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ORIGINAL ARTICLE

Chemical and electrical approaches to anesthesia of Ship sturgeon, *Acipenser nudiventris*: induction and recovery, physiological response to anesthesia

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Abstract In this study, we investigated the induction and recovery times and physiological response of juvenile Ship sturgeon, *Acipenser nudiventris* anesthetized by CO_2 (bar), clove powder (mg/L), and available electric shock (V). The shortest and longest induction time was for electric shock and clove powder, respectively. By contrast, the shortest and longest recovery time took place for electric shock and clove powder, respectively. Cortisol and glucose levels increased in 1 and 6 h after anesthesia. Changes in plasma osmolality and hematological indices were less changed among the anesthesia treatments. Results demonstrated that electric shock was a more effective method for quick induction time and prevented stress response caused by anesthesia, although all anesthetic methods were considered to be safe.

Keywords Fish · CO_2 · Clove powder · Electric shock · Stress

Introduction

Aesthesia is a valuable tool to achieve enough sedation, reduce stress and pain during various procedures such as the manipulation of individuals, measurement, weighing, vaccination, transportation, and blood or biopsy sampling (Iverzen et al. 2003; Mylonas et al. 2005; Kiessling et al. 2009). There are several approaches in use to anesthetize fish. To select an anesthetic method for particular purpose, different properties such as effectiveness, physiological response, cost, and safety to fish, human, and environment should be considered (Pirhonen and Schreck 2003).

Clove oil has traditionally been used as topical anesthetic for toothaches, headaches, and joint pain and is extracted from the buds, leaves, and stems of clove trees, *Eugenia aromatic* (Soto and Burhanuddin 1995), which its active compound is eugenol [2-methoxy-4-(2-propenyl) phenol; 70–90 % by weight] (Weber et al. 2009; Velisek et al. 2005b). It is also used as food additive, organic substance that does not require any withdrawal. Clove oil is an inexpensive and readily available, for instance, from health food stores (Keene et al. 1998); however, its disadvantage included its relatively low therapeutic index (Velisek et al. 2005b) and sensitivity to light (Cho and Heath 2000). Fish (1942) has described carbon dioxide as an anesthetic for the first time. It can be produced by bubbling CO_2 gas or by the soda-acid technique (Post 1979; Summerfelt and Smith 1990). CO_2 is considered as a drug of "low regulatory priority" (USFDA 2002). It can be difficult to use CO_2 uniformly and is usually slow-acting and lethal after repeated exposure (Marking and Meyer 1985). No decrease in blood PO_2 and lowered plasma pH are reported in fish anesthetized with CO_2, which is different from those of benzocaine, 2-phenoxyethanol, MS-222, and metomidate (Iwama et al. 1989). The use of an electric approach in order to anesthetize fish has some advantages than chemical agents such as no production of contaminant made by chemical agents, short recovery time, low cost, and ready for use. Fish response to electricity is dependent on electrical field and shock

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duration. Other factors such as electroconductivity, temperature, size of fish, and species can be involved (Mirzargar and Sepidgar 2005). However, requirement to particular electric equipment and possible physical damages to human and fish are disadvantages of electric approach (Ackerman and Bellwood 2002).

Sturgeons are the most valuable and economic fish, which their population has been led to sharp decline due to overfishing, habitat destruction, environmental pollution, no conservation, and making barrier on their reproduction place (Doukakis et al. 1999). It resulted in these valuable species considered as endangered fish (IUCN 2013). Regarding to the importance of these species, the use of anesthetic during manipulation is highly emphasized to reduce the risk of injuries, facilitate handling, and prevent stress. Although different studies have investigated the effect of CO_2 and electric shock on anesthesia of various species (Erikson et al. 1997; Coyle et al. 2004; Bowzer et al. 2012; Trushenski et al. 2012b), there are limited data on the effect of these approaches on sturgeons.

Thus, this study was carried out to investigate the effect of some anesthetic methods on induction and recovery times and hematological indices as well as biochemical blood indices as stress responses.

