



**HAL**  
open science

## Cardiac and behavioural responses to hypoxia and warming in free-swimming gilthead seabream, *Sparus aurata*

Alexandre Mignucci, Jérôme Bourjea, Fabien Forget, Hossein Allal, Gilbert Dutto, Eric Gasset, David J. Mckenzie

### ► To cite this version:

Alexandre Mignucci, Jérôme Bourjea, Fabien Forget, Hossein Allal, Gilbert Dutto, et al.. Cardiac and behavioural responses to hypoxia and warming in free-swimming gilthead seabream, *Sparus aurata*. *Journal of Experimental Biology*, 2021, 224 (14), pp.jeb242397. 10.1242/jeb.242397 . hal-03415613

**HAL Id: hal-03415613**

**<https://hal.umontpellier.fr/hal-03415613>**

Submitted on 22 Nov 2021

**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 **Cardiac and behavioural responses to hypoxia and warming in free-swimming gilthead seabream**  
2 **(*Sparus aurata*)**

3

4 Alexandre Mignucci<sup>1</sup>, Jérôme Bourjea<sup>1</sup>, Fabien Forget<sup>1</sup>, Hossein Allal<sup>2</sup>, Gilbert Dutto<sup>3</sup>, Eric Gasset<sup>3</sup>,  
5 David J. McKenzie<sup>4</sup>

6

7 <sup>1</sup>MARBEC, Univ Montpellier, CNRS, IRD, Ifremer, 34200 Sète, France

8 <sup>2</sup>CHU de Montpellier, Service Chirurgie Pédiatrique, 34000 Montpellier, France

9 <sup>3</sup>MARBEC, Univ Montpellier, CNRS, IRD, Ifremer, 34250, Palavas-les-Flots, France

10 <sup>4</sup>MARBEC, Univ Montpellier, CNRS, IRD, Ifremer, 34095 Montpellier, France

11

12

13 \*Corresponding author: alexandre.mignucci@ifremer.fr

14

15 KEY WORDS: Heart rate, acceleration, biologging, warming, hypoxia, respirometry, *Sparus aurata*,  
16 Star-Oddi

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38 **SUMMARY STATEMENT**

39 Biologging of cardiac responses to hypoxia and warming in a free-swimming fish revealed that  
40 confinement in respirometer chambers raises heart rate, with consequences for estimates of  
41 metabolic rates.

42

43

44

45

46 **ABSTRACT**

47 Gilthead seabream were equipped with intraperitoneal biologging tags to investigate cardiac  
48 responses to hypoxia and warming, comparing when fish were either swimming freely in a tank with  
49 conspecifics or confined to individual respirometers. After tag implantation under anaesthesia, heart  
50 rate ( $f_H$ ) required 60 hours to recover to a stable value in a holding tank. Subsequently, when  
51 undisturbed under control conditions (normoxia, 21°C), mean  $f_H$  was always significantly lower in the  
52 tank than respirometers. In progressive hypoxia (100 - 15% oxygen saturation), mean  $f_H$  in the tank  
53 was significantly lower than respirometers at oxygen levels until 40%, with significant bradycardia in  
54 both holding conditions below this. Simultaneous logging of tri-axial body acceleration revealed that  
55 spontaneous activity, inferred as the variance of external acceleration ( $VAR_m$ ), was low and invariant  
56 in hypoxia. Warming (21 to 31°C) caused progressive tachycardia with no differences in  $f_H$  between  
57 holding conditions. Mean  $VAR_m$  was, however, significantly higher in the tank during warming, with a  
58 positive relationship between  $VAR_m$  and  $f_H$  across all temperatures. Therefore, spontaneous activity  
59 contributed to raising  $f_H$  of fish in the tank during warming. Mean  $f_H$  in respirometers had a highly  
60 significant linear relationship with mean rates of oxygen uptake, considering data from hypoxia and  
61 warming together. The high  $f_H$  of confined seabream indicates that respirometry techniques may bias  
62 estimates of metabolic traits in some fishes, and that biologging on free-swimming fishes will provide  
63 more reliable insight into cardiac and behavioural responses to environmental stressors by fishes in  
64 their natural environment.

65

66

67

68 *List of symbols and abbreviations:*

69 -  $f_H$ : heart rate frequency

70 -  $\dot{M}O_2$ : rate of oxygen uptake

71 - EA: external acceleration

72 -  $VAR_m$ : Variance of external acceleration

73

74 **INTRODUCTION**

75 Cardiac performance is a core determinant of the ability of fishes to survive and thrive in their  
76 environment, especially under challenging environmental conditions (Eliason and Anttila, 2017;  
77 Farrell and Smith, 2017; Stecyk, 2017). For instance hypoxia, a reduced availability of dissolved  
78 oxygen, is a common stressor in aquatic habitats (Diaz and Rosenberg, 2008) that challenges the  
79 ability of the heart to ensure tissue oxygen supply (Randall, 1982; Taylor, 1992). Most fishes are  
80 ectotherms, so increases in water temperature have direct thermodynamic effects on their  
81 metabolic rate and consequent oxygen demand, which the heart must be able to respond to (Cossins  
82 and Bowler, 1987; Rodgers, 2016; Schulte et al., 2011). Investigating how the fish heart responds to  
83 hypoxia and warming is of increasing relevance, because of the hypoxic episodes and summer  
84 heatwaves that are occurring in many aquatic ecosystems due to global change (Altieri and Diaz,  
85 2019; Costa and Barletta, 2016; Eliason and Anttila, 2017; Stecyk, 2017; Stillman, 2019).

86 The primary cardiac response to progressive hypoxia in fishes is a slowing of heart rate ( $f_H$ ),  
87 known as hypoxic bradycardia (see Farrell, 2007; Stecyk, 2017; Taylor, 1992, for detailed review).  
88 Although bradycardia is a chemoreflex response, there is still debate about its actual functional  
89 significance for hypoxia tolerance (Farrell, 2007; Joyce et al., 2016; McKenzie et al., 2009; Stecyk,  
90 2017). When progressively warmed, fishes exhibit increased  $f_H$ , a tachycardia that may have multiple  
91 contributing mechanisms (Eliason and Anttila, 2017). It presumably serves to meet the increased  
92 oxygen demands caused by thermal acceleration of metabolism, such that intrinsic capacity to raise  
93  $f_H$  may be a determinant of a species' upper temperature tolerance (see Eliason and Anttila, 2017, for  
94 a detailed review). **Although these cardiac responses to hypoxia and warming have been described in**  
95 **multiple species, this has almost exclusively been from acute experiments under controlled**  
96 **conditions with animals confined in some way and instrumented with wires connected to a**  
97 **measurement device (Eliason and Anttila, 2017; Stecyk, 2017). Very little is known about cardiac**  
98 **responses to hypoxia and temperature in free-swimming fishes (Claireaux et al., 1995a; Claireaux et**  
99 **al., 1995b; Lefrançois et al., 1998; Prystay et al., 2017).**

100 Small biologging tags that record  $f_H$  from the electrocardiogram (ECG) are now available,  
101 which can be implanted into fishes to measure their cardiac activity when they are recovered and  
102 free-swimming (Bjarnason et al., 2019; Brijs et al., 2018; Ekström et al., 2018; Prystay et al., 2017). It  
103 is not known whether cardiac responses to progressive hypoxia and warming will differ markedly in  
104 fishes swimming freely compared to when they are confined, but responses in free-swimming  
105 animals should be a more reliable reflection of responses by wild animals in their natural  
106 environment (Claireaux et al., 1995 a,b; Lefrançois et al., 1998). The tags can also log external tri-axial  
107 body acceleration (EA), such that it is possible to interpret cardiac responses of free-swimming  
108 animals against simultaneous measures of spontaneous behaviour (Clark et al., 2010).

109 We implanted biologgers into gilthead seabream (*Sparus aurata*) to compare cardiac  
110 responses to progressive hypoxia and warming when each animal was either shoaling in a tank with  
111 conspecifics, or confined in an individual respirometer chamber. We expected that, although fish  
112 would exhibit hypoxic bradycardia and warming tachycardia when swimming freely in the tank  
113 (Claireaux et al., 1995a,b; Lefrançois et al., 1998), the ability to express spontaneous activity would  
114 alter responses compared to when confined in a respirometer. We expected that  $f_H$  of undisturbed  
115 animals would be higher in the tank in normoxia at acclimation temperatures, due to spontaneous  
116 swimming activity. Progressive hypoxia might cause progressive declines in  $f_H$  in the tank, which  
117 initially and prior to the chemoreflexive hypoxic bradycardia, were due to reduced spontaneous  
118 swimming activity (Remen et al., 2015). In the respirometer, by contrast,  $f_H$  would be low and  
119 relatively stable until onset of chemoreflexive bradycardia (Perry et al., 2009). We expected  $f_H$  to  
120 increase more rapidly in the tank with warming, due to stimulation of activity (Claireaux et al.,  
121 1995a), with a maximum  $f_H$  reached at a lower temperature. We expected to demonstrate that  
122 swimming activity was a determinant of cardiac activity in the tank, by revealing a direct relationship  
123 between logged EA and  $f_H$ . Finally, the respirometers allowed us to investigate whether  $f_H$  was a  
124 predictor of metabolic rate in *S. aurata* during exposure to hypoxia and warming, as it is during  
125 aerobic exercise (Hachim et al., 2020).

