**EFFECTS OF CLOVE OIL, ESSENTIAL OIL OF *Lippia alba* AND 2-PHE
ANAESTHESIA ON JUVENILE MEAGRES (*Argyrosomus regius* Asso 1801)**

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Summary

The objectives of this experiment were i) to determine the efficacy of essential oils of clove (CO) and *Lippia alba* (EOLA) to induce deep anaesthesia in juvenile specimens (49.0 ± 6.2 g body mass, 16.6 ± 0.8 cm; $n = 8$ per treatment) of meagre (*Argyrosomus regius*), and ii) to study the feasibility of these substances, together with 2-phenoxyethanol (2-PHE), as potential sedatives (low concentration: i) EOLA: 12 mg L^{-1} ; ii) CO: 1 mg L^{-1} ; and iii) 2-PHE: 33 mg L^{-1} ; $n = 8$ per treatment) for live fish transport on this species. All test were performed at constant temperature (18°C). Thus, we evaluated the main primary stress indicator (plasma cortisol) and secondary factors (plasma metabolites). In addition, growth hormone (GH) mRNA expression was also evaluated in the pituitary gland. The results indicated that EOLA is considered effective for deep anaesthesia when its concentration is close to 160 mg L^{-1} , while CO produces the same effect when lower concentrations are added ($40\text{-}50 \text{ mg L}^{-1}$). Regarding sedative concentrations, a significant ~ 3 -fold increase in plasma cortisol levels was detected in EOLA group when compared to control specimens. In addition, glucose levels were not reduced and significantly increased (~ 1.6 -fold) for 2-PHE in relation with control fish. None of the anaesthetics promoted a significant difference for GH expression respect to the control group, but a significant ~ 2 -fold increase for 2-PHE treatment with respect to EOLA exposition was found in this gene expression. Our results shown that none of the anaesthetics analysed, at least between the ranges of concentrations used in this study (EOLA 12 mg L^{-1} , CO 1 mg L^{-1} , 2-PHE 33 mg L^{-1}), are recommended for live fish transport, as shown by the absence of inhibition on the stress parameters assessed.

1 **Introduction**

2 In aquaculture, handling and transfer operations are frequently performed using
3 anaesthetics to avoid or diminish stress system activation, which can induce negative
4 effects on fish performance and survival (Cho and Heath 2000). The activation of the
5 stress system in fish induces a primary response increasing plasma catecholamine and
6 cortisol levels. In addition, as secondary responses, modifications in metabolites
7 (glucose, lactate, proteins, triglycerides, etc.) and osmotic balance appear, involving
8 changes in the physiology of the specimen (Wendelaar Bonga 1997; Mommsen et al.
9 1999; Barton 2002; Iwama et al. 2004).

10
11 Generally chronic stress should not cause death of the fish but it may negatively affect
12 physiological processes such as growth, reproduction, osmoregulation and immune
13 capabilities (Barton and Iwama 1991; Wendelaar Bonga 1997; Mommsen et al. 1999;
14 Iwama et al. 2004). Considering the impacts of stress on such aspects, it is of high
15 importance to determine the stress levels induced by aquaculture activities, and to
16 understand how anaesthesia can mitigate stress system activation.

17
18 Besides the traditional anaesthetics used in aquaculture such as 2-phenoxyethanol (2-
19 PHE), metomidate or MS-222, products extracted from plants had demonstrated
20 efficacy in fish anaesthesia. It is the case of the clove oil (CO) (Tort et al. 2002; Wagner
21 et al. 2003; Weber et al. 2009; Javahery et al. 2012), as well as essential oil of *Lippia*
22 *alba* (EOLA) (Cunha et al. 2010, 2011; Becker et al. 2012; Toni et al. 2015b) and
23 *Hesperozygis ringens* (Toni et al., 2015a). Although anaesthetics can be valuable tools
24 to ensure animal welfare during aquaculture practices, these agents can also have
25 unwanted side-effects and should therefore be used with caution (Zahl et al. 2012).

