Surgical implantation of electronic tags does not induce medium-term effect. Insights from growth and stress physiological profile in two marine fish species

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Short communication

Abstract

Background: Telemetry applied to aquatic organisms has recently developed greatly. Physiological sensors have been increasingly used as tools for fish welfare monitoring. However, for the technology to be used as a reliable welfare indicator, it is important that the tagging procedure does not disrupt fish physiology, behaviour and performance. In this communication, we share our medium-term data on stress physiological profile and growth performance after surgical tag implantation in two important marine fish species for European aquaculture, the sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*).

Results: Blood samples after surgical tag implantation (46 days for the sea bream and 95 days for the sea bass) revealed no differences between tagged and untagged fish in cortisol, glucose and lactate levels, suggesting that the tag implantation does not induce prolonged stress in these species. Moreover, the specific growth rates were similar in the tagged and untagged fish of both species.

Conclusion: Surgical tag implantation does not have medium-term consequences for the stress physiology and growth performance of these two marine fish species in a controlled environment. These observations support the use of accelerometer tags as valuable tools for welfare monitoring in aquaculture conditions. This study also shows that tagged fish can be sampled during experiments and considered a representative portion of the population, as they display growth and physiological parameters comparable to those of untagged fish.

Introduction

Over the past decades, telemetry applied to aquatic organisms has greatly developed in terms of tag miniaturization, battery life, software and hardware [1]. These tags are precious tools for the characterization and monitoring of behaviour in a wide range of organisms, including fish [2]. Moreover, electronic tags can also be equipped with environmental sensors that can record diverse data, such as temperature, depth and salinity, while monitoring physiological parameters, such as heart and ventilation rates or muscle activity [3–6]. Although these physiological sensors have mainly been used in the wild in the context of conservation and ecology, they have progressively been employed in aquaculture, serving as welfare indicators of common stressors (e.g. slaughtering practices, water quality and stocking density) [4,7–9].

Telemetry studies assume that tagged fish are physiologically representative of the entire population. Therefore, it is essential that the tag does not negatively affect growth performance, physiology and survival. The implantation method and site and the tag's size are important factors for preventing the disruption of the physiological state, normal movement, and growth performance of tagged fish [10–13] and avoiding bias in the collected data. The maximum tag weight generally considered acceptable is no more than 2% of the fish's body weight in air (the so-called "2% rule") [10,11]. However, in some cases, the "2% rule" is not enough to avoid negative effects on the fish's health and welfare, such as stress,

inflammation or obstruction of internal organs, or on its buoyancy and swimming performance [10,14]. In particular, stress is considered as "a condition induced by a factor (a stressor) that evokes an endocrine response (e.g. cortisol release) that could be beneficial as well as disadvantageous" [15]. Thus, due to many factors listed above, surgical implantation of electronic tag may induce stress for fishes. Most of our knowledge about the link between surgical implantation of electronic tag and stress is mainly based on salmonids [14,16,17]; therefore, more species-specific information is needed.

In this study, we collected data from two different experiments, on the European sea bass (*Dicentrarchus labrax*) and the sea bream (*Sparus aurata*), two of the most important species for European aquaculture [18,19], aiming to evaluate growth performance and the physiological stress profile of tagged fish at least 46 days after intraperitoneal surgical implantation. Their physiological stress profile was assessed by comparing the means of plasma stress indicator values (cortisol, glucose and lactate levels) with those of untagged fish, while growth was assessed by comparing the specific growth rates (SGR) between tagged and untagged fish.

Methods

Animals

Sea breams (mean weight ± SD: 314.6 ± 49.1 g) were obtained from the commercial hatchery Ittica Caldoli (Lesina, Italy). After three weeks of acclimation, ID100 radio frequency identification (RFID) tags (Trovan, Netherlands) were implanted in the fish, which were then separated into three fiberglass tanks of 1.2 m^3 (n= 115 fish per tank; ~30 kg/m^3), forming triplicates. The implantation of pit-tag was performed under anaesthesia conditions (hydroalcoholic clove oil solution; 30 mg/L) under the skin in the region near the first dorsal fin. The fish were reared in marine water at a constant temperature of 18 °C, salinity of 35 PSU and a pH of 7.1. The water was completely replaced three times a day, and the oxygen levels were continuously monitored by an automatic system programmed to maintain the dissolved oxygen concentration above 5 ± 1 ppm.

