

[Research]

## Evaluation of electronarcosis and clove oil for short-term anesthesia in common carp, *Cyprinus carpio* L. 1758

S.F. Monsef Rad<sup>1\*</sup>, S.Y. Paighambari<sup>1</sup>, A. Haji Moradlou<sup>1</sup>, F. Mashayekhi<sup>2</sup>, R. Qorbani<sup>1</sup>

1- Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

2- Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

\* Corresponding author's E-mail: monsefrad@gmail.com

(Received: April. 25. 2016 Accepted: Oct. 15. 2016)

### ABSTRACT

Anesthesia is considered to be the best way to ensure animal welfare during handling etc. Clove oil and electronarcosis are among the most popular chemical and physical anesthetics used in fisheries and biology; however there is a little information available on the effect of anesthesia using direct electric current in fish. In the present study, the impacts of clove oil (30 mg.l<sup>-1</sup>) and electro-anesthesia (constant direct current, 0.5 v.cm<sup>-1</sup>) (DC) were assessed in common carp, *Cyprinus carpio* during narcotic stage in two experimental sets. Experiment 1 was conducted to quantitatively compare induction of anesthesia and recovery time. The biochemical and hematological effects of anesthetics were analyzed as experiment 2. The results revealed that induction and recovery times in fish anesthetized with electric current were significantly lower than clove oil treatment. Significant differences were observed in cortisol concentrations, red blood cells and mean corpuscular hemoglobin concentration between anesthetic methods. Our results suggest that constant direct current electronarcosis was more quickly than clove oil and associated with lower side effects in common carp.

**Key words:** Clove oil, Electro-anesthesia, *Cyprinus carpio*, Hematology, Plasma biochemistry.

### INTRODUCTION

The use of anti-stress agents is a common practice in modern aquaculture. Such substances are utilized to induce sedation or anesthesia during normally stressful procedures such as handling, sorting, tagging, artificial reproduction procedures, and surgery (Velisek *et al.* 2011). Therefore, reducing these substances may lead to changes in metabolism and growth, immune function, and normal behavior (Barton 2002; Velisek *et al.* 2011). Furthermore, notably, all sedative and anesthetic procedures themselves induce the primary stress response and cause side effects which may not be considered desirable (Ross & Ross 2008).

Stress is defined as a natural reaction to a negative environmental stimulus which impairs the normal performance, physiology and activity of an animal (Barton 2002; Ross & Ross 2008). On the other hand, with respect to

animal welfare, fish should be free from physiological discomfort, disease and functional impairment, fear and chronic stress during handling or surgical procedures (Renault *et al.* 2011). Proper use of anesthetics may attenuate the primary stress response and, in turn, minimize the occurrence of negative consequences after veterinary practices (Trushenski *et al.* 2012c).

Basically, there are two types of anesthesia techniques: chemical anesthesia, using plethora of molecules and protocols (Topic Popovic *et al.* 2012; Ghanawi *et al.* 2013; Witeska *et al.* 2014), and physical anesthesia, using electricity, gas pressure or temperature (Vandergoot *et al.* 2011).

Chemical anesthesia in fish may be achieved by different agents, mainly tricaine methane sulphonate (MS-222), benzocaine, quinaldine sulphate, methomidate and clove oil (Velisek *et al.* 2011). At present, clove oil is commonly used

in aquaculture because of its low cost and rapid effect (Renault *et al.* 2011); however this chemical anesthetic and its components are not approved as an anesthetic for fish (FDA 2007). Clove oil is a natural product distilled from stems, leaves and flower buds of *Eugenia caryophyllata*, and its active ingredient, eugenol (4-allyl-2-methoxyphenol), makes up about 80% of the oil by weight (Ross & Ross 2008; Javahery *et al.* 2012).

An interesting alternative to chemical anesthesia is the use of electricity. The constant direct current of electricity may act as a nonchemical anesthetic by generating electronarcosis in fish. Electronarcosis occurs through the inhibition of medullary motor paths and thus spinal reflex. During electronarcosis, no cerebral message reaches the motor paths, the fish loses equilibrium, and muscle tone, but gill ventilation continues (Henyey *et al.* 2002). The fish remains in a state of narcosis with the body relaxed while submerged in water and exposed to direct current (DC) within the appropriate voltage gradient range. It is not possible to physiologically achieve the narcotizing effect with alternative current (AC) or pulsed direct current (PDC). The relatively low DC voltage gradient presumably translates to reduced stress and injury to the fish, and a safer working environment for the handler (Hudson *et al.* 2011).

The stages of anesthesia described by Summerfelt & Smith (1990) are widely referenced (Bowzer *et al.* 2012; Javahery *et al.* 2012; Trushenski *et al.* 2012c). Stage 1 anesthesia refers to light sedation which described by slight loss of reactivity to external stimuli and slightly decreased opercular rates whereas the stage 6 refers to cardiac arrest and asphyxia. According to Summerfelt & Smith (1990), total loss of muscle tone and equilibrium, slow and regular opercular movements, and a loss of spinal reflexes refers to stage 4 anesthesia. This level of anesthesia is consistent with stage 2 anesthesia as described by Mc Farland (1959). The physiological effect of anesthesia and sedation using electrical current and clove oil

have been evaluated in numerous studies (Cooke *et al.* 2004; Velisek *et al.* 2005; Altun *et al.* 2006; Renault *et al.* 2011; Trushenski *et al.* 2012a, b, c), but a direct comparison between anesthesia using clove oil and constant direct current of electricity is lacking in the literature. The objectives of this study were to compare the induction of anesthesia and recovery times in common carp (*Cyprinus carpio*), as a model fish, anaesthetized with clove oil and low - voltage constant direct current of electricity, and to evaluate the effects of two types of anesthesia with regard to some biochemical and hematological parameters.

