Calibrating accelerometer data, as a promising tool for health and welfare monitoring in aquaculture: Case study in European sea bass (*Dicentrarchus labrax*) in conventional or organic aquaculture

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ARTICLE INFO

ABSTRACT

Both the conventional and organic aquaculture sectors have grown rapidly over the past few years. Moreover, welfare has attracted increased attention on the part of both consumers and governments. However, fish welfare assessment is complex and thus needs to adapt measurements that are easily applicable to aquaculture conditions. In this study, in addition to classical welfare indicator measurements (physiological stress indicators and growth performance), we recorded the swimming activity data using acoustic transmitters to evaluate the welfare of European sea bass (*Dicentrarchus labrax*) fed a conventional vs. an organic diet. Prior feeding trial, the swimming activity recorded by tag has been calibrated with water speed during critical swimming speed (U_{crit}) tests. This calibration allowed us to increase the power of the recorded data, providing information on swimming activity with respect to the U_{crit} value and on the metabolic cost of swimming. After a four-month experimental period, physiological stress indicators and growth performance did not differ significantly between the two diet groups. However, we observed a subtle difference in swimming activity: the fish in the organic diet group were more active during the feeding period in the morning. All indicators considered, our results suggest that an organic diet does not incur higher metabolic costs and does not affect the welfare of the European sea bass. Moreover, this study shows that the use of acoustic transmitters previously calibrated with physiological indicators, such as U_{crit}, is a promising tool for welfare monitoring in aquaculture conditions.

1. Introduction

Due to an increasing demand for fish products and a decrease in natural resources, the aquaculture sector has grown rapidly over the past decades, now representing more than 50 % of total fish production (FAO, 2018). Fish production from organic aquaculture has also increased rapidly (Gambelli et al., 2019; Gould et al., 2019). Organic aquaculture may contribute to addressing environmental issues related to the aquaculture sector – for example, by replacing fish protein content with proteins and oils from land-based agriculture, thus preventing overfishing for the production of fish feed (Gould et al., 2019; Turchini et al., 2009). However, the replacement of fish protein in feed formulations must be measured, as total substitution can disrupt physiological

processes and growth performance (Borquez et al., 2011; Geay et al., 2011), causing fish health and welfare issues, mainly in the farming of carnivorous fish species (e.g. sea bass, *Dicentrarchus labrax* and rainbow trout, *Oncorhynchus mykiss*).

Besides the development of aquaculture, fish welfare has also attracted increased attention on the part of both consumers and governments and has become a critical point to consider in the growth of this sector (Ashley, 2007; FSBI, 2002; Huntingford and Kadry, 2008), especially organic aquaculture (Lembo et al., 2019). However, assessing fish welfare is complex and requires an integrative overview, from physiology to behaviour and biological performances (Huntingford et al., 2006; Martos-Sitcha et al., 2020). Overall, fish welfare can be closely linked to stress (Prunet et al., 2012; Schreck and Tort, 2016;

Sneddon et al., 2016). Thus, plasma cortisol, end product of the hypothalamus–pituitary–interrenal (HPI) axis, and secondary stress response indicators, such as blood glucose and lactate levels, may be used as welfare indicators (Barton, 2002). However, stress does not always mean compromised welfare, as it can also be a response to predation, competition or environmental changes (Alfonso et al., 2020a; Barton, 2002; Gorissen and Flik, 2016). Thus, a short-term stress response (e.g. elevation of cortisol levels) can be viewed as adaptive, allowing fish to cope with stressors and preserve both individuals and populations. Chronic or repeated stress, on the other hand, can lead to dysfunctions and compromise welfare. Therefore, stress indicators alone may be insufficient for properly evaluating fish welfare (Ellis et al., 2012; Raposo de Magalhães et al., 2020; Sadoul et al., 2021; Sadoul and Geffroy, 2019). Consequently, many innovative approaches using fish spontaneous swimming activity and/or behaviour as a reliable proxy of fish welfare have been developed (Martins et al., 2012). This can be achieved using classical video recordings (Alfonso et al., 2020b; Sadoul et al., 2017, 2014; Stien et al., 2007) but also acoustic transmitters that record several variables, such as positioning, speed or acceleration. Having previously been widely used for monitoring natural fish populations for conservation purposes (McKenzie et al., 2016), these transmitters are now being increasingly used in aquaculture contexts for fish welfare monitoring (Carbonara et al., 2020a, 2020b, 2019a; Gesto et al., 2020; Muñoz et al., 2020). Indeed, their use appears promising for monitoring welfare, as they allow evaluations of the behaviour of free-swimming fish over long periods (Halachmi et al., 2019) without affecting welfare and biological performance (Alfonso et al., 2020c; Jepsen et al., 2011). It is important to emphasize that tag implantation does not affect fish welfare and biological performances only if some conditions are respected, i.e. the tag has not to exceed 2% of the fish's body weight in air, the position has to be thought to not disturb normal fish movements, and the tag implantation has not to obstruct the internal organs (Bridger and Booth, 2003; Jepsen et al., 2005; Smircich and Kelly, 2014). The signals recorded by the transmitter can be prior calibrated with other physiological variables, such as muscle activity and swimming performance, thereby increasing the power of the physiological data obtained (Clark et al., 2010; Martos-Sitcha et al., 2019; Wilson et al., 2013; Zupa et al., 2015).

