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Effectiveness of Sodium Bicarbonate as an Anaesthetic for different sizes of Nile tilapia (*Oreochromis niloticus* L., 1758) Juveniles

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Abstract: The effectiveness of sodium bicarbonate as an anaesthetic for Nile tilapia (*Oreochromis niloticus*) juveniles ranging from 2 to 27g was investigated. The juveniles were exposed to different concentrations of 0 (control), 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 g/L of sodium bicarbonate solution. Fish were bath immersed in each of the different concentrations of sodium bicarbonate solution for 5 min. Generally, induction time decreased while recovery time increased with increase in sodium bicarbonate concentration. Induction and recovery times increased significantly ($P < 0.05$) with increasing body weight. All fish reached full anaesthesia in less than 2 min in higher concentrations of 50 g/L and no mortality occurred under any of the concentrations tested. The most optimal concentration leading to full anaesthesia in 2 min and recovery in less than 4 min was 45 g/L.

Key Words: *Oreochromis niloticus*, sodium bicarbonate, anaesthetic, induction time

Introduction

Anaesthetics are chemical agents which induce calming effects, then successive loss of equilibrium, mobility, consciousness and finally reflex action in an organism exposed to higher concentration of the agent, or exposed for longer periods of time (Summerfelt and Smith, 1990). In aquaculture, anaesthetics are used to sedate fish when weighing, sorting, tagging, administration of vaccines, live fish transport, grading and spawning. Anaesthesia reduces physiological stressors and behaviour effects during fish handling hence minimizing stress related cortisol release, which can lead to reduced immunity against disease hence high mortalities (Zahl *et al*. 2009). Examples of anaesthetics used in aquaculture include clove

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oil, sodium bicarbonate, carbon dioxide gas, metomidate, benzocaine, tricaine, methanesulphonate (MS-222), 2- phenoxyethanol and quinaldine (Bowser, 2001; Massee *et al.,* 2005; Palić *et al.,* 2006).

Sodium bicarbonate ($NaHCO_3$), commonly known as baking soda, is soluble in water and slowly releases CO_2 which is one of the anaesthetics of fish (Bowser, 2001; Coyle *et al.,* 2004; Altun *et al.,* 2009). Sodium bicarbonate is cheap and readily available in developing countries and is listed by Food and Drug Administration (FDA) as a low regulatory priority compound. It has been successfully used as anaesthetic on rainbow trout (*Oncorhynchus mykiss*) (Keene *et al.,* 1998) and common carp (*Cyprinus carpio*) in cold and warmer conditions (Booke *et al.,* 1978; Altun *et al.,* 2009). No data is available on the efficacy of using sodium bicarbonate on Nile tilapia (*Oreochromis niloticus*) the most commonly cultured fish in Africa. Since optimal dosages of sodium bicarbonate that can induce anaesthesia are reported to be species specific (Altun *et al.*, 2009), the main aim of the current study was to determine the suitability and effective dose of sodium bicarbonate as anaesthetic of *O. niloticus* juveniles.

Materials and Methods

The study was conducted at Kenya Marine and Fisheries Research Institute, NARDTC, Sagana. Nile tilapia juveniles of average weight 2, 4, 8, 14, 18 and 27 g were collected from a nursery pond at the station and acclimatized for 3 days in 10 L aquaria filled with aerated well water. During acclimation, fish were fed twice daily for the first two days with 26% CP formulated diet and starved on the third day for 24 hrs prior to the experiment in order to optimize experimental conditions for anaesthesia study. Average water temperature was maintained at 24.0 ± 1.0°C, pH (7.8 ± 0.2), dissolved oxygen (6.0 ± 1.0) mg/L, and Total Ammonia Nitrogen (0.1 ± 0.1) mg/L in the experimental aquaria.

To determine optimal dosage and effect of size on anaesthetic efficacy of sodium bicarbonate, a group of 10 fish from each weight class were exposed to different concentrations of sodium bicarbonate by bath immersion. Concentrations of sodium bicarbonate used were (0 control), 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 g/L). The concentrations were increased by an interval of 5 based on the ability of the fish to go to stage III anaesthesia as described by Iwama *et al.* (1989). After full anaesthesia, fish were removed from the anaesthetic solution and transferred to other aquaria with clean aerated well water for recovery. All treatments were done in three replicates. The various stages of anaesthesia and recovery used in this study are presented in Table 1. The optimum anaesthetic dose for *O. niloticus* juveniles was established as the minimum dose producing the desired effect of rapid immobility at stage III anaesthesia, defined as total loss of equilibrium and gross loss of body and operculum movement as described by Iwama *et al.* (1989) and Palić *et al.* (2006).

