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## Exposure to chronic moderate hypoxia impacts physiological and developmental traits of European sea bass (*Dicentrarchus labrax*) larvae

Vanderplancke Gwenaëlle <sup>1</sup>, Claireaux Guy <sup>1,2</sup>, Quazuguel Patrick <sup>1</sup>, Huelvan Christine <sup>1</sup>, Corporeau Charlotte <sup>1</sup>, Mazurais David <sup>1</sup>, Zambonino-Infante José-Luis <sup>1,\*</sup>

<sup>1</sup> Ifremer, LEMAR UMR 6539 CNRS-UBO-IRD-Ifremer, ZI de la Pointe du Diable, CS 10070, 29280, Plouzané, France

<sup>2</sup> Université de Bretagne Occidentale, LEMAR UMR 6539 CNRS-UBO-IRD-Ifremer, ZI de la Pointe du Diable, CS 10070, 29280, Plouzané, France

\* Corresponding author : José-Luis Zambonino-Infante, email address : [jose.zambonino@ifremer.fr](mailto:jose.zambonino@ifremer.fr)

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### Abstract :

Since European sea bass (*Dicentrarchus labrax*) larvae occurred in coastal and estuarine waters at early life stages, they are likely to be exposed to reduced dissolved oxygen waters at a sensitive developmental stage. However, the effects of hypoxia at larval stage, which depend in part on fish species, remain very poorly documented in European sea bass. In the present study, the impacts of an experimental exposure to a chronic moderate hypoxia (40 % air saturation) between 30 and 38 days post-hatching on the physiological and developmental traits of European sea bass larvae were assessed. This study was based on the investigation of survival and growth rates, parameters related to energy metabolism [Citrate Synthase (CS) and Cytochrome-c Oxidase (COX) activities], and biological indicators of the maturation of digestive function [pancreatic (trypsin, amylase) and intestinal (Alkaline Phosphatase "AP" and Aminopeptidase-N "N-LAP") enzymes activities]. While condition of hypoxia exposure did not induce any significant mortality event, lower growth rate as well as CS/COX activity ratio was observed in the Hypoxia Treatment group. In parallel, intestinal enzyme activities were also lower under hypoxia. Altogether, the present data suggest that sea bass larvae cope with moderate hypoxia by (1) reducing processes that are costly in energy and (2) regulating mitochondria functions in order to respond to energy-demand conditions. Both these effects are associated with a delay in the maturation of the digestive function.

**Keywords :** Hypoxia, Fish larvae, Energy metabolism, Maturation

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## Abbreviations

AP

Alkaline Phosphatase

C

Control group

COX

Cytochrome-c Oxidase

CS

Citrate Synthase

d.p.h.

Days post-hatching

FI

Feed intake

H

Head

HT

Hypoxia treatment

IS

Intestinal segment

N-LAP

Aminopeptidase-N

NS

No significant differences

PS

Pancreatic segment

RMR

Routine metabolic rate

s.e.m.

Standard error of mean

T

Tail

## 46 **Introduction**

47 Since larvae of marine fish species hatch at an undeveloped stage, their development and  
48 ontogeny are liable to be greatly affected by biotic and abiotic conditions. This is especially  
49 true in coastal areas, which are nurseries for many marine fish species and where ecosystems  
50 are particularly impacted by human activities and global change. Among the environmental  
51 factors affecting the world's coastal waters, hypoxia is a major problem and is forecast to  
52 increase further under the combined effects of spreading coastal eutrophication and global  
53 warming (Diaz, 2001; Diaz and Rosenberg, 1995). Hypoxia episodes often coincide with  
54 periods of annual recruitment and the growth of benthic and pelagic fish species occurring in  
55 late spring and early summer (Breitburg, 1992; Pihl et al., 1991; Pihl et al., 1992).  
56 Understanding the impact of a low oxygen environment on the early life stages of fish is  
57 therefore of great importance, since fishery production relies on larval and juvenile  
58 recruitment.

59 Exposure of juveniles **or adults** from different fish species to hypoxia has been shown to  
60 notably affect fish routine metabolic rate (RMR), feed intake (FI), growth, condition factor  
61 and concentration of protein, *e.g.*, European sea bass (*Dicentrarchus labrax*) (Claireaux and  
62 Lagardere, 1999; Pichavant et al., 2001; Thetmeyer et al., 1999); turbot (*Scophthalmus*  
63 *maximus*) (Pichavant et al., 2001; Pichavant et al., 2000); Amazon oscar (*Astronotus*  
64 *crassipinis*) (Almeida-Val et al., 2011); and mummichog (*Fundulus heteroditus*) (Rees et al.,  
65 2012). It is supposed that some of these effects can be explained by the hypoxia-induced  
66 control of fundamental processes related to energy saving. Long-term exposure to hypoxia in  
67 fish has been also shown to affect egg development and hatching success, resulting in  
68 malformation, lower fitness and high mortality rates at larval and juvenile stages, *e.g.*, brown  
69 trout (*Salmo trutta*) (Massa et al., 1999; Roussel, 2007); black bream (*Acanthopagrus*  
70 *butcheri*) (Hassell et al., 2008); zebrafish (*Danio rerio*) (Padilla and Roth, 2001; Shang and  
71 Wu, 2004); and dogfish (*Scyliorhinus canicula*) (Diez and Davenport, 1990). These effects  
72 occur due to disturbances in a series of programmed, highly intricate and energy-consuming  
73 processes (Ozernyuk, 2011; Wu et al., 2006). Although it is generally accepted that early  
74 embryonic developmental stages are particularly sensitive to stresses in fish (Cameron and  
75 VonWesternhagen, 1997), post-hatching development also has windows of sensitivity to  
76 hypoxia. The effects of hypoxia exposure when applied at larval stages vary widely  
77 depending on fish species, hypoxia severity, exposure period and individual adaptive  
78 capacities (Anjos et al., 2008; Bickler and Buck, 2007). While Barrionuevo et al. (2010)

