple comparisons (Holm–Sidak method) were conducted to detect further differences between substrates or species. Statistical analysis was performed using SIGMAPLOT 12 (Systat Software, Inc.; www.systatsoftware.com).

RESULTS

Body and tissue masses

Because of the much lower body mass of sardines, wet tissue masses were all significantly lower in sardines than in seabream (Table 1). However, relative to their body mass, the proportion of the different tissues varied between species. Sardines had a 2.5-fold higher proportion of red muscle to body mass than seabream, while visceral and white muscles were in similar proportions in both species (Table 1). The wet mass-to-body mass ratios (g/100 g BM) of liver were significantly lower in sardines than in seabream (Table 1).

Muscle fibers bioenergetics

Red and white muscle phosphorylating respiration rate (i.e. ADP-induced respiration rates with pyruvate/malate/succinate as respiratory substrates) were markedly different; white muscle fibers exhibited 6 to 12-fold lower respiration rates than red muscle fibers from sardine and seabream, respectively (Fig. 1A). Phosphorylating respiration rate of white muscle was higher in sardine than in gilthead seabream, while red muscle respiration rate was not statistically different between species (Fig. 1A). Using masses of white and red muscles, we then calculated the total muscle oxidative metabolism per gram of fish (Fig. 1B) and the relative contribution of white and red muscle to this total muscle oxidative metabolism (Fig. 1C). Sardines exhibited a significantly higher body mass specific-muscle oxidative capacity than gilthead seabream (Fig. 1B), and the contribution of its red muscles to whole body muscle oxidative capacity was slightly but significantly greater than gilthead seabream ($67\pm1\%$ versus $58\pm3\%$; Fig. 1C).

Because of the very low level of aerobic capacity in white muscles, the study regarding energy substrates oxidation was conducted on red muscles only. On the whole, red muscle fibers of sardines exhibited higher oxidative capacities than gilthead seabream,

regardless of respiratory substrates (Table 2). In details, the differences in ADP-induced respiration rate observed between species (2 and 2.5-fold higher in sardines than in gilthead seabream fibers respiring on PMS ($p=0.11$) and PCM ($p=0.07$)) were, however, not statistically significant. (Table 2). The rates of FCCP-induced maximal oxygen consumption were significantly higher in sardine than in gilthead seabream fibers, regardless of respiratory substrates (Table 2). The factorial cellular aerobic scope was not significantly different between fishes when fibers respired on PMS, but higher in sardine for a lipid-derived substrate, such as PCM (Table 2).

The higher muscle aerobic metabolism is even more pronounced when the rates of muscle oxygen consumption are expressed per gram of body mass when taking into account the total mass of red skeletal muscle in these two species (Fig. 2A). Despite huge differences in body mass-specific oxidative activities (Fig. 2A), the part of oxygen consumption associated with mitochondrial ATP synthesis was similar in the two species, accounting for about 75% and 87% of phosphorylating respiration rate with PMS and PCM, respectively (Fig. 2B). The remaining 13-25% of oxygen consumption was devoted to counteract proton leakage across the mitochondrial inner membrane (Fig. 2B). Hence, muscle mitochondria of both species allocated the same proportion of oxygen consumed to synthesize ATP. When fibers oxidized PMS, both species exhibited a large mitochondrial extra respiratory capacity (FCCP-induced maximal respiration rate minus ADP-induced phosphorylating respiration rate; Fig. 2B). By contrast, the extra respiratory capacity was reduced in the muscle fibers of gilthead seabream oxidizing a lipid-derived substrate (Fig. 2B). By comparison, sardine muscle fibers retained a large extra respiratory capacity with PCM, which was as high as that calculated with PMS (Fig. 2B).

DISCUSSION

The results obtained at the cellular level show that sardines have muscle biochemical adaptations for a lifestyle of constant sustained aerobic exercise, with a better ability to oxidize lipids than the sedentary gilthead seabream.

