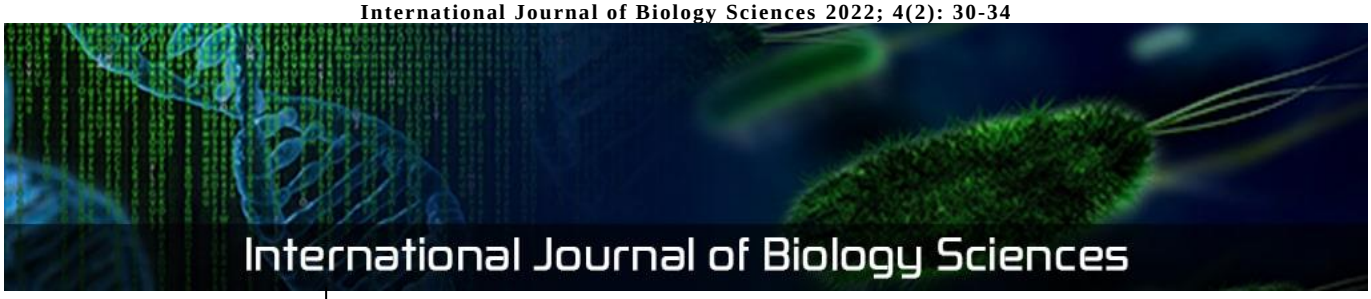

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Assessing the efficacy of tricaine methanesulfonate (MS-222) on sedation of Nile tilapia (*Oreochromis niloticus*) fingerlings during transportation

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Abstract
The efficacy of the anaesthetic agent tricaine methanesulfonate (MS-222) was evaluated in the Nile tilapia (*Oreochromis niloticus*) fingerlings during transportation. MS 222 is an anaesthetic agent that is approved for use in the United States, Canada, the United Kingdom and other European countries. MS-222 is used to suppress the central and peripheral nervous systems of fish to immobilize them for transportation and experimental purposes to avoid mortality and injury.
In the present study, juvenile Nile tilapia of length 7-10 cm were subjected to different concentrations (0-20 mg/L and 100-500 mg/L) of MS-222. *O. niloticus* subjected to 0 and 20 mg/L (trial experiment) of MS-222 in this study did not reach stage IV anaesthesia (Total loss of equilibrium) within 1 hour, indicating that none of the fish exposed to these concentrations of MS-222 was induced. At 20 mg/L, the buffered MS-222 slowly rendered the fingerlings physiologically inactive (calm) for a prolonged time (> 1 h) and the fish were almost instantly reactivated in untreated water. Sedation time for the fingerlings decreased significantly (p<0.05) with an increase in the anaesthetic concentration (100 – 500 mg/L). MS-222 at 100-500 mg/L successfully induced sedation in Nile tilapia fingerlings, but the optimal concentration was established at 250 mg/L at which sedation time was the same as recovery time. The extremely high concentration of 10,000 mg/L in the trial experiment was lethal; sedation was almost instantaneous (< 5 s) but recovery failed. Lethal doses may be applied to kill fish humanely for post mortem studies in places where animal rights are in force. In general, the higher the concentration, the shorter the sedation time and the longer the recovery time. The required amount of concentration of an anaesthetic agent needed to induce fish varies with the concentration of the anaesthetic agent. The times of sedation and recovery of *O. niloticus* at various concentrations of MS-222 in this study were found to differ significantly.
Overall, the stress responses vary widely between species, therefore, it is important to screen effective dosages of anaesthetic agents for each cultured species. The definition of efficacy concerning anaesthetics is more or less subjective. The present study showed that MS-222 is efficient in anaesthetizing *O. niloticus*, an important freshwater fish species in the Tono reservoir.