Swimming performance and aerobic/anaerobic metabolism use are of primary importance in assessing the physiological state of fish and their ability to cope with stressors (Lembo et al., 2007; Zupa et al., 2015). The critical swimming speed (U_{crit}) achieved by a fish during a swimming test provides information on swimming performance, as well as its maximum metabolic rate (MMR) (Norin and Clark, 2016). The MMR indicates the capacity for energy usage by aerobic pathways under different environmental conditions (Brett, 1964; Norin and Clark, 2016). Previous evidences on European sea bass shown that the MMR is generally achieved before the U_{crit} (Claireaux et al., 2006; Zupa et al., 2015), suggesting that near this threshold, the supplementary energy requirement necessary to sustain the increasing swimming activity of fish is mainly fuelled by anaerobic metabolism. As observed by Claireaux et al. (2006), before the U_{crit} was reached, the maximum swimming speed (U_{max}) of sea bass is reached, and fish use anaerobic metabolism to

display a burst swimming mode, and the MO_2 usually levelled off or decreased slightly. This state may be detrimental to fish health and welfare if repeated or sustained for long time, as observed by Carbonara et al. (2015) regarding high stocking density. Moreover the environmental conditions, included the feed, have an impact on the fish activities and performance (Claireaux and Lefrançois, 2007). Therefore, calibrating the acceleration data recorded by acoustic transmitters with swimming performance during a U_{crit} test may offer more precise information on the swimming activity, aerobic/anaerobic metabolism and life energy cost of free-swimming fish (Carbonara et al., 2020b; Zupa et al., 2015). In fish, the activity of red muscles, supported by aerobic metabolism, increases with speed until it reaches a maximum and is maintained at that level even if the swimming speed increases further (Rome et al., 1992). On the other hand, white muscle recruitment, supported by anaerobic metabolism, follows an exponential pattern (Burgetz et al., 1998; Zupa et al., 2015). Muscle recruitment appears to be species-specific, as indicated by the different placement and amount of slow-twitch aerobic (for sustained swimming) and fast-twitch anaerobic muscle fibres (recruited during fast starts) in different species (Ellerby et al., 2000). In the European sea bass, we have previously observed that the activity of red muscles increases with speed to a maximum and is maintained at that level until the end of the U_{crit} (Zupa et al., 2015), following a classical activation pattern. On the other hand, white muscle activation follows an exponential pattern, with the start of increase at approximately 65 % of the U_{crit} to compensate for the reduction of red muscle activity recruitment (Zupa et al., 2015). Thus, information about swimming performances may offer valuable insights into the metabolic costs of swimming related to both aerobic and anaerobic metabolism. This can be valuable in free swimming fish under different aquaculture rearing conditions, including diet regime.

In this study, we first calibrated the values of acceleration recorded by acoustic transmitters with the swimming velocity in a swimming chamber, obtaining a baseline of fish swimming performance to determine the U_{crit}. Then, we used the recorded swimming activity data, as a proxy of the metabolic cost of swimming of European sea bass, and be used for health and welfare monitoring of European sea bass fed a conventional and an organic diet. Indeed, organic aquaculture is a production method that combines preservation of natural resources and the use of renewable products (Lembo and Mente, 2019). Organic feed is composed of a greater percentage of proteins and oils from land-based agriculture (Gould et al., 2019). However, the partial substitution of fishmeal and fish oil with plant proteins and oils was found to be a promising more environmental friendly alternative to fish protein and oil on one hand (Lembo and Mente, 2019), but from the other hand, it can negative impact the natural physiological processes and so also the growth performances (Geay et al., 2011; Montero and Izquierdo, 2010). Therefore, we also measured fish growth performance and classical physiological stress indicators at the end of the experimental period. The results obtained from both kinds of data were considered together in the framework of welfare assessment in organic aquaculture of European sea bass, a key species of European marine aquaculture (Vandeputte et al., 2019).

2. Materials and methods

Care and handling of fish were accomplished in accordance with the recommendations 2007/526/EU C(2007) 2525 on the accommodation and care of animals used for experimental and other scientific purposes. The experimental protocol was approved by the ethic committee on the animal experiments of COISPA.

2.1. Fish holding and diet

Juvenile (approximately 1 year old) European sea bass specimens were purchased from the commercial farm Ittica Caldoli (Lesina, Italy) and maintained at a stocking density of ~10 kg/m³ at salinity of 35 PSU,

Table 1
Compositions of the conventional and organic diets used in the experiment.

Diet composition	Conventional diet	Organic diet
Crude protein (%)	43	43
Crude oils and fats (%)	21	15
Raw cellulose (%)	2.0	2.9
Ashes (%)	9.0	11.5
Vitamin A (UI/kg)	7515	6938
Vitamin D3 (UI/kg)	2505	2313
Phosphorus (%)	1.1	1.5
Sodium (%)	0.2	0.8
Calcium (%)	1.4	2.1

pH of 7.30 ± 0.05, temperature of 18 ± 1 °C and oxygen saturation above 90 % for two weeks in a 12Light:12Dark photoperiod (light from 06:00 am to 06:00 pm). The fish were reared in a flow-through system with water replacement of 25 L/min and fed commercial feed (Skretting Marine 3 P, Italy) amounting to 1 % of their body mass. After this two-weeks period, the fish were randomly separated into six fiberglass tanks of 1.2 m^3 (n = 35 per tank; 10 kg/m^3) with the same water quality and photoperiod (specified above) but fed two different commercial diets (a conventional and an organic diet) in three replicates each. Some additional fish remained in the initial batch and fed commercial feed until the calibration of accelerometer tag (see Section 2.3). The conventional feed used was Performance Mare (NaturAlleva, Italy), and the organic feed was Acquabiomare 15 PLUS (NaturAlleva, Italy) (Table 1).

In the organic feed, 51.13 % of the feed is composed from contents from agricultural origin (80 % of the protein and fats). The fishmeal and fish oil contribute to 18 % and 7% of the total content of the diet, asserting the diet as organic according to EU regulation (Busacca and Lembo, 2019). In comparison, in the conventional feed, the fishmeal and fish oil account for about 30 % and 12 % of the content of the total content of the diet respectively. In both diet groups, the fish were fed feed amounting to 1% of their body mass administered by automatic feeders for six hours a day six days a week throughout the experimental period (133 days) from 07:00 am to 01:00 pm.

Morphometric parameters were assessed, and blood samples were taken from a subsample of fish as a baseline for each diet at the beginning of the experiment (t$_0$; details are provided in the following Sections 2.2. and 2.5). The fish weight in the conventional and organic diet groups was similar at the beginning of the experiment (mean ± standard error of the mean [SEM] = 336.05 ± 19.41 and 356.76 ± 7.83 g, respectively).