Muscles represent a large proportion of the body mass of both gilthead seabream and sardine (approximately 45%), which is in accordance with the classically described range of 40% to 60% (Bone, 1978). Even if the proportion of white muscle is roughly the same between species (~40%), the ratio between red muscle and body mass was significantly higher in sardines (above 10%) than in gilthead seabream (below 5%). Hence, red muscle represents almost 25% of the total muscle mass in sardines and only 10% in gilthead seabream. Our results are in line with the 30% obtained for *Sardina pilchardus* and the 15% in *Pagellus bogaraveo*, a fish closely related to *Sparus aurata* (Greer-Walker and Pull, 1975). Overall, fishes with a more active mode of life have a higher proportion of red fibers (Webb, 1984; Dwyer et al., 2014). Red and white muscle also show different aerobic capacity, with respiration rates 6-fold to 12-fold higher in red than in white muscle of sardine and gilthead seabream, respectively. Even though red muscles contribute to a small proportion of total skeletal muscle masses, its high respiratory capacity explains why its oxidative activity contributes to a major part of total muscle respiration rate (Fig. 1). The high metabolic capacity found in the red muscle of the two species is coherent with the extensive evidence of differences in histochemistry, enzyme activity and respiratory capacity between red and white muscles of many fish species (Bone, 1978, Moyes et al., 1992; Martinez et al., 2003; Morash et al., 2008; Strobel et al., 2013; Zak et al., 2017; Teulier et al., 2018).

The body-mass specific aerobic metabolism of red muscle is markedly higher in *S. pilchardus* than in *S. aurata*. Interestingly, the relative increase in respiration induced by ADP phosphorylation, i.e. the oxygen consumption dedicated to drive ATP synthesis, was the same for both species. This indicates that red muscle fibers of both species allocate the same energy

215 to synthesize ATP and thus to sustain aerobic locomotor performance. However, the FCCP-
216 induced extra respiratory capacity of red muscle fibers differed between species depending on
217 the respiratory substrates. Hence, it was not different when fibers respired on PMS, a mixture
218 of substrates that fully activate the ETS by generating a convergent electron flow at the
219 coenzyme-Q junction. It was, however, significantly lower in gilthead seabream than in
220 sardines when fibers oxidized PCM, a lipid-derived substrate. The FCCP-induced extra
221 respiratory capacity is controlled exclusively by mitochondrial oxidation of substrates, which
222 include the electron transport system, dehydrogenases and translocases. Hence, the results
223 indicate that the capacity to oxidize lipids is significantly higher in the red skeletal muscle of
224 sardine than in gilthead seabream. Indeed, sardine red muscle fibers oxidize PCM at 79% the
225 rate of fully activated ETS, whereas red muscle fibers of seabream oxidize lipids at 47%. This
226 metabolic “specialization” toward lipids oxidation is also supported by a very high value of
227 factorial aerobic scope in sardine red muscle fibers oxidizing a lipid-derived substrate. These
228 results are in accordance with other studies showing that, for instance, red muscles of the
229 pelagic coalfish (Johnston and Moon, 1980) or the endurance swimming rainbow trout
230 (Kiessling and Kiessling, 1993) exhibit a high lipid oxidation ability. And by directly
231 comparing the red muscles of skipjack tuna with the more sedentary freshwater carp, Moyes
232 and collaborators showed that the former contained more active mitochondria with also a
233 greater ability to oxidize lipids than the latter (Moyes et al., 1992).

234 Such difference of lipid aerobic metabolism results from a combination of both a
235 higher respiratory capacity and proportion of red muscle in *S. pilchardus* than in *S. aurata*.
236 However, we cannot completely rule out that part of the muscle metabolism difference
237 between the two species might also be explain by large difference in size, as *S. aurata*
238 weighting 8-fold more than *S. pilchardus*. Indeed, it has been reported that mitochondrial
239 oxidative enzyme activities in both red and white muscles negatively correlate with body

240 mass of fishes (Childress and Somero, 1990; Pelletier et al., 1993; Burness et al., 1999;
241 Almeida-Val et al., 2000; Davies and Moyes, 2007; Young and Egginton, 2009). For instance,
242 the activity of cytochrome-c oxidase, which measure the maximal oxidative capacity of a
243 tissue, correlate negatively with body mass with a scaling coefficient of -0.014 and -0.31 in
244 red muscle of striped bass and white muscle of Atlantic cod, respectively (Pelletier et al.,
245 1993; Young and Egginton, 2009). Based on these equations, body mass would explain
246 between 30% and 60% of the differences in the lipid-supported maximal respiratory capacity
247 between *S. aurata* and *S. pilchardus*. The rest would thus relate to difference in lipid energy
248 metabolic pathway (β -oxidation process and/or mitochondrial translocation system), which
249 might ultimately fit with the lifestyles of the two species. Lipids are well known to be a major
250 fuel for long distance locomotion in animals (Weber, 2011) and to play an important role as
251 energy substrates for sustained aerobic swimming in fishes (Johnston and Moon, 1980; Moyes
252 and West, 1995; McClelland et al., 1995). It is therefore not surprising that pelagic sardines,
253 that swim constantly, exhibit a higher ability to oxidize fat than benthopelagic and less active
254 gilthead seabream. Finally, pelagic fishes are characterized by abundant fat deposits under
255 skin and within red muscle, which are associated with their enhanced capacity to consume
256 lipids as fuel, whereas less active demersal fish typically stock fat in their liver (McClelland et
257 al., 1995; Venugopal and Shahidi, 1996). In this context, the very different lipid stores
258 between *Sardina pilchardus* and *Sparus aurata* would also participate in the differences in the
259 availability of fatty acids to fuel aerobic muscle function (McClelland et al., 1995; Venugopal
260 and Shahidi, 1996).