79 reported that zebrafish did not respond to moderate hypoxia before 30 days post-hatching  
80 (d.p.h.), Pelster (2002) showed that exposure of the same species to 7 days of moderate  
81 hypoxia affected larval cardiac activity, cardiac output, heart rate, organ perfusion and blood  
82 vessel formation. Since hatching of marine fish species occurs at earlier stages of  
83 development compared with freshwater species, their ontogenic processes during the larval  
84 phase are likely more sensitive to hypoxic episodes. Low tolerance to hypoxia has been  
85 observed over the period of metamorphosis in sea bream (*Pagrus major*) (Ishibashi et al.,  
86 2005); Japanese flounder (*Paralichthys olivaceu*) (Ishibashi et al., 2007); and bonefish  
87 (*Albula sp.*) (Pfeiler, 2001). It is supposed that this sensitivity is due to the increase of  
88 metabolic rates just before and just after the flexion stage, which is a period of dramatic  
89 molecular, physiological and behavioural changes for marine fish species such as European  
90 sea bass (Mazurais et al., 2011). The European sea bass is a highly valued fish that migrates  
91 from offshore areas to the coast just after flexion stage, which occurs between 22 and 25  
92 d.p.h. (Dufour et al., 2009; Jennings and Pawson, 1992; Pickett and Pawson, 1994). This  
93 species can therefore be exposed to hypoxic events from this stage onward. However, few  
94 studies have been published on the effects of hypoxia on larval development of this marine  
95 fish species.

96 The purpose of the present study was to evaluate the impact of a chronic (between 30 and 38  
97 d.p.h., a natural hypoxia exposure window) moderate (40% air saturation) hypoxia exposure  
98 on growth, metabolism and maturation in European sea bass larvae. To gain insight into the  
99 energy metabolism of larvae, Citrate Synthase (CS) and Cytochrome c Oxidase (COX)  
100 activities were assessed. CS and COX are key mitochondrial enzymes localized in matrix or  
101 membrane which fluctuations provide information on the properties and the numbers of the  
102 mitochondria present (Guderley, 2007; Lucassen et al., 2003). CS/COX activity ratio was  
103 used in order to estimate changing in mitochondrial size and shape in response to energy-  
104 demand conditions as indicated in studies by Lucassen et al. (2003) and Ibarz et al. (2010).  
105 Finally, digestive enzyme activities [Trypsin, Amylase, Alkaline Phosphatase (AP) and  
106 Aminopeptidase-N (N-LAP)] were used as indicators of the developmental status of the fish  
107 larvae.

108

## 109 **Material and methods**

### 110 **Larval rearing**

111 Post-hatching, larvae of European sea bass were reared under normal oxygen conditions in 8  
112 tanks at  $19.1 \pm 0.4$  °C water temperature and  $35 \pm 0.2$  ‰ salinity, and were fed with *Artemia*  
113 until 45 d.p.h according to Zambonino-Infante et al. (1996). The tanks were then divided into  
114 two groups of four tanks. The Control group (or C group) was maintained under normal  
115 oxygen condition ( $96.6 \pm 1.3$  % air saturation,  $7.10$  mg O<sub>2</sub>. L<sup>-1</sup>), while larvae from the  
116 challenged group, also named Hypoxia Treatment group (or HT group), were subjected to  
117 moderate hypoxia ( $40.1 \pm 2.6$  % air saturation,  $2.95$  mg O<sub>2</sub>.L<sup>-1</sup>) for 8 days (from 30 to 38  
118 d.p.h., **Fig. 1**). These hypoxia conditions had previously been determined to not induce  
119 mortality. During hypoxia exposure larvae of each group were fed with *Artemia*, except 24 h  
120 prior to samplings where larvae were left undisturbed and unfed. After the hypoxia exposure,  
121 larvae continue to be fed with *Artemia* until the end of experimentations. It was not possible  
122 to evaluate feed ingestion throughout this experiment; however, we did not notice any visible  
123 change in feeding activity in HT group during or after hypoxia exposure.

### 124 **Survival rate and larval growth**

125 Survival rate was estimated at 45 d.p.h. by counting the number of remaining larvae in each  
126 group and taking into account the larvae collected for sampling during the experiment.  
127 Growth was evaluated on four pools (1 pool from each tank) of approximately 40 larvae per  
128 sample from the beginning of hypoxia exposure (30 d.p.h.) until 45 d.p.h. (*i.e.* at 30, 32, 35  
129 and 38 d.p.h – during hypoxia exposure – and at 42 and 45 d.p.h. – after the return to  
130 normoxic conditions).

### 131 **Larvae sampling**

132 During hypoxia exposure, five samplings (at 30, 32, 35 and 38 d.p.h. and 42 d.p.h.) were  
133 performed in order to analyse CS and COX activities. For this purpose, in each treatment  
134 group, 4 samplings (1 per tank) of 35 or 40 pooled larvae were for protein concentration and  
135 enzymes activities assays. Larvae were first placed on a glass maintained at 0°C and dissected  
136 under microscope into four parts: head “H” (just behind the eye), pancreatic segment “PS”  
137 (just behind the gills) and tail “T” (point between the end of the digestive tract and the anal  
138 tail) were discarded, and the intestinal segment “IS” conserved (**Fig. 2**). For each group,

139 pooled IS were then weighed, placed in ice-cold lysis buffer and stored at  $-80^{\circ}\text{C}$  until proteins  
140 extraction.

141 For digestive enzymes assays, four samplings (one per tank) of 35 larvae per group were  
142 performed at 38 d.p.h. and stored at  $-20^{\circ}\text{C}$ . Larvae were placed on an ice-chilled glass and  
143 dissected under a microscope into 4 parts: head (H), pancreatic segment (PS) intestinal  
144 segment (IS) and tail (T) (**Fig. 2**). PS and IS were then stored at  $-20^{\circ}\text{C}$  for later assay of their  
145 respective enzymes, as previously described by Cahu and Zambonino-Infante (1994).

#### 146 **Extractions of proteins from larvae for analysis of CS and COX activities**

147 **Proteins were solubilized** by adding 3-6 mL of ice-cold lysis buffer containing 150 mM NaCl,  
148 10 mM Tris, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% Igepal, 1 tablet of complete  
149 EDTA-free protease inhibitor cocktail (Roche Pharma; Basel, Switzerland) in 25 mL buffer,  
150 1% phosphatase inhibitor cocktail III (Sigma-Aldrich®; St. Louis, MO, USA) and **5mM**  
151 NaPPi, pH 7.4. Total proteins and enzymes were then extracted as described by Le Foll et al.  
152 (2007). Briefly, total proteins and enzymes lysates were obtained after homogenization with a  
153 Pro Polytron® (BioBlock Scientific; Illkirch , Bas-Rhin , France) tissue disruptor. The  
154 homogenates obtained were then centrifuged at 3,000 g for 1 h at  $4^{\circ}\text{C}$ . At the end of the  
155 centrifugation, the interphase containing proteins and enzymes was collected by carefully  
156 pipetting and then centrifuged at 10,000 g for 45 min at  $4^{\circ}\text{C}$  in order to ensure total lipid  
157 removal. The new interphase was carefully collected, aliquoted and stored at  $-80^{\circ}\text{C}$ .

