

ISSN 2277-7729

Original Article

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

Pawar H.B., Ingole 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 ( $163.2 \pm 10.2$  mm height and  $12.22 \pm 1.94$  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 50g L<sup>-1</sup> concentrations of sodium bicarbonate. It was observed that very high dose of sodium bicarbonate (more than 30g L<sup>-1</sup>) 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.

© 2013 Universal Research Publications. All rights reserved

**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 anaesthesia to minimize unnecessary stress during handling (Pawar et al. 2011b). The effect of anaesthetic 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 anaesthetic vary with the research objectives however; quick induction of anaesthesia is desirable in most cases (Marking & Meyer, 1985; Stoskopf, 1993). The recovery time from anaesthesia 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, 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 (NaHCO<sub>3</sub>) 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 (Summerfeld 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

### 2.3 Induction and recovery stages of anaesthesia:

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

Stage	Descriptor	Behavioural response of the seahorse
0	Normal	Normal swimming and opercular rate; reacts to the external stimuli.
IS1	Sedation	Opercular rate slightly decreased. Slow swimming with slight loss of reaction to external stimuli.
IS2	Partial loss of equilibrium	Striking the water, erratic swimming; increased opercular rate; reaction only to strong tactile and vibration stimuli.
IS3	Cessation of swimming and loss of reflex reactivity	Completely stops swimming and stands on the bottom of the tank, opercular rate decreases slowly, no reaction to stimuli.
IS4	Modulary collapse	Opercular movement ceases due to over dose or longer immersion in anaesthetic solution. Subsequently death occurs.
RS1	Starts operculum and fin movement	Starts operculum movement, starts erratic movement of body.
RS2	Cannot balance head, tapping head on tank bottom	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.
RS3	Normal	Ability to swim normally and regular opercular rate; Reactive to the external stimuli.

### 2.4 Statistical analysis:

All data are presented as mean  $\pm$  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<sup>-1</sup> 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 ( $R^2$ : 0.7438) was observed between sodium bicarbonate concentration and induction time ( $P < 0.05$ ), whereas scatter

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 $\pm$ 0.2<sup>o</sup>C, dissolve oxygen: 6 $\pm$ 0.2 ppm). Healthy adult seahorses measuring 163.2 $\pm$ 10.2 mm height and 12.22 $\pm$ 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 (NaHCO<sub>3</sub>) (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 5g L<sup>-1</sup> 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<sup>-1</sup> 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 50g L<sup>-1</sup> were tried to find out ideal dose for the yellow seahorse. Three seahorses were exposed to the each anaesthetic concentration.

plot shows inverse exponential relationship (Fig. 1). Regression equation for the time to reach IS1 and concentration of sodium bicarbonate in *H kuda* was  $IS\ 1 = 187.14 e^{-0.0586 C}$

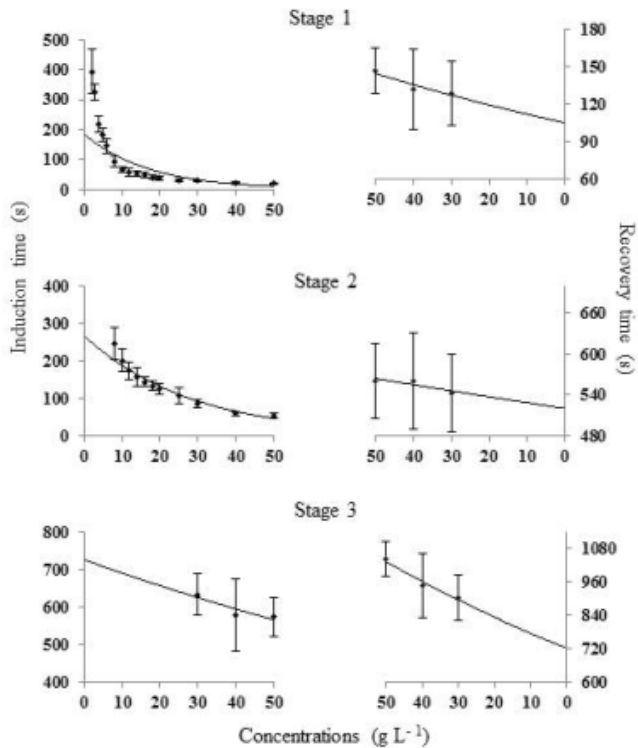
### 3.2 Induction stage 2 (IS 2):

Induction stage 2 occurred at 8 g L<sup>-1</sup> and above concentration (Table 1) as sodium bicarbonate concentrations 6, 5, 4, 3, 2, 1 and 0.5 g was not sufficient to produce IS2. Significant correlation ( $R^2=0.9559$ ) 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 IS2 and concentration of sodium bicarbonate in *H kuda* was  $IS\ 2 = 268.32 e^{-0.0356 C}$

### 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<sup>-1</sup> 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<sup>-1</sup>. Significant correlation (R<sup>2</sup>: 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 *H kuda* was  $IS3=726.15e^{-0.005C}$



