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Original Research Article

Comparative Efficacy of Anesthetic Agents (Clove Oil and Sodium Bicarbonate) on Cultured African Catfish, *Clarias gariepinus* (Burchell, 1822) and Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

The increasing demand for protein in Nigeria has resulted in the culture of many fishes in confinements. This practice has exposed many fish species to some tedious processes, thus the need to sedate fishes to reduce stress during breeding. In this study, the efficacy of two anesthetic agents (clove oil and sodium bicarbonate) was compared on the two most cultured species; *Clarias gariepinus* and *Oreochromis niloticus*. *Clarias gariepinus* (798.9 ± 0.07 g) and *Oreochromis niloticus* (581.8 ± 0.2 g) were subjected to different concentration of clove oil (mg/L) and sodium bicarbonate (g/L) of 20, 40, 60, 80, 100 and 10, 20, 30, 40, 50 respectively. There were significantly decreased induction times as concentrations and size of the species increases in all the anesthetic agents. Similarly, recovery times increases as concentrations and weight of the species increases (P<0.05). A significant correlation (P<0.05) was observed between body weight, anaesthetic concentrations, times to reach complete anesthesia and time to recover. Duration for induction and recovery depended significantly (p < 0.05) on the concentration of the anaesthetic agents. The lowest effective concentrations that induced complete anesthesia in this study were 20 mg/L (15.1 ± 0.8 mins and recovery time 4.2 ± 0.2 mins) for clove oil, 20 g /L (7.0 ± 0.12 mins and recovery time 7.0 ± 0.12 mins) for sodium bicarbonate. No mortality was recorded for all sizes of species exposed to the anaesthetic agents. Clove oil and Sodium bicarbonate have been found to be effective and safe at different concentrations for both species.

Keywords: catfish, clove oil, fish anesthesia, induction time, recovery time, sodium bicarbonate, tilapia

Introduction

Fishes are known generally to exhibit diversity in size,[1] shape, biology and in the habitats they occupy[2,3,4]. Cultivation of finfishes (Catfishes, tilapias) and shellfishes (shrimps, prawns) in confinements using ponds and tanks is one of the rising sources of food production sectors in the world [5] with a projection of worldwide production increase from 179 million tons in the year 2018 to 204 million tons in the year 2030.[6]
In Nigeria, aquaculture helps to provide an appropriate and affordable replacement for nutritional and digestible protein and the commonly cultivated finfishes are the catfishes and the tilapia species.

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Fish (Fin and shellfishes) is a principal supply for humans, especially as source of animal protein[7,8]; digestible minerals [9, 10]; commercial (profit- making) or subsistence (survival) farming [11] and also serve as important by-products for exportation in many regions of the world. [12].
Anesthetics are chemical/physical agents used to calm animals by causing them to gradually lose their equilibrium, mobility, consciousness, and ultimately their reflex action [13]. In laboratories, overdose of anaesthesia is often usually use as euthanasia at the end of experimental procedures and it is not a common practice for fish which is destined for the food chain.[14]
Availability of an anesthetic that is nontoxic, inexpensive, easily administered and able to immobilize fish quickly with good recovery rate make it an ideal candidate for anesthesia[15,16]. Tricaine methane sulphonate (MS-222), benzocaine (ethyl-p-aminobenzoate), ethylen glycol monophenyl ether (eugenol and 2- phenoxyethanol), methomidate and clove oil are the most commonly used anesthetics in aquaculture.[17]
There are many factors influencing the efficacy of anesthetic agent in fishes. These are biological factors, environmental factors; fish species, fish body size, the fish density in the bath solution and the water quality parameters [18,19, 20, 21]. Different species differ greatly in their responses to various anesthetics.[22,23,24]
It is therefore very vital to screen and select the best anesthetic and effective doses for a particular species and procedure. This is because unpremeditated adverse effects can result from a good anaesthetic if not

