A comparative analysis of the anaesthetic effect of sodium bicarbonate (NaHCO₃) on male and female three spotted tilapia (Oreochromis andersonii)

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ABSTRACT
The anaesthetic effect of sodium bicarbonate (NaHCO₃) on male and female three spotted tilapia (Oreochromis andersonii) was investigated. A total of 210 broodstock O. andersonii (weight: 160 ± 10 g) of different sexes were immersed in NaHCO₃ at concentrations of 0, 5, 10, 15, 20, 25 and 30.0 g/L in triplicates. The induction and recovery times were monitored using a stopwatch. The induction and recovery times at all stages of anaesthesia for both sexes were significantly higher at concentrations ≥10 g/L. The induction times were significantly longer in the male fish than in the female at concentrations of 15 g/L for stages 1, 20 g/L for stages 1 and 2, 25 g/L for all the stages and 30 g/L for stage 2. The recovery time for male fish was significantly longer than that of the female fish at concentrations of 10 g/L for stages 2 and 3, 15 g/L for stage 1 and 20–30 g/L for stages 1 and 2. Overall, the induction and recovery times of the three spotted tilapia broodstock exposed to different concentrations of NaHCO₃ could be affected by sex. Therefore, 30 and 25 g/L of NaHCO₃ concentrations are recommended for use in male and female broodstock, respectively.

1. Introduction
In modern aquaculture, the use of anaesthetics is very common during fish handling, especially when conducting routine procedures such as weighing, vaccination, blood sampling, tagging, experimental surgery and veterinary procedures considering the fact that these activities cause stress in fish (Sneddon 2012; Gabriel et al. 2020; Hasimuna et al. 2021). This is to avoid stressing the fish which is a major predisposing factor to disease outbreaks in fish (Hasimuna et al. 2020b; Maulu et al. 2021; Kord et al. 2022). Anaesthetics are a group of pharmaceutical preparations which are reversible, controlled drug-induced intoxication of the central nervous system (CNS) which does not enable the patient to perceive or recall noxious or painful stimuli (Hall et al. 2001; Donohue et al. 2013). The anaesthetics to be used must be effective, safe and inexpensive (Velisek et al. 2007). The most commonly used anaesthetics in aquaculture are MS-222, benzocaine, quinaldine sulphate, clove oil and powder, eugenol, 2-phenoxyethanol, metomidate, aqui-s™ and carbon dioxide (CO₂; Coyle et al. 2004; Hasimuna et al. 2020a; Liu et al. 2021; Rairat et al. 2021). It is important to avoid stress in broodstock as it is a major factor that is capable of adversely affecting eggs’ hatchability and fry survival rates in females (Okanlawon 2010). Recently, there has been much emphasis on the need for safe aquaculture practices, which include the use of environmentally friendly anaesthetics that do not put the health of fish and that of consumers at risk (Hasimuna et al. 2020a). The use of sodium bicarbonate (NaHCO₃) in this study is thought to fulfil the above requirement. Also known as baking soda, NaHCO₃ is a white coloured substance commonly used for baking in many countries (Githukia et al. 2016). It releases CO₂ when dissolved in water which is one of the recommended gases to be used for anaesthetic purposes in cold, cool and warm water fishes (Avillanosa and Caipang 2019), and this enables the handling of large numbers of fish without harming them. NaHCO₃ has been used as an anaesthetic in a number of fish species such as common carp (Cyprinus carpio) in both cold and warm water conditions and on African Catfish (Clarias gariepinus) juvenile (Altun et al. 2009; Githukia et al. 2016). In both studies, animals with higher concentrations of NaHCO₃ attained full anaesthesia faster compared to those exposed to lower concentrations. Due to anaesthetic properties, NaHCO₃ has been recommended for use because of its low cost, wide availability, safety to human beings and low environmental impact (Altun et al. 2009).

