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RESEARCH ARTICLE

An early life hypoxia event has a long-term impact on protein digestion and growth in juvenile European sea bass

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ABSTRACT

Ocean warming, eutrophication and the consequent decrease in oxygen lead to smaller average fish size. Although such responses are well known in an evolutionary context, involving multiple generations, this appears to be incompatible with current rapid environmental change. Instead, phenotypic plasticity could provide a means for marine fish to cope with rapid environmental changes. However, little is known about the mechanisms underlying plastic responses to environmental conditions that favour small phenotypes. Our aim was to investigate how and why European sea bass that had experienced a short episode of moderate hypoxia during their larval stage subsequently exhibited a growth depression at the juvenile stage compared with the control group. We examined whether energy was used to cover higher costs for maintenance, digestion or activity metabolisms, as a result of differing metabolic rate. The lower growth was not a consequence of lower food intake. We measured several respirometry parameters and we only found a higher specific dynamic action (SDA) duration and lower SDA amplitude in a fish phenotype with lower growth; this phenotype was also associated with a lower protein digestive capacity in the intestine. Our results contribute to the understanding of the observed decrease in growth in response to climate change. They demonstrate that the reduced growth of juvenile fishes as a consequence of an early life hypoxia event was not due to a change of fish aerobic scope but to a specific change in the efficiency of protein digestive functions. The question remains of whether this effect is epigenetic and could be reversible in the offspring.

KEY WORDS: Climate change, Digestive enzymes, *Dicentrarchus labrax*, Growth, Hypoxia, Respirometry

INTRODUCTION

One of the most pressing problems for several marine coastal and estuarine ecosystems is the increased occurrence of hypoxia events, particularly in shallow areas (Diaz, 2001; Rabalais et al., 2009). Oxygen depletion in marine waters is mainly the consequence of eutrophication caused by nutrient input through anthropogenic activities, and is exacerbated by warmer conditions (Díaz and Rosenberg, 2011).

Only recently have studies begun to address the effects of warming and reduction in dissolved oxygen on marine fish development and growth (Daufresne et al., 2009; Vanderplancke et al., 2015b), revealing a significant reduction of fish body size (Sheridan and Bickford, 2011).

From an evolutionary point of view, in a given population, warmer conditions should favour survival of smaller sizes, leading over generations to a reduction in the population average size, in accordance with established temperature–size relationships (Daufresne et al., 2009; Sheridan and Bickford, 2011). However, such evolutionary responses occur on long-term scales and thus are incompatible with current rapid environmental change.

Phenotype plasticity could be a more efficient means for marine fish to cope with rapid environmental changes. Such plasticity may also have long-term consequences for population dynamics and evolution (Zambonino-Infante et al., 2013). Warmer seawaters and the co-occurring reduction in dissolved oxygen could negatively influence marine fish size in two ways: first, by increasing metabolic rate, which would undoubtedly reduce body size (unless increased metabolic demands could be met by higher food intake); and second, by decreasing dissolved oxygen concentration, which would make it difficult for several fish species to meet their metabolic needs (Pörtner et al., 2004; Wang and Overgaard, 2007) and potentially have negative consequences for their physiology and fitness (Pörtner and Knust, 2007).

In the present study, we focused in particular on the mechanisms resulting from oxygen depletion. Hypoxia events often occur in late spring, early summer and autumn, which correspond to periods of recruitment and fast growth in several fish species (Breitburg, 2009; Pihl et al., 1992). As demonstrated in a number of studies, early stages of marine fish life are particularly affected by hypoxia episodes, depending on hypoxia severity, duration and individual adaptive capacities (Anjos et al., 2008; Bickler and Buck, 2007; Vanderplancke et al., 2015b). Understanding the long-term impact of a low-oxygen environment at early life stages is therefore crucial to forecast possible effects on juvenile recruitment, which determine fish population structure and dynamics (van der Veer, 2000).