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rationally administered and may create further stress, stimulate negative metabolic responses and perhaps death of the fish .[23]
Clove oil is one of the anesthetic agents experimentally evaluated, and is being used in non-food fish and in research [13]. The major constituent of clove oil is eugenol (70-90% by weight). It is an effective anesthesia that produced sedation sufficient to transport fish, other effective doses gave effective surgical plane anaesthesia. [25]
There is increase of interest in using CO_2 anesthesia by fisheries and aquaculture practitioners based on its gaseous nature, and the fact that it leaves no residues in the tissues. Water acidification using bicarbonate or carbonic acid evolves CO_2, which is similar in producing a hypercapnic condition in the water [26]. Sodium bicarbonate (baking soda) is usually cheap, readily available and a low regulatory priority compound [27]. Its releases carbon dioxide gas which is not toxic to both fish and humans and effective for sedative fish [28]. Carbon dioxide is soluble and introduced bubbled in water when sodium bicarbonate or air stone is added [29]. Sodium bicarbonate has been successfully used as an anaesthetic in common carp and *Tilapia mozambique* of different ages and environmental conditions. [30]
Fish are often handled for weighing, selection, brood-stock management, out of water examination, stripping, and sampling (swabs, biopsies) or for disease treatment especially those involving invasive procedures such as tagging, fin clipping, cardiac excision surgery, amputation, and also liver and ovary biopsy [14]. In fisheries and aquaculture, Anesthetic agents are being employed to reduce activity in fish or achieve general anesthesia (total loss of consciousness).[16] Anesthetics are also used during transportation to prevent physical injury and reduce metabolism (Dissolved oxygen consumption and excretion).[13]
The aim of this study was to determine induction (Total time it takes the fish to be completely sedated / immovable) and recovery (total time it takes for the fish to resume normal swimming activities) time of two commercially and economically important fish on the anesthetic agents (Clove oil and Sodium bicarbonate) and to compare efficiency for use in different sizes of the finfishes, *C. gariepinus* (African catfish) and *Oreochromis niloticus* (Nile Tilapia) under controlled conditions.

Materials and Methods

Study design
Three hundred (300) fish samples comprising one hundred and fifty (150) African catfish (*Clarias gariepinus*) and one hundred and fifty (150) Nile Tilapia (*Oreochromis niloticus*) of three different age categories of mixed sexes (8, 12 and 16 weeks) 100 per age category were cultured at the fish farm complex of the Department of Fisheries and Aquaculture, Bayero University, Kano. They weighed 120.6 g ± 4.1 g, 253.3 ± 5.0 g and 425.4 g ± 2.4 g respectively (*Clarias gariepinus*), 121.3 g ± 3.1 g, 242.3 ± 3.2 g and 418.2 g ± 3.4 g respectively (*Oreochromis niloticus*). The fish were fasted for 24 hours according to [27] and later each category was further randomly divided in to five groups (ten per group) which were tested against five different concentrations of the anesthetics; clove oil (20, 40, 60, 80 and 100 mg/L), and (10, 20, 30, 40 and 50 g/L).

Anesthetic agents
The anesthetic agents clove oil (67% eugenol) sourced from Jiangxi Natural plant company limited and sodium bicarbonate (60%) sourced from the Chemistry Department, Bayero University Kano were used for the present study. Various concentrations of the anesthetics were prepared some minutes before the experiments began. Clove oil is a liquid that does not dissolve in water and so, it was diluted in ethanol at the standard ratio of 1:5 [31]. Sodium bicarbonate was mixed with water (1 g/L) in a reagent bottle according to concentrations used previously by [27] before added to the aquarium (20L rubber plastics that serves as the Anaesthetic chamber).

