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The anesthetic effects of 2-phenoxyethanol on Munzur trout fingerlings (*Salmo munzuricus* Turan *et al*., 2017*)* at different temperatures

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Abstract

In this study, the anesthetic effects of 2-phenoxyethanol (2-PE, with %99 purity, BASF, Germany) on Munzur trout (*Salmo munzuricus.*, a native species) originated from Munzur stream have been studied. 300 fish with the average weight of 4.42±0.62 g and length of 8.10±0.60 cm were used. To determine the anesthetic effects of various concentrations of 2-PE on Munzur trout fingerlings, the experiments were performed in two different temperatures (13 ºC and 18ºC) and five different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 ml L^{-1}). 2-PE was used in this study as an anesthetic agent. In the anesthetic processing we practiced in all concentrations, except for 0.1 ml L^{-1} of 2-PE, loss of reflex (S5) was determined between 68 sec and 276 sec, and total behavioral recovery (R3) was determined between 58 sec and 208 sec. Induction times of anesthesia varied with anesthetic concentrations, decreasing with the increases of 2-PE concentrations. On the other hand, recovery times increased with decreasing of anesthetic concentrations. The lowest effective dose was determined as 0.3 ml L^{-1} at the temperatures 13 ºC and 18ºC. In this study, first time optimum anesthetic doses of 2-PE were determined for fingerling specimens of *Salmo munzuricus* which is endemic to Munzur stream and has an importance for aquaculture.

Keywords: Munzur trout, *Salmo munzuricus*, Anaesthesia, 2-phenoxyethanol, Temperature.

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Introduction

Anesthesia applications are used for different purposes in aquaculture especially reducing the stress of fish, e.g. hormone injection, stripping, vaccination, tagging, weighing, grading and transporting. An ideal anesthetic agent should induce anesthesia or recovery rapidly with minimum hyperactivity or stress, should be easy to use and inexpensive, and should be effective at low doses with being safe for users and consumers (Kaiser *et al*., 2006; Perdikaris *et al*., 2010).

Anesthetics, first applied in the medical field in the 1840s (Summerfelt and Smith, 1990), are also widely used in aquaculture and fisheries facilities. MS222 (*Tricainemethane sulfonate*), benzocaine and 2-phenoxyethanol (2-PE) are the most widely used anesthetics in aquaculture (Burka *et al*., 1997; Mylonas *et al*., 2005; Weber *et al*., 2009; Serezli *et al*., 2011), with anesthesia usually being induced by immersing the fish in a solution of a given concentration. Optimum anesthetic concentrations can minimize the negative impact and thus reduces stress in fish and these concentrations are usually expected to induce anesthesia within 3 min and recover within 10 min (Park *et al*., 2008).

Munzur trout (*Salmo munzuricus*), a species commonly called red spotted trout, their identity was not clear up to now, newly described by Turan *et al*. (2017) is a native Salmonid species with high economic value in Munzur stream where is an important water supply in Turkey and its culturing studies are performed. There is limited studies on Munzur trout and these are about feeding habits, reproduction traits etc. (Kocabas *et al*., 2011a,b). Besides, there is lack of literature about sedative and anesthetic doses at different temperatures. Handling stress can cause bad quality of eggs and also some mortality would be seen after stripping. Anesthesia of fish before stripping is an important issue in aquaculture applications. The water temperature is important factor in anesthesia treatments because the induction and recovery times can vary in relation to water temperature (Zahl *et al*., 2009). The fish anesthesia treatments are often applied in fingerlings size for grading and vaccinating. In this study, the effects of 2-PE at different concentrations were investigated on Munzur trout fingerlings for determination of the effective doses of this anesthetic agent, at different water temperatures (13 ºC and 18 ºC).

Materials and methods

Experiments were conducted on the Fisheries Application and Research Center in Munzur University, Turkey in 2014. In this study, anesthetic effects of five different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 ml L^{-1}) of 2- PE (with %99 purity, BASF, Germany) have been examined on Munzur trout (*S. munziricus,* originated from Munzur stream) at 13 ºC and 18 ºC. Totally 300 fish with the average weight of 4.42±0.62 g (mean±SE) and length of 8.10±0.60 cm (mean±SE) have been used.