## MATERIALS AND METHODS

### Anesthetics

Clove oil (eugenol concentration 75%) obtained from the Giah Essense Company (Giah Essense, Gorgan, Iran). Eugenol is the major active ingredient of clove oil and poorly soluble in water; thus clove oil diluted 1:10 with 95% ethanol to yield a working stock solution of 100 mg.ml<sup>-1</sup> (each ml of clove oil contains ~0.75 g of active ingredient) (Noga 2010).

The portable electronarcosis unit was constructed based on Hudson *et al.* (2011). Briefly, the unit consisted of a holding tank modified from a 70- L marine grade cooler, two electrodes which were installed facing each other 45cm apart at opposite ends of the holding tank, and a 0-30-V DC (Direct Current) power supply (DAZHENG PS- 305D).

### Fish

One hundred and twenty juvenile carp (50.08 ± 1.75 g body weight and 15.03 ± 0.19 cm total body length) were obtained from a local fish farm and after transportation to the laboratory acclimatized for 3 weeks to the aquarium facilities at the University of Guilan. The fish were kept in 1000 liter fiberglass tank; the physiochemical parameters of the water during experiment were: NH<sub>4</sub><sup>+</sup>: 0.06 mg.l<sup>-1</sup>; NO<sub>2</sub><sup>-</sup>: 0.02 mg.l<sup>-1</sup>; NO<sub>3</sub><sup>-</sup>: 0.5 mg.l<sup>-1</sup>; salinity: 2- 3 mg.l<sup>-1</sup>; conductivity: 2.24 mS.cm<sup>-1</sup>; pH 7.5. During the acclimatization fish were offered food at a rate

sufficient to maintain growth. Water temperature ( $21.3 \pm 1.1^\circ\text{C}$ ) was continuously monitored. Fish were starved 24 h before the experiment.

### Experimental procedure

A preliminary experiment was conducted to achieve proper dosage according to the literature (Velisek *et al.* 2005; Hudson *et al.* 2011). Briefly, thirty five fish were randomly collected from the reference stock and divided to six groups; Five groups were exposed to clove oil concentration of 20, 30, 45, 65 & 90  $\text{mg.l}^{-1}$  and the other group, comprised 10 fish, subjected to the voltage gradient increasing gradually to the range of 0.25-0.56  $\text{V.cm}^{-1}$ . In all cases, fish were ensured to be in the stage IV of anesthesia; the clinical signs were identified as total loss of equilibrium, responsiveness to handling with maintenance of a slow, steady, opercular ventilation rate (Bowzer *et al.* 2012). With regard to induction time, recovery time and post-sedation distress, 30  $\text{mg.l}^{-1}$  clove oil and 0.5  $\text{V.cm}^{-1}$  electricity were used for the principal investigation.

### Experiment 1: induction and recovery times

Two groups of 15 fish were randomly collected from the reference stock and transferred to individual holding tanks one day before the experiment. Fish were placed into anesthesia chamber filled with aerated culture water either dosed with clove oil or equipped with the electro-anesthesia unit. Care was taken to monitor fish and determine induction time to attain stage IV of anesthesia. Fish were considered induced to stage IV when they no longer responded to the visual and tactile stimulus, but the opercular rate remained steady and slow. In the case of electro-anesthesia, a tremor was observed immediately following electrical exposure and fish were not responsive to the stimuli during this tremor. Thus, induction was considered complete after the tremor had ceased. After induction fish were weighed and measured to determine total length, and then transferred to an aerated recovery tank. In the tank, fish were monitored to determine recovery of normal equilibrium

according to the literature (Bowzer *et al.* 2012; Trushenski *et al.* 2012c). The survival rate of recovered fish was controlled for 24 h.

### Experiment 2: hematological and biochemical response

Eight groups of fish were used in this experiment. Each treatment group consisted of six fish transferred from the holding tank to an anesthesia chamber. Each anesthesia regime was conducted in triplicate. In each treatment group, one fish sampled immediately after all fish had reached stage IV anesthesia and the rest were returned to a holding tank for further sampling at 0.5, 1, 2, 6 & 24 h post-anesthesia (one fish per group per time point; no fish were sampled more than once). Two control groups out of eight experimental ones were also sampled every hour over the course of the experiment. Since no differences were found between the control fish, the results of these groups were pooled.

The entire capture and blood sampling was conducted within two minutes for each fish. Blood samples collected by caudal vessels puncture with a heparinized syringe and kept on ice (<4 h) until analysis. Subsamples of whole blood were used for determination of hematological parameters; remaining whole blood was centrifuged at 13000 rpm at  $4^\circ\text{C}$  for 10 min and resultant plasma was stored at  $-80^\circ\text{C}$  for further analysis.