Induction of anesthesia
The efficacy of the two anesthetic agents was assessed using different concentrations of the anesthetics. The lowest (Minimum) and highest (maximum) concentrations used were based on previously published works by.[32,33] The following concentrations of clove oil were evaluated; clove oil (20, 40, 60, 80 and 100 mg/L), and (10, 20, 30, 40 and 50 g/L) for sodium bicarbonate. The three group from each species were exposed to five different concentrations of each anesthetic agents. After acclimation for one week, the fish were transferred to the holding aquarium (40L plastic buckets) and half- filled with fresh water in the laboratory. Fish were then individually transferred to the anesthetic aquarium (20 L plastic buckets) containing different concentrations of anesthetic solutions. The complete induction time for all the anesthetic agents was measured using digital stopwatch.

Recovery from anesthesia
Each sample was immediately after the induction transferred to a recovery chamber (filled with fresh aerated water under laboratory conditions) and the complete recovery time for all the anesthetic agents was measured using digital stopwatch.
The induction and recovery duration for each anaesthetic stage were monitored by trained fisheries technicians with digital stopwatches. The fishes were further observed for seven (7) days for any abnormal fish behavior and /or post-exposure mortalities.
Water quality parameter
During this study, water quality parameters (Temperature, Dissolved Oxygen, Hydrogen ion concentration, Ammonia, Nitrite, sulphide and conductivity were monitored, measured and recorded using various standard instruments in the anaesthetic chambers. [27]

Ethical standards
The study was conducted with proper and standards handling of the experimental fish species. The study has followed international standard protocol of animal experimentation.

Statistical analysis
Statistical Package for Social Sciences (SPSS version 23) was the package used to analysis the data obtained. The time differences for the induction and recovery from anesthesia within and in between groups were compared using One-way ANOVA (Kruskal-Wallis) for the different concentrations of the anesthetic agents. Non-linear regression analyses were used to establish the relationship between anesthetic dosage, the induction time and recovery time. Significance difference was tested and represented $P<0.05$.

Results and Discussion

Water quality parameters
The water quality parameters (Table 1) in the experimental tanks of the fish exposed to clove oil and sodium bicarbonate solution shows that the parameters were within the same range in comparison to the control. The water quality parameters detected in this study were in similarity with the reports of [13, 34] but slightly different from the results presented by [27]. The amount of the parameters detected in this study does not have great variation with the control. This means that the anaesthetic agents used for this study does not affect the water parameters or influence the physiological behaviors of the species.

Behavioral descriptions at induction and recovery stages of Clarias gariepinus and Oreochromis niloticus
Physiological changes at induction and recovery due to the anesthesia were assessed in three (3) consecutive stages as shown in Table 2.
The induction and recovery time for both species and varying species sizes at different concentrations are presented in Tables 3 - 8. Both drugs appear to possess anesthetic capacity in both species tested with clove oil more effective than sodium bicarbonate when the minimum effective doses were compared. The induction time was significantly ($P < 0.05$) affected by the concentration and fish body weight. The induction time decreased with increasing concentration and increased as the body weight increases. Although the induction time recorded for the highest concentration was shortest, there was no significant difference when this concentration was compared within the weight group groups.
Three stages of behavioral changes were observed in this study during the period of induction (when the fish is fully sedated) and the recovery time (when the fish is completely out of the effects of the anaesthesia

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and has fully resumed normal activities). The observed stages in this research are in agreement with the reports of [16, 27], who also documented three (3) stages in their researches, although the stages were explained using different terms. While these observed behavioral stages contradict the documentations of [13, 34] who recorded about five (5) different stages for the induction and recovery observations of their researches for various experimental fishes.