Different anaesthetics have been discovered and their mode of operation documented (Coyle et al. 2004). Numerous authors (Hall et al. 2001; Coyle et al. 2004; Velisek et al. 2007; Altun et al. 2009; Sneddon 2012; Zahl et al. 2012; Donohue et al. 2013; Githukia et al. 2016; Küçük and Çoban 2016;
Hasimuna et al. (2020a) have described the effects of different anaesthetic on different species under different conditions. However, all these studies concentrated on the anaesthetic effects of different substances on different fish species without investigating their effect on the different sexes of fish. Just as female and male fish are different physically, so is physiological and genetic makeup different (Šimková et al. 2015). A study by Bhatta et al. (2012) on the differences between male and female growth and sexual maturation in tilapia (Oreochromis mossambicus) showed that during critical periods of development, growth patterns of tilapia vary between males and females as they increase in age and sexual maturation. Other studies including Šimková et al. (2015) and Madenjian et al. (2016) showed differences in fish’s physical and physiological attributes between males and females. Differences observed include length in gynogens, weight of intestines and that males exceeded females in terms of polychlorinated biphenyl concentration due to the difference in physiological activities (higher rate of energy expenditure), stemming from higher resting metabolic rate (or standard metabolic rate) and physical activities (higher swimming activity). Given the differences between female and male fish in the studies above, it is possible that there might be differences in the way each sex may respond to anaesthetics. When fish is not fully anaesthetized it may recover quicker before the intended purpose is achieved. The fish in this situation will start flip-flopping during handling thereby causing bruises which are a suitable substrate for bacterial and fungal infection (Margeirsson et al. 2006; Maulu et al. 2021).

The three spotted tilapia (O. andersonii) is among the most important indigenous fish species commonly cultivated in Zambia (Maulu et al. 2019; Hasimuna et al. 2020a). The species is currently undergoing a genetic improvement programme by the Zambian government in collaboration with the World fish Center with the aim to improve its production. Therefore, it is likely to be a major aquaculture species not only in Zambia but also in the neighbouring countries.

The objective of this study was to determine the right concentration of NaHCO3 to be administered to either female or male broodstock to avoid the stress that may be caused due to partial anaesthesia during any handling procedures to ensure successful hatchery operations. Knowledge of the differences in responding to anaesthesia by different sexes of fish is important as different sexes are handled differently during breeding.

2 Methods and materials

2.1 Experimental fish

A total of 210 broodstock O. andersonii of different sexes with an average weight of 160±10 g were obtained from an outdoor concrete pond at Solwezi Aquaculture Research Station in Solwezi, Zambia. A seine net was used to catch the fish after the pond was partially drained. The fish were grouped into two based on sex and each group had 90 specimens. The male tilapia has a simple papilla, while the female has a slightly wider organ with a wide opening to allow eggs to eject during mating (Fuentes-Silva et al. 2013). The structure of the anal papilla was the main feature used to separate males from females and the opening of the oviduct is distinguishable in the female and is absent in males (Fuentes-Silva et al. 2013).

2.3 Test chemical

NaHCO3, commonly known as Chapa Mandashi baking powder (NaHCO3) was procured from the local supermarket. Its principal contents are cereal filler, phosphate of sodium and the main active ingredient bicarbonate of soda (Hasimuna et al. 2020a). Chapa Mandashi is very common on the Zambian market and is used mainly for baking purposes.

2.4 Experimental design

A randomized complete block design with sex as the blocking factor was used in this study. Each fish sex group had 90 specimens (male and female fish) and, the fish were fasted for 24-hours prior to the experiment to ensure gut evacuation (Hasimuna et al. 2020a, 2021). The selected O. andersonii broodstock of different sexes were exposed to different concentrations of NaHCO3 by bath immersion according to the description of Githukia et al. (2016). The experiment was carried out in 20 L transparent plastic containers in triplicate (Altun et al. 2009) and a total of six containers corresponding to the treatment groups were used. The six concentrations of NaHCO3 used in this study were 0, 5, 10, 15, 20 and 30 g/L, of which 0 g/L concentration served as a control (Githukia et al. 2016). For each concentration, five (5) fish of the same sex were exposed at the same time and the average times were recorded for induction and recovery, all replicated thrice without re-using the solution. Therefore, a total of 180 fish were used: 90 male fish and 90 female fish were used in this study. The concentration levels were based on previous studies done on other species, for example, on Cyprinus carpio (Altun et al. 2009) and Oreochromis macrochir (Hasimuna et al. 2020a). The test solutions were prepared by adding NaHCO3 to the plastic containers filled with water and stirring the mixture continuously until complete dissolution as described by Avillanosa and Caipang (2019). The source of water used in the experiment was the outdoor fish pond where the specimen was collected. Test fish were randomly assigned to each transparent plastic tank (20 L) with each tank containing 5 specimens of the same sex and observed for three different stages of induction and recovery times (Table 1) as described by Githukia et al. (2016) and Hasimuna et al. (2020a). The fish were considered fully

