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Sedative responses of *Clarias gariepinus* and *Heterobranchus longifilis* broodstocks to lime (*Citrus aurantiifolia*) leaf extract after stripping and during transportation

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ABSTRACT: Handling catfish properly has precipitated the use of anaesthesia to minimise stress and prevent injuries. This study aims to show the efficacy of lime leaf extract as a sedative for *Clarias gariepinus* and *Heterobranchus longifilis* female broodstocks, after stripping and during transportation of broodfish to a fish farm in New Bussa. A total of 48 female broodstocks, comprising 24 *C. gariepinus* and 24 *H. longifilis*, were assigned to three lime leaf extract concentrations (0 mg/L, 50 mg/L and 100 mg/L) under two experimental conditions: post-stripping and transportation. Each treatment was conducted in duplicate. *Clarias gariepinus*♀ were sedated at 1.20 minutes for the 50mg/l inclusion level after stripping; and at 1.46minutes during transportation. At 100 mg/L, sedation occurred at 42 seconds and 60 seconds in *C. gariepinus* after stripping and during transportation, respectively. *Heterobranchus longifilis*♀ were sedated at 1.12 minutes under the 50 mg/L inclusion level after stripping and 1.28 minutes during transportation. *Heterobranchus longifilis* ♀ in the 100 mg/L treatment were sedated at 0.30 seconds after stripping and 0.46 seconds during transportation. Recovery time for *Clarias gariepinus*♀ in the 50mg/L holding recorded 3.00 minutes after stripping and 4.15 minutes during transportation. *Clarias gariepinus*♀ in the 100mg/L treatment recorded 26.0 minutes recovery time after stripping; and 35.0 minutes during transportation. *Heterobranchus longifilis*♀ in the 50 mg/L and 100 mg/L treatments, after stripping, reported recovery times as 4.0 minutes and 33.0 minutes, respectively. 5.20 minutes and 41.0 minutes were recovery times during transportation for *Heterobranchus longifilis*. Recovery time increased with extract concentration, with the 100 mg/L treatment producing substantially longer recovery times than the 50 mg/L treatment in both species.

Keywords: Anaesthesia, lime leaves extract, sedation time, recovery time, *Clarias gariepinus, Heterobranchus longifilis,* after stripping, during transportation.

INTRODUCTION

Fish anaesthesia is commonly used during aquaculture operations and research procedures to reduce handling stress, minimise physical injury, and facilitate procedures such as stripping, sorting, sampling and transportation. It is a proven stress reliever in fish and helps prevent injuries caused by routine handling procedures such as netting, sorting, stripping, and transportation, which can expose fish to considerable physical and physiological stress in aquaculture. These mechanical activities include routine handling procedures such as netting, sorting, stripping and transportation, which can expose fish to considerable physical and physiological stress (Priborsky and Velisek, 2018). When using a sedative, it is with the understanding that the fish undergoes clinical assessment, such as egg and sperm stripping in the hatcheries and treatment for ulcerations, fin damage, gill damage and surgical procedures (Schroeder *et al.,* 2021; Priborsky and Velisek, 2018). A wide range of synthetic fish anaesthetics are commonly used. They are: Tricaine (MS-222), benzocaine, metomidate and quinaldine. However, 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). An overdose of an anaesthetic results in the complete euthanasia of fish (Hedayati, 2016).

Herbal anaesthetics from plant extraction are now widely used in aquaculture because they are beneficial to fish health while suppressing oxidative and physiological stress (Hoseini *et al.,* 2018). This is evident as herbs have been used in medieval times to relieve pain caused by disease, injury and even during convalescence (Tsuchiya, 2017). Although several studies have investigated plant-derived anaesthetics in cultured fish, limited information is available on the comparative sedative and recovery responses of *C. gariepinus* and *H. longifilis* broodstocks to lime (*C. aurantiifolia*) leaf preparations, particularly following stripping and during transportation. The present study therefore evaluated the effects of graded lime leaf extract concentrations on sedation time, recovery time and survival under these two handling conditions.

MATERIALS AND METHODS

Study area

The study was carried out in New Bussa, the headquarters of Borgu Local Government Area of Niger State. New Bussa is located at latitude 9°53′N and longitude 4°31′E. New Bussa has a documented population of One hundred and Two Thousand, Three hundred and Seventy (Google Maps, 2026; Wikipedia, 2026).

