**EFFECTS OF NATURAL ANESTHETIC ON FISH BREEDING**

**AND FISH TRANSPORTATION.**

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**ABSTRACT**

This study looked comparatively at the use of lime leaves powder on *Clarias gariepinus* and *Heterobranchus longifilis* 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. There was a significant difference (P<0.05) in the weights of *Clarias gariepinus* and *Heterobranchus longifilis* females in the 0mg/l graded level (non-inclusion of lime leaves powder) after induced breeding. However, there was no significant difference (P>0.05) in the weights of *Clarias gariepinus* and *Heterobranchus longifilis* femalesin the 0mg/l graded level (non-inclusion of lime leaves powder) during transportation. The 50mg/l inclusion levels of lime leaves powder treatments reported a non-significant difference (P>0.05) in timeframes after induced breeding and during transportation 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. The higher the concentration of lime leaves powder extract used (100mg/l), the slower the recovery time (26.0, 35.0 minutes recovery time). Survival was 100% throughout the study for *Clarias gariepinus* and *Heterobranchus longifilis* females used for induced breeding and transportation.

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). This study will comparatively show the efficacy of powdered lime leaves on *Clarias gariepinus* and *Heterobranchus longifilis* broodstocks, after 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) [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 unit 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 were 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 subjected to One-way Analysis of Variance (ANOVA), Duncan multiple range test and the differences 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**

There was a significant difference (P<0.05) in the weights of *Clarias gariepinus* and *Heterobranchus longifilis* females in the 0mg/l graded level (non-inclusion of lime leaves powder) after induced breeding. However, there was no significant difference (P>0.05) in the weights of *Clarias gariepinus* and *Heterobranchus longifilis* femalesin the 0mg/l graded level (non-inclusion of lime leaves powder) during transportation. The anesthesized 50mg/l graded levels of lime leaves powder treatments showed a significant difference (P<0.05) in the weights of *Clarias gariepinus* and *Heterobranchus longifilis* femalesafter induced breeding and during transport. The 100mg/l inclusion levels of lime leaves powder treatments depicted also, a significant difference (P<0.05) in the weights of *Clarias gariepinus* and *Heterobranchus longifilis* females after induced breeding and during transport. Sedation times 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 leave 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. Clove seed extract sedated *Clarias gariepinus* fingerlings within 5 minutes at 2.5g/l concentration (Jegede, 2014). This result was completely at variance with the sedation times of 1.10, 1.46 minutes for *Clarias gariepinus* and 1.12, 1.28 minutes for *Heterobranchus longifilis* broodstocks recorded in 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 (26.0, 35.0 minutes recovery time). This observation was in agreement with Usman *et al.* (2019) whose earlier study reported sedation to be significantly slower (23.40, 24.32 and 24.45 minutes) in *Clarias gariepinus* broodstock treated with 40g/l of lime, lemon, sweet orange leaves extracts. Survival was 100% throughout the study for females 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 pooled water quality parameter results investigated in this study reported 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 however reported a significant difference (P<0.05) in the 0mg/l and 100mg/l lime leaves powder treatments 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 for were non-significantly different (P>0.05).

**CONCLUSION AND RECOMMENDATION**

There was a dearth of information for *Heterobranchus longifilis* fishes (male and female) anesthesized with plant extracts. There was also little information for *Clarias gariepinus* females anesthesized with natural plant extracts; hence the need for this study. It is therefore recommended that natural plant extracts at concentration 50mg/l be used to anesthetize fish after induced breeding to relieve pain, stress and during transportation. Water quality parameters remained at normal ranges throughout this study.

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