Hematocrit was measured using microhematocrit capillaries filled with blood, centrifuged at 3000 rpm for 5 min and expressed as percentage of total blood volume. Hemoglobin was determined with a spectrophotometer (UV- 2100, Unico, USA) at 540 nm absorbance using the cyanomethemoglobin method (Dacie & Lewis 1975). Red blood cell (RBC) and white blood cell (WBC) counts were conducted with Neubauer chamber using Dacie's dilution fluid (3 g sodium citrate, 99 ml distilled water and 1 ml formalin). Differential leukocyte count was performed with blood smears stained with Giemsa solution. The smears were observed by light microscopy (Olympus, Tokyo, Japan). The RBC indices including mean corpuscular

hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Pradhan *et al.* (2014).

Biochemical indices in plasma included cortisol, glucose and lactate. Plasma cortisol levels were measured by ELISA reader (ELx800, Bio Tek, USA), and glucose and lactate were colorimetrically assessed using an auto-analyzer (Technicon RA- 1000, USA).

### Statistical analysis

In experiment 1, individual fish were considered as experimental unit (n = 15). Data were first tested for normality (the Shapiro - Wilk test) and any abnormally distributed data were transformed logarithmically. Then, induction and recovery times were analyzed using one-way analysis of variance (ANOVA) to reveal significant differences in measured variables among the experimental groups.

Data from experiment 2 were analyzed by one-way, repeated measures ANOVA. For this experiment, replicate groups were considered experimental unit (n = 3). Although each anesthetic was used to triplicate groups, each composed of six fish, it is determined that

groups, not individuals, should be served as experimental units. Thus, experimental units represent independent observations. Fish sampled at each time point represented repeated observation made on the same experimental unit.

Data are expressed as mean  $\pm$  SEM. Statistical analyses were conducted with SPSS 17.0 for windows where significance level was set at 0.05.

## RESULTS

### Induction and recovery time

Induction and recovery times differed significantly between anesthetics ( $p < 0.05$ , Table 1). Fish were anaesthetized within  $110.47 \pm 4.20$  sec and  $37.73 \pm 4.58$  sec using clove oil and electric current, respectively. The recovery pattern was to regain equilibrium, then tactile responsiveness in rapid succession. These stages were achieved rapidly in the constant DC when electricity was turned off; the mean times were  $3.07 \pm 0.41$  sec and  $4.40 \pm 1.27$  sec to regain equilibrium and tactile responsiveness, respectively. These figures were  $99.14 \pm 14.46$  sec and  $159.41 \pm 18.02$  sec respectively, for fish exposed to clove oil ( $p < 0.05$ ).

**Table 1.** Duration of induction and recovery times for clove oil and electroanesthesia (mean  $\pm$  SEM, n= 30).

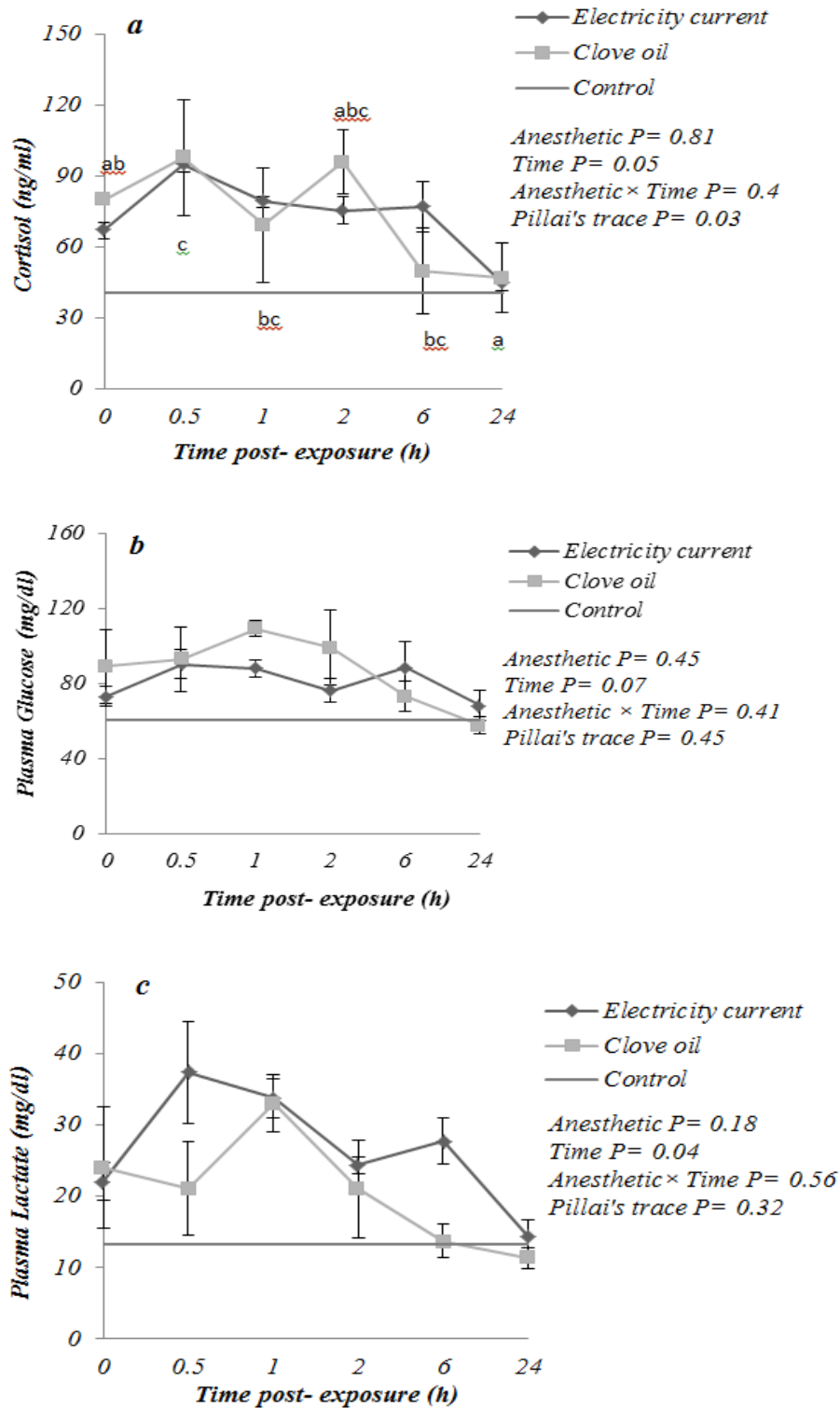
Anesthetic	Induction time (sec)	Recovery time (sec)	
		Maintain equilibrium	Respond to tactile stimulus
	$p < 0.05$	$p < 0.05$	$p < 0.05$
Clove oil	$110.47 \pm 4.20$	$99.14 \pm 14.64$	$159.41 \pm 18.02$
Electro-anesthesia	$37.73 \pm 4.58$	$3.07 \pm 0.41$	$4.40 \pm 1.27$