European sea bass larvae generally enter shallow coastal areas just after the flexion stage, between 22 and 25 days post-hatching (dph), and they may then be exposed to hypoxic episodes, while their larval development is not totally completed (Dufour et al., 2009). Vanderplancke et al. (2015b) reported that early life hypoxia had a negative impact on juvenile growth rate. Their study revealed that such reduced growth was associated with a long-lasting down-regulation of some metabolic pathways, particularly at the hepatic level, even though food intake remained unchanged. In other words, juvenile fish ingested the same amount of food (or energy) whether or not they had previously been exposed to hypoxic conditions, but those previously exposed did not use it for growth. This could be due to a different metabolic use of the ingested energy or to lower digestive/transport efficiency.

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The present study aimed to understand why juvenile fish growth was reduced in response to a hypoxic signal experienced during the early stages of life. To do so, we first investigated the fate of the ingested energy by evaluating fish standard and maximum metabolic rates, and the digestion-related increase in metabolic rate (specific dynamic action, SDA). We then examined the functioning of four intestinal enzymes involved in digestion.

MATERIALS AND METHODS

Animals and diets

European sea bass, *Dicentrarchus labrax* (Linnaeus 1758), used in this study were from the same experimental population as used in a previous experiment (Vanderplancke et al., 2015b). Sea bass juveniles were randomly taken at day 450 dph (nearly 15 months old) in the control group (n=24) and in the early life hypoxia treatment (ELHT) group (n=23 fish after the accidental death of one individual). The mean (±s.d.) mass was 441±70.1 and 397±76.6 g for the control and ELHT groups, respectively. The early life hypoxia treatment corresponded to a moderate hypoxia episode (40% air saturation) at the end of the larval period, from 30 to 38 dph (Vanderplancke et al., 2015a). Experimental conditions tested here mimic field conditions; indeed, sea bass larvae arrive in coastal areas approximatively 1 month after hatching (Dufour et al., 2009) and are likely to experience short hypoxia episodes as evidenced for an 8 day period along the coast of South Brittany (Coastal Observations and Forecast, http://marc.ifremer.fr/en). In addition, a moderate hypoxia level was applied to avoid any significant larva mortality that could induce a potential genetic selection (Vanderplancke et al., 2015a). Apart from this hypoxia episode, control and ELHT fish experienced identical experimental conditions (water temperature, salinity, oxygenation, food, etc.) throughout their whole lives. During all manipulations (and all phases in between), fish were maintained in normoxic conditions, at 20°C and fed a commercial feed (Neo Grower Extra Marin, Le Gouessant Aquaculture, France) containing in particular 43% protein and 20% lipid (with 1.5% eicosapentaenoic acid +docosahexaenoic acid).

All fish experiments were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals (NRC, 2010). The present work conforms to the French legal requirements concerning welfare of experimental animals (APAFIS permit no. 5173-2016042515065062 v2).

Standard metabolic rate (SMR) and SDA

Four intermittent-flow respirometers (volume 8 l) submerged in a thermoregulated (20°C; Seachill TR20, Teco, USA) and aerated water reservoir (2 m×0.6 m and 0.4 m deep) were used. The water from the reservoir was re-circulated to each of the respirometers using computer-controlled flush pumps (Compact 600, EHEIM, Germany), relays and software (AquaResp, University of Copenhagen, Helsingør, Denmark). Before being introduced into the respirometry chambers, fish (n=24 and n=23 for control and ELHT groups, respectively) were anaesthetized (ethyl-*m*-aminobenzoate; MS-222, 100 mg l^{-1}), weighed and fed via a gastric tube. The feeding mixture consisted of 1 g of feed (Neo Grower Extra Marin) mixed with 1.5 g of water. Preliminary experiments had determined that the sea bass stomach is completely filled with 4% body mass of our mixture. To avoid regurgitation, the initially targeted amount of mixture for injection via the gastric tube was 3% of fish body mass. *A posteriori* calculation showed, however, that mean stomach fullness was slightly lower than expected (2.88% of fish body mass). Each fed fish was then placed in a respirometry chamber and $\dot{M}_{O_2}$ measurement cycles immediately started. Measurement cycles consisted of a flushing period (5 min) followed by a stabilization (1 min) and measurement period (4 min) during which respirometers were sealed (flush pump turned off). Fish were left undisturbed for 72 h in a shaded, quiet room, after which they were removed from their respirometers and replaced in their original rearing tank. Once emptied, the background $\dot{M}_{O_2}$ of each respirometer was measured (15 min) and the entire system was then disinfected (bleach). Bacterial $\dot{M}_{O_2}$ typically accounted for less than 5% of fish $\dot{M}_{O_2}$ and was taken into account in the calculations.