#### 158 **Enzymatic activities assays (aerobic metabolism and larval maturation)**

159 CS activity was assessed in Nunc™ 96-well microplates (Thermo Scientific Inc.; Waltham,  
160 MA, USA). Data were obtained with a microplate reader (Bio-Tek® Synergy™ HT; Colmar,  
161 Haut-Rhin, France) and then treated with KC4 v3 software. The assay was adapted from  
162 Guderley et al. (2011). Briefly, **CS activity** was assayed for 10 min at wavelength  $\lambda = 412$  nm  
163 and temperature  $T = 25^{\circ}\text{C}$ . The microplate was prepared by mixing 20  $\mu\text{L}$  of sample with 160  
164  $\mu\text{L}$  of a mix consisting of 0.25 mM Acetyl-coA, 0.125 mM DTNB and 86.25 mM Tris-HCl,  
165 pH = 8. The reaction was initiated by adding 20  $\mu\text{L}$  5 mM oxaloacetate to each well. The  
166 control used for the assay was a commercial enzyme of CS (Sigma-Aldrich® Inc.; St. Louis,  
167 MO, USA).

168 In parallel, COX activity was assessed using Cytochrome c Oxidase Assay Kit, CYTOCOX1  
169 (Sigma-Aldrich® Inc.; St. Louis, MO, USA) following manufacturer's instructions. Briefly,

170 **COX activity** was assayed for 1 min at wavelength  $\lambda = 550$  nm and temperature  $T = 25^{\circ}\text{C}$ .  
171 The reaction was prepared by mixing 350  $\mu\text{L}$  of assay buffer (10 mM Tris-HCl, 120 mM KCl,  
172 pH = 7.0), 80  $\mu\text{L}$  of enzyme buffer (10 mM Tris-HCl, 250 mM sucrose, pH = 7.0) and 20  $\mu\text{L}$   
173 of sample. The reaction was then initiated by adding 50  $\mu\text{L}$  of 0.22 mM Ferrocycytochrome c  
174 substrate solution.

175 Larval development was evaluated as described by Cahu and Zambonino-Infante (1995).  
176 Protocols for the homogenisation of the pancreatic segments and intestine, brush border  
177 purification, protein dosages and enzymatic assay procedures for Amylase, Trypsin, AP and  
178 N-LAP are described in Zambonino-Infante et al. (1997). Enzyme activities were expressed as  
179 specific activities, *i.e.*, units per mg of proteins.

## 180 **Statistical analyses**

181 Data are presented as means  $\pm$  s.e.m. (Standard Error of Mean). Statistical analyses were  
182 performed using R software (R Core Team, 2013). For all analyses, all response variables  
183 were checked for normality with the Shapiro test and for equality of variances using the  
184 Levene test. A regression was used in order to determine the influence of oxygen treatment on  
185 survival. **A two-way ANOVAs were then used (except for survival and maturation analyses,**  
186 **for which a regression and a one-way ANOVA were used, respectively) to determine the**  
187 **influence of oxygen treatment, age of larvae (as main effects) and their interaction on each**  
188 **response variable. When the interaction between oxygen treatment and the age of larvae was**  
189 **significant, Student-test were used to determine significant differences among groups within**  
190 **each sampling time.** An ANCOVA was used for CS/COX activity ratio analyses in order to  
191 determine the influence of oxygen treatment (as main effect) with age of larvae as co-factor.  
192 In addition, a Student-test was used to determine significant differences among groups within  
193 each sampling time for CS/COX activity ratio and at 30, 38 and 45 d.p.h. for growth rate.  
194 Finally, a one-way ANOVA was used in order to determine the influence of oxygen treatment  
195 on larval development. Differences were considered significant at the 95% confidence level  
196 (p-value < 0.05).

197



## 198 **Results**

199 **No variable differed significantly between** the Control (C) and the Hypoxia Treatment (HT)  
200 groups prior to the hypoxia exposure, *i.e.* **at 30 d.p.h. just before the beginning** of exposure.

### 201 **Survival and growth of larvae**

#### 202 *Survival*

203 No significant mortality events were observed in the tanks during and after the period of  
204 exposure to hypoxia. This was confirmed by the survival rate estimated at 45 d.p.h., which  
205 was not significantly different between the two groups (C =  $44.9 \pm 7.2\%$ , HT =  $44.7 \pm 5.3\%$  ;  
206  $p = 1$ ).

#### 207 *Larval growth*

208 Growth of larvae was monitored from the beginning of hypoxia exposure (30 d.p.h.) until 45  
209 d.p.h. (after the return to normoxic conditions). Data were transformed using cubic root  
210 following recommendations of Bureau et al. (2000) (**Fig. 3**). Globally, in both groups, the  
211 larval growth rate increased with the age of larvae. **Statistical analyses revealed that larval**  
212 **growth was affected by oxygen treatment (two-way ANOVA:  $p < 0.001$ ) and by the age of**  
213 **larvae (two-way ANOVA:  $p < 0.001$ ). The interaction between oxygen treatment and the age**  
214 **of larvae was significant (two-way ANOVA:  $p < 0.01$ ).** While the mean larval masses were  
215 similar between the two groups at the beginning of hypoxia exposure (30 d.p.h.) (C =  $4.66 \pm$   
216  $0.17$  mg ; HT =  $4.67 \pm 0.16$  mg ; Student-test:  $p > 0.5$ ), they were found significantly different  
217 at the end of hypoxia exposure (38 d.p.h.) (C =  $13.26 \pm 2.29$  mg ; HT =  $9.23 \pm 1.73$  mg ;  
218 Student-test:  $p < 0.01$ ). This data can be related to the significant impact of hypoxia exposure  
219 on the growth rate (**two-way ANOVA interaction:  $p < 0.01$** ). **Furthermore**, growth tend to be  
220 higher in HT group after hypoxia exposure resulting in similar mass between the two groups  
221 at 45 d.p.h. (C =  $31.40 \pm 2.97$  mg ; HT =  $27.79 \pm 4.18$  mg ; Student-test:  $p > 0.09$ ).

