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Efficacy of clove oil as anesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichlidae) juveniles

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ABSTRACT. This work evaluated the efficiency of clove oil as anaesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). In the first experiment, safety concentrations of clove oil were assessed by measuring induction times to anaesthesia. The second experiment evaluated exposure times to anaesthetic. Clove oil efficiency during transportation was evaluated in a 24 h experiment using three concentrations of the anaesthetic (0, 9, and 18 $mg.L^{-1}$). The most appropriate clove oil concentration to induce surgical anaesthesia was 90 $mg.L^{-1}$. To biometry or other brief handling, the recommended concentration is 50-60 $mg.L^{-1}$ as it provides fast recovery. Maximum anaesthesia time should be 10 min. The mortality rate of fish transported using 18 $mg.L^{-1}$ of anaesthetic was significantly higher than that of the control group at 24 h of transportation and at 96 h after transportation. The fish transported using clove oil as anaesthetic presented more significant Na^+ and K^+ disorders as compared to the control group. As an anaesthetic, clove oil is efficient in the handling of Nile tilapia in routine fish hatchery procedures, although it should be avoided in the transportation.

KEY WORDS. Eugenol; net ion flux; stress; tropical fish.

In Brazil, tilapia farming is usually carried out in two distinct sites: the hatchery station, where reproduction and sex reversion are conducted and juveniles grow up to 5 cm, and growing station, where the 5 cm fish reach harvest size. Anaesthesia is required in several procedures conducted in hatchery and growth stations (Ross & Ross 2008).

In tilapia hatcheries, the handling and transportation of large numbers of fish are the procedures in which anaesthesia is especially required. In general, the handling of fish is an extreme procedure, but of short duration, which leads to acute stress (Kiessling *et al.* 2009). In turn, transportation is an essential step in the production process and likewise is considered to increase stress in fish. In spite of the advancements in farming techniques, fish mortality caused by poor management and transportation strategies still lead to losses in the tilapia hatcheries.

In Brazil, the most widely utilized chemical anaesthetics are obtained with difficulty, and for many producers costs are prohibitive (Roubach *et al.* 2005). In this scenario, the use of essential oils extracted from plants has proved to be a feasible alternative to chemical anaesthetics during fish handling and transportation (Kaiser *et al.* 2006, Pálic *et al.* 2006, Simões & Gomes 2009). The main substance used is clove oil, extracted from the leaves and buds of the tree *Eugenia caryophyllata* (Linnaeus). The active principle is eugenol which concentration in clove oil is between 70 and 90%. Clove oil is considered an appropriate anaesthetic for fish because of its low costs, simple obtaining, and considerable anaesthetic efficiency. Also, the substance apparently does not exert any toxic effect (Ross & Ross 2008). Clove oil has been extensively used in several fish species, and the results show that the substance is a good economic alternative to the chemicals normally used in fish anaesthesia (reviewed by Ross & Ross 2008). The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), is the fish most commonly grown in Brazil, and one of the most cultured in the world (FAO 2007). Nevertheless, there was no literature on the use of anaesthetics with juveniles of this species in hatcheries. The present study was designed to test the efficiency of clove oil as an anaesthetic in handling and transportation procedures of Nile tilapia juveniles.

MATERIAL AND METHODS

Clove oil as anaesthetic during handling

Ninety Nile tilapia juveniles (1.46 ± 0.38 g, 4.33 ± 0.30 cm) were obtained from a commercial supplier and transported to our research laboratory, where the individuals were acclimated in two 150 L tanks equipped with continuous water inflow and aeration. Acclimation lasted 10 days, throughout which period water quality parameters were monitored every three days, with values as follows: dissolved oxygen (5.99 ± 1.23 $mg.L^{-1}$); temperature (26.7 ± 0.96 ºC); conductivity (66.49 ± 3.09 $\mu S.cm^{-1}$); hardness (23.62 ± 3.29 $mgCaCO_3.L^{-1}$); water flow (1.43.20 ± 0.41.13 $L.min^{-1}$) and pH (6.70 ± 0.15). During acclimation fish were fed daily with specific commercial fish feed containing 36% of crude protein.