Fish oxygen consumption ($\dot{M}_{O_2}$ in mg O_2 kg^{-1} h^{-1}) was calculated as follows:

$$\dot{M}_{O_2} = \Delta C_{O_2,w} \times \Delta t^{-1} \times V_{resp} \times M^{-1}, \quad (1)$$

where $\Delta C_{O_2,w}$ is the variation in water oxygen concentration (mg O_2 l^{-1}), Δt is the duration of the measurement period (h), V_{resp} is the volume of the respirometer minus the volume of the fish (l) and M is fish body mass (kg). Oxygen consumption values were only taken into account when linear regression analyses of the oxygen decline in the respirometer chamber versus time yielded r^2>0.95.

A typical example of the time course of fish oxygen consumption over the 3 day monitoring period is given in Fig. 1. This biphasic response results from the combination of (1) an initial decrease in oxygen demand due to the stress recovery phase, which is linked to fish recovery from manipulation, including feeding and introduction into the respirometers (first 8 h; solid grey line in Fig. 1); (2) a subsequent progressive increase and decrease in oxygen consumption, related to post-prandial digestive processes (solid black line in Fig. 1). To deconvolute these signals, the first phase of the metabolic response was modelled using Eqn 2 (Scarabello et al., 1991):

$$\dot{M}_{O_2} = Ae^{-K_1 t} + Be^{-K_2 t} + C, \quad (2)$$

where K_1, K_2, A, B and C are constants and t is time (min).

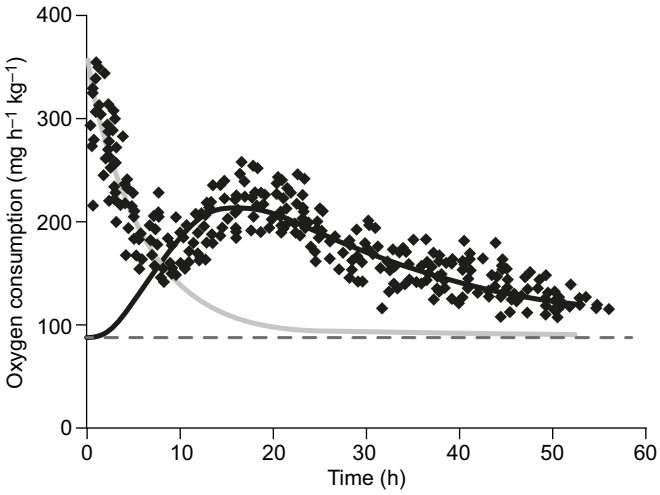

Fig. 1. Typical example of the time course of sea bass post-prandial oxygen consumption. Two phases can be distinguished, the stress recovery phase (from 0 to 8 h; grey solid line) and the specific dynamic action phase (from 8 to 53 h; black solid line). The dashed line is fish standard metabolic rate. The equations used to model the phases of the response are given in Materials and methods.

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The second phase of the response, from 8 h onward, was modelled using Eqn 3 (Motulsky and Christopoulos, 2003):

$$\dot{M}_{O_2} = A + ((B - A)/(1 + N_1 \times 10^{(\log K_1 - \log t)})) + ((B - A)/(1 + N_2 \times 10^{(\log t - \log K_2)})), \quad (3)$$

where A corresponds to peak of $\dot{M}_{O_2}$, B corresponds to SMR, K_1, K_2, N_1 and N_2 are constants and t is time (min).