European sea bass fish (mean weight ± SD: 335.5 ± 62.4 g) were obtained from the commercial hatchery Panittica Pugliese SpA (Torre Canne, Italy). After three weeks of acclimation, RFID tags (ID100) were implanted in the fish, which were then separated into three fiberglass tanks of 1.2 m^3 (n= 35 fish per tank; ~10 kg/m^3), forming triplicates. The implantation of pit-tag in sea bass was performed under similar conditions (anaesthesia and area of implantation) as for sea bream. The fish were left undisturbed for two months before the start of the experiment. The water parameters (temperature, salinity and oxygen) were constant and similar to those for the sea breams.

Throughout the experimental period, all fish were exposed to a 12L:12D photoperiod and were fed 1% of their body mass using commercial feed (Skretting Marine 3P, Italy) dispensed by automatic feeders for 3 h every morning.

Experimental procedure

At the beginning of the experiment (t$_0$; Fig. 1), the fish were gently removed from their rearing tanks and anaesthetized with a hydroalcoholic clove oil solution (30 mg/L) [16, 17]. Morphometric parameters (body weight and total length) were recorded to calculate the SGR (see the "*Growth measurements and SGR calculations*" section).

Tag implantation

At the beginning of the experiment (Day 0) for sea bass and 18 days later for sea breams (Day 18) (Fig. 1), V9AP acoustic accelerometer tags (Vemco Systems Inc., Nova Scotia, Canada) were implanted in nine randomly selected sea bass and five randomly selected sea breams (at least two fish from each tank, except one fish from one tank for the sea bream experiment), as described in Carbonara et al. [7]. Briefly, the fish were subjected to fasting for 24 h before implantation and were anaesthetized using a hydroalcoholic clove oil solution in doses of 30 mg/L [20,21]. The transmitter was inserted into the body cavity through a 1.5-cm incision. The incision was then carefully sutured, and the fish were injected with antibiotic (sodic ampicillin−cloxacillin; 1 mg/kg 24 h^{-1}) [22] before being returned to their home tanks until the end of the experiment (t$_1$; Fig. 1). The mean tag weight in air accounted for 1.63% ± 0.32 and 0.90% ± 0.21 of the sea bream and sea bass body mass, respectively. All tagged fish recovered within a few days, and no mortality linked to the surgical procedure was observed [7]. To evaluate possible tag effects, 12 untagged sea breams and 9 untagged sea bass were randomly selected as controls (at least three fish per tank; Table 1) and were monitored during the experimental period.

Table 1. Sample sizes and mean masses of tagged and untagged sea breams and European sea bass

Species	Status	N	Mass at t$_0$ (g)	Mass at t$_1$ (g)
Sea bream (*Sparus aurata*)	Tagged	5	312.6 ± 48.2	407.8 ± 52.4
	Untagged	12	309.4 ± 65.3	389.5 ± 90.8
European sea bass (*Dicentrarchus labrax*)	Tagged	9	423.8 ± 80.7	466.9 ± 79.5
	Untagged	9	425 ± 76.4	479.2 ± 71.4

t$_0$: beginning of experiment; t$_1$: end of experiment

Growth measurements and SGR calculations

At t$_1$ (Days 46 and 95 after tagging the sea breams and sea bass, respectively; Fig. 1), the tagged and untagged fish were once again gently removed from their rearing tanks and anaesthetized with clove oil solution as described above. Their body weight was measured (in grams) to calculate the differences in SGR between t$_0$ and t$_1$. The SGR was calculated according to the following equation [23]: (see Equation 1 in the Supplementary Files)

where *W* is the total weight at the end (*t$_1$*) and the beginning of the experiment (*t$_0$*), and *T* is the number of feeding days between *t$_0$* and *t$_1$*.

Blood sampling and stress indicator analysis

After the morphometric measurements (2–3 minutes after anaesthesia inducement), blood samples of 0.5 mL were immediately taken from the first branchial arch of the tagged and untagged fish using a heparinized syringe. The samples were then centrifuged at 15,000 g for 3 min, and plasma was collected and stored at −20 °C until further processing, described below.

The plasmatic cortisol, glucose and lactate concentrations were measured as described in Carbonara et al. [7]. Briefly, the cortisol concentration was determined using solid-phase competitive chemiluminescent enzyme immunoassays with a cobas Cortisol II kit (Roche, Switzerland). The glucose and lactate concentrations were determined using kits 17630H and 17285 (Sentinel Diagnostics, Italy), respectively, based on the enzymatic colorimetric Trinder reaction (GOD/PAP for glucose and PAP for lactate).