After acclimation, the tests were carried out in 6 L static aquaria containing 2 L water. Recovery was always conducted in 45 L plastic aquaria filled with 20 L water and constant aeration. Water quality parameters during tests were as follows: dissolved oxygen (8.15 ± 0.44 $mg.L^{-1}$); temperature (27.5 ± 0.34 ºC); conductivity (67.73 ± 1.00 $\mu S.cm^{-1}$); hardness (25.02 ± 1.42 $mgCaCO_3.L^{-1}$); and pH (6.52 ± 0.10). Prior to use, a 1:10 (v/v) stock solution of clove oil (Petite Marie; SP, Brazil; density 1,03 $g\ cm^{-3}$, 90% de eugenol) was prepared with ethanol 95%. The water in each aquarium was replaced at the end of each test.

In the first experimental segment, six different clove oil concentrations were tested (50, 60, 70, 80, 90, and 100 $mg.L^{-1}$). For each concentration, 10 fish were individually exposed to the anaesthetic for 10 min. The aim was to observe the induction time in seconds for each anaesthesia stage. After exposure, each fish was transferred to the recovery aquarium. The different induction stages were assessed according to the criteria proposed by Stoskopf (1993). Recovery was defined as the full-range, active swimming of fish inside the recovery aquarium.

The second experimental segment evaluated the safety concentration of the anaesthetic based on the results of the first experiment. Recovery time was measured after the induction of anaesthesia at different exposure times with 90 $mg.L^{-1}$ clove oil. The concentration of 90 $mg.L^{-1}$ was chosen as the most appropriate because it caused the interruption of oppercular beat rates at the shortest time and the induction of all anaesthesia stages. The previously acclimated fish were individually exposed to the anaesthetic for 10, 20, and 30 min (n = 10 for each exposure time). After exposure, each fish was transferred to the recovery aquarium and the recovery time measured.

Behavioral events and recovery time results are expressed as mean and standard deviation. The times to reach the different anaesthesia stages under the different clove oil concentrations, as well as the recovery times after exposure to the anaesthetic for different anaesthesia times were evaluated using an analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$) (Zar 1999).

Clove oil as anaesthetic during transportation

For this experiment, 2,700 Nile tilapia (0.43 ± 0.11 g; 3.27 ± 0.21 cm) provided by the fish farm Bioalevinos (Ibiraçú, ES, Brazil) were used. The fish were captured in a breeding tank and transferred to a 500 L depuration tank equipped with constant water inflow and kept there for 24 h. The water quality parameters measured were: dissolved oxygen (6.85 $mg.L^{-1}$); temperature (25.3 ºC); conductivity (45.8 $\mu S.cm^{-1}$); hardness (10.01 $mgCaCO_3.L^{-1}$); water inflow (4.94 $L.min^{-1}$); CO_2 (8.8 $mg.L^{-1}$); ammonia (below the detection limit) and pH 7.58.

Fish were transported in 27 6-L plastic bags containing 2 L water and pure oxygen, to a final volume that took up 90% of the bag's total capacity. Next, all bags were sealed with rubber stringers. Fish density was 50 fish/L (100 fish per bag). The transportation experiment was done in a factorial design with three clove oil concentration (0, 9 and 18 $mg.L^{-1}$ clove oil) and three transportation times (6, 12 and 24 h). These concentrations are 10 and 20% of the ideal concentration for surgical anaesthesia, choose in the first experiment. Three replicates were conducted for each combination of time versus anaesthetic concentration. Transport trial started 8 o'clock in the morning and were done in paved road, with six hours of actual transportation until arrival in the laboratory, where it was held over 18 hours of simulated transport, totaling 24 hours of transport.

At the end of the transportation period, all bags were opened and mortality, water parameters and Na^+ and K^+ flux values measured. The fish from each bag were transferred to 30 L tanks with constant aeration and monitored for 96 h to evaluate accumulated mortality rate.