Fish SMR was estimated as the mean of the lowest 10% of measurements obtained during the third night of the trial (black hatched line in Fig. 1).

Maximum metabolic rate (MMR)

Following SDA and SMR measurements, fish were transferred to a 30 l, swim-tunnel respirometer (Loligo Systems, Tjele, Denmark; swim chamber: 50×14×14 cm) and left undisturbed for 2 h at a water velocity of 10 cm s^{-1}. Following acclimation to the experimental device, water velocity was increased by 7 cm s^{-1} every minute until exhaustion, which was indicated by the fish being unable to remove itself from the grid placed downstream from the swim chamber. This water velocity corresponded to the critical swimming speed (U_{crit}). At this point, the water velocity was rapidly reduced to 10 cm s^{-1} and the respirometry monitoring cycle quickly started. The $\dot{M}_{O_2}$ measuring device and protocol were the same as described for the static respirometry, and fish $\dot{M}_{O_2}$ was monitored for 5 h post-exhaustion. The MMR was estimated as the highest recorded $\dot{M}_{O_2}$. Note that the relationship between the rpm of the motor that propelled the water and the velocity of the water in the swimming chamber was established using a velocimeter (Höntzsch, Waiblingen, Germany). As for static respirometry, background bacterial oxygen consumption was measured and systematically subtracted from fish $\dot{M}_{O_2}$ (approximately 7%). To avoid excessive bacterial colonization, the swim tunnel was also cleaned with a bleach solution once a week and oxygen probes were calibrated daily.

Faeces collection and analysis

From 600 to 620 dph (at nearly 20 months old), faeces were collected over a 2 week period using a faeces settling column system (four replicate tanks of six fish per experimental group; note that one ELHT tank contained five fish) similar to the one described by Cho and Kaushik (1990) but adapted for cylindro-conical tanks. Fish were fed by hand 3 times a day to visual satiety (visual observation of first feed refusal) over 30 min periods. The quantity of feed distributed per day was recorded, normalized to the biomass in each group and corresponded to the normalized food ingestion rate (NFIR, %). Before daily feeding, faeces from each tank were collected and centrifuged for 6 min at 1500 rpm. After centrifugation, the dry matter of the solid faeces was determined (24 h in an oven at 110°C). Pooled faeces from the same group were then ground in a mixer mill. Gross energy in faeces was assayed using an adiabatic bomb calorimeter (IKA C4000, Staufen, Germany).

Sampling for biochemical analyses

After all these manipulations, fish were left undisturbed until day 823. After 12 h of fasting, fish from control (n=24) and ELHT (n=23) groups were first lightly anaesthetized (0.05 g l^{-1}), weighed and then killed with a lethal dose (0.25 g l^{-1}) of MS-222. The fish intestines were quickly removed, flushed with 5 ml of ice-cold NaCl 0.9%, and the mucosa of each intestine was scraped and stored at −80°C until assays.

Analytical methods

The frozen intestinal mucosa from each fish was homogenized in mannitol (50 mmol l^{-1} with 2 mmol l^{-1} Tris, pH 7) according to Crane et al. (1979). Leucine-alanine peptidase, a cytosolic peptidase, was assayed in the mucosa homogenate according to Nicholson and Kim (1975). The purification of brush border membranes was performed according to Crane et al. (1979). Alkaline phosphatase, aminopeptidase N and sucrase-isomaltase were assayed in the brush border membrane fraction according to Bessey et al. (1946), Maroux et al. (1973) and Dahlqvist (1970), respectively. Enzyme activity is expressed as specific activity, i.e. mU mg^{-1} protein. Protein was determined by the Bradford procedure (Bradford, 1976).