Experimental fish

Clarias gariepinus and *Heterobranchus longifilis* broodstocks were obtained from the Fish Breeding and Culture Program of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria.

Water source

The Kigera Dam situated on the grounds of the Institute, supplied water for this research.

Purchase and preparation of lime leaves extract

Leaves of lime (*Citrus aurantiifolia*) were bought from the market in New Bussa, Niger State, Nigeria. The plant material was authenticated by a qualified botanist, and a voucher specimen (NIFFR/REC/2026/JBBD/003) was deposited in the herbarium (laboratory) for future reference. Leaves were air-dried for seven (7) days in the Fish Breeding and Culture Program (Hatchery unit of NIFFR). Then the leaves were blended into powder using a kitchen blender (Euro-Premium Blender- Tango DX Mixer Grinder 750 watts). The powder was then sieved using a fine nylon mesh of the 0.1µ variety. The known mass of dried lime leaves powder, already macerated, was poured into a defined volume of 95% ethanol at a 1:10 w/v ratio for three hours. The mixture was filtered, and the resulting extract was used to prepare final concentrations of 50 and 100 mg/L by adding calculated volumes of the stock solution to the experimental water (El-Dakar *et al.,* 2021; Can and Sumer, 2019). Graded concentration levels for the lime leaves extract in this study were: 0mg/L, 50mg/L and 100mg/L and were stored in labelled airtight plastic bottles.

Acclimatising broodstocks after stripping and during transportation

The experiment consisted of three extract concentrations (0 mg/L, 50 mg/L and 100 mg/L), two fish species and two handling conditions (post-stripping and transportation). Each treatment combination was replicated two times, with two fish per replicate. The experimental unit was a cylindrical 1000 L drum. Twenty-four (24) females (12 *C. gariepinus* and 12 *H. longifilis*) were acclimatised for induced breeding. Six males of *C. gariepinus* were held in a cylindrical 55-gallon drum measuring 57.15 cm, while the females were held in three plastic 1000 L cylindrical drums (2 per drum), cut open at the top with a diameter of 1.2 m and a height of 0.9 m. The same procedure was repeated for *H. longifilis*. The ratio was two males to four females for *C.gariepinus* and *H. longifilis*. These tanks holding the female fish were also labelled 0 mg/L, 50 mg/L and 100 mg/L, respectively. Drums were covered with nets and placed in the indoor hatchery of the institute (NIFFR). After the milt from the males were obtained, eggs from each of the twelve (12) female of *C. gariepinus* and eggs from each of the twelve (12) female of *H. longifilis* were fertilized with the milt from their males, and the fish were returned to their holding drums and anesthetized; by pouring the stock solution of Lime leaf extract into graded levels of 0 mg/L, 50 mg/L and 100 mg/L, respectively, to relieve them of their pain and stress during the stripping. Treatments were duplicated. Recovered fishes were transferred to 2 X 2 m^2 outdoor concrete tanks after recording their recovery time.

Thereafter, another set of Twenty-four (24) (12 *C. gariepinus* and 12 *H. longifilis*) females, which had also followed acclimatisation procedures as the broodstocks which were used for induced breeding, were placed in labelled drums at the back of a truck, duplicated and anaesthetised in the labelled drums using Lime leaf extract of 0 mg/L, 50 mg/L and 100mg/L. These fish were transported to Asian Fisheries, a fish farm in New Bussa, which was twenty minutes away from NIFFR. Fish were transported for 20 minutes in labelled 1000 L cylindrical

drums containing 400 L of water, with two fish per container. The containers were secured on the vehicle, and water temperature and dissolved oxygen were measured before and immediately after transportation.

Water quality parameters

The water quality parameters measured during the study, based on standard methods of APHA *et al*. (2017), were water temperature, dissolved oxygen and pH.

Statistical analysis

Data from this study were analysed using One-way Analysis of Variance (ANOVA). To test for differences, the Duncan multiple range test was used to differentiate the significance. A t-test was used to test for similarities among the treatments. Excel 21 (Version 16.0) was used as a statistical tool.

