INDUCTION AND RECOVERY TIMES IN THREE SIZES OF *Clarias gariepinus* EXPOSED TO ANAESTHETIC TRICAINE METHANESULFONATE (MS-222)

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ABSTRACT

The efficacy of tricaine methanesulfonate (MS 222) in three sizes of African catfish (Clarias gariepinus) was investigated. Three sizes: fingerlings (mean length 12. 14 cm ± 3.62 SD; mean weight 18.62 g ± 1.68 SD); juveniles (mean length 25:68 cm ± 3.14 SD, mean weight 354.64 g ± 11.24 SD) and adult (mean length 51.68 cm ± 11.81 SD; mean weight 1102.41 g ± 40.68 SD), were exposed by immersion to different concentrations: 0.00 ml/l (control) 50.00, 100.00, 150.00 and 200.00 ml/l of MS 222 solution. The induction time (time taken for the fish to get anaesthetized) reduced significantly (P < 0.05), with increasing concentrations of the anaesthetics, while the recovery time (time taken for the fish to resume active swimming) increased considerably as the concentrations of MS-222 increased. The induction, recovery time, and survival of C. gariepinus to the anaesthetics were higher in adult size compared to fingerlings and juveniles. Hence MS-222 can be used to sedate all sizes of this fish species with optimum dosage at 50 ml/l for fingerlings and juveniles and 150.0 ml/l for adults.

Keywords: aquaculture, tranquilizer, stress, sedative effect

INTRODUCTION

Aquaculture production in Nigeria is becoming more intensive, with corresponding increase in different methods to enhance fish production in the culture medium. These production processes as crucial as they are elicit some stress related responses in fish. Moreover, applications of anaestethics, according to Akinrotimi *et al.* (2013a) have been reported to reduce the incidence of stress in teleost fish.

Generally, anaesthetics in fish are mainly taken up by the gills, although some enters through the skin (Ferreira *et al.*, 1984). The gills are ideal sites for transfer of small lipophilic, neutral molecules as they receive virtually all the cardiac output. They are composed of very thin layers of lipid rich cells that facilitate exchange of material with the immediate environment (Meinertz *et al.*, 1991). The rate of uptake and hence the induction time, should be proportional to the products of branchial surface area, ventilation rate, blood flow through the gills and anaesthetic concentration (Hill and Foster, 2004).

The induction time of fish to anaesthetics is affected by a number of factors such as species, size, age, previous history, sexual maturity, gill surface area, environmental factors, and the nature of anaesthetics (Ross and Ross, 2008). Not all of the research findings on anaesthetic application and efficacy in aquaculture agree with each other and the effects of some of the factors seems to be quite species dependent. For this reason, Akinrotimi *et al.* (2013b) suggests that when working with a new species or new anaesthetics, the anaesthetic agent should be tested on a small number of fish, so as to work out the correct anaesthetic doses as it relates to the induction and recovery times.

Induction time in fish anaesthetics can be described as the time taken for the fish to become completely immobilized. Investigators in fish response to anaesthetics have used different methods to assess the degree of anaesthesia depth in fish (Oikawa *et al.*, 1994; Detar and Mattingly, 2004). The criteria employed include activities such as reactivity to stimuli, equilibrium (righting reflex), jaw tone, muscle tone, respiratory and heart beat rates. Broad stages of anaesthesia include sedation, loss of equilibrium and anaesthesia, with each stage subdivided into light and deep plane (Stamper, 2007). Depending on the species, drug and dosage, some stage components are not noticeable (Stetter, 2001).

Recovery from anaesthesia occurs when the anaesthetic stops interacting with the active sites. This can occur by way of removal or inactivation of the drug. Which of these that has the greatest impact depends on the type of anaesthetics and the species of

fish involved (Ross and Ross, 2008). This usually takes place when the fish is placed in drug- free water, it excretes drugs or their metabolites via the gills and presumable accessing respiratory organs; some elimination also occurs through the kidney and skin (AVMA, 2007). In mammals, anaesthetics gases tend to be relatively inert and are generally expelled unaltered (Barreto *et al.,* 2007). In contrast, intravenous anaesthetics tend to be metabolized to an active form, which allows recovery and are then excreted. However, in fish anaesthetics are removed from fish body through metabolism (Pramod *et al.*, 2010).