26
27 The meagre, *Argyrosomus regius* (Asso 1801), is a teleost fish of the family Sciaenidae
28 that lives in inshore and shelf waters at depths between 15-200 m. This species has an
29 attractive fish shape and good processing yield providing high quality products of high
30 nutritional value, excellent taste and firm texture (Monfort 2010). Moreover, it has been
31 reported to reach fast growth rates, providing high potential as a good candidate for
32 marine diversification on aquaculture (Jiménez et al., 2005; Cárdenas 2011).

To our knowledge, information on the effects produced by CO and EOLA anaesthesia on *A. regius* is not available, while these aspects have been partially addressed by using 2-PHE (Serezli et al. 2011). In the present study, different anaesthetics were tested in *A. regius* juveniles covering two main objectives in order to define the appropriate sedative concentrations and the potential stress reduction capability of them. For this purpose, two different approaches were used to i) determine the concentration-response effectiveness of the anaesthetics CO and EOLA assessing both induction and recovery times after anaesthesia, and ii) to evaluate physiological changes in different plasmatic and tissue players of the stress pathways, in specimens maintained under low anaesthesia (CO, EOLA and 2-PHE) dosage in combination with a stress situation induced by a simulated transport.

Materials and Methods

Anaesthetics

2-PHE was purchased from Sigma (St. Louis, MO, USA). The EOLA, provided by CESNORS (Campus of Frederico Westphalen, Brazil), was extracted from the fresh leaves of the plant by hydrodistillation for 2 h, using a Clevenger type apparatus (European Pharmacopoeia, 2007). The EOLA was stored at -4°C in amber glass bottles until the composition analysis by gas chromatography coupled to mass spectrometry (GC-MS; as described by Silva et al., 2012). The clove oil (CO) was obtained from the Muñoz Gálvez distillery (Murcia, Spain).

Fish

Immature juveniles of meagre, *Argyrosomus regius* (49.0 ± 6.2 g body mass, 16.6 ± 0.8 cm; $n = 88$), were provided by MARESA S.A. (Cartaya, Huelva, Spain) and transferred to *Servicios Centrales de Investigación de Cultivos Marinos* (SCI-CM) (CASEM, University of Cádiz, Puerto Real, Cádiz, Spain; Operational Code REGA ES11028000312). Here, fish were kept in two different 400-L tanks ($n = 44$ per tank) in an open system circuit (water renewal rate of $200\% \text{ day}^{-1}$) containing seawater (38 ppt salinity, 7.5 pH, oxygen saturation above 80%) and constant aeration for a 2-week acclimation period. During this period, fish were maintained under a natural photoperiod (April/May, 2013) for our latitude ($36^{\circ} 31' 44''$) and constant temperature (18°C). Water quality was checked in each tank twice a day to affirm their stability.

Fish were fed daily with commercial dry pellets (AQUASOJA Perform 18; crude protein: 42%, crude lipids: 8%, ash: 11%, crude fiber: 4%, calcium: 1.7%, phosphorous: 1.3%, sodium: 0.4%) at 1% of their body mass. Each fish was used only once to avoid effects caused by handling or anesthetics in subsequent experiments.

Anaesthetic efficacy: induction and recovery times

CO was first dissolved with ethanol (96 %) at a ratio of 1:10 (clove oil: ethanol) and then diluted in seawater through agitation. The solution was added to the experimental tank before introducing the fish. The concentrations of 30, 40 and 50 mg L⁻¹ were used for CO, in accordance with the range of concentrations typically used for other teleost species (*Dicentrarchus labrax*, *Sparus aurata*, *Solea senegalensis*) in the SCI-CM of the Cádiz University (Cádiz, Spain; personal communication). Similar concentrations of this compound were previously used with *S. senegalensis* specimens (Weber et al. 2009).

