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Research Article

Anesthetic Efficiency of 2-Phenoxyethanol on Broodstock of *Salmo munzuricus*, a New Trout Species Originating from Munzur Stream [1]

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Abstract

Salmo munzuricus is a newly-named species of trout that spreads through rivers flowing along the Munzur Valley National Park. The efficacy of anaesthetic 2-phenoxyethanol was evaluated in indigenous brown trout in the present study. Fish were divided into three weight classes: 100-200 g (W1), 200-400 g (W2) and 400-600 g (W3) and they were exposed to four concentrations of anaesthetic (0.2, 0.3, 0.4 and 0.5 mL L^{-1}). The lowest induction time was identified in the group W3 while the highest one was in the group W1. The lowest recovery time occurred after 73.2±3.83 sec in the group W3 and the highest one was 136±31.46 sec in the group W1. The recovery times were increased with increasing the concentration of 2-phenoxyethanol in the group W1. However, recovery times in the groups W2 and W3 were significantly longer than observed in W1, generally. Besides, it was indicated that fish size affected induction times and recovery times. As a result of the present study, the most appropriate concentrations were determined as 0.4 mL L^{-1} at weights of 100-400 g while 0.5 mL L^{-1} at weights of 400-600 g for 2-phenoxyethanol in *Salmo munzuricus* broodstock.

Keywords: *Fish anesthesia, Recovery, Brown trout, Salmo munzuricus, Broodstock, 2-phenoxyethanol, Anesthetic, Effective concentration, Fish size*

Munzur Akarsuyu Orijinli Yeni Bir Alabalık Türü Olan *Salmo munzuricus*'un Anaç Bireyleri Üzerinde 2-Fenoksietanol'ün Anestezik Etkinliği

Öz

Salmo munzuricus Munzur Vadisi Milli Parkı boyunca akan akarsularda yayılım gösteren yeni isimlendirilmiş bir alabalık türüdür. Bu çalışmada, anestetik madde olarak kullanılan 2-fenoksietanolün etkinliği Munzur akarsuyundan yakalanan yerli kahverengi alabalık üzerinde değerlendirilmiştir. Balıklar, 100-200 g (W1), 200-400 g (W2) ve 400-600 g (W3) olmak üzere üç ağırlık sınıfına ayrılmıştır ve çalışma 0.2, 0.3, 0.4 ve 0.5 mL L^{-1} olmak üzere 4 farklı anestetik konsantrasyonda yürütülmüştür. En kısa indüksiyon zamanı W3 grubunda, en uzun indüksiyon zamanı W1 grubunda bulunmuştur. En kısa anesteziden uyanma süresi W3 grubunda 73.2±3.83 sn sonra ortaya çıkmış ve en uzun uyanma süresi W1 grubunda 136±31.46 sn olarak tespit edilmiştir. W1 grubundaki 2-fenoksietanol konsantrasyonunun artması ile iyileşme süreleri artış göstermiştir. Bununla birlikte, W2 ve W3 gruplarındaki uyanma sürelerinin, W1 grubunda gözlemlenenden genellikle anlamlı derecede daha uzun olduğu belirlenmiştir. Ayrıca balık büyüklüğünün indüksiyon sürelerini ve uyanma sürelerini etkilediği de tespit edilmiştir. Bu çalışmanın sonucu olarak, 2-fenoksietanol için *Salmo munzuricus* anaçlarında en uygun konsantrasyonlar 100-400 g ağırlık aralığında 0.4 mL L^{-1}, 400-600 g aralığında ise 0.5 mL L^{-1} olarak belirlenmiştir.