261 In conclusion, our results show that pelagic sardine exhibits a higher percentage of red
262 muscle than benthopelagic gilthead seabream, with a higher oxidative capacity at the cellular
263 level. Sardine are also able to oxidize lipids at nearly 80% the rate of fully ETS activity,
264 whereas gilthead seabream reach only 47% of the maximum respiratory capacity. However

265 even if muscle bioenergetics clearly show different patterns between these two species,
266 directly linking the locomotion performances to the cellular energetics needs further
267 investigations at the individual scale.

268

269 **Acknowledgements**

270 Sardine experiments were funded through the MONALISA project, which was co-
271 funded by the European Union and the French Ministry of Agriculture in the framework of the
272 European Maritime and Fisheries Fund (grant number PFEA280017DM0910001) and by
273 France Filière Pêche (France). The work on seabreams was funded by IFREMER – Site
274 Politic Initiative. Thanks to Philippe Joris for all his investment in managing the seabream for
275 so long as well as Hachim Mouniboudine for all the help in the lab analysis.

276

277 **References**

- 278 Almeida-Val, V. M. F., Val, A. L., Duncan, W. P., Souza, F. C. A., Paula-Silva, M. N. and
279 Land, S. (2000). Scaling effects on hypoxia tolerance in the Amazon fish *Astronotus*
280 *ocellatus* (Perciformes: Cichlidae): contribution of tissue enzyme levels. Comp.
281 Biochem. Physiol. 125B, 219–226.
- 282 Beamish, F. W. H. (1978). Swimming capacity. In Fish Physiology (ed. W.S. Hoar and D. J.
283 Randall), New York: Academic Press, 7, 101–187.
- 284 Bergen, D. J., Kague, E., & Hammond, C. L. (2019). Zebrafish as an emerging model for
285 osteoporosis: a primary testing platform for screening new osteo-active compounds.
286 Front. Endocrinol. 10, e6.
- 287 Bone, Q. (1978). Locomotor muscle. In Fish Physiology (ed. W.S. Hoar and D.J. Randall),
288 New York: Academic Press, 7, 361-424.
- 289 Brett, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye
290 salmon. Journal of the Fisheries Board of Canada, 21(5), 1183-1226.
- 291 Brett, J.R. (1971). Energetic Responses of Salmon to Temperature. A Study of Some Thermal
292 Relations in the Physiology and Freshwater Ecology of Sockeye Salmon
293 (*Oncorhynchus nerka*), Integr. Comp. Biol. 11 , 99–113.
- 294 Burness, G. P., Leary, S. C., Hochachka, P. W. and Moyes, C. D. (1999). Allometric scaling of
295 RNA, DNA, and enzyme levels: an intraspecific study. Am. J. Physiol. 277, R1164–
296 R1170.
- 297 Childress, J. J. and Somero, G. N. (1990). Metabolic Scaling: A New Perspective Based on
298 Scaling of Glycolytic Enzyme Activities. Integr. Comp. Biol. 30, 161–173.
- 299 Davies, R. and Moyes, C. D. (2007). Allometric scaling in centrarchid fish: origins of intra-
300 and inter-specific variation in oxidative and glycolytic enzyme levels in muscle. J.
301 Exp. Biol. 210, 3798-3804.

302 Drucker, E. G. (1996). The Use of Gait Transition Speed in Comparative Studies of Fish
 303 Locomotion. *Integr. Comp. Biol.* 36, 555–566.

304 Dwyer, G. K., Stoffels, R. J. and Pridmore, P. A. (2014). Morphology, metabolism and
 305 behaviour: responses of three fishes with different lifestyles to acute hypoxia.
 306 *Freshwater Biol.* 59, 819-831.