Tab. 1: Stages of anaesthesia and recovery employed in the present study

Stages of anaesthesia	Description
I	Loss of equilibrium
II	Loss of gross body movements but continued opercula movement
III	Same as stage II but opercula movement ceases
Stages of recovery	
I	No body movements but opercula movements start
II	Regular opercula movements and body movements start
III	Equilibrium regained with pre-anaesthetic appearance

*Adapted from Iwama *et al.,* 1989.

Time of induction to anaesthesia and recovery time were recorded. Fish were held for 3 days after exposure to the anaesthetic to observe their behavior and possible mortality. The differences among means were analyzed by two-way ANOVA followed by Duncan's multiple range tests. Differences were considered significant at P <0.05. All analyses were performed with the SPSS version 17.0 statistical package.

Results

The time taken for different sizes of *O. niloticus* juveniles to become immobile differed for different concentrations of sodium bicarbonate (Tab. 2). Induction times ranged from 0.79 to 5.02 min. The control and 5 g/L concentration did not elicit any anaesthetic effect. However, induction of anaesthesia began to occur at concentrations of 10 g/L except for juveniles of 18 and 27 g, which became anaesthetized from 15 g/L concentration. Sodium bicarbonate concentration had a significant effect (P < 0.01) on the induction time to anaesthesia. Size of fish significantly affected the induction time to anaesthesia and recovery time (P < 0.01). Fish of an average weight of 2 to 8g took the least time (0.79 to 0.84 min) to reach anaesthesia in 50 g/L concentration of sodium bicarbonate. Fish of 2 to 14g reached the required stage III anaesthesia in less than 2 min when subjected to higher concentrations of 30 g/L to 50 g/L while at low concentrations of 10 and 15 g/L, the fish took more than 2 min to reach full anaesthesia.

After exposure to the anaesthetic, all the fish recovered and were feeding and swimming normally within the first day of observation. No mortality occurred in the fish exposed to all the sodium bicarbonate concentrations following 3 days of post anaesthesia observation period.

Tab. 2: Induction time (min) to anaesthesia of different sizes of *O. niloticus* in different concentrations of sodium bicarbonate.

Concentration (g/L)	Fish weight (g)					
	2	4	8	14	18	27
0	-	-	-	-	-	-
5	-	-	-	-	-	-
10	2.9±0.02[a]	3.99±0.01[a]	4.00±0.02[a]	5.02±0.02[a]	-	-
15	2.62±0.3[b]	3.01±0.01[b]	3.43±0.01[b]	3.85±0.02[b]	4.6±0.02[a]	4.99±0.01[a]
20	1.69±0.01[c]	2.13±0.00[c]	2.19±0.01[c]	2.48±0.01[c]	2.64±0.03[b]	3.23±0.02[b]
25	1.54±0.03[d]	1.98±0.02[d]	2.01±0.01[d]	2.11±0.01[d]	2.4±0.02[c]	2.92±0.01[c]
30	1.20±0.00[e]	1.58±0.01[f]	1.68±0.02[e]	1.94±0.01[e]	2.01±0.01[d]	2.22±0.01[d]
35	1.16±0.01[f]	1.39±0.01[e]	1.62±0.01[f]	1.76±0.01[f]	1.91±0.01[e]	1.94±0.01[e]
40	1.12±0.03[g]	1.32±0.01[g]	1.41±0.01[g]	1.47±0.01[g]	1.81±0.00[f]	1.85±0.02[f]
45	0.93±0.01[h]	0.99±0.01[h]	1.09±0.02[g]	1.16±0.01[h]	1.17±0.01[h]	1.57±0.01[g]
50	0.79±0.01[i]	0.81±0.01[i]	0.84±0.01[i]	0.96±0.01[i]	1.01±0.02[h]	1.15±0.02[h]

-Data are presented as Mean values ± SE of three determinations

-Different superscript in each column indicate significant differences (P <0.05).

-The sign -, means no change in the fish

Recovery time varied from 0.32 to 4.76 min (Tab. 3) and increased with increase in sodium bicarbonate concentration in all sizes of fish. Recovery in less than 2 min was only experienced in fish exposed to concentrations of 10 to 25 g/L with exception of 18g and 27 g fish, which recovered in more than 2 min at 25 g/L. The time of recovery differed significantly (P < 0.05) between the concentration levels in each of the fish weight classes (Tab. 3). Recovery time was related to sodium bicarbonate concentration and size of fish (Fig. 1). Small fish of 2g exposed to 10 g/L sodium bicarbonate took 0.32 min to recover while the big fish (27 g) took 4.76 min to recover when exposed to 50 g/L sodium bicarbonate (Tab. 3).

