Utilization of Sodium Bicarbonate as Anesthetic During Routine Husbandry Activities in Ornamental Fish

C M A Caipang¹,²*, J E Deocampo Jr.¹,², R V Pakingking Jr.³, I Suharman⁴, J T Fenol¹, F B Onayan¹

¹ Department of Biology, College of Liberal Arts, Sciences, and Education, University of San Agustin, Iloilo City 5000, Philippines
² Center for Chemical Biology and Biotechnology, University of San Agustin, Iloilo City 5000, Philippines
³ Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC/AQD), Tigbauan, Iloilo 5021, Philippines
⁴ Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Riau, Pekanbaru-Riau, Indonesia

*cmacaipang@yahoo.com

Abstract. The tropical ornamental fish industry requires the stock to be reared in captivity before being sold; thus, exposing them to various handling- and transport-related stressors. A number of commercially available anesthetics are used during fish husbandry procedures, but these products are expensive and not readily available to small-scale ornamental fish operators. The use of sodium bicarbonate as an inexpensive anesthetic during routine husbandry activities in molly, Poecilia sp., a freshwater ornamental fish was assessed in terms of its effect on sedation, recovery and survival post-exposure in three independent experimental runs. Juvenile molly were exposed to sodium bicarbonate at a concentration of 100 g L⁻¹ and the time to sedation and recovery were monitored. It took longer for the fish to be fully sedated than their recovery. Survival of the fish a week post-exposure ranged 40-60%. Simulated transport of fish for 6 hrs in water containing 1 g L⁻¹ of sodium bicarbonate showed survival ranging 70-100% a week after transport. These findings demonstrated that sodium bicarbonate could be potentially used as a low-cost anesthetic during handling and short-term transport of ornamental fish. Future studies shall focus on discovering the underlying physiological mechanisms in fish following sedation with this chemical.

1. Introduction

Fish are routinely handled when carrying out certain husbandry operations including sorting, weighing, and treatment for diseases. These activities are stressful to fish and to reduce the effects of these stressors, there are a number of chemicals and anesthetics that are routinely administered to fish or added to their holding containers prior to handling [1, 2].

Anesthetic agents are used to immobilize the fish prior to doing any husbandry procedure on them (Ross and Ross, 2008). Fish can be anesthetized in several ways including light sedation to mitigate stress during handling and when performing non-invasive procedures to general anesthesia that
prevents pain during surgery [3, 4]. When added to the water in the holding container, the fish gills absorb the anesthetic agent then transported to the central nervous system via the arterial blood [2].

An ideal anesthetic should induce anesthesia rapidly with minimal hyperactivity or stress in fish. It must be easy to administer and should maintain the condition of the animal in the desired state. Recovery should be rapid, as soon as the animal is removed from the water with the anesthetic. The anesthetic should also be effective at low doses, and must have a wide margin of safety. Moreover, anesthetics must not produce lasting physiological effects in fish, should have high solubility in both fresh- and salt water and must be safe for humans [3, 5]. Gilderhus and Marking [6] suggested the criteria of a good anesthetic agent for fish: full anesthesia must be attained within 3 min, recovery should take place in less than 10 min, and all anesthetized fish must survive.

Commercially available anesthetics include tricaine methane sulphonate (MS-222), benzocaine and quinaldine, however, these are expensive and not readily available to most fish growers and fish hobbyists. In order to address issues on cost and availability of anesthetics, there have been research initiatives of developing low-cost anesthetics using crude plant extracts [7, 8]. For example, clove oil have been shown to be effective as an anesthetic at low concentrations [9, 10]. Crude water extracts of tobacco also showed potential as an anesthetic agent in fish [8]. Aside from using plant extracts, another chemical that is readily available to be used as anesthetic is sodium bicarbonate or baking soda. The efficacy of the chemical as an anesthetic has been tested in a number of fish species including cichlids, salmonids, carps and some marine fish with encouraging results [11-18].

While most of the studies geared towards developing low-cost and highly effective anesthetics for aquaculture fish species, such efforts have lagged behind in the case of the ornamental fish industry. Although ornamental fish keeping is the second largest hobby in the world with at least 2,000 species of ornamental fish being traded annually with 65% of the production coming from Asia [19, 20], proper handling and welfare of ornamental fish are sometimes overlooked. Hence, this study aimed to test the efficacy of using sodium bicarbonate or baking soda as an inexpensive anesthetic that can be used for routine husbandry activities and short-distance transport of ornamental fish, using molly, *Poecilia* sp. as the test species. This species of ornamental fish is popular among first time and amateur fish hobbyists and are subjected to various husbandry procedures.