Statistical analyses

Results for respirometry and enzymes are given as means±s.e.m. (n=24 for the control group; n=23 for the ELHT group). Data on feed and faeces are given as means±s.e.m. (n=4, i.e. four replicate tanks of six fish per dietary group; note that one ELHT tank contained five fish). As data complied with normality and homoscedasticity, they were compared using an independent two-group t-test (Statistica software).

RESULTS

SMR and MMR were not significantly different between the two groups (P>0.05; Table 1). The post-prandial $\dot{M}_{O_2}$ increase (SDA_{scope}) was nearly 2.1 and 2.3 times above the SMR values for the ELHT and control groups, respectively. SDA_{scope} was 8% lower in the ELHT group compared with the control group (P<0.05) and this lower value in the ELHT group was accompanied by a 14% increase in the time taken for the $\dot{M}_{O_2}$ to return to the pre-feeding level ($SDA_{duration}$; P<0.04).

No significant difference in food intake was observed between the two groups (NFIR-control=1.3±0.001%; NFIR-ELHT=1.3 ±0.001%). In addition, the total amount of food distributed and faeces recovered over 15 days was not significantly different between control and ELHT groups (P>0.05; Table 2). However, faeces of the ELHT group had a 4% higher energy content per gram (P=0.02) than those of the control group.

Out of the four different intestinal enzymes studied, only sucrase did not show a difference in specific activity between ELHT and control groups (P=0.31), while the specific activities of alkaline phosphatase, aminopeptidase N and leucine-alanine were, respectively, 19% (P=0.0012), 13% (P=0.0001) and 25%

Table 1. Metabolic parameters in control and ELHT groups

	Control (n=24)	ELHT (n=23)	P-value
SMR (mg O_2 kg^{-1} h^{-1})	93.8±4.40[a]	89.5±4.00[a]	0.29
MMR (mg O_2 kg^{-1} h^{-1})	530±21.4[a]	550±28.8[a]	0.28
SDA_{scope} (mg O_2 kg^{-1} h^{-1})	209±9.8[a]	193±5.3[b]	0.11
t_{peak} (h)	13.2±0.72[a]	14.0±1.08[a]	0.21
$SDA_{duration}$ (h)	35.8±1.58[b]	40.8±1.8[a]	0.02

Data are means±s.e.m. Different superscript letters in the same row indicate a significant difference.

ELHT, early life hypoxia treatment; SMR, standard metabolic rate; MMR, maximum metabolic rate; SDA_{scope}, the maximum $\dot{M}_{O_2}$ value recorded during the specific dynamic action (SDA) process minus SMR; t_{peak}, the time from feeding to SDA_{scope}; $SDA_{duration}$, the time taken for the $\dot{M}_{O_2}$ to return to the pre-feeding level.

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Table 2. Total amount of feed distributed and faeces recovered

	Control (*n*=4)*	ELHT (*n*=4)*	*P*-value
Feed (g)	22.7±0.66	22.1±1.65	0.32
Faeces (g dry matter)	4.6±0.46	4.5±0.70	0.35
Energy in faeces (kJ g^{-1} dry matter)	18.7±0.09[b]	19.5±0.23[a]	0.02

Data are means±s.e.m. for four replicate tanks of six fish per dietary group; note that one ELHT tank contained five fish. Different superscript letters in the same row indicate a significant difference.

(*P*=0.0001) lower in the ELHT group than in the control group (Table 3).

DISCUSSION

This study was conducted to elucidate one intriguing result reported in Vanderplancke et al. (2015b), where fish had different life history traits following early environmental conditioning. Fish that had experienced moderate hypoxia during a short period of their larval life showed a growth depression at the juvenile stage that was not observed in the control group (Vanderplancke et al., 2015b). This lower growth was not a consequence of lower food intake or a modification of growth-related gene expression, *igf-1*, but was associated with a down-regulation of some metabolic processes that consume energy. It should be pointed out that, in the present study, this difference in growth was still observable between the two groups at 450 dph, with the control group exhibiting a significantly higher (+11%) mass than the ELHT group. If fish ingest the same amount of food (or energy), and do not use it for growth, one can ask what becomes of this ingested energy. Is this energy used to cover higher metabolic costs of maintenance, digestion or activity due to an altered metabolic rate?

