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Comparison of efficacy of four anaesthetic agents in the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852)

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ABSTRACT

To meet the demands of traditional Chinese medicine and marine ornamentals, there has been a commercial interest stimulated in culturing many seahorse species. In modern aquaculture, anaesthetics play an important role in reducing handling stress and mortality. In this investigation, the efficacy of four anaesthetic agents (MS-222, benzocaine, clove oil and 2-phenoxyethanol) was compared in captive-bred yellow seahorse, *Hippocampus kuda* (Bleeker, 1852). Minimum effective concentrations determined were 125 mg L⁻¹ (induction 115±16 s and recovery time 246±36 s) for MS-222, 175 mg L⁻¹ (induction 175±19 and recovery time 354±55 s) for benzocaine, 50 mg L⁻¹ (induction 115±28 and recovery time 385±37 s) for clove oil, 1000 µl L⁻¹ (induction 176±22 and recovery time 271±37 s) for 2-phenoxyethanol. Induction and recovery times for adult *H. kuda* anaesthetised with anaesthetic agents were dose-dependent ($P<0.05$). The onset of individual phases of anaesthesia and recovery times depended significantly on the concentration of the anaesthetic used. An inverse exponential relationship was observed between concentrations of anaesthetic and induction time, whereas exponential relationships were observed between concentrations and recovery time for all anaesthetic agents evaluated. Although all anaesthetics were considered safe, MS-222 and clove oil were proved to be most effective for use in husbandry of the *H. kuda*. The study being the first one that compared the efficacy of different anaesthetic agents in adult seahorses has practical significance with regards to seahorse husbandry and production efficiency.

Key words: MS-222, benzocaine, clove oil, 2-phenoxyethanol, induction time, recovery time

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1. Introduction

Use of anaesthetic agents is a common in modern aquaculture and has practical relevance when these are required for diverse husbandry manipulations such as selection of fish, their measurement, sampling, tagging, transportation, artificial reproduction and surgery procedures (Roubach et al., 2005; Weber et al., 2009). These routine operations often induce a physiological stress response which may result in undesirable outcomes such as immunosuppression and growth retardation (Roubach et al., 2005; Heo and Shin, 2010). The use of anaesthetic agents may reduce stress-induced damage to the fish, but may also attenuate the physiological response to stress (Weber et al., 2009). A number of chemicals have proved effective in the anaesthetisation of fish (Velíšek et al., 2006), each having its own merits and demerits. The most commonly used anaesthetics in aquaculture are MS-222 and 2-phenoxyethanol (PE) (Svoboda and Kolářová, 1999). Recent studies have evaluated the anaesthetic efficacy of clove oil, the main active component (70 to 90%) of which is eugenol [2-methoxy-4-(2-propenyl) phenol], in various fish species (Walsh and Pease, 2002; Iversen et al., 2003; King et al., 2005; Mylonas et al., 2005; Roubach et al., 2005; Hajek et al., 2006), as well as benzocaine (ethyl aminobenzoate) (Heo and Shin, 2010; Pramod et al., 2010).

Choosing an appropriate anaesthetic depends mainly on its effectiveness in immobilizing fish with good recovery rates (Gilderhus and Marking, 1987; Burka et al., 1997). An ideal anaesthetic should possess several attributes. They should be non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery (Treves-Brown, 2000). The efficacy is conditioned by environmental (temperature, pH and salinity) and biological factors (size, weight, lipid content and fish species) (Burka et al., 1997; Ross and Ross, 1999). 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).