Material and methods

Fish and experimental facilities

Ship sturgeons hatched and raised at the International Sturgeon Research Institute, Guilan, Iran, were used for this study in 2014. Fish with the range body weight of 60–100 g were initially maintained in 2-m diameter × 0.5-m deep circular fiberglass tanks supplied with 900 l aerated freshwater and acclimated to the experimental condition for 2 weeks. Fish were kept under natural 12 L:120-day light regime. Mean water temperature was 25.5 ± 0.1 °C. Fish were fed daily with standard pellets (no. 1.9, Biomar, Nersac, France) at 1–2 % of their body weight and deprived for 24 h before starting the experiment.

Induction and recovery times

To determine induction and recovery times, 45 juvenile Ship sturgeons were caught from acclimation tanks and divided randomly into three treatments in three triplicates for each (15 fish per treatment). A fresh solution of clove powder (obtained from a health food store) was prepared a few minutes before start of the trial, which 0.5 g of clove powder was dissolved into 1 l of fresh water. Fish of three treatments were anesthetized under followed anesthesia treatments as 0.5 g/l of clove powder, 0.3 bar of CO_2, and 130 V of electric shock. Anesthesia treatments were based on a preliminary experiment so that the exposure time for different anesthetics was 3 min. Fish were monitored every minute after transferring into 20-l observation tanks (filled with aerated fresh water) to which anesthetic had already been added. At the point when total loss of equilibrium, mussel tune, was obtained and opercular movements became irregular, at which stage IV of anesthesia (Summerfelt and Smith 1990), time was recorded and considered as induction time of anesthesia. Then, anesthetized fish were transferred quickly to 20-l recovery tanks with pure well water. Fish were monitored to determine the time to recovery of normal equilibrium and tactile responses, stage IV of recovery outlined by Summerfelt and Smith 1990. Once fish reached to normal equilibrium and respond to the tactile, fish were considered fully recovered. Recovered fish were returned to acclimation tanks and monitored for 24 h.

Physiological response to anesthesia

To determine the physiological response of juvenile Ship sturgeon to anesthesia, 72 fish were netted from acclimation tanks and distributed randomly into three anesthesia treatments with three replicates for each (24 fish for each treatment). Anesthesia treatments were prepared as previously described. Single working treatments of clove powder, CO_2, and electric shock were used to anesthetize all groups of fish in this part. To investigate how the physiological parameters would change in response to anesthesia, prior to start the experiment, two fish were removed from each replicate and blood was taken using 2-ml heparinized syringe from the caudal vein; bled fish were return to their

Table 1 Induction and recovery times of juvenile Ship sturgeon, *Acipenser nudiventris*, anesthetized with different concentrations of three anesthetics

Treatments	Time (min)	
	Induction	Recovery
Electric shock	0.2 ± 0.1^{c}	3.8 ± 0.3^{a}
Clove powder	4.3 ± 0.4^{a}	2.5 ± 0.1^{b}
CO_2	1.5 ± 0.1^{b}	2.3 ± 0.1^{b}

Values are mean ± SE. Different superscript letters in the same column show significant difference among treatments by Duncan's range test ($P < 0.05$). $n = 15$ per each treatment

