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ANAESTHETIC EFFECTS OF SODIUM BICARBONATE AT DIFFERENT CONCENTRATIONS ON AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES

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Abstract:

In the present study, anaesthetic effects of different sizes (5, 10, 15, 20, 25, 30, 35 and 40g) of African catfish (*Clarias gariepinus*) juveniles was investigated at different concentrations (0/control, 10, 15, 20, 25, 30, 35, 40, 45, 50 g/L) of sodium bicarbonate. Juveniles were bath immersed in each of the different concentrations of sodium bicarbonate solution for 5 minutes and recovery times in clean aerated water was established. Juveniles took between 0.67 to 5.44 minutes to reach full anaesthesia and 0.66 to 4.25 minutes to recover fully from anaesthesia. An inverse exponential relationship was observed between sodium bicarbonate concentrations and induction time, whereas exponential relationship was observed between sodium bicarbonate concentrations and recovery time. Size of *C. gariepinus* juveniles significantly affected the induction time ($P < 0.05$) with smaller juveniles getting to full anaesthesia faster than bigger juveniles at the same concentration of sodium bicarbonate. In addition, recovery time was size dependent where smaller *C. gariepinus juveniles* took significantly longer time ($P < 0.05$) to recover from anaesthesia than bigger juveniles. All juveniles reached anaesthesia and recovered in less than 5 minutes except for 30 g juveniles at 15g/L of sodium bicarbonate which was anaesthetized at 5.44 minutes.

Keywords: Immersion, Induction, Recovery, Anaesthesia

Introduction

Anaesthetics are used to minimize stress associated with handling live fish when marking, tagging, counting, injecting, transporting, weighing, artificial reproduction and surgical operations among others (Hajek et al., 2006, Altun et al., 2009, Yildiz et al., 2013, Opiyo et al., 2013). The need to handle fish without impairing their health or commercial value has led to the development of many techniques to anaesthetize fish (Yildiz et al., 2013). Different anaesthetics act with various intensity driving fish in to general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity, and reduction in skeletal muscle tone (Hajek et al., 2006). Quick induction and recovery from anaesthesia is desirable in most cases (Marking & Meyer, 1985; Stoskopf, 1993). However, long recovery time is desirable while collecting fish from the wild or where fish must be handled for a longer time in the laboratory. An ideal anaesthetic should possess several attributes such as; non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery (Pawar et al., 2011). It is of paramount importance to identify the effective dose of anaesthetic for specific fish species since response to the same anaesthetic vary depending on the concentration used and the species of fish (Tytler and Hawkins, 1981; Pawar, et al., 2011). Common fish anaesthetics include clove oil, sodium bicarbonate, carbon dioxide gas, metomidate, benzocaine, tricaine, methanesulphonate (MS-222), 2-phenoxylethanol and quinaldine (Massee et al., 1995; Palić et al., 2006). Regardless of the agent, the process of anaesthesia in fish develops in a similar way and runs in a progressive pattern (McFarland, 1959).

Sodium bicarbonate (Na_2CO_3) also known as baking soda, is a white substance that gives carbon dioxide when dissolved in water (McFarland and Klontz, 1969). Its main advantages lie in its low cost, wide availability and safety to both fish and humans (Altun et al., 2009). Sodium bicarbonate has been effectively used as an anaesthetic in common carp (*Cyprinus carpio*) in both cold and warm water conditions (Booke et al., 1978, Altun et al., 2009), Rainbow trout (*Oncorhyncus mykiss*) (Keen et al., 1998) and in Nile tilapia (*Oreochromis niloticus*) (Opiyo et al., 2013).

The African catfish (Clarias gariepinus), is found in several countries throughout Africa, where it has a native distributional range as well as in Europe (the Netherlands, Germany and Belgium), Asia (Indonesia, Thailand) and South America (Brazil) (de Graaf and Janssen, 1996; Brummett, 2008). *C. gariepinus* is the second most important freshwater cultured fish (after tilapia) in Africa (Barasa et al., 2014) with the exception of Nigeria where its production far exceeds that of tilapia production and accounts for 70–80 % of the total freshwater fish production (Ponzoni and Nguyen, 2008). Successful culture of *C. gariepinus* requires artificial propagation whereby there is need of injection of the fish with gonadotropin to spawn and the juveniles are often moved from one place to another during sorting and grading at the larval growth phases (de Graaf and Janssen, 1996). These procedures may induce stress in the fish hence require use of anaesthetics to induce calming effects and reduce the mobility of the aggressive fish. *C. gariepinus* is therefore an ideal species to examine the suitability of sodium bicarbonate as an anaesthetic. The aim of the current study is to determine the efficacy and optimum concentration of sodium bicarbonate in different sizes of *C. gariepinus* which hitherto has not been reported.