### 222 **Regulation of Citrate Synthase and Cytochrome c Oxidase activities**

223 CS and COX activities in the IS of larvae were assessed from the beginning of hypoxia  
224 exposure (30 d.p.h.) until **42 d.p.h.** (after the return to normoxic conditions) (**Fig. 4**). Overall,  
225 CS and COX specific activities increased during larval development in both groups (**Fig. 4 A**

226 **and Fig. 4 B)** (ANCOVA:  $p < 0.001$  and  $p = 0.001$ , respectively). During hypoxia exposure,  
227 *i.e.* from 30 to 38 d.p.h., CS specific activity was not impacted by hypoxia exposure while  
228 COX specific activity was higher in the HT group (ANCOVA:  $p < 0.001$ ). Globally, the  
229 CS/COX activity ratio exhibit significant fluctuations throughout the experiment (**Fig. 4 C**)  
230 (ANCOVA:  $p < 0.001$ ). During hypoxia exposure, *i.e.* from 30 to 38 d.p.h., CS/COX activity  
231 ratio was lower in the HT group (ANCOVA:  $p < 0.001$ ). The effect of the oxygen treatment  
232 on CS/COX activity ratio is particularly marked at 32, 35 and 38 d.p.h. (Student-test:  $p <$   
233  $0.001$ ,  $p < 0.001$  and  $p < 0.05$ , respectively). There was no more difference in CS/COX  
234 activity ratio between C and HT groups 96h (42 d.p.h.) after the return to normoxic conditions  
235 (Student-test:  $p > 0.05$ ).

### 236 **Maturation of larvae digestive functions**

237 Activities of digestive enzymes were determined in both the C and HT groups at 38 d.p.h.  
238 (**Table 1**). For pancreatic enzymes, larvae from the HT group exhibited 25% lower trypsin  
239 specific activity and showed a significantly 150% higher amylase specific activity than the C  
240 group (one-way ANOVA:  $p_{\text{Trypsin}} < 0.01$ ,  $p_{\text{Amylase}} < 0.05$ ). For the intestinal enzymes, larvae  
241 exposed to hypoxia exhibited AP and N-LAP specific activities significantly lower than those  
242 from the C group by 78% and 53%, respectively (one-way ANOVA:  $p_{\text{AP}} = 0.01$ ,  $p_{\text{N-LAP}} <$   
243  $0.01$ ).

244

## 245 Discussion

246 Since they arrive on the coastline around one month after hatching (Dufour et al., 2009;  
247 Jennings and Pawson, 1992), European sea bass (*Dicentrarchus labrax*) are likely to  
248 experience hypoxia episodes at the end of the larval period. However, the impact of hypoxic  
249 events on their physiology at this developmental stage is still largely unknown. The present  
250 study was intended to assess the effects of moderate hypoxia (40% air saturation) applied for  
251 a duration of 8 days on 30 day-old sea bass larvae. The hypoxia treatment did not induce any  
252 significant mortality. Moreover no significant impact on skeletal deformity rate was observed  
253 in juveniles originated from larvae of HT group (data not shown). The absence of effect on  
254 survival and malformation rates in the preset study differs from previous data showing that  
255 moderate hypoxia induces lower survival rates and higher frequency of vertebral column  
256 deformities in first feeding yellowfin tuna (*Thunnus albacares*) (Wexler et al., 2011) and  
257 Atlantic salmon alevins (*Salmo salar*) (Sanchez et al., 2011) respectively. This apparent  
258 discrepancy of response is consistent with the well admitted species-specific as well as stage-  
259 specific response of fish to hypoxia constraint (Richards et al., 2009).

260 We observed a lower mass in larvae from the HT group at the end of hypoxia exposure  
261 resulting from a reduction of growth during the exposition compared to the C group.  
262 Evaluation of food intake in larvae is not really easy and necessitates the use of <sup>14</sup>C-labelled  
263 feeds (Kolkovski et al., 1997), which was not possible to perform during this experiment.  
264 However, it has been well described that hypoxia usually induces reduction in food ingestion  
265 (Lakani et al., 2013; Pichavant et al., 2001; Pichavant et al., 2000; Thetmeyer et al., 1999).  
266 Seven days after the return under normoxic conditions (*i.e.* at 45 d.p.h.), larvae from HT  
267 group recovered the same weight as those from the C group (Fig. 3) suggesting a potential  
268 compensatory growth. Yet, such a compensatory growth after hypoxia exposure was  
269 previously shown in Chinese shrimp (*Fenneropenaeus chinensis*) (Wei et al., 2008); it is  
270 likely that a higher number of sampling points after the return under normoxic conditions  
271 would have permitted to point out compensatory growth in our study.

272 In the present study, the lower trypsin specific activity in larvae originated from the HT group  
273 suggested that one week moderate hypoxia exposure impaired ingestion of fish. As mentioned  
274 by Zambonino-Infante and Cahu (2001), pancreatic maturation in sea bass larvae has been  
275 completed by 25 d.p.h. and trypsin activity can be directly related to the dietary protein intake  
276 from 35 d.p.h. Therefore, a decrease in trypsin specific activity observed after 35 d.p.h. is  
277 generally related to undernutrition (Zambonino-Infante and Cahu, 2001). Again, in the present

278 study, even if we observed that larvae exposed to 40% air saturation hypoxia continued to  
279 have a certain feeding activity, we cannot exclude that they reduced their food consumption,  
280 compared to control larvae. Furthermore, in our study, the larvae which have experienced  
281 hypoxia exposure exhibited higher amylase specific activity at the same stage of development  
282 than larvae from C group. It is well documented that amylase specific activity decrease  
283 throughout the larval stage (Péres et al., 1996; Ribeiro et al., 1999; Zambonino-Infante and  
284 Cahu, 2001). This difference indicates that the normal decrease of amylase specific activity in  
285 larvae was delayed in the HT group. In parallel, the lower AP and N-LAP specific activities in  
286 larvae from the HT group revealed that the maturation of the enterocytes was impaired by the  
287 hypoxia episode. In marine fish species, particularly European sea bass, it is well admitted  
288 that the settlement of an efficient intestinal membrane digestion (characterised by the  
289 concomitant increase in AP and N-LAP specific activities) occurred around 30 d.p.h. at 19°C  
290 (Cahu and Zambonino-Infante, 1994; Zambonino-Infante and Cahu, 2001). In the present  
291 study, these developmental delays could also have contributed to a growth depression as long  
292 as the sea bass larvae were exposed to hypoxia, as already observed in fish larvae (Perez-  
293 Dominguez and Holt, 2006; Wexler et al., 2011).

