The Anesthetic Effects of Clove Oil and 2-Phenoxyethanol on Rainbow Trout (*Oncorhynchus mykiss*) at Different Concentrations and Temperatures

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Abstract

In this study, anesthetic effects of five different concentrations of 2-phenoxyethanol (0.2, 0.3, 0.4, 0.5 and 0.6 ml/L) and clove oil (0.50, 0.75, 1.00, 1.25 and 1.50 ml/L) on rainbow trout (*Oncorhynchus mykiss*) were studied at temperatures 7, 13 and 18ºC. For this purpose, 900 fish (39.08 ± 1.13 g and 15.48 ± 0.21 cm) were used in the experiment. Induction time of 2-phenoxyethanol and clove oil varied between 1.05 and 3.36 min at all concentrations, except for 0.2 ml/L (for 2-phenoxyethanol only) and at every temperature application. Full recovery time occurred between 2.44 and 7.14 min for 2-phenoxyethanol and 3.23 – 6.11 min for clove oil. It was found that full recovery times significantly increased with increase in 2-phenoxyethanol concentrations (r^2=0.81). The same increasing trend was observed in clove oil, but the increase was not strong compared to 2-phenoxyethanol (r^2=0.21). On the other hand, full induction times of 2-Phenoxyethanol and clove oil significantly declined with the increase in concentrations (r^2=0.74; r^2=0.84 for 2-phenoxyethanol and clove oil, respectively). Based on the ideal induction (less than 3 min) and recovery (less than 5 min) time criteria, it can be suggested that the most appropriate concentrations for rainbow trout were 0.3, 0.4 and 0.5 ml/L for 2-phenoxyethanol and 0.50, 0.75 and 1.00 ml/L for clove oil.

Keywords: Anesthetic, Clove oil, *Oncorhynchus mykiss,* Temperature, 2 phenoxyethanol

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Introduction

Anesthetics are very important for aquaculture studies and used to minimize the stress due to aquaculture procedures. The necessity of transporting and handling live fish (marking, counting, injection, stripping, weighing) within various fish enhancement programmes, commercial fisheries and the fish-farming industry has led to the development of techniques to anaesthetize fish without impairing their health or commercial value. Chemical anaesthetics have a wide variety (Bell, 1964, 1987; Iwama and Ackerman, 1994; Altun and Danabaş, 2006; Altun et al., 2009). The use of chemical anaesthetics in food is or phenoxetol) is colorless and contains 1.11 g/ml fat density. In addition, 2-phenoxyethanol is cheaper than other anesthetics. The safety and efficacy of 2-phenoxyethanol were tested on many fish species (Gilderhus and Marking, 1987; Hseu et al., 1994; Weyl et al., 1996; Weber et al., 2009; Uçar and Atamanalap, 2010). As a result, the anesthetic is widely used for transporting live fish (Teo et al., 1989, Teo and Chen, 1993; Guo et al., 1995).

Clove oil is one of the alternatives anaesthetics and a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Soto and Burhanuddin, 1995). According to Isaacs (1983), Briozzo et al. (1989) and Keene et al. (1998), ingredient of clove oil, makes up 70 to Gholipour kanani et al., (2011) but there is a limited information in the literature on effect of clove oil at different temperatures.

The objective of this study was to examine anesthetic effects of five different concentrations of 2-Phenoxyethanol and clove inappropriate due to economic, safety and especially regulatory considerations (Carpenter, 1994; Iwama and Ackerman, 1994; Prince et al., 1995; Terzioglu, 2001). Effect of biotic and abiotic factors on fish is one of the most important factors that change the effectiveness of anesthetics (Endo et al., 1972, Sylvester, 1975; Houston and Corlett, 1976; Smith and Hattingh, 1979; Amend et al., 1982; Limsuwan et al., 1983; Hseu et al., 1994; Weyl et al., 1996).

2-phenoxyethanol (2-PE, ethylene glicol monophenyle ether, 1hydroxy2phenoxyethane,

90% of clove oil by weight. Clove oil also contains eugenol acetate (> 17%) and kariofilen 5 (> 12%). Although reports of clove oil use as a potential fish anesthetic dates back 35 years (Endo et al., 1972), the intensive use of clove oil as an anesthetic is designated in recent years (Soto and Burhanuddin, 1995; Keene *et al*., 1998; Wagner et al., 2003, Cho and Heath, 2000; Kanyilmaz et al., 2007; Gullian and Villanueva, 2009; Sudagara et al., 2009; Zahl et al., 2009; Imanpoor et al., 2010; Akbulut et al., 2011a,b; Doleželová et al., 2011; Akbulut et al., 2012). A great deal of past research has focused on anesthetic effect of clove oil in rainbow trout Akhlaghi and Brojerdi, 1999; Holloway et al., 2004; Velisek et al., 2005; Perdikaris et al., 2010; Uçar and oil on rainbow trout (*Oncorhynchus mykiss*) at different temperatures.