We will first examine the respirometry results (rate of oxygen consumption) for SMR and MMR before considering SDA. MMR provides an integrative measurement of energy expenditure for maintenance and activity, while SDA corresponds to the energy expenditure associated with ingestion, absorption and assimilation of a meal (McCue, 2006). We did not observe any significant difference in SMR or MMR between the two fish groups. This finding markedly contrasts with some results reported in salmon (Millidine et al., 2009), suggesting that a high potential for growth correlates with a high SMR. Our result instead suggests that the different growth potential between the two groups of fish with different life trajectories is not related to their metabolic scope, which corresponds to the difference between MMR and SMR (Fry, 1947). Our findings clearly show that several different metabolic cost strategies could be proposed for fish having life trajectories based on high growth potential. As a consequence, fish from the two treatments exhibited a similar capacity to mobilize oxygen to cover their energetic needs for swimming and growth under aerobic conditions (Neill and Bryan, 1991).

Table 3. Specific activity of alkaline phosphatase, aminopeptidase N, sucrase and leucine-alanine peptidase in enterocytes of control and ELHT groups

	Control (*n*=24)	ELHT (*n*=23)	*P*-value
Alkaline phosphatase (mU mg^{-1} protein)	4599±208.5[a]	3860±254.5[b]	0.0012
Aminopeptidase N (mU mg^{-1} protein)	2363±57.8[a]	2067±57.0[b]	0.0001
Sucrase (mU mg^{-1} protein)	223±10.8	233±12.1	0.31
Leucine-alanine peptidase (U mg^{-1} protein)	1936±70.9[a]	1449±59.3[b]	0.0001

Alkaline phosphatase, aminopeptidase N and sucrase were measured in the brush border membranes; leucine-alanine peptidase was measured in the cytosol.
Data are means±s.e.m. Different superscript letters in the same row indicate a significant difference.

Post-prandial $\dot{M}_{O_2}$ peaked at a mean of 13–14 h post-feeding and rose to more than twice SMR in both fish groups, which is comparable to previous measurements of European sea bass SDA at 20°C (Dupont-Prinet et al., 2010). Interestingly, Dupont-Prinet et al. (2010) revealed that a particular lower-growth fish phenotype (that had a lower growth rate after food deprivation but higher tolerance to fasting) was also associated with longer SDA duration, together with lower SDA amplitude, as reported in our present study. This lower growth phenotype corresponded to a lower physiological capacity to exploit dietary resources when available, but was less costly to sustain during fasting periods.

More recently, McKenzie et al. (2014), working on fish exhibiting the same phenotype as those of Dupont-Prinet et al. (2010), revealed that the difference in fasting tolerance did not depend on the routine energy expenditure during fasting; rather, it relied on a lower use of proteins as metabolic fuel, particularly during fasting.

Vanderplancke et al. (2015b) showed that in sea bass juveniles that experienced the same moderate hypoxia at the larval stage as in the present experiment, the growth reduction was not due to a lower feed ingestion, and was associated with a metabolic depression. In particular, they revealed a decrease in blood glycaemia of fish and, in the liver, a down-regulation of expression of several genes involved in energy-consuming pathways (glucose transporter and fatty acid synthesis) together with activation of the anaerobic metabolism (lactase dehydrogenase). In the present experiment, we also did not observe any difference in either feed ingestion or the amount of faeces produced, but fish with lower growth potential were losing dietary energy in their faeces. In consequence, we wondered whether such a metabolic depression reported by Vanderplancke et al. (2015b) had also impacted on the functioning of the digestive tract. This consideration prompted us to analyse the intestinal enzyme capacity of the two fish groups, especially proteases and disaccharidases, as lipases are mainly synthetized in the pancreas.