294 We also found a down-regulation of CS/COX activity ratio in the HT group during hypoxia  
295 exposure. This down-regulation disappeared after the return in normoxic conditions (**Fig. 4**  
296 **C**). **This down-regulation of CS/COX activity ratio was mainly due to a significant increase in**  
297 **COX specific activity (Fig. 4 B) more than a decrease in CS specific activity (Fig. 4 A).** The  
298 CS is a key enzyme localized in the mitochondrial matrix and is involved in the Krebs' cycle  
299 whereas COX is the terminal electron acceptor of the electron transport chain located in the  
300 mitochondrial inner membrane. These two enzymes are of great importance in aerobic  
301 metabolism and provide information on respiration metabolism capacity. The fluctuations of  
302 these two key mitochondrial enzymes provide information on the properties and the numbers  
303 of the mitochondria present (Guderley, 2007; Lucassen et al., 2003). CS/COX activity ratio  
304 could be used in order to estimate changes in mitochondrial size and shape in response to  
305 energy-demand conditions (Ibarz et al., 2010). In consequence, the decrease in CS/COX  
306 activity ratio observed in our study during the hypoxia period could reflect a lower citrate  
307 synthesis over respiratory chain capacities of mitochondria in the larvae from the HT group  
308 (Lucassen et al., 2003) as well as a modification of the size and the shape of mitochondria  
309 (Ibarz et al., 2010). The similar CS/COX activity ratio observed at 42 d.p.h. indicated that  
310 such mitochondrial changes were transient.

311 It is interesting to note that the decrease in CS/COX activity ratio in HT group coincided with  
312 a growth depression, but we were not able to establish a significant relationship between  
313 growth rate and CS/COX activity ratio. Such relationship has yet been reported by Mathers et  
314 al. (1992) in the saithe (*Pollachius virens*).

### 315 **Conclusion**

316 Altogether, the results obtained suggest that sea bass larvae are able to implement  
317 physiological regulations in order to cope with a moderate decrease in ambient oxygen.  
318 However, these regulations seemed to have a metabolic cost that impacted growth and  
319 development, but not survival. Such consequences could have a strong impact on fish larvae  
320 activities, especially on their capacities to escape predation, which could adversely affect the  
321 recruitment of sea bass.

322

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325 **Author contributions**

326 Study conception and design: G. Vanderplancke, D. Mazurais, J-L. Zambonino-Infante

327 Animal experiments: G. Vanderpancke, P. Quazuguel

328 Acquisition and analysis of data: G. Vanderplancke, C. Huelvan

329 Interpretation of data: G. Vanderpancke, C. Corporeau, D. Mazurais, J-L. Zambonino-Infante

330 Drafting of manuscript: G. Vanderplancke, D. Mazurais, J-L Zambonino-Infante

331 Critical revision: D. Mazurais, J-L. Zambonino-Infante, G. Claireaux

332

333 **Competing interests**

334 No competing interests declared.

335

336

337 **References List**

338

339 **Almeida-Val, V. M. F., Oliveira, A. R., da Silva, M. D. P., Ferreira-Nozawa, M.**  
340 **S., Araujo, R. M., Val, A. L. and Nozawa, S. R.** (2011). Anoxia- and hypoxia-induced  
341 expression of LDH-A\* in the Amazon Oscar, *Astronotus crassipinis*. *Genetics and Molecular*  
342 *Biology* **34**, 315-322.

343 **Anjos, M. B., De Oliveira, R. R. and Zuanon, J.** (2008). Hypoxic environments as  
344 refuge against predatory fish in the Amazonian floodplains. *Brazilian Journal of Biology* **68**,  
345 45-50.

346 **Barrionuevo, W. R., Fernandes, M. N. and Rocha, O.** (2010). Aerobic and  
347 anaerobic metabolism for the zebrafish, *Danio rerio*, reared under normoxic and hypoxic  
348 conditions and exposed to acute hypoxia during development. *Brazilian Journal of Biology*  
349 **70**, 425-434.

350 **Bickler, P. E. and Buck, L. T.** (2007). Hypoxia tolerance in reptiles, amphibians, and  
351 fishes: Life with variable oxygen availability. *Annual Review of Physiology* **69**, 145-170.

352 **Breitbart, D. L.** (1992). Episodic hypoxia in Chesapeake Bay : Interacting effects of  
353 recruitment, behavior, and physical disturbance. *Ecological Monographs* **62**, 525-546.

354 **Bureau, D. P., Azevedo, P. A., Tapia-Salazar, M. and Cuzon, G.** (2000). Pattern  
355 and cost of growth and nutrient deposition in fish and shrimp: Potential implications and  
356 applications. *Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de*  
357 *Nutrición Acuícola*, 19-22.

358 **Cahu, C. L. and Zambonino-Infante, J. L.** (1994). Early weaning of sea bass  
359 (*Dicentrarchus labrax*) larvae with a compound diet : Effect on digestive enzymes.  
360 *Comparative Biochemistry and Physiology a-Physiology* **109**, 213-222.

361           **Cahu, C. L. and Zambonino-Infante, J. L.** (1995). Maturation of the pancreatic and  
362 intestinal digestive functions in sea bass (*Dicentrarchus labrax*): effect of weaning with  
363 different protein sources. *Fish Physiology and Biochemistry* **14**, 431-437.

364           **Cameron, P. and VonWesternhagen, H.** (1997). Malformation rates in embryos of  
365 North Sea fishes in 1991 and 1992. *Marine Pollution Bulletin* **34**, 129-134.

366           **Claireaux, G. and Lagardere, J. P.** (1999). Influence of temperature, oxygen and  
367 salinity on the metabolism of the European sea bass. *Journal of Sea Research* **42**, 157-168.

368           **Diaz, R. J.** (2001). Overview of hypoxia around the world. *Journal of Environmental*  
369 *Quality* **30**, 275-281.

