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Efficacy of 2-phenoxyethanol as an Anesthetic for Two Size of Persian Sturgeon, *Acipenser persicus*

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Abstract

The purpose of this research was to appraise the efficacy of 2-phenoxyethanol as an anesthetic for two sizes of Persian sturgeon, *Acipenser persicus* (100 and 400 g) to find the minimum concentration for desirable anesthetic effects on them. Fish were exposed to concentrations varying from 0.1 to 1.10 mL/L for a 10 min period. At concentrations of 0.1 and 0.3 mL/L, 2-phenoxyethanol was unsuccessful to cause deep anesthesia in small fish, whereas at a concentration of 1.1 mL/L all the fish were anesthetised within 3 min of exposure. In larger fish, low concentrations of 2-PE (0.1 mL/L) did not cause deep anesthesia, whereas at concentrations of 0.9 and 1.1 ml/L all the fish were anesthetised within 3 min of exposure. Increasing the concentration of 2-phenoxyethanol decreased the induction time, contrary to this trend recovery time from anesthesia increased. At all tested concentrations, induction time was significantly weight-dependent ($p < 0.05$), but recovery time was not different between the two size classes at 0.7 mL/L. 2-Phenoxyethanol proved to be an efficient and careful anesthetic in both sizes of Persian sturgeon permitting quick and not eventful induction and recovery after a 10 min exposure period.

Keywords: 2-Phenoxyethanol, anesthesia, Persian sturgeon, recovery, induction time

Introduction

Numerous stressful situations are produced during the manipulations carried out in aquaculture and related studies, making the use of anesthetics necessary under such circumstances. Use of anesthetics during usual tasks (weighing, sampling, vaccination, marking etc.) not only impedes or minimizes physical injury, but also minimizes stress.

When selecting an anesthetic, a number of considerations are prominent, such as effectiveness, cost, attainability and comfort of use, as well as toxicity to fish, humans and the environment [1], and the nature of the research and species of fish [2,3]. So far, a number of distinctive anesthetics have been used or appraised for aquaculture implementation [2,4-8], of which the serious ones are tricaine methane sulphonate (MS-222), benzocaine, metomidate, 2-phenoxyethanol and quinaldine. Still, none of these meet the ideal anesthetic depiction, which according to Marking and Meyer [5] should let a rational duration of exposure, product anesthesia within 3 min or less, allow recovery within 5 min or less, cause no toxicity to fish at treatment levels, present no mammalian safety problems, leave low tissue remainder after a retreat time of 1 h or less, and be reasonable in cost. The efficient anesthetic concentrations of 2-phenoxyethanol in some species of fish have been reported and range from about 0.2 to 0.6 mL/L [6,9-14]. Persian sturgeon (*Acipenser persicus*) is an imperiled species [15]. It is spawned artificially in aquaculture facilities with the aim of restocking to increase its population. So some research has been done on the

effects of anesthetic agents on brood stocks and fries [16]. Elevating sturgeons for producing brood stocks in order to decrease dependency on natural populations is very encouraging [17]. During culturing and breeding exercise, stressful roles such as handling and transportation might affect its survival and growth, so using anesthetic agents could be helpful [8].

Materials and methods

Two groups of 60 specimens of Persian sturgeon weighing 100 ± 5.40 g and 400 ± 20.32 g, respectively, were obtained from the Shahid Marjani proliferation and culture centre for sturgeon fish, Gorgan, Iran. Fish were transferred to the Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources and acclimatized to the laboratory conditions for 2 wk. Fishes were randomly parceled in 24 fiberglass tanks (400 L). The average values for aerated and dechlorinated tap water used in the both acclimatization and experiments was pH 7.21 ± 0.50, dissolved oxygen 8.80 ± 0.06 mg/L, temperature at 19 ± 1 °C and total hardness 290 ± 2.35 mg/L as $CaCO_3$. The water quality parameters mentioned above were assessed in the experimental period. Throughout the acclimatization period and experiment periods, fish were held under a photoperiod of 12 h of light and 12 h of darkness. Each size was kept in separate open flow-through system fiberglass tanks (400 L). All fish were starved for 24 h before the experiments. Fish were observed daily to evaluate their health state as indicated by their activity and external appearance. The various doses of 2-Phenoxyethanol (SIGMA, 99.5 %, d = 1.107 - 1.108 g/L) were first dissolved completely in 5 ml ethanol. Experimental fish were exposed to concentrations of 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 ml/L 2-Phenoxyethanol dissolved in aerated and dechlorinated tap water. Ten fish were individually exposed to each of the aforementioned concentrations and induction times (surgical or deep anesthesia phase; total loss of reactivity, lay on the tank bottom and did not respond to handling) were recorded. After 10 min, the fish were transferred to fresh and aerated tanks and recovery times were recorded. Recovery time was recorded as the time needed for fish to regain equilibrium and begin active swimming. Following recovery, fish were transferred to large fiberglass tanks and were observed for 48 h for adverse effects. In the present study an effective concentration is defined as a concentration that induces deep anesthesia within 3 min.

