**EFFECT OF NATURAL ANESTHETIC ON FISH BREEDING**

**AND FISH TRANSPORTATION.**

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**ABSTRACT**

This study evaluated the use of lime leaves powder as sedative for *Clarias gariepinus* and *Heterobranchus longifilis* female broodstocks, after induced breeding and during transportation of broodfish to a fish farm in New Bussa, Niger state, Nigeria. A total of seventy-two (72) broodstocks (36 females anesthetized in plastic holding receptacles after induced breeding and 36 females anesthesized during transportation to a fish farm) were used in the study. Graded levels of lime leaves powder used in the study were 0, 50mg/l and 100mg/l respectively. *Clarias gariepinus* females were sedated at 1.20 minutes for the 50mg/l inclusion level after induced breeding; and at 1.46minutes during transportation. *Clarias gariepinus* females were sedated at 0.42 seconds after induced breeding and at 0.60 seconds during transportation for the 100mg/l inclusion level. *Heterobranchus longifilis* females were sedated at 1.12 minutes under the 50mg/l inclusion level after induced breeding; and 1.28 minutes during transportation. *Heterobranchus longifilis* females in the 100mg/l treatment were sedated at 0.30 seconds after induced breeding and 0.46 seconds during transportation. Recovery time for *Clarias gariepinus* females in the 50mg/l holding recorded 3.00 minutes after induced breeding and 4.15 minutes during transportation. *Clarias gariepinus* females in the 100mg/l treatment recorded 26.0 minutes recovery time after induced breeding; and 35.0 minutes during transportation. *Heterobranchus longifilis* females in the 100mg/l treatment after induced breeding reported recovery time as 33.0 minutes and 41.0 minutes during transportation. The higher the concentration of lime leaves powder extract used (100mg/l), the slower the recovery time (26.0, 35.0 minutes recovery time).

Keywords: natural, anesthesia, lime leaves powder, concentration, sedation time, recovery time, *Clarias gariepinus, Heterobranchus longifilis*

**INTRODUCTION**

During research activities and proclivities, fishes are given anesthetics for sedation purposes. When using anesthetics as a sedative, it is with the understanding that the fish as a subject, is to undergo clinical assessment, egg and sperm stripping in the fish hatcheries and treatment for ulcerations, fin damage, gill damage and surgical procedures (Schroeder *et al.,* 2021; Pribosky and Velisek, 2018).

Anesthesia is a proclaimed stress reliever in fish while preventing injuries brought upon by mechanical activities in aquaculture. These mechanical activities include: dragging, netting, sorting and transportation (Pribosky and Velisek, 2018). There are a range of synthetic fish anesthesia commonly used. They are: Tricaine (MS-222), benzocaine, metomidate and quinaldine. These agents are expensive, scarce and known to leave residues in the body of the fish bringing about lengthy withdrawal periods (Usman *et al.,* 2019; Akinrotimi and Achilike, 2019). The overdose of an anesthetic is with the intention to euthanize fish completely (Hedayati, 2016). Herbal anesthetics of plant extraction are now widely coveted for use in aquaculture because they are beneficial to fish health while suppressing oxidative and physiological stress (Hoseini *et al.,* 2018). Herbs have been used in medieval times to relieve pain caused by disease, injury and even during convalescence (Tsuchiya, 2017). There is a dearth of information on *Heterobranchus longifilis* fishes (male and female) anesthesized with plant extracts. This study comparatively showed the efficacy of powdered lime leaves on *Clarias gariepinus* and *Heterobranchus longifilis* broodstocks, post induced breeding and during transportation of broodfish to a fish farm in New Bussa, Niger state, Nigeria.

**MATERIALS AND METHODS**

**Study Area**

The study was carried out in New Bussa. New Bussa is located at [9°53′N 4°31′E](https://tools.wmflabs.org/geohack/geohack.php?pagename=New_Bussa&params=9_53_N_4_31_E_region:NG_type:city%2824449%29) map [coordinates](https://en.wikipedia.org/wiki/Geographic_coordinate_system) (Robert *et al.,* 2021) in Niger state, Nigeria. *Clarias gariepinus* and *Heterobranchus longifilis* broodfish were obtained from the Fish Breeding and Culture Program of NIFFR, New Bussa. The Kigera Dam situated on the grounds of the National Institute for Freshwater Fisheries Research (NIFFR), supplied water for this research.