Anahtar sözcükler: *Balık anestezisi, Uyanma, Kahverengi alabalık, Salmo munzuricus, Anaç balık, 2-phenoxyethanol, Anestetik, Etkili konsantrasyon, Balık boyutu*

INTRODUCTION

Anesthetics, first applied in the medical field in the 1840 [1] are also widely used in aquatic and terrestrial animals [2-6]. Tricaine methanesulfonate (MS222), benzocaine and 2-phenoxyethanol (2-PE) are the most widely used anesthetics in aquaculture [7-9], with anesthesia usually being induced by immersing the fish in a solution of a given concentration. After MS222, 2-PE is the most commonly used anesthetic in aquaculture. Permissible anesthetic agents are very limited in fishes which are consumed as food, despite the variety of anesthesia applications. Optimum anesthetic

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concentrations can minimize the negative impact and thus reduce stress in fish. Optimum anesthetic concentrations are usually expected to induce anesthesia within 3 min and recovery within 10 min [10-12]. 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 concentrations with being safe for users and consumers [13,14].

The mass-specific basal metabolic rate -oxygen consumption divided by body mass- decreases with increase in body mass of an animal and thus larger animals have lower oxygen consumption relative to body size than do smaller animals [3,15]. Results of the numerous studies on anesthetic agents revealed that the effective concentrations for anesthesia vary with fish body size and water temperature [3,7] with the smaller fish being more responsive than the larger one [14].

Trouts of the genus Salmo are present in almost all cold streams and rivers of Anatolia where they have a high diversity. The Munzur Stream arises from the Munzur Mountains in the north of Ovacik and joins the Pülümür Stream and flows into the Keban Dam Lake. *Salmo munzuricus,* their identity was not clear up to now, newly described by Davut et al.[16], commonly known as red spotted trout is distinguished from other Anatolian Salmo species by having a large adipose fin in male (almost as large as dorsal or anal fins in older males), with a very narrow white margin, then red submarginal band, then a white stripe or spots, then red again in males. It is an endangered salmonid species with high economic value in Munzur Stream which is an important water supply in Turkey. Developing the breeding program is so important to sustain natural population and introduction of the species to aquaculture and to protect its natural population, as well. Red spotted trout breeding studies are ongoing by catching them from the wild. Because this is a new species there is lack of data referring to anesthesia concentrations which are important topic for future studies. Handling stress can cause bad quality of eggs and also some mortality after stripping in trout. Sedation of fish before manipulating is an important issue in aquaculture studies. The objective of this study is to investigate the effects of 2-PE at different concentrations on broodstock specimens of *Salmo munzuricus* at different body weights.

MATERIAL and METHODS

Fish Holding and Experimental Design

The trout broodstock used in the study were caught from the Munzur stream and stocked in 300 L of stock tanks before the treatments. After 3 days of each fish stocking they were randomly taken from the tanks and transferred immediately into the aquariums of 40 L tap water which contains 2-phenoxyethanol at different concentrations, one by one. Surveys of total lengths and weights were performed as quickly as possible on anaesthetized fish which lost reflex to external stimuli. The study was approved from the Fırat University Animal Experiments Local Ethics Committee (FÜ-HADYEK/2016-35, Desicion Number: 47).

In the experiment, fish were sorted by size into three groups as W1, W2 and W3 of the average weight of 150±70.71 g, 300±141.42 g, 500±141.42 and length of 22.68±0.60 cm, 31.87±0.32 cm and 37.98±0.17 cm of, respectively. Twenty animals were used in each group of weight in the study. The experiments were conducted independently for each group, using a completely randomized design, with four concentrations (0.2, 0.3, 0.4 and 0.5 mL L^{-1}) of 2-PE (with %99 purity, BASF, Germany). Each anesthetic treatment consisted of 5 repetitions (each fish was considered a replicate) in a total of 60 animals. All of the fish were starved for 48 h prior to experiment.

The tap water used in the aquariums was aged for a day before in the large tanks while fixing the water temperature using 100 watt power heater at 15ºC with continuous aeration. Then it was transferred to the aquariums just before the anesthesia treatments. Temperature was measured by 0.1ºC sensitivity digital thermometer while dissolved oxygen was measured by hand-held oxygen meter (YSI Professional Plus). Firstly, 2-PE was diluted with the water from the aquarium by mixing with glass stripe at calculated portions until it was fully dissolved and then added to the treatment aquarium.