Statistical analyses were carried out using the computerized package PASW 18.0 for Windows. Normality of data was first estimated using a Kolmogorov-Smirnov's test and homogeneity of variance was assessed with Levene's test. To evaluate the effect of different concentrations of 2-Phenoxyethanol on induction and recovery times for both sizes, all data were subjected to one-way ANOVA followed by Tukey's test at a 5% significance level ($p < 0.05$). The Independent-Samples T-Test was used to assess the statistical significance of the differences between induction time means and between recovery time means of the two weight groups.

Results

At concentrations of 0.1 and 0.3 mL/L, 2-Phenoxyethanol failed to induce deep anesthesia in small fish within the 10 min exposure period. At a concentration of 1.1 mL/L all the fish reached a deep anesthesia phase within 3 min of exposure. In larger fish, 2-phenoxyethanol failed to induce deep at a 0.1 ml/L concentration, whereas at concentrations of 0.9 and 1.1 mL/L all the fish were anesthetised within 3 min of exposure (**Tables 1**). Mortality was not observed at all concentrations in both sizes during the 10 min exposure period. Induction of anesthesia was uneventful in all fish. Furthermore, all the reported recoveries were also uneventful.

Table 1 Efficacy of several concentrations of 2-phenoxyethanol on anesthetising 100 g and 400 g Persian sturgeon to total loss of equilibrium (deep anesthesia) and recovery time after 10 min exposure (n = 10).

Concentration (mL/L)	Induction time (s)		Recovery time (s)	
	100g	400g	100g	400g
0.1	$0.0 \pm 0.0^{d,A}$	$0.0 \pm 0.0^{e,A}$	$0.0 \pm 0.0^{e,A}$	$0.0 \pm 0.0^{e,A}$
0.3	$0.0 \pm 0.0^{d,B}$	$488.50 \pm 60.08^{a,A}$	$0.0 \pm 0.0^{e,B}$	$71.10 \pm 26.95^{d,A}$
0.5	$466.80 \pm 52.24^{a,A}$	$372.0 \pm 21.76^{b,B}$	$72.20 \pm 17.16^{d,B}$	$129.50 \pm 12.06^{c,A}$
0.7	$349.60 \pm 55.70^{b,A}$	$262.60 \pm 22.29^{c,B}$	$175.80 \pm 26.41^{c,A}$	$162.60 \pm 20.16^{b,A}$
0.9	$202.30 \pm 28.63^{c,A}$	$135.40 \pm 18.30^{d,B}$	$246.60 \pm 31.94^{b,A}$	$184.80 \pm 27.61^{b,A}$
1.1	$171.40 \pm 27.29^{c,A}$	$113.20 \pm 17.32^{d,B}$	$291.70 \pm 28.68^{a,A}$	$232.70 \pm 26.93^{a,B}$

Data in the same column with the same superscript small letter are not different from each other ($p < 0.05$).
Data in the same row, for the induction or recovery time, respectively, with the same superscript capital letter are not different from each other ($p < 0.05$).
* Data are given as mean (S.D.).

Induction time decreased and recovery time increased within increasing concentrations, being significantly concentration-dependent for both sizes. There were strong correlations between 2-phenoxyethanol concentrations and induction as well as recovery times (**Figures 1** and **2**).