Materials and methods

Rainbow trout (n=900; 39.08±1.13g, 15.48±0.21 cm mean±SD) were obtained from

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Alpoğlu Fisheries Research Station, aquariums with a rearing volume of 25 L (25 x 25 x 40 cm). Fish were equally allotted to groups (10 fish) with three replicates for each treatment. The fish were fed commercial trout food. The fish were acclimated for 24 h in a 25 L aquarium prior to trial, in aerated water. Temperature was measured with a digital thermometer. The oxygen has been measured by OXYGUARD model oxygen- meter as 7.0-7.5 mg/L. pH in the groups was 7.9-8.2. Anesthetic effects of 2- phenoxyethanol (0.2, 0.3, 0.4, 0.5 and 0.6 ml/L) and clove oil (0.50, 0.75, 1.00, 1.25 and 1.50ml/L) were determined at temperatures 7, 13 and 18ºC. Due to its incomplete solubility in water at temperatures below 15°C, clove oil was first dissolved in ethanol at a ratio of 1:10 (clove oil: ethanol 95%). The density of clove oil is approximately 100 mg/ml (Lewbart, 2001). 2-phenoxyethanol is soluble in water (26.7 g/l) at 25°C but readily soluble in ethanol. The anesthetic was added to the test container and thoroughly mixed, and then one fish was randomly selected from the acclimation aquarium and transferred by net to the anesthetic bath. The air supply to the anesthetic bath was removed immediately before introduction of a fish so that clear observations could be made on fish behavior during the induction period.

The four stages of induction of anesthesia caused by exposure to clove oil, 2-phenoxyethanol under identical experimental conditions are described as follows:

Induction 1, partial loss (50%) of equilibrium and erratic swimming;

Induction 2, total loss of equilibrium;

Uzunburun, Tokat. Fish were reared in

Recovery1, partial recovery (50%) of induction; and

Recovery 2, total recovery of induction.

Induction and recovery times within groups were judged visually and measured with a stopwatch to the nearest second.

All data are presented as mean ±SE. Statistical analyses were performed using SAS software. Two-way analysis of variance (ANOVA) was conducted to compare differences among anesthesia treatments. Regression analysis was performed to test significant differences in relationship between concentrations of anesthetic and effect durations. The significance level for all statistical tests was ($P<0{\cdot}05$).

Results

The mean times of the duration of anesthesia and recovery at different concentrations are presented in Figure 1. At concentrations of 0.50 ml, the longest induction time, total loss of equilibrium (induction 2) was 3.36±0.19 min at 18°C, the shortest time was 2.41±0.11 min at 13°C. The longest induction time total recovery of equilibrium (Recovery 2) was 5.17±0.04 min. at 7°C and the shortest time was 3.23±0.10 min at 18°C (Fig. 1a). The clove oil at the concentration of 0.75 ml, the longest induction time total loss of equilibrium (Induction 2) was 2.16±0.06 min at 18°C, the shortest time was 1.56±0.28 min at 13°C. The longest induction time total recovery of equilibrium (Recovery 2) was 5.43 ± 0.11 min at 13 °C and the shortest time was 3.53 ± 0.42 min at 18 °C (Fig. 1b). At the concentration of 1.00 ml, the longest induction time total loss of equilibrium

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(Induction 2) was 1.55 ± 0.28 min at 18 °C, the shortest time was 1.31 ± 0.05 min at 13 °C. In the recovery process, the longest induction time total recovery of equilibrium (Recovery 2) was 4.57 ± 0.26 min at 18 °C and the shortest time was 5.54 ± 0.06 min at 7 °C (Fig. 1c). At clove oil concentration of 1.25 ml, the longest induction time total loss of equilibrium (Induction 2) was 1.30±0.04 min at 18°C, the shortest time was 1.09±0.03 min at 7°C. The longest induction time total recovery of equilibrium (Recovery 2) was 4.47±0.10 min at 18°C and the shortest time was 5.54±0.06 min at 7°C (Fig. 1d). The clove oil at the concentration of 1.50 ml, the shortest induction time total loss of equilibrium was (Induction 2) 1.05±0.31 min at 13°C. The longest induction time total recovery of equilibrium (Recovery 2) was 5.47±0.09 min at 7°C (Figure. 1e).