| Table 1. Different stages of anaesthesia and description considered for Greenhead tilapia (O. andersonii). |
|---|---|---|
| Anaesthetic stages | Induction | Recovery |
| I | Loss of equilibrium | No body movements but opercula movements start |
| II | Loss of entire body movements but continued opercula movement | Regular opercula movements and body movements start |
| III | Same as in stage II but opercula movement ceases | Equilibrium regained with a pre-anaesthetic appearance |

Note: Adapted from Hasimuna et al. (2019) and Githukia et al. (2016).
anaesthetized when the entire body movements including the operculum movements were completely lost (Githukia et al. 2016). After the fish were fully anaesthetized, they were moved to tanks with clean fresh water to allow them to recover (Hasimuna et al. 2020a, 2021).

2.5 Water quality analysis

Water quality parameters such as temperature, oxygen, nitrite, ammonia and pH were determined before administering the test chemical using a water test kit (SUNPU TEST ZL 2010 2 0,681,385. X – BEIJING SUNPU BIOCHEM. TECH. CO., LTD).

2.6 Statistical analysis

The data on induction and recovery times were subjected to the test of normality and homogeneity of variance using Shapiro–Wilk and Levene’s test, respectively. Since the collected data met the assumptions of analysis of variance (ANOVA), which allows for use of a parametric test, a two-way ANOVA was performed. This was necessary because we were interested in observing the anaesthetic effect of different concentrations of NaHCO₃ on the different sexes of O. andersonii broodstock and the interactions of the two factors (Patel 2015). Turkey’s honestly significant difference test was used to ascertain where differences among the means existed. All the tests were performed using SPSS software version 23.0 and differences were considered significant at $p < 0.05$.

3. Results

Induction times for male and female O. andersonii broodstock exposed to different concentrations of NaHCO₃ are shown in Table 2. Generally, males took between 0.39 and 7.85 min and females took between 0.25 and 7.44 to attain stage 3 at different concentrations of NaHCO₃. Anaesthesia of the fish was faster with the increase in the concentration of NaHCO₃ for both sexes. As shown in the table, the fish got anaesthetized only with concentrations ≥ 10 g/L of bicarbonate ($P < 0.05$). Furthermore, the induction times were significantly longer in the male fish with NaHCO₃ concentrations of 15 g/L at stage 1, 20 g/L at stages 1 and 2, 25 g/L at all stages and 30 g/L for stage 2 compared with the female fish. Therefore, the sex of fish had a significant effect on the induction time of fish ($P < 0.05$).

Recovery times for male and female O. andersonii broodstock exposed to different concentrations of NaHCO₃ are shown in Table 3. Besides, no adverse behavioural effect was observed with the fish. As indicated, recovery time varied from 0.44 to 7.78 min for males and 0.45 to 7.95 min for females. The concentration of NaHCO₃ significantly ($P < 0.05$) affected the recovery time from anaesthesia with fish (both male and female) exposed to a higher concentration taking longer to recover than those exposed to a lower concentration. The recovery time for male fish was significantly longer ($P < 0.05$) than that of the female fish at concentrations of 10 g/L for stages 2 and 3, 15 g/L for stage 1 and 20–30 g/L for stages 1 and 2.

During the experiment and 3 days of the post-recovery period, no mortality was recorded in any concentration of NaHCO₃. All the fish recovered and resumed normal feeding and swimming within the first few hours of removal from the NaHCO₃ solution.

The water quality parameters such as temperature, oxygen, ammonia and pH were determined in the water containing NaHCO₃ just before immersing the fish and the values are presented in Table 4.