EOLA was also dissolved with ethanol at a rate of 1:10 and diluted in seawater. Concentrations of 54, 96 and 160 mg L⁻¹ were used in accordance with other results obtained through previous experiments made in our laboratory using different fish species (*S. aurata*: Toni et al. 2015b; *D. labrax*: unpublished results).

In the anaesthetic induction test, the criteria established by Ross and Ross (2008) were followed to detect induction. That is, stage 1 is characterised by partial loss of equilibrium and erratic swimming; stage 2 is achieved by complete loss of equilibrium with specimens showing very slow movements; and in stage 3 induction is reached when fish remain at the bottom of the tank, with no apparent movement, except for regular ventilation, and no reaction to handling process.

Eight fish per concentration were individually introduced (time $t = 0$ s) in 5-L aerated aquaria containing the anaesthetics and kept there until stage 3. At this stage, induction time was recorded. After that, fish were measured, weighed and transferred into the recovery tank (7-L aerated tanks) to measure recovery times. After that, specimens were transferred to a 30-L tank and observed for 4 hours to check mortality. To ensure that ethanol was not involved in the anaesthetic process, another set of eight fish was introduced in aquaria with the application of 1420 mg L⁻¹ ethanol alone. This quantity was selected as it was the maximum used to dissolve the EOLA, that is, used for the 160

mg L⁻¹ group. No anaesthetic effects were found for ethanol. Data recorders in each experiment, as well as samples for physiological parameter analysis were taken at the same hour (between 9.00 and 11.00 pm, or between 13.00 and 14.00 pm, respectively) to avoid variations caused by possible circadian rhythms. Both experiments were performed following the Guidelines of the European Union (2010/63/UE) and the Spanish legislation (RD 1201/2005 and law 32/2007) for the use of laboratory animals.

Determine the physiological effects of CO, EOLA and 2-PHE at low concentration

Meagre specimens were distributed in the following groups (in duplicate): i) control (without anaesthetic), ii) EOLA (12 mg L⁻¹), iii) CO (1 mg L⁻¹), and iv) 2-PHE (33 mg L⁻¹). Each tank contained four specimens ($n = 8$ per group). The anaesthetics concentrations were determined in previous experiments (data not shown) and chosen because they did not cause deep anaesthesia, i.e. stage 2 was not reached (Cooke et al. 2004; Ross and Ross 2008; Cunha et al. 2010) after 30 min of application. All groups remained in the experimental tanks during four hours and were submitted to stress (including control) by means of the following process: 40 minutes without stressor, then 1 minute being chased by a fishnet followed by 30 seconds without stressor, and finally 1 additional minute of stress produced by chase again. This process was repeated until the four hours of exposure were completed. This methodology had been described by Weber et al. (2009) and Toni et al. (2015b) for other fish species. Then, after four hours of exposure, all specimens were sampled. Blood was collected from the caudal peduncle into 1-mL ammonia-heparinized syringes and centrifuged (3 min at 10,000 g) to obtain plasma, snap-frozen in liquid nitrogen afterwards and stored at -80 °C until further analysis. Pituitary glands were put in a 1/10- relation w/v of RNAlater™ stabilization solution (Ambion®) for 24 h at 4 °C and then stored at -20 °C.

Analytical techniques

Plasma cortisol levels were measured by indirect enzyme immunoassay (ELISA) as described by Martos-Sitcha et al. (2014) for other teleost species. Plasma osmolality was measured with a vapour pressure osmometer (Fiske One-Ten Osmometer, Fiske-VT, USA) and expressed as mOsm kg⁻¹. Glucose, lactate and triglycerides concentrations were measured using commercial kits from Spinreact (Barcelona, Spain) (Glucose-HK Ref. 1001200; Lactate Ref. 1001330; Triglycerides ref. 1001311) adapted to 96-well microplates. Plasma protein concentrations were measured on a 50-fold

plasma dilution using the bicinchonic acid method with the BCA protein kit (Pierce P.O., Rockford, USA), with bovine serum albumin serving as standard. All the assays were run on an Automated Microplate Reader (PowerWave 340, BioTek Instrument Inc., Winooski, USA) controlled by KCjunior™ software. Standards and samples were measured in duplicate.