RESULTS AND DISCUSSION

Sedation was considered achieved when fish exhibited loss of equilibrium, cessation of active swimming and reduced response to external stimulation according to the predefined anaesthesia scoring criteria after 1.20 minutes when female *C. gariepinus* were sedated with 50 mg/L after stripping and 1.46 minutes during transportation. However, the fish were completely sedated at 0.42 seconds and 0.60 seconds after stripping and during transportation, respectively, for the 100 mg/L inclusion level. *Heterobranchus longifilis* females in this study were sedated at 1.12 minutes under the 50 mg/L inclusion level after stripping and at 1.28 minutes during transportation. This was documented in Table 1. In the 100 mg/L treatment reported on Table 1, the fish were sedated at 0.30 seconds after stripping and 0.46 seconds during transportation. Clove seed extract in an earlier study sedated *Clarias gariepinus* fingerlings within 5 minutes at 2.5 g/L concentration (Jegede, 2014). The shorter sedation times observed in the present study compared with the study of Jegede (2014) may be related to differences in fish developmental stage, anaesthetic type, concentration and experimental conditions.

The results of this study further suggest that the higher the concentration of natural anaesthesia, the faster the sedation time. This statement agrees with Palomera *et al.* (2016) that anaesthetic effects (sedation) of 300 µL/L, 600 µL/L and 900 µL/L Clove concentrations on *Heterobranchus bidorsalis* juveniles produced anaesthesia at exactly 17, 12 and 8 minutes, respectively. The sedation times of *Clarias gariepinus* and *Heterobranchus longifilis* females, completely immobilised and calm, revealed a non-significant difference ($p>0.05$) after stripping and during transportation. However, slightly different values, depicting a non-significant difference ($p>0.05$) after stripping and during transportation for *C. gariepinus* and *H. longifilis* females that were completely immobilised, were reported at the 50 mg/L inclusion levels of lime leaves extract. Sedation times reported a significant difference ($p<0.05$) after stripping and during transportation for *C. gariepinus* and *H. longifilis* females in the study using inclusion levels of 100 mg/L of lime leaf extract.

Recovery times were clearly evident as documented in Table 1, when fish started wriggling and swimming, and there was no calmness throughout the holding tanks of *C. gariepinus* and *H. longifilis* females stripped after being used for induced breeding and during transportation. This observation agrees with Correia *et al.* (2018), whose findings on the use of Basil, tea tree and clove essential oils in *Amphiprion clarkii* as analgesics and anaesthetics reported recovery time as a state whereby the animals responded to visual stimuli and reached a horizontal swimming position. The recovery times for *C. gariepinus* females subjected to 50 mg/L were recorded as 3.00 minutes after stripping and 4.15 minutes during transportation, while those in the 100 mg/L treatments recorded a higher recovery time of 26.0 minutes after stripping and 35.0 minutes during transportation.

H. longifilis females, on the other hand, recorded longer recovery times than *Clarias gariepinus* females. *Heterobranchus longifilis* females in the 100 mg/L treatments after stripping reported recovery times 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 anaesthetic properties in juveniles *Macrobrachium tenellum* reported total recuperation times in 900 µL/L, 600 µL/L and 300 µL/L concentrations of clove oil as 34 minutes, 27 minutes and 20 minutes, respectively. It is suggested, therefore, that *C. gariepinus* females recovered faster than *H. longifilis* females during this study. The recovery times showed that 0 mg/L, 50 mg/L and 100 mg/L treatments for *C. gariepinus* and *H. longifilis* females did not differ significantly ($p>0.05$) after stripping and during transportation. The higher the concentration of lime leaf extract used, the slower the recovery time after induced breeding and during transportation. Prolonged or slower recovery times could reflect deeper anaesthesia and increased uptake of active compounds. This observation was in agreement with Olufayo and Ojo (2018), whose earlier study reported recovery times to be significantly slower in higher concentrations of Clove oil used as anaesthetic.

Survival reported throughout the study in Table 1 for female broodstocks of *C. gariepinus* and *H. longifilis* stripped, after being used for induced breeding and transportation, was 100%. Akinrotimi and Achilike (2019) reported 100% survival of *Sarotherodon melanotheron* juveniles anaesthetised with 40 mg/l nutmeg extracts. This is in consonance with the findings of this study.