Statistical analysis

Statistical analyses were performed using the R software version 3.6.2 [24] at a 95% level of significance. Homoscedasticity of the data was a priori tested using the Shapiro-Wilk test. The appropriate statistical test (either the Wilcoxon test or the *t*-test) was then performed to compare the SGRs and physiological stress indicators (cortisol, glucose and lactate) between the tagged and untagged fish of each species.

Results

In terms of growth performance, the SGR was similar between the tagged and untagged fish for both the sea bream (W = 38, p = 0.44) and the sea bass (t = −0.58, p = 0.56; Fig. 2) between t_0 and t_1, which correspond to a period of 64 days for the sea breams and 95 days for the sea bass.

At t_1, the plasma concentrations of stress indicators were overall similar between the tagged and untagged fish of both species (Fig. 3). More specifically, the plasma cortisol concentration showed no statistically significant differences either in the sea breams (W = 32, p = 0.88) or in the sea bass (t = 0.94, p = 0.36; Fig. 3a). The levels of the secondary stress indicators (i.e. glucose and lactate) were also similar both in the sea breams (W = 25.5, p = 0.67 for glucose and t = 1.04, p = 0.33 for lactate) and in the sea bass (W = 39, p = 0.93 for glucose and t = 1.18, p = 0.26 for lactate; Figs. 3a, 3b).

Discussion

Our results show that after a relatively long period (46 days for the sea bream and 95 days for the sea bass) following surgical implantation of accelerometer tags, the tagged fish were comparable with the untagged fish in terms of both growth and stress physiology in aquaculture conditions. To our knowledge, this is the first report concerning stress physiological indicators for the sea bream and the European sea bass, two important species for European marine aquaculture. These findings support the use of accelerometer tags in these two species in aquaculture conditions.

Surgical implantation of accelerometer tags is perceived as a stressor for fish, causing cortisol release into the blood [25], which is the main stress hormone in teleost fishes [26]. It is a relatively acute response of organisms coping with stressors before regaining homeostasis, but it may last only a few days, depending on the species. For instance, in rainbow trout (*Oncorhynchus mykiss*), a heart rate increase was observed during the first 72 h following surgical implantation of a heart rate sensor, after which it was stabilized [27], suggesting that fish regain homeostasis relatively quickly after this stressful event. Jepsen et al. [25] reported similar observations in Chinook salmon, where physiological stress indicators were higher up to 24 h following tag implantation but were comparable with those of untagged fish at most seven days later. In our experiments, 46 and 95 days after tag implantation in sea breams and sea bass, respectively, the levels of all monitored stress indicators (cortisol, glucose and lactate) were found to be similar to those of untagged fish and consistent with the levels reported in the literature regarding these species [7,28]. Our results confirm that tag implantation does not induce chronic stress in either the sea bream or the sea bass, as observed in various other fish species [25,29]. It is thus important to emphasize that tag implantation does not exert long-term adverse effects on a high-stress responder species such as the European sea bass [30–32].

Nonetheless, although we did not directly investigate the acute stress response to tag implantation by measuring physiological stress indicators after the surgical procedure, we did observe that generally, the tagged fish did not eat for two to four days post-operatively (personal observations), probably because of surgery-induced stress. Indeed, stress and growth are closely related; stress is known to inhibit food intake and, consequently, limit the energy available for biological processes, including growth [33]. Therefore, it appears that acute stress is indeed induced by tag implantation, but it only lasts a few days in these species. Moreover, this period of no food intake has no long-term consequences on growth, as shown by the similar SGRs between the tag and untagged fish of both species. It has been demonstrated in different fish species that when the "2% rule" is applied, growth performance is generally not impacted [11,25,34]. The similar growth rates between tagged and untagged fish can be explained by compensatory growth, which is a period of unusually rapid growth following a period of undernutrition [35]. It is noteworthy that we observed similar growth rates between the tagged and untagged fish in two different stocking densities (~10 kg/m^3 for the sea bass and ~30 kg/m^3 for the sea bream), which suggests that tagged fish can compensate growth and continue their normal life under different rearing conditions.