Discussion

The present study demonstrated that sodium bicarbonate could be used as an effective anaesthetic for *O. niloticus* juveniles. An appropriate anaesthetic is chosen based on its effectiveness in immobilizing fish, thereby allowing the fish to be manipulated (Gilderhus and Marking, 1987; Burka *et al.,* 1997). Sodium bicarbonate has previously been used as an effective anaesthetic for *Oncorhynchus mykiss*, *Cyprinus carpio* and *Salvelinus fontinalis* (Booke *et al.,* 1978; Altun *et al.,* 2009).

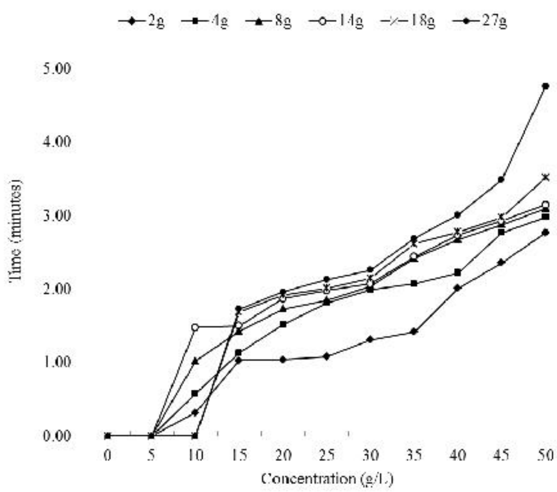

Fig. 1: Recovery of different sizes of fish exposed to concentrations of sodium bicarbonate.

An induction time of less than 3 min with complete recovery in 5 min is considered acceptable for fish handling (Marking and Meyer, 1985; Hseu *et al.,* 1998; Ross and Ross, 1999; King *et al.,* 2005). In the present study, the induction time varied from 0.79 to 5.02 min depending on the concentration and fish body weight. In the size range used in this study, induction times of less than 3 min were realized for fish exposed to concentrations of 15 to 50 g/L. Using 0.2 to 2 g/L sodium bicarbonate, Altun *et al.* (2009) obtained higher induction times of 4 to 9 min for fish of 7.7 g. Although direct comparison may not be possible since we are dealing with two different species, taken together Altun *et al.* (2009) and the present study indicate that higher concentrations of sodium bicarbonate may lead to faster induction times in fish.

In the present study, larger fish experienced longer induction and recovery times. This is similar to Tsantilas *et al.* (2006) who found that larger (60 g) white sea bream (*Diplodus sargus*) took longer to anaesthetize than smaller (30 g) fish when exposed to 2-phenoxyethanol. In contrast, larger fish experienced shorter induction and recovery times in Atlantic salmon (*Salmo salar)* anaesthetized with metomidate (Olsen *et al.,* 1995), rainbow trout (*O. mykiss)* anaesthetized with MS-222 (Houston *et al.,* 1976) and *O. mykiss* anaesthetized with quinaldine and 2-phenoxyethanol (Gilderhus and Marking, 1987). The size differential effect of sodium bicarbonate may be as a result of low levels of CO_2 which was insufficient to induce anaesthesia in bigger fish (Pirhonen and Schreck, 2003).

Several authors indicated that concentration of anaesthetic may lead to differences in induction and recovery times (e.g. Booke *et al.,* 1978; King *et al.,* 2005; Palić *et al.,* 2006; Weber *et al.,* 2009). In the present study, fish exposed to lower anaesthetic concentration of 5 g/L took longer time to reach anaesthesia for all

Tab. 3: Recovery time (min) for different sizes of *O. niloticus* in different concentrations of sodium bicarbonate.