Keywords: MS-222, anaesthetic, transportation, sedation, recovery, fingerlings

1. Introduction
With the development of commercial fish farming practices, the transportation of fish from one place to another has become an inevitable part of the aquaculture industry. Handling fish especially out of their natural habitat for transportation had always been difficult and fish are subjected to various stress during the period of capture, handling and packaging procedures (Maule *et al*., 1988; Nikinmaa *et al*., 1983; Specker and Schreck, 1980) [15, 20, 25]. Struggling fishes have been documented to have damaging effects on their physiology and behaviour, and may lead to death during successive transportation (Ross and ROSS, 1999) [21]. Nile tilapia is an important freshwater fish which is of high demand in both local and foreign markets. Tilapia fish is mainly exposed to constant stress during transportation which has a negative physiological effects on the wellbeing of the fish.

The main problem encountered in the transportation is the accumulation of toxic gases like carbon dioxide and ammonia in the transport medium which are mainly attributed to fish metabolic activities (McFARLAND, 1959; Sindhu and Ramachandran, 2015) [16, 22]. Chow *et al*. (1994) [4] opined that the mortality rate increases when the temperature of the transport medium increases (Chow *et al*., 1994) [4]. For this The main problem encountered in the transportation is the accumulation of toxic gases like carbon dioxide and ammonia in the transport medium which are mainly attributed to fish metabolic activities (McFARLAND, 1959; Sindhu and Ramachandran, 2015) [16, 22]. Chow *et al*. (1994) [4] opined that the reason, the use of anaesthetics has become important to prevent injury to the fish, which may also attenuate the physiological stress response.

The uses of anaesthetics or tranquillizing agents play a major role in the live fish transportation for fish culture. Anaesthetic is a biological state induced by an external agent, which results in a partial or complete loss of sensation or loss of voluntary neuromotor control through chemical means (Wedemeyer *et al*., 1990) [32]. A variety of anaesthetics have proven their ability to minimize the negative effects on the physiology and behaviour of fish due to stress. Examples of some of the anaesthetics employed in fish transportation are lidocaine, benzocaine, metonidate, tricaine methanesulphonate, 2-phenoxyethanol, quinidine sulphate etc. (Burka *et al*., 1997) [1].

Among these broad spectra of anaesthetics, the tricaine methanesulfonate, $C_9H_{11}O_2N + CH_3SO_3H$, known also as MS-222, ethyl 3-aminobenzoate methanesulfonic acid, tricaine mesilate, metacaine, methanesulfonate appears to be the most widely used anaesthetic and tranquillizing agent for poikilotherms (such as fish) worldwide since its introduction in 1967 (Topic Popovic *et al*., 2012) [28]. MS-222 is currently recognized and approved by the United States Food and Drug Administration and many other European countries for use in food fish production (Carter *et al*., 2011; Daniel, 2009) [2, 6]. Various researchers have evaluated the efficacy of MS-222 in many fish species for different operations including; selection of fish, sorting, grading, weight/length measurements, sampling, labelling, transportation, broodstock anaesthesia, gamete collection, physiological data collection, blood sampling, health monitoring, vaccination, etc. (Chambel *et al*., 2015; Cotter and Rodnick, 2006; Lewbart *et al*., 2005; McKim *et al*., 1999; Nikinmaa *et al*., 1983; Späth and Schweickert, 1977; Wagner *et al*., 2003; Weber *et al*., 2009) [3, 5, 20, 17, 20, 24, 29, 31, 12]. MS-222 acts systemically when absorbed through the gills and skin of fish in an anaesthetics bath. The potency of MS-222 is conditioned by environmental factors (temperature, oxygen content, pH, hardness and salinity of water), biological factors (age, sex, size, weight, lipid content), fish species and density of biomass (Ross and Ross, 1999; Topic Popovic *et al*., 2012) [21, 28]. An effective dose of a successful anaesthetic hugely depends on the differences among fish species, size/length, maturity, and between individual fish (King *et al*., 2005) [11].

Given the growing concern and interest in the culturing of Nile tilapia, the present study aims to investigate the effective dose of MS-222 in sedating *O. niloticus* fingerlings in the field for transportation.