307 Fry, F. E. J. (1957). The aquatic respiration of fish. In *The Physiology of Fishes* (ed. M. E.
 308 Brown), New York: Academic Press, 1, 1-63.

309 Greer-Walker, M. and Pull, G. (1975). A survey of red and white muscle in marine fish. *J.*
 310 *Fish Biol.* 7, 295-300.

311 Grigorakis, K., Alexis, M. N., Taylor, K. D. A. and Hole, M. (2002). Comparison of wild and
 312 cultured gilthead sea bream (*Sparus aurata*): composition, appearance and seasonal
 313 variations. *Int. J. Food Sci. Techn.* 37, 477-484.

314 Jayne, B. and Lauder, G. (1994). How swimming fish use slow and fast muscle fibers:
 315 implications for models of vertebrate muscle recruitment. *J. Comp. Physiol. A* 175,
 316 123-131.

317 Johnston, I. A. (1983). Dynamic properties of fish muscle. In *Fish biomechanics*, eds. P. W.
 318 Webb and D. Weihs), pp. 36-67. New York: Praeger.

319 Johnston, I. A. and Moon, T. W. (1980). Exercise Training in Skeletal Muscle of Brook Trout
 320 (*Salvelinus fontinalis*). *J. Exp. Biol.* 87, 177-194.

321 Johnston, I. A. and Moon, T. W. (1980). Endurance exercise training in the fast and slow
 322 muscles of a teleost fish (*Pollachius virens*). *J. Comp. Physiol. B* 135, 147–156.

323 Kiessling, K. H. and Kiessling, A. (1993). Selective utilization of fatty acids in rainbow trout
 324 (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. *Can. J. Zool.* 71, 248–
 325 251.

326 Krishnan, J. and Rohner, N. (2019). Sweet fish: Fish models for the study of hyperglycemia
 327 and diabetes. *J. Diabet.* 11, 193-203.

328 Kuznetsov A, Lassnig B, Stadlmann S, Rieger G, Gnaiger E. (1998). Selected media and
 329 chemicals for respirometry with mitochondria and permeabilized cells. *Mitochondr.*
 330 *Physiol. Network* 3, 1–10.

331 Lasserre, G. (1976). Dynamique des populations ichtyologiques lagunaires —
 332 application à *Sparus aurata*. PhD thesis, Université des Sciences et Techniques
 333 du Languedoc, Montpellier

334 Leary, S. C., Lyons, C. N., Rosenberger, A. G., Ballantyne, J. S., Stillman, J. and Moyes, C.
 335 D. (2003). Fiber-type differences in muscle mitochondrial profiles. *Am. J. Physiol.*
 336 285, R817-R826.

337 Li, M., Hromowyk, K. J., Amacher, S. L., & Currie, P. D. (2017). Muscular dystrophy
 338 modeling in zebrafish. *Methods Cell Biol.* 138, 347-380.

339 McClelland, G., Weber, J.-M., Zwingelstein, G. and Brichon, G. (1995). Lipid composition of
 340 tissue and plasma in two Mediterranean fishes, the gilt-head sea bream
 341 (*Chrysophrys auratus*) and the European seabass (*Dicentrarchus labex*). *Can. J.*
 342 *Fish. Aquat. Sci.* 52, 161-170.

343 Martinez, M., Guderley, H., Dutil, J.D., Winger, P.D., He, P. and Walsh, S.J. (2003).
 344 Condition, prolonged swimming performance and muscle metabolic capacities of
 345 cod *Gadus morhua*. *J. Exp. Biol.* 206, 503-511.

346 McKenzie, D. J. (2011). Swimming and other activities - energetics of fish swimming. In
 347 Encyclopedia of Fish Physiology: From Genome to Environment, vol. 3 (ed. A. P.
 348 Farrell). San Diego, CA: Academic press.

349 Mercier, L., Mouillot, D., Bruguier, O., Vigliola, L., & Darnaude, A. M. (2012). Multi-
 350 element otolith fingerprints unravel sea– lagoon lifetime migrations of gilthead sea
 351 bream *Sparus aurata*. Mar. Ecol. Progr. Ser. 444, 175-194.

352 Morash, A.J., Kajimura, M. and McClelland, G.B. (2008). Intertissue regulation of carnitine
 353 palmitoyltransferase I (CPTI): Mitochondrial membrane properties and gene
 354 expression in rainbow trout (*Oncorhynchus mykiss*). Biochim. Biophys. Acta 1778,
 355 1382-1389.