Dissolved oxygen, temperature and electric conductivity were measured using a YSI 85 multiparameter monitor (Yellow Spring Inc., Yellow Spring, USA). pH was monitored using a digital potentiometer (Quimis Q-400, Quimis, São Paulo, Brazil). Water hardness and CO_2 were evaluated by titration, total ammonia was measured according to the endophenol technique, all measurements were conducted in accordance with the guidelines defined by APHA (1998).

The concentrations of Na^+ and of K^+ in water were measured directly using a flame photometer (Analyser 910, Analyser, São Paulo, Brazil). Net ion fluxes (Jnet) were calculated from the changes in the ion concentration of the water of transportation over the sampling periods according to the equation of Gonzalez *et al.* (1998): $Jnet = V([ion]_1 - [ion]_2).(Mt)^{-1}$, where $[ion]_1$ and $[ion]_2$ are the bath ion concentrations at the beginning and end of the flux period, respectively, V is the bath volume in liters, M is the mass of the fish in kg, and t is the duration of the flux period in hours. In transportation water, the mean initial Na^+ concentrations were 350, 315.6 and 317.8 $\mu mol.L^{-1}$, while for K^+ these concentrations were 18, 12.1 and 12 $\mu mol.L^{-1}$ for fish transported in 0, 9, and 18 $mg.L^{-1}$ clove oil, respectively.

Results are expressed as means ± SD and data from mortality and accumulated mortality were log+1 transformed before statistical analysis. The mortality, accumulated mortality, water quality and ion flux results were calculated based on a two-way ANOVA followed by the Tukey test ($p < 0.05$) (Zar 1999).

RESULTS AND DISCUSSION

Clove oil as anaesthetic during handling

Clove oil as used in the concentrations 80, 90, and 100 $mg.L^{-1}$ induced all anaesthesia stages in fish; however, the fish anaesthetized with 90 $mg.L^{-1}$ reached total loss of equilibrium and had lower oppercular movement rates at a significantly shorter time, as compared to the those using the lower concentrations tested. At 100 $mg.L^{-1}$, clove oil led to a 20% mortal-

ity rate in the anaesthetized fish. Therefore, 90 mg.L^{-1} should be considered as the ideal clove oil concentration indicated for the induction of surgical anaesthesia (total loss of movement and minimum oppercular movement, no reaction to stimulus) in Nile tilapia juveniles (Tab. I).

The concentrations required for induction of anaesthesia vary with fish species and size, both for clove oil and for other anaesthetics, and are probably related to the particular species metabolism (Ross & Ross 2008). Ideal clove oil concentrations lie within 10 and 50 mg.L^{-1} for a wide variety of fish species, such as the Atlantic salmon, *Salmo salar* Linnaeus, 1758 (Iversen *et al*. 2003); the largemouth bass, *Micropterus salmoides* (Lacepède, 1802) (Cooke *et al*. 2004); the gilthead seabream, *Sparus aurata* Linnaeus, 1758 (Mylonas *et al*. 2005); and the fathead minnow, *Pimephales promelas* Rafinesque, 1820 (Pálic *et al*. 2006). On the other hand, species like the eel, *Anguilla reinhardtii* Steindachner, 1867 (Walsh & Pease 2002) and tambaqui, *Colossoma macropomum* (Cuvier, 1816) (Roubach *et al*. 2005) require higher concentrations, which vary between 65 and 80 mg.L^{-1} for the induction of surgical anaesthesia.

In brief procedures, the adequate anaesthetic concentration should promote total loss of equilibrium without necessarily inducing all anaesthesia stages (see Tab. I to all anaesthetic stages details). In the present study, the concentrations of 50, 60, 70 and 80 mg.L^{-1} clove oil promoted total loss of equilibrium at similar intervals, and promote the fast recovery of fish anaesthetized with the compound. Therefore, 50-60 mg.L^{-1} is the optimal concentration range to induce anaesthesia in handling of the Nile tilapia (Tab. I).