Pituitary GH was analyzed by real-time-qPCR. Total RNA was isolated from pituitary glands using NucleoSpin®RNA XS kit (Macherey-Nagel) and the on-column RNase-free DNase digestion, according to manufacturer's protocol. The amount of RNA was spectrophotometrically measured at 260 nm with the BioPhotometer Plus (Eppendorf) and its quality was measured in a 2100 Bioanalyzer using the RNA 6000 Nano Kit (Agilent Technologies). Total RNA (250 ng) was reverse-transcribed in a 20 µL reaction using the qScript™ cDNA synthesis kit (Quanta BioSciences). Real-time PCR was carried out with a Fluorescent Quantitative Detection System (Eppendorf Mastercycler ep realplex² S). Each reaction mixture (10 µL) contained 0.5 µL of each specific forward and reverse primers at a final concentration of 200 nM, 5 µL of PerfeCTa SYBR® Green FastMix™ (Quanta Biosciences), and 4 µL of cDNA. β-actin was used as a reference gene owing its low variability (less than 0.10 cycles) under our experimental conditions. Relative gene quantification was performed using the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001).

Statistical analysis

Results are presented as means $\pm$ SEM. After normality and homogeneity of variance were checked, comparison between groups was analyzed as appropriate using one-way analysis of variance (ANOVA) taking i) water quality parameters (pH, temperature, oxygen saturation) in each replicate tank, ii) the anesthetic concentration, or iii) the type of anesthetic used, in each case, as main factor, followed by post-hoc comparison with Tukey's test. Significance was taken at $p < 0.05$. All tests performed were carried out using the GraphPad Prism® (v.6.0b) software for Macintosh.

Results

No statistical differences were found in any of the parameters studied both related to water quality between all experimental tanks, as well as in those biological results

obtained between different replicated tanks. In addition, no mortality occurred during any of the experiments performed.

Anaesthetic efficacy, induction and recovery times

Concentrations of 40 and 50 mg L⁻¹ of CO achieved general anaesthesia induction in less than 3 minutes, while specimens held at 30 mg L⁻¹ did not completely reach stage 3 of anaesthesia after 15 minutes of exposure. In addition, recovery time for all CO concentrations tested (30, 40 and 50 mg L⁻¹) was below 5 minutes (Figure 1). Regarding EOLA, all concentrations used (54, 96 and 160 mg L⁻¹) induced stage 3 of anaesthesia in less than 3 minutes, and specimens completely recovered their normal behavior in < 5 minutes (Figure 2). However, with the lowest concentration (54 mg L⁻¹), fish still expressed movements during the biometric measurements, without completely reaching full induction of anaesthesia after 5 min of exposure.

Physiological effects of CO, EOLA and 2-PHE at low concentrations

Specimens submitted for four hours to EOLA had significantly higher plasma cortisol levels than the control group (Figure 3). Regarding with glucose levels, 2-PHE group significantly increased its plasma values when compared to those specimens maintained in the control group (Figure 4). However, other plasma parameters assessed did not show significant differences between different treatments (Table 2).

Pituitary GH mRNA expression presented significant differences between EOLA and 2-PHE groups, showing those fish exposed to EOLA the lowest gene expression levels. No differences were found when the three experimental groups were compared with the control fish (Figure 5).

