

Are tagged fish like others? Insights from growth and stress physiological profile of two marine fish species

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Short communication

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Abstract

Background

Recently, telemetry applied to the aquatic organisms had a great development. Progressively, physiological sensors were used, as tools for fish welfare monitoring. However, it is important that tagging procedure does not disrupt fish physiology, behavior and performances to be used as a reliable non-invasive welfare indicator. In this communication, we share our mid-term data about stress physiological profile and growth performances following tag implantation in two important marine fish species of the European aquaculture, sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*).

Results

Mid-term blood samples post-tag implantation (46 days for sea bream and 95 days for sea bass) revealed no difference between tagged and untagged fish in the cortisol, glucose and lactate levels, suggesting that the tag implantation does not induce prolonged stress in these species. Moreover, the specific growth rate was similar for tagged and untagged fish in both species.

Conclusion

As a conclusion, the tag implantation does not induce mid-term consequences on the stress physiology and the growth performances of these two marine fish species under controlled environment. These observations first support accelerometer tags as useful tools for welfare monitoring in aquaculture condition because they do not affect the welfare and health of implanted fish. Secondly, this study shown that tagged fish can be sampled during experiments and be considered as a representative portion of the population, displaying similar growth and physiological parameters compared to untagged fish.

Introduction

During the last decades, telemetry applied to the aquatic organisms had a great development in term of tag miniaturization, battery life, software and hardware [1]. These tags are precious tools for the characterization and monitoring of behaviors in a wide range of organisms, including fishes [2]. In addition, electronics tag can be equipped with several kinds of environmental sensors (*e.g.* temperature, depth, salinity), while monitoring physiological parameters, such as heart and ventilation rates or muscle activity [3–6]. These physiological sensors were mainly used for monitoring in the wild in the context of conservation and ecology, but they were progressively transferred toward aquaculture, serving as welfare indicators in response to common stressors (*e.g.* slaughtering practices, water quality or stocking density) [4, 7–9].

Telemetry studies assume that the tagged fish is physiologically representative of the entire population. Thus, it is fundamental that the tag does not negatively influence growth performance, physiology state and/or survival. In this sense, the implantation method, the location size of the tag is important to prevent the disruption of normal movement, physiological state and growth performance of tagged fish [10–13], thus avoiding bias in the observations/data collected from electronics device implanted. Overall, the threshold limit generally considered acceptable is below 2% of the body weight of the fish in the air (so-called '2% rule') [10–12]. Nevertheless, in some cases, the '2% rule' is not enough to avoid some negative effects on buoyancy and swimming performance, or leads to negative consequences on the fish health and welfare (*e.g.* stress, inflammation, obstruction of internal organs) [10]. Most of our knowledge about the link between implantation of tag and stress are available for salmonids, therefore more specie specific insights are needed.

In this context, we collected data from two different experiments respectively performed in European sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), two of the most important species for the European aquaculture [14, 15], aiming to evaluate growth performances and the physiological stress profile of tagged fish at least 46 days *post*-surgical procedure. The physiological stress profile was assessed by the mean of measurement of plasma stress indicators (cortisol, glucose and lactate levels), while the growth was assessed by measuring the Specific Growth Rate (SGR) and compared with untagged fish.

Material And Methods

The experiments were performed in accordance with Italian national legislation (D. lgs. 26/2014) and EU recommendations (Directive 2010/63/EU), with the authorization of the Italian Ministry of Health number code 665/2016-PR.

Animals

Sea breams were obtained from the commercial hatchery Ittica Caldoli s.r.l. (Lesina, Italy). After 3 weeks of acclimation, fish (mean weight \pm SD: 314.6 \pm 49.1 g) were implanted with ID100 radio frequency identification (RFID) tags (Trovan, The Netherlands) and separated into three fiberglass tanks of 1.2 m³ (n= 115 fish per tank; 30 kg/m³), forming triplicates. Fish were reared in marine water at a constant temperature of 18 °C, a salinity of 35 PSU and pH of 7.1. Three complete water replacements per day were guaranteed, and the oxygen levels were continuously monitored by an automatic system programmed to keep the dissolved oxygen concentration higher than 5.0 \pm 1.0 ppm.