126

## 127 MATERIAL AND METHODS

### 128 Ethical approval

129 Experimental procedures were approved by the ethics committee for animal experimentation n° 036  
130 of the French Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation, with  
131 reference number APAFIS #20294.

### 132 Animals

133 Experiments were performed on  $n = 12$  seabream with a mass of approximately 500 g and age of  
134 approximately 18 months. Sex was not verified, but fish were most probably male, as *S. aurata* is a  
135 protandrous hermaphrodite species. The seabream were obtained from the Ferme Marine du  
136 Douhet (La Brée les Bains, France) as post-larvae then reared at the Ifremer Aquaculture Research  
137 Station in Palavas-les-Flots, in indoor cylindrical tanks (vol 2 m<sup>3</sup>) under seasonal photoperiods,  
138 provided with a flow of biofiltered and UV-treated seawater at 21°C. Fish were fed daily with  
139 commercial pellets (B-Grower Marin, Le Gouessant, www.legouessant.com) but fasted for 24 h prior  
140 to surgery.

### 141 Surgery

142 Fish were anesthetized by immersion in 0.1 g l<sup>-1</sup> benzocaine (Benzocaine ethyl 4-Aminobenzoate,  
143 VWR, www.vwr.com) in aerated seawater, until active ventilation ceased, then weighed and placed

144 on an operating table with their gills irrigated with aerated seawater containing 0.05 g l<sup>-1</sup> benzocaine.  
145 Heart rate loggers (DST milli HRT-ACT, 13 mm × 39.5 mm, 12 g, Star-Oddi, Iceland, www.star-oddi.  
146 com) were implanted in the intraperitoneal space, via an a ~2 cm longitudinal incision along the  
147 ventral midline, ~0.5 cm posterior to the pectoral fins. Loggers were advanced as close as possible to  
148 the pericardium and fixed with sutures (silk suture and non-absorbable monofilament) such that  
149 their ECG electrodes lay against the body wall, with the incision then closed with sutures (non-  
150 absorbable monofilament). Fish were left to recover in a 1 m<sup>3</sup> cylindrical tank provided with a flow of  
151 aerated, biofiltered and UV-treated seawater at 21°C. The tank was isolated in a room dedicated  
152 exclusively to the study, with a natural photoperiod through skylights. The tank was shielded behind  
153 an opaque black plastic curtain. During recovery fish were checked by visual observation through  
154 small holes in the curtain (McKenzie et al., 2007) in the morning (08:30) and evening (17:00). Fish  
155 were not fed during recovery or subsequent experiments.

#### 156 **Hypoxia and temperature challenges**

157 Fish were instrumented and studied in two groups of six individuals. To allow us to compare each  
158 individual's responses to the challenges when either swimming freely in the tank or confined in a  
159 respirometer, we used a protocol where they were sequentially exposed in one holding condition  
160 and then in the other.

161 At 90h after surgery (Fig 1), three of the six fish were netted from the 1 m<sup>3</sup> tank and placed in  
162 individual rectangular clear plastic respirometer chambers (vol. 9 l) which were submerged in a small  
163 raceway in the same room, shielded behind an opaque black curtain and provided with the same  
164 water as the tank. The other three fish were left in the tank without any handling. After 24h recovery  
165 from this disturbance (hence at 5 days after surgery for all animals, Fig 1), experiments were  
166 conducted over five days. Over the first two days, the fish were exposed to warming and hypoxia  
167 challenges, with overnight recovery between the stressors. After this, fish were exchanged into their  
168 reciprocal holding condition and allowed to recover for 24h. Then, they were once again exposed to  
169 the two challenges over two successive days. The exact order in which each fish experienced  
170 warming and hypoxia, in the tank or in a respirometer, is provided in Tab S1. Fish were observed  
171 throughout all exposure protocols, at all exposure levels, through small holes in the curtains  
172 (McKenzie et al., 2007b). Care was taken to reduce all disturbance to a minimum during experiments,  
173 therefore experimenters entered at 08:20 to set up the trials then gave fish 30 min to recover from  
174 any disturbance before commencement.

175 For progressive hypoxia, oxygen partial pressure in the tank and raceway was decreased  
176 simultaneously by bubbling with 100% nitrogen, from 100% (normoxia) to 80%, then in steps of 10%  
177 from 80% to 20%, then finally 15%. Each step had a duration of 30 min, water oxygen levels were  
178 recorded using optical oxygen probes (Firesting sturdy dipping probes, Pyrosience,

179 www.pyroscience.com) and meter (FireSting FSO2-4), with data displayed and recorded in the Pyro  
180 Oxygen Logger software, with nitrogen flow and setpoints controlled manually. For acute warming,  
181 temperature was raised simultaneously in the tank and respirometers, in steps of 1°C every 30 min,  
182 from 21 to 31°C, using automated temperature control systems (AquaMedic T controller twin,  
183 www.aqua-medic.de) that reached incremental setpoints precisely by controlling activity of a  
184 submerged pump, in the tank or the raceway, that generated a flow of water through heat exchange  
185 coils immersed in a reservoir (1 m<sup>3</sup>) of tapwater held at 40°C. All fish were exposed to these levels of  
186 hypoxia and warming. The limit of 15% saturation in hypoxia was chosen because seabream of a  
187 similar size have been reported to be able to tolerate exposure to below 10% at 21°C (Remen et al.,  
188 2015). A limit of 31°C in warming is some degrees below CT<sub>max</sub> for *S. aurata*, which is at least 34°C (Kir  
189 et al., 2020; Madeira et al., 2014; Madeira et al., 2016). Thus, these exposures were sublethal but  
190 should nonetheless have engendered significant cardiac and behavioural responses (Claireaux et al.,  
191 1995a,b. Remen et al., 2015).

192 Rates of oxygen uptake ( $\dot{M}O_2$ , mmol kg<sup>-1</sup> h<sup>-1</sup>) were measured on the fish in the respirometers,  
193 using automated intermittent stopped-flow respirometry (Steffensen, 1989), over a 15 min cycle  
194 with a 6 min closure and 9 min flush period, providing two measures of  $\dot{M}O_2$  for each 30 min step of  
195 hypoxia or warming. Water oxygen concentrations were recorded continuously in each respirometer  
196 using Firesting sturdy dipping probes and meter, with data displayed and recorded in Pyro Oxygen  
197 Logger. Each fish's  $\dot{M}O_2$  was then calculated considering rate of decline in oxygen concentration in  
198 the chamber, chamber volume and the mass of the fish (McKenzie et al., 1995; McKenzie et al.,  
199 2007a). Background measurements, on empty chambers, were made prior to placing the fish and at  
200 the end of each series. These were always negligible and so no corrections were applied.

#### 201 **Logger programming and data processing**

202 The loggers were programmed with Mercury software (Star-Oddi) according to the manufacturer's  
203 instructions. Fish were all operated in the morning, with tags activated at 07:00 the following day.  
204 For  $f_H$ , the ECG data were sampled at 200 Hz for 4 s. During recovery from surgery and at night during  
205 experiments,  $f_H$  was measured once every two hours. It was measured once every 5 min from 07:00  
206 to 18:00 during the exposure trials. An ECG trace was saved with each measure of  $f_H$ , for visual  
207 confirmation of data quality. For EA, data were sampled at 10 Hz for 1 min, only during experiments,  
208 once every 5 min from 07:00 to 16:00 for the first series, and from 09:00 to 17:00 for the second  
209 series. Water temperature, date and time were also recorded with each  $f_H$  and EA measurement.