2.2. Experimental procedure, growth performance and sampling

Growth performance was measured at three sampling points over the experimental period: the first day of the experiment (t$_0$), the day 74 (t$_1$) and 133 (t$_2$) after the beginning of the experiment in all fish (n = 35 per tank). For each measurement, fish were gently caught from their rearing tanks and anaesthetized with a hydro-alcoholic clove oil solution (30 mg/L) (Iversen et al., 2003). The morphometric parameters measured were body weight and total length. The mean specific growth rate (SGR) in each tank was calculated based on the mean fish mass as follows:

$$SGR = 100 * \frac{(lnW_{t2} - lnW_{t0})}{(t_2 - t_0)} \qquad (1)$$

where *W* is the mean fish weight (in grams) per tank at t$_0$ (Wt$_0$) and t$_2$ (Wt$_2$). The feed conversion ratio (FCR) was calculated as the ratio of the feed supplied (in kilograms of dry weight) per biomass of weight gained (in kilograms), and the protein efficiency ratio (PER) was calculated as the ratio of the total biomass in each tank to the total proteins assumed.

2.3. Tag calibration using a critical swimming speed (U$_{crit}$) trial

For the calibration of the accelerometer tags, 10 fish from the batch fed commercial feed (i.e. Skretting Marine 3 P) were subjected to a U$_{crit}$ trial. The mean body weight of the specimens subjected to the U$_{crit}$ trial (mean ± SEM = 398.64 ± 25.93 g) did not differ significantly from that of the fish in which tags were implanted (see Section 2.4) during the diet experiment (mean ± SEM = 447.61 ± 16.57 g; p > 0.05). In addition, the tag did not exceed 2% of the fish's body weight in air (i.e., 1.53 and 1.36 % for calibration and diet experiment respectively), in order to do not alter fish physiology and normal behaviour of tagged fish during the experiment (Bridger and Booth, 2003; Jepsen et al., 2005; Smirich and Kelly, 2014). The U$_{crit}$ tests were performed in a Blazka-type respirometer as previously described (Carbonara et al., 2006). The chamber volume was 120 L with a respirometer 123 cm long with an inner

diameter of 24 cm. This swimming chamber was built to generate a laminar water flow in the inner part of the tube (Carbonara et al., 2006). After the V9A acoustic accelerometer tags (VEMCO, Nova Scotia, Canada; see Section 2.4 for details on the surgical procedure) were implanted, the fish were left undisturbed for at least five days to ensure full recovery prior to the U$_{crit}$ trial. Twenty-four hours before the trial, the fish were subjected to fasting to ensure a post-absorptive state during the test (McFarlane et al., 2004). On the trial day, the fish were gently caught from their rearing tanks and placed in the chamber for a maximum acclimatization time of one hour. After acclimatization, the fish were left at a velocity of 0.1 m/s for 30 min before the start of the trial. The protocol consisted in an increase in water velocity of 0.1 m/s every 15 min until fatigue ensued and the test was terminated. Fatigue was indicated by the caudal fin touching the grid for at least 5 s (Carbonara et al., 2010). The U$_{crit}$ was calculated as described by Brett (1964), and a solid blocking effect correction was performed according to the equation Smit et al. (1971). During the swimming trial, the accelerometer tags were programmed to record data at a sampling rate of 10 Hz (10 measurements per second), which were sent to an acoustic receiver (VR2W; VEMCO) located near the swimming chamber. The tag returned an 8-bit value that represents the root mean square (RMS) acceleration resulting from the contribution of two axes (vertical and lateral directions of movement), every 30 s on average. The tag algorithm was designed to provide data with a more precise measurement of tailbeat activity, excluding the forward/backward component of the movement. The adimensional value obtained can be converted in acceleration using the following equation (acceleration (m/s^2) = 0.01955 (x), where x is the adimensional value returned by tags). In this study, adimensional value of acceleration data (RMS) were provided without conversion to real acceleration values, and later called swimming activity (expressed in arbitrary units; AU). To calibrate the swimming activity data with the water velocity, the recorded data were averaged for each water velocity increment (from 0.1 m/s to fatigue).

2.4. Tag implantation and recording of swimming activity

At t$_1$ (74 days after the beginning of the experiment), following the morphological measurements, a subsample of three fish per tank (n = 9 per diet) was randomly selected for implantation of acoustic accelerometer tag (V9A; Vemco) to measure swimming activity as described in Alfonso et al. (2020c). Briefly, fish were subjected to a 24-h fast before tag implantation. The gills were continuously irrigated with a hydro-alcoholic clove oil solution (30 mg/L) during the entire surgical procedure. The transmitter was inserted into the body cavity through an incision 1.5 cm long. The body cavity was then carefully closed using sutures. The fish then received antibiotic injections (sodic-ampicillin–cloxacillin; 1 mg/kg 24 h^{-1}) (Lembo et al., 2008) before being returned to their home tanks. All fish recovered within a few days, and no mortality related to the surgical operation was observed, regardless of the diet treatment.

The accelerometer tags were programmed to record data with a sampling rate of 10 Hz (10 measurements per second) during the whole experiment duration. The tag IDs and coded acceleration values were stored in the memory of submergible acoustic receivers (VR2W) located in each tank. Swimming activity (AU) were recorded for 43 days, from 82 to 125 days after the beginning of the diet treatments. The fish were allowed an interval of eight days between the surgical procedure and the start of data collection to fully recover from the surgery and display normal behaviour during data collection.

2.5. Physiological measurements

At t$_0$ and t$_2$, a subsample of fish (n = 9 fish at t$_0$ and n = 5 fish per tank, 15 per diet at t$_2$) was randomly selected for blood sampling. After 2–3 min under anaesthetic, the blood samples were taken from the first branchial arch using a heparinized syringe. The samples were analysed

to assess the basal levels of the following physiological indicators: cortisol, glucose, lactate, haematocrit (HCT), haemoglobin (Hb), red blood cell count (RBCC) and lysozyme.