Induction and recovery times of fish to MS – 222, have been investigated in a number of species such as channel catfish, *Ictalurus punctatus* (Welker *et al.,* 2007); steel head trout, *Oncorhynchus mykiss (*Pirohen and Schreck, 2003); common carp, *Cyprinus carpio* (Hikasa *et al*., 1986) Zebra fish, *Danio rerio* (Collymore *et al*., 2014); black sea bass, *Centropritis striata* (King *et al.,* 2005); rainbow trout, *Salmo gairdneri* (Kleinow *et al.,* 1986*;* Laidley *et al.,* 1988) but information on the induction and recovery time of MS – 222 in *C. gariepinus* a popular culturable fish in Nigeria and sub- Saharan Africa is scanty, thus necessitating the need to carry out this work.

This study therefore, examined the responses (induction and recovery time) in three sizes of *C. gariepinus* to MS-222 application, so that fish farmers and aquaculturists can utilize this information in anaesthetics application in *C. gariepinus.*

MATERIALS AND METHODS

The work was conducted at the Genetic Family Testing Unit Hatchery in African Regional Aquaculture Center, (ARAC), Aluu, Port Harcourt, Rivers State, Nigeria. *Clarias gariepinus,* numbering 60 each of fingerlings (mean length 12. 14 cm ± 3.62 SD; mean weight 18.62 g ± 1.68 SD); juveniles (mean length 25.68 cm ± 3.14 SD; mean weight 354.64 g ± 11.24 SD); and adult (mean length 51.68 cm ± 11.81 SD; mean weight 1102.41 g ± 40.68 SD); were collected from ARAC production tanks adjacent to the hatchery. The fish were later acclimated to the hatchery conditions for a period of three days (Gabriel *et al.,* 2004). During this period the fish were fed daily with ARAC feed (40.0% CP) at 5% body and the water in the holding tanks were renewed on daily basis.

Tricaine methanesulfonate, MS – 222 is a white crystalline solid (Manufactured by Sigma Chemical A 5040, St Louis, MO, USA). The stock solution was prepared according to the methods of Gullian and Villanueva (2009). The aliquots of the stock solution were then used to achieve the experimental concentrations of 0.00 ml/l (control), 50.00, 100.00, 150.00 and 200.00 ml/l. In each size, four fish were exposed to each concentration in triplicates.

The induction time (time taken for the fish to get sedated and become immobilized) was monitored and recorded with stop watch, following the description of Coyle *et al. (*2004) in Table 1. After the anaesthesia, fish was removed individually using a scoop net and transferred into a clean water tank without anaesthetics. Recovery time (time taken for the fish to resume normal swimming) was equally recorded following various stages described by Coyle *et al.* (2004) Table 1. During the study, water quality parameters such as water temperature, pH, dissolved oxygen, nitrite, ammonia and sulphide were monitored in the experimental tanks (APHA 1998).

Data from the study were collated and analyzed with two way analysis of variance (ANOVA), while differences among means if any, were detected by Tukey's multiple comparison test (Zar, 1996).

RESULTS

The water quality parameters evaluated during the trial in the experimental tanks of the exposed fish, were within the same range (Table 2). However, significant reductions ($P < 0.05$) were observed in the values of dissolved oxygen and ammonia at 200 m/l concentration of the anaesthetic (Table 1). The induction time in the fingerlings size of the fish reduced significantly as the level of the concentrations of the anaesthetics increased with the lowest value (70.65 ± 8.20) at 200.0 ml/l and the highest (121.64 ± 11.41) recorded at 50.0ml/l of the anaesthetics, while the induction times in the juvenile size of the exposed fish reduced steadily with increasing concentrations with the lowest value (81.46 ± 10.115) also at 200ml/l^{-1} and the highest (133.33 ± 25.325) recorded at 50ml/l concentration of the anaesthetic (Table 4). In the adult size of *C. gariepinus* (Table 5), the highest value of induction time (278.33 ± 9.07) was observed at 50.0ml/l concentration of the anaesthetic while the lowest induction time (153.33 ± 12.66) at 200.00 ml/l. The recovery time in the juvenile fish (Table 4), increased steadily as the concentrations of the anaesthetic rises, with the highest (305.33 ± 11.015) at 200.0 ml/l and the lowest (0.00 ± 0. 00) in the control. Similar trends were equally observed in adult of *C. gariepinus* with the 200.00 ml/l of MS-222 concentration having the highest (340.34 ± 16.10) and the lowest (0.00 ± 0.00) in the control (Table 5).