2. Materials and Methods

2.1 Study Area and Fish Species

Juvenile Nile tilapia (*O. niloticus)* were seined in a pond from the Tono Fish Farm in Navrongo, Upper East Region of Ghana, and transported to an experimental aquarium. The fish were held in a net cage inside an irrigation canal for 2 h to recover from the stress of seining. The fish had not been fed for up to 3 days before the capture to allow them to evacuate their bowels which is necessary to avoid faecal contamination of the experimental water.

2.2 Anaesthetic Agent

The anaesthetic agent, tricaine methanesulphonate (MS-222), was used and prepared by dissolving 10g in deionised water and buffered with Sodium bicarbonate ($NaHCO_3$). The buffering was necessary because unbuffered MS-222 is known to induce stress to fish.

2.3 Experimental Design

A stock solution of the MS-222 was prepared by dissolving 10g in deionised water and buffered with sodium bicarbonate ($NaHCO_3$) in a ratio of 1:1 (i.e. 10g MS-222: 10g $NaHCO_3$). The selected test concentrations of MS-222 were 100, 200, 300, 400 and 500 mg/L based on the study of Weber *et al*. (2009) [31] (Weber *et al*., 2009) [31] and following the recommendations of Hicks (1998) (Hicks, 1989).

A preliminary study was conducted using 0, 20, 100 and 10,000mg/L of the buffered MS-222 solution to assess the full range of anaesthesia (slight sedation to death) for Nile tilapia fingerlings.

Five 3 L capacity transparent plastic containers were each filled with 1 L of water from the irrigation canal where the fingerlings were held in a net cage. Four fingerlings (7 – 10 cm TL) were transferred from the net cage inside the canal and placed in MS-222 test baths starting from 100 to 500 mg/L at intervals of 30 minutes.

The time of sedation was recorded for each fingerling until all the fingerlings in each test bath were sedated. Sedation in this study referred to fish that showed a slight loss to external stimuli indicated by a slight decrease in opercular and fin movement, which corresponds to stage I of anaesthesia (light sedation) (Marking and Meyer, 1985) [14]. Sedated fish were removed from the MS-222 baths one after the other into a plastic bowl containing clean water from the canal to recover. Recovery was indicated by restoration of normal opercular and fin movements. The time for each sedated fish to recover was also recorded. The total length (TL) of the recovered fish was measured and the fish was returned to the pond. None of the doses used caused mortality in the fish. The experimental treatments were replicated three times.

3. Statistical Analysis

Sedation and recovery times were calculated for the various sizes of the fingerlings and the concentrations of the anaesthetic baths. The effects were tested statistically using one-way ANOVA at a 95% level of confidence. SPSS package (Windows version 20.0) was used to perform the analysis. Graphs were drawn to illustrate the response of the test concentrations using an Excel spreadsheet (Windows version 13.0)

Table 1: Stages of induction of anaesthesia and recovery in Nile tilapia (*Oreochromis niloticus)*

Stages	Description	General behaviour response of fish
0	Normal	Reactive to external stimuli, opercular rate and muscle normal
I	Light sedation	Slightly lost to external stimuli and the opercular rate decreased
II	Deep sedation	Total loss of reactivity to all but strong external stimuli slightly decreases in opercular rate and equilibrium normal
III	Partial loss of equilibrium	Partial loss of muscle tone, swimming erratic, the opercular rate increased and reactive to only strong tactile
IV	Total loss of equilibrium	Total loss of equilibrium and muscle tone, slow but regular opercular rate
V	Medullary collapse	Respiratory movement ceases

4. Results and Discussions

In the trial, fish exposed to 0 mg/L (untreated water) were not sedated at all as expected. At the concentration of 20 mg/L, only slight anaesthesia described was evident within 1 h of exposure, and the fish remained calm till the study was terminated. From 100 mg/L various stages of advanced anaesthesia were induced at increasing rapidity until mortality occurred in less than 5 s at the highest concentration of 10,000 mg/L. No initial hypersensitivity (or hyperactivity) to the anaesthetic was exhibited by the fish at the low concentrations of 20 and 200 mg/L. However, from the concentration of 300 mg/L hyperactivity occurred in increasing intensity. Fish exposed to 20 mg/L MS-222 concentration remained calm or physiologically inactive in Stage I anaesthesia for a long time (> 1 h) and did not reach Stage IV anaesthesia before the termination of the study. This concentration could be suitable for transportation of live fingerlings of *O. niloticus* on long journeys exceeding 1 h.