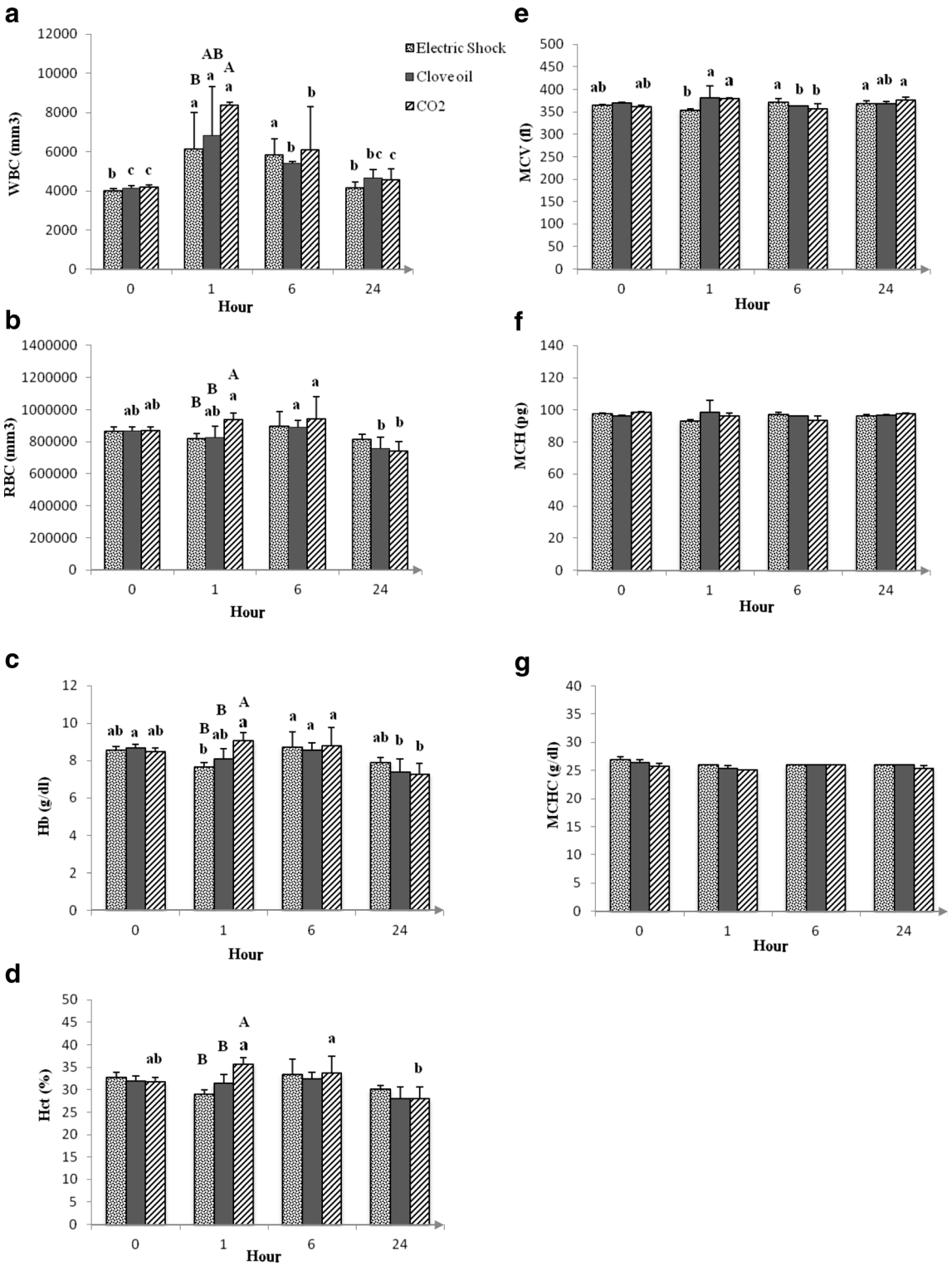
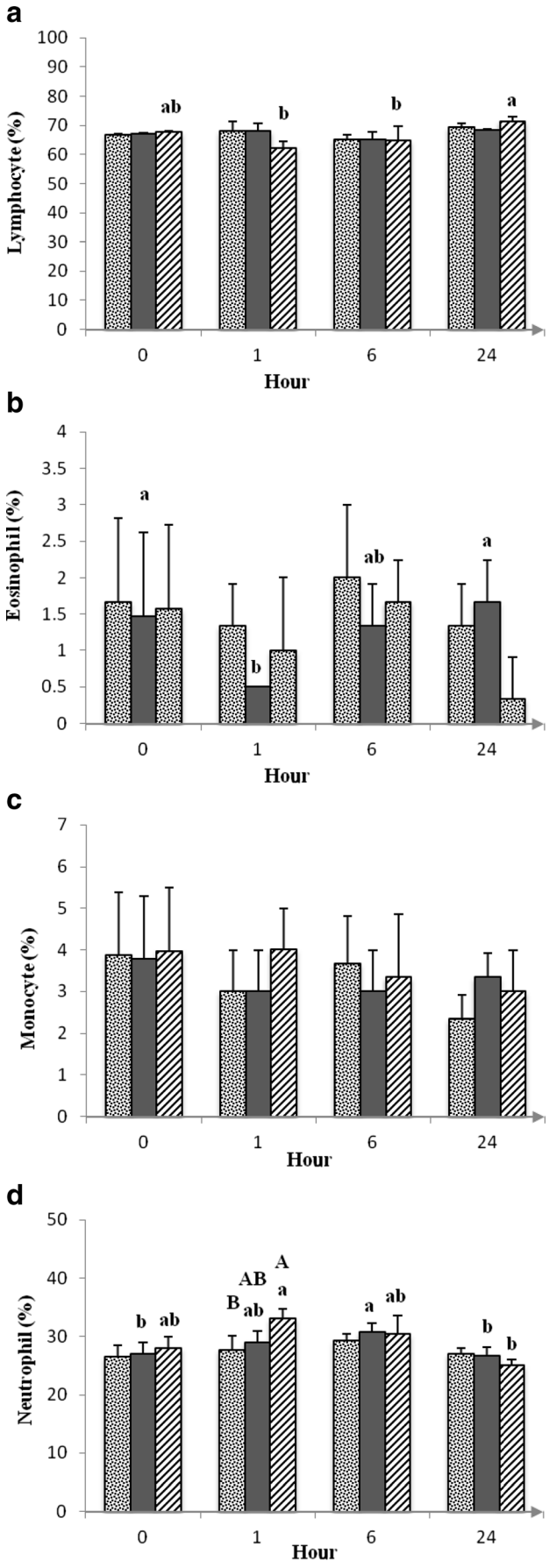