210 Heart rate was returned in beats min<sup>-1</sup>, calculated by the manufacturer's Patternfinder  
211 v.1.16.0 software from R-R intervals in the QRST wave of the ECG (Altimiras et al., 1997). Each  
212 measure was confirmed by visual inspection of the ECG trace and manual calculation of R-R interval  
213 within Patternfinder. The variance of EA (VAR<sub>m</sub>) was used to identify periods where variation in



214 acceleration indicated bouts of activity or agitation. It was calculated as the variance of the 600 EA  
215 measurements per minute, which indicated when the sensor was measuring acceleration above 1 g,  
216 in units of mg (1000 mg = 1 g), where EA = 0 is equal to 1 g and EA = 1000 is equal to 2 g. Each  
217 measure of  $f_H$  or VAR<sub>m</sub> was associated with temperature, date and time recorded on the logger. Date  
218 and time information on the logger were used to establish the associated oxygen levels for the  
219 hypoxia trials, based on the oxygen probe recordings.

## 220 **Data and statistical analysis**

221 Statistics were performed with R version 3.5.3 (R Core Team 2019) with  $p = 0.05$  taken as the fiducial  
222 level for statistical significance. To evaluate  $f_H$  across recovery days following surgery, data for each  
223 individual were averaged into 6h bins (3 2-hourly measures), starting at tag activation until 84h post-  
224 surgery, when fish were handled for experimentation. A one-way analysis of variance ANOVA with  
225 repeated measures was used to compare the sequential 6h values, using the aov function from the  
226 stats package (R Core Team 2019). Holm-Bonferroni post-hoc tests were used to identify where  
227 significant differences lay. For the five-day experimental protocol, 'undisturbed'  $f_H$  at 21°C in  
228 normoxia was evaluated from a single measure at 07:00 for each fish each morning. A two-way  
229 ANOVA was used to compare the undisturbed values with one factor being holding condition and the  
230 second being the sequential days of the protocol. The overall mean for each individual was compared  
231 between the tank and the respirometers by paired t-test. For these tests, normality,  
232 homoscedasticity and independence of residuals were verified visually.

233 Effects of progressive hypoxia or warming on  $f_H$  were evaluated and compared between  
234 holding conditions by two-way ANOVA with repeated measures using the aov\_car function from the  
235 afex package (Singmann et al., 2021) where one factor was holding condition and the other was  
236 either oxygen level or temperature. Since measures were repeated, both factors were treated as  
237 within subject factors. Undisturbed values at 07:00 prior to each trial were included in the ANOVA. As  
238 a two-way ANOVA with repeated measures does not tolerate missing data, 1.7% of the  $f_H$  measures  
239 were imputed using either nearest neighbours' or linear regression method, using the method kNN  
240 function from DMwR package (Torgo 2010). Linear regression models were always significant and  
241 calculated  $f_H$  was always plausible. Normality of the data was verified with a Shapiro-Wilk test.  
242 Sphericity of the data was not met, therefore a Greenhouse-Geisser correction was applied.  
243 Homoscedasticity of the residuals was verified visually by plotting them as a function of their fitted  
244 values. Holm-Bonferroni post-hoc tests were used to identify where significant differences occurred.  
245 A paired t-test was also used to compare maximum and minimum  $f_H$  during trials and the oxygen  
246 partial pressure or temperatures at which these occurred, with normality, homoscedasticity and  
247 independence of the residuals verified visually.

248 As  $VAR_m$  data were not normal, effects of the stressors and comparison between holding  
249 conditions were evaluated with a generalized mixed linear model, with fixed factors being holding  
250 condition and either oxygen partial pressure or temperature, and individuals as a random effect.  
251 Tukey post-hoc tests were used to identify where any significant differences lay. Models were  
252 calculated with the function `glmer` from `lme4` package (Bates et al., 2020) and post-hoc analysis was  
253 performed with `emmeans` package (Lenth et al., 2021).

254 A linear relationship between  $VAR_m$  and  $f_H$  was established for all individuals using a  
255 generalized mixed linear model, and between  $f_H$  and  $\dot{M}O_2$  using a mixed linear model, with  
256 individuals as a random effect. Regression slopes between temperature and hypoxia trials were  
257 compared using the function `lstrends` from the `lsmeans` R package (Lenth, 2018). A linear relationship  
258 was also established between  $f_H$  and  $\dot{M}O_2$  for each individual, using a linear model. Homoscedasticity  
259 and independence of the residuals were verified visually.

260

## 261 RESULTS

262 A complete dataset was collected for  $n = 10$  seabream (four from first and six from second series),  
263 with a mean ( $\pm$  S.D.) mass of  $534 \pm 86$  g, ranging from 363 to 801 g.

### 264 Undisturbed $f_H$

265 Over 72h of recovery with absolutely minimal disturbance (Fig 1), all individuals showed a  
266 progressive decline in  $f_H$  (Fig 1). The mean ( $\pm$  S.E.)  $f_H$  declined significantly until 60h, after which it  
267 showed no further significant change (Fig 1). At 90h of recovery, corresponding to 15:00 in the  
268 afternoon, fish were disturbed by netting and transfer of individuals to chambers, to start  
269 experiments. Over the ensuing five-day protocol, the mean undisturbed  $f_H$  (i.e. the mean  $f_H$  at 07:00  
270 each day) was significantly lower in the tank than in the respirometers. This was irrespective of the  
271 days elapsed or individual exposure history during the protocol (Tab 1, Figs 2 and 3, Fig S1).

### 272 Responses to hypoxia

273 During progressive hypoxia, *S. aurata* displayed bradycardia in both the tank and respirometers (Fig  
274 2). Within each condition,  $f_H$  did not vary significantly from undisturbed normoxia down to 40%, but  
275 then  $f_H$  decreased significantly at 30%, 20% and 15%. There was, however, a significant difference in  
276  $f_H$  between the tank and the respirometers during hypoxia trials ( $p < 0.01$ ) and a significant  
277 interaction between holding condition and oxygen level ( $p < 0.01$ ). Thus, mean  $f_H$  was significantly  
278 lower in the tank than in the respirometers at all oxygen steps between 80% and 40%. Although  
279 undisturbed normoxic  $f_H$  differed significantly (Tab 1, Fig 2), this was not true of the measures taken  
280 in normoxia at the first step of the exposure trial, likely because of increased individual variation in  $f_H$   
281 in the respirometers (Fig 2). This was presumably because the fish had been slightly disturbed by  
282 presence of experimenters. Once bradycardia occurred, namely at 30%, 20% and 15%, there were no

283 significant differences in  $f_H$  between the tank and the respirometers (Fig 2). These different patterns  
284 of  $f_H$  during hypoxia, between the two holding conditions, were reflected in the fact that mean  
285 maximum  $f_H$ , whenever this might have occurred during hypoxia trials, was significantly lower ( $p <$   
286  $0.01$ ) in the tank than in the respirometers (Tab 1). On the other hand, the mean minimum  $f_H$  was  
287 similar and occurred at a similar very low oxygen saturation (Tab 1).

288 The  $VAR_m$  of all individuals was generally low in hypoxia, with no significant differences  
289 between the tank and the respirometers at any level of hypoxia. (Fig S2). Visual inspection of the  
290 tank revealed that the seabream were moving slowly around the perimeter in hypoxia and tended to  
291 stop swimming entirely and rest on the bottom of the tank at hypoxic levels that caused bradycardia.  
292 Inspection of the respirometers showed no signs of agitation at any of the hypoxic levels.

293 Oxygen uptake in hypoxia showed a typical teleost response, where it was regulated at levels  
294 similar to normoxia until 40%, from where there was a significant and progressive decline in mean  
295  $\dot{M}O_2$  (Fig S3).

#### 296 **Responses to warming**

297 During progressive warming, *S. aurata* displayed tachycardia in both the respirometers and in the  
298 tank (Fig 3). Mean  $f_H$  was statistically similar between holding conditions at all temperatures, despite  
299 having been different when undisturbed at 21°C (Tab 1, Fig 3). Once again,  $f_H$  at the initial 21°C step  
300 of the exposure protocol was variable among individuals, presumably due to mild disturbance. There  
301 was, however, a significant interaction between holding condition and temperature ( $p < 0.01$ ). Thus,  
302  $f_H$  was similar between undisturbed and 21°C steps, but then increased significantly from 21 up until  
303 27°C in the tank, but only increased from 21 up until 23°C in respirometers (Fig 3). Furthermore, the  
304 mean temperature at which maximum  $f_H$  occurred was significantly higher in the tank ( $30.3 \pm 0.34^\circ\text{C}$ )  
305 than in the respirometers ( $28.6 \pm 0.68^\circ\text{C}$ ), being closer to the maximum temperature tested ( $31^\circ\text{C}$ ) in  
306 the tank (Tab 1). One fish in a respirometer (Fish n. 4, Tab S1) showed an aberrant cardiac response  
307 to temperature, with a drastic drop in  $f_H$  at 28°C, which then remained low until 31°C (Fig 3), which  
308 probably contributed to the interaction between holding condition and temperature. Visual  
309 inspection revealed that the fish was very dark coloured and striped at 28°C, and then showed signs  
310 of losing equilibrium at the end of trial. It was immediately removed and transferred to the tank. It  
311 then performed normally in subsequent trials, so we had no reason to exclude this animal from  
312 analyses.