Stress during handling weakens fish immune system thereby predisposing fish to diseases which may eventually lead to the death of the fish. To reduce the stress to fish, anesthetics plays a vital role.[30,35] An ideal anesthetic agent should be readily available, nontoxic, inexpensive, easily administered and able to immobilize fish quickly with good recovery rate [15,16]. Many factors influence the efficacy of an anesthetic agent in fishes, including fish species (fin fishes – scaleless / scaled fishes or shell fishes) and fish body size – weight. [18, 19, 20, 21] Different species differ greatly in their responses to various anesthetics. [22, 23, 24]

Clove oil as an anesthetic agent on *Clarias gariepinus* and *Oreochromis niloticus* at varying body weight

Tables 3 - 5 shows the frequency in induction time and recovery time in *Clarias gariepinus* and *Oreochromis niloticus* when exposed to Clove oil as an anesthetic agent. For both *Clarias gariepinus* and *Oreochromis niloticus*, increase in concentration (mg/l) of Clove oil with increase in the body weight (g) of the species exposed consequently leads to decrease in the time it takes the species to be completely anesthetized (induction time) while the recovery process took more time to recover completely from the effect of the anesthetic (recovery time). But it was observed that with increase in concentration of Clove oil on *Clarias gariepinus* and *Oreochromis niloticus* at 425.4 ± 2.4 g and 418.2 ± 3.4 g respectively, there was decrease in the recovery time (Table 5).

Induction time tends to significantly ($p<0.05$) decrease as the concentration increases in both species. However, there is also a slight increase in the induction time and decrease recovery time across group as the fish body weight increases. Increased induction time and decreased recovery time may be attributed to the species- specific differences and perhaps increase surface area of the body which reduced the bioavailability of the anesthetic agent. This result is in line with the report of [36] who reported significant differences in fish species sensitivity to Clove oil.

Sodium Bicarbonate as an anesthetic agent on *Clarias gariepinus* and *Oreochromis niloticus* at varying body weight

At 10g/L concentration of Sodium Bicarbonate, there was no observed effect on all the finfishes exposed at mean body weight of 120.6 g ± 4.1 g; 253.3 ± 5.0 g and 425.4 g ± 2.4 g for *Clarias gariepinus* and 121.3 g ± 3.1 g; 242.3 ± 3.2 g and 418.2 g ± 3.4 g for *Oreochromis niloticus* (Tables 6 – 8). Increased exposure of Sodium bicarbonate on *Clarias gariepinus* led to decrease in the induction time while the recovery time increases as the concentration increases (Table 6 – 8).

Table 1: Summary of water quality parameters in the control and anaesthetic chambers of *Clarias gariepinus* and *Oreochromis niloticus*

		Anaesthetic chambers	
Parameters	**Control 0.00mg/l**	**Clove oil (100mg/l) ***	**Sodium bicarbonate (50g/l) ***
Temperature (ºC)	26.8	26.70 ± 0.036	26.10 ± 1.64
pH	6.81	6.80 ± 0.16	6.82 ± 0.13
Dissolved Oxygen (mg/l)	7	6.93 ± 0.35	6.80 ± 0.025
Nitrite (mg/L)	0.3	0.033 ± 0.02	0.30 ± 0.03
Ammonia (mg/L)	less than 0.1	less than 0.1	less than 0.1
Sulphide (mg/L)	0.02	0.02 ± 0.01	0.027± 0.01
Conductivity (mS/cm)	381	383	379

* = anaesthetic agents in the chambers for the three age categories for both Catfish and Tilapia

Table 2: Behavioral reactions observed with the fish species at the Anaesthetic chambers

Stages	Induction	Recovery
I	Partial resistance to external stimuli, loss of equilibrium	Fish moving gradually moving but still at the bottom
II	Total loss of balance, fish response to strong stimuli	Breathing normally, respond to strong stimuli but still unbalanced
III	Total loss of reflexes and movement	Regained equilibrium, swim normally and respond to slight stimuli

Table 3: Clove oil complete Anesthesia mean induction and recovery time for 120.6 g ± 4.1 g *Clarias gariepinus* and 121.3 g ± 3.1 g *Oreochromis niloticus*

