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

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Summary statement: This study examines several physiological pathways in juvenile fish involved in metabolic rate in order to identify the strongest drivers underlying growth reduction in response to an early-life hypoxia.

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Abstract

Ocean warming, eutrophication and consequent decrease in oxygen lead to smaller average fish size. Although such responses are well-known in an evolutionary context, involving multiple generations, it appears to be incompatible with current rapid environmental change. Rather, 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 to 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 feed intake.

We measured several respirometry parameters and we only found a higher SDA (Specific Dynamic Action) 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, European sea bass, 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 nutrients inputs through anthropogenic activities, and is exacerbated by warmer conditions (Diaz 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 this population average size, in accordance with established temperature-size relationships (Daufresne et al., 2009; Sheridan and Bickford, 2011). However, such evolutionary responses occur at 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 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).

The present study will particularly focus 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, 1992; 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, 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. This 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.

The present study aims to understand why juvenile fish growth was reduced in response to a hypoxic signal experienced during 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). Then, we also examined the functioning of four intestinal enzymes involved in digestion.

Materials and Methods

Animals and diets

European sea bass (*Dicentrarchus labrax*) used in this study were from the same experimental population as a previous experiment (Vanderplancke et al., 2015b). Sea bass juveniles were randomly taken at day 450 dph (days post hatching; nearly 15 months old) in the control group (n = 24) and in ELHT (Early Life Hypoxia Treatment) group (n = 23 fish after the accidental loss of one individual). The mean mass and standard deviation were 441 ± 70.1 g 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., 2015 a). Experimental conditions tested here mimic field conditions; indeed, sea bass larvae arrive in coastal areas approximatively one month after hatching (Dufour et al. 2009). They are likely to experience short hypoxia episodes as evidenced for an eight day period along the coasts of South Brittany (Coastal Observations and Forecast, http://www.previmer.org/en). In addition, a moderate hypoxia level was applied to avoid any significant larvae 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…) throughout their whole lives. During all manipulations (and all phases in between) fish were maintained in normoxic condition, at 20°C and fed a commercial feed (Neo Grower Extra Marin; Le Gouessant Aquaculture, France) containing in particular 43% proteins and 20% lipids (with 1.5% EPA+DHA).

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. Its permit number is APAFIS#5173-2016042515065062 v2.

Standard metabolic rate (SMR) and Specific dynamic action (SDA)

Four intermittent-flow respirometers (volume = 8 L) submerged in a thermoregulated (20°C; Teco, Seachill TR20) an aerated water reservoir (2 m x 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 in 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; Le Gouessant Aquaculture, France) mixed with 1.5 g of water. Preliminary experiments had determined that sea bass stomach is completely filled with 4% body mass of our mixture. To

avoid regurgitation, the initially targeted amount of mixture to inject 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 were 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 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 (MO_2 in mg O_2 kg^{-1} h^{-1}) was calculated as follows:

$$\dot{M}O_2 = \Delta C_w O_2 \, x \, \Delta t^{-1} \times VOL_{resp} \times M^{-1}$$

where $\Delta C_w O_2$ is the variation in water oxygen concentration (mg O_2 L^{-1}), Δt the duration of the measurement period (h), VOL_{resp} the volume of the respirometer minus the volume of 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 an $r^2 > 0.95$.

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

$$\dot{M}O_2 = Ae^{-K1t} + Be^{-K2t} + C,$$

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

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

$$\dot{M}O_2 = A + ((B-A)/(1+ N_1 \times 10^{(\log K1 - \log t)})) + ((B-A)/(1+ N_2 \times 10^{(\log t - \log K2)})),$$

where A corresponds to peak of MO_2, B corresponds to SMR, K_1, K_2, N_1 and N_2 are constants and t is time (min).

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

Maximum metabolic rate (MMR)

Following SDA and SMR measurements, fish were transferred in a 30-L, swim-tunnel respirometer (Loligo Systems, Tjele, Denmark; swim chamber: 50×14×14 cm) and left undisturbed for two hours at a water velocity of 10 cm s^{-1}. Following acclimation to experimental device, water velocity was increased by 7 cm sec^{-1} every minute until exhaustion which was indicated by the fish being unable to remove himself from the grid placed downstream from the swim chamber. This water velocity corresponded to the critical swimming speed (U_{crit}). At that time 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 during 5h post-exhaustion. The maximum metabolic rate (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 MO_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 two-week-period using a faeces settling column system (four replicate tanks of 6 fish per experimental group; note that one ELHT tank contained 5 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 minutes 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 grounded 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 hours of fasting, fish from control (n = 24) and ELHT (n = 23) groups were first lightly anesthetized (0.05g.L^{-1}), weighted and then killed with a lethal dose (0.25g.L^{-1}) of MS-222. The fish intestines were quickly removed, flushed with 5 ml of iced 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 mM-Tris 2 mM, 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 (BBM) was performed according to Crane, Bogé and Rigal (1979). Alkaline phosphatase, aminopeptidase N, sucrase-isomaltase were assayed in the BBM fraction according to Bessey et al. (1946), Maroux et al., (1973) and Dalqvist (1970), respectively. Enzyme activities were expressed as specific activities, i.e., mU/mg 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. 4 replicate tanks of 6 fish per dietary group; note that one ELHT tank contained 5 fish). As data complied with normality and homoscedasticity, they were compared using an independent 2-group t-test (STATISTICA software).