313 During the warming trials,  $VAR_m$  was highly variable in the tank but generally showed a  
314 progressive increase (Fig 4). It increased significantly from 21 to 23°C ( $p < 0.01$ ), the first warming  
315 step, and then showed various further significant changes, being highest overall at 31°C, the highest  
316 temperature ( $p < 0.001$ ) compared to 21°C (Fig 4). As would be expected in confined fish,  $VAR_m$  was  
317 usually low in the respirometers and did not vary significantly with temperature (Fig 4). As a result,

318 mean  $VAR_m$  in the tank was significantly higher than in the respirometer at many temperatures (Fig  
319 4). Visual inspection of the tank showed that the fish were swimming actively around the perimeter  
320 at high temperatures, with occasional bursts of speed, especially at 31°C. Inspection of the  
321 respirometers only revealed signs of activity in one individual, starting at 26°C, which was reflected in  
322 a high  $VAR_m$  at 26 and 28°C (Fig 4). Unfortunately,  $VAR_m$  data were not collected on this animal at  
323 higher temperatures due to an error in our programming of the Star-Oddi tag.

324 During warming, animals in respirometers generally showed an increase in  $\dot{M}O_2$  with  
325 warming that was significant at temperatures above 23°C (Fig S4). The individual that showed the  
326 aberrant cardiac response also showed an aberrant metabolic response, with a decline in  $\dot{M}O_2$  from  
327 28°C (Fig S4).

### 328 Relationships between $VAR_m$ and $f_H$ , and $f_H$ and $\dot{M}O_2$

329 There was a significant linear relationship between  $VAR_m$  and  $f_H$  in the tank during the warming trials  
330 ( $p < 0.001$ ), which was the only condition where animals showed significant activity (Fig 5). There was  
331 no relation of  $f_H$  to  $VAR_m$  under any other condition.

332 There was a significant positive linear relationship between  $f_H$  and  $\dot{M}O_2$  in the respirometers  
333 during both hypoxia ( $\dot{M}O_2 = f_H (0.034) + 0.4$ ; marginal  $R^2 = 0.31$ ; conditional  $R^2 = 0.65$ ;  $p < 0.001$ ) and  
334 warming trials ( $\dot{M}O_2 = f_H (0.02) + 3.37$ ; marginal  $R^2 = 0.085$ ; conditional  $R^2 = 0.72$ ;  $p < 0.001$ ). There  
335 was no significant difference between these two slopes, so a single linear relationship between  $f_H$   
336 and  $\dot{M}O_2$  was fitted for all hypoxia and warming values plotted together, which was highly significant  
337 ( $p < 0.001$ ; Fig 6). Heart rate was also a predictor of metabolic rate for each individual fish (Tab S2).

338

## 339 DISCUSSION

340 This study provides the first explicit demonstration of cardiac responses to progressive hypoxia and  
341 progressive warming in a free-swimming fish, and comparison to responses by the same individuals  
342 confined in a respirometer. The cardiac loggers have, however, already been used on several species  
343 (Arvén Norling, 2017; Brijs et al., 2018; Brijs et al., 2019a; Brijs et al., 2019b; Davidsen et al., 2020;  
344 Prystay et al., 2017; Skeeles et al., 2020), including to demonstrate that  $f_H$  is significantly affected by  
345 water temperature in sockeye salmon (*Oncorhynchus nerka*) migrating in the wild (Prystay et al.,  
346 2017). A major unexpected result was that the  $f_H$  of undisturbed seabream was higher when they  
347 were confined compared to free-swimming. As a consequence, cardiac responses to hypoxia and  
348 warming in the free-swimming animals did not follow our predictions, although they did exhibit some  
349 interesting differences when compared to the fish when confined.

### 350 Undisturbed heart rates and the effects of confinement

351 The relatively high  $f_H$  after surgery presumably indicates an acute stress response, which may have  
352 included a release of circulating catecholamines (Reid et al., 1998, Gallo and Civinini 2003) and/or

353 removal of inhibitory cholinergic neural control (Randall 1982, reviewed in Farrell, 1984). The  
354 progressive decline in  $f_H$  during ensuing recovery presumably indicates an associated decline in stress  
355 and recovery of autonomic control (Campbell et al., 2004; McKenzie et al., 2007b; Sandblom and  
356 Axelsson, 2011; Taylor et al., 2010). Recovery of mean  $f_H$  by 60h is somewhat faster than studies on  
357 salmonids. Brijis et al., (2019b) reported that a minimum of three days was necessary for free-  
358 swimming rainbow trout (*Oncorhynchus mykiss*) to recover a stable  $f_H$  after implantation of these  
359 loggers, while Føre et al., (2021) found that an average of four days was required in free-swimming  
360 Atlantic salmon (*Salmo salar*). The undisturbed mean  $f_H$  of the seabream following 60h recovery, at  
361 around 60 beats  $\text{min}^{-1}$  at 21°C, are amongst the lowest resting values reported for this species  
362 (Aissaoui et al., 2000; Aissaoui et al., 2005; Altimiras et al., 1997; Hachim et al., 2020).

363 Throughout the five-day exposure protocol, the seabream were necessarily subjected to  
364 daily disturbance from presence of experimenters, plus the exposures to hypoxia and warming.  
365 Nonetheless, when undisturbed in the morning, seabream consistently had higher  $f_H$  when in the  
366 respirometers, irrespective of whether they had been handled or exposed to stress on the previous  
367 day. The most obvious explanation would be a stress response to confinement, as stress is known to  
368 increase heart rate in fishes (Farrell, 1991; Lefrançois et al., 1998; Rabben and Furevik, 1993; Sopinka  
369 et al., 2016; Svendsen et al., 2021). The proximate mechanism for the high  $f_H$  in seabream confined in  
370 chambers requires further investigation. This implies, nonetheless, that allowing the seabream to  
371 shoal with conspecifics was less stressful than being confined alone. This finding indicates that  
372 confinement may introduce bias into studies of physiological responses by fishes to environmental  
373 stressors, in a manner that may differ among species. Notably, it could bias measures of metabolic  
374 rate by static respirometry, given that  $f_H$  can be a predictor of  $\dot{M}O_2$ , which is the case for *S. aurata*  
375 (Hachim et al., 2020). The experiments also revealed how sensitive seabream were to disturbance as,  
376 despite taking great care, our simple presence was enough to obscure differences in  $f_H$  between the  
377 tank and the respirometers at trial commencement in normoxia at 21°C.

### 378 **Responses to hypoxia**

379 The data demonstrate that hypoxic bradycardia is observed in free-swimming fish but the cardiac  
380 response did not comprise a progressive decline linked to a progressive drop in spontaneous activity.  
381 Instead, it was very similar to 'typical' responses reported for many species under confined  
382 experimental conditions (Perry et al., 2009). Hypoxic bradycardia is a reflex response in teleosts, the  
383 sensory arm being chemoreceptor nerve cells in and around the gills that sense oxygen levels in  
384 ventilatory water and blood streams and transmit this information to the brainstem. The reflex  
385 response occurs via cholinergic fibres in the cardiac branch of the vagus nerve, which slow the heart  
386 (reviewed by Farrell and Smith, 2017; Stecyk, 2017; Taylor, 1992). The functional significance of  
387 hypoxic bradycardia is still debated but it may protect function of the cardiac pump, a purely aerobic

388 organ, by conserving contractility and reducing myocardial energy requirements when oxygen supply  
389 in the blood is below a critical level (Farrell, 2007; Iversen et al., 2010; Joyce et al., 2016; McKenzie et  
390 al., 2009).

391 It is noteworthy that, although  $f_H$  was significantly lower in the tank compared to the  
392 respirometers at oxygen levels above the threshold for hypoxic bradycardia, this threshold did not  
393 differ, being between 40 and 30% in both conditions. The higher  $f_H$  in seabream confined in a  
394 respirometer should, presumably, have been accompanied by a higher  $\dot{M}O_2$  than when swimming  
395 freely in the tank, given the direct relationship between these two variables. It might be expected,  
396 therefore, that the threshold for bradycardia would be higher in respirometer than in the tank. The  
397 fact that the threshold was the same and that, once bradycardia did occur,  $f_H$  was similar between  
398 tank and respirometer, requires further investigation.