An inverse relationship was established between the concentration of the anaesthetic MS-222 and the time of sedation in 7-10 cm *O. niloticus* fingerlings (Fig. 1). Sedation time decreased significantly ((P<0.05)) as the concentration was increased. The longest sedation time (397.50±190 seconds) occurred when the fish were exposed to the concentration of 100 mg/L while the shortest time (41.50±30 seconds) occurred at the concentration of 500 mg/L Sedation in this study was defined as a slight decrease in the movement of the opercula (breathing) and movement of the fins (swimming) following exposure of the fish to the external stimulus (MS-222). Recovery time however increased significantly (P<0.05) with increasing concentrations of the anaesthetic (Fig. 1). Recovery in this study was defined as the restoration of normal movement of the opercula (breathing) and fins (swimming). The threshold or optimal concentration of the buffered anaesthetic MS-222 in this study is theoretically established at 250 mg/L, which corresponds to the point of intersection of the sedation curve and the recovery curve (Fig.1). At the optimal concentration, the sedation time is theoretically the same as the recovery time (approximately 160 s) in this study.

Sedation time for 7-10 cm fingerlings decreased significantly (p<0.05) with an increase in the anaesthetic concentration. This is consistent with previous studies by different researchers (Gullian and Villanueva, 2009; Heo and Shin, 2010; Hseu *et al*., 1998; Mercy *et al*., 2013; Mylonas *et al*., 2005; Wajsbrot *et al*., 1991; Weber *et al*., 2009) [7, 8, 10, 18, 31]. In the present study, MS-222 at 20 mg/L has been recommended for transportation of *O. niloticus* fingerlings, similar to the results made by Lin *et al*. (2012) in *Culter mongolicus* juvenile (20 – 40 mg/L) (Lin *et al*., 2012), but lower than those obtained for temperate species such as *Salmo gairdneri*, *Cyprinus carpio* and *Pimephales promelas* (Ross and ROSS, 1999; Sylvester and Holland, 1982) [21]. *O. niloticus* subjected to 0 and 20 mg/L of MS-222 in this study did not reach stage IV anaesthesia (Total loss of equilibrium) within one hour, indicating that none of the fish exposed to these concentrations of MS-222 was induced. Similar results were reported by Sladky *et al*. (2001) in *Piaractus brachypomus* (Sladky *et al*., 2001) and Mercy *et al.* (2013) [18] in *Puntius denisonii* (Mercy *et al*., 2013) [31].

Altogether, the required concentration of an anaesthetic agent required to sedate fish varies with the concentration of chemical required to bring them to a given level of anaesthesia, their tolerance of a given chemical and their recovery time (Summerfelt, 1990) [26]. The times of sedation and recovery of *O. niloticus* at various concentrations of MS-222 in this study differed significantly (P<0.05).

Stress responses vary widely between species, therefore, it is often necessary to screen effective dosages of anaesthetic agents for each cultured species (Ross and ROSS, 1999) [21]. The definition of efficacy concerning anaesthetics is more or less subjective (Marking and Meyer, 1985) [14]. The present study demonstrated that MS-222 is efficient in anaesthetizing *O. niloticus*, an important freshwater fish species in the Tono reservoir.