The water temperature in the holding drums for *C.*

Table 1. Responses of *Clarias gariepinus* and *Heterobranchus longifilis* female broodstocks treated with different concentrations of lime leaves extract.

Parameter	0 (S)	0 (T)	50 (S)	50 (T)	100 (S)	100 (T)
Fish weight (g)						
Clarias gariepinus	2000.0^{a}	2100.0^{b}	2000.0^{a}	1820.0^{c}	2100.0^{b}	1600.0^{d}
Heterobranchus longifilis	1500.0^{d}	1800.0^{b}	1600.0^{c}	1500.0^{d}	1900.0^{a}	1800.0^{b}
ST (mins/secs)						
Clarias gariepinus	5.0^{a}	5.10^{a}	1.20^{b}	1.46^{b}	0.42^{c}	0.60^{c}
Heterobranchus longifilis	5.27^{a}	5.30^{a}	1.12^{b}	1.28^{b}	0.30^{c}	0.46^{c}
RT (mins/secs)						
Clarias gariepinus	67.0^{a}	80.0^{a}	3.00^{c}	4.15^{c}	26.0^{b}	35.0^{b}
Heterobranchus longifilis	89.2^{a}	94.3^{a}	4.00^{c}	5.20^{c}	33.0^{b}	41.0^{b}
Survival (%)						
Clarias gariepinus	100.0^{a}	100.0 ^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Heterobranchus longifilis	100.0^{a}	100.0 ^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}

S = After stripping, T = During transport, ST = Sedation time, RT = Recovery time. Graded levels 0, 50, 100 measured in mg/l. Within each species and response variable, means bearing different superscript letters differ significantly at $p<0.05$.

Table 2. Water quality parameters of the experiment setup.

Parameter	WT (S)	WT (T)	DO (S)	DO (T)	ρH (S)	ρH (T)
Clarias gariepinus						
0 mg/L	27.00^{a}	27.80^{a}	5.00^{a}	5.30^{a}	7.50^{a}	7.50^{a}
50 mg/L	27.00^{a}	27.50^{a}	5.21^{a}	6.00^{b}	7.00^{a}	7.35^{a}
100 mg/L	27.50^{a}	27.90^{a}	5.50^{a}	5.70^{a}	7.21^{a}	7.50^{a}
Heterobranchus longifilis						
0 mg/L	27.10^{a}	28.00^{b}	4.59^{a}	4.50^{a}	8.00^{a}	8.00^{a}
50 mg/L	27.00^{a}	27.31^{a}	5.50^{a}	5.78^{a}	7.10^{a}	7.60^{a}
100 mg/L	27.80^{a}	28.01^{b}	5.23^{a}	5.60^{a}	7.21^{a}	7.40^{a}

S = After stripping, T = During transport. WT = Water temperature (^{0}C), DO = Dissolved oxygen (ppm). Within each species and response variable, means bearing different superscript letters differ significantly at $p<0.05$.

gariepinus was reported as 27.0°C after stripping and 27.50°C during transportation to reach anaesthesia in the 50 mg/l treatment, while that of *H. longifilis* was 27.0°C after stripping and 27.31°C during transportation to reach anaesthesia. The 100 mg/L treatment for *C. gariepinus* and *H. longifilis* reported water temperature results of 27.50°C and 27.80°C after stripping and 27.90°C and 28.01°C during transportation, respectively. These results are documented in Table 2. Results for DO in this study reported 5.21 and 6.00 to reach anaesthesia, after stripping and during transportation, respectively, in the 50 mg/L treatment for *C. gariepinus*. The 100 mg/L treatment also reported DO values of 5.50 and 5.70 to reach anaesthesia after stripping and during transportation, respectively, for *C. gariepinus*. Results for DO in this study for *H. longifilis,* however, reported 5.50 and 5.78 to reach anaesthesia after stripping and during transportation, respectively, in the 50 mg/L treatment. The 100 mg/L treatment for *H. longifilis* reported DO values of 5.23 and 5.60 to reach anaesthesia after stripping and during transportation, respectively. The ρH in the holding drums for *C. gariepinus was* reported as 7.10 after stripping and 7.60 during transportation to reach anaesthesia in the 50 mg/L treatment; while that of *H. longifilis was* reported as 7.21 after stripping and 7.40 during transportation to reach anaesthesia. The 100 mg/L treatment also documented 7.21 after stripping and 7.50 during transportation for *C. gariepinus;* and 7.21 after stripping and 7.40 during transportation for *H. longifilis.*

