Efficacy of sodium bicarbonate as anaesthetic for yellow seahorse, *Hippocampus kuda* (Bleeker, 1852)

Pawar H.B., Ingle B.S. and Sreepada R.A
Aquaculture Laboratory, CSIR-National Institute of Oceanography, Council of Scientific & Industrial Research (CSIR), Dona Paula, Goa–403 004 (India).

Received 12 June 2013; accepted 10 July 2013

Abstract

In the present investigation anaesthetic efficacy of sodium bicarbonate on yellow seahorse *Hippocampus kuda* was studied. Captive bred two years old (163.2±10.2 mm height and 12.22±1.94 g weight) yellow seahorses were exposed to 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40 and 50 g L⁻¹ concentrations of sodium bicarbonate. It was observed that very high dose of sodium bicarbonate (more than 30 g L⁻¹) was needed to completely anaesthetise adult seahorses. The onset of individual phases of anaesthesia and recovery times depended significantly on the concentration of the anaesthetic used (P<0.05). An inverse exponential relationship was observed between sodium bicarbonate concentrations and induction time, whereas exponential relationships were observed between concentrations and recovery times for sodium bicarbonate. Results showed that sodium bicarbonate could not be a good anaesthesia for adult yellow seahorses.

Key words: Anaesthesia, sodium bicarbonate, induction, recovery, yellow seahorse, *Hippocampus kuda*

1. Introduction

Seahorses are expensive and highly demanded fish; nearly 80 countries of the world are involved in its trade. Asia alone contributed for trade of dried seahorses was more than 45 tons (till 1995) which was mainly used for Traditional Chinese Medicine (TCM). Apart from that seahorses are used for curious and live seahorses are traded for aquarium purpose especially in Europe and America (Vincent, 1996). Overexploitation brought down the wild population of seahorses by 25-75% during the short period of five years (Vincent, 1996). In the year 2001 Govt. of India impose ban on wild catch of seahorse, till the date India was one of the major exporter of dried seahorses and use to contribute about 30% of the global trade of seahorse (Salin and Nair, 2006).

The Yellow seahorse, *Hippocampus kuda* is one of the 46 reported species from Indo-Pacific waters (Koldewey and Martin-Smith, 2010). This species is well known for its special medicinal composition and used as one of the most famous, heavily traded and expensive materials for traditional Chinese medicine (Qian et al. 2008). The *H. kuda* has fairly smooth appearance and often exhibits a yellow pale colour, traits that are preferred by overseas markets of live aquarium and souvenir trades on a global scale (Marichamy et al. 1993; Vincent 1996; Lourie et al. 1999). The *H. kuda* had been exploited extensively from wild for several years in India mainly for the traditional Chinese medicine market. Seahorse aquaculture has received widespread attention due to concerns over decline in wild seahorse populations and its high economic value and marketability (Koldewey and Martin-Smith, 2010).

Wild *H. kuda* and other seahorses from Southeast Asia are the only source the Traditional Chinese Medicine (Foster & Vincent 2004), therefore seahorse aquaculture could play big role and will help to reduce pressure on wild stocks as well as will provide an alternative subsistence livelihood option to fisher men who are currently engage in seahorse fishing (Koldewey and Martin-Smith, 2010, Pawar et al. 2011a).

Though seahorse is slow swimming fish and comparatively easy to handle; it needs anesthesia to minimize unnecessary stress during handling (Pawar et al. 2011b). The effect of anesthetic varies with species, size, condition of the fish, dosage rate, period of immersion in anaesthetic solution, water temperature etc. (Ross and Ross, 1999). The properties required of an anesthetic vary with the research objectives however; quick induction of anesthesia is desirable in most cases (Marking & Meyer, 1985; Stoskopf, 1993). The recovery time from anesthesia is equally important because, long recovery time is desirable while collecting fish in the wild or where fish must be handled for some time in the laboratory; else a rapid recovery is better (Marking & Meyer, 1985; Stoskopf, 1993).

An ideal anaesthetic should possess several attributes such as:...
as, non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery (Pawar et al., 2011b). It is often advisable to identify the lowest effective doses of different anaesthetics in a specified species, as the responses to the same anaesthetic may vary considerably among different species (King et al., 2005). Sodium bicarbonate (NaHCO₃) commonly known as baking soda, is white colour powder which readily dissolves in water and release carbon dioxide. Carbon dioxide gas is a recommended anaesthesia for the fishes; it is used to produce sedation during transport and allow handling (Bowser, 2001). Sodium bicarbonate is easily available, cost effective, nontoxic and safe for human beings and there is no any ban or restrictions in its usages (Summerfield and Smith, 1990). Given the growing interest in the culture of seahorses and lack of detailed practical information on the administration of anaesthesia, the objective of the present study was to determine the efficacy of sodium bicarbonate for yellow seahorse, Hippocampus kuda.