Concentration (g/L)	Fish weight (g)					
	2	4	8	14	18	27
0	-	-	-	-	-	-
5	-	-	-	-	-	-
10	0.32±0.02[a]	0.58±0.02[a]	1.02±0.03[a]	1.48±0.01[a]	-	-
15	1.03±0.00[b]	1.13±0.01[b]	1.43±0.01[b]	1.50±0.03[b]	1.69±0.03[a]	1.73±0.01[a]
20	1.04±0.01[c]	1.52±0.01[c]	1.73±0.02[c]	1.87±0.01[c]	1.92±0.01[b]	1.96±0.01[b]
25	1.08±0.02[d]	1.81±0.01[d]	1.85±0.03[d]	1.98±0.02[d]	2.01±0.01[c]	2.13±0.00[c]
30	1.31±0.01[e]	1.99±0.01[e]	2.04±0.01[d]	2.07±0.01[e]	2.15±0.02[d]	2.26±0.01[d]
35	1.42±0.01[f]	2.07±0.02[f]	2.42±0.03[e]	2.44±0.01[f]	2.62±0.01[e]	2.69±0.01[e]
40	2.01±0.01[g]	2.22±0.01[g]	2.68±0.01[f]	2.74±0.03[g]	2.78±0.01[f]	3.01±0.01[f]
45	2.36±0.03[h]	2.77±0.00[h]	2.88±0.02[g]	2.93±0.01[h]	2.98±0.01[g]	3.49±0.01[g]
50	2.77±0.02[i]	2.98±0.02[i]	3.10±0.00[h]	3.15±0.03[i]	3.52±0.02[h]	4.76±0.01[h]

-Data are presented as Mean values ± SE of three determinations

-Different superscript in each column indicate significant differences ($P<0.05$).

-The sign -, means no change in the fish

the fish sizes and recovered within the shortest time (less than 1.50 min). This indicates that at low concentrations, low amounts of sodium bicarbonate may have diffused into the body of the fish hence the fast recovery recorded. This is consistent with the results of Altun *et al.* (2009) who recorded increased recovery time with increase in sodium bicarbonate concentration for *C. carpio*. Higher concentration have also been reported to result in shorter induction times and longer recovery times when clove oil was used as anaesthesia in black sea bass (*Centropristis striata*) (King *et al.,* 2005). According to Oikawa and Itazawa (1985), the rate of uptake by diffusion of the anaesthetic in relation to weight is slower in larger fish due to the smaller gill surface area in relation to body weight in larger fish hence a smaller area for drug diffusion relative to size.

Increase in concentration led to decrease of induction time to anaesthesia perhaps due to faster diffusion rate caused by high concentration gradient. Similar observations were made by Mylonas *et al.* (2005) who attributed the decrease of time to anaesthesia to contact with high concentrations of the chemical that makes the fish to reach anaesthesia quickly. The faster recovery at low concentrations could also be attributed to the fact that CO_2 produced by sodium bicarbonate does not induce analgesia and hence has a ‘’shallow effect’’ of anaesthesia as reported by Pirhonen and Schreck (2003).

The efficacy and response of any anaesthetic depends on the environment (temperature, pH, salinity of the water) and the biological factors of the fish (the size, sex, weight and fish species (Burka *et al.,* 1997; Ross and Ross, 1999; Zahl *et al.,* 2009). In the present study, fish used were between 2 to 27g while average water temperature was maintained at 24.0 ± 1.0°C, pH 7.8 ± 0.2, dissolved oxygen 6.0 ± 1.0 mg/L, and Total Ammonia Nitrogen 0.1± 0.1 mg/L. Since there was no variation in water quality parameters in this study, it is not possible to predict the effect of a change in these parameters. Furthermore, in most fish farming conditions, it is often necessary to handle larger tilapia. Since Nile tilapia is cultured across a range of environmental conditions, it would be interesting to determine the efficacy and dosage of sodium bicarbonate as anaesthetic across different conditions, sex and sizes.

Conclusions

The present study indicated that sodium bicarbonate can be an effective anaesthetic for *O. niloticus* juveniles of different weight classes. Smaller fish were more sensitive to the anaesthetic than larger fish. None of the concentrations used caused mortalities among the fish. A concentration of 45 g/L induced the stage of anaesthesia required for handling in less than 2 min with recovery times of less than 4 min. Sodium bicarbonate at a concentration of 45 g/L can be a good alternative to more expensive anaesthesia. Sodium bicarbonate has the advantages of low cost, no withdrawal time requirements, ease of availability and being relatively safe to fish, users and the environment (Altun *et al.,* 2009). It is therefore recommended for use when anesthetizing *O. niloticus* juveniles for handling especially in developing countries.

Data in the present study was carried out under similar temperatures, only varying the concentration levels, and therefore may not be applicable under all range of conditions where tilapia are reared. Further studies are required on the effects of temperature on the efficacy of Sodium bicarbonate as anaesthesia for *O. niloticus* juveniles.

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