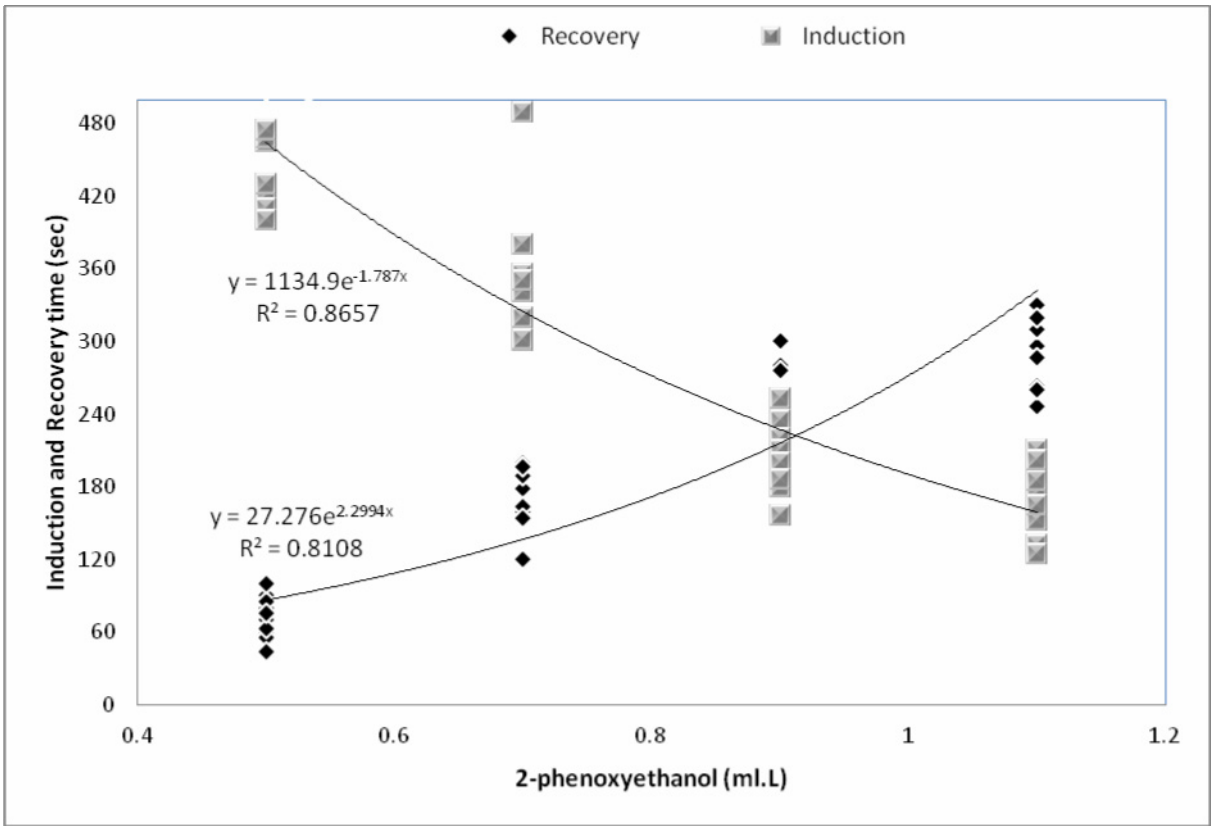

Figure 1 Correlation between induction and recovery times with different concentrations of 2-phenoxyethanol in Persian sturgeon (100 g).