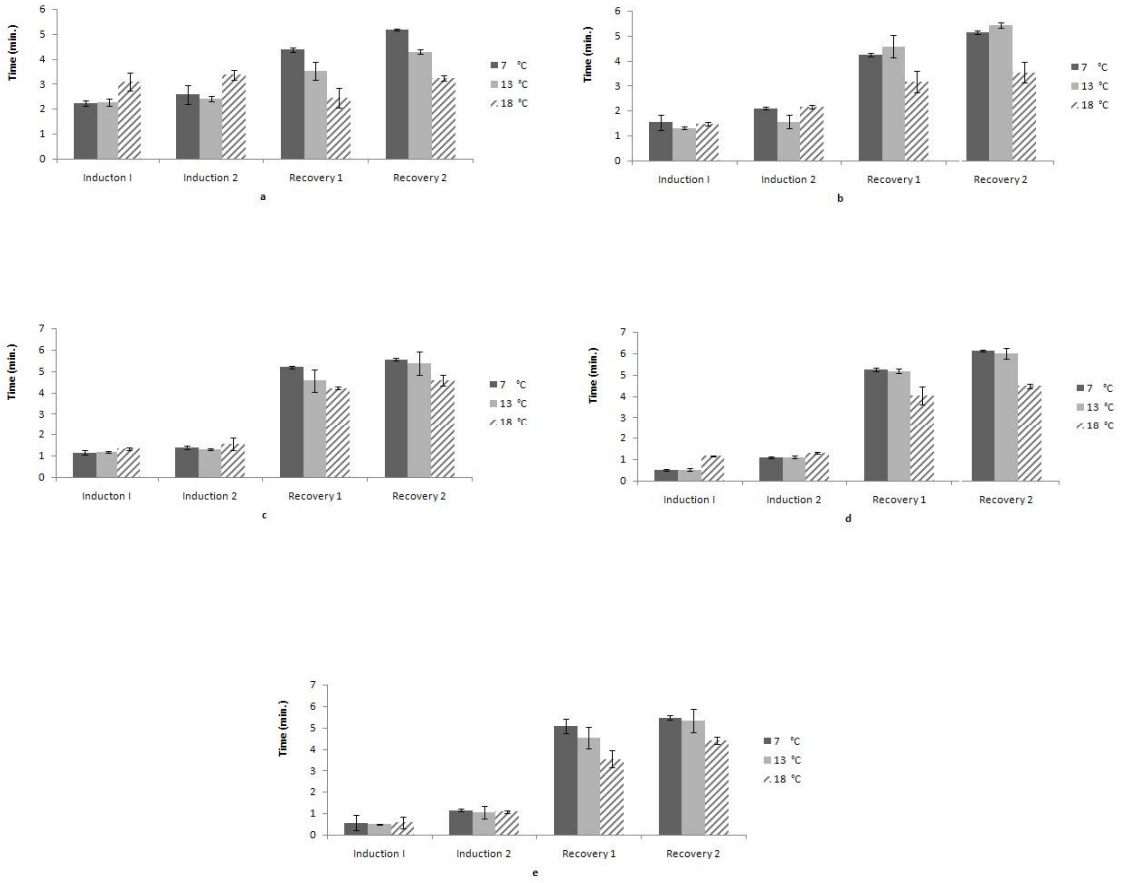

Figure 1: Induction and recovery times for rainbow trout (*Oncorhynchus mykiss*) anesthetized with clove oil at concentrations of 0.50, 0.75, 1.00, 1.25 and 1.50 ml/L and at temperatures 7, 13 and 18°C.