### Stress and plasma analyses

Plasma concentrations of cortisol varied significantly between treatments (Pillai's trace = 1.00,  $F(4, 1) = 623.3$ ,  $p = 0.03$ ). Plasma cortisol levels were significantly elevated at 0.5 h, 2 h and again at 24 h post-anesthesia among fish anaesthetized with clove oil, whereas cortisol concentration of electro-anesthetized fish increased at 0.5 h and then stabilized and decreased during the sampling period.

In the clove oil treatment, the highest value of glucose and lactate were observed 1 h after

treatment and returned to below resting levels within 24 h post-anesthesia; while the peak of these biochemical responses under electric current treatment were observed 0.5h after anesthesia and returned to the control value by 24 h. There were no significant difference between fish anaesthetized with clove oil and electro-anesthesia (glucose: Pillai's trace = 0.900,  $F(4, 1) = 2.250$ ,  $p = 0.459$ ; lactate: Pillai's trace = 0.954,  $F(4, 1) = 5.161$ ,  $p = 0.317$ ; Fig. 1). The plasma factors for fish in stress - free condition were reported for the reference population.



**Fig. 1.** Time course of cortisol (a), glucose (b) and lactate (c) in common carp after anesthesia to stage IV with clove oil and constant direct current of electricity. Data are presented as means  $\pm$  SEM. Different letters stand for significant differences within the same group (one-way repeated measures ANOVA:  $p < 0.05$ ).

**Table 2.** Hematological parameters in common carp anesthetized with clove oil and electricity constant direct current (mean  $\pm$  SEM).

Group	RBC <sup>a, b, c</sup> (10 <sup>5</sup> mm <sup>3</sup> )	WBC <sup>a, b, c</sup> (mm <sup>3</sup> )	Hb <sup>c</sup> (g/dL)	HCT <sup>b, c</sup> (%)	MCV (fL)	MCH (pg)	MCHC <sup>b</sup>
	p< 0.01	p= 0.3	p= 0.3	p= 0.8	p= 0.8	p= 0.2	p< 0.01
Controls	5.965 $\pm$ 0.237	4100 $\pm$ 724.33	6.35 $\pm$ 0.23	31.5 $\pm$ 1.384	527.666 $\pm$ 4.233	106.539 $\pm$ 0.571	20.198 $\pm$ 0.22
Clove oil 0	6.47 $\pm$ 0.241	4750 $\pm$ 550	6.773 $\pm$ 0.236	31.333 $\pm$ 1.763	487.002 $\pm$ 43.597	104.654 $\pm$ 0.341	21.825 $\pm$ 1.88
Electricity 0	5.78 $\pm$ 0.337	2933.33 $\pm$ 88.191	6.067 $\pm$ 0.338	28.667 $\pm$ 2.027	495.479 $\pm$ 16.795	104.938 $\pm$ 0.581	21.234 $\pm$ 0.808
Clove oil 0.5	7.5 $\pm$ 0.125	4300 $\pm$ 200	6.683 $\pm$ 1.18	30.333 $\pm$ 4.70	405.290 $\pm$ 64.48	89.292 $\pm$ 16.063	21.850 $\pm$ 0.604
Electricity 0.5	5.79 $\pm$ 0.174	2966.667 $\pm$ 145.29	6.067 $\pm$ 0.145	30.667 $\pm$ 0.881	529.702 $\pm$ 1.742	104.822 $\pm$ 0.842	19.788 $\pm$ 0.105
Clove oil 1	6.87 $\pm$ 0.029	5600 $\pm$ 600	7.037 $\pm$ 0.230	31 $\pm$ 2.082	450.993 $\pm$ 29.964	102.373 $\pm$ 3.25	22.811 $\pm$ 0.844
Electricity 1	5.76 $\pm$ 0.195	3350 $\pm$ 150	6.05 $\pm$ 0.35	30 $\pm$ 2	537.929 $\pm$ 8.545	105.429 $\pm$ 1.997	19.614 $\pm$ 0.573
Clove oil 2	6.05 $\pm$ 0.103	4750 $\pm$ 750	6.1 $\pm$ .264	27.33 $\pm$ 1.201	451.944 $\pm$ 17.175	100.851 $\pm$ 3.612	22.319 $\pm$ 0.088
Electricity 2	6.96 $\pm$ 0.518	3500 $\pm$ 115.47	7.1 $\pm$ 0.404	34.33 $\pm$ 0.881	496.475 $\pm$ 22.96	103.281 $\pm$ 2.703	20.648 $\pm$ 0.648
Clove oil 6	5.78 $\pm$ 0.200	4150 $\pm$ 350	6.02 $\pm$ 0.271	26.667 $\pm$ 2.027	460.085 $\pm$ 20.002	104.078 $\pm$ 1.158	22.688 $\pm$ 0.760
Electricity 6	6.04 $\pm$ 0.0926	3666.67 $\pm$ 425.57	6.433 $\pm$ 0.088	31.33 $\pm$ 0.881	518.278 $\pm$ 6.703	106.472 $\pm$ 1.210	20.552 $\pm$ 0.401
Clove oil 24	5.46 $\pm$ 0.214	4300.00	6.10	28.00	431.198 $\pm$ 25.47	105.588 $\pm$ 0.735	24.668 $\pm$ 1.53
Electricity 24	5.26 $\pm$ 0.134	3100 $\pm$ 100	5.7 $\pm$ 0.1	27 $\pm$ 2	514.012 $\pm$ 22.160	109.895 $\pm$ 4.066	21.417 $\pm$ 0.726