The quantification of these parameters was performed as described by Carbonara et al. (2019a). Briefly, HCT was determined using a heparinized micro-haematocrit tube filled with blood directly from the syringe needle, which was then centrifuged at $15,000 \times g$ for 3 min and immediately read. HCT was expressed as the red blood cell percentage of the entire blood volume. The RBCC was performed in a Bürker counting chamber under a light microscope (Nikon 400E, Japan). Hb was measured using a commercial kit (H7379; Sigma, USA). The remaining blood was centrifuged at $15,000 \times g$ for 3 min to obtain plasma samples, which were stored at $-20\,^{\circ}\mathrm{C}$ until further analysis. Plasma cortisol was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (InterMedical, Italy) for microplate readers (k = 450 nm) following the manufacturer's instructions. Plasma glucose and lactate concentrations were measured using commercial kits (17630H and 17285, respectively; Sentinel, Italy) based on enzymatic colorimetric Trinder reactions (GOD/PAP for glucose and PAP for lactate). Plasma lysozyme concentrations were measured using a turbidimetric assay modified for a microplate reader (Carbonara et al., 2019b).

2.6. Statistical analyses

Statistical analyses were performed using R software (R Core Team, 2021). All analyses were performed at a 95 % significance level. Values were expressed as means ± standard errors (SE) unless otherwise indicated. Data normality was assessed using the Shapiro–Wilk test, following which an appropriate statistical test was performed.

The Wilcoxon rank sum test was used to investigate differences in the SGR, FCR and PER between the two diet groups. For the calibration of swimming activity data as a function of water speed during the U_{crit} trial, linear and exponential models were tested. Model showing the lowest AIC has been selected for the calibration (Akaike, 1973). A generalized linear mixed model (GLMM) was used to compare the swimming activity between the two diet groups, with the diet (conventional or organic) and the time of day (day- or night-time) as fixed factors and the fish ID as a random factor, using the package lme4 (Bates et al., 2014). An analysis of frequency distribution between the two diets has been carried out on the whole data set of swimming activity values recorded by tags during the experiment, merging by slot of 10 the swimming activity values (i.e., 0–10, 11–20, [...], 241–250, 251–255). The statistical analysis has been carried out using Pearson's Chi-squared test, and pairwise comparisons have been then carried out to compare slot by slot the two diets using comparisons using pairwise chi-squared tests with p value adjusted by the Bonferroni method. The proportion of acceleration values above the U_{crit} value during the experiment was also analysed using the diet and time of day as fixed factors and the fish ID as a random factor. Both GLMM analyses were performed using the gamma distribution family and logarithmic link. If significant, the GLMM analyses were followed by Tukey's honest significant difference (HSD) post-hoc test (Lenth et al., 2019). A visual inspection of the residuals showed no violation of the statistical assumptions by the two models. It should be noted that one individual in the conventional diet group was excluded from the statistical analyses because of accelerometer tag acquisition defect, resulting in 17 fish (eight in the conventional and nine in the organic diet group).

Finally, analysis of variance (ANOVA) was performed to evaluate differences between the two diet groups (conventional or organic) and the control group at the start of experiment t_0, in normally distributed physiological parameters (RBCC, HCT, Hb and lysozyme), and the Kruskal–Wallis test was used for non-normally distributed variables (cortisol, glucose and lactate). When significant, ANOVA or Kruskal-Wallis was followed by Tukey's HSD test.

Table 2

Growth performance in the conventional and organic diet groups (n = 3 tanks per diet).

Diet	Mass at t_0 (g)	Mass at t_1 (g)	Mass at t_2 (g)	SGR*	FCR*	PER*
Conventional	336.05 ± 19.41	378.37 ± 29.66	412.79 ± 26.80	0.20 ± 0.02	4.99 ± 0.39	2.15 ± 0.17
Organic	356.76 ± 7.83	402.96 ± 30.58	440.02 ± 3.95	0.20 ± 0.03	5.45 ± 1.68	2.15 ± 0.72

All values are means ± standard errors.
t_0, Day 1 of the experiment; t_1, Day 74 of the experiment; t2, Day 133 of the experiment; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio.
* Calculated between t_0 and t_2.

Table 3

Estimations of the parameters and Standard Error of the exponential model ($y = \alpha * e^{\beta * x}$) for swimming activity as a function of water speed (BL s^{-1}) during the U_{crit} test ($R^2 = 0.67$).

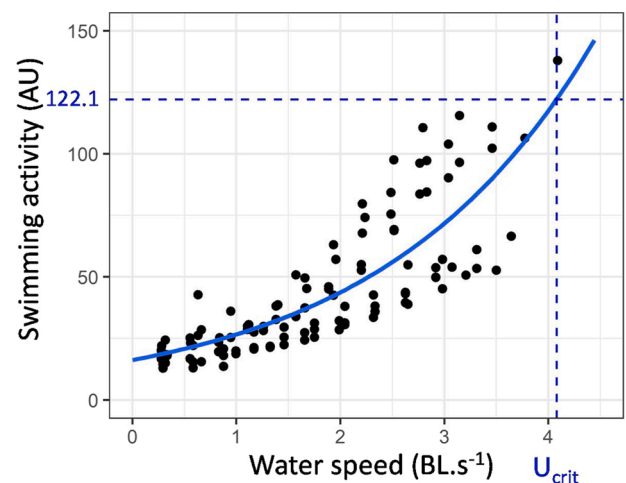| Parameter	Estimate	Standard Error	t-value	p-value
α	16.264	1.648	9.869	<0.001
β	0.494	0.036	13.772	<0.001

Fig. 1. Exponential model fitting the swimming activity (in arbitrary units; AU) recorded by accelerometer tags as a function of water speed (Body length, BL s^{-1}) during the U_{crit} trial (n = 10 fish). The black dots represent individual values, and the blue line represents the model curve based on the parameters displayed in Table 3. The dashed blue lines represent the correspondence of the U_{crit} value to the swimming activity according to the exponential model.

3. Results

3.1. Growth performance

There were no statistically significant differences between the two groups in SGR, FCR and PER at the end of the experimental period (Table 2; p > 0.05 for all).

3.2. Calibration of swimming activity values with water velocity during the U_{crit} test

The mean absolute U_{crit} value was 1.32 ± 0.11 m/s, which is corresponding to a relative U_{crit} of 4.08 ± 0.15 BL/s. Exponential model showed the lowest AIC value compare to linear one (926.9 for exponential vs. 934.4 for linear). Thus, the recorded swimming activity was

Table 4

Outputs of the generalized linear mixed model (GLMM) for swimming activity as a function of diet (conventional or organic) and time of day (day- or night-time).