356 Moyes, C. D., Mathieu-Costello, O. A., Brill, R. W. and Hochachka, P. W. (1992).
 357 Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonus*
 358 *pelamis*) and a slow (*Cyprinus carpio*) fish. Can. J. Zool. 70, 1246-1253.

359 Moyes, C. D. and West, T. G. (1995). Exercise metabolism of fish. In Biochemistry and
 360 molecular biology of fishes, vol. 4 (eds. P. W. Hochachka and T. P. Mommsen), pp.
 361 367-392: Elsevier.

362 Pavlidis, M. A., & Mylonas, C. C. (Eds.). (2011). Sparidae: Biology and aquaculture of
 363 gilthead sea bream and other species. John Wiley & Sons. Blackwell Publishing Ltd.,
 364 UK.

365 Peake, S. J., Farrell, A. P. (2004). Locomotory behaviour and post-exercise physiology in
 366 relation to swimming speed, gait transition and metabolism in free-swimming
 367 smallmouth bass (*Micropterus dolomieu*). J. Exp. Biol, 207, 1563-1575.

368 Pelletier, D., Guderley, H. and Dutil, J.-D. (1993). Does the aerobic capacity of fish muscle
 369 change with growth rates? Fish Physiol. Biochem. 12, 83-93.

370 Queiros, Q., Fromentin, J.-M., Gasset, E., Dutto, G., Huiban, C., Métral, L., Leclerc L., Schull
 371 Q., Mckenzie D. J. and Saraux C. Food in the Sea: size also matters for pelagic fish.
 372 (submitted)

373 Roussel, D., Salin, K., Dumet, A., Romestaing, C., Rey, B. and Voituron, Y. (2015). Oxidative
 374 phosphorylation efficiency, proton conductance and reactive oxygen species
 375 production of liver mitochondria correlates with body mass in frogs. *J. Exp. Biol.*
 376 218, 3222-3228.

377 Saraux, C., Van Beveren, E., Brosset, P., Queiros, Q., Bourdeix, J.-H., Dutto, G., Gasset, E.,
 378 Jac, C., Bonhommeau, S. and Fromentin, J.-M. (2019). Small pelagic fish dynamics:
 379 A review of mechanisms in the Gulf of Lions. *Deep Sea Res. Pt II* 159, 52-61.

380 Steinhausen, M. F., Fleng Steffensen, J. and Gerner Andersen, N. (2010). The effects of
 381 swimming pattern on the energy use of gilthead seabream (*Sparus aurata L.*). *Mar.*
 382 *Freshwater Behav. Physiol.* 43, 227-241.

383 Strobel, A., Leo, E., Pörtner, H.O. and Mark, F.C. (2013). Elevated temperature and PCO₂
 384 shift metabolic pathways in differentially oxidative tissues of *Notothenia rossii*.
 385 *Comp. Biochem. Physiol.* 166B, 48-57.

386 Teulier, L., Guillard, L., Leon, C., Romestaing, C. and Voituron, Y. (2018). Consequences of
 387 electroshock-induced narcosis in fish muscle: from mitochondria to swim
 388 performance. *J. Fish Biol.* 92, 1805-1818.

389 Venugopal, V. and Shahidi, F. (1996). Structure and composition of fish muscle. *Food Rev.*
 390 *Int.* 12, 175-197.

391 Webb, P. W. (1984). Body Form, Locomotion and Foraging in Aquatic Vertebrates. *Am. Zool.*
 392 24, 107-120.

393 Weber, J.-M. (2011). Metabolic fuels: regulating fluxes to select mix. *J. Exp. Biol.* 214, 286-
 394 294.

395 Weber, J.-M. and Haman, F. (1996). Pathways for metabolic fuels and oxygen in high
 396 performance fish. *Comp. Biochem. Physiol.* 113A, 33–38.

397 Weiss, J., Demaneche, S., Evano, H., Guyader, O., Bourjea, J., Derridj, O., Reynal, L.,
 398 Mansuy, E., Berthou, P., Leonardi, S., Rostiaux, E., Leblond, E. and Le Blond, S.
 399 (2018). Synthèse 2017 de l'observation des efforts et débarquements des pêcheries
 400 côtières. Rapport annuel Convention socle halieutique DPMA -Ifremer.

401 Young, S. and Egginton, S. (2009). Allometry of skeletal muscle fine structure allows
 402 maintenance of aerobic capacity during ontogenetic growth. J. Exp. Biol. 212, 3564–
 403 3575.