399 The low  $VAR_m$  during progressive hypoxia, and absence of differences between the tank and  
400 respirometers, presumably indicates that movements in the tank did not involve changes in speed,  
401 which are necessary to engender variation in acceleration (Hinch et al., 2002; Kawabe et al., 2003;  
402 Palstra et al., 2021; Tanaka et al., 2001). Thus, the gentle movements observed during hypoxia in the  
403 tank were clearly below the sensitivity of the accelerometer in the tag.

#### 404 **Responses to warming**

405 Although these tags have been used to study cardiac responses to acute warming in an anaesthetised  
406 fish (Skeeles et al. 2020), this is the first report of responses to progressive warming in a fully-  
407 recovered free-swimming animal. As for hypoxia, cardiac responses were generally similar between  
408 the tank and respirometer, with a pronounced tachycardia in both cases. Warming tachycardia in  
409 fishes presumably represents a response to increased oxygen demand when metabolism is  
410 accelerated by warming, as demonstrated by the linear relationship of  $f_H$  and  $\dot{M}O_2$  during warming in  
411 the respirometer. In terms of the heart itself, this response may reflect both direct effects of  
412 temperature on pacemaker function and modulation of autonomic control (see Eliason and Anntilla,  
413 2017, for a detailed review). In the seabream, the maximum  $f_H$  observed during warming, 205 beats  
414  $\text{min}^{-1}$  at a temperature of 31°C, was about double the maximum achieved during forced exercise in a  
415 swim-tunnel at 16°C in this species (Hachim et al., 2020). These high  $f_H$  in the seabream were all  
416 confirmed by visual inspection of the traces, with clear ECG waveforms.

417 It is interesting that, unlike in hypoxia, the accelerometer detected activity in the tank during  
418 warming, especially at the higher temperatures. The consistently higher  $VAR_m$  in the tank relative to  
419 the respirometer, for most warming steps, would explain why  $f_H$  did not differ significantly between  
420 the holding conditions. Thus, the  $VAR_m$  data confirm that, in free-swimming individuals, the  
421 tachycardia was not only due to the warming itself, but also to behavioural responses to increasing  
422 temperature (Claireaux et al., 1995a). When free to express spontaneous behaviour, the seabream

423 were extremely sensitive: the significant increase in  $VAR_m$  upon initial exposure to 23°C may have  
424 been because the fish were reared their whole post-larval life at 21°C. The fact that the mean  
425 temperature for maximum  $f_H$  was reached at a lower temperature in the respirometer than in the  
426 tank was contrary to our expectation, but entirely consistent with the fact that the confined fish  
427 already exhibited tachycardia when undisturbed at 21°C. The activity observed in the tank, especially  
428 the bursts of speed at high temperatures, may have reflected attempts to escape the conditions,  
429 although fish did not become agitated at the same temperature when confined in the respirometer.  
430 Overall, the behavioural responses to warming may provide useful indicators of tolerance that are  
431 much more sensitive than, for example, loss of equilibrium at a critical thermal maximum ( $CT_{max}$ )  
432 (McDonnell and Chapman, 2015). In *S. aurata*,  $CT_{max}$  ranges from about 34.3°C to 36.6°C, depending  
433 on acclimation temperature (Kir, 2020; Madeira et al., 2014; Madeira et al., 2016).

#### 434 **Relationships of heart rate to acceleration and metabolic rate**

435 The significant dependence of  $f_H$  on  $VAR_m$  during warming in the tank is further proof that  
436 spontaneous activity was responsible for raising  $f_H$  of the free-swimming fish. The relationship was,  
437 nonetheless, rather noisy with low predictive power. This may be because increases in  $VAR_m$ ,  
438 especially at high temperatures, reflect agitation and burst swimming movements powered by fast-  
439 twitch glycolytic muscle (Webb, 1978). The metabolic costs of such movements are paid during  
440 recovery, rather than during the activity itself (Webb, 1978; Kieffer\*, 2000), so changes in  $f_H$  may  
441 have been out of phase with changes in  $VAR_m$ . Palstra et al. (2021) concluded that acceleration was  
442 most reliable as an index of unsteady burst swimming activity in the seabream, for example when  
443 fish are feeding. Although measures of body acceleration have also been used to predict metabolic  
444 rate in fishes (Bouyoucos et al., 2017; Gleiss et al., 2010; Metcalfe et al., 2016; Wilson et al., 2013;  
445 Wright et al., 2014), it seems unlikely they will ever have the same predictive power as  $f_H$ , not least  
446 because movement is only one component of metabolic activity in fishes.

447 The data demonstrate that  $f_H$  is a predictor of metabolic rate in *S. aurata* during hypoxia and  
448 warming, as it is during aerobic swimming (Hachim et al., 2020). The fact that a single linear  
449 relationship between mean  $f_H$  and mean  $\dot{M}O_2$  could be described, irrespective of whether data  
450 derived from exposure to hypoxia or warming, demonstrates a tight coupling of cardiac pumping  
451 activity to metabolic oxygen demand under diverse environmental conditions in this species. **The**  
452 **relationships for individual animals were highly significant, which Thorarensen et al., 1996 cite as a**  
453 **necessary condition to use  $f_H$  as a predictor for metabolic rate. However, their predictive power**  
454 **differed markedly among fish, with variation in  $f_H$  explaining less than 70% of variation in  $\dot{M}O_2$  in six**  
455 **of the ten seabream. Also, the relationship may break down under multiple stressors and elevated**  
456 **workloads, or at high temperatures where heart rate can become thermally limited (Thorarensen et**  
457 **al., 1996, Brijs et al., 2019) For this reason, we did not perform the exercise of predicting individual**

458  **$\dot{M}O_2$  from their  $f_H$  when in the tank.** Further research is required to establish the extent to which  
459 this variation among individuals is methodological, for example because  $f_H$  and  $\dot{M}O_2$  were measured  
460 over different time scales, or is physiological. Nonetheless, the results are promising in terms of  
461 calibrating the relationship of  $f_H$  to  $\dot{M}O_2$  using respirometry and then using logged  $f_H$  data to estimate  
462 patterns of energy use by free-swimming seabream (Clark et al., 2010; Cooke et al., 2016; Lucas,  
463 1994; Treberg et al., 2016). The need to retrieve the tag is still a limitation on performing such  
464 studies on fish released into their natural environment (Prystay et al., 2017, 2019).

465

## 466 **CONCLUSIONS**

467 The results demonstrate that hypoxic bradycardia and warming tachycardia are observed in fish  
468 whether they are free to shoal in a tank or confined in a respirometer. The fact, however, that  
469 confining *S. aurata* in a respirometer raised their  $f_H$ , presumably due to stress, and that  $f_H$  is a  
470 predictor of metabolic rate, has clear implications for estimating metabolic traits by static  
471 respirometry in some fish species. Tachycardia in free-swimming fish during warming was due, to  
472 some degree, to increased spontaneous activity. Thus, the combined measures of  $f_H$  and  $VAR_m$  in  
473 free-swimming fish provided novel insight into drivers of cardiac responses to temperature, and  
474 revealed highly sensitive behavioural responses to warming. Overall, the results demonstrate that  
475 biologging of physiological and behavioural responses to hypoxia and warming, in free-swimming  
476 fish, can provide more valid and reliable data than on confined fish, and has potential to reveal  
477 sensitive sub-lethal thresholds for impacts of these stressors.

478

479

480

481

482

483

484

485

486

487

488

489

490

491

492



493 **FUNDING**

494 This work was supported by the French National program EC2CO 2019 (Ecosphère Continentale et  
495 Côtière) N°DEC20045DR16, Région Occitanie PhD funding initiative (ALDOCT 00374 – 2018001408)  
496 and IFREMER.

497

498

499

500

501

502

503

504

505

506

507

508

509

510

511

512

513

514

515

516

517

518

519

520

521

522

523

524

525

526

527

528 **ACKNOWLEDGMENTS**

529 The authors are grateful to Marc Vandeputte and Ferme du Douhet for donating the fish. The  
530 authors are also grateful Ásgeir Bjarnason of Star-Oddi Ltd for technical advice and assistance, and to  
531 Germain Salou and Aurélien Leddo of Ifremer Palavas-les-Flots for help in setting up the experiments.  
532 The authors also thank Tobias Wang for stimulating discussions about the experiments.