Conclusion

In conclusion, surgical implantation of accelerometer tags does not cause medium-term changes in the stress physiological profile and growth of either sea breams or sea bass reared in a controlled environment. Future studies are needed to investigate exactly how long these species take to recover from stress induced by tag implantation and thus be considered "normal" fish, displaying normal behaviour (e.g. feeding) and basal levels of stress indicators. Our study confirms (i) that the implanting process of accelerometer tags does not affect the basic growth and stress physiological indicators of

tagged fish and (ii) that tagged fish can be sampled 46 or 95 days post-surgery for sea bream and seabass respectively during experiments and considered representative of the population, as they display growth and physiological parameters comparable to those of untagged fish.

Declarations

Ethics approval and consent to participate

The sea bream experiment was performed in accordance with Italian national legislation (Legislative Decree 26/2014) and EU Directive 2010/63/EU with authorization from the Italian Ministry of Health (No. 665/2016-PR). The sea bass experiment was performed in add date of Italian national legislation, however the experiment was done in accordance with European Commission recommendation 2007/526/EC C(2007) 2525 and EU Directive 2010/63/EU. In both experiments, all fish recovered fully from the tag implantation procedures, and no associated mortality was observed.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

WZ and PC performed the tag implantations in both species. PC, WZ, EF, AM, MDa, MDi, MC and PL performed the blood sampling and the analysis of physiological parameters. SA performed the statistical analyses and prepared the figures. PC and SA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Figures
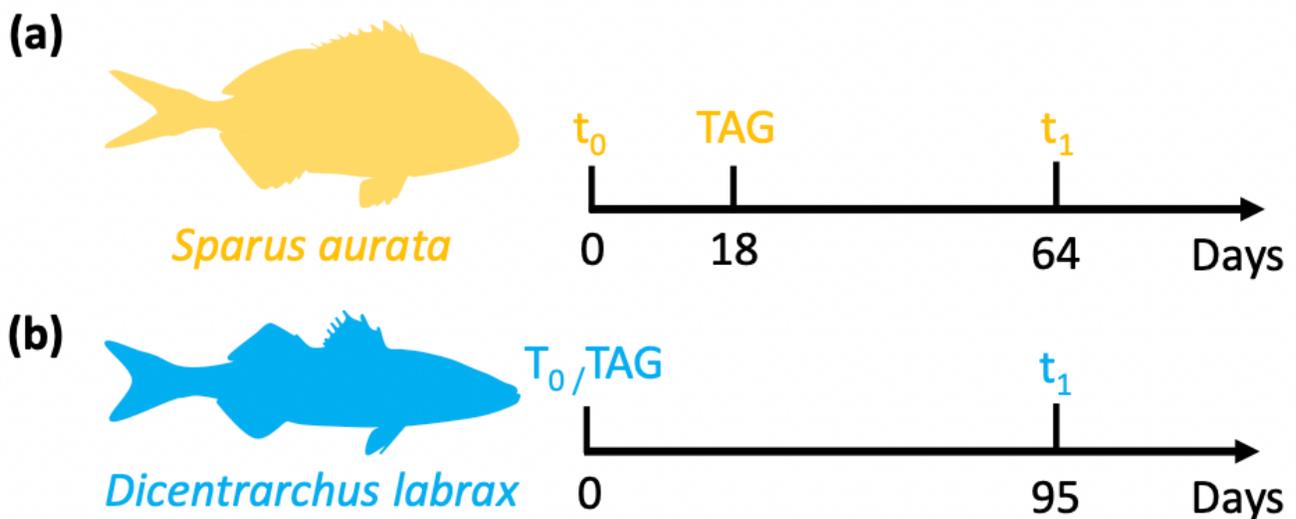
Figure 1