**Purchase, Preparation Of Lime Leaves Powder**

Leaves of lime were sourced from Monday market in New Bussa, Niger state, Nigeria. Lime leaves were then identified in the Fish Breeding and Culture Unit (Hatchery unit of NIFFR). Leaves were air-dried for 7 days. Then the leaves were blended into powder using a kitchen blender (Euro-Premium Blender- Tango DX Mixer Grinder 750 watts). The blended leaves now powder, were then sieved using a fine nylon mesh of the 0.1µ variety. Graded levels for the powdered lime in this study were: 0mg/l, 50mg/l and 100mg/l. Powdered lime leaves were stored in airtight plastic bottles for use under this study. The lime leaves powder was prepared into a solution by diluting the powder in ethanol (95%) at a ratio of 1:10. The stock solution was 100 µL/mL (El-Dakar, *et al.,* 2021; Can & Sumer, 2019). The female broodfishes were then taken to the eighteen concrete tanks, dropped in and anesthesized to relieve them of their pain and stress during the induced breeding.

**Acclimatizing Brood Fishes**

Seventy-two broodfishes (2 males and 2 females in nine holding receptacles for *Clarias gariepinus* labelled 0, 50 and 100mg/l; *a*nd 2 males, 2 females in nine holding receptacles for *Heterobranchus longifilis* labelled 0, 50 and 100mg/l) were acclimatized in eighteen (18) big plastic holding receptacles with netting as cover, in readiness for induced breeding.

**Experimental fishes**

After the males were stripped and sacrificed, eighteen (18) females for *Clarias gariepinus a*nd eighteen (18) females for *Heterobranchus longifilis* remained. These female broodfishes were then returned to the eighteen holding plastic receptacles and anesthesized; by pouring the stock solution of Lime leaves powder from a 2L bucket in graded levels of 0, 50 and 100mg/l respectively; to relieve them of their pain and stress during the induced breeding. Treatments were triplicated. Recovered fishes were transferred to 2 X 2 m2 concrete tanks after recording of the recovery time. Thereafter, another selection of eighteen (18) *Clarias gariepinus* and eighteen (18) *Heterobranchus longifilis* already acclimatized, were transported to a fish farm in New bussa, fifteen minutes away from NIFFR. Transportation was done using eighteen yellow plastic jerrycans and anesthesized with lime leaves powder occurring in graded levels of 0, 50 and 100mg/l respectively. Treatments were triplicated.

**Experimental Design**

Experimental design was a completely randomized design.

**Statistical Analysis**

Data from this study were analysed using One-way Analysis of Variance (ANOVA), Duncan multiple range test and the differences reported in the study were tested using T-test.

**Water Quality Parameters**

The water quality parameters measured during the study were: water temperature, dissolved oxygen and ρН.

**RESULTS**

**Table 1 Effect of Lime leaves powder on *Clarias gariepinus* and *Heterobranchus longifilis* broodstocks after induced breeding and during transportation within New Bussa, Niger state.**

**Parameters 0(B) 0(T) 50(B) 50(T) 100 (B) 100(T)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Fish weight (kg)**  ***Clarias gariepinus*** | **2000.00a** | **2100.00b** | **2000.00a** | **1820.00c** | **2100.00b** | **1600.00d** |
| **Fish weight (kg)**  ***Heterobranchus longifilis*** | **1500.00d** | **1800.00b** | **1600.00c** | **1500.00d** | **1900.00a** | **1800.00b** |
| **ST(mins/secs) *Clarias gariepinus*** | **5.00a** | **5.10a** | **1.20b** | **1.46b** | **0.42c** | **0.60c** |
| **ST (min/secs) *Heterobranchus longifilis*** | **5.27a** | **5.30a** | **1.12b** | **1.28b** | **0.30c** | **0.46c** |
| **RT (mins/secs) *Clarias gariepinus*** | **67.0a** | **80.0a** | **3.00c** | **4.15c** | **26.0b** | **35.0b** |
| **RT (mins/secs) *Heterobranchus longifilis*** | **89.2a** | **94.3a** | **4.00c** | **5.20c** | **33.0b** | **41.0b** |
| **Survival (%) *Clarias gariepinus*** | **100.00a** | **100.00 a** | **100.00 a** | **100.00 a** | **100.00 a** | **100.00 a** |
| **Survival (%) *Heterobranchus longifilis*** | **100.00a** | **100.00 a** | **100.00 a** | **100.00 a** | **100.00 a** | **100.00 a** |