Four enzymes were evaluated. Alkaline phosphatase, known to be intimately associated with the hydrophobic core of the membrane, is very sensitive to changes in membrane ultrastructure; this enzyme was therefore used as a model to assess the effects of changes in lipid membrane composition on membrane function (Cahu et al., 2000). Aminopeptidase N and leucine-alanine peptidase are both involved in peptide hydrolysis, but are located in two different intestinal cell compartments, the brush border membrane and the cytosol, respectively (Zambonino-Infante et al., 1997). Sucrase-isomaltase is another enzyme of the brush border membrane of intestinal absorptive cells, which specifically hydrolyses sucrose into glucose and fructose (Hunziker et al., 1986). These enzymes are commonly used as indicators of intestine functioning in fish (Cyrino et al., 2008).

Our data (Table 2) did not reveal any effect of early life hypoxia on carbohydrate digestive potential, and clearly showed that the digestive potential for dietary proteins was specifically and significantly impacted in enterocytes of fish with lower growth. This effect could be associated with changes in membrane ultrastructure of enterocytes as suggested by the different alkaline phosphatase specific activities; however, only histological examination of the intestinal villi, combined with analyses of fatty acid composition of enterocyte membranes, would provide a firm conclusion on this aspect. From a nutritional point of view, a lower protein digestive capacity is somewhat similar to a situation with a lower dietary protein supply. Protein is the main dietary component

of fish feeds as carnivorous fish have particularly high protein requirements, either for maintenance or for growth (Mambrini and Guillaume, 2001). Therefore, it is not surprising that a situation leading to a lower protein supply would have negative effects on fish growth. It remains to be explained why these intestinal protein hydrolases exhibited lower activities in fish that had experienced hypoxia during their larval stage. Vanderplancke et al. (2015a) showed that the hypoxia episode during the larval stage induced a delay in growth (due to lowered feed ingestion during exposure) and maturation of the digestive function, the latter resulting in 25% lower trypsin activity in the pancreas together with lower aminopeptidase N (−53%) and alkaline phosphatase (−78%) activity in the intestine. It is therefore likely that the hypoxia episode during early ontogenesis led to a nutritional disturbance in terms of protein supply, with a possible adverse effect on the metabolic programming of enzymatic digestive functions. Recent studies suggest that besides the initiation of a gene transcription programme via hypoxia-inducible factor (HIF), hypoxia can also modulate histone methylation via an epigenetic mechanism (Yang et al., 2009). Metabolic programming of digestive enzymes by carbohydrate or lipid conditioning has already been demonstrated in fish (Geurden et al., 2007; Vagner et al., 2007); however, the present study suggests for the first time that such nutritional programming could also occur for proteins, even though this effect was indirectly due to a hypoxia-induced diet restriction. Interestingly, similar long-term consequences have been reported in a higher vertebrate: differences in intestinal enzyme activities were induced by a nutritional protein deficit during the weaning period of rat pups (Egorova et al., 2008; Timofeeva et al., 2009).

The present study addresses the long-term effect of a moderate hypoxia episode on the physiology and growth of European sea bass. It contributes to the understanding of observed size declines in response to climate change. Recent studies have underlined the necessity to focus research on the effect of climate change on size trends, and to identify the drivers of declining size (Sheridan and Bickford, 2011). The present study clearly demonstrates that the growth reduction of juvenile fish as a consequence of an early life hypoxic episode is not due to a change of fish aerobic scope, and suggests a specific change in the efficiency of protein digestive functions. The question remains as to whether this effect is epigenetic and could be reversible in the next generation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
J.L.Z.-I. and D.M. conceived, designed and coordinated the study. G.C. supervised and interpreted the respirometry measurements. J.L.Z.-I. and C.C. interpreted the enzymatic results. N.L.B. and C.H. conducted the fish experiment and enzyme analyses, respectively. A.D. and P.Q. performed the respirometry measurements. G.V. was involved in all experimental and analytical aspects of this study. J.L.Z.-I., D.M., A.S., C.C. and G.C. contributed to manuscript drafting.

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