The fingerlings were stocked in 300 L tank for 15 days before the

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treatments. Feeding was stopped 2 days before the anesthesia treatments. Trout samples were randomly caught from the holding tanks and transferred immediately into the total 20 L top water which contains 2-PE at 5 different concentrations in triplicate. Total lengths and weights were measured at the S5 and R1 stages, quickly.

The water in the aquaria of 20 L were conditioned by resting for a day before the anesthesia treatments while fixing the water temperature using 100 watt aquarium heater at required ranges with continuous aeration. Thus, the adaptation of fish to the aquariums was achieved and the study was conducted at triplicate for both temperature and concentrations. Rested water was used during the treatments and it was renewed every after each study. Temperature and dissolved oxygen were measured by oxygen meter (YSI Professionals Plus, USA).

Fish were kept in the aquaria contain of 20 L water with 10 fish. Firstly, the anesthetic material 2-PE were diluted with water and added to the aquarium by mixing with glass stripe at calculated concentration. Homogenization of anesthetic material was achieved in the all treatments. Air stones were used for aeration of the aquariums and the oxygen level was maintained at 7.0-7.5 mg L^{-1} during the treatments by this way. pH was measured between 7.9 and 8.2. After the treatment, fish were transferred to the stock tanks and observed for 24 hours and monitoring if there could be any mortality (Yildiz *et al*., 2013).

The stages were specified by observing and recorded times as the seconds by chronometer. The criteria were based on the Table 1 to identify the certain phase. The time for each phase fixed after 50 % of fish entered the phase.

Determination of induction (sedation=S) and recovery (R) stages modified from Keene *et al.* (1998) are shown in Table 1. Analysis of data was carried out using SPSS 15.0. One-way ANOVA followed by the Duncan test was used to determine significant differences among means. Statistically significant differences were expressed as $p<0.05$.

Table 1: Anesthesia phases (modified from Keene *et al*., 1998).

Phase	Code	Fish behavior characteristics
Light sedation	S1	equilibrium normal, opercular rate slightly decreased,
Deep sedation	S2	equilibrium normal, slight decrease in opercular rate no respond to weak external stimulus,
Partial loss of equilibrium	S3	swimming erratic, opercular movements fast, no respond to strong external stimulus,
Total loss of equilibrium	S4	Total loss of equilibrium, regular opercular movement but slow
Loss of reflex	S5	No reflex, opercular movements irregular and slow
Partial recovery of equilibrium	R1	partial equilibrium and swimming, opercular movements starting,
Total recovery of equilibrium	R2	Total recovery of equilibrium, swimming erratic starting
Total behavioral recovery	R3	Normal swimming starting

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Results

Figs. 1 and 2 present the results of induction and recovery times of Munzur trout exposed to different doses of the 2-PE for two temperatures. Only 5 individual of 100 fish died after treatment during the observation of 24 hours. They were observed unhealthy while the others were healthy. However, no mortalities were observed during the treatment.

Induction times of anesthesia varied with anesthetic concentrations, decreasing with the increase of 2-PE concentrations. On the other hand, recovery times increased with decreasing of 2-PE concentrations (p<0.05; Figs. 1 and 2).

During the study, at the dose of 0.1 ml L^{-1} the total loss of equilibrium was not observed in both 2 temperatures. Loss of reflex activity (S5) was induced faster at higher concentrations of anesthetic than 0.1 ml L^{-1} for both temperature (p<0.05) (Figs. 1 and 2). At 13 ºC and 18 ºC for 0.2 ml L^{-1} dose of anesthesia loss of reflex (S5) were lasted the longest time and it resulted as 227±1.73 sec and 276±10.23 sec of treatment, respectively. For two temperatures at 0.5 ml L^{-1} dose of anesthesia S5 time was the shortest 68±1.63 sec and 80±4.49 sec respectively. At the duration of recovery ability of swimming was observed as shortest at the dose of 0.2 ml L^{-1} with 85±3.6 sec and 58.5±1.08 sec at 13 ºC and 18 ºC, respectively. The longest time for recovery stage was 0.5 ml L^{-1} dose of anesthetic treatment for both two temperatures. It was completed at 208±10.23 sec for 13 ºC and 107±10.61 sec for 18 ºC (Figs. 1 and 2).