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.
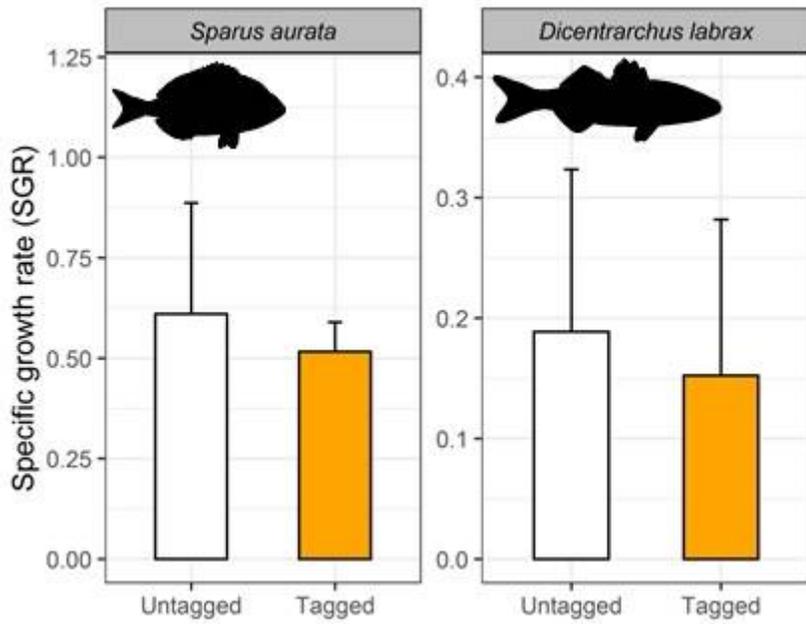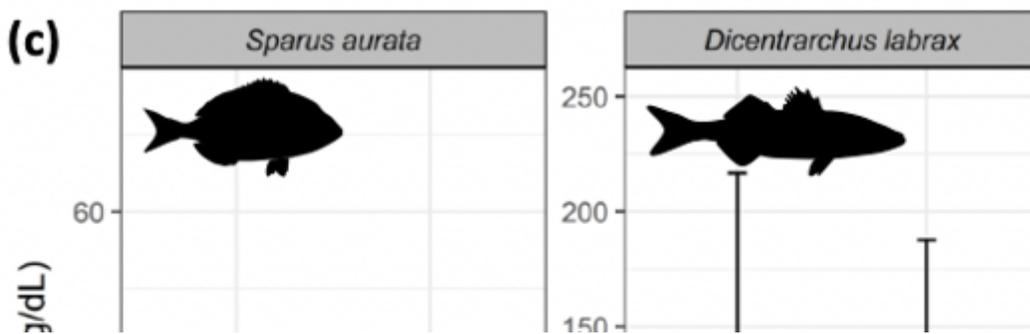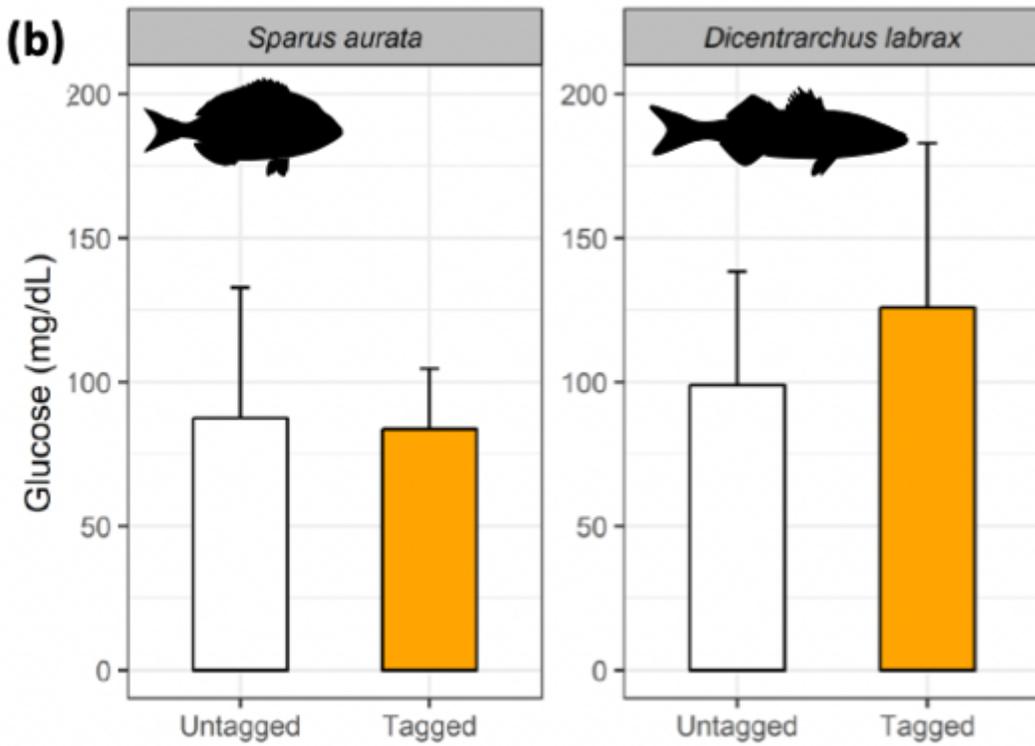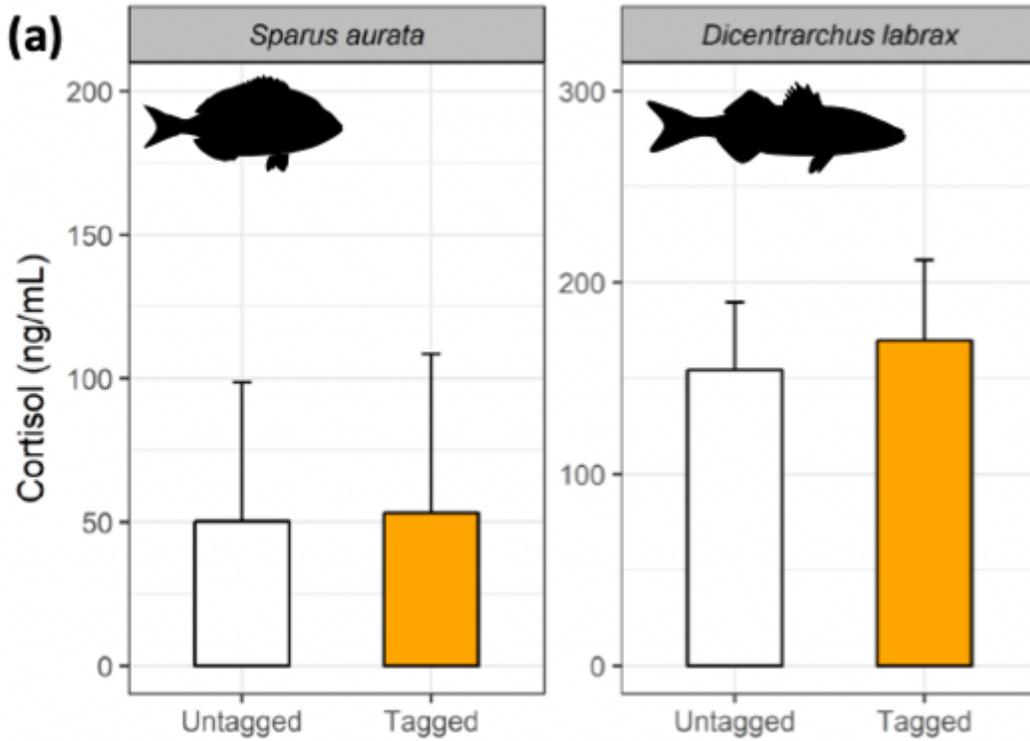
Figure 2