	***Clarias gariepinus* (n=25)**		***Oreochromis niloticus* (n=25)**	
Conc (mg/L)	**Induction time (mins)**	**Recovery time (mins)**	**Induction time (mins)**	**Recovery time (mins)**
20	13.6 ± 0.2	5.1 ± 0.3	14.2 ± 0.2	5.2 ± 0.2
40	12.3± 0.4	6.3 ± 0.12	13.7 ± 0.5	6.2 ± 0.1
60	10.4 ± 0.3	8.2 ± 0.4	11.9 ± 0.2	7.7 ± 0.3
80	7.2 ± 0.1	9.7± 0.1	7.0 ± 0.3	9.9 ± 0.2
100	5.4 ± 0.1	12.2 ± 0.2	5.8 ± 0.4	12.4 ± 0.4

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Table 4: Clove oil complete Anaesthesia mean induction and recovery time for 253.3 ± 5.0 g *Clarias gariepinus* and 242.3 ± 3.2 g *Oreochromis niloticus*

	***Clarias gariepinus* (n=25)**		***Oreochromis niloticus* (n=25)**	
Conc (mg/L)	**Induction time (mins)**	**Recovery time (mins)**	**Induction time (mins)**	**Recovery time (mins)**
20	15.1 ± 0.4	4.2 ± 0.2	15.5 ± 0.4	4.2 ± 0.2
40	13.5± 0.5	6.0 ± 0.1	13.1 ± 0.3	6.8 ± 0.1
60	11.4 ± 0.6	7.2 ± 0.5	12.9 ± 0.2	7.0 ± 0.3
80	9.8 ± 0.1	8.7± 0.3	12.0 ± 0.7	8.2 ± 0.2
100	8.3 ± 0.3	9.5 ± 0.1	10.8 ± 0.4	8.9 ± 0.3

Table 5: Clove oil complete anaesthesia mean induction and recovery time for 425.4 g ± 2.4 g *Clarias gariepinus* and 418.2 g ± 3.4 g *Oreochromis niloticus*

	***Clarias gariepinus* (n=25)**		***Oreochromis niloticus* (n=25)**	
Conc (mg/L)	**Induction time (mins)**	**Recovery time (mins)**	**Induction time (mins)**	**Recovery time (mins)**
20	16.5 ± 0.7	3.9 ± 0.3	18.3 ± 0.6	3.9 ± 0. 8
40	14.2 ± 0.6	2.5 ± 0.6	16.1 ± 0.3	2.8 ± 0.5
60	12.8 ± 0.16	2.3 ± 0.3	13.9 ± 0.4	2.4 ± 0.2
80	12.2 ± .04	1.7 ± 0.4	12.0 ± 0.7	1.2 ± 0.3
100	11.3 ± 0.4	1.2 ± 0.5	11.8 ± 0.7	1.0 ± 0.1

Table 6: Sodium bicarbonate complete anesthesia mean induction and recovery time for 120.6 g ± 4.1 g *Clarias gariepinus* and 121.3 g ± 3.1 g *Oreochromis niloticus*

	***Clarias gariepinus* (n=25)**		***Oreochromis niloticus* (n=25)**	
Conc (g/L)	**Induction time (mins)**	**Recovery time (mins)**	**Induction time (mins)**	**Recovery time (mins)**
10	0	0	0	0
20	7.2 ± 0.02	0.6 ± 0.02	4.7 ± 0.02	2.7 ± 0.11
30	6.82. ± 0.01	1.2 ± 0.03	2.9 ± 0.02	3.9 ± 0.03
40	4.3 ± 0.01	2.05± 0.01	2.0 ± 0.03	4.7 ± 0.02
50	3.5 ± 0.13	2.42 ± 0.02	1.8 ± 0.04	5.4 ± 0.04

There was no visible effect observed on all the samples tested at the lowest concentration (10 g/L) of Sodium bicarbonate. Also, the concentration and fish body weigh directly affected the recovery time significantly ($P < 0.05$) as it increased with an increase in concentration and decreased with an increase in fish body with. No mortality was recorded during or after the experiment.