**Fig.1.** Induction (IS1, IS2 and IS3) and recovery (RS1, RS2 and RS3) stages in relations to sodium bicarbonate concentrations for *Hippocampus kuda*

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

Stages	Concentrations g L <sup>-1</sup>																	
	0.5	1	2	3	4	5	6	8	10	12	14	16	18	20	25	30	40	50
IS 1	-	-	394±74	328±26	217±27	181±22	146±25	94±18	68±9	57±12	54±8	49±10	41±8	38±6	31±4	29±4	24±4	20±3
IS 2	-	-	-	-	-	-	245±42	201±30	173±24	156±25	143±13	133±12	125±14	106±20	85±6	58±6	54±7	-
IS 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	633±56	578±97	573±53
RS 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	128±25	132±32	146±18
RS 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	543±56	560±70	561±55
RS 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	901±82	948±115	1041±63

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,

### 3.4 Recovery Stage 1 (RS 1):

Recovery stages were observed at concentration of 30, 40 and 50 g L<sup>-1</sup> of sodium bicarbonate. Mean and standard deviation values for RS1 are presented in Table 1. Significant correlation (R<sup>2</sup>:0.7438) 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 *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<sup>-1</sup> concentrations of sodium bicarbonate are presented in Table 1. Significant correlation (R<sup>2</sup>: 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 RS2 and concentration of sodium bicarbonate in *H kuda* was  $RS2=519.69e^{0.0016C}$

### 3.6 Recovery Stage 3 (RS 3):

At concentration of 30, 40 and 50 g L<sup>-1</sup> of sodium bicarbonate RS3 were observed and mean ± standard values presented in Table 1. Significant correlation (R<sup>2</sup>:0.971) 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 *H kuda* was  $RS3=720.97e^{0.0072C}$

Post treatment mortality of seahorse was not observed at any concentration of sodium bicarbonate.

## 4. Discussion:

Seahorse aquaculture has been expanding considerably in terms of both number and size of aquaculture operations and in the number of species cultured to sustain the ever-increasing trade in traditional medicine, aquarium fishes and curios (Koldewey and Martin-Smith, 2010). Demand

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 researches 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 *H. kuda* and recommended 125 mg L<sup>-1</sup>, 50

mg L<sup>-1</sup>, 175 mg L<sup>-1</sup> and 1000 µL<sup>-1</sup> of MS222, clove oil, benzocaine and 2-phenoxyethanol respectively. Castro et al. (2008) used clove oil (0.05%) to anaesthetise adult *H. reidi* whereas, Olivotto et al., 2008 anaesthetised juveniles of *H. reidi* with 50 mg L<sup>-1</sup> concentration of MS-222. Otero-Ferrer et al., 2010 used 25 mg L<sup>-1</sup> clove oil to anaesthetize *H. hippocampus* juveniles. Wilson et al. (2006) and Martins et al. (2010) used benzocaine 50 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> respectively to anaesthetise adults of *H. reidi* and *H. abdominalis* juveniles. In the present investigation vary high dosages of sodium bicarbonate >30 g L<sup>-1</sup> 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 NaHCO<sub>3</sub>, 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 the in marine water (salinity 32 ppt) and it was observed that pH decreases as addition of NaHCO<sub>3</sub> 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-phenoxyethanol 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-phenoxyethanol and benzocaine.

Many researchers studied the efficacy of sodium bicarbonate of various fishes. Keene et al. (1998) used sodium bicarbonate (dosage 1000mg L<sup>-1</sup>) 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<sup>-1</sup> at 10<sup>0</sup> C temperature with 5.5 pH was most suitable. In the present investigation very high dosage (>30 g L<sup>-1</sup>) 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:

1. Altun, T., Bilgin, R., Danabas, D., 2009. Effects of sodium bicarbonate on anaesthesia of common carp (*Cyprinus carpio* L., 1758) juveniles. Turk J Fish Aquat Sc. 9, 29-31.
2. Booke, H. E., Hollender, B. and Lutterbie, G. 1978.