2. Materials and Methods

2.1. Fish and experimental set-up

This study was carried out at the Biology laboratory of the College of Liberal Arts, Sciences, and Education, University of San Agustin in March, 2021. Mixed sexes of molly (Gold Dust variety) with average weight of 0.71 ± 0.30 g were purchased from a local ornamental fish supplier and reared in the laboratory for the experiment. Prior to the experiment, the fish were acclimatized for 15 days in 100 L rectangular glass aquaria (150 fish/tank) supplied with dechlorinated freshwater and adequately aerated (dissolved oxygen: > 5.0 ppm, ammonia-N: < 0.1 ppm, pH: 7.6 – 8.2 and water temperature: 27-29°C). The fish were fed twice daily with commercial ornamental fish feed pellets until satiation.

Commercial grade sodium bicarbonate was used in this study. For the experiment, a concentration of 100 g L$^{-1}$ was used following the procedures described by Opiyo et al. [13] and Avillanosa and Caipang [16]. This concentration was utilized because earlier tests using concentrations ranging 10 - 50 g L$^{-1}$ did not result in full sedation of the fish within 3 min.

After acclimation for 15 days, ten (10) 1-L plastic aquaria were filled with water. The water quality parameters in all aquaria were more of less similar. In each aquarium, 100 g L$^{-1}$ of sodium bicarbonate was added and this set of aquaria was designated as the anaesthesia group. The water was stirred continuously until complete dissolution of sodium bicarbonate. The experiment commenced 30 min after complete dissolution of the sodium bicarbonate [16, 18]. Another set consisting of ten (10) 1-L plastic aquaria was filled with water and aerated. This set of aquaria consisted the recovery group for the fish. Three independent experimental runs were done for this experiment. Water quality parameters in all experimental runs were recorded and no significant differences in the values were
observed. Handling of fish and all procedures that were used in the study adhered to the principles of proper fish handling and welfare set by institutional and national competent authorities.

2.2. Induction to anesthesia and recovery
The anesthetic response of molly to sodium bicarbonate was determined by placing one fish in each anesthesia aquarium and observed until it reached stage III anesthesia [21, 22]. A fish at stage III anesthesia exhibits complete loss of equilibrium and loss of body and cessation of opercular movement (Table 1). Once stage III was reached, the fish was immediately scooped out of the anesthesia aquarium and rapidly transferred to the recovery aquarium. The fish was observed until it has attained stage II recovery. Trained laboratory technicians monitored and recorded the time for each fish to reach stage III anesthesia and the time for it to attain stage II recovery. At the end of each experimental run, all fish were transferred to a 10-L glass aquarium and provided with adequate aeration. The survival rate and other post-exposure behavior of the fish following anesthesia were monitored for one week.

Table 1. Stages of anesthesia and recovery in fish.

| Stages of anesthesia | Description                                      |
|----------------------|--------------------------------------------------|
| I                    | Fish starts to exhibit loss of equilibrium        |
| II                   | Low of body movement; slow opercular movement    |
| III                  | Cessation of body and opercular movement          |

| Stages of recovery   | Description                                      |
|----------------------|--------------------------------------------------|
| I                    | No body and opercular movement                   |
| II                   | Body movement starts to normalize; regain opercular movement |
| III                  | Full body equilibrium                             |

Adapted from Iwama et al. (1989) and Iwama and Ackerman (1994)

2.3. Simulated transport of ornamental fish
A simulated transport experiment was done to determine the efficacy of sodium bicarbonate as a sedative in ornamental fish. Simulations were done using an open container transport system. Ten (10) ornamental fish were placed in a 5-L plastic container and added with 1 g L⁻¹ sodium bicarbonate as sedative. Adequate aeration was provided during the simulation experiment. The container was placed on a slow-moving shaker to simulate movement during transport. Water pH was monitored every hour and after the 6th hour, all fish were placed in a new 5-L plastic container for recovery. Survival of the fish was determined one week after the simulated transport. There were three independent experimental runs for the simulated transport in ornamental fish.
2.4. Statistical analyses
Means and standard deviations for time to anesthesia, recovery of the fish and survival were obtained in the three experimental runs. The time to induction and recovery in each experimental run were compared using Student’s t test for independent samples. All statistical computations were performed at the 0.05 probability level using the statistical package of Microsoft Excel 2010.