370           **Diaz, R. J. and Rosenberg, R.** (1995). Marine benthic hypoxia: A review of its  
371 ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and*  
372 *Marine Biology - an Annual Review* **33**, 245-303.

373           **Diez, J. M. and Davenport, J.** (1990). Energy exchange between the yolk and  
374 embryo of dogfish (*Scyliorhinus canicula* L.) eggs held under Normoxic, Hypoxic and  
375 Transient Anoxic conditions. *Comparative Biochemistry and Physiology B-Biochemistry &*  
376 *Molecular Biology* **96**, 825-830.

377           **Dufour, V., Cantou, M. and Lecomte, F.** (2009). Identification of sea bass  
378 (*Dicentrarchus labrax*) nursery areas in the north-western Mediterranean Sea. *Journal of the*  
379 *Marine Biological Association of the United Kingdom* **89**, 1367-1374.

380           **Guderley, H.** (2007). Temperature and growth rates as modulators of the metabolic  
381 capacities of fish muscle. *Cold ocean physiology* **66**, 58.

382           **Guderley, H., Brokordt, K., Cortes, H. M. P., Marty, Y. and Kraffe, E.** (2011).  
383 Diet and performance in the scallop, *Argopecten purpuratus*: force production during escape  
384 responses and mitochondrial oxidative capacities. *Aquatic Living Resources* **24**, 261-271.



385 **Hassell, K. L., Coutin, P. C. and Nugegoda, D.** (2008). Hypoxia impairs embryo  
386 development and survival in black bream (*Acanthopagrus butcheri*). *Marine Pollution*  
387 *Bulletin* **57**, 302-306.

388 **Ibarz, A., Blasco, J., Gallardo, M. A. and Fernandez-Borras, J.** (2010). Energy  
389 reserves and metabolic status affect the acclimation of gilthead sea bream (*Sparus aurata*) to  
390 cold. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **155**,  
391 319-326.

392 **Ishibashi, Y., Inoue, K., Nakatsukasa, H., Ishitani, Y., Miyashita, S. and Murata,**  
393 **O.** (2005). Ontogeny of tolerance to hypoxia and oxygen consumption of larval and juvenile  
394 red sea bream, *Pagrus major*. *Aquaculture* **244**, 331-340.

395 **Ishibashi, Y., Kotaki, T., Yamada, Y. and Ohta, H.** (2007). Ontogenic changes in  
396 tolerance to hypoxia and energy metabolism of larval and juvenile Japanese flounder  
397 *Paralichthys olivaceus*. *Journal of Experimental Marine Biology and Ecology* **352**, 42-49.

398 **Jennings, S. and Pawson, M. G.** (1992). The origin and recruitment of bass,  
399 *Dicentrarchus labrax*, larvae to nursery areas. *Journal of the Marine Biological Association*  
400 *of the United Kingdom* **72**, 199-212.

401 **Kolkovski, S., Arieli, A. and Tandler, A.** (1997). Visual and chemical cues stimulate  
402 microdiet ingestion in sea bream larvae. *Aquaculture International* **5**, 527-536.

403 **Lakani, F. B., Sattari, M. and Falahatkar, B.** (2013). Effect of different oxygen  
404 levels on growth performance, stress response and oxygen consumption in two weight groups  
405 of great sturgeon *Huso huso*. *Iranian Journal of Fisheries Sciences* **12**, 533-549.

406 **Le Foll, C., Corporeau, C., Le Guen, V., Gouygou, J. P., Berge, J. P. and Delarue,**  
407 **J.** (2007). Long-chain n-3 polyunsaturated fatty acids dissociate phosphorylation of Akt from  
408 phosphatidylinositol 3'-kinase activity in rats. *American Journal of Physiology-Endocrinology*  
409 *and Metabolism* **292**, E1223-E1230.

- 410           **Lucassen, M., Schmidt, A., Eckerle, L. G. and Portner, H. O.** (2003).  
411 Mitochondrial proliferation in the permanent vs. temporary cold: enzyme activities and  
412 mRNA levels in Antarctic and temperate zoarcid fish. *American Journal of Physiology-*  
413 *Regulatory Integrative and Comparative Physiology* **285**, R1410-R1420.
- 414           **Massa, F., Delorme, C., Bagliniere, J. L., Prunet, P. and Grimaldi, C.** (1999).  
415 Early life development of brown trout (*Salmo trutta*) eggs under temporary or continuous  
416 hypoxial stress : Effects on the gills, yolk sac resorption and morphometric parameters.  
417 *Bulletin Francais De La Peche Et De La Pisciculture*, 421-440.
- 418           **Mathers, E. M., Houlihan, D. F. and Cunningham, M. J.** (1992). Nucleic acid  
419 concentrations and enzyme activities as correlates of growth rate of the saithe *Pollachius*  
420 *virens*: growth-rate estimates of open-sea fish. *Marine Biology* **112**, 363-369.
- 421           **Mazurais, D., Darias, M., Zambonino-Infante, J. L. and Cahu, C. L.** (2011).  
422 Transcriptomics for understanding marine fish larval development. *Canadian Journal of*  
423 *Zoology-Revue Canadienne De Zoologie* **89**, 599-611.
- 424           **Ozernyuk, N. D.** (2011). Adaptive specific features of energy metabolism in fish  
425 ontogenesis. *Russian Journal of Developmental Biology* **42**, 201-205.
- 426           **Padilla, P. A. and Roth, M. B.** (2001). Oxygen deprivation causes suspended  
427 animation in the zebrafish embryo. *Proceedings of the National Academy of Sciences of the*  
428 *United States of America* **98**, 7331-7335.
- 429           **Pelster, B.** (2002). Developmental plasticity in the cardiovascular system of fish, with  
430 special reference to the zebrafish. *Comparative Biochemistry and Physiology a-Molecular*  
431 *and Integrative Physiology* **133**, 547-553.
- 432           **Perez-Dominguez, R. and Holt, G. J.** (2006). Interrenal and thyroid development in  
433 red drum (*Sciaenops ocellatus*): Effects of nursery environment on larval growth and cortisol  
434 concentration during settlement. *General and Comparative Endocrinology* **146**, 108-118.

435 **Pfeiler, E.** (2001). Changes in hypoxia tolerance during metamorphosis of bonefish  
436 leptocephali. *Journal of Fish Biology* **59**, 1677-1681.