3. Sodium bicarbonate, an inexpensive fish anesthetic for field use. Progressive Fish Culturist., 40, 11-13.
4. Bowser, P.R., 2001. Anesthetic options for fish. In: R.D. Gleed and J.W. Ludders (Eds.), Recent advances in veterinary anaesthesia and analgesia: Companion animals, international veterinary information service (www.ivis.org), Ithaca, New York, USA.
5. Castro, A.L.C., Diniz, A.F., Martins, I.Z., Vendel, A.L., Oliveira, T.P.R., Rosa, I.L., 2008. Assessing diet composition of seahorses in the wild using a nondestructive method: *Hippocampus reidi* (Teleostei: Syngnathidae) as a study-case. Neotrop. Ichthyol. 6, 637-644.
6. Foster, S.J., Vincent, A.C.J., 2004. Life history and ecology of seahorses: implications for conservation and management. J. Fish Biol. 65, 1-61.
7. Keene, J.L., Noakes, D.L.G., Moccia, R.D., Soto, C.G., 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 29, 89-101.
8. King, W.V., Hooper, B., Hillsgrave, S., Benton, C., Berlinsky, D., 2005. The use of clove oil, metomidate, tricaine methanesulphonate and PE for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.) Aquac. Res. 36, 1442-1449.
9. Koldewey, H.J., Martin-Smith, K.M., 2010. A global review of seahorse aquaculture. Aquaculture 302, 131-152.
10. Lourie, S.A., Vincent, A.C.J., Hall, H.J., 1999. Seahorses: an identification guide to the world's species and their conservation. Project seahorse, London, UK. 214 pp.
11. Marichamy R., Lipton A.P., Ganapathy A., Ramalingam J.R., 1993. Large scale exploitation of seahorse (*Hippocampus kuda*) along the Palk Bay coast of Tamil Nadu. Mar. Fish. Inf. Serv., 119, 17-21.
12. Marking, L.L., Meyer, F.P., 1985. Are better anaesthetics needed in fisheries? Fisheries. 10, 2-5.
13. Martins, M.L., Mouriño, J.L., Fezer, G.F., Buglione Neto, C.C., Garcia, P., Silva, B.C., Jatobá, A., Vieira, F.N., 2010. Isolation and experimental infection with *Vibrio alginolyticus* in the sea horse, *Hippocampus reidi* Ginsburg, 1933 (Osteichthyes: Syngnathidae) in Brazil. Braz. J. Biol. 70, 205-209.
14. Olivotto, I., Avella, M.A., Sampaolesi, G., Piccinetti, C.C., Navarro, R.P., Carnevali, O., 2008. Breeding and rearing the longsnout seahorse *Hippocampus reidi*: rearing and feeding studies. Aquaculture 283, 92-96.
15. Otero-Ferrer, F., Molina, L., Socorro, J., Herrera, R., Fernández-Palacios, H., Izquierdo, M.S., 2010. Live prey first feeding regimes for short-snouted seahorse *Hippocampus hippocampus* (Linnaeus, 1758) juveniles. Aquac. Res. 1-12.
16. Pawar H.B., Sanaye S.V., Murugan A., Sreepada R.A., Tanu, 2011a. Effect of Background Color of Tanks on Growth and Survival of Juvenile Yellow Seahorse, *Hippocampus kuda* (Bleeker 1852), in the Pelagic Phase. Isr. J. Aquacult. - Bamidgeh. 63, 1-6.
17. Pawar H. B., Sanaye S. V., Sreepada R.A., Harish V.,

- Suryavanshi U., Tanu, Ansari. Z.A., 2011b. Comparative efficacy of four anaesthetic agents in the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852). *Aquaculture*. 311, 155-161.
18. Peake S., 1998. Sodium Bicarbonate and Clove Oil as Potential Anesthetics for Nonsalmonid Fishes. *North Am. J. Fish. Manage.* 18, 919–924.
  19. Qian, Z.J., Ryu, B.M., Kim, M.M., Kim, S.K., 2008. Free Radical and Reactive Oxygen Species Scavenging Activities of the Extracts from Seahorse, *Hippocampus kuda*, Bleeler. *Biotechnol. Bioprocess Eng.* 13, 705-715.
  20. Ross, L.G., Ross, B., 1999. *Anaesthetic and Sedative Techniques for Aquatic Animals*. Blackwell Science, Oxford. 159 pp.
  21. Salin, K.R., Nair, M.C., 2006. Resources and biodiversity of seahorses and the need for their conservation in India. *Aquacult. Asia* 10, 3–8.
  22. Sonawane, U.D., Kulkarni, G.N., 2001. Anaesthetic effects of clove oil and sodium bicarbonate on the fry of *Liza parsia*. *Iran J Fish Sci.*, 3(2), 49-62.
  23. Stoskopf, M.K., 1993. Anesthesia. In: *Aquaculture for Veterinarians: Farmed Fish Husbandry and Medicine*, L. Brown, (Editor), Pergamon Press, Oxford England. 161-168.
  24. Summerfelt, R.C., Smith, L.S., 1990. Anaesthesia, surgery and related techniques. In: Schreck, C.B., Moyle, P.B. (Eds.), *Methods for fish biology*. American Fisheries Society, Bethesda, MD, pp. 213–272.
  25. Vincent, A.C.J., 1996. *The international trade in seahorses*. TRAFFIC International, Cambridge, UK. 163 pp.
  26. Wilson, Z., Carter, C.G., Purser, G.J., 2006. Nitrogen budgets for juvenile big-bellied seahorse *Hippocampus abdominalis* fed *Artemia*, mysids or pelleted feeds. *Aquaculture* 255, 233–241.
  27. Zar, J. H., 2005. *Biostatistical analysis*, Fourth addition, Pearson education (Singapore) Pvt. Ltd., Indian Branch, Delhi: 633 p.

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