The survival of the size of the exposed reduced significantly ($P < 0.05$) with in creasing concentrations of the anaesthetics (Table 3 and 4) reverse was the case in the adult size where 100.0% survival was recorded in all concentrations except at 200.00 ml/l where 96.40 ± 1.21% survival was recorded (Table 5).

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, 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

Adopted from (Coyle *et al*., 2004)

Table 2: Water quality parameters in experimental tanks of *C. gariepinus* exposed to MS-222 (Mean ± SD)

	Concentrations (ml/l)				
Parameters	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Temperature (°C)	28.90± 0.20[a]	28.70± 0.45[a]	29.30 ± 0.75[a]	28.83± 0.13[a]	**29.86± 050[a]**
pH	6.91± 0.96[a]	6.88± 0.10[a]	6.981± 0.11[a]	6.92± 0.12[a]	**6.86±0.12[a]**
Dissolved Oxygen (mg/l)	6. 89± 0.40[a]	6.84±2.11[a]	6.89± 0.18[a]	6.88± 0.31[a]	**6.93± 0.37[a]**
Nitrite (mg/l)	0.0047± 0.02[a]	0.053± 0.02[a]	0.005± 0.01[a]	0.007± 0.01[a]	**4.87±1.12[b]**
Ammonia (mg/l)	0.28± 0.04[a]	0.28± 0.04[a]	0.30± 0.05[a]	0.31± 0.03[a]	**0.007± 0.01[a]**
Sulphide (mg/l)	**0.04± 0.01[a]**	**0.04± 0.02[a]**	**0.04± 0.02[a]**	**0.43± 0.052[a]**	**0.43± 6.05[a]**

Mean within the row with different superscripts are significant ($P < 0.05$)

Table 3: Induction, recovery and survival of fingerlings of *C. gariepinus* exposed to MS– 222 (Mean ± SD)

	Concentration (ml/l)				
Parameters	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Induction time (s)	0.00 ± 0.00[a]	121.64 ± 11.41[c]	110.80	78.64 ± 9.81[b]	**70.65 ± 8.2[b]**
Recovery time (s)	0.00 ± 0.00[a]	158.82 ± 10.21	179.61 ± 11.11[c]	199.81 ± 14.21[c]	**236.11 ± 21.68[d]**
Survival %	**100 ± 0.00[a]**	**100.00 ± 0.01[c]**	**96.46 ± 8.11[b]**	**96.46 [b] ± 11.22**	**90.40 ± 6.81**

Mean within the row with different superscripts are significant ($P < 0.05$)

Table 4: Induction, recovery and survival of juvenile *C. gariepinus* exposed to MSS-222 (Mean ± SD)

	Concentrations (ml/l)				
Parameters	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Induction time (s)	0.00± 0.00[a]	133.33± 25.32[c]	123.66±34.99[ab]	83.00± 11.26[b]	**81.46± 90.11[b]**
Recovery time (s)	0.00± 0.00[a]	173.42± 15.22[b]	242.00±4.58[ab]	268.16± 12.01[c]	**305.33±11.01[c]**
Survival (%)	**100.00± 0.0[c]**	**100.00±0.1[c]**	**96.40± 6.51[ab]**	**90.80±9.87[ab]**	**80.42± 1.21[a]**

Mean within the row with different superscripts are significant ($P < 0.05$)

Table 5: Induction, recovery and survival of adult *C. gariepinus* exposed to MSS-222 (Mean ± SD)