The European sea bass were obtained from the commercial hatchery Panittica Pugliese SpA (Torre Canne, Italy). After 3 weeks of acclimation, fish (mean weight \pm SD: 335.5 \pm 62.4 g) were implanted with RFID tags (ID100), and separated into three fiberglass tanks of 1.2 m³ (n= 35 per tank; 10 kg/m³), forming triplicates. Fish were left without disturbance during 2 months before the start of the experiment,

thereafter presented. Water parameters (temperature, salinity and oxygen) were constant and similar to those for sea breams.

During all the experimental duration, both sea bream and sea bass were reared with a constant photoperiod of 12L:12D and fish were fed 1% of the body mass using commercial food (Skretting Marine 3P, Italy) administered by automatic feeders for three hours in the morning.

Experimental procedure

At the beginning of experiment (t_0 ; **figure 1**), fish were gently caught from the rearing tank and anesthetized with a hydroalcoholic clove oil solution (with a 30 mg/L; [16,17]). Morphometric parameters (body weight and total lengths) were recorded to further evaluate the fish growth performance: specific growth rate (SGR, see "*Growth measurement and SGR calculation*" section).

Tag implantation

Eighteen days after the beginning of the experiment for sea bream and at the beginning of the experiment for sea bass ($d(\text{days})=18$ and $d=0$; see **figure 1**), $n=5$ and $n=9$ fish were randomly selected for sea bream and sea bass respectively and implanted with VEMCO V9AP acoustic accelerometer tag (AMIRIX Systems Inc., Nova Scotia, Canada), as described in Carbonara et al. [7] (at least $n=2$ fish per tank, except $n=1$ fish in one tank for the sea bream experiment). Briefly, for both species, fish were fasted 24 h before the implantation and were anaesthetized using 30 mg/L of a hydroalcoholic clove oil solution [16,17]. The transmitter was inserted into the body cavity through a 1.5 cm incision. The body cavity was then carefully closed using sutures and fish were treated with antibiotic injection (sodic-ampicillin–cloxacillin $1 \text{ mg/kg } 24 \text{ h}^{-1}$; [18]) before sending back to their home tank until further processing at t_1 . The tag represented $1.63 \pm 0.32 \%$ and $0.90 \pm 0.21 \%$ (mean \pm SD) of the sea bream and sea bass mass respectively. All operated fish recovered in few days and no mortality linked to the surgical operation was observed for both species [7]. For evaluating the possible tag effects, $n=12$ untagged sea bream and $n=9$ untagged sea bass were randomly chosen from the group to serve as control (at least $n=3$ per tank; **Table 1**) and followed during the experimental duration.

Table 1. Sample size and mass (g; mean \pm SD) of tagged and untagged fish for sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) used in the present study at both t_0 and t_1 .

Species	Treatment	N	Mass at t_0 (g)	Mass at t_1 (g)
<i>Sparus aurata</i>	Untagged	12	309.4 ± 65.3	389.5 ± 90.8
	Tagged	5	312.6 ± 48.2	407.8 ± 52.4
<i>Dicentrarchus labrax</i>	Untagged	9	425 ± 76.4	479.22 ± 71.4
	Tagged	9	423.8 ± 80.7	466.9 ± 79.5

Growth measurement and SGR calculation

At the end of the experiment, (t_1 ; *i.e.* $d=46$ and $d=95$ days after marking for sea bream and sea bass respectively; see **figure 1**), tagged and untagged fish were gently caught once again from their rearing tank and anesthetized with clove oil solution as already described above. The body weight (g) was measured for evaluating the Specific Growth Rate (SGR) between the end (t_1) and the beginning of the experiment (t_0). For both sea bream and sea bass, the SGR was calculated according to the following equation [19]:

$$SGR = 100 * (\ln W_{t_1} - \ln W_{t_0}) * T^{-1}$$

where W is the total weight of the fish (g) respectively at the end of the experiment t_1 (W_{t_1}) and the beginning of the experiment t_0 (W_{t_0}) respectively, and T is the number of feeding days between these two dates of the experiment.

