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The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858)

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ABSTRACT

The efficacy of four anaesthetic agents (2-phenoxyethanol, metomidate, clove oil and MS-222) was evaluated in the Senegalese sole (*Solea senegalensis*). It was assumed that stage II of anaesthesia is sufficient to carry out routine aquaculture procedures in less than 3 min, with recovery in less than 5 min. The following optimal doses were determined: 600 mg L^{-1} of 2-phenoxyethanol (induction 1.50±0.37 and recovery time 1.94±0.56 min), 5 mg L^{-1} of metomidate (induction 1.50±0.22 and recovery time 3.70±1.18 min), 30 mg L^{-1} of clove oil (induction 3.16±0.40 and recovery time 3.76±1.01 min) and 75 mg L^{-1} of MS-222 (induction 2.42±0.20 and recovery time 0.56±0.14 min). The induction times decreased with increasing doses for all of the anaesthetic agents evaluated. Finally, the ability of each anaesthetic agent to prevent a reflex reaction in less than 3 min during simulated blood sampling was evaluated in Senegalese soles of different weights (74±4 g; 213±15 g; 300±12 g), being only achieved in the following cases: 600 mg L^{-1} of 2-phenoxyethanol and 6 and 8 mg L^{-1} of metomidate, with fish of 74±4 g, and 600 mg L^{-1} of 2-phenoxyethanol, 8 mg L^{-1} of metomidate and 200 mg L^{-1} of MS-222 with fish of 213±15 g. The most effective of the four anaesthetic agents studied was 2-phenoxiethanol, although all were considered acceptable for use in culture of Senegalese sole.

1. Introduction

In aquaculture, the use of anaesthetics takes on special importance when these agents are required for diverse routine operations such as the selection of fish, their measurement, sampling, labelling and transportation, amongst many others. Such manipulations often induce a physiological stress response. For this reason, the use of an anaesthetic will not only help to prevent damage to the fish, but may also attenuate the physiological response to stress. Two of the most widely used anaesthetics in research and in fish aquaculture are 2-phenoxyethanol, whose active ingredient is ethylene glycol monophenyl ether, and MS-222 (tricaine methanesulphonate), both of which act as general anaesthetics in fish (Burka et al., 1997). Recent studies have evaluated the anaesthetic efficacy—in various fish species—of clove oil (Walsh and Pease, 2002; Iversen et al., 2003; King et al., 2005; Mylonas et al., 2005; Hajek et al., 2006), the main active component (70 to 90%) of which is eugenol [2-methoxy-4-(2-propenyl) phenol], and of metomidate (DL-1-(1-phenyl-ethyl)-5-(metoxycarbonyl) imidazolole hydrochloride) (Burka et al., 1997; Iversen et al., 2003; King et al., 2005; Palić et al., 2006), a nonbarbiturate hypnotic agent with potential cortisol suppressing properties (Small, 2003).

The choice of an appropriate anaesthetic depends mainly on its effectiveness in immobilizing fish, thereby allowing the fish to be manipulated (Gilderhus and Marking, 1987; Burka et al., 1997). The efficacy is conditioned by environmental (temperature, pH and salinity) and biological factors (size, weight, lipid content and fish species) (Burka et al., 1997; Ross and Ross, 1999). It is known that the responses to the same anaesthetic can vary considerably among different species, so the characterization of the effective dose of the different anaesthetics in a determined species is a rather advisable practice (King et al., 2005).

Given the growing interest in the culture of flat fish and that anaesthetic agents have only been partially evaluated in such species (Bourne, 1984; Malmstrøm et al., 1993; Ribas et al., 2007), the aim of the present work was to provide practical information on the lowest effective dose of these four commonly used anaesthetic agents for use in Senegalese sole.

2. Materials and methods

2.1. Fish

Juvenile Senegalese sole (*Solea senegalensis*) were obtained from the Instituto Español de Oceanografía (Canido, Vigo), and transported to the experimental aquarium (Faculty of Biology, University of Santiago de Compostela). The fish were acclimated in six 300 L storage tanks (mean

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Table 1
Stages of induction of anaesthesia and recovery in the *Solea senegalensis*

Stage of anaesthesia	Description
I	Partial loss of equilibrium Fish retain some body movements
II	Total loss of equilibrium and muscular tone Low frequency of opercular movements and strongly attenuated reflexive responses
III	Imperceptible opercular movements Total loss of spinal reflexes.
Stage of recovery	**Description**
I	Lack of equilibrium Fish do not show any body movements Fish begin to recover opercular movements
II	Recovery of equilibrium and some body movement Recovery of the frequency of opercular movements
III	Similar to pre-anaesthesia Opercular frequency slightly higher than in pre-anaesthesia

density 2.6–2.8 kg m^{-2}) supplied with constantly running and aerated seawater (by a recirculation system) for at least 4 weeks, under aquarium conditions: pH: 7.3±0.1, temperature: 14±1 °C, salinity: 36 g L^{-1}, dissolved oxygen: above 80% saturation level and a photoperiod 12 h light: 12 h dark. The fish were fed to satiety once daily, at 17:00 h, with commercial dry pellets (Skretting LE-2), and were fasted 24 h prior to sampling. The tanks were daily cleaned, and the levels of oxygen, ammonia and nitrite in the water were measured twice a week, with no substantial changes being observed during the study period. Fish from duplicate tanks were used to avoid a possible tank effect. The experiments described comply with the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

2.2. Anaesthetic agents

The anaesthetic agents 2-phenoxyethanol (Panreac Química SA, Barcelona, Spain), metomidate (Aquacalm; Syndell International Inc., Vancouver, Canada), clove oil (Omya Peralta Gmbh, Hamburg, Germany) and MS-222 (Sigma Aldrich Co., St. Louis, USA) were used. The doses of the anaesthetic agents were prepared a few minutes before each experiment. Clove oil is poorly soluble in cold water, and therefore was initially dissolved in 94% ethanol (ratio of clove oil: ethanol, 1:9). Preliminary trials confirmed that the volume of ethanol used in each trial did not have a visible anaesthetic effect on fish for at least 15 min.