Results

SMR (Standard Metabolic Rate) and MMR (Maximum Metabolic rate) were not significantly different between the two groups ($p>0.05$; Table 1). The postprandial MO_2 increase (SDA_{scope}) was nearly 2.1 and 2.3 times above the SMR values for ELHT and Control groups, respectively. SDA_{scope} was 8% lower in the ELHT group compared with the control ($p<0.05$) and this lower value in the ELHT group was accompanied by an SDA duration that was 14% longer ($p<0.04$).

No significant difference in feed intake was observed between the two groups (NFIR-Control = 1.3 ± 0.001 %; NFIR-ELHT = 1.3 ± 0.001 %). In addition, total amount of food distributed and faeces recovered over 15 days were not significantly different between control and ELHT groups (p

>0.05;Table 2). However, faeces of 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 specific activities were 19% (p=0.0012), 13% (p=0.0001) and 25% (p=0.0001) respectively lower in the ELHT group compared to the control (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 feed intake, nor a modification of the 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 feed (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 for maintenance, digestion or activity due to an altered metabolic rate?

We will first examine the respirometry results (rates of oxygen consumption) for standard (SMR) and maximum (MMR) metabolism before considering SDA (Specific Dynamic Action). MMR provides an integrative measurement of energy expenditures for maintenance and activity, while SDA corresponds to the energy expenditures 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 rather suggests that the different growth potential between the two groups of fish with different life trajectories could not therefore be related to their metabolic scope, which corresponds to the difference between MMR and SMR (Fry 1947). Our findings clearly showed 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).

Post-prandial MO_2 peaked at mean of 13-14 hours 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 lower growth rate after food-deprivation but higher tolerance to fasting) was also associated with higher 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 expenditures during fasting; rather, it relied on a lower use of proteins as metabolic fuels, particularly during fasting.

In sea bass juveniles having experienced the same moderate hypoxia at larval stage as in the present experiment, Vanderplancke et al. (2015b) showed that the growth reduction was not due to a lower feed ingestion, and was associated with a metabolic depression. They particularly 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 an activation of the anaerobic metabolism (Lactase dehydrogenase). In the present experiment, we also did not observe any difference neither in feed ingestion nor in the amount of faeces produced, but fish with lower growth potential were losing dietary energy in their faeces. In consequence, we could wonder 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, since lipases are mainly synthetized in the pancreas.

Four enzymes have been 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 is therefore used as a model to assess the effects of changes in lipid membrane composition on membrane functions (Cahu et al., 2000). Aminopeptidase N and leucine-alanine peptidase are both involved in peptide hydrolyse, 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 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 since carnivorous fish have particularly high protein requirements, either for maintenance or 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 later resulting in 25% lower trypsin activity in the pancreas together with lower aminopeptidase N (-53%) and alkaline phosphatase (-78%) activities 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 been already 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 be observed 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 physiology and growth of European sea bass. It contributes to the understanding of observed size declines in response to climate change. Recent studies have indeed 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 fishes 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 interest

No competing interests declared

Author contributions

J.L.Z.I. and D.M. conceived, designated and coordinated the study. G.C. supervised and interpreted respirometry measurements. J.L.Z.I. and C.C. interpreted enzymatic results. N.L.B. and C.H. conducted the fish experiment and enzyme analyses respectively. 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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Tables

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]	*p = 0.29*
MMR (mg O_2.kg^{-1}.h^{-1})	530 ± 21.4 [a]	550 ± 28.8 [a]	*p = 0.28*
SDA_{scope} (mg O_2.kg^{-1}.h^{-1})	209 ± 9.8[a]	193 ± 5.3[b]	*p = 0.11*
t_{peak} (h)	13.2 ± 0.72 [a]	14.0 ± 1.08 [a]	*p = 0.21*
$SDA_{duration}$ (h)	35.8 ± 1.58[b]	40.8 ± 1.8[a]	*p = 0.02*

Means ± s.e.m. with a different superscript letter in same row are significantly different.