The recovery times after exposure to a 90 mg.L^{-1} solution of clove oil for 10 and 20 min were respectively 359.1 ± 41.9 s and 560.8 ± 123.35 s, with no statistically significant difference. However, 50% of the fish that were anaesthetized for 20 min died, and the mortality rate of fish anaesthetized for 30 min reached 100%. Waterstrat (1999) observed similar results with anaesthetized channel catfish juveniles, *Ictalurus punctatus* (Rafinesque, 1818) using 100 mg.L^{-1} clove oil, in an experiment that showed that exposure times of over 20 min increase recovery time and mortality rates. Ross & Ross (2008) are cautious and believe 10 min to be the maximum anaesthesia time – a finding that was corroborated in the present study.

Clove oil as anaesthetic during transportation

As part of the handling procedures in intensive fish farming, transportation times may vary considerably, depending on the distance covered. As a rule, juveniles are transported from a hatchery to growth stations. In this process, the fish should arrive in good physiological conditions to meet the criteria demanded by the buyer (Carneiro *et al*. 2002).

All the concentrations tested in the present study caused hyperactivity in fish, as of the first moment of exposure to the anaesthetic. This, was manifested as the swift movements of the animals inside the plastic bags, which decreased as the anaesthetic effect of clove oil increased. After the 6 h transportation period, the fish exposed to 9 mg.L^{-1} clove oil presented reduced response to stimuli, which included slight sedation, differently from the fish anaesthetized with 18 mg.L^{-1}, in which the majority of the individuals presented total loss of equilibrium. After 12 and 24 h of transportation, fish transported in clove oil at the two concentrations tested lost response to stimuli. According to Cooke *et al*. (2004), the anaesthesia stages desired in transportation are manifested as the partial loss of response to stimuli and partial loss of equilibrium. The authors studied different clove oil concentrations with *M. salmoides*, and maintain that optimal anaesthetic concentrations for the transportation of this fish species are within the 5-8.5 mg.L^{-1} range.

Water temperature was similar across concentration treatments in the 6 and 12 h transportation periods. In the 24 h transportation period, water temperature was the same across the groups treated with different clove oil concentrations, but this temperature was significantly higher than the value measured at 6 and 12 h (Tab. II). Throughout this experiment, temperature variation was under 1ºC.

Table I. Induction times, to the different anaesthesia stages in *O. niloticus* juveniles exposed to different concentrations of clove oil. Behavioral events adapted from Stoskopf (1993). Different letters in the columns indicate statistical difference between concentrations in one same anaesthesia stage by the ANOVA and Tukey test (5%).

Clove oil (mg.L^{-1})	Behavioral events (seconds)					
	Loss of reaction to stimulus	Partial loss of equilibrium	Total loss of equilibrium	Reduction oppercular movements	Total loss of oppercular movements	Recovery
50	24.7 ± 1.0 ^{a}	43.7 ± 2.3 ^{a}	90.9 ± 7.0 ^{a}	344.1 ± 14.2 ^{a}	–	177.4 ± 21.2 ^{a}
60	20.2 ± 1.0 ^{b}	32.0 ± 1.7 ^{b}	90.0 ± 9.8 ^{a}	219.0 ± 12.4 ^{b}	–	163.0 ± 15.9 ^{a}
70	16.9 ± 1.1 ^{bc}	28.8 ± 1.4 ^{bc}	83.6 ± 4.8 ^{ab}	348.7 ± 6.7 ^{a}	–	287.5 ± 18.1 ^{ab}
80	17.5 ± 0.5 ^{b}	34.0 ± 1.5 ^{b}	95.6 ± 5.3 ^{a}	212.5 ± 7.7 ^{b}	545.0 ± 25.0 ^{a}	220.0 ± 15.5 ^{a}
90	13.4 ± 0.8 ^{cd}	23.5 ± 2.1 ^{cd}	86.6 ± 10.8 ^{ab}	147.0 ± 14.0 ^{c}	281.0 ± 15.1 ^{b}	379.0 ± 71.8 ^{b}
100 †	9.6 ± 0.7 ^{d}	17.6 ± 1.2 ^{d}	56.6 ± 3.7 ^{b}	126.8 ± 6.9 ^{c}	199.2 ± 6.5 ^{c}	377.1 ± 35.6 ^{b}

† Mortality rate of 20%.