2.3. Experimental design

In a pilot study it was found that the characteristics that define the induction of different stages of anaesthesia and recovery are similar to those previously reported for non-flat fish. For practical reasons, three stages of induction and three stages of recovery were considered (Iwama et al., 1989). In the pilot study, 2-phenoxyethanol (300 mg L^{-1}) and sole weighing 65±6 g were used (n=10). Each fish was transferred from storage tanks into a 20 L observation tank (made of methacrylate and filled with fresh aerated sea water) to which the anaesthetic had already been added. The fish were observed every minute (to check opercular movements, equilibrium and absence of response to tactile stimulus) until the opercular movements were imperceptible (stage III of anaesthesia). As soon as the fish reached this stage, they were immediately netted from the observation tank, weighed and placed in a 100 L recovery tank (filled with re-circulating fresh aerated sea water) with the white ventral side upwards. The same previously mentioned parameters were evaluated. Owing to the characteristics of the Senegalese sole, it was assumed that loss of equilibrium takes place when the fish placed with its white ventral side upwards is unable to recover its normal posture. In the same way, recovery of equilibrium is established when the fish is able to recover its normal posture.

In Experiment I several doses of each anaesthetic were tested and the times taken to reach stage II of induction and recovery were determined. The following doses of each agent were evaluated: 2-phenoxyethanol (300, 400, 500, 600 mg L^{-1}), metomidate (4, 5, 6 mg L^{-1}), clove oil (20, 30, 40 mg L^{-1}) and MS-222 (50, 75, 100 mg L^{-1}). The experiment was performed as indicated for the pilot study, but determining the times needed for the fish to reach stage II of anaesthesia and to recovery, respectively. In this experiment, fish weighing 99±2.5 g were used.

Experiment II was performed to evaluate the depth of stage II of anaesthesia by simulating a blood sampling, and recording the presence or absence of a reflexive response (King et al., 2005). This was carried out bearing in mind the fish weight (74±4 g, 213±15 g and 300±12 g). The following doses were evaluated: 500 and 600 mg L^{-1} for 2-phenoxyethanol, 6 and 8 mg L^{-1} for metomidate, 40 and 80 mg L^{-1} for clove oil and 100 and 200 mg L^{-1} for MS-222. The experiment was performed as indicated in the pilot study. Once a fish had reached stage II of anaesthesia, it was netted and placed carefully on a moist cloth. A heparinised syringe (1 ml 25 GA) was then used to simulate extraction of blood from the caudal vein. If the fish reacted, it was returned to the observation tank, and the process was repeated (each minute), and the time taken until the fish did not react was recorded. None of the doses used caused mortality in the fish.

2.4. Statistical analysis

Data are presented as means±standard error of the mean (SEM). The differences among means were analysed by one- and two-way ANOVA followed by a Student's–Newman–Keuls' multiple range test. Differences were considered significant at $P<0.05$. All analyses were performed with the Sigma Stat 2.0 statistical package.

3. Results and discussion

In the pilot study it was observed that the characteristics that define the different stages (I, II, III) of anaesthesia and recovery in Senegalese sole (Table 1) are similar to those reported by Iwama et al. (1989) for a non-flat fish. The results of experiment I are shown in Table 2. In general it was observed that with all the anaesthetics, the induction times decreased significantly as the doses increased. Similar results were observed in non-flat fish (Mattson and Riple 1989; Hseu

Table 2
Induction and recovery times for juveniles of *Solea senegalensis* anaesthetized with various concentrations of four anaesthetic agents

Anaesthetic	Concentration (mg L^{-1})	n	Time (min)	
			Induction	Recovery
2-Phenoxyethanol	300	6	13.2±1.30^{a}	5.35±1.03^{a}
	400	8	8.37±0.73^{b}	6.09±0.92^{a}
	500	8	4.37±0.67^{c}	5.47±1.52^{ab}
	600	8	1.50±0.37^{d}	1.94±0.56^{b}
Metomidate	4	9	3.12±0.39^{a}	9.09±1.44^{a}
	5	6	1.50±0.22^{b}	3.70±1.18^{b}
	6	8	1.50±0.26^{b}	4.30±1.20^{b}
Clove oil	20	6	3.50±0.34^{a}	3.20±0.75^{a}
	30	7	3.16±0.40^{a}	3.76±1.01^{a}
	40	6	1.66±0.33^{b}	3.59±1.10^{a}
MS-222	50	4	7.50±1.19^{a}	2.76±1.09^{c}
	75	7	2.42±0.20^{b}	0.56±0.14^{c}
	100	8	2.25±0.31^{b}	2.99±0.68^{c}

Induction time: time necessary to reach stage II of anaesthesia. Recovery time: time necessary to return to stage II of recovery.
Data are presented as mean values±SEM. For each anaesthetic agent, values in the same column indicated with different superscript letters indicate significant differences ($P<0.05$). n=number of fishes.