*B=After induced breeding, T=During transport, ST=Sedation time, RT=Recovery time. Graded levels 0, 50, 100 measured in mg/l. Means in the same row (for each section) with different superscript are statistically significant (P<0.05).*

**Table 2 Pooled water quality parameters of Lime leaves powder on *Clarias gariepinus* and *Heterobranchus longifilis* broodstocks after induced breeding and during transportation within New Bussa, Niger state.**

**Parameters WT (B) WT(T) DO(B) DO(T)** ρН**(B)** ρН **(T)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Clarias gariepinus***  ***0mg/l*** | **27.00a** | **27.80a** | **5.00a** | **5.30a** | **7.50a** | **7.50a** |
| ***50mg/l*** | **27.00 a** | **27.50 a** | **5.21 a** | **6.00 b** | **7.00 a** | **7.35 a** |
| ***100mg/l*** | **27.50 a** | **27.90a** | **5.50 a** | **5.70 a** | **7.21 a** | **7.50 a** |
| ***Heterobranchus longifilis***  ***0mg/l*** | **27.10a** | **28.00b** | **4.59a** | **4.50a** | **8.00a** | **8.00a** |
| ***50mg/l*** | **27.00 a** | **27.31 a** | **5.50 a** | **5.78 a** | **7.10 a** | **7.60 a** |
| ***100mg/l*** | **27.80 a** | **28.01 b** | **5.23 a** | **5.60 a** | **7.21 a** | **7.40 a** |

*B=After induced breeding, T=During transport. WT=0C, DO=ppm. Means in the same row (for each section) with different superscript are statistically significant (P<0.05).*

**DISCUSSION**

The fishes showed no activity at jumping or wriggling when their mouths were prised open by hands at exactly 1.20 minutes for the 50mg/l inclusion level, which sedated *Clarias gariepinus* immediately after induced breeding; and at 1.46minutes during transportation. *Clarias gariepinus* females were however completely sedated at 0.42 seconds after induced breeding and at 0.60 seconds during transportation for the 100mg/l inclusion level of lime leaves powder extract. *Heterobranchus longifilis* females in this study were sedated at 1.12 minutes under the 50mg/l inclusion level of lime leaves powder extract after induced breeding. *Heterobranchus longifilis* females also reached sedative state at 1.28 minutes during transportation under the 50mg/l inclusion level of lime leaves powder extract. *Heterobranchus longifilis* females in the 100mg/l treatment receptacles were sedated at 0.30 seconds after induced breeding and 0.46 seconds during transportation. Clove seed extract in an earlier study sedated *Clarias gariepinus* fingerlings within 5 minutes at 2.5g/l concentration (Jegede, 2014). This result was completely at variance with the sedation times recorded in this study. The results of this further suggest that, the higher the concentration of natural anesthesia, the faster the sedation time. This statement agrees with Palomera *et al.* (2016) whose earlier study on anesthetic effects of 900µL/L Clove concentration on *Heterobranchus bidorsalis* juveniles produced anesthesia at exactly 8 minutes; anesthetic effects of 600µL/L Clove concentration on *Heterobranchus bidorsalis* juveniles produced anesthesia at 12 minutes, while a 300µL/L Clove concentration on *Heterobranchus bidorsalis* juveniles produced anesthesia at 17 minutes. Sedation times therefore, for when *Clarias gariepinus* and *Heterobranchus longifilis* females were completely immobilized and calm revealed a non-significant difference (P>0.05) in the after induced breeding time frame and the transportation timeframe. The 50mg/l inclusion levels of lime leaves powder however reported slightly different values also depicting a non-significant difference (P>0.05) in the after induced breeding and during transportation timeframes for when *Clarias gariepinus* and *Heterobranchus longifilis* females were completely immobilized. Sedation times reported a significant difference (P<0.05) after induced breeding and during transportation for *Clarias gariepinus* and *Heterobranchus longifilis* females in the study using inclusion levels of 100mg/l of lime leaves powder as anesthesia.