Fixed effects	Estimate	Standard error	t-value	p-value
Intercept	3.844	0.021	187.222	<0.001
Diet	0.086	0.028	3.048	0.002
Time of day	−0.038	0.002	−16.636	<0.001
Diet–time of day interaction	−0.046	0.003	−13.880	<0.001

Random effects	Estimate	Standard error
Fish ID (n = 17)	0.003	0.058
Residuals	0.249	0.499

The reference factor level for diet is conventional. The reference factor level for the time of day is daytime.

fitted with an exponential model using the water velocity ($p < 0.001$; R^2 = 0.71; Table 3).

Using this model, the swimming activity value at which the fish reached the U_{crit} value was determined to be 122.1 (Fig. 1). This value was then used for further analysis of the swimming activity data of the tagged fish in the two diet groups during the experiment (see Section 3.3).

3.3. Swimming activity

Daytime and diet, as well as the interaction between the two parameters, had significant effects on the swimming activity of the fish (Table 4). The sea bass showed a diurnal activity pattern, even though the differences in swimming activity between day and night were relatively small (Fig. 2A). During the daytime, the fish in the organic diet group displayed more intense swimming activity than those in the conventional diet group (Fig. 2B), especially during the first hours of daylight (Fig. 2A). In both groups, the mean swimming activity levels were far below the corresponding U_{crit} value (46.3 ± 22.5 AU and 51.4 ± 24.2 AU for the conventional and organic diet respectively), representing 37.9 % of the value in the conventional diet group and 42.1 % of the value in the organic diet group during the daytime, but overall displaying high variability throughout the experiment (Fig. 2C).

However, the frequency distributions were found different between the two diets during the whole experiment (χ^2 = 4310, df = 25, $p <$ 0.001; Fig. 3A). In more details, higher data frequency for low swimming activity values (<20 AU) has been found in fish fed organic diet ($p <$ 0.05; Fig. 3C). For values ranged from 21 to 50 AU, higher data frequency has been observed in fish fed conventional diet ($p <$ 0.05; Fig. 3C), while from 51 to 120 AU higher data frequency has been again recorded in fish fed organic diet than fish fed with conventional one ($p <$
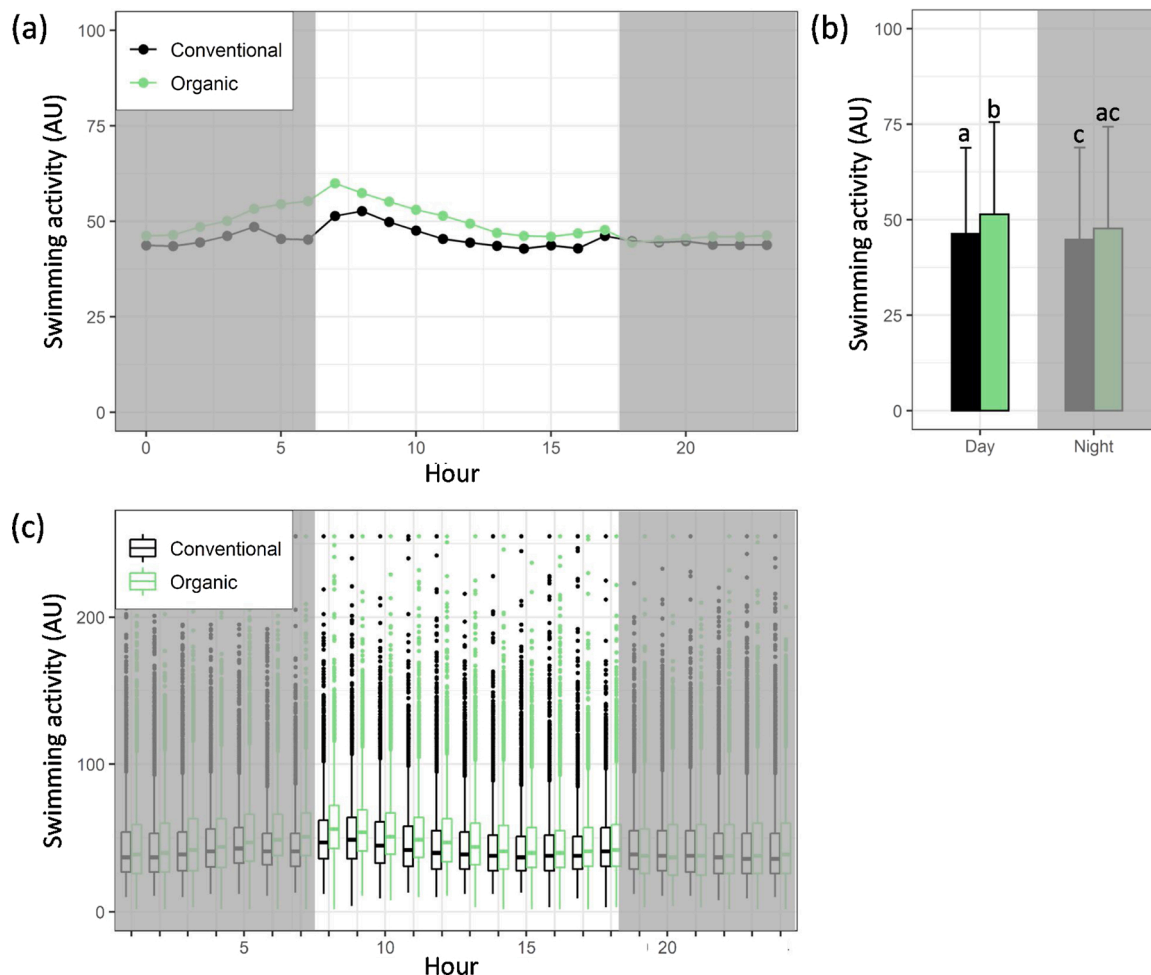