<sup>a</sup> significant effect of anesthesia (repeated- measures ANOVA with Bonferroni correction): p< 0.05

<sup>b</sup> significant effect of time (repeated- measures ANOVA with Bonferroni correction): p< 0.05

<sup>c</sup> significant effect of anesthesia  $\times$  time (repeated- measures ANOVA with Bonferroni correction): p< 0.05

### Hematological responses

The levels of RBC and MCHC were significantly higher in fish anaesthetized with clove oil than with electro-anaesthetized fish (RBC: Pillai's trace = 1.00,  $F(4, 1) = 9862.9$ ,  $p = 0.008$ ; MCHC: Pillai's trace = 1.00,  $F(4, 1) = 22079$ ,  $p = 0.005$ ; Table 2). The somewhat similar pattern was observed for WBC values; whereas the changes were not statistically significant (Pillai's trace = 0.594,  $F(4, 1) = 0.366$ ,

$p = 0.826$ ; Tables 2 & 3). Hematocrit and hemoglobin also decreased during the course of sampling (Hct: Pillai's trace = 0.594,  $F(4, 1) = 0.366$ ,  $p = 0.826$ ; Hb: Pillai's trace = 0.958,  $F(4, 1) = 5.712$ ,  $p = 0.303$ ). During these two experiments only one mortality was observed after 24h - observation period and general behavior of the experimental population seemed normal.

**Table 3.** Differential leukocyte in common carp anesthetized with clove oil and constant direct electric current (mean  $\pm$  SEM)\*.

Group	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Controls	28.66 $\pm$ 1.52	66.83 $\pm$ 1.85	3.33 $\pm$ 0.42	1.16 $\pm$ 0.16
Clove oil 0	23.5 $\pm$ 0.5	73.5 $\pm$ 0.5	1.5 $\pm$ 0.5	1.5 $\pm$ 0.5
Electricity 0	25.66 $\pm$ 2.33	71 $\pm$ 2.64	3 $\pm$ 0.57	1.00
Clove oil 0.5	22.5 $\pm$ 0.5	73 $\pm$ 1.1	3.5 $\pm$ 0.5	1.00
Electricity 0.5	26.667 $\pm$ 2.66	47.00	2 $\pm$ 0.5	1.00
Clove oil 1	25.5 $\pm$ 3.5	69.5 $\pm$ 3.5	3.00	2.00
Electricity 1	33.00	61.5 $\pm$ 1.5	4 $\pm$ 1	1.5 $\pm$ 0.5
Clove oil 2	24.5 $\pm$ 2.5	73 $\pm$ 3	2.00	1.00
Electricity 2	31.66 $\pm$ 1.45	63.33 $\pm$ 2.02	3.66 $\pm$ 0.88	2.00
Clove oil 6	23.5 $\pm$ 2.5	73.5 $\pm$ 1.5	2.5 $\pm$ 0.5	1.00
Electricity 6	31.66 $\pm$ 0.88	64.33 $\pm$ 1.20	3.33 $\pm$ 0.66	1.00
Clove oil 24	25.00	70.00	3.00	2.00
Electricity 24	27 $\pm$ 2	69.5 $\pm$ 2.5	2.5 $\pm$ 0.5	1.00

\* $p > 0.05$  (significant difference at  $p = 0.05$ ).