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In this study, 10 fish died during post-experimentation period. The mean times of the duration of anesthesia and recovery at different concentrations are presented in Figure 2. During the experiment, at the concentration of 0.2 ml/L, the longest induction time total loss of equilibrium (Induction 2) was 7.17 ±0.77 min. at 13°C, the shortest time was 4.01 ± 0.32 min at 7°C. The longest induction time total recovery of equilibrium (Recovery 2) was 3.11±0.07 min at 7°C and the shortest time was 2.44±0.05 min. at 18°C (Figure 2a). At the concentration of 0.3 ml/L, the longest induction time total loss of equilibrium (Induction 2) was 3.19±0.12 min at 18°C, the shortest time was 2.34±0.13 min at 7°C. The longest induction time total recovery of equilibrium (Recovery 2) was 3.58±0.43 min at 7°C and the shortest time was 3.40±0.13 min at 18°C (Figure 2b). For the clove oil at the concentration of 0.4 ml/L, the longest induction time total loss of equilibrium (Induction 2) was determined as 2.54±0.07 min at 13°C, the shortest time was 1.38±0.07 min at 18°C. The longest induction time total recovery of equilibrium (Recovery 2) was 4.14±0.07 min at 13°C and the shortest time was 4.45±0.42 min at 7 °C (Figure. 2c). At clove oil concentration of 0.5 ml/L, the longest induction time total loss of equilibrium (Induction 2) was determined as 2.54±0.07 min at 13°C, the shortest time was 1.38±0.07 min at 18°C. the longest period of regaining equilibrium was observed at 13°C as 4.14±0.07 min and the shortest time was 4.45±0.42 min at 7°C (Fig. 2d). At the concentration of 0.6 ml/L, the shortest induction time total loss of equilibrium (Induction 2) was 1.09±0.32 min at 18°C. The longest induction time total recovery of equilibrium (Recovery 2) was 4.14±0.07 min at 13°C and the shortest time was 7.14±0.39 min at 13°C (Figure. 2e).

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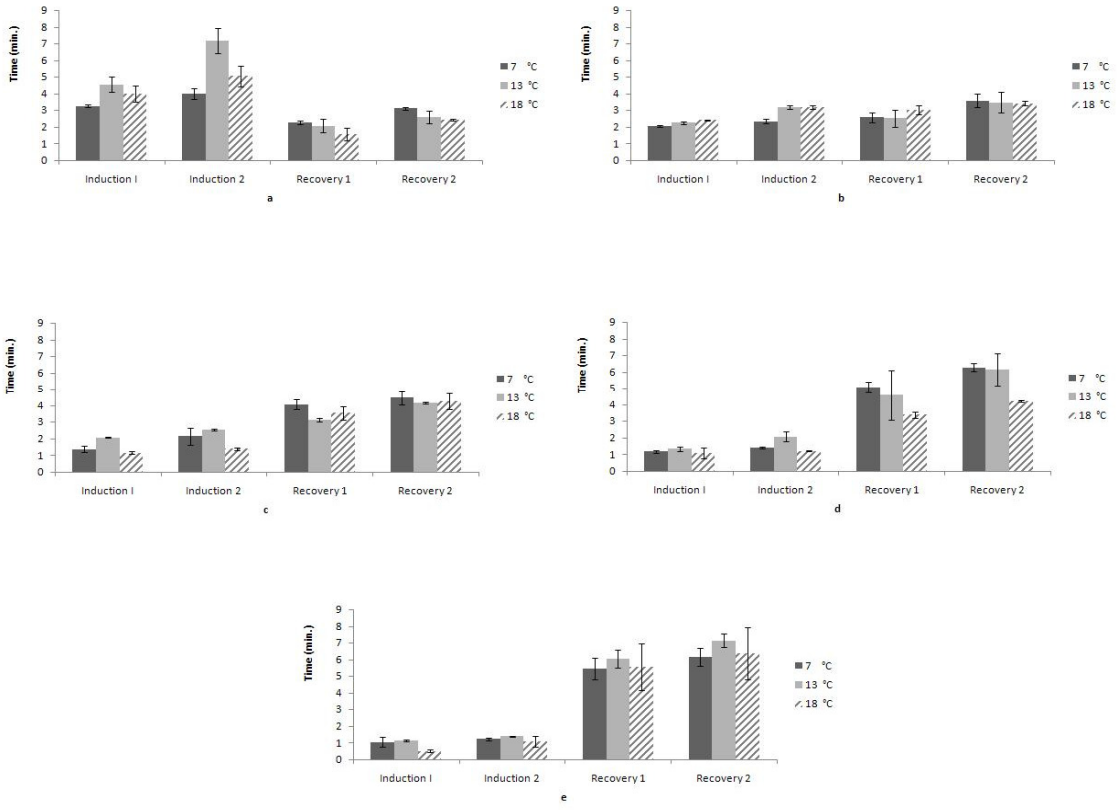

Figure 2: Induction and recovery times for rainbow trout (Oncorhynchus mykiss) anesthetized with 2-phenoxyethanol at concentrations of 0.2, 0.3, 0.4, 0.5 and 0.6 ml/L and at temperatures 7, 13 and 18ºC.