Specific growth rate (SGR; mean ± SD) 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) in Sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax). See main text for statistics.
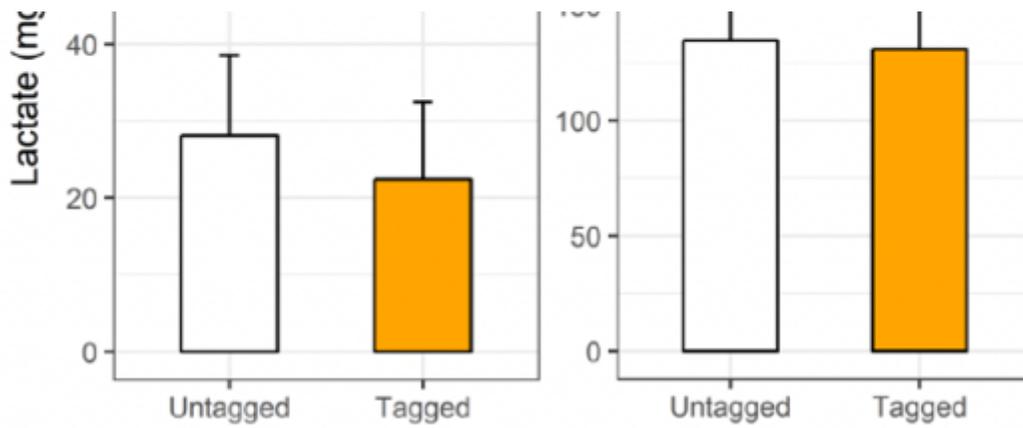
Figure 3

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 ± SD. See main text for statistics.

Supplementary Files

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- Equation1.pdf