Materials and Methods

C. gariepinus juveniles of average weight 10, 15, 20, 25, 30, 35 and 40 g were collected from the nursery ponds at Kenya Marine and Fisheries Research Institute, Kegati Aquaculture Research Station, Kenya. The juveniles were starved for 24 hours to allow for gut evacuation prior to experiment (Weyl et al., 1996). Average water temperature was at 23.5 ±1.2°C, pH (7.8 ±0.18), dissolved oxygen (4.5 ±1.7) mg/L, and Total Ammonia Nitrogen (0.1 ±0.1) mg/L in the experimental aquaria. To determine the optimal dosage and effect of size on anaesthetic efficacy of sodium bicarbonate, a group of 5 juveniles from each weight class were exposed to different concentrations of sodium bicarbonate by bath immersion. The experiment was carried out in 15 L glass aquaria in triplicates. 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) and Palić et al., (2006). Concentrations of sodium bicarbonate used were (0/control, 10, 15, 20, 25, 30, 35, 40, 45, 50 g/L). The juveniles were observed for three different stages of induction and recovery time (Table 1) as described by Iwama et al., (1989) and Palić et al., (2006).

Table 1. Stages of induction and recovery

Stage of induction	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	**Description**
I	No body movement but opercula movement start
II	Regular opercula movements and body movements start
III	Equilibrium regained with pre-anaesthetic appearance

*Adapted from Iwama et al. (1989) and Palić et al. (2006).

After full anaesthesia, juveniles were removed from the anaesthetic solution and transferred to another aquarium with clean aerated well water for recovery. The time of induction to anesthesia and recovery were recorded and fish were maintained there for 48 hours in order to observe their behavior and possible mortality.

Differences among means were analyzed by two-way ANOVA followed by Duncan's multiple range tests. All analyses were performed with SPSS version 17.0 statistical package. Differences were considered significant at $P< 0.05$.

Results and Discussion

Induction and recovery time of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate are shown in table 2 and 3. In general juveniles took between 0.67 to 5.44 minutes to reach full anaesthesia in different concentrations of sodium bicarbonate (Table 2). Juveniles exposed to 0 and 10g/L concentration of sodium bicarbonate did not undergo anaesthesia. In addition, 35 and 40 g juveniles did not undergo anesthesia when exposed to 15g/L concentration of sodium bicarbonate. The recovery time for the juveniles which did not undergo anaesthesia was not recorded. Induction time decreased with increase in the concentration of sodium bicarbonate while recovery time increased with increase in the concentration of sodium bicarbonate (Figure 1 and 2). Concentration of sodium bicarbonate solution had a significant effect ($P<0.05$) on the induction time to anaesthesia with juveniles attaining full anaesthesia faster at a higher concentration than at a lower concentration. Juveniles weighing 10g took 0.67 minutes to attain anaesthesia at 50 g/L concentration of sodium bicarbonate and 3.04 minutes at 15 g/L of sodium bicarbonate. Size of *C. gariepinus* juveniles significantly affected the induction time to anaesthesia ($P<0.05$) with smaller juveniles getting to full anaesthesia faster than bigger juveniles at the same concentration of sodium bicarbonate. At a concentration of 20g/L, 10g juveniles took 2.78 minutes to attain anaesthesia while 40 g juveniles took 4.98 minutes to attain anaesthesia.

C. gariepinus juveniles generally took between 0.66 to 4.25 minutes to fully recover from the anaesthetic solution. Sodium bicarbonate concentration significantly ($P < 0.05$) affected the recovery time from anaesthesia with juveniles exposed to a higher concentration taking longer to recover than those exposed to a lower concentration (Table 3). 10 g juveniles took 4.25 minutes to recover from anaesthesia at 50g/L of sodium bicarbonate concentration and 1.66 minutes at 15g/L of sodium bicarbonate concentration. Size of juveniles also significantly ($P < 0.05$) affected the recovery time from anaesthesia with smaller juveniles taking longer time to recover than bigger juveniles at the same concentration of sodium bicarbonate. 10 g juveniles took 1.88 minutes to recover from anaesthesia while 40 g juveniles took 0.66 minutes to recover from anaesthesia when exposed to 20g/L concentration of sodium bicarbonate. No mortality was recorded in any concentration of sodium bicarbonate during the application and post recovery period.