Discussion

Anaesthetic efficacy: induction and recovery times

According to Marking and Meyer (1985) the adequate induction time of an anaesthetic for fish shall be below 15 minutes with a recovery time of < 5 minutes. Previous studies have tested the use of CO as anaesthetic for different fish species, showing that anaesthetic efficacy of this compound is clearly i) species-specific, ii) size-dependent, and/or iii) affected by the water temperature in which the specimens are acclimated (see Table 1). Our results showed that CO concentrations that induced anaesthesia in A.

regius are in accordance with those concentrations reported in other species, either with CO (95% of eugenol) or AQUI-S, which also has eugenol as the active ingredient (Iversen et al. 2003; Keene et al. 1998; Stehly and Gingerich 1999; Cho and Heath 2000). However, 30 mg L⁻¹ is not recommended for situations in which anaesthesia is necessary, because *A. regius* specimens did not completely reach stage 3, while any of the other concentrations used provided fast induction. Concerning EOLA, its anaesthetic effect has been confirmed for silver catfish (*Rhamdia quelen*) (Cunha et al. 2010; Toni et al. 2014), seahorse (*Hippocampus reidi*) (Cunha et al. 2011) and gilthead sea bream (*Sparus aurata*) (Toni et al. 2015b). Cunha et al. (2010) reported that rapid anaesthesia in *R. quelen* was achieved using concentrations of 240-400 mg L⁻¹ while Toni et al. (2015b) indicated that *S. aurata* specimens reached stage 3 with concentrations ranging between 80–240 mg L⁻¹. We found that in *A. regius* the concentration of 160 mg·L⁻¹ was sufficient to reach this stage, being the unique recommended concentration, in the range covered in the present study, since fish still showed movements at lower concentrations; that is, fish were not fully induced with concentrations of 56 and 96 mg L⁻¹. These results with EOLA suggest a species-dependent concentration and, therefore, the need to test it in each teleost species of interest.

The use of correct concentrations for CO between 40 and 50 mg L⁻¹ and EOLA within a range of 160 mg·L⁻¹ in *A. regius* are effective enough to be used as anaesthetics for deep induction when conducting many activities in aquaculture (biometric samplings, blood extraction, surgery, etc.).

Physiological effects of CO, EOLA and 2-PHE at low concentration

In all experimental groups, including control fish, cortisol and glucose levels were higher compared to that obtained in studies on non-stressed *A. regius* specimens (Fanouraki et al. 2011; Millán-Cubillo et al. 2016). Therefore, although our experimental procedure was conceived as a short-time protocol, changes in the internal homeostasis of undisturbed fish clearly validates the stress source produced by a transport simulation. In addition, the activation of the stress system also in disturbed fish, together with the absence of reducing these parameters described as important players in the stress response in fish, suggest that the concentrations of anaesthetics used in this study would not be advisable for sedation during transport situations.

For CO, all parameters assessed (plasma cortisol and metabolites, as well as GH mRNA expression) were similar to those found in specimens from the control group. Studies performed with CO as an anaesthetic indicated that, at low concentrations, there is no reaction of the stress response (Strange et al. 1978; Olsen et al. 1995; Davidson et al. 2000; Iversen et al. 2003). In addition, the EOLA group showed a significant cortisol increase, but without any plasma glucose change, as was found in *S. aurata* juveniles under a similar experimental procedure (Toni et al., 2015b). Contrary, 2-PHE provided a similar level of cortisol when compared to control group, but there was a significant glucose increase. Other authors have not shown a correlation between cortisol and glucose levels using other fish species and anaesthetics in their experiments (Iversen et al. 2003).

Regarding GH mRNA expression, cortisol can negatively impact fish somatic growth by decreasing plasma GH levels and inhibiting hepatic GH receptors as well as reducing IGF-I production (Pérez-Sánchez and Le Bail 1999; Kajimura et al. 2003). Therefore, it makes sense to study the stress impact on the GH pathways (Laiz-Carrión et al. 2009). For GH expression, although no significant difference was found between any anaesthetics with respect to the control specimens, a significant increase in fish treated with 2-PHE when compared to the EOLA group was found. In *S. aurata*, GH hormone promoted hepatic glucose production through enhancement of the glycogenolytic potential (Sangiao-Alvarellos et al. 2006). Similarly, cortisol also increased plasma glucose levels in this species (Laiz-Carrión et al. 2003; Cádiz et al. 2015). Therefore, when the anaesthetics affect plasma cortisol values and GH expression levels, an impact on plasma glucose concentration could be a direct consequence. Fish exposed to CO provided similar values for plasma cortisol and glucose as well as pituitary GH expression when compared to control the group, suggesting that this anaesthetic did not affect the production of GH in the pituitary cells. Similarly, studies performed in *Oncorhynchus mykiss* with MS-222 and eugenol also demonstrated that plasma levels of GH and glucose were not affected when compared to those untreated fish maintained as control group (Holloway et al. 2004). However, 2-PHE provided the highest glucose level and GH expression but showing similar cortisol levels than those found in control fish. Contrary, EOLA treatment induced the highest plasma cortisol concentrations, together with a similar glucose level when compared to the control fish, and also the lowest GH expression. In addition to our results, a study performed on *S. aurata*