Fig. 1 Changes in WBC (**a**), RBC (**b**), Hb (**c**), Hct (**d**), MCV (**e**), MCH (**f**), and MCHC (**g**) levels during 24 h in juvenile Ship sturgeon, *Acipenser nudiventris* exposed to three anesthetics. Values are mean ± SE. $n = 24$ fish per treatment. *Capitalized letters* show significant difference among treatments at the same time, and *small letters* show significant difference in each treatment during time by Duncan's range test ($P < 0.05$)

original acclimation tanks. When fish were anesthetized, each replicate was individually transferred into recovery tanks with 40-l water volume filled with aerated fresh water.



◀ **Fig. 2** Changes in lymphocyte (**a**), eosinophil (**b**), monocyte (**c**), and neutrophil (**d**) levels during 24 h in juvenile Ship sturgeon, *Acipenser nudiventris* exposed to three anesthetics. Values are mean ± SE. $n = 24$ fish per treatment. *Capitalized letters* show significant difference among treatments at the same time, and *small letters* show significant difference in each treatment during time by Duncan's range test ($P < 0.05$)

After 1, 6, and 24 h, blood samples were taken from the rest of fish.

Blood was divided into two aliquots; one was transferred to an Eppendorf tube and used for the determination of hematocrit (Hct) value, hemoglobin (Hb) concentration, and numbers of red blood cells (RBC) and white blood cells (WBC). Afterwards, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured according to the below formulas (Klinger et al. 1996):

$$\mathrm{MCV(fl)} = 10 \times (\mathrm{Hct}) \Big/ \mathrm{RBC}$$
$$\mathrm{MCH(pg)} = 10 \times (\mathrm{Hb}) \Big/ \mathrm{RBC}$$
$$\mathrm{MCHC}(\%) = 100 \times (\mathrm{Hb}) \Big/ \mathrm{Hct}$$

The blood was centrifuged in heparinized microhematocrit capillary tubes after centrifugation in a standard microhematocrit centrifuge at 3500 *g* for 7 min to determine Hct. Hb was measured using a commercial kit (cyanmethemoglobin method; Sigma, St. Louis, MO, USA). Numbers of WBC and RBC were counted with an improved Neubauer hemocytometer (Houston 1990).