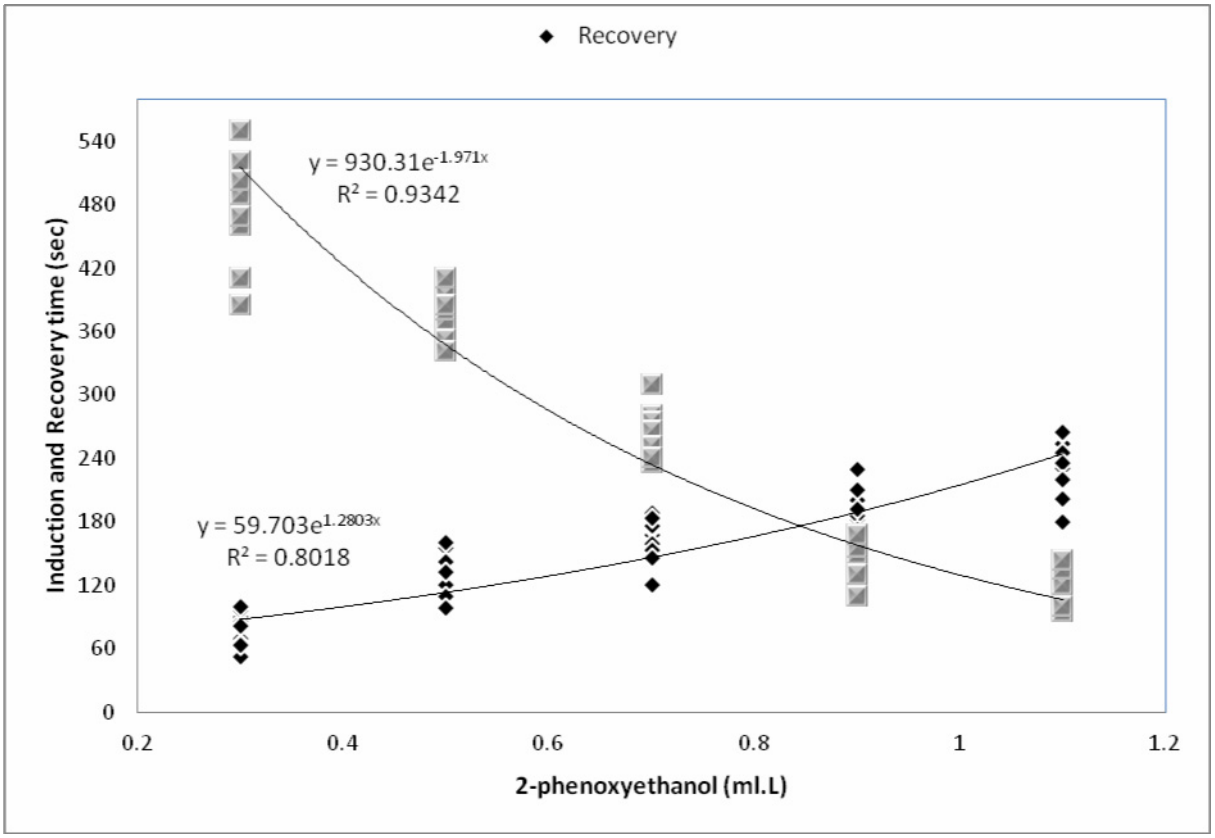

Figure 2 Correlation between induction and recovery times with different concentrations of 2-phenoxyethanol in Persian sturgeon (400 g).

Discussion

An excellent anesthetic for fish should cause anesthesia in less than 3 to 5 min, with entire loss of equilibrium and muscle tone, should permit a non-eventful and quick (less than 10 min) recovery, should leave low tissue remainder, and be unharmful to users as well as be economical and comfortable to use [6-11]. Although its style of activity is poorly understood [14], 2-phenoxyethanol is known to act quickly and, at low concentrations when exposure to the drug is not protracted, recovery is also quick and not eventful in a number of species [1,11-13,18]. However, guesses from data applicable to one species, in order to accept an anesthetic concentration as efficient and safe in another, may lead to perilous supposition. As a result, studies remain the only safe way to appraise the effectiveness of an anesthetic in a particular species to find the minimum concentration that has the desirable anesthetic effects on it. The criterion for determining efficacy of an anesthetic are based on behavioral responses that have been delineated and modified more than once. In this research, total loss of equilibrium and muscle tone is a situation equivalent to stage 4 anesthesia of a six-stage system [2,19]. Recent research shows that 2-phenoxyethanol acts as an anesthetic in two sizes of Persian sturgeon, and could be used in aquaculture applications. Both fish exposed to the anesthetics proceed consecutively through the typical stages of anesthesia [2,8] and recover in a short period of time following removal from the anesthetic bath.

In the present research 2-phenoxyethanol at concentrations of 1.1 and 0.9 mL/L induced anesthesia quickly in two sizes of Persian sturgeon and allowed quick and non-eventful recovery without any deaths after a 10 min exposure period.

At lower concentrations (0.1 and 0.3 mL/L) 2-phenoxyethanol failed to induce anesthesia. Therefore, 0.5, 0.7, 0.9 and 1.1 mL/L concentrations of 2-phenoxyethanol induced safe and efficient anesthesia. However, even the lowest concentration should not be considered absolutely safe when used with fish of a compromised health status, as it is well known that anesthetic animals that have infections generally results in enlarged mortality and morbidity [20]. In such a case, the exposure time should be as small as possible and fish should be transferred to a comparably large recovery tank in order to minimize the contamination of the water with anesthetic transferred from the body of the fish [19]. According to our results concentrations of 2-phenoxyethanol, 0.5 or 0.7 mL/L, could be used as sedatives in Persian sturgeon but a true anesthetic effect should not be expected. In the present research, induction time reduced and recovery time increased with increasing concentrations. This is consistent with other reports on the effectiveness of 2-phenoxyethanol in different species [1,11-12]. However, Hseu *et al*. [11] reported that, although induction time reduced with increasing concentrations of 2-phenoxyethanol as well as another four anesthetics, recovery time was concentration-independent in the gold lined sea bream, *Sparus sarba*.

It seems that, with regard to the recovery time, the anesthetic concentration plays a more prominent function than the duration of anesthesia [12]. This is encouraged by Bonath [21] who observed that the recovery time remained uninfluenced after a 5-fold increase in the duration of anesthesia. It is believed that the independence of the recovery time from the duration of anesthesia is due to the anesthetic being taken up by the fish through a concentration gradient at the gill interface; therefore, after the fish has attained a level of equilibrium with the anesthetic solution, this equilibrium is sustained. During recovery, the anesthetic agent is lost through this gradient; therefore, the recovery time is governed by the anesthetic concentration and remains unaffected by the duration of anesthesia [12,21].

Conclusion

In summary, under the indicated trial conditions, during our research, 2-phenoxyethanol was demonstrated to be an efficient and careful anesthetic in two sizes, 100 and 400 g of Persian sturgeon resulting in a quick and uneventful induction and recovery after a 10 min exposure period. The minimum concentration to induce the desirable anesthetic effect in these two sizes was 0.9 mL/L. The effect of body weight on both recovery and induction time is also intriguing. In our research, induction time decreased in larger fish.

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