437 **Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A. and**  
438 **Boeuf, G.** (2001). Comparative effects of long-term hypoxia on growth, feeding and oxygen  
439 consumption in juvenile turbot and European sea bass. *Journal of Fish Biology* **59**, 875-883.

440 **Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A.,**  
441 **Quemener, L., Maxime, V., Nonnotte, G. and Boeuf, G.** (2000). Effects of hypoxia on  
442 growth and metabolism of juvenile turbot. *Aquaculture* **188**, 103-114.

443 **Pickett, G. D. and Pawson, M. G.** (1994). Sea Bass: Biology, Exploitation and  
444 Conservation. *Fish and Fisheries* **12**.

445 **Pihl, L., Baden, S. P. and Diaz, R. J.** (1991). Effects of periodic hypoxia on  
446 distribution of demersal fish and crustaceans. *Marine Biology* **108**, 349-360.

447 **Pihl, L., Baden, S. P., Diaz, R. J. and Schaffner, L. C.** (1992). Hypoxia-induced  
448 structural changes in the diet of bottom-feeding fish and crustacea. *Marine Biology* **112**, 349-  
449 361.

450 **Péres, A., Cahu, C. L., Zambonino-Infante, J. L., LeGall, M. M. and Quazuguel,**  
451 **P.** (1996). Amylase and trypsin responses to intake of dietary carbohydrate and protein  
452 depend on the developmental stage in sea bass (*Dicentrarchus labrax*) larvae. *Fish Physiology*  
453 *and Biochemistry* **15**, 237-242.

454 **R Core Team.** (2013). R: A language and environment for statistical computing. R  
455 Foundation for Statistical Computing, Vienna, Austria: URL : <http://www.R-project.org/>.

456 **Rees, B. B., Targett, T. E., Ciotti, B. J., Tolman, C. A., Akkina, S. S. and Gallaty,**  
457 **A. M.** (2012). Temporal dynamics in growth and white skeletal muscle composition of the  
458 mummichog *Fundulus heteroclitus* during chronic hypoxia and hyperoxia. *Journal of Fish*  
459 *Biology* **81**, 148-164.

460 **Ribeiro, L., Zambonino-Infante, J. L., Cahu, C. and Dinis, M. T.** (1999).  
461 Development of digestive enzymes in larvae of *Solea senegalensis*, Kaup 1858. *Aquaculture*  
462 **179**, 465-473.

463 **Richards, J. G., Farrell, A. P. and Brauner, C. J.** (2009). *Fish Physiology*, Vol. 27:  
464 Hypoxia: Academic Press, Amsterdam : Elsevier.

465 **Roussel, J. M.** (2007). Carry-over effects in brown trout (*Salmo trutta*): hypoxia on  
466 embryos impairs predator avoidance by alevins in experimental channels. *Canadian Journal*  
467 *of Fisheries and Aquatic Sciences* **64**, 786-792.

468 **Sanchez, R. C., Obregon, E. B. and Rauco, M. R.** (2011). Vertebral Column  
469 Deformity and Hypoxia in *Salmo salar*. *International Journal of Morphology* **29**, 1291-1295.

470 **Shang, E. H. H. and Wu, R. S. S.** (2004). Aquatic hypoxia is a teratogen and affects  
471 fish embryonic development. *Environmental Science & Technology* **38**, 4763-4767.

472 **Thetmeyer, H., Waller, U., Black, K. D., Inselmann, S. and Rosenthal, H.** (1999).  
473 Growth of European sea bass (*Dicentrarchus labrax* L.) under hypoxic and oscillating oxygen  
474 conditions. *Aquaculture* **174**, 355-367.

475 **Wei, L. Z., Zhang, X. M., Li, J. and Huang, G. Q.** (2008). Compensatory growth of  
476 Chinese shrimp, *Fenneropenaeus chinensis* following hypoxic exposure. *Aquaculture*  
477 *International* **16**, 455-470.

478 **Wexler, J. B., Margulies, D. and Scholey, V. P.** (2011). Temperature and dissolved  
479 oxygen requirements for survival of yellowfin tuna, *Thunnus albacares*, larvae. *Journal of*  
480 *Experimental Marine Biology and Ecology* **404**, 63-72.

481 **Wu, R., Shang, E. and Zhou, B. S.** (2006). Endocrine disrupting and teratogenic  
482 effects of hypoxia on fish, and their ecological implications. In *International Symposium*  
483 *Chongqing, China October 12-14, 2004*, pp. 75.

484           **Zambonino-Infante, J. L. and Cahu, C. L.** (2001). Ontogeny of the gastrointestinal  
485 tract of marine fish larvae. *Comparative Biochemistry and Physiology C-Toxicology &*  
486 *Pharmacology* **130**, 477-487.

487           **Zambonino-Infante, J. L., Cahu, C. L. and Peres, A.** (1997). Partial substitution of  
488 di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval  
489 development. *Journal of Nutrition* **127**, 608-614.

490           **Zambonino-Infante, J. L., Cahu, C. L., Peres, A., Quazuguel, P. and Le Gall, M.**  
491 **M.** (1996). Sea bass (*Dicentrarchus labrax*) larvae fed different Artemia rations: growth,  
492 pancreas enzymatic response and development of digestive functions. *Aquaculture* **139**, 129-  
493 138.

494

495 **Figures captions:**

496 **Fig. 1 Schema of hypoxic conditioning at 40% air saturation.** Sea bass larvae were divided  
 497 into two groups : “C” represents the Control group and “HT” represents the Hypoxia  
 498 Treatment group in which the level of oxygen was reduced to 40% air saturation for 8 days.  
 499 Samplings were performed during hypoxia exposure (*i.e.* at 30, 32, 35 and 38 d.p.h.) and after  
 500 the end of exposure phase (*i.e.* at 42 and 45 d.p.h.)