The second aliquot was centrifuged at 3000 *g* for 10 min, then plasma was separated and stored in Eppendorf tubes at −20 °C for subsequent assays of biochemical factors including cortisol and glucose, and osmolality was measured by Vapro 5520 osmometer (Wescor, Inc., Logan, UT).

Plasma cortisol concentrations was measured by radioimmunoassay method based on the competitive reaction (Redding et al. 1984) using a commercial kit (Immunotech Co., Marseille, France). Plasma glucose levels were determined by glucose-oxidase (GOD-PAP) method (Bayunova et al. 2002) using a commercial kit (Pars Azmoon diagnostics kit, Tehran, Iran).

Data analyses

One-sample Shapiro-Wilks and Levene's tests were used to check normality of data and homogeneity of variances, respectively. Data are presented as means ± standard error (SE). One-way analysis of variance (ANOVA) was used to evaluate the effects of the different concentrations of three anesthetics on induction and recovery times. To determine differences among treatments, during time, two-way ANOVA was used. When differences were detected,

Duncan's range test was used to determine which treatments were significantly different. The level of significance was chosen at $P < 0.05$. All analyses were performed using SPSS software (SPSS. 16.0, Chicago, IL).

Results

Induction and recovery times

The mean times of induction and recovery are presented in Table 1. There are significant difference between both induction and recovery times of three anesthetics ($P < 0.05$), of which the shortest induction time belonged to fish anesthetized with electric shock. By contrast, the longest recovery time occurred for electric shock treatment. On the other hand, fish anesthetized with clove powder had both the longest time of induction and shortest time of recovery. In the case of the electric shock treatment, muscle spasm was observed following electrical exposure.

Hematological parameters

There were significant differences in WBC, RBC, Hb, and Hct values among three treatments, only 1 h after induction ($P < 0.05$, Fig. 1a, b, c, d). The most elevated value of these parameters was for fish treated with CO_2 compared to other treatments. The lowest value of these parameters was found at time 0, at which no significant difference was observed among treatments ($P > 0.05$). Comparing the effects of different treatments on MCV, MCH, and MCHC revealed that for all treatments, only MCV changed significantly during the time ($P < 0.05$, Fig. 1e), whereas no significant differences were observed in MCH and MCHC for three treatments ($P > 0.05$, Fig. 1f, g).

Differential leucocyte count

The percent value of lymphocyte changed significantly only for fish exposed to CO_2 ($P < 0.05$, Fig. 2a) during the time. Only fish anesthetized with clove powder indicated significance in eosinophil as the experiment progressed ($P < 0.05$, Fig. 2b). Monocyte showed no significant difference ($P > 0.05$, Fig. 2c) for all three treatments during the time. The significant highest percent value of neutrophil was found in CO_2 treatment 1 h after induction ($P < 0.05$, Fig. 2d). The percent value of neutrophil did not change significantly during the time for fish exposed to electric shock ($P > 0.05$, Fig. 2d).

Biochemical parameters

Plasma cortisol concentration revealed significant differences among all treatments at 1, 6, and 24 h ($P < 0.05$, Fig. 3a), of which the highest level belonged for fish treated with CO_2, then significant decrease trend was observed. No significant change in cortisol level was