### DISCUSSION

The present study indicates that electric current (DC) and clove oil are effective in anesthesia of common carp to deep narcosis stage; however the induction and recovery time in electro-anesthesia were less than 1 min which were significantly lower than that of clove oil (about 1.8 min) in examined doses. Our results are in line with the use of low - voltage constant direct current to immobilize juvenile lake sturgeon (*Acipenser fulvescens*) and shortnose sturgeons (*A. brevirostrum*) conducted by Henyey *et al.* (2002). According to Gholipour Kanani *et al.* (2013), the induction and recovery times in rainbow trout anesthetized by clove oil (25 ppm) were longer than by electro-anesthesia; however there were no significant difference

between the treatments. Their finding is similar to the result reported by Sattari *et al.* (2009) about induction and recovery times induced by clove oil (100 ppm) and electric current in rainbow trout. According to the literature (Cunha & Rosa 2006; Trushenski *et al.* 2012c), it is likely that higher doses of chemical anesthetics like clove oil would have been used to attain more quick induction time, all the same, the greater concentration of these agents can result in a longer recovery time.

The influences of anesthesia upon fish biochemical and hematological parameters have been investigated (Velisek *et al.* 2005; Trushenski *et al.* 2012a, b, c). However, these literatures have mainly evaluated the effects of

sedation in the case of electric current, and little information is available on hematological changes. Our results revealed alteration in biochemical and hematological parameters of anaesthetized fish with clove oil and electric direct current. The cortisol circulating level is commonly used as indicator of degree stress (Barton 2002). In the present study, the highest plasma cortisol levels was observed at about 0.5h post - treatment with values elevated to  $94.67 \pm 2.85 \text{ ng.ml}^{-1}$  &  $97.6 \pm 24.41 \text{ ng.ml}^{-1}$  by the electric current and clove oil treatments respectively. These findings are in accordance with the reports suggesting that in most fish species the highest plasma cortisol occurs within 0.5- 1h after stressful disturbance (Barton 2002; Bowzer *et al.* 2012; Trushenski *et al.* 2012c). According to Roques *et al.* (2010), increasing cortisol up to  $60 \text{ ng.ml}^{-1}$  are typically referred to as a mild response, while rapid increase above  $100 \text{ ng.ml}^{-1}$  are generally considered to reflect a severe stress response. In our experiment, as indicated by large standard error, the cortisol concentration of one fish anaesthetized by clove oil was above  $140 \text{ ng.ml}^{-1}$  which is referred to as a severe stress. However, variation in stress responses within a single strain or population can be related to genetic component; and it is ambiguous whether fish that indicate high or low corticosteroid stress responses are really more or less stressed than others or have different capacities to react to stressors (Barton 2002). Our results also indicate that cortisol levels returned to reference values by 24 h which shows complete recovery from procedure. In addition to circulating level of cortisol, glucose and lactate pulses are used as indicator of acute stress experienced by fish (Martinez-Porchas *et al.* 2009). Plasma glucose levels followed the changes observed in cortisol levels; however, the highest level of glucose ( $109.3 \pm 3.92$ ) observed 1h after the clove oil treatment. The opposite pattern was observed for plasma lactate such that the highest value of lactate ( $37.3 \pm 7.1$ ) was found at 0.5h post - anesthesia by electric current. Bowzer *et al.* (2012) reported greater plasma glucose and lactate pulses in

grass carp exposed to pulsed DC electro-sedation. In another experiment, relatively weak glucose and lactate response were observed in largemouth bass sedated with eugenol in comparison with electro-sedation (pulsed DC) (Trushenski *et al.* 2012c). Cortisol can activate glycogenolysis and gluconeogenesis process in fish which lead to increase in glucose as substrate levels in the blood to provide enough energy for the organism (Bowzer *et al.* 2012). On the other hand, circulating lactate levels can be increased in fish as a result of cortisol-stimulated lactate production; moreover, increasing lactate may be a consequence of some physical effects of anesthesia such as hyperactivity during induction, reduced ventilation and tetanic muscle in the case of electro-anesthesia (Trushenski *et al.* 2012 b). This may explain why electro-anaesthetized carp, experienced greater post-treatment lactate pulses than the other group.

Hematological measurements are important for investigation of changes in physiological status of fish (Gholipor Kanani & Ahadzadeh 2013). Furthermore, hematological profiles have been used as stress indicators (Gharache *et al.* 2012). Results of our experiment indicated significant difference in RBC between the two treatments. Increase in RBC counts after short- term handling stress has been reported in literatures (Abdolazizi *et al.* 2011; Souza Nevens *et al.* 2014). As shown in Table 2, the hematocrit levels in clove oil treatment were higher than in electro anesthesia; however, no statistically significant difference was observed between these anesthetics. Similar results were reported by Trushenski *et al.* (2012c) in largemouth bass. This increase presumably is due to the direct effect of higher metabolic demand during stress which leads to elevation of hematocrit and the red blood cell counts to increase oxygen-carrying capacity (Gharache *et al.* 2012). In the present study, we found significant difference in MCHC between the treatments. This difference may be explained by increasing in the Hb levels and erythrocyte volume (%) in blood of fish anaesthetized by clove oil.



Furthermore, the lower concentration of Hb in electro-anesthetic treatment could be explained by haemodynamic alterations and re-distribution of blood cellular elements in the vascular bed (GholipourKanani & Ahadizadeh 2013).

In conclusions, two anesthetic methods were associated with alterations in blood biochemical and hematological parameters; although varying in magnitude of effects in two treatments. On the basis of our results, considering lower induction and recovery times and changes in blood parameters, electro-anesthesia was more relevant than clove oil. In addition, as clove oil is not approved for use in food fish, we do not recommend its use on any fish until Food and Drug Administration (FDA) standards and proper licensing is acquired.