Table II. Water quality after transportation of *O. niloticus* juveniles for different times and at different clove oil concentrations. Data were analyzed by Two-way ANOVA and Tukey test ($p < 0.05$). Lower case letters indicate statistical difference of the different treatments at one same transportation time. Upper case letters indicate statistical difference of different transportation times for one same treatment.

Transportation time (hour)	Clove oil concentration ($mg.L^{-1}$)		
	0	9	18
Temperature (°C)			
6	25.3 ± 0.09 ^{aa}	25.3 ± 0.06 ^{aa}	25.3 ± 0.15 ^{aa}
12	25.3 ± 0.09 ^{aa}	25.2 ± 0.13 ^{aa}	25.3 ± 0.12 ^{aa}
24	25.8 ± 0.03 ^{aB}	25.8 ± 0.06 ^{aB}	25.7 ± 0.09 ^{aB}
Dissolved Oxygen ($mg.L^{-1}$)			
6	16.1 ± 1.25 ^{aa}	13.2 ± 0.73 ^{aa}	13.6 ± 0.24 ^{aa}
12	13.4 ± 1.44 ^{aaB}	9.2 ± 0.56 ^{ba}	13.7 ± 1.14 ^{aa}
24	10.9 ± 1.97 ^{aB}	5.0 ± 0.48 ^{bB}	4.1 ± 1.03 ^{bB}
Conductivity ($\mu s.cm^{-1}$)			
6	94.2 ± 1.87 ^{aa}	98.9 ± 0.74 ^{aa}	104.9 ± 2.24 ^{aa}
12	129.9 ± 3.80 ^{abB}	126.7 ± 2.49 ^{aB}	145.2 ± 4.38 ^{bB}
24	198.8 ± 3.99 ^{aC}	178.9 ± 0.28 ^{bC}	162.0 ± 12.89 ^{bB}
pH			
6	6.4 ± 0.10 ^{aa}	6.6 ± 0.08 ^{ba}	6.7 ± 0.04 ^{ba}
12	6.1 ± 0.02 ^{aB}	6.1 ± 0.01 ^{aB}	6.2 ± 0.03 ^{aB}
24	6.0 ± 0.02 ^{aB}	5.9 ± 0.04 ^{abB}	5.7 ± 0.11 ^{bC}
CO_2 ($mg.L^{-1}$)			
6	58.7 ± 1.47 ^{aa}	51.3 ± 5.29 ^{aba}	41.9 ± 2.10 ^{ba}
12	48.4 ± 6.60 ^{aa}	50.6 ± 0.00 ^{aa}	41.8 ± 1.27 ^{aa}
24	80.7 ± 0.73 ^{aB}	90.2 ± 5.54 ^{abB}	98.3 ± 1.94 ^{bB}
Total ammonia ($mg.L^{-1}$)			
6	4.8 ± 1.42 ^{aa}	4.7 ± 0.84 ^{aa}	3.1 ± 3.37 ^{aa}
12	6.3 ± 0.84 ^{aa}	4.7 ± 0.65 ^{aa}	6.9 ± 0.26 ^{aaB}
24	10.1 ± 3.37 ^{aa}	11.7 ± 0.20 ^{aB}	9.3 ± 1.23 ^{aB}

Oxygen levels were higher in the 6 h transportation time followed by a continuous decrease in the parameter, up to 24 h transportation time in all groups. At the end of the transportation experiment, dissolved oxygen was significantly higher in the control group as compared to the groups transported using clove oil. A pronounced consumption of oxygen was seen in fish treated with the anaesthetic in the 12 and in the 24 h measurements. This may be linked with the hyperactivity in fish, at the first moment of exposure to the anaesthetic, especially in the 18 $mg.L^{-1}$ clove oil concentration. Therefore, considering fish anaesthetized with clove oil at concentrations between 9 and 18 $mg.L^{-1}$ (at a density of 50 fish/L maximum) transportation times should be 24 h, since oxygen levels in this experimental configuration reached values near the critical survival threshold.

