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Assessment of Efficacy and Egg Hatchability in African Catfish (*Clarias Gariepinus)* Exposed to Anaesthetic Metomidate

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Abstract: *The efficacy of metomidate as an anaesthetic agent and its effects on egg hatchability of African catfish (Clarias gariepinus) female brood fish were assessed using various concentrations (0.00 – control; 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00 and 12.00mlL^{-1}) of the anaesthetics in triplicates. A total of 110 (10 fish per each concentration) gravid female C. gariepinus (mean length 64.23±0.36cm and mean weight (2780±1.30g) were used for the trial. The results from the study indicated that the induction time (time taken for the fish to get sedated) decreased as the concentrations of metomidate increased, with the longest time (30.12±1.12mins) observed at 0.25mlL^{-1}, while the shortest time (2.74± 0.81min) was recorded in fish exposed to 12.10mlL^{-1} concentration. The recovery time (time taken for the fish to become active) increased as the concentrations of the anaesthetic decreased and the lowest (3.12 ± 0.11mins) was observed in fish exposed to 0.25mlL^{-1} of metomidate and the highest recovery time (28.10±1.18mins) recorded at 12.00 mlL^{-1}. The ideal concentration of metomidate for brood female C. gariepinus is 8.00mlL^{-1}. These results will be useful to the aquaculture industry, where anaesthestics is required for a range of fish handling activities.*

Keywords: *Anaesthesia, Fish, Aquaculture, Metomidate, Stress.*

1. INTRODUCTION

Modern aquaculture practices frequently expose fish to a variety of handling procedures which always involves physical activity [1, 2]. The characteristic struggling exhibited by fishes during capture and handling impairs their physiology and behaviours, which negatively affects performance and survival in the culture medium [3, 4, 5]. Therefore, it is often necessary to immobilize fish before attempting to perform even the simplest task [6]. Conversely, aquaculture, operations such as netting, weighing, grading, stripping and transport can cause primary stress responses in fish, including elevated of plasma cortisol concentrations [7, 8]. This is considered undesirable because it has been associated with depressed growth, distorted reproduction and decreased immune responses [9, 10]. Hence there is the need to mitigate the effects of these stress responses in cultured fish.

One method commonly used to minimize the effect of stress in fish during handling procedures is the use of anaesthetics [11].The utilization of anaesthetics is becoming more popular in recent times due to the intensive nature of aquaculture practices. Anaesthetics are physical or chemical agents that act on an animal by initially inducing a calming effect and subsequently inducing loss of equilibrium, mobility, consciousness and reflex action [12]. The use of an anaesthetic during handling or relocation of fish may not only protect the fish and the fish handler from physical disturbances but also reduce the perception of the stressor and thus the magnitude of the stress response [13]. When choosing an anaesthetic, a number of considerations are important. These include efficiency, cost, availability and ease of use as well as toxicity to fish, humans and the environment [14].The choice may also depend on the nature or type of the experiment and species of fish [15]. The major anaesthetics used or evaluated for aquaculture applications included tricaine methane-sulphonate (MS-222), 2-phenoxyethanol, quinaldinne, benzocaine and metomidate [16].

The anaesthetic metomidate is a rapid acting, water-soluble and non- barbiturate hypnotic, that is being used in several species of fish [17]. Its mode of action include blockage of cortisol synthesis and prevent handling-related glucose increase in fish, with a good safety margin. [18]. Much attention should be paid to careful and proper handling of brood fish, especially during artificial spawning procedures, which subject brood fish to repeated handling during hatchery operations and thus exposed the fish to mechanical injury. Hence, anaesthetics can be used to facilitate the handling of fish during hatchery operations and prevent mechanical injury to fish [19].

A good anaesthetic should induce anaesthesia in 3 to 4 minutes and recovery should occur within 10 minutes after placing the fish in clean water [20]. Therefore, many studies have focused on the effects of metomidate on immobilization and recovery times [21], swimming performance and cardiovascular function [22]. However, few studies have investigated the effects of anaesthetics on egg hatchability in female brood fish during spawning in aquaculture. Therefore, the aim of this study was to determine the optimum concentration of anaesthetic metomidate for *C. gariepinus* over a range of varying concentrations. The effects of application of this anaesthetic on the egg hatchability of this species were also investigated.