SDA_{scope} (the maximum MO_2 value recorded during the SDA process – SMR); t_{peak}: the time from feeding to SDA_{scope}; $SDA_{duration}$: the time taken for the MO_2 to return to pre-feeding level

ELHT: Early Life Hypoxia Treatment.

Table 2: Total amount of feed distributed and faeces recovered (in dry matter)

	Control (n = 4)*	ELHT (n = 4)*	*p value*
Feed (g)	22.7 ± 0.66	22.1 ± 1.65	*p = 0.32*
Faeces (g)	4.6 ± 0.46	4.5 ± 0.70	*p = 0.35*
Energy in faeces (kJ/g)	18.7 ± 0.09[b]	19.5 ± 0.23[a]	*p = 0.02*

Means ± s.e.m. with a different superscript letter in same row are significantly different.

* Four replicate tanks of 6 fish per dietary group; note that one ELHT tank contained 5 fish.

Table 3: Specific activities 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*
Brush border membranes (mUnit/mg protein)			
Alkaline phosphatase	4599 ± 208.5[a]	3860 ± 254.5[b]	*p = 0.0012*
Aminopeptidase N	2363 ± 57.8[a]	2067 ± 57.0[b]	*p = 0.0001*
Sucrase	223 ± 10.8	233 ± 12.1	*p = 0.31*
Cytosol (Unit/mg protein)			
Leucine-alanine peptidase	1936 ± 70.9[a]	1449 ± 59.3[b]	*p = 0.0001*

Means ± s.e.m. with a different superscript letter in same row are significantly different.

Figures

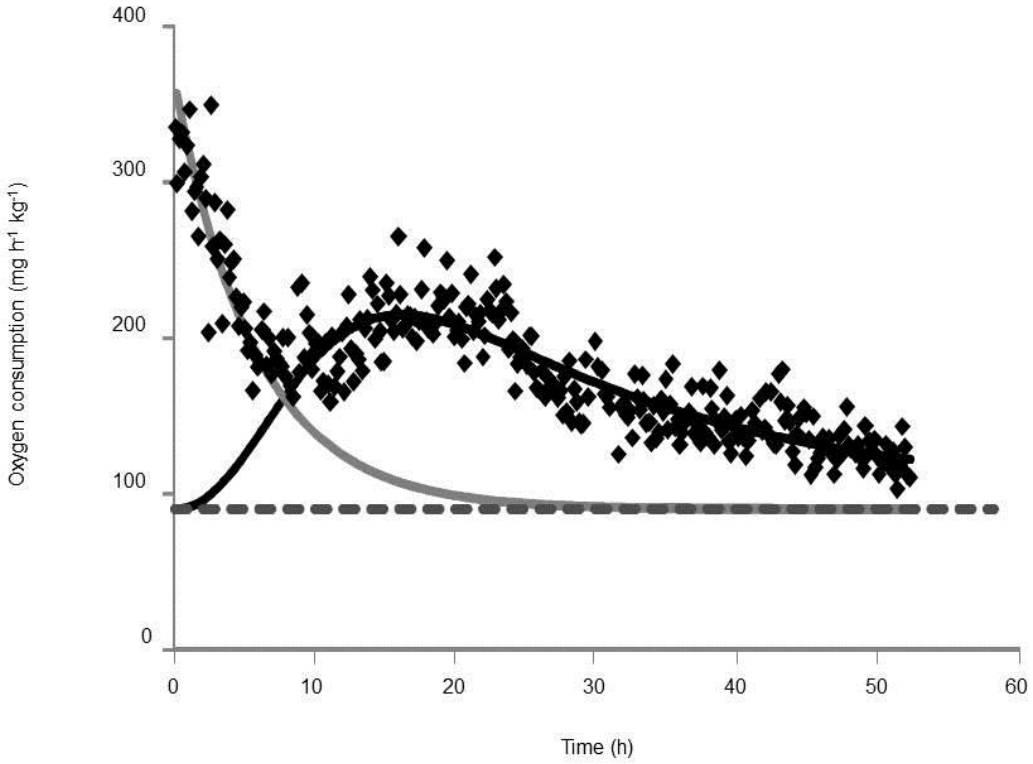

Figure 1: Typical example of the time course of sea bass post-prandial oxygen consumption (black dots). Two phases can be distinguished, the stress recovery phase (from 0 to 8h; grey solid line) and the specific dynamic action phase (8 to 53h; black solid line). The equations used to model the phases of the response are given in the text.