As for electric conductivity, values exhibited significant increases across transportation times in all clove oil concentrations tested (Tab. II). According to Gomes *et al.* (2009), this increase is imputable mainly to the escalation of ammonia levels in the water. This pattern of increase in conductivity with transportation time has been observed during the carrying of the cardinal tetra, *Paracheirodon axelrodi* (Schultz, 1956) (Gomes *et al.* 2009).

In turn, pH and CO_2 exhibited opposite behaviors, with pH falling during transportation, in all clove oil concentrations, whilst CO_2 values were observed to increase (Tab. II). At the end of the transportation experiment, pH was significantly higher in the control group as compared to the fish anaesthetized with 18 $mg.L^{-1}$ clove oil, while CO_2 exhibited an opposite trend, with values significantly higher in the 18 $mg.L^{-1}$ treatment group when evaluated against the control. This results was expected since was well established that increase in CO_2 cause a decrease in pH. Inoue *et al.* (2005) also reported that pH decreased after 4 h of transportation of juvenile matrinxã (*Brycon cephalus,* Gunther) anaesthetized with 5 $mg.L^{-1}$ clove oil, probably due to the increase in CO_2 values.

Ammonia measurements in the control group did not diverge significantly across the different clove oil concentrations tested. In the treatments with the anaesthetic, ammonia concentration was markedly higher in the 24 h transportation period (Tab. II). The ammonia values at 24 h of transportation (10-11 $mg.L^{-1}$) were below the lethal concentration threshold for Nile tilapia juveniles (Benli & Küksal 2005). Tilapia juveniles tolerate high ammonia levels in the water (Benli & Küksal 2005, Benli *et al.* 2008).

No mortality was observed in any of the clove oil anaesthesia concentrations in the 6 h transport measurement. Mortality of control fish and of the 9 $mg.L^{-1}$ treatment group, after 12 and 24 h of transportation was only 0.67% (Tab. III). In the 18 $mg.L^{-1}$ clove oil treatment, the mortality at 24 h of transportation (3.67%) was significantly higher than the values observed for the other concentrations used. The Lake Victoria cichlid, *Haplochromis obliquidens* (Hilgendorf, 1888), anaesthetized with 18 ¼$L.L^{-1}$ of clove oil also revealed higher mortality rates after long transportation times (48 h) (Kaiser *et al.* 2006). Accumulated mortality, measured 96 h after transportation presented a similar tendency as compared to the mortality after the end of the transportation period, and was significantly higher in fish transported in 18 $mg.L^{-1}$ clove oil for 24 h. Total mortality for control fish and for fish anaesthetized with 9 $mg.L^{-1}$ clove oil was only 1.33%.

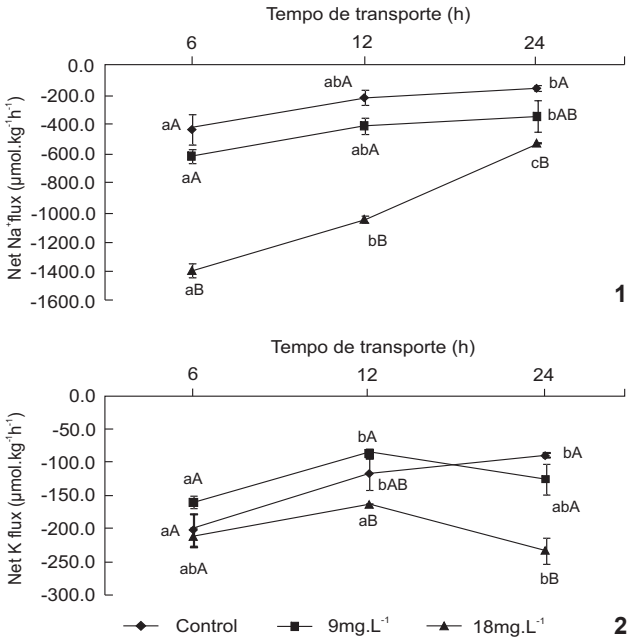
Deriggi *et al.* (2006) reported that short-term exposures to eugenol did not affect the ion balance in Nile tilapia juveniles (60 g). However, in the treatments and transportation times tested in the present study, all fish presented Na^+ and K^+ net eflux.