	Concentrations (ml/l)				
Parameters	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Induction time (s)	0.00± 0.00[a]	278.33± 9.07 [d]	225.67±9.07[c]	184.67± 13.[05ab]	**153.33± 12.66**
Recovery time (s)	0.00± 0.00 [a]	298.66± 6.71 [b]	325.66±12.50[ab]	315.33± 3.05 [ab]	**340.34±16.10**
Survival (%)	**100.00± 0.0 [a]**	**100.00±0.1 [a]**	**100.1± 0.1[a]**	**100.00± 0.1[ab]**	**96.40± 1.21 [a]**

Mean within the row with different superscripts are significant (P<0.05

DISCUSSION

High commercial demand for catfish coupled with dwindling fish catch from the wild have stimulated a new dimension of intensity in breeding, rearing and transportation of *C. gariepinus* in the country. Akinrotimi *et al.* (2013b) reported that the intensive culture of fish in aquaculture may impose a stress related responses in the cultured fish. Hence there is the need for the use of anti stress agents such as anaesthetics in modern day aquaculture operations.

The general trend in this study observed in all the three sizes of *C. gariepinus* exposed to anaesthetic MS – 222, indicated that the induction times reduced significantly as the concentrations of the anaesthetics increased. This is in line with the findings of Gullian and Villanueva (2009) in two sizes of cobia (*Rachycentron canadium*); King *et al.* (2005) in black sea bass (*Centropritsts striata*); Heo and Shin (2010) in Crucian carp (*Carassius carassius*); and Akinrotimi *et al*. (2013b) in two species of mullets, (*Liza falcipinnis* and *Liza grandisquamis*). This may be due to the fact that at higher concentrations of the anaesthetics in solution, more of the anaesthetic diffused and enter the system of the fish via the gills and hence results in lower induction times (Opiyo *et al.,* 2013; Akinrotimi *et al.,* 2014).

On the other hand, longer recover time with increasing dosage of the anaesthetics were observed in all the sizes of *C. gariepinus* exposed to anaesthetic MS-222. This results aggress with that of Velisek *et al*. (2005) in common carp (*Cyprinus carpio*) and that of Park *et al*. (2008) in kelp grouper (*Epinephelus bruneus)* but contradict that of Mylonass *et al.* (2005) who reported a shorter recovery times with increasing concentrations of anaesthetics in European sea bass (*Dicentrarchus labarax*) and gilthead sea bream *(Sparus aurata)* exposed to clove oil and 2 – phenoxy ethanol. Also Collymore *et al*. (2014*)* reported the same trend in two sizes of Senegalese sole (*Solea senegalensis*) treated with MS – 222. This may be due to the fact that with the high doses, the fish is not in contact with the anaesthetic for a long time, which would allow faster recovery (Burka *et al.,* 1997). Conversely, the longer recovery time observed in this study may be as a result of MS – 222 persistent natures on the surface of the gill, which inadvertently increase recovery time of anaesthetic in fish (Castro *et al., 2008;* Weber *et al*., 2009; Marino *et al.,* 2010).

The survival of the exposed fish to MS – 222 in all the sizes indicated that there was higher survival in adult than the other size groups; this result is in tandem with that of Akinrotimi *et al.* (2013b) in exposure of two species of mullets to clove seed extracts. They noted that the bigger sized fish (juveniles) survived more than fingerlings exposed to the same concentration of anaesthetics, this may be due to the fact that bigger fish have a small gill surface area in relation to body size and consequently a small area for anaesthetics absorption compare to small sizes (Opiyo *et al*., 2013).

CONCLUSION

The relevance and importance of anaesthetics application in fisheries and aquaculture is well established, their administration and efficacy in catfish culture especially *C.gariepinus* has been limited. This study demonstrated that MS – 222 is efficacious in sedating three sizes of *C. gariepnus.* It can be concluded that the anaesthetic MS – 222 was effective and had a good margin of safety especially in adult sizes, with optimum dosage of 50 ml/l for fingerlings and juveniles, while 150 ml/l is appropriate for adults.

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