533

534

535

536

537

538

539

540

541

542

543

544

545

546

547

548

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563 LITERATURE CITED

- 564 **Aissaoui, A., Tort, L. and Altimiras, J.** (2000). Circadian heart rate changes and light-dependence in  
565 the Mediterranean seabream *Sparus aurata*. *Fish Physiol. Biochem.* **22**, 89–94.
- 566 **Aissaoui, A., Altimiras Corderroue, J. and Tort, L.** (2005). Cardiac conduction times in *Sparus*  
567 *auratus* at different heart rates. Influence of body weight. *J. Fish Biol.* **52**, 1154–1164.
- 568 **Altieri, A. H. and Diaz, R. J.** (2019). Chapter 24 - Dead Zones: Oxygen Depletion in Coastal  
569 Ecosystems. In *World Seas: an Environmental Evaluation (Second Edition)* (ed. Sheppard, C.),  
570 pp. 453–473. Academic Press.
- 571 **Altimiras, J., Aissaoui, A., Tort, L. and Axelsson, M.** (1997). Cholinergic and Adrenergic Tones in the  
572 Control of Heart Rate in Teleosts. How Should They be Calculated? *Comp. Biochem. Physiol. A*  
573 *Physiol.* **118**, 131–139.
- 574 **Arvén Norling, T.** (2017). Remotely monitoring heart-rate and feeding behaviour of fish by using  
575 electronic sensor-tags.
- 576 **Bates, D., Maechler, M., Bolker [aut, B., cre, Walker, S., Christensen, R. H. B., Singmann, H., Dai, B.,**  
577 **Scheipl, F., Grothendieck, G., et al.** (2020). *lme4: Linear Mixed-Effects Models using “Eigen”*  
578 *and S4*.
- 579 **Bjarnason, Á., Gunnarsson, A., Árnason, T., Oddgeirsson, M., Sigmarsson, A. B. and Gunnarsson, Á.**  
580 (2019). Validation of ECG-derived heart rate recordings in Atlantic cod (*Gadus morhua* L.)  
581 with an implantable data logging system. *Anim. Biotelemetry* **7**, 13.
- 582 **Bouyoucos, I. A., Montgomery, D. W., Brownscombe, J. W., Cooke, S. J., Suski, C. D., Mandelman, J.**  
583 **W. and Brooks, E. J.** (2017). Swimming speeds and metabolic rates of semi-captive juvenile  
584 lemon sharks (*Negaprion brevirostris*, Poey) estimated with acceleration biologgers. *J. Exp.*  
585 *Mar. Biol. Ecol.* **486**, 245–254.
- 586 **Brijs, J., Sandblom, E., Axelsson, M., Sundell, K., Sundh, H., Huyben, D., Broström, R., Kiessling, A.,**  
587 **Berg, C. and Gräns, A.** (2018). The final countdown: Continuous physiological welfare  
588 evaluation of farmed fish during common aquaculture practices before and during harvest.  
589 *Aquaculture* **495**, 903–911.
- 590 **Brijs, J., Sandblom, E., Axelsson, M., Sundell, K., Sundh, H., Kiessling, A., Berg, C. and Gräns, A.**  
591 (2019a). Remote physiological monitoring provides unique insights on the cardiovascular

592 performance and stress responses of freely swimming rainbow trout in aquaculture. *Sci. Rep.*  
593 **9**, 9090.

594 **Brijs, J., Sandblom, E., Rosengren, M., Sundell, K., Berg, C., Axelsson, M. and Gräns, A. (2019b).**  
595 Prospects and pitfalls of using heart rate bio-loggers to assess the welfare of rainbow trout  
596 (*Oncorhynchus mykiss*) in aquaculture. *Aquaculture* **509**, 188–197.

597 **Campbell, H. A., Taylor, E. W. and Egginton, S. (2004).** The use of power spectral analysis to  
598 determine cardiorespiratory control in the short-horned sculpin *Myoxocephalus scorpius*. *J.*  
599 *Exp. Biol.* **207**, 1969–1976.

600 **Claireaux, G., Webber, D., Kerr, S. and Boutilier, R. (1995a).** Physiology and behaviour of free-  
601 swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. *J. Exp.*  
602 *Biol.* **198**, 49–60.

603 **Claireaux, G., Webber, D., Kerr, S. and Boutilier, R. (1995b).** Physiology and behaviour of free-  
604 swimming Atlantic cod (*Gadus morhua*) facing fluctuating salinity and oxygenation  
605 conditions. *J. Exp. Biol.* **198**, 61–69.

606 **Clark, T. D., Sandblom, E., Hinch, S. G., Patterson, D. A., Frappell, P. B. and Farrell, A. P. (2010).**  
607 Simultaneous biologging of heart rate and acceleration, and their relationships with energy  
608 expenditure in free-swimming sockeye salmon (*Oncorhynchus nerka*). *J. Comp. Physiol. B*  
609 **180**, 673–684.

610 **Cooke, S. J., Brownscombe, J. W., Raby, G. D., Broell, F., Hinch, S. G., Clark, T. D. and Semmens, J.**  
611 **M. (2016).** Remote bioenergetics measurements in wild fish: Opportunities and challenges.  
612 *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **202**, 23–37.

613 **Cossins, A. R. and Bowler, K. (1987).** Rate compensations and capacity adaptations. In *Temperature*  
614 *Biology of Animals* (ed. Cossins, A. R.) and Bowler, K.), pp. 155–203. Dordrecht: Springer  
615 Netherlands.

616 **Costa, M. F. and Barletta, M. (2016).** Special challenges in the conservation of fishes and aquatic  
617 environments of South America. *J. Fish Biol.* **89**, 4–11.

618 **Davidson, J. G., Dong, H., Linné, M., Andersson, M. H., Piper, A., Prystay, T. S., Hvam, E. B.,**  
619 **Thorstad, E. B., Whoriskey, F., Cooke, S. J., et al. (2020).** Effects of sound exposure from a

620 seismic airgun on heart rate, acceleration and depth use in free-swimming Atlantic cod and  
621 saithe. *Conserv. Physiol.* **7**,.

622 **Diaz, R. J. and Rosenberg, R.** (2008). Spreading Dead Zones and Consequences for Marine  
623 Ecosystems. *Science* **321**, 926–929.

624 **Ekström, A., Axelsson, M., Gräns, A., Brijs, J. and Sandblom, E.** (2018). Importance of the coronary  
625 circulation for cardiac and metabolic performance in rainbow trout (*Oncorhynchus mykiss*).  
626 *Biol. Lett.* **14**, 20180063.

627 **Eliason, E. J. and Anttila, K.** (2017). 4 - Temperature and the Cardiovascular System. In *Fish*  
628 *Physiology* (ed. Gamperl, A. K.), Gillis, T. E.), Farrell, A. P.), and Brauner, C. J.), pp. 235–297.  
629 Academic Press.

630 **Farrell, A. P.** (1991). From Hagfish to Tuna: A Perspective on Cardiac Function in Fish. *Physiol. Zool.*  
631 **64**, 1137–1164.

632 **Farrell, A. P.** (2007). Tribute to P. L. Lutz: a message from the heart – why hypoxic bradycardia in  
633 fishes? *J. Exp. Biol.* **210**, 1715–1725.

634 **Farrell, A. P.** (1984). A review of cardiac performance in the teleost heart: intrinsic and humoral  
635 regulation. *Can. J. Zool.*

636 **Farrell, A. P. and Smith, F.** (2017). 4 - Cardiac Form, Function and Physiology. In *Fish Physiology* (ed.  
637 Gamperl, A. K.), Gillis, T. E.), Farrell, A. P.), and Brauner, C. J.), pp. 155–264. Academic Press.

638 **Føre, M., Svendsen, E., Økland, F., Gräns, A., Alfredsen, J. A., Finstad, B., Hedger, R. D. and Uglem,**  
639 **I.** (2021). Heart rate and swimming activity as indicators of post-surgical recovery time of  
640 Atlantic salmon (*Salmo salar*). *Anim. Biotelemetry* **9**, 3.

641 **Gallo, V. P., & Civinini, A.** (2003). Survey of the adrenal homolog in teleosts. *International Review of*  
642 *Cytology*, 89-187.

643 **Gleiss, A. C., Dale, J. J., Holland, K. N. and Wilson, R. P.** (2010). Accelerating estimates of activity-  
644 specific metabolic rate in fishes: Testing the applicability of acceleration data-loggers. *J. Exp.*  
645 *Mar. Biol. Ecol.* **385**, 85–91.