Blood sampling and stress indicators analysis

After the morphometric measurements (~ after 2-3 minutes in anesthetic), a blood sample of 0.5 mL was immediately taken from the first branchial arch of the tagged and untagged fish using a heparinized syringe. Blood was then centrifuged (15,000 g for 3 min), and plasma was collected and stocked at -20°C until further processing, described below.

For both species, the cortisol, glucose and lactate plasmatic concentrations were measured as described in Carbonara et al. [7]. Briefly, the plasma cortisol concentration was determined using a Cortisol II kit (cobas®) based on a solid-phase, competitive chemiluminescent enzyme immunoassay. Plasma glucose and lactate concentrations were determined using a commercial kit 17630H and 17285 for glucose and lactate respectively (Sentinel, Italy) based on the enzymatic colorimetric Trinder reactions (GOD/PAP for glucose and PAP for lactate).

Statistical analysis

Statistical analyses were carried out using the R software version 3.6.2 [20] at the level of significance of 95%. Homoscedasticity of the data was *a priori* tested using a Shapiro test and then the appropriate statistical test was performed to compare the SGR and the physiological stress indicators (cortisol, glucose and lactate) between tagged and untagged fish (*i.e.* either Wilcoxon or t-test) for each species.

Results

From the growth performances point of view, the SGR was similar between tagged and untagged fish for both sea bream ($W = 38$, $p = 0.44$) and sea bass ($t = -0.58$, $p = 0.56$; Fig. 2) between the two monitored dates, which is corresponding to a period of 64 days for sea bream and 95 days for sea bass.

At the end of the experiment (t_1), *i.e.* 46 and 95 days after the tag implantation in sea bream and sea bass respectively, the plasma concentration of stress indicators was overall similar between tagged and untagged fish regardless of the species (Fig. 3). In more details, the plasma cortisol concentration was not different between tagged and untagged fish in sea bream ($W = 32$, $p = 0.88$) and sea bass ($t = 0.94$, $p = 0.36$; Fig. 3.A). Also, the levels of the secondary stress indicators (*i.e.* glucose and lactate) resulted similar between the tagged and untagged sea bream ($W = 25.5$, $p = 0.67$ and $t = 1.04$, $p = 0.33$ for glucose and lactate respectively) and sea bass ($W = 39$, $p = 0.93$ and $t = 1.18$, $p = 0.26$ for glucose and lactate respectively; Fig. 3.A,B).

Discussion

In our experiments, we observed that after a relatively long period following surgical implantation of accelerometers tags (*i.e.* 46 for sea bream and 95 days for sea bass), tagged fish were comparable with untagged fish in aquaculture conditions, both in term of growth and stress physiology. As far we know, it is the first time this is reported for these two important species for European marine aquaculture, and this may also be important for supporting the use of the physiological tags for these species in aquaculture conditions.

Surgical implantation of accelerometers tag is generally perceived as a stressor for fishes, inducing a cortisol release into the blood [21], which is the major stress hormone in teleost fish [22]. Overall, this is a relatively acute response for the organism which is coping with stressors before regaining homeostasis, but this condition may last few days depending on the species. For instance, in rainbow trout (*Oncorhynchus mykiss*), the surgery and implantation of a heart-beat rate sensor induced an increase of the heart rate during the 72 h following the procedure before to be stabilized [23], suggesting that fish regain homeostasis relatively quickly after this stressful event. Same observations were done in Chinook salmon by Jepsen et al. [21], where physiological stress indicators were higher up to 24 h following the tag implantation but were comparable with untagged fish at least 7 days following the tag implantation. In our experiments, after 46 days for bream and 95 days for sea bass following the tag implantation, the levels of all stress indicators monitored (cortisol, glucose and lactate) were found to be similar to untagged fish, and consistent with the literature for these species [7, 24]. Our results support the fact that the implantation of a tag does not induce chronic stress in sea bream and sea bass, as already observed for various fish species [21, 25]. It is important to empathize that tag implantation does not induce long term consequences for a high-stress responder species, such as European sea bass [26–28].