404 Zak, M.A., Regish, A.M., McCormick, S.D. and Manzon, R.G. (2017). Exogenous thyroid
 405 hormones regulate the activity of citrate synthase and cytochrome c oxidase in warm-
 406 but not cold-acclimated lake whitefish (*Coregonus clupeaformis*). Gen. Comp.
 407 Endocrinol. 247, 215-222.

408
 409

Figure legends

Figure 1: Aerobic metabolism of red and white muscles in sardine and gilthead

seabream. A) Phosphorylating respiration rate of white (white bars, n=2-4) and red (red bars, n=6) muscle fibers. B) Contribution of white and red muscles to total body mass-specific respiration rate. C) Relative contribution of white and red muscles to whole body muscle oxidative activity. Values are means $\pm$ sem. $\dagger p < 0.05$, significantly different from white muscle within the same fish species; $*p < 0.05$, significantly different from gilthead seabream.

Figure 2: Bioenergetics status of red muscle fibers. Fibers were respiring either on pyruvate/malate/succinate to fully activate the electron transport system (ETS) or palmitoyl-carnitine/malate (a lipid-derived substrate). A) Contribution of mitochondrial proton leak (basal non-phosphorylating respiration), mitochondrial ATP synthesis (ADP-induced phosphorylating respiration minus basal non-phosphorylating respiration), and mitochondrial respiratory reserve (FCCP-induced maximal respiration minus ADP-induced phosphorylating respiration) to body mass-specific respiration rates of fibers. B) Contribution of mitochondrial proton leak, ATP synthesis and respiratory reserve expressed as percentage of phosphorylating respiratory capacity. Values are means $\pm$ SEM for n=6 independent fiber preparations. $*p < 0.05$, significantly different from seabream.

431 **Table 1: Fish biometrics and tissue masses**

Tissues	Parameters	Gilthead Seabream	Sardine
	Body mass, g	340 ± 20	40 ± 5*
	Body length (mm)	270 ± 6	162 ± 5*
Red muscle	Fresh mass, g	15.5 ± 1.2	4.5 ± 0.5*
	Relative mass, g/100g BM	4.6 ± 0.5	11.3 ± 0.4*
White muscle	Fresh mass, g	137 ± 14	14 ± 2*
	Relative mass, g/100g BM	40 ± 2	36 ± 2
Liver	Fresh mass, g	9.5 ± 0.8	0.4 ± 0.1*
	Relative mass, g/100g BM	2.8 ± 0.2	0.8 ± 0.1*
Visceral tissues	Fresh mass, g	25.3 ± 2.7	3.3 ± 0.8*
	Relative mass, g/100g BM	7.4 ± 0.5	7.7 ± 0.9

432 Values are means ± sem for n=6. * $p < 0.05$, significantly different from gilthead seabream.

433

434 **Table 2: Respiratory variables of red muscle fibers from gilthead seabream and sardines.**

Substrates	Respiratory parameters	Gilthead seabream	Sardine
Pyruvate/Malate/Succinate	Basal non-phosphorylating rate	3.3 ± 0.8	6.1 ± 1.4
	ADP-induced phosphorylating rate	14.1 ± 3.5	27.4 ± 6.8
	FCCP-induced maximal oxidative rate	26.4 ± 4.1	47.6 ± 7.8*
	Factorial cellular aerobic scope	10.2 ± 2.5	8.5 ± 0.9
Palmitoyl-carnitine/Malate	Basal non-phosphorylating rate	1.5 ± 0.1	2.7 ± 0.5 †
	ADP-induced phosphorylating rate	9.4 ± 3.1	23.2 ± 6.1
	FCCP-induced maximal oxidative rate	12.5 ± 3.3 †	37.8 ± 5.9*
	Factorial cellular aerobic scope	7.0 ± 0.9	15.4 ± 1.7*†

435 Oxygen consumption rates are expressed in pmol O₂ s⁻¹ mg of tissue⁻¹ and were determined at 12°C. Basal state, basal non-phosphorylating
436 respiration measured in the presence of respiratory substrate alone; Phosphorylation state, ADP-induced phosphorylating respiration determined
437 after addition of 1 mM ADP; Maximal respiratory state, FCCP-induced maximal uncoupling respiration determined after addition of 1 µM
438 FCCP; Factorial cellular aerobic scope is the ratio of FCCP-induced maximal uncoupling respiration rate to basal non-phosphorylating
439 respiration rate. Values are means ± sem for n=6 independent fibers preparations. **p*<0.05, significantly different from seabream; † *p*<0.05,
440 significantly different from pyruvate/malate/succinate within the same fish species.