Induction time partial and total loss of equilibrium showed statistically significant difference in different concentrations [F4, 30=157.40, P<0.0001 (partial loss of equilibrium), F4, 30 = 150.50, P <0.0001 (total loss of equilibrium)] and temperatures [F8, 30 = 4.11, P = 0.0021 (partial loss of equilibrium), F8, 30 = 3.26, P = 0.0087 (total loss of equilibrium)]. In general, induction time partial and total loss of equilibrium decreased with increasing concentration (Figure 3). At 18°C, induction time partial and total loss of equilibrium was occurred in earlier periods than 7°C and 13°C (Figure. 1).

Significant (P<0.05) linear regressions were found between concentrations of anesthetic and effect durations. It was determined that relationship between induction time partial and total loss of equilibrium was logarithmically decreasing. [time = -1.6847 Ln (concentration) +1.1922, r2=0.8352 (partial loss of equilibrium) (Fig. 3a), Length =-1.6593 Ln (concentration) +1.5818, r2 = 0.838 (total loss of equilibrium)] (Figure. 3b).

The results indicated that there was a statistically significant interaction of temperature and concentration on induction time partial and total loss of equilibrium

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[F8, 30=4.11, P=0.0021 (partial loss of equilibrium), F8, 30=3.26, P=0.0087 (total loss of equilibrium)]. In all concentrations of clove oil, induction time partial loss of equilibrium (Induction1) increased with increasing temperature. However, this was not observed at 0.75 ml/L and 1.50ml/L concentrations. Induction time at 7 °C was longer than other temperatures at 0.75 ml/L concentrations. At 1.50 ml/L concentration, partial loss of equilibrium was earlier than other concentrations at temperature of 13 °C (Figure. 1).

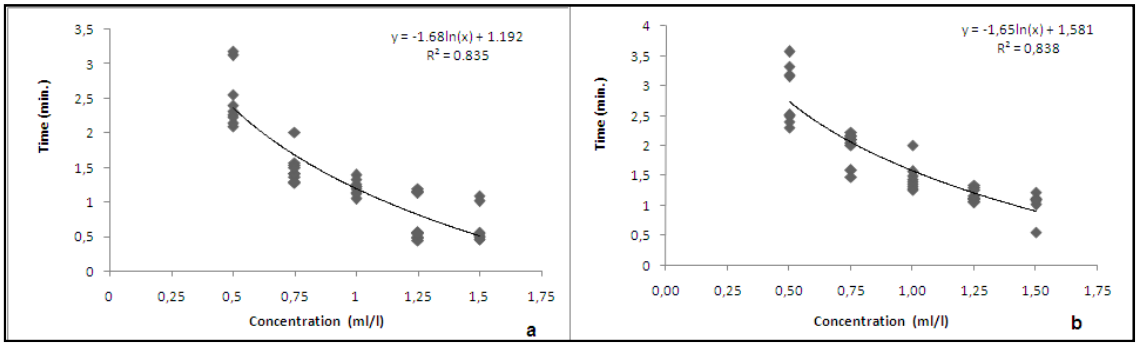

Figure 3: Relationship between concentrations of clove oil and induction time partial loss of equilibrium (a) and total loss of equilibrium (b).

Induction time partial and total recovery of equilibrium (Figure. 4) showed statistically significant difference at concentrations [F4, 30 = 20.94, P<0.0001 (partial recovery of equilibrium), F4, 30 = 31.54, P<0.0001 (total recovery of equilibrium)] and temperature [F8, 30 = 2.47, P = 0.0349 (partial recovery of equilibrium), F8, 30=4.14, P=0.0020 (total recovery of equilibrium)]. In general, induction time partial and total recovery of equilibrium increased with increasing concentration.

Significant (P<0.05) linear regressions were found between concentrations of anesthetic and effect durations. Relationship between induction time partial and total recovery of equilibrium was weakly exponential with

increasing concentration [Time=4.2802 (concentration), 0.2588, r2= 0.2188 (partial recovery of equilibrium) (Fig. 4b), Time=4.9478 (concentration) 0. 2082, r2=0.2092 (partial total recovery of equilibrium) (Figure. 4a)].

The results showed that there was a statistically significant interaction of temperature and concentration on induction time partial and total recovery of equilibrium [F8, 30 = 2.47, P = 0.0349 (partial recovery of equilibrium), F8, 30 = 4.14, P=0.0020 (total recovery of equilibrium)]. In all concentrations of clove oil, induction time partial recovery of equilibrium (Recovery 1) decreased with increasing temperature. However, this was not observed at 0.75 ml/L concentrations. At concentrations of 0.75 ml/L, partial recovery of equilibrium was later than other concentrations at temperature of 13°C (Figure. 1).