3. Results and Discussion
The time to induction of anesthesia (stage III) and recovery (stage II) of molly following immersion in water containing 100 g L\(^{-1}\) of sodium bicarbonate is shown in Fig. 1. The average time to induction of anesthesia in molly was 83.2 s (median: 67 s), 104.6 s (median: 105 s) and 103.2 s (median: 103 s) in experimental runs 1, 2 and 3, respectively. The average time for recovery of the fish was 47 s (median: 41 s) in Experiment 1, 62.5 s (median: 61 s) in Experiment 2 and 53 s (median: 53 s) in Experiment 3. There were no significant differences in the time to induction of anesthesia and recovery in the three experimental runs. The fish took a longer time (p < 0.05) to be anesthetized than their recovery following exposure to sodium bicarbonate at a dose of 100 g L\(^{-1}\) with water temperature of 27-29\(^\circ\)C and pH of 8.4. Monitoring of the fish for one week after exposure to sodium bicarbonate showed survival ranging 40-60% (60% in Trial 1, 50% in Trial 2 and 40% in Trial 3) in the three experimental runs.

![Figure 1](image-url)

**Figure 1.** Induction of anesthesia and recovery of molly following immersion in freshwater containing 100 g L\(^{-1}\) of sodium bicarbonate. Three independent trials were conducted. An asterisk (*) on top of the column bar indicates significant difference (p < 0.05) for the particular experimental run. N= 10

A simulated transport of the fish for 6 h in an open container transport system added with 1 g L\(^{-1}\) sodium bicarbonate showed no mortality after the duration of transport in the three experimental runs (Table 2). Water pH during the simulated transport ranged 7.9 – 8.4 and no significant differences were observed in the pH levels in experimental runs and time of exposure. Moreover, monitoring of the fish a week after simulated transport showed survival of 90, 100 and 70% in Trial 1, 2 and 3, respectively.
Table 2. Water pH during simulated transport and survival of fish one week post-recovery.

| Trial | 0-h | 1-h | 2-h | 3-h | 4-h | 5-h | 6-h | Survival (%) after one week |
|-------|-----|-----|-----|-----|-----|-----|-----|----------------------------|
| 1     | 8.1 | 7.9 | 8.2 | 8.2 | 8.3 | 8.3 | 8.4 | 90.0                       |
| 2     | 8.0 | 8.0 | 8.0 | 8.0 | 8.1 | 8.1 | 8.2 | 100.0                      |
| 3     | 8.1 | 8.0 | 8.2 | 8.3 | 8.3 | 8.4 | 8.4 | 70.0                       |

The induction of anesthesia in molly progressed in stages, similar to what was observed in the juveniles of red tilapia hybrids [16]. Upon immersion in water with the anesthetic, the fish exhibited rapid and erratic swimming behaviour, followed by gradual loss of equilibrium and slower movements of the opercula. Upon induction of stage III anesthesia, the fish was not moving, no opercular movement and was oriented on one side of its body (Fig. 2). Upon transfer to the recovery aquarium, the fish slowly regained equilibrium and the opercula and pectoral fins start to move slowly (Fig. 3). There was full recovery of the fish, when it exhibited the characteristics in the pre-anesthetic stage (stage III).

Figure 2. Molly showing Stage III anesthesia
In the present study, we demonstrated that sodium bicarbonate can be used as an effective anesthetic for routine husbandry operations and short-term transport of ornamental fish using molly as the model species. These results add further to existing data on the benefits of using this low-cost sedative in cichlids [13, 16-18], in salmonids, carps and catfish [11-12, 15] and even in some marine fish species [14]. To the best of our knowledge, this is the first report to use sodium bicarbonate as an anesthetic in an ornamental fish species. Mollies appeared to have a positive response to this substance when used as an anesthetic as no mortality were observed immediately after recovery and during the short-term simulated transport. There was complete knock-out of the fish when they were immersed in freshwater containing 100 g L\(^{-1}\) sodium bicarbonate, and because of that they can be easily handled to perform any husbandry-related procedures without causing any unwarranted stress [23].

A good anesthetic candidate for fish must induce full sedation within 3 min and must attain complete recovery in 5 min [24, 25]. During the time of full sedation, any husbandry-related activities can be carried out without compromising the health and survival of the fish. Similar to previous studies in cichlids [13, 16, 18], the use of sodium bicarbonate as anesthetic in mollies satisfied these requirements.