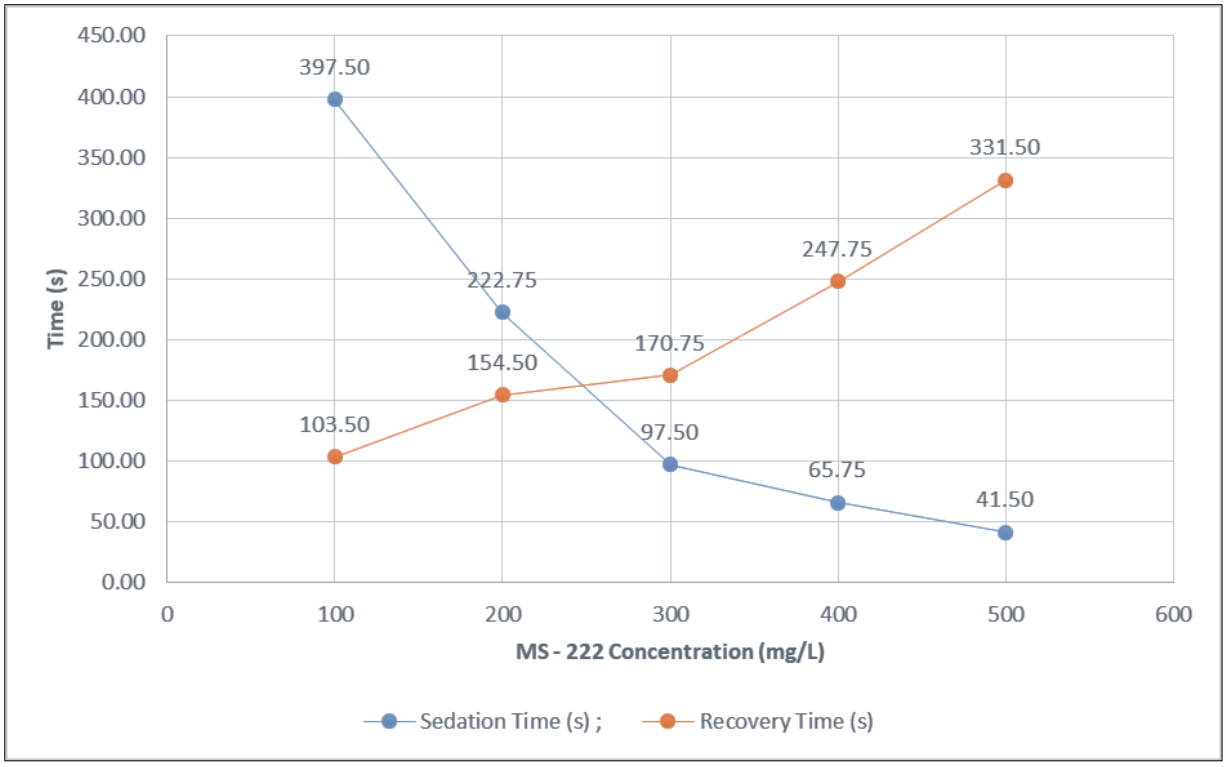

Fig 1; The effects of the anaesthetic MS-222 concentrations on sedation and recovery in Nile tilapia (*O. niloticus*) fingerlings.

5. Conclusion

MS-222 induced various stages of anaesthesia in 7-10 cm fingerlings of *O. niloticus*. At 20 mg/L, the buffered MS-222 slowly rendered the fingerlings physiologically inactive (calm) for a prolonged time (> 1 h) and the fish were almost instantly reactivated in untreated water. This dosage is recommended for live fish transportation over a long distance.

The extremely high concentration of 10,000 mg/L was lethal: sedation was almost instantaneous (< 5 s) but recovery failed. Lethal doses may be applied to kill fish humanely for post mortem studies in places where animal rights are in force.

In general, the higher the concentration, the shorter the sedation time and the longer the recovery time. MS-222 at 100-500 mg/L successfully induced sedation in Nile tilapia fingerlings, but the optimal concentration was established at 250 mg/L at which sedation time was the same as recovery time.

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