Nonetheless, in our experiments, even if the acute stress response to tag implantation was not directly investigated by measuring physiological stress indicators, we observed that tagged fish generally did not eat 2–4 days following the surgical procedure (personal observations), probably because of stress state induced by surgery. Indeed, stress and growth are closely related; stress being generally known to inhibit food intake and so the energy acquired by fish for biological processes, including growth [29]. Therefore, we can suggest that the acute stress, resulting from the tag implantation, lasts only a few days for these species. However, this period without food intake for the tagged has no long-term consequences on

growth, since we recorded similar SGR between tag and untagged fish for both species. It was demonstrated in different fish species that when the “2% rule” is followed, the growth performances of fish are generally not impacted [12, 21, 30]. The similar growth rate between tagged and untagged fish over the experimental period can be explained by compensatory growth, which is a phase of unusually rapid growth following a period of undernutrition [31]. It is important to emphasize that we observed similar growth performances between tagged and untagged fish in two different stocking density (*i.e.* 10 kg/m³ for sea bass and 30 kg/m³ for sea bream), suggesting that tagged fish can compensate growth and continue their normal life under different rearing conditions.

As a conclusion, we observed that the surgical implantation of accelerometer tags does not trigger mid-term changes of stress physiological profile and growth of both sea bream and sea bass reared under a controlled environment. Future studies are needed to investigate precisely how long these species take to recover from stress-induced by tag implantation, and thus can be considered equivalent as “normal” fish, displaying normal behavior (*e.g.* feeding) and basal level of stress indicators. This short communication supports the fact that (i) accelerometer tags are useful tools for welfare monitoring, because they do not affect welfare and health of implanted fish and that (ii) tagged fish can be sampled during experiments and be considered as representative of the population, by displaying similar growth and physiological parameters compared to untagged fish.

Declarations

Ethics approval and consent to participate

The experiment in sea bream was performed in accordance with Italian national legislation (D. lgs. 26/2014) and EU recommendations (Directive 2010/63/EU), with the authorization of Health Ministry number code 665/2016-PR. The experiments in sea bass were performed in add date in accordance with the European Commission recommendations 2007/526/EU C2007 2525. For both experiments, all fish recovered well from the tag implantation and no mortality due to the marking procedure was observed.

Consent for publication

Not applicable

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

WZ and PC carried out the implantation of tags in both species. PC, WZ, EF, AM, MDa, MDi, MC and PL carried out the blood samplings and the analyzes of the physiological parameters. SA carried out the statistical analyzes and the draw of the figures. PC and SA both wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