2. Materials and Methods

2.1 Experimental animals and facilities

The experimental animals used in this study were about two years old hatchery bred yellow seahorse (Hippocampus kuda) maintained in the seahorse hatchery at Aquaculture laboratory of CSIR-National Institute of Oceanography, Goa, India. Experiment was conducted in Borosil glass tank (25 X 11.5 X 25 cm) filled with five litter fresh filtered seawater (Salinity: 32 ppt, pH: 8.2, temperature: 29±0.2°C, dissolve oxygen: 6±0.2 ppm). Healthy adult seahorses measuring 163.2±10.2 mm height and 12.22±1.94 g weight were selected. Seahorses were starved 24 h prior to the initiation of anaesthetic experiment.

2.2 Anaesthetic agents and dosages:

Anaesthetic agent sodium bicarbonate (NaHCO₃) (HiMedia, India) was used for the present investigation. On the basis of available literature (Altun et al, 2009; Sonawane and Kulkarni 2001; Peake, 1998) six different dosages of sodium bicarbonate 0.5, 1, 2, 3, 4 and 5 g L⁻¹ were formulated but for the present investigation these concentrations could not attain complete induction. Therefore, subsequently higher dosages 6, 8, 10, 12, 14, 16 and 18 g L⁻¹ were used but even these higher dosages were also unable to produce complete induction. Finally, very high sodium bicarbonate dosages of 20, 25, 30, 40 and 50 g L⁻¹ were tried to find out ideal dose for the yellow seahorse. Three seahorses were exposed to the each anaesthetic concentration.

2.3 Induction and recovery stages of anaesthesia:

Stages of anaesthesia and recovery in seahorse from Pawar et al., 2011b

<table>
<thead>
<tr>
<th>Stage</th>
<th>Descriptor</th>
<th>Behavioural response of the seahorse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal swimming and opercular rate; reacts to the external stimuli.</td>
</tr>
<tr>
<td>IS1</td>
<td>Partial loss of equilibrium</td>
<td>Ocular rate slightly decreased. Slow swimming with slight loss of reaction to external stimuli.</td>
</tr>
<tr>
<td>IS2</td>
<td>Cessation of swimming and loss of reflex reactivity</td>
<td>Striking the water, erratic swimming; increased opercular rate; reaction only to strong tactile and vibration stimuli.</td>
</tr>
<tr>
<td>IS3</td>
<td>Modulatory collapse</td>
<td>Completely stops swimming and stands on the bottom of the tank, opercular rate decreases slowly, no reaction to stimuli.</td>
</tr>
<tr>
<td>RS1</td>
<td>Starts operculum and fin movement</td>
<td>Opercular movement ceases due to over dose or longer immersion in anaesthetic solution. Subsequently death occurs.</td>
</tr>
<tr>
<td>RS2</td>
<td>Cannot balance head, tapping on tank bottom</td>
<td>Starts operculum movement, starts erratic movement of body. Cannot balance the head, most of the time keep head down trying to get up with tail force. Mouth tapping on the bottom of aquarium tank.</td>
</tr>
<tr>
<td>RS3</td>
<td>Normal</td>
<td>Ability to swim normally and regular opercular rate; Reactive to the external stimuli.</td>
</tr>
</tbody>
</table>

2.4 Statistical analysis:

All data are presented as mean ± standard deviation. Correction coefficients were calculated and regression analyses were used to establish the relationship between dosage and induction time, as well as dosage and recovery time. Significance difference was tested at a 5.00% level, represented as P<0.05 (Zar, 2005).

3. Results:

3.1 Induction Stage 1 (IS 1):

At concentrations 0.5 and 1 g L⁻¹ of sodium bicarbonate seahorses could not attain induction stage 1 as theses concentrations were too low. The estimated mean and standard deviation values are presented in Table 1. Induction stage 1 was attained faster as dosages of sodium bicarbonate goes increasing Fig. 1. Significant correlation (R²: 0.7438) was observed between sodium bicarbonate concentration and induction time (P<0.05), whereas scatter plot shows inverse exponential relationship (Fig. 1). Regression equation for the time to reach IS 1 in H kuda was IS 1 = 187.14 e⁻⁰.⁰⁵⁸⁶ C⁻¹.