The pooled water quality parameters investigated in this study (Table 2) therefore suggested non-significant differences ($p>0.05$) in the holding drums for *C. gariepinus*

at 0 mg/L, 50 mg/L and 100 mg/L lime leaf extract treatments after being used for induced breeding and during transportation for water temperature, dissolved oxygen and ρH. This could be as a result of the same source of water and biological activities (Abowei, 2010). *H. longifilis* females in the 0 mg/L and 100 mg/L treatments, however, reported a significant difference ($p<0.05$) after being used for induced breeding and during transportation for water temperature. This could be as a result of natural environmental processes and human activities (Coulter *et al.,* 2015). Dissolved oxygen and ρH values for the 0 mg/L, 50 mg/L and 100 mg/L lime leaves powder treatments after being used for induced breeding and during transportation were not significantly different ($p>0.05$).

Summarily, therefore, *Clarias gariepinus*♀ were sedated at 1.20 minutes for the 50 mg/L treatment after stripping and at 1.46 minutes during transportation. *Clarias gariepinus*♀ were also sedated at 0.42 seconds after stripping and at 0.60 seconds during transportation for the 100 mg/L treatment. *Heterobranchus longifilis*♀ in this study reported a sedation time of 1.12 minutes for the 50 mg/L treatment after stripping and 1.28 minutes during transportation. *Heterobranchus longifilis* ♀ also reported a sedation time of 0.30 seconds in the 100 mg/L treatment after stripping and 0.46 seconds during transportation. Recovery time for *Clarias gariepinus*♀ in the 50 mg/L holding drum was recorded as 3.00 minutes after stripping and 4.15 minutes during transportation. *Clarias gariepinus*♀ in the 100 mg/L holding drum recorded 26.0 minutes recovery time after stripping and 35.0 minutes during transportation. *Heterobranchus longifilis*♀ in the 50 mg/L and 100 mg/L holding drums after stripping reported recovery times as 4.0 minutes and 33.0 minutes, respectively. 5.20 minutes and 41.0 minutes recovery times were reported for *H.longifilis* during transportation. Considering the balance between rapid sedation and shorter recovery time, the 50 mg/L treatment appeared more suitable than 100 mg/L for the handling conditions evaluated in this study. This result does not agree with Carneiro *et al.* (2019), whose study compared Clove oil anaesthetic efficacy for Tambacu juveniles at 25 mgL^{-1} while considering water temperature at 25.0°C.

Conclusion and Recommendation

Under the conditions evaluated, 50 mg/L lime leaf extract provided effective sedation with shorter recovery times than 100 mg/L in female *C. gariepinus* and *H. longifilis* broodstocks. No mortality was observed during the experimental period, while the measured water quality parameters remained within the reported experimental ranges. Further controlled studies should evaluate the suitability of 50 mg/L lime leaf extract for short-duration transportation of catfish broodstocks under different stocking densities, transport durations and environmental conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abowei, J. F. N. (2010). Salinity, dissolved oxygen, pH and surface water temperature conditions in Nkoro River, Niger Delta, Nigeria. *Advanced Journal of Food Science and Technology*, *2*(1), 36-40.

Akinrotimi, O. A., & Achilike, N. M. (2019). Efficacy of nutmeg (*Myristica fragrans*) extracts as anaesthetics in transportation of *Sarotherodon melanotheron. Proceedings of the 34th Annual Conference of The Fisheries Society of Nigeria (FISON*), held at the Law Auditorium, Ebonyi State University, (CAS CAMPUS), on October 28th -November 1st, Abakaliki, Ebonyi State University, Nigeria. pp.35-39.

American Public Health Association, American Water Works Association and Water Environment Federation (2017). *Standard methods for the examination of water and wastewater* (23rd edition), In: Lipps, W. C., Baxter, T. E., & Braun-Howland, E. (eds.). APHA Press.