Oberg et al. [14] stressed that the differences in the anesthetic response of fish to sodium bicarbonate may differ across species and these are dependent on the biological requirements of a particular species of fish, especially the availability of oxygen. We observed that a higher dose (100 g L\(^{-1}\)) is needed to achieve this purpose and using a lower dose (50 g L\(^{-1}\)) resulted in a much longer time (< 3 min) to attain complete anesthesia. In contrast, lower dose (50 g L\(^{-1}\)) of sodium bicarbonate are sufficient to trigger complete sedation in cichlids [16, 18]. It is apparent that using low concentration (≤ 50 g L\(^{-1}\)) of sodium bicarbonate may not be suitable for use during anaesthetization in mollies.

Furthermore, we demonstrated that a dilute concentration of sodium bicarbonate can be potentially utilized as an anesthetic during transport of ornamental fish for a short period of time. This was evident from the results that we obtained in a simulated transport experiment of the fish by immersing them in freshwater that contained 1 g L\(^{-1}\) of sodium bicarbonate for 6 h. The efficacy of sodium bicarbonate to induce anesthesia in fish during transport is likely due to its ability to liberate carbon dioxide in the water [18]. When sodium bicarbonate is dissolved in water, it produces carbon dioxide gas. This gas, in turn, has an anesthetic effect in fish and is utilized to sedate fish during transport or to mass handling of fish [26]. The loss of consciousness in molly following sodium bicarbonate exposure...
could be due to the presence of high levels of carbon dioxide in fish blood (hypercapnia) and low blood pH, which subsequently reduces oxygen transport to the brain [27]. Earlier studies in cichlids showed similar effects [13, 16, 18]. In addition, the anesthetic response in fish could be the result of a pH-controlled carbon dioxide release from sodium bicarbonate when dissolved in water [11]. Such mechanism might be true of ornamental fish, because we have noticed that the pH of the water did not significantly alter during the 6 h of simulated transport.

Although carbon dioxide can be used to immobilize fish, it also poses some inherent disadvantages. These include: difficulty in controlling the concentration of this gas in water, high oxygen levels in the holding tanks, and the disruption in the concentration of blood gases and acid–base balance in fish [28-30]. Any of these preceding conditions could have taken place in mollies after exposure to sodium bicarbonate either during anesthetization or during simulated transport because we observed mortalities within one week during recovery. This contradicts the results in cichlids that demonstrated 100% survival up to one week after exposure to sodium bicarbonate [16, 18]. Contrasting findings could be due to physiological differences in how fish respond to carbon dioxide production as a consequence of adding sodium bicarbonate. Cichlids are able to tolerate low oxygen as well as high carbon dioxide levels in the water, and this could likely explain why there was no mortality in cichlids during recovery.

When fish recovers in a sodium bicarbonate-free water, the carbon dioxide in the blood reduces through diffusion, the blood pH elevates, and the fish gain its equilibrium and normal swimming [29]. We have observed this in mollies during their recovery after sodium bicarbonate exposure, and the time to recovery is much shorter than the time to anesthesia. The shorter recovery time in mollies is mostly likely related to the size of the fish: smaller fish had shorter recovery time following sodium bicarbonate anesthetization. This was also demonstrated in previous studies involving cichlids [13, 16, 18]. Shorter recovery times in smaller-sized fish is possibly due to their faster ventilation and metabolism [31].

Our study provides baseline information on how to better utilize this chemical as anesthetic for ornamental fish and to further elucidate physiological mechanisms in fish following exposure to sodium bicarbonate. Until a sustainable and inexpensive fish anesthetic is readily available to the ornamental fish industry, sodium bicarbonate may be adopted for use as a suitable anesthetic agent. Careful attention is needed in determining the optimum dose to be administered, which is based on the physiological requirements of the particular fish species.

4. Conclusion
Aqueous solution of sodium bicarbonate at a concentration of 100 g L\(^{-1}\) can be utilized as a safe, low-cost, and readily available anesthetic agent for molly, a popular ornamental fish. The effectiveness of this substance as an anesthetic ensures the carrying out of short-term husbandry activities without causing severe stress to the fish. In addition, using very dilute concentration of sodium bicarbonate (1 g L\(^{-1}\)) during transport for a short period of time (≤ 6 h) is also effective in ensuring high survival rates in fish after transport. Future studies shall focus in obtaining sufficient information on the physiological effects of this substance and the mechanisms that take place in molly and other species of ornamental fish.

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