2. MATERIALS AND METHODS

2.1 Experimental Fish

A total of 110 gravid female fish ready to spawn brood fish (mean total length 60.23±0.36cm and mean weight 2780.64±1.30g) were obtained from water recirculatory system of water shed Fish farms, Rumuodara, Port Harcourt, Nigeria. The recirculatory culture system consisted of ten 2,000-L rectangular tanks and one 2000-L filtering tank and an aeration system. Culture water is renewed on a regular basis, and replaced every week.

2.2 Anaesthetics

The anaesthetics used, metomidate hydrochloride. Tranquil®, Manufactured by Syndell Aqua Ltd, Canada was purchased off shelf from Gabrovic Agric Ltd, Rumuodara, Port Harcourt, Rivers State, Nigeria.

2.3 Acclimation of Experimental Fish

C. gariepinus brood fish were maintained in eleven 200-L aquaria (10 fish/aquarium) supplied with bore hole water, at a flow rate of 1L/min. The fish were fed with 36% crude protein floating catfish feed daily during the 2-week acclimation period.

2.4 Investigation of anaesthetic effect

The anaesthetic effect of metomidate was investigated at 0.00 – control, 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00 and 12.00 mlL^{-1}, in 30L aquaria. The experimental solution was prepared following the method of Cho and Heath [23]. The mixture after preparation was then stirred with a glass rod for homogenous mixing. Within 10 mins the fish were stocked in 30L aquaria with one brood fish in a tank, with each treatment replicated ten times. Physicochemical parameters such as pH, dissolved oxygen, temperature, ammonia, nitrite and sulfide were monitored in all the experimental tanks, using the methods described by APHA [24].

2.5 Determination of Anaesthetic Efficacy

The anaesthetic efficacy of metomidate in brood *C. gariepinus* was determined based on behavioural responses described by Park *et al.* [25]. Induction time was determined from the time when the fish were stocked in different concentrations of anaesthetized water to the time of the state in which opercula movement ceased. Recovery time was determined from the time when the fish were stocked in clean water to the time of the state in which normal swimming and quick response to visual stimulation recommenced in the fish.

2.6 Evaluation of egg hatchability

After the recovery the fish were kept individually in fresh water and were later induced using ovaprim, after eight hours of incubation. The fish were again exposed to anaesthetics at various concentrations similar to earlier experiment. After the fish has been anaesthetized, they were then removed from water and then stripped. The numbers of eggs were estimated using hand lens. They

were later fertilized with the milt from the sacrificed male and incubated [26]. After 24 hours, the egg hatchability was then determined by estimating the number of hatched fry with the aid of hand lens.

2.7 Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for the significance ($P < 0.05$) of concentration of metomidate on the efficacy (induction and recovery time) and egg hatchability. The differences among groups were analyzed by ANOVA using the SPSS Statistical Package (SPSS 9.0, SPSS Chigago, IL, USA) and comparisons were performed using Tuckey comparative test.

Table 1. *Anaesthetic Stages in Fish*

Stages of Anaesthesia		**Description**
Induction	I	Slow swimming
	II	Slight increase in opercula beat frequency
	III	Loss of equilibrium
	IV	Loss of reflexes and movement
	V	Deep anaesthesia, and fish lies on one side
Recovery	I	Reappearance of opercula movement
	II	Partial recovery of equilibrium
	III	Irregular balance
	IV	Total recovery of equilibrium
	V	Normal swimming

Adapted from Park *et al*.[25]

3. RESULTS

Introduction of metomidate in the experimental tanks did not cause any significant change ($P > 0.05$) in the water quality variables before and after the trial (Table 2). However, it causes a drop in the dissolved oxygen level and slight increase in ammonia concentration. The induction time (time taken for the fish to be anaesthetized) and recovery time (time taken for the fish to become active) was shown in Table 3. The induction time decreased as the concentration of metomidate increased, with the longest time (30.12mins) observed in $0.25mlL^{-1}$, while the shortest time (2.74mins) was recorded in the fish exposed to metomidate at $12.00mlL^{-1}$ concentration. The recovery time increased as the concentration of the anaesthetic decreased and the lowest (3.12mins) was observed at $0.25mlL^{-1}$, while $12.00mlL^{-1}$ had the highest duration of 28.10mins (Table 3). The numbers of eggs obtained from the females were similar, but hatchability decreased significantly as the concentration of metomidate increased (Table 4). Highest percentage hatchability (98.84%) was recorded in the control, while the lowest 76.38% was observed in the fish exposed to $12.00mlL^{-1}$ (Table 4).