- 646 **Hachim, M., Rouyer, T., Dutto, G., Kerzerho, V., Bernard, S., Bourjea, J. and McKenzie, D. J.** (2020).  
647 Oxygen uptake, heart rate and activities of locomotor muscles during a critical swimming  
648 speed protocol in the gilthead sea bream *Sparus aurata*. *J. Fish Biol.* jfb.14621.
- 649 **Hinch, S. G., Standen, E. M., Healey, M. C. and Farrell, A. P.** (2002). Swimming patterns and  
650 behaviour of upriver-migrating adult pink (*Oncorhynchus gorbuscha*) and sockeye (*O. nerka*)  
651 salmon as assessed by EMG telemetry in the Fraser River, British Columbia, Canada. In  
652 *Aquatic Telemetry: Proceedings of the Fourth Conference on Fish Telemetry in Europe* (ed.  
653 Thorstad, E. B.), Fleming, I. A.), and Næsje, T. F.), pp. 147–160. Dordrecht: Springer  
654 Netherlands.
- 655 **Iversen, N. K., Dupont-Prinet, A., Findorf, I., McKenzie, D. J. and Wang, T.** (2010). Autonomic  
656 regulation of the heart during digestion and aerobic swimming in the European sea bass  
657 (*Dicentrarchus labrax*). *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **156**, 463–468.
- 658 **Joyce, W., Simonsen, M., Gesser, H. and Wang, T.** (2016). The effects of hypoxic bradycardia and  
659 extracellular HCO<sub>3</sub><sup>-</sup>/CO<sub>2</sub> on hypoxic performance in the eel heart. *J. Exp. Biol.* **219**, 302–305.
- 660 **Kawabe, R., Kawano, T., Nakano, N., Yamashita, N., Hiraishi, T. and Naito, Y.** (2003). Simultaneous  
661 measurement of swimming speed and tail beat activity of free-swimming rainbow trout  
662 *Oncorhynchus mykiss* using an acceleration data-logger. *Fish. Sci.* **69**, 959–965.
- 663 **Kieffer\*, J. D.** (2000). Limits to exhaustive exercise in fish. *Comp. Biochem. Physiol. A. Mol. Integr.*  
664 *Physiol.* **126**, 161–179.
- 665 **Kır, M.** (2020). Thermal tolerance and standard metabolic rate of juvenile gilthead seabream (*Sparus*  
666 *aurata*) acclimated to four temperatures. *J. Therm. Biol.* **93**, 102739.
- 667 **Lefrançois, C., Claireaux, G. and Lagardère, J.-P.** (1998). Heart rate telemetry to study environmental  
668 influences on fish metabolic expenditure. *Hydrobiologia* **371**, 215–224.
- 669 **Lenth, R.** (2018). *Ismeans: Least-Squares Means*.
- 670 **Lenth, R. V., Buerkner, P., Herve, M., Love, J., Riebl, H. and Singmann, H.** (2021). *emmeans:*  
671 *Estimated Marginal Means, aka Least-Squares Means*.
- 672 **Lucas, M. C.** (1994). Heart rate as an indicator of metabolic rate and activity in adult Atlantic salmon,  
673 *Salmo salar*. *J. Fish Biol.* **44**, 889–903.

- 674 **Madeira, D., Vinagre, C., Costa, P. M. and Diniz, M. S.** (2014). Histopathological alterations,  
675 physiological limits, and molecular changes of juvenile *Sparus aurata* in response to thermal  
676 stress. *Mar. Ecol. Prog. Ser.* **505**, 253–266.
- 677 **Madeira, D., Vinagre, C. and Diniz, M. S.** (2016). Are fish in hot water? Effects of warming on  
678 oxidative stress metabolism in the commercial species *Sparus aurata*. *Ecol. Indic.* **63**, 324–  
679 331.
- 680 **McDonnell, L. H. and Chapman, L. J.** (2015). At the edge of the thermal window: effects of elevated  
681 temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of  
682 a widespread African cichlid. *Conserv. Physiol.* **3**,.
- 683 **McKenzie, D. J., Piraccini, G., Steffensen, J. F., Bolis, C. L., Bronzi, P. and Taylor, E. W.** (1995). Effects  
684 of diet on spontaneous locomotor activity and oxygen consumption in Adriatic sturgeon  
685 (*Acipenser naccarii*). *Fish Physiol. Biochem.* **14**, 341–355.
- 686 **McKenzie, D. J., Pedersen, P. B. and Jokumsen, A.** (2007a). Aspects of respiratory physiology and  
687 energetics in rainbow trout (*Oncorhynchus mykiss*) families with different size-at-age and  
688 condition factor. *Aquaculture* **263**, 280–294.
- 689 **McKenzie, D. J., Campbell, H. A., Taylor, E. W., Micheli, M., Rantin, F. T. and Abe, A. S.** (2007b). The  
690 autonomic control and functional significance of the changes in heart rate associated with air  
691 breathing in the jeju, *Hoplerythrinus unitaeniatus*. *J. Exp. Biol.* **210**, 4224–4232.
- 692 **McKenzie, D. J., Skov, P. V., Taylor, E. W. T., Wang, T. and Steffensen, J. F.** (2009). Abolition of reflex  
693 bradycardia by cardiac vagotomy has no effect on the regulation of oxygen uptake by  
694 Atlantic cod in progressive hypoxia. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **153**,  
695 332–338.
- 696 **Metcalfe, J. D., Wright, S., Tudorache, C. and Wilson, R. P.** (2016). Recent advances in telemetry for  
697 estimating the energy metabolism of wild fishes. *J. Fish Biol.* **88**, 284–297.
- 698 **Palstra, A. P., Arechavala-Lopez, P., Xue, Y. and Roque, A.** (2021). Accelerometry of Seabream in a  
699 Sea-Cage: Is Acceleration a Good Proxy for Activity? *Front. Mar. Sci.* **8**,.
- 700 **Perry, S. F., Esbaugh, A., Braun, M. and Gilmour, K. M.** (2009). Gas Transport and Gill Function in  
701 Water-Breathing Fish. In *Cardio-Respiratory Control in Vertebrates: Comparative and*

702 *Evolutionary Aspects* (ed. Glass, M. L.) and Wood, S. C.), pp. 5–42. Berlin, Heidelberg:  
703 Springer.

704 **Prystay, T. S., Eliason, E. J., Lawrence, M. J., Dick, M., Brownscombe, J. W., Patterson, D. A.,**  
705 **Crossin, G. T., Hinch, S. G. and Cooke, S. J.** (2017). The influence of water temperature on  
706 sockeye salmon heart rate recovery following simulated fisheries interactions. *Conserv.*  
707 *Physiol.* **5**,

708 **Rabben, H. and Furevik, D. M.** (1993). Application of heart rate transmitters in behaviour studies on  
709 Atlantic halibut (*Hippoglossus hippoglossus*). *Aquac. Eng.* **12**, 129–140.

710 **Randall, D.** (1982). The control of respiration and circulation in fish during exercise and hypoxia.  
711 *Journal of Experimental Biology*, **100(1)**, 275-288.

712 **Reid, S. G., Bernier, N. J. and Perry, S. F.** (1998). The adrenergic stress response in fish: control of  
713 catecholamine storage and release1Communicated by Dr P.W. Hochachka, Editor.1. *Comp.*  
714 *Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* **120**, 1–27.

715 **Remen, M., Nederlof, M. A. J., Folkedal, O., Thorsheim, G., Sitjà-Bobadilla, A., Pérez-Sánchez, J.,**  
716 **Oppedal, F. and Olsen, R. E.** (2015). Effect of temperature on the metabolism, behaviour and  
717 oxygen requirements of *Sparus aurata*. *Aquac. Environ. Interact.* **7**, 115–123.

718 **Rodgers, G. G.** (2016). Climate change in a stable thermal environment: effects on the performance  
719 and life history of coral reef fish.

720 **Sandblom, E. and Axelsson, M.** (2011). Autonomic control of circulation in fish: A comparative view.  
721 *Auton. Neurosci.* **165**, 127–139.

722 **Schulte, P. M., Healy, T. M. and Fangué, N. A.** (2011). Thermal Performance Curves, Phenotypic  
723 Plasticity, and the Time Scales of Temperature Exposure. *Integr. Comp. Biol.* **51**, 691–702.

724 **Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M. S., Højsgaard, S., Fox, J., Lawrence,**  
725 **M. A., Mertens, U., Love, J., et al.** (2021). *afex: Analysis of Factorial Experiments*.

726 **Skeeles, M. R., Winkler, A. C., Duncan, M. I., James, N. C., van der Walt, K.-A. and Potts, W. M.**  
727 (2020). The use of internal heart rate loggers in determining cardiac breakpoints of fish. *J.*  
728 *Therm. Biol.* 102524.