### Table 2. Induction time for male and female O. andersonii.

| Concentration (g/L) | Stage 1 | Stage 2 | Stage 3 | Stage 1 | Stage 2 | Stage 3 |
|---------------------|---------|---------|---------|---------|---------|---------|
| 0                   | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* |
| 5                   | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* | 0.00 ± 0.00* |
| 10                  | 1.71 ± 0.39d | 2.49 ± 0.07f | 7.85 ± 0.32c | 1.15 ± 0.03d | 2.42 ± 0.06f | 7.44 ± 0.12c |
| 15                  | 1.36 ± 0.06e* | 2.18 ± 0.08d | 3.35 ± 0.07f | 1.10 ± 0.03e* | 2.07 ± 0.04d | 3.25 ± 0.09d |
| 20                  | 1.21 ± 0.03c* | 2.23 ± 0.06e* | 3.46 ± 0.06e* | 0.50 ± 0.09e* | 1.35 ± 0.16d | 3.26 ± 0.13e |
| 25                  | 0.51 ± 0.06b* | 1.46 ± 0.03c* | 2.52 ± 0.04e* | 0.35 ± 0.03b* | 0.52 ± 0.05c* | 3.09 ± 0.04e* |
| 30                  | 0.39 ± 0.07b | 1.08 ± 0.04b* | 2.26 ± 0.04b | 0.25 ± 0.04b | 0.43 ± 0.08b | 2.26 ± 0.05b |

Notes: Data are mean values and standard deviation (±SD) in minutes; values in the same column (stages) with different alphabetical superscripts are significantly different ($P < 0.05$), and means across sex groups with a star superscript in the same row (concentrations) were significantly different within the same stage ($P < 0.05$).

### Table 3. Recovery time for male and female O. andersonii.

| Concentration (g/L) | Stage 1 | Stage 2 | Stage 3 | Stage 1 | Stage 2 | Stage 3 |
|---------------------|---------|---------|---------|---------|---------|---------|
| 0                   | 0.44 ± 0.12b | 1.53 ± 0.05b | 4.30 ± 0.07b | 0.45 ± 0.04b | 2.06 ± 0.05b | 2.71 ± 0.28b |
| 5                   | 1.23 ± 0.02c* | 1.92 ± 0.45b | 4.41 ± 0.10c | 1.16 ± 0.02c* | 1.46 ± 0.09c | 4.09 ± 0.46c |
| 10                  | 3.06 ± 0.41d | 4.74 ± 0.49f | 5.39 ± 0.08d | 2.09 ± 0.04d | 2.94 ± 0.43f | 4.99 ± 0.55d |
| 15                  | 3.40 ± 0.08d* | 5.26 ± 0.05b* | 6.35 ± 0.38c* | 2.54 ± 0.03d* | 4.45 ± 0.05b* | 6.27 ± 0.20c |
| 20                  | 3.89 ± 0.27c* | 5.46 ± 0.15f | 7.78 ± 0.46f | 2.17 ± 0.08c* | 4.64 ± 0.39d* | 7.95 ± 0.39c |

Notes: Data are mean values and standard deviation (±SD) in minutes; values in the same column (stages) with different alphabetical superscripts were significantly different ($P < 0.05$), and means across sex groups with a star superscript in the same row (concentrations) were significantly different within the same stage ($P < 0.05$).
The present study has demonstrated that NaHCO₃ is ideal as an anaesthetic material (Hall et al. 2001; Coyle et al. 2004; Altun et al. 2009; Donohue et al. 2013; Hasimuna et al. 2020a). Several anaesthetics have been used and the results are promising (Hall et al. 2001; Coyle et al. 2004; Velisek et al. 2007; Altun et al. 2009; Sneddon 2012; Zahl et al. 2012; Donohue et al. 2013; Githukia et al. 2016; Küçük and Çoban 2016; Hasimuna et al. 2020a). The present study has demonstrated that NaHCO₃ is ideal as an effective anaesthetic for both male and female O. andersonii broodstock. Determining an appropriate anaesthetic is dependent on how effective it is in sedating the fish to allow for ease of handling (Hasimuna et al. 2020a). NaHCO₃ has been successfully used as an anaesthetic material in diverse fish species such as Common Carp (Cyprinus carpio) (Altun et al. 2009), Nile tilapia (Oreochromis niloticus) (Opiyo et al. 2013), yellow seahorse (Hippocampus kuda) (Pawar et al. 2012), African catfish (C. gariepinus) (Githukia et al. 2016), Red Tilapia (Oreochromis niloticus) (Avillanosa and Caipang 2019), Mozambique tilapia (Oreochromis mossambicus) (Gabriel et al. 2020) and Greenhead tilapia (Oreochromis macrochir) (Hasimuna et al. 2020a, 2021).