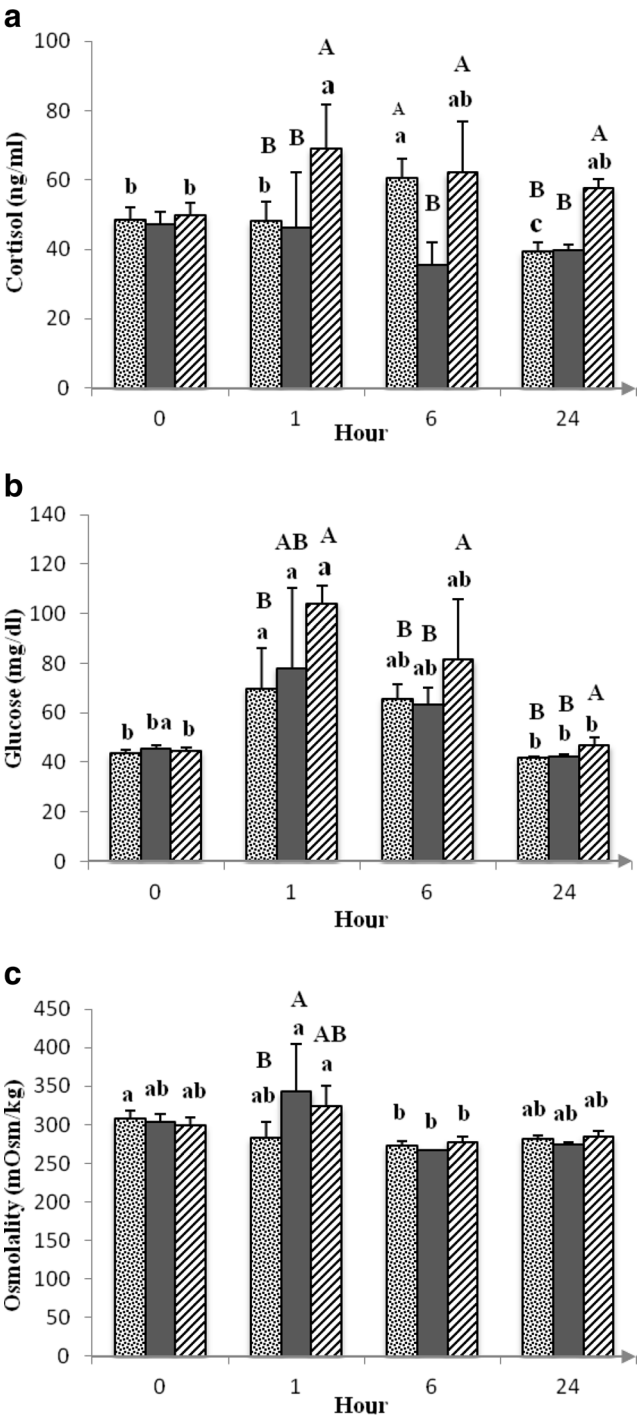


Fig. 3 Changes in cortisol (**a**), glucose (**b**), and osmolality (**c**) levels during 24 h in juvenile Ship sturgeon, *Acipenser nudiventris* exposed to three anesthetics. Values are mean ± SE. $n = 24$ fish per treatment. *Capitalized letters* show significant difference among treatments at the same time, and *small letters* show significant difference in each treatment during time by Duncan's range test ($P < 0.05$)

found at the baseline ($P > 0.05$). Fish exposed to clove powder indicated no significant difference in plasma cortisol concentration ($P > 0.05$). Cortisol concentration indicated the highest level after 6 h for fish anesthetized with electric shock ($P < 0.05$).

All treatments showed significant change in glucose level over time ($P < 0.05$, Fig. 3b), of which the highest level was observed at time 1, then a decrease trend was found. Plasma glucose concentration revealed significant differences among all treatments at all sampling steps except for time 0 ($P < 0.05$, Fig. 3b).

Fish exposed to clove powder and CO_2 indicated the significant highest level of osmolality, then a decrease trend was found ($P < 0.05$, Fig. 3c). The highest level of osmolality for fish treated with electric shock was at time 0, then decreased significantly ($P < 0.05$). Osmolality concentration showed significant differences among all treatments only at time 1 ($P < 0.05$).