Anesthetic bath was constantly mechanically aerated and the oxygen concentration was maintained at 7.25±2.5 mg L^{-1} during the treatments by this way. pH level was measured 8.05±1.5. After anesthetic assessments, fish from each treatment were placed in a large tank and monitored for 24 h before feeding was resumed. The specified stages of anesthesia were recorded as the second by chronometer [17].

Anesthesia Phases and Fish Behavior Characteristics

The criteria to identify each particular stage are presented in *Table 1*. Determination of induction (sedation = S) and recovery (R) stages modified from Keene et al.[18] .

Statistical Analysis

Analysis of data was carried out using SPSS 15.0. In comparison of the groups, nonparametric Kruskal-Wallis variance analysis was applied. The Mann-Whitney U test was used to compare differences between two independent groups. Statistically significant differences were expressed as $P<0.05$.

RESULTS

Fig. 1, Fig. 2 and *Fig. 3* present the results of induction and recovery of *Salmo munzuricus (Fig. 4)*, exposed to different concentrations of the 2- phenoxyethanol for three different

Table 1. *Anesthesia phases and fish behavior characteristics*			
Main Stages	**Definite Stages**	**Fish Behavior Characteristics**	**Code**
Induction	*Light sedation*	Equilibrium normal, opercular rate slightly decreased,	*S1*
	Deep sedation	Equilibrium normal, slight decrease in opercular rate no respond to weak external stimulus,	*S2*
	Partial loss of equilibrium	Swimming erratic, opercular movements fast, no respond to strong external stimulus,	*S3*
	Total loss of equilibrium	Total loss of equilibrium, regular opercular movement but slow	*S4*
	Loss of reflex	No reflex, opercular movements irregular and slow	*S5*
Recovery	*Partial recovery of equilibrium*	Partial equilibrium and swimming, opercular movements starting,	*R1*
	Total recovery of equilibrium	Total recovery of equilibrium, swimming erratic starting	*R2*
	Total behavioral recovery	Normal swimming starting	*R3*

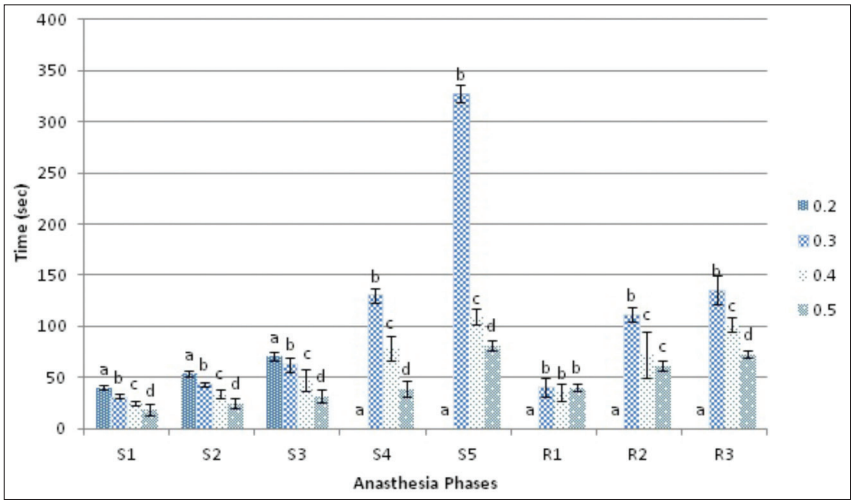
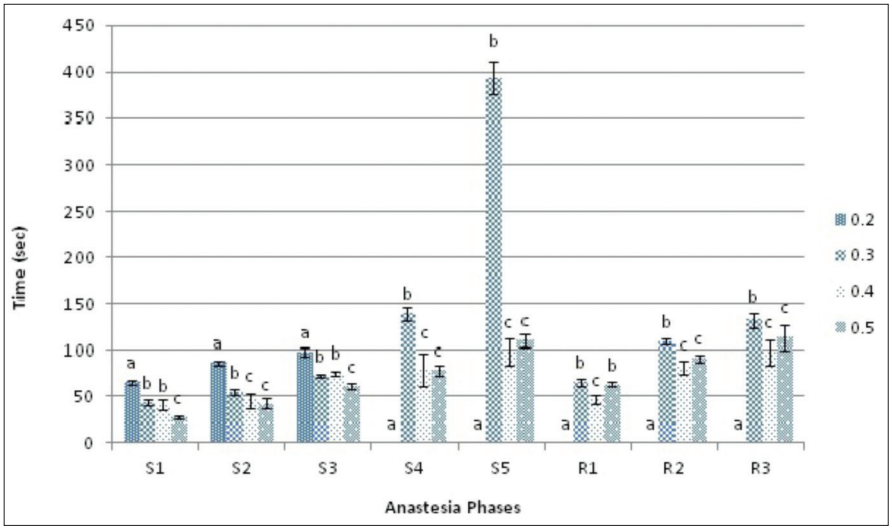