Fig. 2. (a) Means of swimming activity (in arbitrary units; AU) as a function of hours per day in the conventional (black; n = 8 fish) and organic diet (green; n = 9 fish) groups. (b) Means ± standard errors of swimming activity as a function of the time of day (day- and night-time) and diet. The light grey areas indicate the night periods. The different letters indicate statistically significant differences between the two diet groups and/or time of day (GLMM followed by Tukey's HSD post-hoc test; $p <$ 0.05; see Table 4 for details). (c) Boxplot of the swimming activity (in arbitrary units; AU) as a function of hours per day in the conventional (black; n = 8 fish) and organic diet (green; n = 9 fish) groups. The central line in each boxplot indicates the median, and the boxes on either side represent the quartiles, with the whiskers covering 95 % of the values. Outliers are represented by points.
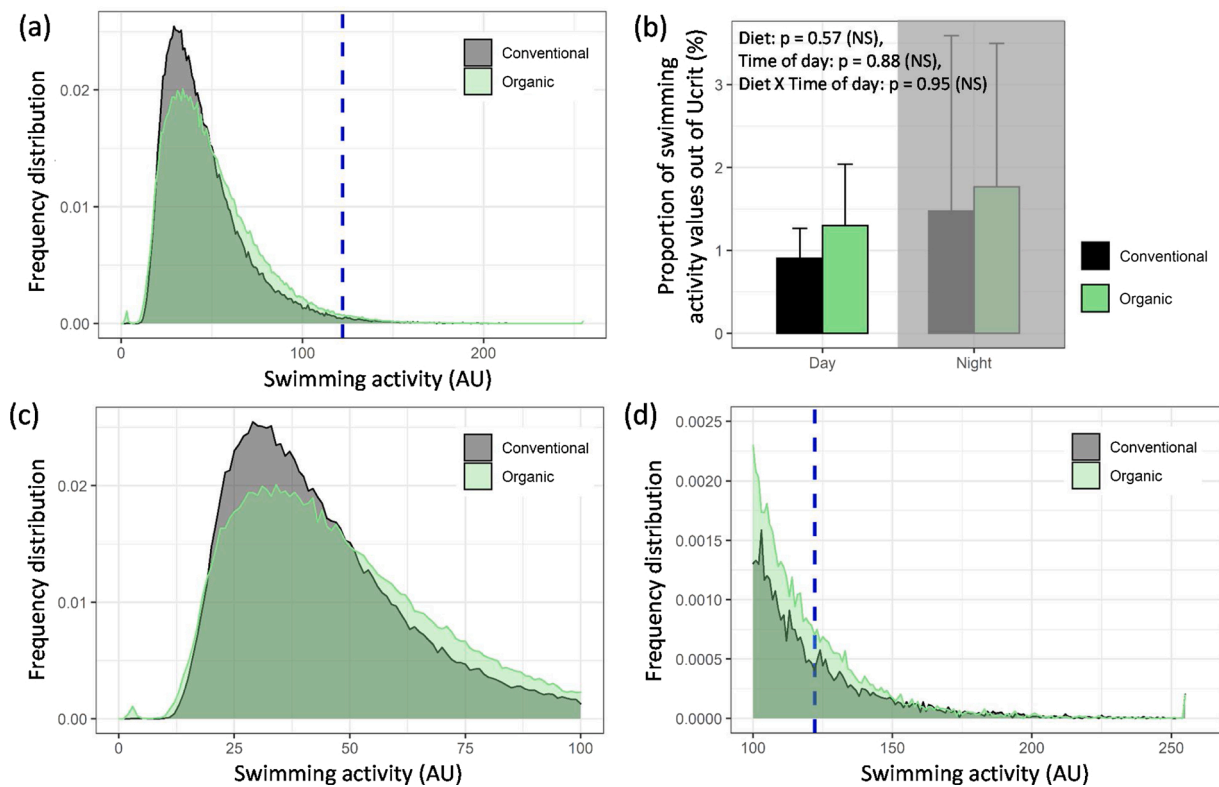
Fig. 3. (a) Frequency distribution of the swimming activity values (in arbitrary units; AU) as a function of conventional (black distribution; n = 8 fish) and organic (green distribution; n = 9 fish) diets. The dashed blue line corresponds to the swimming activity value at the U_{crit}. i.e., 122.1 AU). (b) Mean ± standard error of the percentage of swimming activity values above the U_{crit} as a function of the time of day (day- and night-time), diet and interaction of both factors. P values are indicated for each factor, as well as the significance. NS = not significant (GLMM; $p < 0.05$). Panels (c) and (d) are zooms to the frequency distribution of swimming activities values displayed in panel (a) from 0 to 100 for panel (c) and 100 to 260 AU for panel (d).

0.05; Fig. 3A, D). For high swimming activity values (>140 AU), no difference in data distribution has been observed between the two diet regimes ($p > 0.05$; Fig. 3D). Indeed, the recorded swimming activity values above the calibrated U_{crit} value (122.1 AU) represented a small proportion of all recorded values (approximately 1.4 %; Fig. 3A), and did not differ significantly between the two diet groups (GLMM, t = 0.57, $p = 0.57$), regardless of the time of day (GLMM, t = 0.15, $p = 0.88$) or interaction of both factors (GLMM, t = -0.07, $p = 0.95$; Fig. 3B).

3.4. Physiological parameters

No significant differences were observed between the two diet groups in any physiological indicators of stress and welfare at the end of the experiment (Fig. 4; $p > 0.05$ for all). However, the RBCC, lactate, Hb and lysozyme levels were significantly higher at the end than at the beginning of the experiment in both groups (Fig. 4; $p < 0.05$ for all), The HCT levels were significantly higher only in the organic diet group ($p < 0.05$).

4. Discussion

In this study, we evaluated some physiological indicators of health and welfare, swimming activity and growth performance of European sea bass under two different feed regimes (conventional and organic). Swimming activity was measured using acoustic transmitters previously calibrated with the swimming performances during U_{crit} trial, allowing better qualification of swimming activity during the experimental period according to the diet. In particular, due to a recent increase in fish production from organic aquaculture and the importance of fish welfare in this context, it is important to address this question in one of the most important farmed fish species of European marine aquaculture, the European sea bass.