Table III. Accumulated mortality (96 h after transportation), of *O. niloticus* juveniles for different times and at different clove oil concentrations. Data were analyzed by two-way ANOVA and Tukey test ($p < 0.05$). Lower case letters indicate statistical difference of the different treatments at one same transportation time. Upper case letters indicate statistical difference of different transportation times for one same treatment.

Clove oil Concentration ($mg.L^{-1}$)	Mortality after transport (%)			Accumulated mortality 96 h (%)		
	Transportation time (h)			Transportation time (h)		
	6	12	24	6	12	24
0	0.00 ^{aa}	0.67 ^{aa}	0.67 ^{aa}	0.67 ^{aa}	1.00 ^{aa}	1.33 ^{aa}
9	0.00 ^{aa}	0.00 ^{aa}	0.67 ^{aa}	1.00 ^{aa}	0. 67 ^{aa}	1.33 ^{aa}
18	0.00 ^{aa}	0.00 ^{aa}	3.67 ^{bB}	0.33 ^{aa}	0.33 ^{aa}	5.67 ^{bB}

Na^+ net eflux in fish transported in 18 $mg.L^{-1}$ clove oil was significantly higher than in fish anaesthetized with all other concentrations, in the different transportation times (6, 12, and 24 h) (Fig. 1). The only exception was observed at the 24 h measurement, in which Na^+ net eflux in fish transported in 9 $mg.L^{-1}$ anaesthetic was similar as that in the fish transported in 18 $mg.L^{-1}$. In control fish and in the fish transported in 9 $mg.L^{-1}$ clove oil, Na^+ net eflux was between two and three times as low as the value measured for fish transported in 18 $mg.L^{-1}$ anaesthetic. The highest net flux values occurred in the 6 h transportation time, in all treatments; in the other transportation times a trend towards homeostasis was observed in fish. The fish also presented K^+ net flux in all treatments. The K^+ net flux pattern was similar to the one observed for Na^+ net flux. In the 6 h transportation measurement, the control group exhibited higher K^+ net eflux, which in the subsequent measurements tended to be zero. Such trend has previously been observed for the pirarucu, *Arapaima gigas* (Schinz, 1822) (Gomes *et al*. 2006). The treatment with anaesthetic presented higher K^+ net eflux in the 6 h measurement, which was followed by a drop in the parameter in the 12 h measurement and a subsequent increase in the 24 h value (Fig. 2). Similar result was observed by Gomes *et al*. (2008) for the marbled hatchedfish, *Carnagiella strigata* (Günther, 1864), in which the animals revealed high Na^+ and K^+ net eflux, which decreased significantly after 24 h of transportation, reaching values near zero. The main reason to the observed Na^+ and K^+ efflux in transported fish is an increase in gill permeability, which can cause a temporary hydromineral imbalance in the fish. In freshwater fishes, this can be manifested during acute stress as a loss in major blood ions (Barton *et al*. 2002). This result suggests that 18 $mg.L^{-1}$ clove oil induce additional stress in tilapia juvenile, as fish transported with this clove oil concentration presented a more evident ion efflux.

In conclusion, clove oil is an efficient anaesthetic for routine fish farming handling procedures for the Nile tilapia that require anaesthesia for up to 10 min. The appropriate clove oil concentration to induce surgical anaesthesia is 90 $mg.L^{-1}$, while for biometry procedures the best concentration of the anaesthetic is between 50 and 60 $mg.L^{-1}$. As for transportation procedures, this anaesthetic should be avoided for Nile tilapia juveniles, as this anaesthetic induce a greater osmorregulatory disturbance and mortality rate.



Figures 1-2. Net Na^+ (1) and K^+ (2) flux during 6, 12 and 24 h of transportation of *O. niloticus* juveniles exposed to 0 (control), 9 and 18 $mg.L^{-1}$ of clove oil. Data were analyzed by two-way ANOVA and Tukey test ($p < 0.05$). Upper case letters indicate statistical difference of the different treatments at one same transportation time. Lower case letters indicate statistical difference of different transportation times for one same treatment.

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