Fig 1. The timing of anesthesia phases at different concentrations of 2-phenoxyethanol for the group of W1 (100-200 g); * Values (mean± SE) with different superscripts within the same phase are significantly different (P<0.05)

Fig 2. The timing of anesthesia phases at different concentrations of 2-phenoxyethanol for the group of W2 (200-400 g); * Values (mean± SE) with different superscripts within the same phase are significantly different (P<0.05)



size classes. No died fish was found during the observation of 24 h post exposure.

The induction times of anesthesia varied with anesthetic concentrations, decreasing with the increase of 2-phenoxyethanol concentrations. On the other hand, the recovery times increased with decreasing of 2- phenoxyethanol concentrations (P<0.05). During the study, at the concentrations of 0.2 mL L^{-1} the total loss of equilibrium was not observed at all groups. Loss of reflex activity (S5) was induced faster at higher concentrations of anesthetic than 0.2 mL L^{-1} at all weights (P<0.05) *(Fig. 1, Fig. 2, Fig. 3)*. Loss of reflex activity (S5) was induced faster at anesthetic concentrations higher than 0.2 mL L^{-1} at all weights. S5 was between 73.00±3.16 sec and 476.80±28.09 sec in W3 and W1 group, respectively. On the other hand, the lowest time value of total behavioral recovery (R3) was determined as 73.2±3.83 sec at the

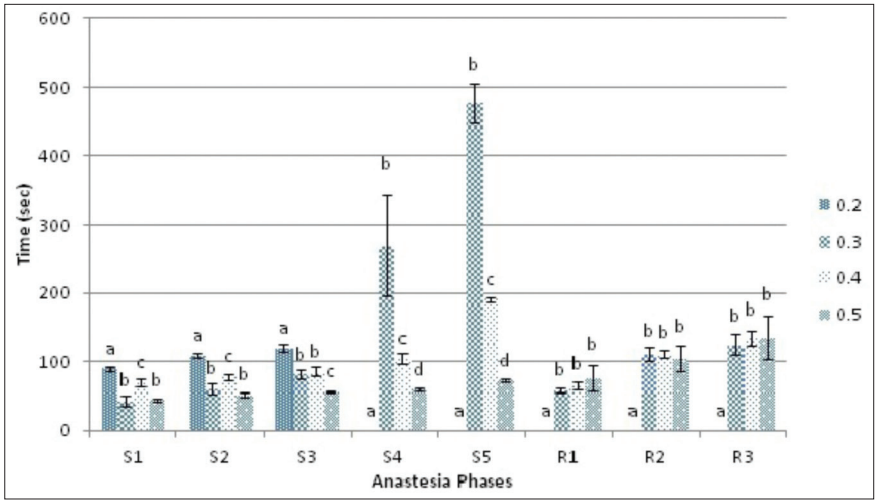


Fig 3. The timing of anesthesia phases at different concentrations of 2-phenoxyethanol for the group of W3 (400-600 g); * Values (mean± SE) with different superscripts within the same phase are significantly different (P<0.05)