specimens exposed to 2-PHE and EOLA did not show a significant difference for GH expression between both groups, but a significant difference of cortisol was found with respect to the control fish (Toni et al. 2015b). In conclusion, these results suggest that GH expression could be driven by a different impact of anaesthetic depending on the species.

The rest of parameters studied did not show any significant change compared to the control group. For lactate, although the literature suggests that both glucose and lactate levels are typically correlated with cortisol increase (Wendelaar Bonga 1997; Barton 2002), this was not observed in the present study. Our results for lactate are in accordance with those reported by other authors, who failed when trying to find a relationship between cortisol and lactate values (Iversen et al. 2003; Small 2004; Toni et al. 2015a, 2015b), including *A. regius* juveniles (Millán-Cubillo et al., 2016). Iversen et al. (2003) suggested that plasma lactate level could be dependent on secondary effects of anaesthesia, related with oxygen insufficiency for cell aerobic metabolism. For instance, longer periods of anaesthesia or even more delayed sampling points would be necessary to observe variations of this plasma metabolite. Following such a hypothesis, we suggest that the concentrations used in our experiment were low enough to avoid a negative impact in aerobic pathways at least during our experimental period. Plasma protein concentrations and osmolality alterations are also considered secondary stress responses (Wendelaar Bonga 1997; Iwama et al. 2004). However, to our knowledge, there are not many studies assessing changes in these parameters due to the effect of anaesthetics in fish (Knoph and Olsen 1994; Velíšek et al. 2004; Bernatzeder et al. 2008).

Conclusion

Our results indicated, in comparison with the induction and recovery times reported for other anaesthetics, that CO and EOLA can be considered good enough to induce anesthesia in fish. This makes them good candidates for anaesthetics as they presented safe characteristics for operators and, potentially, for future fish consumption. Secondly, our investigation concluded that, regarding the use of low sedative concentrations of CO (1 mg L⁻¹), 2-PHE (33 mg L⁻¹) and EOLA (12 mg L⁻¹) for transport, these anaesthetics produced changes in physiological parameters, requiring caution in their use.

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FIGURE LEGENDS

Figure 1. Induction and recovery times of the anaesthesia with clove oil (CO) acting as anaesthetic in juveniles of *A. regius* ($n = 8$ for each concentration tested) maintained at 18 °C water temperature. Data are presented as mean (columns) $\pm$ SEM (bars). Different letters indicated significantly differences between experimental groups ($p < 0.05$).

Figure 2. Induction and recovery times of the anaesthesia with essential oil of *Lippia alba* (EOLA) acting as anaesthetic in juveniles of *A. regius* ($n = 8$ for each concentration tested) maintained at 18 °C water temperature. Data are presented as mean (columns) $\pm$ SEM (bars). Different letters indicated significantly differences between experimental groups ($p < 0.05$).

Figure 3. Plasma cortisol levels in *A. regius* juveniles exposed to control (without anaesthesia) and low anaesthetic concentration (CO: 1 mg L⁻¹, EOLA: 12 mg L⁻¹, 2-PHE: 33 mg L⁻¹), and submitted to a simulated transport stress situation for 4 hours. All test were performed at constant temperature (18 °C). Data are presented as mean (columns) $\pm$ SEM (bars) ($n = 8$ per group). Different letters indicated significantly differences between experimental groups ($p < 0.05$).