3.2 Induction stage 2 (IS 2):

Induction stage 2 occurred at 8 g L⁻¹ and above concentration (Table 1) as sodium bicarbonate concentrations 6, 5, 4, 3, 2, 1 and 0.5 g was not sufficient to produce IS 2. Significant correlation (R²=0.9559) was observed between sodium bicarbonate concentration and induction time (P<0.05), whereas scatter plot shows inverse exponential relationship (Fig. 1). Regression equation for the time to reach IS 2 and concentration of sodium bicarbonate in H kuda was IS 2=268.32e⁻¹.⁰³⁵⁶C⁻¹.

3.3 Induction stage 3 (IS 3):

Sodium bicarbonate concentrations of 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20 and 25 g L⁻¹ were not sufficient to...
attain the induction stage 3. Mean and standard deviation values for IS3 are shown in Table 1. Seahorse reached IS 3 at higher concentrations of 30, 40 and 50 g L\(^{-1}\). Significant correlation (R\(^2\): 0.8229) was observed between sodium bicarbonate concentration and induction time (P<0.05), whereas scatter plot shows inverse exponential relationship (Fig. 1). Regression equation for time to reach IS3 and concentration of sodium bicarbonate in \textit{H kuda} was IS3=726.15e\(^{-0.005C}\).

3.4 Recovery Stage 1 (RS 1):
Recovery stages were observed at concentration of 30, 40 and 50 g L\(^{-1}\) of sodium bicarbonate. Mean and standard deviation values for RS1 are presented in Table 1. Significant correlation (R\(^2\): 0.7773) was observed between sodium bicarbonate concentration and recovery time (P<0.05), whereas scatter plot shows direct exponential relationship (Fig. 1). Regression equation for time to reach RS1 and concentration of sodium bicarbonate in \textit{H kuda} was RS1 = 104.72e\(^{0.0064C}\).

3.5 Recovery Stage 2 (RS 2):
Mean and standard deviation values for RS2 exposed to 30, 40 and 50 g L\(^{-1}\) concentrations of sodium bicarbonate are presented in Table 1. Significant correlation (R\(^2\): 0.9711) was observed between sodium bicarbonate concentration and recovery time (P<0.05), whereas scatter plot shows direct exponential relationship (Fig. 1). Regression equation for time to reach RS2 and concentration of sodium bicarbonate in \textit{H kuda} was RS2 = 519.69 e\(^{0.0070C}\). Post treatment mortality of seahorse was not observed at any concentration of sodium bicarbonate.

3.6 Recovery Stage 3 (RS 3):
At concentration of 30, 40 and 50 g L\(^{-1}\) of sodium bicarbonate RS3 were observed and mean ± standard deviation values presented in Table 1. Significant correlation (R\(^2\): 0.9711) was observed between sodium bicarbonate concentration and recovery time (P<0.05), whereas scatter plot shows direct exponential relationship (Fig. 1). Regression equation for time to reach RS3 and concentration of sodium bicarbonate in \textit{H kuda} was RS3 = 720.97e\(^{0.0072C}\).

4. Discussion:
Seahorse aquaculture has been expanding considerably in terms of both number and size of aquaculture operations and the number of species cultured to sustain the increasing trade in traditional medicine, aquarium fishes and curios (Koldewey and Martin-Smith, 2010). Demand for captive bred seahorse for aquarium trade is considerably increasing which resulted in starting of commercial seahorse culture of at least 13 species (Koldewey and Martin-Smith, 2010). Historically anaesthetics are used by aquaculturists to aid the capture, handling, for thorough examination, removal of external parasite, taking length-weight measurements, individual marking or tagging as well as during transportation (Ross and Ross, 1999, Pawar et al., 2011b). Anaesthesia plays a vital role in modern day aquaculture as it helps to reduce the stress which hamper growth and makes animal more susceptible to diseases. Generally, stress response varies from species to species therefore; it has become necessary to standardise each anaesthetic agent for each cultured species (Ross and Ross, 1999). Although, the anaesthetic administration minimize the stress during transportation and handling, its use and effectiveness in seahorse aquaculture has received very less attention. Because of high price and demand for the seahorses as well as its IUCN status renewed interest among the aquacultureist and researchers in breeding and rearing of seahorses.

Pawar et al., 2011b first time, differentiate the stages of anaesthetic induction and recovery in seahorse species and found out the lowest effective concentrations (LEC) of different anaesthetic agents based on the anaesthetic induction and recovery times using a wide range of doses. They standardized four anaesthetic dosages on culture yellow seahorses \textit{H. kuda} and recommended 125 mg L\(^{-1}\), 50

---

**Table 1.** Induction and recovery time (s) for \textit{Hippocampus kuda} anaesthetised with different concentration of Sodium Bicarbonate. Data is presented as Mean ± s.d. “...” denotes non-attainment of stage.