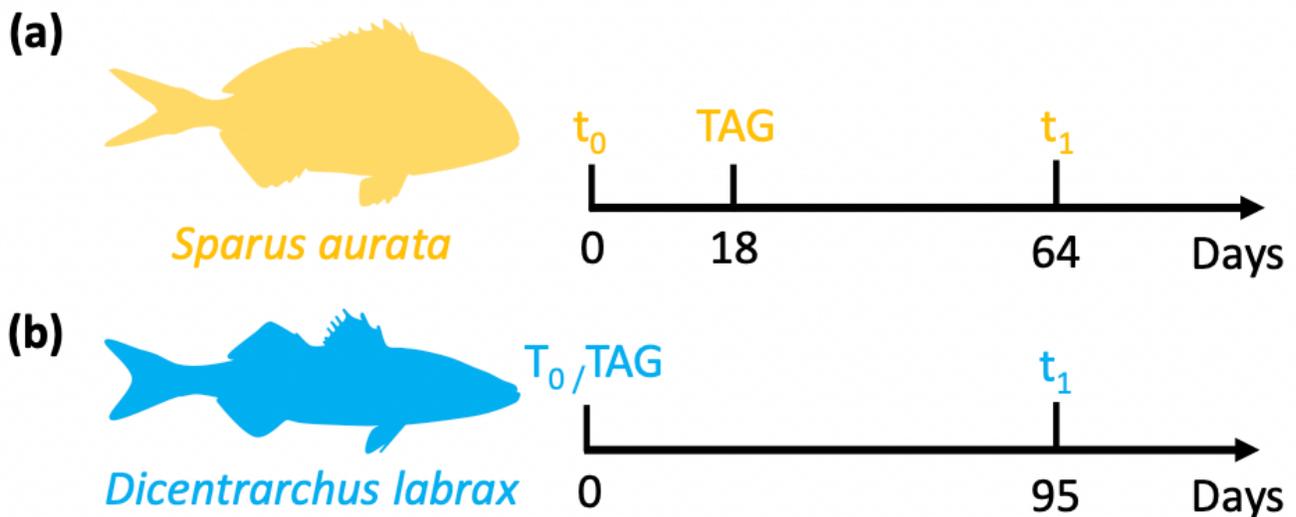


Figure 1

Time course schedule (days) of the experimental procedure for Sea bream (*Sparus aurata*; yellow) and European sea bass (*Dicentrarchus labrax*; blue). T0 and t1 represent the beginning and the end of the experiment, corresponding to the first and final measurement for SGR calculation. TAG represents the period of implantation of accelerometers tag.

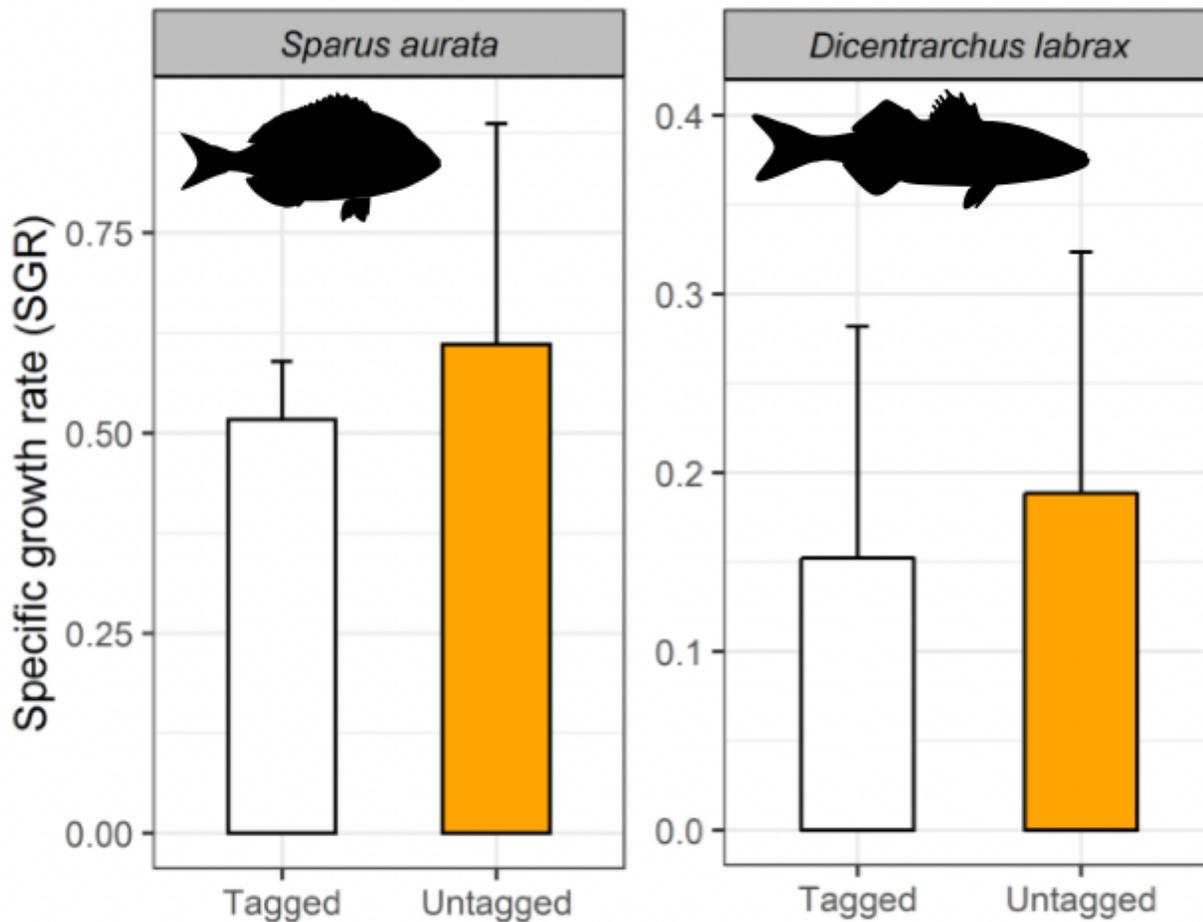


Figure 2

Specific growth rate (SGR) of untagged (white bars; n=12 sea bream and n=9 European sea bass) and tagged fish (orange bars; n=5 Sea bream and n=9 European sea bass). Values are mean \pm SD. See main text for statistics.