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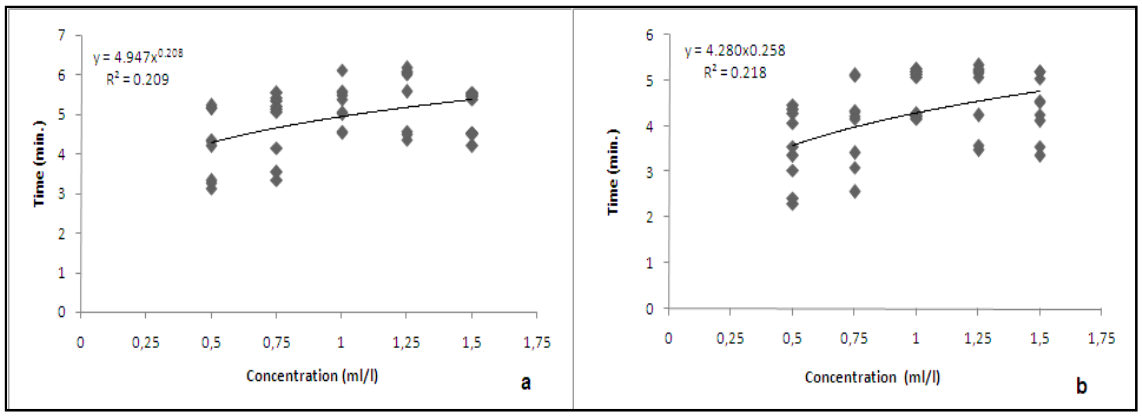

Figure 4: Relationship between concentrations of clove oil and induction time partial recovery of equilibrium (a) and total recovery of equilibrium (b).

Induction time partial and total loss of equilibrium showed statistically significant difference in different concentrations [F4, 30=281.12, P<0.0001 (partial loss of equilibrium), F4, 30 = 218.66, P <0.0001 (total loss of equilibrium)] and temperatures [F8, 30=7.83, P<0.0001 (partial loss of equilibrium), F8, 30=14.46, P<0.0001 (total loss of equilibrium)]. At 7 and 18°C, induction time partial and total loss of equilibrium occurred in earlier periods than 13°C (Figure. 2).

Significant (P<0.05) linear regressions were found between concentrations of anesthetic and effect durations. It was determined that relationship between induction time partial and total loss of equilibrium was logarithmically decreasing [Time = -2.7709 Ln (concentration) -0.7886, r2=0.837 (partial loss of equilibrium), Time=-3.6394 Ln(concentration) -1.0266, r2= 0.7385 (total loss of equilibrium)] (Fig. 5). The results showed that there was a statistically significant interaction of temperature and concentration on induction time partial and total recovery of equilibrium [F8, 30 = 7.83, P<0.0001 (partial recovery of equilibrium), F8, 30 = 14.46, P<0.0001 (total recovery of equilibrium)]. In all concentrations of 2-phenoxyethanol, induction time partial recovery of equilibrium increased with increasing temperature (Figure. 2).

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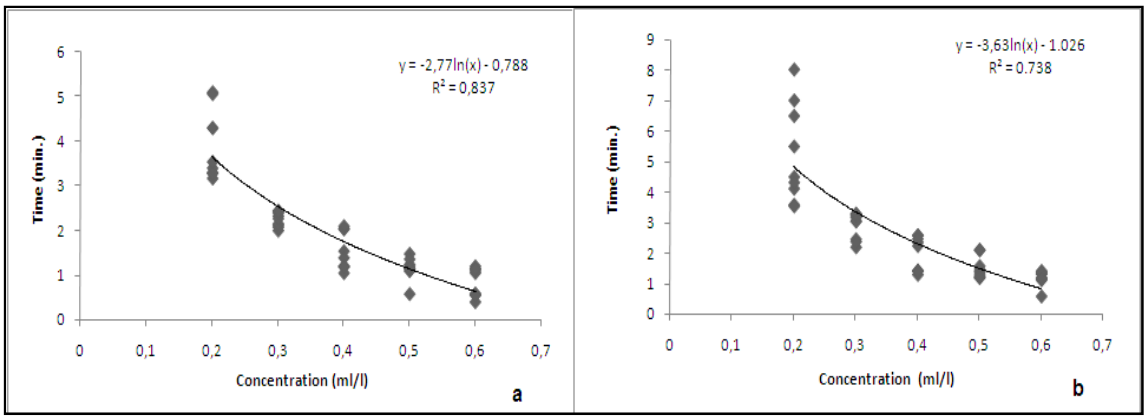

Figure 5: Relationship between concentrations of 2-phenoxyethanol and induction time partial loss of equilibrium (a) and total loss of equilibrium (b).