<table>
<thead>
<tr>
<th>Stages</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS 1</td>
<td>994±74</td>
<td>326±26</td>
<td>217±27</td>
<td>181±22</td>
<td>166±25</td>
<td>941±18</td>
<td>689±14</td>
<td>570±12</td>
<td>494±10</td>
<td>416±8</td>
<td>358±10</td>
<td>310±8</td>
<td></td>
</tr>
<tr>
<td>IS 2</td>
<td>928±41</td>
<td>301±30</td>
<td>237±23</td>
<td>212±20</td>
<td>191±15</td>
<td>116±13</td>
<td>83±12</td>
<td>70±3</td>
<td>56±5</td>
<td>45±5</td>
<td>37±4</td>
<td>30±3</td>
<td></td>
</tr>
<tr>
<td>IS 3</td>
<td>853±36</td>
<td>270±26</td>
<td>210±21</td>
<td>183±18</td>
<td>159±15</td>
<td>105±13</td>
<td>75±12</td>
<td>61±3</td>
<td>49±4</td>
<td>40±4</td>
<td>33±4</td>
<td>27±3</td>
<td></td>
</tr>
<tr>
<td>RS 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RS 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RS 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
mg L⁻¹, 175 mg L⁻¹ and 1000 µL⁻¹ of MS222, clove oil, benzocaine and 2-phenoxethanol respectively. Castro et al. (2008) used clove oil (0.05%) to anaesthese adult H. reidi whereas, Olivotto et al., 2008 anaesthetised juveniles of H. reidi with 50 mg L⁻¹ concentration of MS-222. Otero-Ferrer et al., 2010 used 25 mg L⁻¹ clove oil to anaesthetize H. hippocampus juveniles. Wilson et al. (2006) and Martins et al. (2010) used benzocaine 50 mg L⁻¹ and 10 mg L⁻¹ respectively to anaesthesia adults of H. reidi and H. abdominalis juveniles. In the present investigation vary high dosages of sodium bicarbonate >30 g L⁻¹ could only produce complete induction H. kuda.

Booke et al., 1978 and Altun et al., 2009 adjusted pH level with HCl of the water after addition of NaHCO₃, but in the present experiment pH was not adjusted. Booke et al., 1978 and Altun et al., 2009 set experiment in fresh water whereas, present study was performed in the marine water (salinity 32 ppt) and it was observed that pH decreases as addition of NaHCO₃ in marine water therefore it was not found necessary to adjust pH during the experiment.

Pawar et al., 2011b reported the induction time decreases with increase in concentrations of MS-222, benzocaine, clove oil and 2-phenoxethanol with H. kuda similar trend was observed in present study as sodium bicarbonate concentration increase, the induction time decrease. On the other hand, in the present investigation results shows recovery times increases progressively with increasing concentration sodium bicarbonate in adult H. kuda similar results were reported by Pawar et al., 2011b with MS222, clove oil, 2-phenoxethanol and benzocaine.

Many researchers studied the efficacy of sodium bicarbonate of various fishes. Keene et al. (1998) used sodium bicarbonate (dosage 1000mg L⁻¹) to anaesthetize adult sockeye salmon Oncorhynchus nerka with 6 min. Booke et al., 1978 used sodium bicarbonate to anaesthetize common carp juveniles and found 642 mg L⁻¹ at 10°C temperature with 5.5 pH was most suitable. In the present investigation very high dosage (>30 g L⁻¹) of sodium bicarbonate was required to anaesthetize the animal which was about 30 to 100 times more the previous reports of sodium bicarbonate with different fish species. Though post treatment mortality did not happen present results suggest that sodium bicarbonate could not be used as anaesthetics for the adult yellow seahorse H. kuda.

Acknowledgements

The authors are grateful to The Director, CSIR-National Institute of Oceanography (CSIR-NIO), Goa (India) for facilities and encouragement. First author (HBP) is thankful to CSIR for providing Senior Research Fellowship for pursuing Ph.D. Permission granted by the Ministry of Environment & Forests, Government of India for collection of seahorses is gratefully acknowledged. This paper represents contribution No. XXXX of the CSIR-NIO, Goa (India).

References:

17. Pawar H. B., Sanaye S. V., Sreepada R.A., Harish V.,


Source of support: Nil; Conflict of interest: None declared