Discussion

Our results indicated that electric shock, CO_2, and clove powder are all effective in anesthetizing juvenile Ship sturgeons according to Marking and Meyer (1985) who suggested that the ideal anesthetic would have an induction time of 3 min or less and a recovery time of less than 5 min. In this study, electric shock made faster induction times and longer recovery than other anesthetics evaluated. Anesthetizing fish to the desired endpoint with higher voltage of anesthesia may result in a longer recovery period. Similar results were found in previous studies in which fish were anesthetized to stage IV in 0.1–0.2 min using electrosedation and in 2.5 min using CO_2 (Trushenski et al. 2012b; Jennings and Looney 2011; Bowzer et al. 2012). Since there is variability in induction time due to size, water temperature (Trushenski et al. 2012b), mechanism of functioning, and pharmacokinetics of each anesthetic (Weber et al. 2009), it is difficult to compare induction and recovery times during the experiments.

In general, to reduce stressor severity, anesthesia is commonly used (Iverzen et al. 2003; Wagner et al. 2003; Small 2004; Palić et al. 2006); however, there are indications that anesthesia itself can induce a mild to moderate stress response and induce departures from normal physiological states (Kiessling et al. 2009). In the study presented here, only fish anesthetized with CO_2 indicated significant increase in RBC, Hb, and Hct. On the other hand, these parameters had no significant increase for fish treated with electric shock and clove powder. Adrenergic and cholinergic innervations contract the spleen when stimulated by stress and that this event increases RBC, Hb, and Hct (Nilson and Grove 1984). Exposure to CO_2 may cause high metabolic demand, so this is as a possible strategy in response to increased oxygen-carrying capacity of the blood or a result of increased swimming activity during the stressful condition (Caruso et al. 2005). Trushenski et al. (2012b) reported increased value of Hb for hybrid striped bass following CO_2 exposure. Also, the low change of Hb was found in grass carp, *Ctenopharyngodon idella*, anesthetized with electric sedation (Bowzer et al. 2012) which is in accordance with our study. WBC increased significantly for all treatments. Migration of WBC from the spleen to blood circulation (Barcellos et al. 2004) can be the reason for the increase in WBC following stress. There are similar results in *Limanda limanda* (Pulsford et al. 1994) and *Rhamdia quelen* (Barcellos et al. 2004).

Elevation of plasma cortisol concentrations has been used as an indicator of stress in fish (Barton 2002). Some anesthetics can induce a measurable increase in fish cortisol stress responses (Iwama et al. 1989; Small 2004). In the present study, CO_2 indicated significant elevated cortisol levels in 1 h after exposure, whereas fish anesthetized with clove powder had no significant change in cortisol level. Increased level of cortisol happened 6 h after induction for juvenile Ship sturgeons exposed to electric shock. Clove powder was revealed to have stress-reducing potential, and no significant change in cortisol level in some species including channel catfish, Atlantic salmon, and fathead minnows was found (Iverzen et al. 2003; Small 2004; Small and Chatakondi 2005; Palić et al. 2006). Similar results were observed in hybrid striped bass following electric sedation and CO_2 exposure (Trushenski et al. 2012b). Stress reaction varies between different anesthetics (Kiessling et al. 2009); probably, the mode of function of various anesthetics affect on stress reactions (Zahl et al. 2010). Similarly, plasma glucose is also commonly associated with exposure to anesthesia (Cho and Heath 2000; Wagner et al. 2003). Elevated levels of glucose were found in juvenile Ship sturgeons followed with anesthesia treatments. This is in agreement with previous studies in which an increased level of glucose was found after exposure to CO_2, electric sedation, and clove oil (Trushenski et al. 2012b; Gaus et al. 2012). Rapid increases in plasma glucose are mediated by the release of catecholamines, which increase in the plasma of anesthetized fish and occur in response to the hypoxia caused by cessation of respiration (Gingerich and Drottar 1989; Iwama et al. 1989). Plasma osmolality appeared to vary somewhat among anesthesia and over time. The lack of substantial change in osmolality may suggest a relatively minor and brief acute stress response following sedation.

Conclusion

Considering the results, CO_2 induced the most stress response, of which the amount of cortisol released was greater compared to other anesthetics. Electric shock may be a suitable tool for quickly inducing anesthesia in juvenile ship sturgeon.

However, all of the anesthesia options evaluated were effective in anesthetizing fish within reasonable time frames at the doses or strengths used.

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