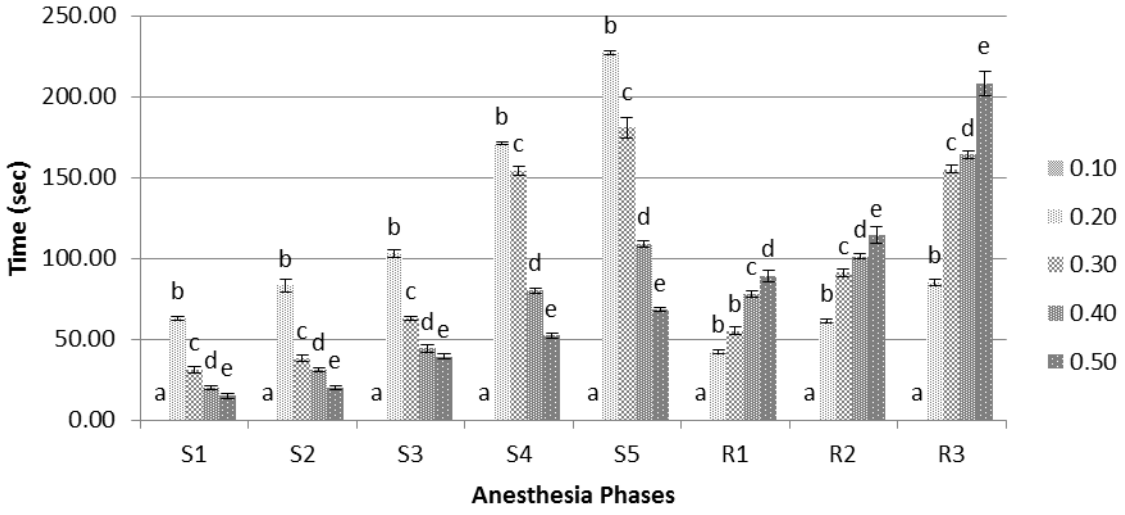

Figure 1: The time of anesthesia phases at different concentrations of 2-PE at 13 °C

*The letters with different superscripts within the same stage are significantly different (p<0.05).

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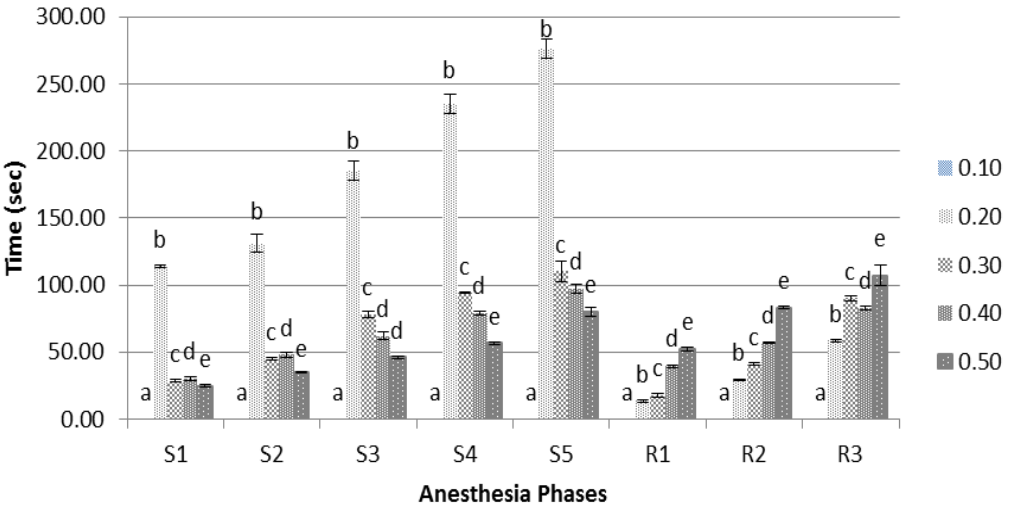

Figure 2: The time of anesthesia phases at different concentrations of 2-PE at 18 °C.
*The letters with different superscripts within the same stage are significantly different (p<0.05).

Discussion

Fish welfare is over against by stress and has become an increasing concern in the operation of capture, rearing and research operations (Huntingford *et al*., 2006; Browman and Skiftesvik., 2007). In aquaculture, sedative and anesthetic agents are very useful for reducing the stress caused by handling, sorting, transportation, stripping, tagging, administration of vaccines and surgical procedures (Mylonas *et al*., 2005; Altun and Danabaş, 2006; Weber *et al*., 2009).