Fig 4. *Salmo munzuricus* during the anesthesia treatment

group of W3 and the highest one was 136±31.46 sec at the W1 group.

DISCUSSION

Animal welfare is compromised by stress and has become an increasing concern in the operation of capture, rearing and research operations [19,20]. In aquaculture, sedative and anesthetic agents are very useful for reducing the stress caused by handling, sorting, transportation, artificial reproduction, tagging, administration of vaccines and surgical procedures [7,8,21].

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 [22,23].

In this study, the effective anesthetic concentrations of 2-phenoxyethanol for *Salmo munzuricus,* a fish species new to aquaculture, were determined. Effective concentration of 2- phenoxyethanol ranged from about 0.2 to 0.5 mL L^{-1} at different size class. An ideal anesthetic should produce anesthesia rapidly (e.g., less than 3 min), allow a speedy recovery, not be toxic to fish and users, leave low tissue residues, and be inexpensive [24]. 2-phenoxethanol was found to be effective anesthetic for *Salmo munzuricus* broodstock in the present study. However, total loss of equilibrium was not observed despite 10 min of waiting at all groups at 0.2 mL L^{-1} concentration. After this period, the exposure to this concentration was finished assuming that the concentration of phenoxyethanol in the bath was too low. However, the loss of reflex was occurred at all concentrations between 73.00±3.16 and 476.80±28.09 sec in W3 and W1 group, respectively.

Recovery times were observed in the range of 73.2±3.83 and 136±31.46 sec at all concentrations except for 0.2 mL L^{-1}. According to Weyl et al.[4] recovery time positively correlated with concentration of anesthetics in goldfish. In the present study, recovery times were increased with increasing the concentration of 2- phenoxyethanol at the group of W1, as reported in previous studies [4,7,25] although some researchers determined that increasing the concentration did not affect the recovery time [2,26]. However, the group W2 and W3 was different from the W1. However there is actually an insignificant difference between recovery times of W1 and W2. This finding suggests that the fish size may be related to the anesthetic activity. So, the group of W3 was the biggest one. It is because the rate of oxygen consumption, ratio of body volume to gill surface area and rate of gill perfusion are important factors affecting uptake and elimination of anesthetic agents [27], and gill surface area decreases in relation to increased body weight [27,28]. Fish size had an effect on induction times of 2-P phenoxyethanol in other fish [8,17,29], and in the present study, as well.

According to the results of the study with rainbow trout *(Oncorhynchus mykiss)* the authors reported that anaesthetic concentration of 0.3 mL.L^{-1} 2-phenoxyethanol is safe for rainbow trout [30]. Our optimum effective concentrations are higher than theirs although they were lower than the concentration of 0.8 mL.L^{-1} in common carp

(Cyprinus carpio) [28]. This difference may also be due to the effect of anesthetic is species specific since anesthetic absorption through gills can be varied in different species.

Additionally, it was determined that induction and recovery times are related to the anesthetic concentration. However, it was observed that it is difficult to precisely distinguish the anesthesia phases from each other. This leads to confusion among the evaluations of various researchers.

Consequently, our results indicated that 2-phenoxyethanol could be used to anesthetize indigenous brown trout *(Salmo munzuricus)* broodstock, it is important for the future studies of this species, at the concentrations of 0.4 mL L^{-1} at weights of 100-400 g, and 0.5 mL L^{-1} concentrations at weights of 400-600 g, safely.

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