Before immersing the specimen in the NaHCO₃-containing water, some water quality parameters including temperature, oxygen, ammonia and pH were determined, and the results are shown in Table 4. According to Martins et al. (2016), the temperature of the water when administering an anaesthetic reagent could significantly affect the induction and recovery time for the fish. The lower temperature has been associated with prolonged induction and recovery times and the opposite is true (Aydin et al. 2015). Anaesthetics reduce the rate of oxygen usage by the fish due to a reduction in the metabolic processes of the fish (Neiffer and Stamper 2009). This was also supported by the results of a recent study conducted by Gabriel et al. (2020). Due to this, when anaesthesia is successful there will be more oxygen available in the water (Neiffer and Stamper 2009). Nitrogenous compounds (e.g., ammonia and nitrite) can damage or alter gill morphology and these changes can have several impacts in that they may affect the uptake and clearance of inhalant anaesthetics (Neiffer and Stamper 2009). The pH of the anaesthetic solutions influences their efficacy; specifically, acidic pH generally reduces efficacy because the increased ionization interferes with absorption (Neiffer and Stamper 2009). For example, in a study by Gabriel et al. (2020), the addition of NaHCO₃ to the water raised the pH, a reduction in acidity. Furthermore, the concentration of NaHCO₃ did not appear to affect the pH. Overall, studies that have investigated the effect of water quality parameters on the anaesthetic effect of NaHCO₃ are limited. Particularly, there is a need to investigate how the water quality parameters could change at different points during the experiment throughout the experiment.

In this study, induction time for both males and females was significantly affected by the concentration as it was decreasing with increasing concentration and the opposite is true for recovery. The reason for this may be because of the increase in CO₂ production as the concentration increases thereby reducing the availability of oxygen. These results are in agreement with those reported by Hasimuna et al. (2020a) and Avillanosa and Caipang (2019) on the anaesthetic effect of NaHCO₃ on O. macrochir and in O. niloticus, respectively. However, it is important to note that NaHCO₃ anaesthesia may be associated with negative health impacts in fish, although this requires investigation. According to Altun et al. (2009), some potential health risks to fish include hypercapnia (increased systemic CO₂) which may lead to fish mortalities and hyperactivity which may impact fish health (Altun et al. 2009). Future studies should critically investigate the physiological effect of NaHCO₃ in fish for better application.

The present study also consents with the observations made by Hasimuna et al. (2020a) that the observed differences in recovery time are due to having more active constituents of the anaesthetic containing CO₂ accumulating in the CNS of the fish with the increase in the concentration. Probably this is what might have led to the fish becoming inactive through the three stages of anaesthesia, which can be explained in terms of suppressing the activity of the CNS to a substantial degree as opposed to lower concentrations thereby subsequently extending the recovery time of the fish from anaesthesia (Githukia et al. 2016). The variability that may exist in the ingredients of NaHCO₃ as baking soda could be a major limitation to the use of this chemical as an anaesthetic in aquaculture. Therefore, the ingredients and concentrations of the NaHCO₃ should always be checked to ensure they are within standard levels prepared for baking as any deviation could lead to inconsistent results.

This study showed that the induction time varied from 0.39 to 7.85 min for males and from 0.25 to 7.44 for females from stage 1 to stage 3. The recovery time varied from 0.44 to 7.78 min for males and from 0.45 to 7.95 min for females. In a study done by Hasimuna et al. (2020a) on the anaesthetic effect of NaHCO₃ on O. macrochir, the induction and recovery time ranged from 0.70 to 11.20 min and 0.85 to 22.22 min, respectively. This study has a high induction and recovery range as opposed to the current study. This may have been due to the difference in species used.