Induction time partial and total recovery of equilibrium showed statistically significant difference at concentrations [F4, 30 = 45.75, P<0.0001 (partial recovery of equilibrium), F4, 30=57.90, P<0.0001 (total recovery of equilibrium)] and temperature [F8, 30 =1.45, P=0.2180 (partial recovery of equilibrium), F8, 30=2.51, P=0.0322 (total recovery of equilibrium)]. In general, induction time partial and total recovery of equilibrium increased with increasing concentration (Fig. 2). At 0.6 ml/L concentration, partial recovery of equilibrium (Recovery 1) was later than other concentrations at temperature 13 °C (Fig. 2e). Significant (p<0.05) linear regressions were found between concentrations of anesthetic and effect durations. It was determined that relationship between induction time partial and total recovery of equilibrium was exponential [Time = 8.4636 Ln (concentration) -0.9189, R2 = 0.8235 (partial recovery of equilibrium), Time = 9.1236 Ln (concentration) - 0.7585, R2 = 0.8138 (total recovery of equilibrium)] (Fig. 6).

The results showed that there was a statistically significant interaction of temperature and concentration on induction time partial and total recovery of equilibrium [F8, 30=1.45, P=0.2180 (partial recovery of equilibrium), F8, 30=2.51, P=0.0322 (total recovery of equilibrium)]. At 7°C, partial recovery of equilibrium was later than other concentrations at 0.2, 0.4, 0.5 ml/L concentration (Fig. 2).

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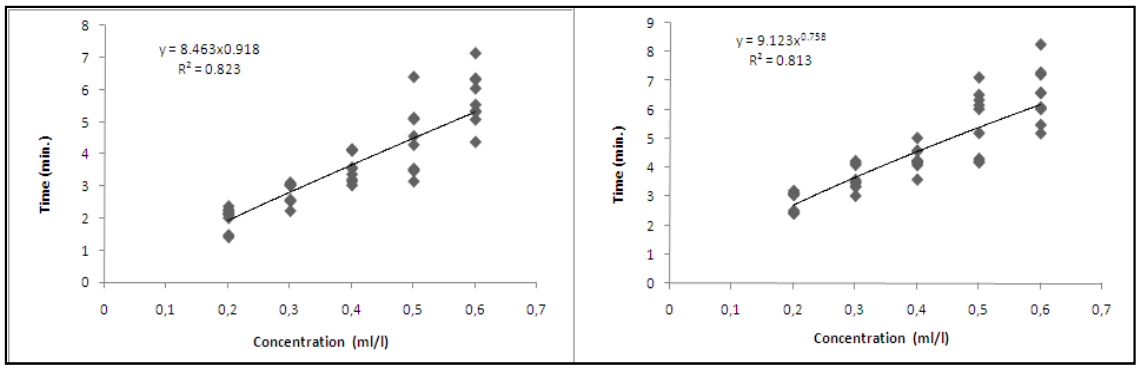

Figure 6: Relationship between concentrations of 2-phenoxyethanol and induction time partial recovery of equilibrium (a) and total recovery of equilibrium (b).

The findings of the present study showed that there were significant differences on duration of partial loss of equilibrium (Induction 1) by anesthetic ($F_{1, 78} = 70.51$, $P<0.0001$). Fish treated with 2-phenoxyethanol reached to total loss of equilibrium (Induction 2) later than clove oil.

The results showed that there were significant differences on duration of partial recovery of equilibrium by anesthetic ($F_{1, 78}=24.10$, $P<0.0001$). Fish treated with clove oil reached to total loss of equilibrium later than 2-phenoxyethanol.

The results indicated that there were significant differences on duration of partial recovery of equilibrium by anesthetic ($F_{1, 78}=11.62$, $P<0.0001$). Fish treated with clove oil reached to total loss of equilibrium later than 2-phenoxyethanol.