#### ACKNOWLEDGEMENT

We thank Mrs. Heidary, Mr. Akhavan and Ms. Avarjeh for their invaluable assistance during the experiments. Special thanks are also given to Dr. Soleimani, the head of Giah Essence Company, and Mr. Rezaei who kindly contributed to conduct this study.

This study was funded by Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, and the research is included in the doctoral dissertation of S. F. M. R., Department of Fishery, G.U.A.S.N.R., Gorgan, Iran. The present research performed with the approval of an appropriate ethics committee INTL K3525.A35 B37 2000.

#### REFERENCES

- Abdolazizi, S, Ghaderi, E, Naghdi, N & Kamangar, BB 2011, Effects of clove oil as an anesthetic on some hematological parameters of *Carassius auratus*. *Journal of Aquaculture Research & Development*, 2: 108.
- Altun, T, Hunt, AÖ & Usta, F 2006, Effects of clove oil and eugenol on anesthesia and some hematological parameters of European eel *Anguilla anguilla*, L., 1758. *Journal of Applied Animal Research*, 30: 171-176.
- Barton, BA 2002, Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42: 517-525.
- Bowzer, JC, Trushenski, JT, Gause, BR & Bowker, JD 2012, Efficacy and physiological responses of grass carp to different sedation techniques: II. Effect of pulsed DC electricity voltage and exposure time on sedation and blood chemistry. *North American Journal of Aquaculture*, 74: 567-574.
- Cunha, FEA & Rosa, IL 2006, Anaesthetic effects of clove oil on seven species of tropical reef teleosts. *Journal of Fish Biology*, 69:1504- 1512.
- Cooke, SJ, Suski, CD, Ostrand, KG, Tufts, BL & Wahl, DH 2004, Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting largemouth bass (*Micropterus salmoides*). *Aquaculture*, 239: 509-529.
- Dacie, JV & Lewis, SM 1975, Practical Haematology. Churchill Livingstone, Edinburgh. U. S. Food and Drug Administration 2007, Guidance for industry: concerns related to the use of clove oil as an anesthetic for fish. FDA, GuideP150.<http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052520.pdf>. Accessed 28 Feb 2015.
- Ghanawi, J, Monzer, S & Saoud, IP 2013, Anaesthetic efficacy of clove oil, benzocaine, 2-phenoxyethanol and tricaine methanesulfonate in juvenile marbled spinefoot (*Siganus rivulatus*). *Aquaculture Research*, 44: 359-366.
- Gharache, MH, Paighambari, SY & Golpour, A 2013, Hematological and biochemical indices of *Rutilus frisii kutum*, associated with capture methods. *Comparative Clinical Pathology*, 23: 979-982.
- GholipourKanani, H & Ahadizadeh, S 2013, Use of propofol as an anesthetic and its efficacy on some hematological values of

- ornamental fish *Carassius auratus*. *Springer Plus* 2: 76.
- Gholipourkanani, H, Soltani, M & Mirzargar, SS 2013, Effect of tricaine methane sulfonate (MS222), clove oil and electro-anaesthesia on respiratory burst activity in whole blood and serum alternative complement response in rainbow trout (*Oncorhynchus mykiss*), during the narcosis stage. *Fish & Shellfish Immunology*, 34: 692-696.
- Henyey, E, Kynard, B & Zhuang, P 2002, Use of electronarcosis to immobilize juvenile lake and shortnose sturgeons for handling and the effects on their behavior. *Journal of Applied Ichthyology*, 18: 502-504.
- Hudson, JM, Johnson, JR & Kynard, B 2011, A portable electronarcosis system for anesthetizing salmonids and other fish. *North American Journal of Fisheries Management*, 31: 335-339.
- Javahery, S, Nekoubi, H & Moradlu, AH 2012, Effect of anaesthesia with clove oil in fish (review). *Fish Physiology and Biochemistry*, 38: 1545-1552.
- J. Noga, Edward, 2010, Fish disease: Diagnosis and treatment. Wiley-Blackwell.
- Martínez-Porchas, M, Martínez-Córdova, LR & Ramos-Enriquez, R 2009, Cortisol and glucose: reliable indicators of fish stress. *Pan-American Journal of Aquatic Sciences*, 4: 158-178.
- McFarland, WN 1959, A study of the effects of anesthetics on the behavior and physiology of fishes. *Publication of the Institute of Marine Science*, 6: 23-55.
- Pradhan, SC, Patra, AK & Pal, A 2014, Hematological and plasma chemistry of Indian major carp, *Labeo rohita* (Hamilton, 1822). *Journal of Applied Ichthyology*, 30: 48-54.
- Renault, S, Daverat, F, Pierron, F, Gonzalez, P, Dufour, S, Lanceleur, L, Schafer, J & Baudrimont, M 2011, The use of Eugenol and electro-narcosis as anaesthetics: Transcriptional impacts on the European eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety*, 74: 1573-1577.
- Roques, JA, Abbink, W, Geurds, F, van de Vis, H & Flik, G 2010, Tailfin clipping, a painful procedure: studies on Nile tilapia and common carp. *Physiology & Behavior*, 101: 533-540.
- Ross, LG, Ross, B & Ross, B 2008, Anaesthetic and sedative techniques for aquatic animals. Blackwell, London.
- Sattari, A, Mirzargar, SS, Abrishamifar, A, Lourakzadegan, R, Bahonar, A, Mousavi, HE & Niasari, A 2009, Comparison of electroanesthesia with chemical anesthesia (MS222 and clove oil) in rainbow trout (*Oncorhynchus mykiss*) using plasma cortisol and glucose responses as physiological stress indicators. *Asian Journal of Animal and Veterinary Advances*, 4: 306-313.
- Souza Neves, M, Barbas, LAL & Fujimoto, RY 2014, Hematology and recovery response in jacundá, *Crenicichla saxatilis* (Linnaeus, 1758) after short-term handling stress. *Journal of Applied Ichthyology*, 30: 42-47.
- Summerfelt, RC & Smith, LS 1990, Anesthesia, surgery, and related techniques. Methods for fish biology. American Fisheries Society, Bethesda, Maryland, 8(9.6), 2.
- Topic-Popovic, N, Strunjak-Perovic, I, CozRakovac Barisic, J, Jadan, M, Persin Berakovic, A & Sauerborn Klobucar, R 2012, Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology*, 28: 553-564.
- Trushenski, JT & Bowker, JD 2012a, Effect of voltage and exposure time on fish response to electro-sedation. *Journal of Fish and Wildlife Management*, 3: 276-287.
- Trushenski, JT, Bowzer, JC, Bowker, JD & Schwarz, MH 2012b, Chemical and electrical approaches to sedation of cobia: induction, recovery, and physiological responses to sedation. *Marine and Coastal Fisheries*, 4: 639-650.
- Trushenski, JT, Bowker, JD, Mulligan, BL & Gause, BR 2012c, Induction, recovery, and hematological responses of largemouth