Figure 4. Plasma glucose levels in *A. regius* juveniles exposed to control (without anaesthesia) and low anaesthetic concentration (CO: 1 mg L⁻¹, EOLA: 12 mg L⁻¹, 2-PHE: 33 mg L⁻¹), and submitted to a simulated transport stress situation for 4 hours. All test were performed at constant temperature (18 °C). Data are presented as mean (columns) $\pm$ SEM (bars) ($n = 8$ per group). Different letters indicated significantly differences between experimental groups ($p < 0.05$).

Figure 5. Pituitary growth hormone mRNA expression levels in *A. regius* juveniles exposed to control (without anaesthesia) and low anaesthetic concentration (CO: 1 mg L⁻¹, EOLA: 12 mg L⁻¹, 2-PHE: 33 mg L⁻¹), and submitted to a simulated transport stress situation for 4 hours. All test were performed at constant temperature (18 °C). Data are presented as mean (columns) $\pm$ SEM (bars) ($n = 8$ per group). Different letters indicated significantly differences between experimental groups ($p < 0.05$).

Table 1. Induction and recovery times in different teleost species when exposed to clove oil (CO) as anaesthetic. ¹Tort et al. 2002; ²Mylonas et al. 2005 ; ³García-Gómez et al. 2002; ⁴Silveira and Martínez 2003; ⁵Cooke et al. 2004; ⁶Keene et al. 1998; ⁷Iversen et al. 2003; ⁸Current experiment.

Specie	Temperature (°C)	Weight (g)	CO (mg L ⁻¹)	Induction time (stage 3) (min)	Recovery time (min)
<i>Sparus aurata</i> ¹	15	90	50	4	4
<i>Sparus aurata</i> ²	25	40	40	3	5
<i>Sparus aurata</i> ²	15	70	55	3	7
<i>Dicentrarchus labrax</i> ²	25	30	40	3	3
<i>Dicentrarchus labrax</i> ²	15	40	30	2.5	8
<i>Dicentrarchus labrax</i> ³	17	100	40	5.5	2.5
<i>Diplodus sargus sargus</i> ³	16	400-1000	40	4.5 - 5	5
<i>Oreochromis niloticus</i> ⁴	--	20	50	5	7.4
<i>Micropterus salmoides</i> ⁵	21	90	20	11	28
<i>Oncorhynchus mykiss</i> ⁶	9.1	20	60	4	13
<i>Salmo salar</i> ⁷	5.4	40	30-100	8.1-2.2	-
<i>Argyrosomus regius</i> ⁸	19	50	40	2.2	3.2
<i>Argyrosomus regius</i> ⁸	19	50	50	1.5	3.4

Table 2. Plasma metabolite levels (lactate, proteins, triglycerides) and osmolality in *A. regius* juveniles exposed to control (without anaesthesia) and low anaesthetic concentration (CO: 1 mg L⁻¹, EOLA: 12 mg L⁻¹, 2-PHE: 33 mg L⁻¹), and submitted to a simulated transport stress situation for 4 hours. All test were performed at constant temperature (18 °C). Data are presented as mean ± SEM (*n* = 8 per group). No significant differences were found between experimental groups.

Parameter	Control	CO	EOLA	2-PHE
Lactate (mM)	0.71 ± 0.13	0.56 ± 0.08	0.84 ± 0.20	0.48 ± 0.12
Proteins (mg mL⁻¹)	18.42 ± 0.58	19.15 ± 0.35	19.73 ± 0.67	17.78 ± 0.50
Triglycerides (mM)	2.24 ± 0.18	2.27 ± 0.15	2.05 ± 0.18	2.08 ± 0.14
Osmolality (mOsm kg⁻¹)	275.3 ± 1.7	268.71 ± 1.6	274.71 ± 3.3	279.85 ± 4.3