In teleost fishes, cortisol is the main stress hormone end product of the HPI axis (Sadoul and Geffroy, 2019). It triggers physiological and behavioural responses, and is involved in other physiological functions, such as growth and reproduction (Sadoul and Vijayan, 2016; Schreck and Tort, 2016). In our experiment, neither cortisol levels nor levels of the secondary stress indicators (glucose, lactate and haematological parameters) were affected by either feeding regime. However, it is important to note that lactate decreased in both groups compared to the levels measured at the beginning of the experiment, whereas the haematocrit, haemoglobin and lysozyme concentrations and RBCC increased. Since the water temperature and photoperiod remained constant throughout the experimental period, these changes may be attributed to stress caused by the transfer of the fish to our facility. In line with the physiological data, the growth performance indicators (SGR, FCR and PER) during the experimental period were similar in the two groups, suggesting that the organic diet did not induce chronic stress, with long-term detrimental effects on growth (Sadoul and Vijayan, 2016).

It is well known that plasma stress physiological indicators, such as cortisol, glucose and lactate, and haematological parameters may be insufficient for a proper assessment of fish welfare under experimental conditions that may induce chronic stress (Ellis et al., 2012; Raposo de Magalhães et al., 2020; Sadoul and Geffroy, 2019). Moreover, rearing procedures, including feed, may change the energy demands (Attia et al., 2012; Carbonara et al., 2020b), which are, in turn, reflected in changes in swimming behaviour (Martins et al., 2012). Therefore, data from accelerometer tags, if appropriately calibrated, could be used as a measure of swimming behaviour in captivity and thus as an indicator of fish well-being (Carbonara et al., 2020a, 2019b, 2015; Zupa et al., 2021). For this reason, besides monitoring classical welfare indicators,
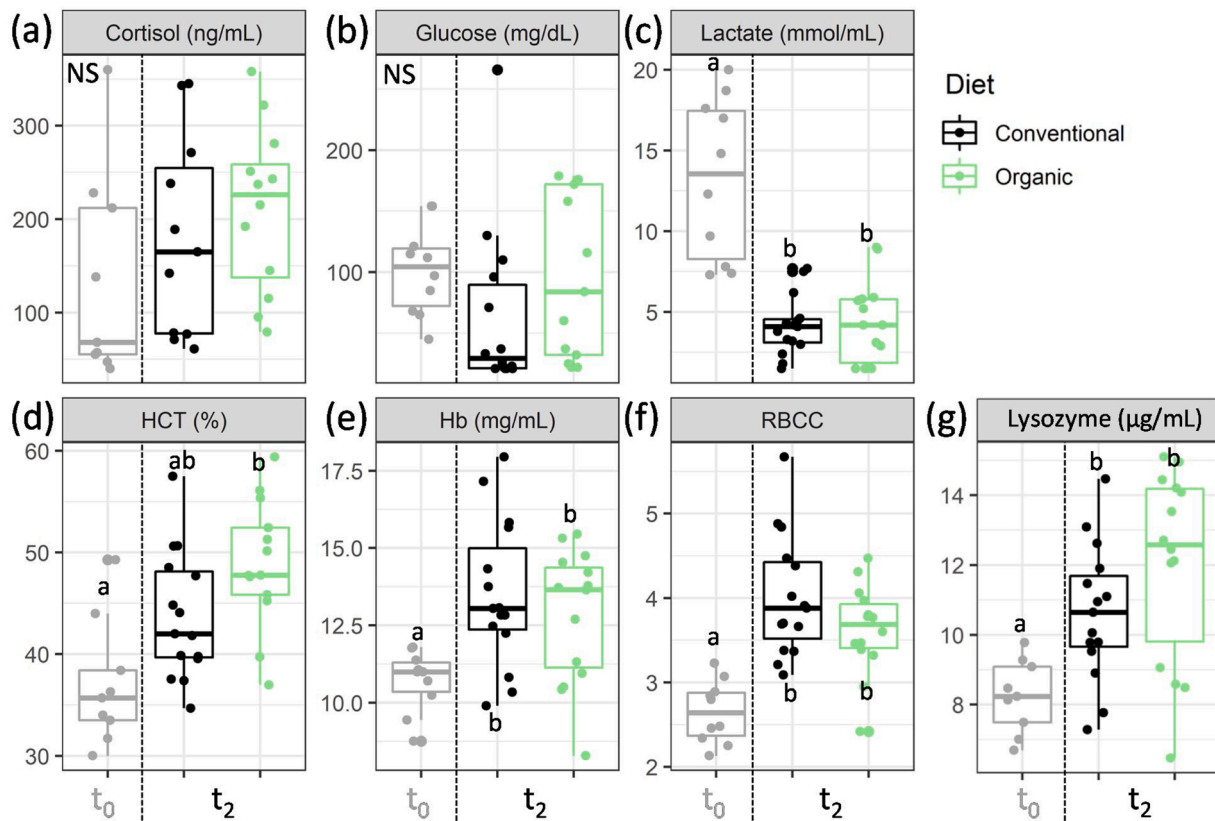
Fig. 4. Physiological parameters measured in blood samples at the start (t_0, grey; n = 9) and the end of the experiment (t_2) in the conventional (black; n = 15) and organic diet (green; n = 15) groups. (a) Cortisol (ng/mL), (b) Glucose (mg/dL), (c) Lactate (mmol/mL), (d) Haematocrit (HCT; %), (e) Haemoglobin (Hb; mg/mL), (f) Red blood cell count (RBCC; 10^6 cells/mm^3) and (g) Lysozyme (µg/mL). The central line in each boxplot indicates the median, and the boxes on either side represent the quartiles, with the whiskers covering 95 % of the values. All values are represented by coloured points. The letters indicate statistically significant differences between the groups (i.e., control at t_0, and conventional and organic at t_2). NS = not significant (ANOVA or Kruskal–Wallis test followed by Tukey's HSD post-hoc test; $p < 0.05$).

we also monitored the swimming activity of the fish using acoustic transmitters.