- 729 **Sopinka, N. M., Donaldson, M. R., O'Connor, C. M., Suski, C. D. and Cooke, S. J.** (2016). 11 - Stress  
730 Indicators in Fish. In *Fish Physiology* (ed. Schreck, C. B.), Tort, L.), Farrell, A. P.), and Brauner,  
731 C. J.), pp. 405–462. Academic Press.
- 732 **Stecyk, J. A. W.** (2017). 5 - Cardiovascular Responses to Limiting Oxygen Levels. In *Fish Physiology*  
733 (ed. Gamperl, A. K.), Gillis, T. E.), Farrell, A. P.), and Brauner, C. J.), pp. 299–371. Academic  
734 Press.
- 735 **Steffensen, J. F.** (1989). Some errors in respirometry of aquatic breathers: How to avoid and correct  
736 for them. *Fish Physiol. Biochem.* **6**, 49–59.
- 737 **Stillman, J. H.** (2019). Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact  
738 Animals, Ecosystems, and Human Communities. *Physiology* **34**, 86–100.
- 739 **Svendsen, E., Føre, M., Økland, F., Gräns, A., Hedger, R. D., Alfredsen, J. A., Uglem, I., Rosten, C. M.,**  
740 **Frank, K., Erikson, U., et al.** (2021). Heart rate and swimming activity as stress indicators for  
741 Atlantic salmon (*Salmo salar*). *Aquaculture* **531**, 735804.
- 742 **Tanaka, H., Takagi, Y. and Naito, Y.** (2001). Swimming speeds and buoyancy compensation of  
743 migrating adult chum salmon *Oncorhynchus keta* revealed by speed/depth/acceleration data  
744 logger. *J. Exp. Biol.* **204**, 3895–3904.
- 745 **Taylor, E. W.** (1992). 6 Nervous Control of the Heart and Cardiorespiratory Interactions. In *Fish*  
746 *Physiology* (ed. Hoar, W. S.), Randall, D. J.), and Farrell, A. P.), pp. 343–387. Academic Press.
- 747 **Taylor, E. W., Leite, C. a. C. and Skovgaard, N.** (2010). Autonomic control of cardiorespiratory  
748 interactions in fish, amphibians and reptiles. *Braz. J. Med. Biol. Res.* **43**, 600–610.
- 749 **Thorarensen, H., Gallagher, P. E. and Farrell, A. P.** (1996). The limitations of heart rate as a  
750 predictor of metabolic rate in fish. *Journal of Fish Biology* **49**, 226–236.
- 751 **Torgo, L.** (2010). Data Mining with R, learning with case studies Chapman and Hall/CRC. URL:  
752 <http://www.dcc.fc.up.pt/~ltorgo/DataMiningWithR>
- 753 **Treberg, J. R., Killen, S. S., MacCormack, T. J., Lamarre, S. G. and Enders, E. C.** (2016). Estimates of  
754 metabolic rate and major constituents of metabolic demand in fishes under field conditions:  
755 Methods, proxies, and new perspectives. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*  
756 **202**, 10–22.

757 **Webb, P. W.** (1978). Temperature Effects on Acceleration of Rainbow Trout, *Salmo gairdneri*. *J. Fish.*  
758 *Board Can.*

759 **Wilson, S. M., Hinch, S. G., Eliason, E. J., Farrell, A. P. and Cooke, S. J.** (2013). Calibrating acoustic  
760 acceleration transmitters for estimating energy use by wild adult Pacific salmon. *Comp.*  
761 *Biochem. Physiol. A. Mol. Integr. Physiol.* **164**, 491–498.

762 **Wright, S., Metcalfe, J. D., Hetherington, S. and Wilson, R.** (2014). Estimating activity-specific energy  
763 expenditure in a teleost fish, using accelerometer loggers. *Mar. Ecol. Prog. Ser.* **496**, 19–32.

764

765

766

767

768

769

770

771

772

773

774

775

776

777

778

779

780

781

782

783

784

785

786

787

788

789

**TABLE**

790 Tab 1. Elements of mean ( $\pm$  S.E.) heart rate ( $f_H$ , beats  $\text{min}^{-1}$ ) of *Sparus aurata* fitted with biologging  
 791 tags and exposed to hypoxia or warming, comparing when swimming as groups of three in a tank or  
 792 confined individually in a respirometer chamber ( $n = 10$ ). Undisturbed  $f_H$  indicates as measured  
 793 between 07:00 and 08:00 in normoxia at 21°C, prior to the respective trial. Maximum refers to the  
 794 mean of the highest, and minimum to mean of the lowest,  $f_H$  observed in each fish in each trial. For  
 795 hypoxia,  $\text{PO}_2$  at max or min refers to the mean oxygen partial pressure at which maximum or  
 796 minimum measures occurred, respectively. For warming, T at max or min refers to the mean  
 797 temperature at which these measures occurred. Asterisks indicate difference between holding  
 798 conditions for that variable, \*  $< 0.05$ , \*\*\*  $< 0.001$

799

Stressor	Holding condition	
	Tank	Respirometer
Hypoxia		
Undisturbed	75 $\pm$ 4.4 ***	105 $\pm$ 4
Maximum	103 $\pm$ 3.2 **	118 $\pm$ 3.4
$\text{PO}_2$ at max	71 $\pm$ 7.7	62 $\pm$ 4.5
Minimum	37 $\pm$ 1.9	39 $\pm$ 1.9
$\text{PO}_2$ at min	17 $\pm$ 1.1	19 $\pm$ 1.7
Warming		
Undisturbed	72 $\pm$ 4.8 ***	103 $\pm$ 3.4
Maximum	172 $\pm$ 6.3	166 $\pm$ 5.5
T at max	30.3 $\pm$ 0.34 *	28.6 $\pm$ 0.68
Minimum	71.2 $\pm$ 6.9	82 $\pm$ 5.3
T at min	21.4 $\pm$ 0.58	22.3 $\pm$ 0.83

800

801

802

803 **FIGURES CAPTIONS**

804 Fig 1. **Heart rate ( $f_H$ ) of *Sparus aurata* as a function of time elapsed after surgery.** Each line is a  
805 single fish ( $n = 10$ ) and each point corresponds to the mean of heart rate values collected once every  
806 two hours over 6 hours. The mean  $f_H$  dropped significantly until 60h (black dashed line) and then  
807 showed no further significant change (One-way ANOVA with repeated measures,  $p < 0.001$ ).

808 Fig 2. **The effect of progressive hypoxia on heart rate ( $f_H$ ) of *S. aurata* ( $n = 10$ ) showing individual**  
809 **responses when in a tank (A) or box (B), and the mean ( $\pm$  S.E.) response in both holding conditions**  
810 **(C). “U100” refers to  $f_H$  of fish when undisturbed in normoxia (21°C) at 07:00, before hypoxia trials.**  
811 The vertical dashed line indicates the beginning of the trial. In (C) a common superscript indicates no  
812 significant difference in the mean (Two-way ANOVA with repeated measures,  $p < 0.05$ ).

813 Fig 3. **The effect of progressive warming on heart rate ( $f_H$ ) of *S. aurata* ( $n = 10$ ) showing individual**  
814 **responses when in a tank (A) or box (B), and the mean ( $\pm$  S.E.) response in both holding conditions**  
815 **(C). “U21” refers to  $f_H$  of fish when undisturbed at 21°C, at 07:00 before warming trials. The vertical**  
816 dashed line indicates the beginning of the trial. In (C) a common superscript indicates no significant  
817 difference in the mean (Two-way ANOVA with repeated measures,  $p < 0.05$ ).

818 Fig 4. **The effect of progressive warming on the variance of external acceleration ( $VAR_m$ ) of *S.***  
819 ***aurata* ( $n = 10$ ) showing individual responses when in a tank (A) or box (B), and the mean ( $\pm$  S.E.)**  
820 **response in both holding conditions (C). In (C) a common superscript indicates no significant**  
821 **difference in the mean (generalized mixed linear model,  $p < 0.001$ ,).**

822 Fig 5. **The relationship of variance of acceleration ( $VAR_m$ ) to heart rate ( $f_H$ ) in *S. aurata* during**  
823 **warming trials in the tank.** Points are  $VAR_m$  calculated over 60 s after the corresponding  $f_H$  value  
824 logged for each fish ( $n = 10$  in all cases). The relationship is described by  $VAR_m = f_H (0.87) + 41.2$   
825 (generalized mixed linear model,  $p < 0.001$ , marginal  $R^2 = 0.061$ ; conditional  $R^2 = 0.36$ )

826 Fig 6. **The relationship of oxygen consumption ( $\dot{M}O_2$ ) to heart rate ( $f_H$ ) in *S. aurata* confined in**  
827 **respirometers, during exposure to hypoxia and warming.** Each point represents the mean oxygen  
828 consumption as a function of mean  $f_H$  for a single fish at a given oxygen partial pressure or  
829 temperature ( $n = 10$  in all cases). The relationship is described by  $\dot{M}O_2 = f_H (0.045) - 0.23$  (mixed  
830 linear model,  $p < 0.001$ , marginal  $R^2 = 0.52$ ; conditional  $R^2 = 0.76$ ) for all trials combined, represented  
831 by the black dashed line.