In the study done by Opiyo et al. (2013) on O. niloticus exposed to 15–50 g/L, the induction time ranged from 0.79 to 5.02 min, which was dependent on the concentration and the bodyweight of the fish. In another study done by Githukia et al. (2016) on the anaesthetic effect of NaHCO₃ on C. gariepinus weighing between 10 and 40 g and the concentration ranging from 10 to 50 g/L, the induction time was found to range from 0.63 to 5.44 min. The induction time in the current study seemingly has a higher range compared to other studies. The reason for this may have been due to the fact that most of these studies have been carried out on

| No. | Parameter       | Mean ± SD       |
|-----|----------------|----------------|
| 1   | Temperature    | 22.5 ± 2°C     |
| 2   | Dissolved oxygen | 6.3 ± 0.5 mg/L |
| 3   | pH             | 7.5 ± 0.2      |
| 4   | Ammonia        | <0.1 mg/L      |

4. Discussion

Studies on anaesthetics have increased in recent years due to the need to minimize stress on the fish in aquaculture (Hall et al. 2001; Coyle et al. 2004; Altun et al. 2009; Donohue et al. 2013; Hasimuna et al. 2020a, 2021).
juvenile fish as opposed to this study which focused on broodstock (adult fish). Besides, the current study looked at one age group with different sexes contrary to that of Opiyo et al. (2013) which focused on fish of the same sex with different age groups against different concentrations of NaHCO₃.

According to the results shown, there was a significant difference between the male and female induction and recovery time at some concentrations. Induction time for male fish was longer than that of female fish. Furthermore, males showed a longer recovery time compared to females. These observed differences may be related to the faster metabolic rate of male fish which may have led to faster absorption of sodium carbonate thereby increasing the induction and recovery times (Madenjian et al. 2016). This study has shown that the anaesthetic concentration of male broodstock should be higher than that of females to avoid the stress that may be caused due to partial anaesthesia during any handling procedures. When fish is not fully anaesthetized it may recover quicker before the intended purpose is achieved. The fish in this situation will start flip-flopping during handling thereby causing bruises which are a suitable substrate for bacterial and fungal infection (Margeirsson et al. 2006). It is important to understand the right concentration to administer to broodstock to ensure successful hatchery operations.

Anaesthetics are very vital in modern-day aquaculture as they help to reduce stress which may make fish more susceptible to diseases as a result of resistance during handling which weakens their immune system (Altun et al. 2009; Githukia et al. 2016). Using NaHCO₃ as an anaesthetic in fish has proved to be relatively cheap due to its affordability and ease of application such that even small-scale farmers can afford it (Opiyo et al. 2013; Avillanosa and Caipang 2019). NaHCO₃ is believed to be safe for use in animals entering the human food chain without any withdrawal period. This is probably because NaHCO₃ acts mainly through CO₂ suggesting that there are no residues left in the animal body, although this requires further investigation to guarantee public health safety. The effectiveness of NaHCO₃ is affected by the weight of the fish and the concentration of the anaesthetic administered (Githukia et al. 2016; Hasimuna et al. 2020a, 2021).

5. Conclusion

The current study has demonstrated that NaHCO₃ can effectively be used as a cheaper anaesthetic on *O. andersonii* broodstock during routine handling procedures at fish farms and research stations. The results obtained in the present study showed that induction time for both males and females decreased with an increase in NaHCO₃ concentration and recovery time increased with increasing concentration. Furthermore, the study showed that the sex of fish has a significant effect on the induction and recovery time of *O. andersonii*. From the results obtained in this study, 25–30 g/L NaHCO₃ concentration has been recommended for use in female and male broodstock, respectively. No mortalities were observed in experimental fish during and after the experiment. This implies that NaHCO₃ can be used as an alternative for expensive anaesthetics during handling especially in developing countries such as Zambia, as it is cheaper and readily available.

More research has been recommended to be done on the same species with respect to factors such as temperature, pH, sizes and age groups since they may have an effect on the induction and recovery time of the fish when NaHCO₃ is used as an anaesthetic material.

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