Table 2. *Physicochemical Parameters of Water in the Experimental Tanks*

Parameters	**Before Trial (Mean ± SD)**	**After Trial (Mean ± SD)**
pH	6.56 ± 0.39^{a}	6.60 ± 0.40^{a}
DO (mgL^{-1})	5.22 ± 0.78^{a}	4.99 ± 1.16^{a}
Temperature (^{0}C)	28.12 ± 0.71^{a}	28.14 ± 0.22^{a}
Ammonia (mgL^{-1})	0.38 ± 0.01^{a}	0.40 ± 0.11^{a}
Nitrite (mgL^{-1})	0.0039 ± 0.01^{a}	0.0043 ± 0.12^{ab}
Sulfide (mgL^{-1})	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}

***** Values in the same row with the same superscripts are not significantly different ($P > 0.05$)

Table 3. *Induction and Recovery Times in Female C. gariepinus Exposed to Anaesthetic Metomidate*

CONC (Mg/L)	**Induction Time (min)**	**Recovery Time (min)**
0.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
0.25	30.12 ± 1.12^{a}	3.12 ± 0.11^{a}
0.50	28.14 ± 1.08^{a}	5.68 ± 0.81^{a}
0.75	25.61 ± 1.11^{a}	7.14 ± 0.21^{a}
1.00	20.81 ± 1.08^{a}	9.31 ± 0.17^{a}
2.00	15.32 ± 1.12^{a}	13.22 ± 0.86^{b}
4.00	10.14 ± 1.08^{a}	15.72 ± 1.01^{b}

6.00	8.67±0.88[a]	17.66±1.17[b]
8.00	6.24± 0.24[a]	21.71±0.89[b]
10.00	4.98 ± 0.32[a]	25.88±1.14[b]
12.00	2.74 ± 0.81[a]	28.10±1.18[b]

Means within the row with the different superscript are not significantly different (P>0.05)

Table 4. *Effects of Anaesthetics on Hatchability of Female C. gariepinus*

CONC (mg/L)	No. of Eggs	Hatchability	% Hatchability
0.00	164212.500±29225.78[a]	160043.25±37412.23[a]	98.84
0.25	164451.25±28943.92[a]	155300.50±2095.71[a]	97.43
0.50	169652.50±32787.75[a]	143212.75±1402.48[a]	95.41
0.75	163140.00±27672.40[a]	130614.24±1864.95[a]	93.10
1.00	154200.20±28854.05[a]	119699.25±21156.81[a]	90.63
2.00	16126.00±1174.51[a]	116100.64±1121.21[b]	88.86
4.00	158390.35±1.7317[a]	111102.24±1001.02[b]	86.84
6.00	161523.66±1499.74[a]	88770.00±1017.94[b]	85.14
8.00	163913.33±2527.07[a]	78670.60±724.42[b]	84.24
10.00	164916.66±4085.48[a]	54121.66±871.63[b]	80.34
12.00	165911.99±2137.26[a]	41221.64±909.46[b]	76.38

Means within the row with the same superscript are not significant (p>0.05)