- bass to chemo-and electrosedation. *North American Journal of Aquaculture*, 74: 214-223.
- Vandergoot, CS, Murchie, KJ, Cook, SJ, Dettmers, JM, Bergstedt, RA & Fielder, DG 2011, Evaluation of two forms of electroanesthesia and carbon dioxide for short-term anesthesia in walleye. *North American Journal of Fisheries Management*, 31: 914-922.
- Velisek, J, Svobodova, Z, Piackova, V, Groch, L & Nepejchalova, L 2005, Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). *Vet. Med-Czech*, 50: 269-275.
- Velisek, J, Stara, A, Li, ZH, Silovska, S & Turek, J 2011, Comparison of the effects of four anaesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. *Aquaculture*, 310: 369-375.
- Witeska, M, Dudyk J & Jarkiewicz, N 2014, Hematological effects of 2-phenoxyethanol and etomidate in carp (*Cyprinus carpio* L.). *Veterinary Anaesthesia and Analgesia*, doi: 10.1111/vaa.12242.

## بررسی بیهوشی کوتاه مدت توسط جریان الکتریکی و اسانس میخک در ماهی کپور معمولی (*Cyprinus carpio*)

س. ف. منصف راد<sup>۱\*</sup>، س. ی. پیغمبری<sup>۱</sup>، ع. حاجی مرادلو<sup>۱</sup>، ف. مشایخی<sup>۲</sup>، ر. قربانی<sup>۱</sup>

۱- گروه شیلات، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران

۲- گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه گیلان، رشت، ایران

(تاریخ دریافت: ۹۵/۰۲/۰۶ تاریخ پذیرش: ۹۵/۰۷/۲۴)

### چکیده

استفاده از بیهوشی به عنوان بهترین راه برای اطمینان از امنیت ماهی در طول دستکاری در نظر گرفته می‌شود. اسانس گل میخک و روش الکتریکی از جمله متداول‌ترین روش‌های بیهوشی شیمیایی و فیزیکی در آبی‌پروری و زیست‌شناسی محسوب می‌شوند؛ اگرچه در مورد اثرات استفاده از بیهوشی با جریان الکتریکی مستقیم، اطلاعات بسیار کمی موجود است. در مطالعه حاضر، اثر بیهوشی با اسانس گل میخک (۳۰ میلی‌گرم بر لیتر) و روش الکتریکی (جریان ثابت مستقیم با شیب ولتاژ ۰/۵ ولت بر سانتی‌متر) بر روی ماهی کپور معمولی (*Cyprinus carpio*) در دو آزمایش مورد بررسی قرار گرفت. آزمایش اول شامل مقایسه زمان القاء و بازگشت بود؛ اثرات بیهوشی بر فاکتورهای هماتولوژی و بیوشیمیایی خون در آزمایش دوم بررسی شد. نتایج نشان داد زمان القاء و بازگشت در ماهیان بیهوش شده با جریان الکتریکی به طور معنی‌داری کمتر از تیمار اسانس گل میخک است ( $p < 0/05$ ). همچنین تفاوت معنی‌داری در غلظت کورتیزول، مقدار گلبول قرمز و میانگین هموگلوبین گلبول قرمز بین دو روش بیهوشی مشاهده شد. به‌طور کلی نتایج مطالعه حاضر نشان داد بیهوشی با جریان الکتریکی مستقیم ثابت در مقایسه با اسانس گل میخک سریع‌تر بوده و همراه با اثرات جانبی کمتری می‌باشد.

\* مولف مسئول