For the Sodium bicarbonate, the increased induction time and decreased recovery time may be due to accumulation of more active carbon (iv) oxide constituents of the anesthetic in the fish Central Nervous System (CNS) as the concentration increased.[37] This might have suppressed the CNS substantially as opposed to smaller concentrations thereby extending the recovery time of the fish.[35,37]

Catfish appears to be more sensitive to the anesthetic agents as significant correlation was observed between species, induction and recovery time at 0.01 level of significance. The finding in this study that induction times decreased significantly ($p<0.05$) with the increasing Clove oil and Sodium bicarbonate concentration agrees with the report of[38, 39, 40,41, 33, 42, 25, 43, 37]

In contrast with a study by Munday and Wilson,[18] who worked on *Pomacentrus amboinensis*, to this study, clove oil exhibited significant ($p<0.05$) variation in induction and recovery times across the effective concentrations. Perhaps this is associated with the variation in species, age and location of the study.

However, Clove oil is more effective than sodium bicarbonate at lower concentrations when the minimum effective doses were compared. Also, Clove oil gives a significantly ($p<0.05$) longer recovery time than Sodium bicarbonate and fish appeared to be calm with clove oil, perhaps due to less irritation compared with Sodium bicarbonate.

Although, some of these chemicals are not fully utilized in developing countries[43], the survival rate was excellent for both chemicals tested which qualifies them to be very good anesthetic for fish. Mortality was noticed only when a higher dose of alcohol was used as a diluent for the clove oil.

Conclusion

From this study, Clove oil and Sodium bicarbonate have been found to be effective and safe at different mild concentrations for both species tested. This study also revealed that the clove oil and sodium bicarbonate can be applied at 20.0 mg/l and is sufficient to completely sedate the fish. There was no record of any mortalities from different anaesthetic concentration of the different age categories during and after the study.

Future studies from the study area need to assess higher doses of the same anesthetic agents and possible toxicity in the species tested afterwards and whether significant difference exist in relation to other factors such as sex.

Table 7: Sodium bicarbonate complete anaesthesia mean induction and recovery time for 253.3 ± 5.0 g *Clarias gariepinus* and 242.3 ± 3.2 g *Oreochromis niloticus*

	Clarias gariepinus **(n=25)**		***Oreochromis niloticus*** **(n=25)**	
Conc (g/L)	**Induction time (mins)**	**Recovery time (mins)**	**Induction time (mins)**	**Recovery time (mins)**
10	0	0	0	0
20	9.4 ± 0.12	0.7 ± 0.12	8.8 ± 0.02	1.1 ± 0.14
30	8.7. ± 0.05	1.6 ± 0.04	8.0 ± 0.01	1.8 ± 0.02
40	7.3 ± 0.02	1.7± 0.11	7.0 ± 0.01	2.7 ± 0.05
50	6.1 ± 0.13	2.2 ± 0.03	6.9 ± 0.14	3.4 ± 0.01

Table 8: Sodium bicarbonate complete anaesthesia mean induction and recovery time for 425.4 g ± 2.4 g *Clarias gariepinus* and 418.2 g ± 3.4 g *Oreochromis niloticus*

	Clarias gariepinus **(n=25)**		***Oreochromis niloticus*** **(n=25)**	
Conc (g/L)	**Induction time (mins)**	**Recovery time (mins)**	**Induction time (mins)**	**Recovery time (mins)**
10	0	0	0	0
20	9.7 ± 0.02	0.8 ± 0.02	10.8 ± 0.03	1.0 ± 0.01
30	9.5. ± 0.06	1.4 ± 0.05	10.1 ± 0.14	1.9 ± 0.03
40	8.7 ± 0.12	1.7± 0.13	9.0 ± 0.02	2.2 ± 0.04
50	8.1 ± 0.01	2.0 ± 0.01	8.5 ± 0.04	2.7 ± 0.11

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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