Can, E., & Sümer, E. (2019). Anaesthetic and sedative efficacy of peppermint (Mentha piperita) and lavender (Lavandula angustifolia) essential oils in blue dolphin cichlid (*Cyrtocara moorii*). *Turkish Journal of Veterinary & Animal Sciences*, *43*(3), 334-341.

Carneiro, W. F., Melo, N., Da Silva, K. C. D., Ferreira, I. S., Paula, D. A. D. J., Ferrante, M., & Murgas, L. D. S. (2019). Clove oil as anaesthetic for tambacu juveniles (♂*Piaractus mesopotamicus*× *Colossoma macropomum*♀). *Advances in Animal and Veterinary Sciences*, *7*(11), 969-976.

Correia, A. M., Pedrazzani, A. S., Mendonca, R. C., Massucatto, A., Ozorio, R. A., & Tsuzuki, M. Y. (2018). Basil, tea tree and clove essential oils as analgesics and anaesthetics in *Amphiprion clarkii* (Bennett, 1830). *Brazilian Journal of Biology, 78*(3), 436-442.

Coulter, D. P., Höök, T. O., Mahapatra, C. T., Guffey, S. C., & Sepulveda, M. S. (2015). Fluctuating water temperatures affect development, physiological responses and cause sex reversal in fathead minnows. *Environmental Science & Technology*, *49*(3), 1921-1928.

El-Dakar, Y. (2021). Using clove and mint oils as natural sedatives to increase the transport quality of the Nile tilapia (*Oreochromis niloticus*) broodstock. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(4), 437-446.

Google Maps (2026). New Bussa. Retrieved from https://www.google.com/maps

Hedayati, A. (2018). Effect of 2-phenoxyethanol anaesthesia on some haematological and biochemical indices of silver carp (*Hypophthalmichthys molitrix*). *Iranian Journal of Fisheries Sciences*, *17*(1), 1-10.

Hoseini, S. M., Mirghaed, A. T., & Morteza, Y. (2018). Application of herbal anaesthetics in aquaculture. *Reviews in Aquaculture, 11*(3), 550-564.

Jegede, T. (2014). Anaesthetic potential of tobacco (*Nicotiana tobaccum*) on *Clarias gariepinus* (Burchell, 1822) fingerlings. *Journal of Agricultural Science,* 6(5), 68-90.

Olufayo, M. O., & Ojo, O. M. (2018). Anaesthetic effects and haematological responses of *Heterobranchus bidorsalis* juveniles exposed to clove oil. *International Journal of Fisheries and Aquaculture,* 10(9), 116-121.

Palomera, M. A. A., Del Rio Zaragoza, O. B., Galvan, S. R. G., & Villasante, F. V. (2016). Evaluation of natural extracts with anaesthetic properties in juveniles *Macrobrachium tenellum. Pan-American Journal of Aquatic Sciences,*11(3), 251-257.

Priborsky, J., & Velisek, J. (2018). A review of three commonly used fish anaesthetics. *Reviews in Fisheries Science & Aquaculture*, *26*(4), 417-442.

Schroeder, P., Lloyd, R., McKimm, R., Metselaar, M., Navarro, J., O'Farrell, M., Readman, G. D., Spielberg, L., & Mocho, J. P. (2021). Anaesthesia of laboratory, aquaculture and ornamental fish: Proceedings of the first LASA-FVS Symposium. *Laboratory Animals*, *55*(4), 317-328.

Tsuchiya, H. (2017). Anaesthetic agents of plant origin: a review of phytochemicals with anaesthetic activity. *Molecules*, *22*(8), 1369.

Usman, M., Abari, M. A., & Yusuf, K. J. (2019). Comparative study on the use of lime, lemon and sweet orange leaves powder as natural anaesthesia in catfish (*Clarias gariepinus*) broodstock. *Proceedings of the 34th Annual Conference of The Fisheries Society of Nigeria (FISON*), held at the Law Auditorium, Ebonyi State University *(*CAS CAMPUS*),* on October 28th -November 1st, Abakaliki, Ebonyi State University, Nigeria. pp.51-54.

Wikipedia (2026). New Bussa. Retrieved from https://en.wikipedia.org/wiki/New_Bussa.