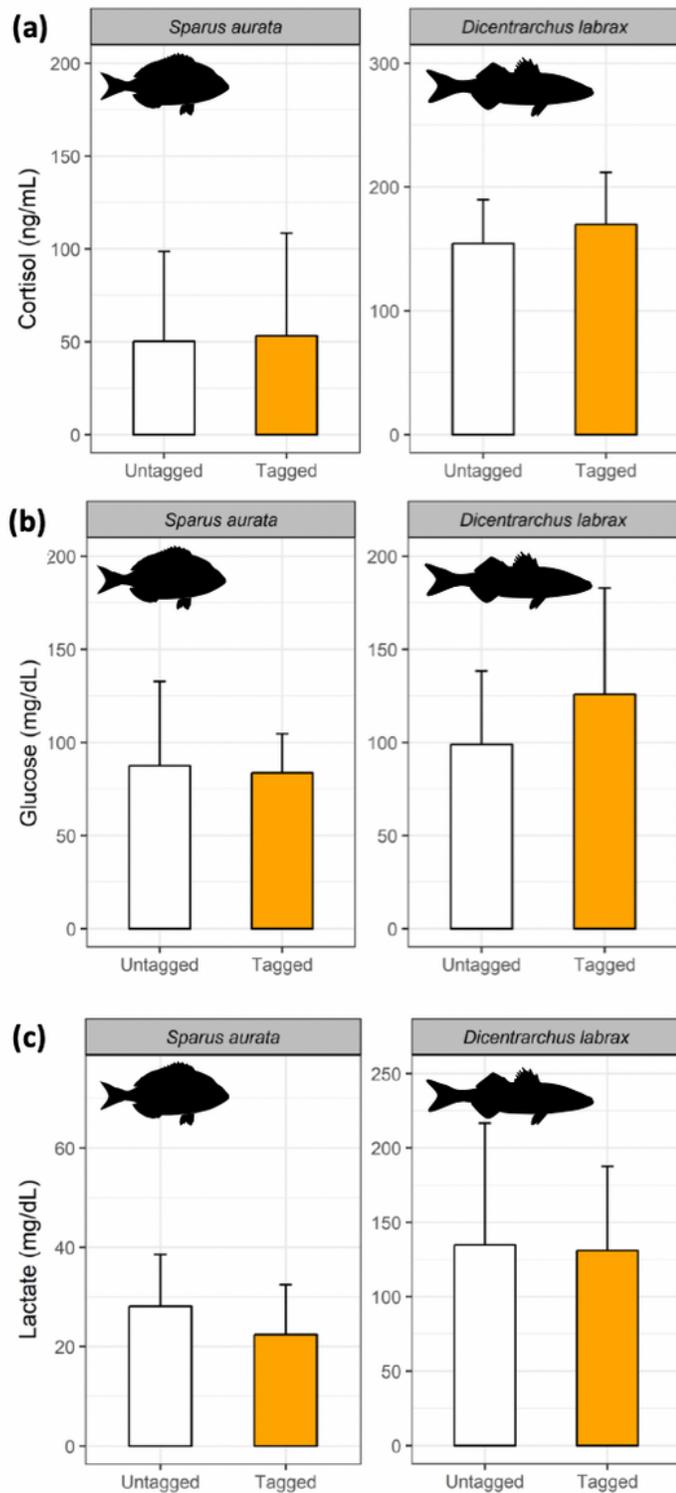


Figure 3

Stress physiological profile of untagged (white bars; n=12 sea bream and n=9 European sea bass) and tagged fish (orange bars; n=5 sea bream and n=9 European sea bass) at t1. (A) Cortisol (ng/mL), (B) Glucose (mg/dL) and (C) Lactate (mg /L). Values are mean \pm SD. See main text for statistics.