Anaesthetics play a vital role in modern day aquaculture. It helps to reduce the stress which makes fish more susceptible to diseases due to resistance during handling (Ross and Ross, 1999). The use of sodium bicarbonate from baking powder as an anaesthetic in fish is a relatively cheap technique because of its affordability and ease of application (Opiyo et al., 2013). It is safe for human and there is no banning or restriction in its use as it leaves no tissue residues (Altun et al., 2009). Consequently, it can be used on animals entering the human food chain without any withdrawal period (Ross and Ross, 1999). The effectiveness of sodium bicarbonate depends on the weight of the fish and the concentration of administration as reported by Booke et al., (1978) and Altun et al., (2009). Similar phenomenon was realized in the present study.

Table 2. Induction time (minutes) for different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate

	Fish weight (g)						
Concentration (g/L)	10	15	20	25	30	35	40
0	−	−	−	−	−	−	−
10	−	−	−	−	−	−	−
15	3.04 ± 0.01[aA]	3.42 ± 0.00[bA]	3.81 ± 0.05[cA]	4.38 ± 0.01[dA]	5.44 ± 0.01[eA]	−	−
20	2.78 ± 0.00[aB]	3.06 ± 0.02[bB]	3.14 ± 0.00[cB]	3.34 ± 0.01[dB]	4.12 ± 0.00[eB]	4.89 ± 0.01[fA]	4.98 ± 0.01[gA]
25	2.31 ± 0.01[aC]	1.97 ± 0.01[bC]	2.19 ± 0.01[cC]	2.45 ± 0.00[dC]	3.67 ± 0.00[eC]	4.00 ± 0.00[fB]	4.66 ± 0.00[gB]
30	2.04 ± 0.00[aD]	1.22 ± 0.01[bD]	1.42 ± 0.01[cD]	1.88 ± 0.01[dD]	3.04 ± 0.03[eD]	3.12 ± 0.00[fC]	3.21 ± 0.00[gC]
35	1.16 ± 0.03[aE]	1.08 ± 0.08[bE]	1.27 ± 0.06[cE]	1.35 ± 0.01[dE]	1.99 ± 0.00[eE]	2.35 ± 0.01[fD]	2.77 ± 0.00[gD]
40	1.01 ± 0.03[aF]	0.91 ± 0.01[bF]	1.13 ± 0.01[cF]	1.23 ± 0.01[dF]	1.55 ± 0.00[eF]	1.75 ± 0.01[fE]	2.11 ± 0.01[gE]
45	0.85 ± 0.01[aG]	0.85 ± 0.01[aG]	0.99 ± 0.01[bG]	1.14 ± 0.01[cG]	1.25 ± 0.01[dG]	1.33 ± 0.00[eF]	1.60 ± 0.00[fF]
50	0.67 ± 0.01[aH]	0.76 ± 0.04[bH]	0.90 ± 0.00[cH]	1.01 ± 0.01[dH]	1.06 ± 0.00[eH]	1.12 ± 0.01[fG]	1.23 ± 0.00[gG]

* Data are presented as Mean values ± SE of five determinations
** Means identified by different small letters in the rows or capital letters in the columns were significantly different ($P < 0.05$).
*** The sign −, means no change in the fish

Table 3. Recovery time (minutes) for different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate

	Fish weight (g)						
Concentration (g/L)	10	15	20	25	30	35	40
0	−	−	−	−	−	−	−
10	−	−	−	−	−	−	−
15	1.66 ± 0.15[aA]	1.65 ± 0.01[bA]	1.65 ± 0.12[cA]	1.36 ± 0.02[dA]	0.98 ± 0.01[eA]	−	−
20	1.88 ± 0.03[aB]	1.62 ± 0.15[bB]	1.70 ± 0.01[cB]	1.55 ± 0.01[dB]	1.37 ± 0.03[eB]	1.02 ± 0.03[fA]	0.66 ± 0.01[gA]
25	2.33 ± 0.01[aC]	2.27 ± 0.02[bC]	2.04 ± 0.01[cC]	1.87 ± 0.01[dC]	1.78 ± 0.03[eC]	1.58 ± 0.02[fB]	1.04 ± 0.00[gB]
30	2.72 ± 0.01[aD]	2.62 ± 0.05[bD]	2.17 ± 0.02[cD]	2.46 ± 0.02[dD]	2.50 ± 0.01[eD]	1.90 ± 0.01[fC]	1.61 ± 0.02[gC]
35	3.28 ± 0.03[aE]	3.13 ± 0.03[bE]	2.74 ± 0.02[cE]	3.00 ± 0.01[dE]	2.60 ± 0.10[eE]	2.18 ± 0.01[fD]	2.04 ± 0.02[gD]
40	3.62 ± 0.01[aF]	3.47 ± 0.06[bF]	3.24 ± 0.00[cF]	3.13 ± 0.01[dF]	2.94 ± 0.05[eF]	2.40 ± 0.01[fE]	2.28 ± 0.01[gE]
45	3.95 ± 0.01[aG]	3.54 ± 0.16[bG]	3.40 ± 0.02[cG]	3.27 ± 0.01[dG]	3.10 ± 0.02[eG]	2.79 ± 0.01[fF]	2.56 ± 0.01[gF]
50	4.25 ± 0.01[aH]	4.21 ± 0.05[bH]	3.93 ± 0.02[cH]	3.45 ± 0.01[dH]	3.25 ± 0.01[eH]	3.05 ± 0.01[fG]	2.71 ± 0.01[gG]

* Data are presented as Mean values ± SE of five determinations
** Means identified by different small letters in the rows or capital letters in the columns were significantly different ($P < 0.05$).
***The sign −, means no change in the fish

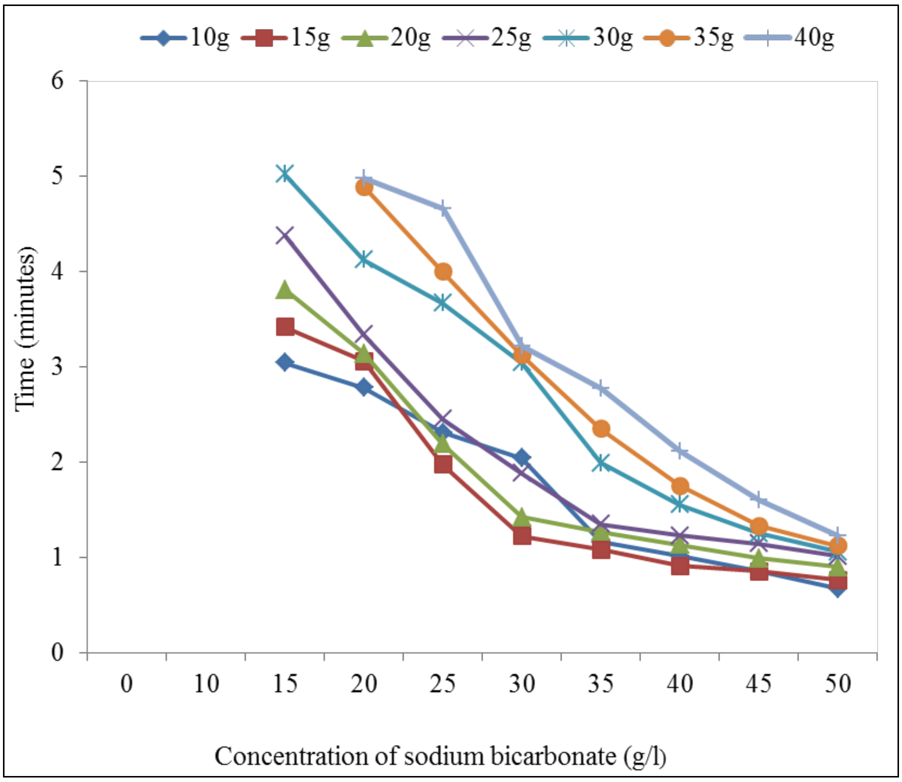

Figure 1. Induction of different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate.