4. DISCUSSION

The efficiency of metomidate for brood *C. gariepinus* during stressful procedures such as stripping and establishing a minimum dose necessary for producing an optimal anaesthetic state was examined in this study. However, Wood *et al*. [27], reported that there was no simple definition of efficacy of anaesthetics in fish, but most authors regard efficacy, as the ability to handle fish without much stress [28]. This is highly subjective, dependent on the handler, the fish, the procedure to be carried out and a number of other parameters [29]. Biological and environmental factors must also be considered when administering or conducting studies dealing with efficacy of anaesthetic agents [30]. Biological factors include species, life cycle, age, size and weight, lipid content, body condition and disease status. All these factors according to Congletion [31] affect the metabolic rate and the pharmacokinetics of the anaesthetic compound. Also, environmental factors such as temperature and pH have been reported to affect fish metabolic activity, in addition to changing the uptake across the gills, and therefore increase or decrease the efficacy of an anaesthetic agent [32]. In this work, physiochemical parameters of water before and after the trial were not significantly different (P>0.05), as noted by Velisek and Svobodova [33] in the exposure of rainbow trout, *Oncorhynchus mykiss* to anaesthetic 2-phenoxy ethanol in the laboratory. The minor decline in the dissolved oxygen concentration suggest that metomidate does not cause severe dissolved oxygen problem and hence can be safely used in handling of *C. gariepinus.*

Desirable anaesthetic properties for fin fish were defined by Schoettger and Juline [34], while investigating the efficacy of MS-222 as an anaesthetic for salmonids. The authors reported the desired effects of rapid immobility, rapid recovery and brief immersion time, as might be required during handling, they cite the criterion for effective concentration as that which produces loss of reflex between 3- 4mins. Gomulka *et al*. [35], modified these criteria as a result of differences in behavioural responses of fish anaesthetized with quinaldinne, incorporating the loss of equilibrium and cessation of locomotion in place of loss of reflex. Full anaesthesia was obtain in *C. gariepinus* within 3 minutes at $8mlL^{-1}$ this was similar to the result obtained in channel catfish, *Ictalurus punctatus* using metomidate [36] rainbow trout, *Oncorhyncus mykiss* using benzocaine [37], cod, *Gadus morhua* [38], and Atlantic Salmon, *Salmo salar* [39], full anaesthesia was within 3minutes but at $6.0mlL^{-1}$. According to Di Marco *et al*. [40] small size fish are more susceptible to the effect of anaesthesia than the bigger ones. The recovery time (time taken for the fish to be active after anaesthesia) is very crucial to the performance of the fish, as fish that recover more rapidly have increased metabolic scope for engaging in other activities such as feeding, movement, predator avoidance or preparation for successive stressors . The recovery time obtained in this study increased with the concentration of metomidate. Fish that were exposed to higher concentrations had a longer recovery time than those exposed to lower concentration as was observed in sockeye salmon [41], rainbow trout [42] and white

sturgeon [43].This observation according to Weber *et al*. [44], may be attributable to the effects of the anaesthetics on the physiology of the fish, as behavioural recovery followed similar patterns as physiological recovery. Fish exposed to higher concentrations, yielding deeper levels of anaesthesia exhibited slower behavioural recovery.

Treatment with excessive anaesthesia is very stressful to fish, causing abnormal metabolic rates, which may lead to distorted reproductive functions [45, 46]. The effects of anaesthetics at high concentration on the hatchability of *C. gariepinus* in this study corroborates the findings of Iwama *et al*.[47], on exposure of rainbow trout to five different types of anaesthetics at higher concentrations. Velisek and Svobodova [48] revealed that anaesthetics 2-phenoxy ethanol at higher doses reduced the hatchability of rainbow trout. This also supports the findings of Lardley *et al*.[49] on the exposure of Atlantic salmon, *Salmon geindnes* to higher doses of metomidate, the observed that the hatchability reduced as the concentrations of the anaesthetics increase. This may be due to delay in the on-set of vitellogenic substances which holds the egg as a result of low metabolic rate [50].

5. CONCLUSION

In summary meomidate is a potent anaesthetic for *C. gariepinus* with good induction and recovery times. The minimum desirable concentrtation for anaesthesia which resulted in a total loss of equilibrium within 3 mins, was determined to be at $8mlL^{-1}$. This concentration proved to be effective for catfish of 1.50 to 3.00kg average body weight. Higher doses of metomidate anaesthesia were found to reduce the egg hatchability of female catfish, with the lowest value at $12mlL^{-1}$. Therefore, future investigation should focus on the comparative effects on egg hatchability in catfish using other fish anaesthetics.

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