Water quality needs to be carefully controlled during an anesthesia procedure, the main problems involved being those faced by all aquatic animals: control of temperature, dissolved oxygen concentration, ammonia levels and other solids in the baths (Ross and Ross, 2008; Serezli *et al*., 2011), who developed work around our study area, reported water parameters similar to those observed in our study.

In this study, the fish species, new for aquaculture, the Munzur trout (*S. munziricus*) was used as treatment material to determine the effective anesthetic concentrations of 2-PE, at a concentration of 0.1 ml L^{-1} to 0.5 ml L^{-1}. 2-PE was found to be effective anesthetic for Munzur trout juveniles. An ideal anesthetic should produce anesthesia rapidly (e.g., less than 3 or 5 min), allow a speedy recovery, not be toxic to fish and users, leave low tissue residues, and be inexpensive (Marking and Meyer, 1985; Ross and Ross, 2008). In the present study, loss of equilibrium was not observed despite 10 minutes of waiting at a dose of 0.1 L^{-1} at 13 and 18 ºC. After this duration, the experiments in this concentration were ended assuming that the concentration of 2-PE in the medium falls due to the time course of active compound. However, the loss of reflex was occurred at the all concentrations; except for 0.2 ml L^{-1} between 68±1.63 sec and 276±11 sec.

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In general, higher water temperatures appear to augment both the anesthetic effects of a chemical, but also the recovery of the fish (Weyl *et al*., 1996; Mylonas *et al*., 2005; Yildiz *et al*., 2013). This is probably related to the higher metabolism and opercular ventilation rates of fish maintained at higher temperatures. Similarly, in the present study, for all doses of 2-PE recovery time at 18 °C was lower than at 13 °C. Recovery times were in the range of 58.5-208 sec at all concentrations except for 0.1 ml L^{-1} concentration at 13 °C and 18 °C. Recovery time positively correlated with concentration of anesthetics (Weyl *et al*., 1996; Kizak *et al*., 2013). In the present study, recovery times were increased with increasing the concentration of 2-PE, as reported Terzioglu (2001) and Mylonas *et al*. (2005) although some researchers determined that increasing the concentration did not affect the recovery time (Mattson and Riple, 1989; Malmstrøm *et al*., 1993). Additionally, it was observed that induction and recovery times are related to the anesthetic concentration. Whereas it has been seen that it is difficult to precisely distinguish the anesthesia phases from each other. This leads to confusion among the evaluations of various researchers.

Summary of 2-PE applications for fish species and comparisons with the present study results were shown in Table 2.

Table 2: Comparison of lowest effective 2-PE concentrations reported for some aquaculture fishes.

Species	Weight (g)	Concentration (ml L^{-1})	Temperature (ºC)	Reference
Sea bass (*Dicentrarchus labrax*)	32.8±1.8	0.35	25	Mylonas *et al*. (2005)
Gilthead sea bream *(Sparus aurata)*	44.1±2.2	0.30	25	Mylonas *et al*. (2005)
Senegalese sole *(Solea senegalensis)*	99 ± 2.5	0.60	14±1	Weber *et al*. (2009)
White sea bream (*Diplodus sargus)*	30-60	0.167	20±1	Tsantilas *et al*. (2006)
Sharpsnout sea bream, (*Diplodus puntazzo*)	15-30	0.167	20±1	Tsantilas *et al*. (2006)
Meagre *(Argyrosomus regius)*	1.3±0.03	0.3	20±1	Serezli *et al*. (2011)
Rainbow trout (*Oncorhynchus mykiss*)	39.08 ± 1.13	0.3	7-18	Yıldız *et al*. (2013)
Shabbout *(Barbus grypus)*	750.26±126.97	0.5	25	Öğretmen *et al*. (2016)
Munzur trout *(Salmo munzuricus)*	4.42±0.62	0.3	13-18	Present study

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Our results indicated that 2-PE could be used to minimize the stress on fingerling Munzur trout (*S. munzuricus*) ongoing cultural studies with the 0.3 ml L^{-1} dose, at 13 and 18 ºC, safely. Not only this study is the first one being for determining the influence of 2-PE on Munzur trout unique to this region on species based, but also it is important for the studies to cultivate this species.

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