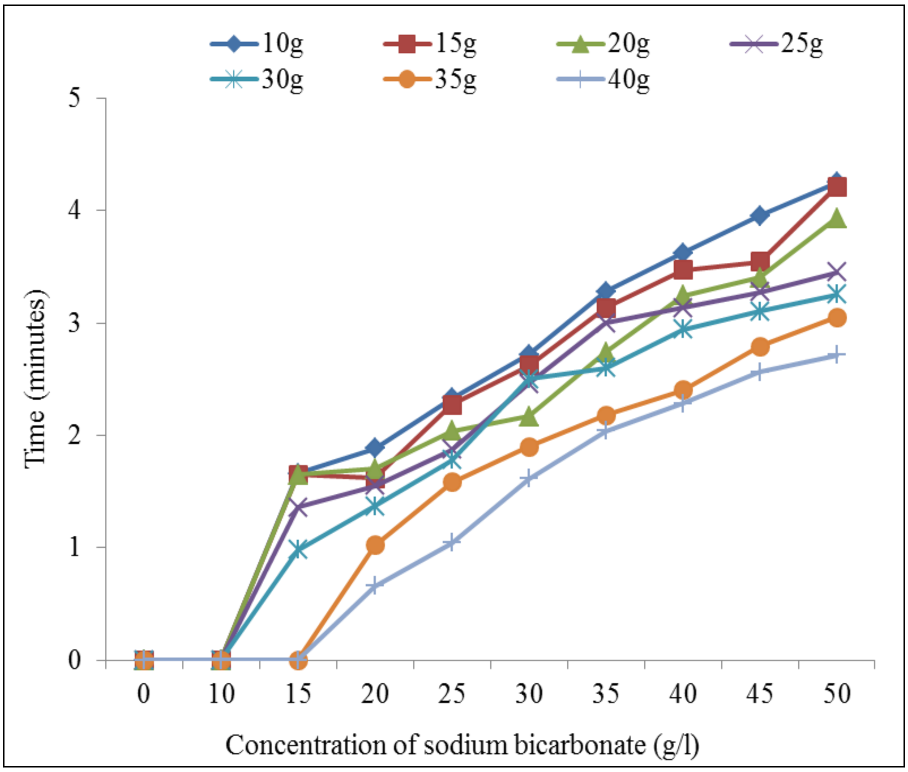

Figure 2. Recovery of different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate.

It took between 0.67 to 5.44 minutes for *C. gariepinus* juveniles to reach anaesthesia. Fish of the same weight reached anaesthesia faster at higher concentrations of sodium bicarbonate than at lower concentrations. This could be attributed to the fact that more anaesthesia diffused in to the body of the fish at higher concentration than those subjected to a lower concentration. This is consistent with Opiyo et al., (2013) and Altun et al., (2009) who found that higher concentration of sodium bicarbonate resulted to faster anaesthesia in Nile tilapia and Common carp respectively. In addition, smaller juveniles were also anaesthetized faster than bigger ones at the same concentration of sodium bicarbonate. This could be explained by the fact that smaller fish have a bigger surface area to volume ratio than bigger fish and therefore absorbed the anaesthetic faster. The induction time from the present study is in agreement with several authors (Marking and Meyer, 1985; Iversen, 2003; Coyle et al., 2004; Mylonas et al., 2005; Opiyo et al., 2013) who recommended a period of between 3-5 minutes for an effective induction time to anaesthesia.

The concentration of the anaesthetic solution significantly affected recovery time from anaesthesia. Juveniles exposed to higher concentration of sodium bicarbonate took longer to recover from anaesthesia than those at a lower concentration. These results are comparable with previously published work by Solomon et al. (2014) who found that when anaesthetic was in higher concentration, more of it was absorbed and accumulated in the Central Nervous System (CNS) of the fish thus suppressing the activity of the CNS to a greater degree than at lower concentrations and consequently prolonging the recovery time. King et al., (2005) also reported similar results when clove oil was used as anaesthesia in black sea bass (*Centropristis striata*). Moreover, bigger juveniles took shorter period of time to recover from anaesthesia than smaller juveniles at the same concentration. This could be linked to the fact that bigger fish have a smaller surface area to volume ratio than smaller fish and is comparable to (Yildiz et al., 2013). Similar results were recorded by Altun et al., (2009) on Common carp juveniles, Zaikov et al., (2008) on Pike and Opiyo et al., (2013) on Nile tilapia juveniles. Solomon et al., (2014) and Hseu et al., (1998) also noted that higher drug concentration increase recovery time. However, Booke et al., (1978) recorded much lower concentrations of sodium bicarbonate at 1,000 mg/L and 600 mg/L at pH 6.5 and 7.7 respectively.

Conclusion

In conclusion, 50g/L concentration of sodium bicarbonate is recommended to anaesthetize *C. gariepinus* juveniles since it led to anaesthesia in less than 2 minutes for all the sizes of fish.

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