Calibrating the swimming activity recorded by acoustic transmitters with the water speed in swimming tunnel allowed us to determine swimming activity value corresponding to the critical swimming speed (the U_{crit}) of the fish. One could say that values obtained in swimming tunnel may be different from spontaneous activity, since swim tunnel conditions may differ from natural swimming conditions, and may cause abnormal behaviour or induce stress (Nelson et al., 2002). Nevertheless, it was previously reported that the range of activity levels achieved during the swim trials successfully spanned the range of activity exhibited by the fish in the holding tank in sea bass (Wright et al., 2014). In addition, we also observed that values of MO_2 in free-swimming fish estimated from the calibration of accelerometer tag signal in swimming tunnel were in the range of what has been measured in the swimming tunnel in rainbow trout (Zupa et al., 2021). Even these results are promising for using the calibration of signals from accelerometer tag in swimming tunnel, it has to be emphasized that forced swimming trials is not always representative of natural swimming conditions, and MO_2 can be overestimated, due do possible stress induced by such swimming conditions. Further studies have to elucidate in which way values can differ from forced to free-swimming conditions.

In this study, during the feeding trial, the European sea bass displayed a diurnal pattern of swimming activity regardless of diet, with the highest activity observed at the beginning of the day during feeding, but also in anticipation of feeding before sunrise (~2 h before feeding). This diurnal pattern has already been described in individuals of this species living in groups (Anras et al., 1997), although different activity patterns may be observed depending on environmental factors (Anras

et al., 1997; Helfman, 1978). However, in our study, the average swimming activity of the fish fed the organic diet was more intense than that of the fish fed the conventional diet during the daytime, while no differences were observed during the night-time.

In addition, the analysis of the frequency distribution of swimming activity values recorded by the tag in free-swimming fish, revealed that higher data frequency has been found for low swimming activity values (<21 AU) in fish fed organic diet. For medium values (from 21 to 50 AU), higher data frequency has been observed in fish fed conventional diet, while from medium/high value (from 51 to 120 AU), higher data frequency has been again recorded in fish fed organic diet than fish fed with conventional one. For high swimming activity values (>140 AU), no difference in data distribution has been observed between the two groups, suggesting near and above swimming activity values corresponding to the U_{crit} value (122.1 AU), no difference exist between fish fed two diets. Near and above the U_{crit} value, swimming activity is mainly fuelled by anaerobic metabolism in many species, including in sea bass (Zupa et al., 2015), which may affect the health and welfare of the fish if repeated or sustained for long time.

More intense swimming activity requires greater use of anaerobic metabolism, reducing the metabolic energy reserves available for growth, reproduction, or coping with stressful situations. In a previous study on the European sea bass, we observed that white muscle (i.e. anaerobic metabolism) activity starts at approximately 65 % of the U_{crit} value (corresponding to ~0.85 m/s or 2.7 BL/s) (Zupa et al., 2015). Indeed, before the U_{crit} was reached, the MO_2 usually levelled off or decreased slightly, and sea bass starts to use anaerobic metabolism to display a burst swimming mode (U_{max}; ~0.9 m/s) (Claireaux et al., 2006). Values of 0.85−0.9 m/s corresponds to swimming activity values

recorded by the tag of ~62−65 AU. In our experiment, we observed that fish fed the organic diet displayed higher frequency of values for this swimming activity range, but were on average similar between the two diets. As, explained above, more intense swimming activity, especially at this level, implies higher metabolic costs (Carbonara et al., 2015, 2006), which may affect growth or stress coping abilities. Nevertheless, in this study, we observed neither decrease in growth, or physiological disruptions in the organic diet group, which suggests that the metabolic costs are relatively low and/or compensated by the organic diet, provided that it is well balanced in terms of protein, lipid and amino acid contents (Carbonara et al., 2020b; Mente et al., 2019).

Calibrating the accelerometer tags also allowed us to estimate the amount of energy reserves (anaerobic metabolism) that the fish could use to cope with stress, which is crucial for animal welfare (Huntingford et al., 2006; Korte et al., 2007). Further, it allowed us to identify the acceleration values above the U_{crit}, providing information about events of intense swimming activity potentially related to stress during the experimental period. By isolating these values, we were able to determine whether the fish in the two diet groups faced a similar number of stress events. Even if, higher frequency of medium-high swimming activity value has been observed near the U_{crit} threshold, the fish in the organic diet group did not show a higher proportion of swimming activity values above the U_{crit}. This suggests that the more intense swimming activity observed in this group was not linked with increased anaerobic metabolism due to stress. The similar lactate levels in the two groups lend weight to this conclusion.

In terms of day/night patterns of swimming activity, the main difference between the two diet groups in this study was observed during the feeding period in the morning, while the swimming activity is overall similar the rest of the day. This suggests that the difference was probably due to more intense competition for organic feed. Overall, we also observed that the frequency of swimming activity values recorded by tag differed between the two diets but were on average similar between the two diets. That did not seem to affect sea bass health and welfare since the physiological indicators and biological performance were similar in the two diet groups throughout the experimental period.

In summary, two main conclusions can be drawn from this study's findings. First, the use of acoustic transmitters previously calibrated with physiological indicators, such as the U_{crit}, appears to be promising for real-time welfare monitoring in aquaculture. The precision of such calibrations of swimming activity may be enhanced by including other parameters, such as oxygen consumption and muscle activity, or other indicators such as U_{max} to better link the swimming performances with aerobic and anaerobic metabolism. Real-time monitoring of fish's behaviour and physiological state offers new possibilities for welfare monitoring in the aquaculture sector (e.g. Brijs et al., 2019, 2018; Gesto et al., 2020; Muñoz et al., 2020), especially with recent advancements in data transmission through acoustic instead of radio channels, which provides greater applicability on production scales (Halachmi et al., 2019). Second, based on all the indicators considered, well-balanced organic diet does not seem to negatively affect the health and welfare of the European sea bass, which suggests that organic aquaculture may address challenges of the sector without compromising fish welfare.

Author statement

Pierluigi Carbonara: Conceptualization, Methodology, Original draft preparation, Reviewing and Editing; **Sébastien Alfonso**: Data analysis, Writing- Original draft preparation, Reviewing and Editing. **Maria Dioguardi** Data curation, Investigation. **Walter Zupa**: Investigation, Data analysis, Reviewing and Editing. **Mirella Vazzana**: Data curation, Investigation. **Mariano Dara:** Data curation, Reviewing and Editing. **Maria Teresa Spedicato:** Conceptualization, Methodology. **Giuseppe Lembo**: Validation, Conceptualization. **Matteo Cammarata:** Conceptualization, Supervision.

Declaration of Competing Interest

Acknowledgements

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