Discussion

In the present study, induction time partial and total loss of equilibrium decreased with increasing concentration of 2-phenoxyethanol and clove oil. The present results are in agreement with other studies on fish regarding effect of 2-phenoxyethanol (Mattson and Ripl, 1989; Weyl et al., 1996; Hseu et al., 1997). In comparison to other anesthetics, induction to anesthesia was rapid contrary to recovery time for fish exposed to clove oil. According to Keene et al. (1998) and Kanyılmaz et al. (2007), the reason for the situation is that clove oil has high lipid solubility and removing of clove oil from fish body takes a long time because of the deceleration of respiratory rate.

Marking and Meyer (1985) stated desirable time scales for the induction and recovery from anesthesia for fish as 3 and 5 min, respectively. Our results indicated that induction and partial and total loss of equilibrium at all temperatures and concentrations of 2-phenoxyethanol, except 0.2 ml/L, were 1.05 to 3.36 min. Recovery time of 2-phenoxyethanol and clove oil were 2.44 to 7.14 min. and 3.23 to 6.11 min., respectively. In addition, it was observed that recovery time increased with increasing concentrations of 2-phenoxyethanol and clove oil.

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Induction and recovery times varied in relation to water temperature (Zahl et al., 2009). In literature, some researchers suggested that induction time total loss of equilibrium increased in low water temperature (Endo et al., 1972; Sylvester, 1975; Amend et al., 1982; Limusuwan et al., 1983). On the other hand, Hseu et al. (1997) reported that the effects of temperature in Ictalurus nebulosus were insignificant. The effects of anesthetics depend on chemical structure of anesthetic and fish species. Locke (1969) and Limsuwan et al. (1983) stated that recovery time and effect duration of concentration of quinaldine depends on temperature. Schoettger and Steucke (1970) examined the effect of synergic mixtures of MS-222 and quinaldine as anesthetics for Northern pike and it was determined that 50 ppm and 60 ppm concentrations of the mixture in Northern pike is effected at 12 and 17 °C, respectively. Terzioglu (2001) reported that the effects of temperature on induction and recovery times of 2-phenoxyethanol were insignificant in sea bream. The present results agree with other studies on fish regarding effect of 2-phenoxyethanol and clove oil.

Recovery time positively correlated with concentrations of anesthetics (Smith and Hattingh, 1979; Limsuwan et al., 1983; Hseu et al., 1994; Weyl et al., 1996; Velisek et al. 2005; Sudagara et al., 2009; Gullian and Villanuera, 2009). Terzioglu (2001) found that recovery times increased with increasing concentration of 2-phenoxyethanol. On the other hand, some researchers determined that increasing concentration was not effected on recovery time (Mattson and Ripl, 1989; Malmstrom et al., 1993). In this study, it was found a strong relationship between increasing concentration and recovery time for 2-phenoxyethanol, whereas there were a weak relationship for clove oil.

Clove oil (Eugenol) is rapidly absorbed into the metebolism when it is taken orally. Gu'enette et al. (2007) stated that clove oil concentration in blood dropped down below 50%, after a hour from anesthesia in rainbow trout (*Onchorynchus mykiss*). Fischer and Dengler (1990) determined that after 24 h from anesthesia, there was no residue and clove oil is removed with urine. Doleželová et al. (2011) stated that fish did not show different sensitivities to clove oil in fish Danio rerio and Poecilia reticulate. In the present study, no side-effects of anesthetics were observed and total recovery of equilibrium occurred in fish.

Main advantage of 2-phenoxyethanol and clove oil is low price, their relatively low therapeutic index and ease of use. In addition, Marking and Meyer (1985) stated that clove oil meets seven out of eight criteria for an ideal anesthetic. Several studies conducted on anesthetic effect of 2-phenoxyethanol and clove oil in fish determined that both could be used in fish (Anderson et al., 1997; Akhlaghi and Brojerdi, 1999; Holloway et al., 2004; Velisek et al., 2005; Weber et al., 2009; Perdikaris et al., 2010; Uçar and Atamanalap, 2010; Gholipour kanani et al., 2011).

At the end of experiments, the best results for 2-phenoxyethanol and clove oil were obtained from 0.3, 0.4 and 0.5 ml/L; 0.50, 0.75, and 1.00 ml/L concentrations,

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respectively. Because interaction and recovery time in these concentrations is ideal.

In conclusion, the results obtained in the present study indicated that clove oil and 2-phenoxyethanol satisfies these criteria and suggests that they be considered as a fish anesthetic. Therefore, our results indicated that clove oil and 2-phenoxyethanol could be used to minimize the stress associated with aquaculture procedures.

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