501  
 502 **Fig. 2 Dissection performed on sea bass larvae at each sampling.** Larvae were dissected  
 503 into 4 parts: Head (*H*), Pancreatic Segment (*PS*), Intestinal Segment (*IS*) and Tail (*T*). H and  
 504 T were systematically removed. PS were only sampled on 38-day-old sea bass larvae for  
 505 pancreatic enzymes activity measurements. IS were used for all enzyme assays

506  
 507 **Fig. 3 Sea bass larvae mass for Control “C” (–) and Hypoxia Treatment “HT” (– –)**  
 508 **groups.** Results are expressed as mean mass larvae  $\pm$  s.e.m. (n = 4 pools of 40 larvae).  
 509 Statistical analyses revealed that larval growth rate was affected by the oxygen treatment  
 510 (Two-way ANOVA:  $p < 0.001$ ) and the age of larvae (Two-way ANOVA:  $p < 0.001$ ).  
 511 Significant interaction between the oxygen treatment and the age of larvae (Two-way  
 512 ANOVA:  $p < 0.01$ ) indicated that the effects of time on growth (*i.e.* the growth rate) depend  
 513 upon the oxygen treatment. Additional statistical analyses (represent by asterisks) indicated  
 514 significant differences in mass between the Control and Hypoxia Treatment groups (Student-  
 515 test: significant codes : \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , NS = No Significant differences). Refer to  
 516 “Material and methods” section for more details on the statistical analyses

517  
 518 **Fig. 4 Changes in Citrate Synthase (CS) and Cytochrome c Oxidase (COX) activities** for  
 519 Control “C” (white bars) and Hypoxia Treatment “HT” (grey bars) groups. **A) Specific**  
 520 **enzymatic activity of CS, B) Specific enzymatic activity of COX and C) Ratio between**  
 521 **enzymatic activity of CS and enzymatic activity of COX.** Results are given  $\pm$  s.e.m. (n = 4  
 522 pools of 40 larvae). Statistical analyses revealed that COX specific activity and CS/COX  
 523 activity ratio was affected by the oxygen treatment (ANCOVA:  $p < 0.001$  and  $p < 0.001$ ,  
 524 respectively) and by the age of larvae (ANCOVA:  $p = 0.001$  and  $p < 0.001$ , respectively). In  
 525 parallel, CS specific activity was not affected by the oxygen treatment (ANCOVA:  $p > 0.7$ )  
 526 but was affected by the age of larvae (ANCOVA:  $p < 0.001$ ). Additional statistical analyses

527 (represent by asterisks) indicated significant differences between the Control and Hypoxia  
528 Treatment groups (Student-test: significant codes : \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , NS =  
529 No Significant differences)  
530

531 **Tables**

532 **Table 1: Specific activities of digestive enzymes.** Results are given in milli-units per mg of protein ( $\text{mU}\cdot\text{mg protein}^{-1}$ )  $\pm$  s.e.m. ( $n = 4 * 35$   
 533 larvae). Within any one column, means with the same superscript letter are not significantly different ( $p$ -value  $> 0.05$ ). C = Control group; HT =  
 534 Hypoxia treatment group; AP = Alkaline Phosphatase; N-LAP = Aminopeptidase-N.

	Pancreatic enzymes		Intestinal enzymes	
	Trypsin	Amylase	AP	N-LAP
C	$42.03 \pm 4.00^a$	$243.89 \pm 22.56^a$	$1242.59 \pm 253.57^a$	$204.12 \pm 43.10^a$
HT	$31.41 \pm 2.21^b$	$608.16 \pm 112.74^b$	$276.96 \pm 89.67^b$	$96.84 \pm 6.81^b$

535



←  $T = 19.1 \pm 0.4^{\circ}\text{C}$ ,  $S = 35.0 \pm 0.2\text{‰}$ , *Artemia* →

**Normoxia**

$\text{O}_2 = 96.6 \pm 1.3\%$  air sat.

**Normoxia**

$\text{O}_2 = 96.6 \pm 1.3\%$  air sat.

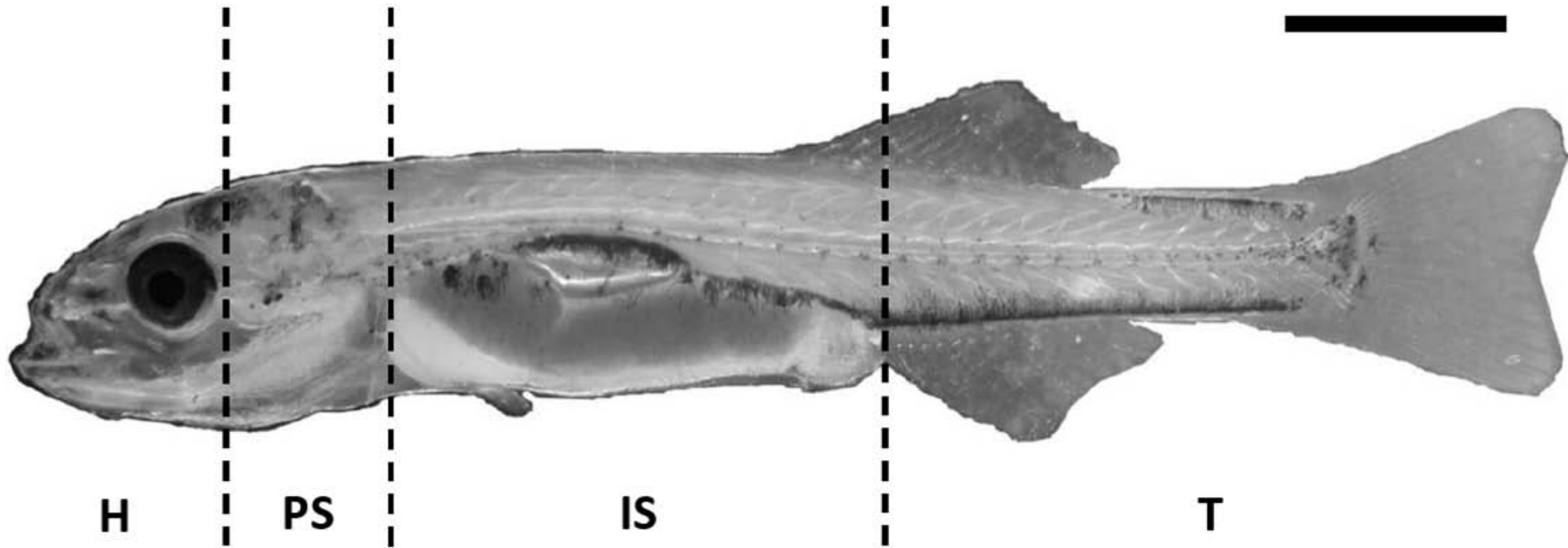
**Hypoxia**

$\text{O}_2 = 40.1 \pm 2.6\%$  air sat.

**Normoxia**

$\text{O}_2 = 96.6 \pm 1.3\%$  air sat.

5 mm



H

PS

IS

T