Recovery times were clearly evident when fish started wriggling, swimming and there was no calmness throughout the holding receptacles of *Clarias gariepinus* and *Heterobranchus longifilis* used for induced breeding and during transportation. This observation agrees with Correia *et al.* (2018) whose previous study on the use of Basil, tea tree and clove essential oils in *Amphiprion clarkii* as analgesics and anesthetics reported recovery time as a state whereby the animals responded to visual stimuli and reached a horizontal swimming position.

Recovery times for *Clarias gariepinus* females in the 50mg/l holding receptacles recorded 3.00 minutes after induced breeding and 4.15 minutes during transportation. *Clarias gariepinus* females in the 100mg/l treatments however recorded 26.0 minutes recovery time after induced breeding and 35.0 minutes recovery time during transportation. *Heterobranchus longifilis* females on the other hand, recorded longer recovery times than the *Clarias gariepinus* females. *Heterobranchus longifilis* females in the 100mg/l treatments after induced breeding reported recovery time of 33.0 minutes and 41.0 minutes during transportation. This result agrees with the recuperation times reported by Palomera *et al.* (2016) whose study on evaluation of natural extracts with anesthetic properties in juveniles *Macrobrachium tenellum* reported total recuperation times in 900µL/L, 600µL/L and 300µL/L concentrations of clove oil as 34 minutes, 27 minutes and 20 minutes respectively. It is suggested therefore, that *Clarias gariepinus* females recovered faster than *Heterobranchus longifilis* females during this study. Recovery times showed throughout the 0mg/l, 50mg/l and 100mg/l treatments for *Clarias gariepinus* and *Heterobranchus longifilis* females, a non-significant difference (P>0.05) after induced breeding and during transportation. The higher the concentration of lime leaves powder extract used (100mg/l), the slower the recovery time after induced breeding (26.0, 35.0 minutes recovery time) and during transportation (35.0, 41.0 minutes). This observation was in agreement with Olufayo and Ojo(2018) whose earlier study reported recovery times to be significantly slower in higher concentrations (1.2ppm, 1.1ppm, 1.0ppm, 0.9ppm and 0.8ppm) of Clove oil as (906.50, 675.00, 514.00, 305.00 and 180.00 seconds) respectively.

Survival reported 100% throughout the study for female broodstocks of *Clarias gariepinus* and *Heterobranchus longifilis* used for induced breeding, and transportation to a fish farm within New Bussa, fifteen minutes away from NIFFR. *Sarethorodon melanotheron* juveniles anesthesized with 40mg/l nutmeg extracts revealed 100% survival values throughout their study (Akinrotimi and Achilike, 2019). Their result was in agreement with the survival results of this study.

The water temperature results for *Clarias gariepinus* reported that it took 27.0 0C after induced breeding and 27.50 0C during transportation, to reach anesthesia in the 50mg/l treatment. Water temperature results for *Heterobranchus longifilis* reported also that it took 27.0 0C after induced breeding and 27.31 0C during transportation to reach anesthesia in the 50mg/l treatment. This result proves the efficiency of lime leaves powder at 50mg/l than at 100mg/l for *Clarias gariepinus* and *Heterobranchus longifilis* female broodstocks when considering temperature of the mediums to which the fishes were held. This result does not agree with Carneiro *et al.* (2019) whose study compared Clove oil anesthetic efficacy for Tambacu juveniles at 25 mg L-1 while considering water temperature at 25.0 0C.

The pooled water quality parameter results investigated in this study therefore, suggested a non-significant difference (P>0.05) in the *Clarias gariepinus* 0mg/l, 50mg/l and 100mg/l lime leaves powder treatments after being used for induced breeding and during transportation for Water temperature, Dissolved oxygen and ρН.

The *Heterobranchus longifilis* females in the 0mg/l and 100mg/l lime leaves powder treatments however reported a significant difference (P<0.05) after being used for induced breeding and during transportation for Water temperature. Dissolved oxygen and ρН values for the 0mg/l, 50mg/l and 100mg/l lime leaves powder treatments after being used for induced breeding and during transportation were non-significantly different (P>0.05).

**CONCLUSION AND RECOMMENDATION**

Natural plant extracts at concentration 50mg/l proved effective as anesthetic for fish after induced breeding to relieve pain, stress after induced breeding and during transportation. Water quality parameters remained at normal ranges, throughout this study. This is therefore recommended for use in fish farms during breeding and transportation of fish. Further studies should be carried out to compare synthetic and natural anesthetics while ascertaining and documenting the effectiveness of using synthetic and natural anesthetics.

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