Seahorses and other Syngnathid fishes are in great demand worldwide. They form an important ingredient in traditional Chinese medicine, as well as popular ornamental fish and curios. Different seahorse species are harvested on a large scale, and traded by at least 77 nations in high volumes and various product forms (McPherson and Vincent, 2004). It has been reported that at least 25 million seahorses (>70 metric tonnes, dry weight) are traded globally (Salin and Mohankumran, 2006; Project Seahorse, 2006). In addition, more than one million live seahorses are caught for the aquarium trade, mostly destined for sale in North America, Europe, Japan and Taiwan (Vincent, 1996). Seahorse aquaculture has been expanding considerably in the number and size of aquaculture operations and number of species cultured to sustain increasing trade in traditional medicine,

aquarium fishes and curios (Koldewey and Martin-Smith, 2010). Some researchers have cited the use of anaesthetics in different seahorse species (Woods, 2002; Woods and Martin-Smith, 2004; Koldewey, 2005; Morgan and Bull, 2005; Castro et al., 2008; Mattle and Wilson, 2009; Otero-Ferrer et al., 2010), however practical details on their efficacy were seldom outlined. Before recommending the use of a particular anaesthetic, a range of induction and recovery times must be established to assess its efficacy. Such information would therefore be necessary whenever a culture technology for a potential species is being developed.

Given the growing interest in the culture of seahorses and lack of detailed practical information on the administration of anaesthetics, the overall aim of the present study was to determine the minimum optimal concentrations (based on anaesthetic induction and recovery times) of four most common fish anaesthetic agents (MS-222, benzocaine, clove oil and PE) that could be efficiently used in the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852) under controlled conditions. Information on stages of induction and recovery associated with exposure of *H. kuda* to a wide range of concentrations of anaesthetic agents provided here should be of practical interest to seahorse aquaculturists.

2. Material and methods

2.1 Fish and experimental facilities

The study was carried out at the seahorse hatchery adjoining the aquaculture laboratory complex of the National Institute of Oceanography, Goa (India), where techniques for the standardization of captive breeding, rearing and culture of Indian seahorse species are being established. New born juveniles (pelagic phase) released from a single *Hippocampus kuda* spawner from the F2 generation were reared in rectangular light blue background fibreglass reinforced plastic (FRP) tanks (capacity, 100 L) at a juvenile density of 2 fish L⁻¹ until they attained settlement phase. Thereafter, juveniles were reared in large FRP tanks (capacity, 500 L) secured with different types and sizes of holdfasts depending upon the growth of juveniles. Various husbandry practices as described in Murugan et al. (2009) were followed. The seawater used was treated by rapid sand filtration, bio-filtration and then passed through ultraviolet radiation. Adequate aeration was provided to the FRP tanks using air blowers and a photoperiod of 12 h L (0700–1900 h):12 h D (1900–0700 h) was maintained using a fluorescent bulb (100 W Philips build) providing a light intensity ~800 lx at the water surface. Juveniles were fed *ad libitum* three times per day (0600, 1400 and 2200 h) with different live prey organisms such as copepodites, *Artemia nauplii* and mysids (*Mesopodopsis orientalis*). The tanks

were cleaned daily, and important water quality parameters measured twice a week. The measured physico-chemical parameters of seawater in the rearing tanks fell within the optimum levels recommended for culture of seahorses (Murugan et al., 2009): temperature (28.5 ± 0.5 °C), salinity (31 ± 1.5 ppt), dissolved oxygen (6.1 ± 0.6 mg L⁻¹), pH (7.9 ± 0.3), NO₂-N (<0.02 mg L⁻¹) and NH₃/NH₄ (0 mg L⁻¹). Altogether, 120 healthy adults measuring 155.1 ± 9.8 mm Ht (as in Lourie et al., 1999) and weighing, 11.15 ± 1.68 g wet weight were selected. Seahorses from two different FRP tanks were used to avoid a possible tank effect and were starved 24 h prior to the initiation of anaesthetic experiments.

2.2. Anaesthetic agents

The anaesthetic agents MS-222 (tricaine methanesulphonate, HiMedia, India), 2-phenoxyethanol (ethylene glycol monophenyl ether, Sigma Aldrich Chemie, Germany), benzocaine (Ethyl-p-aminobenzoate, HiMedia, India) and clove oil (Sigma Aldrich Co. USA) were used. Doses of the anaesthetic agents were prepared a few minutes prior to anaesthetic induction experiments. Since clove oil does not readily dissolve in water (Woody et al., 2002), it was initially diluted in ethanol (ratio of clove oil to ethanol: 1:9) to prepare a stock solution (100 mg ml⁻¹). Similarly, to increase the solubility of benzocaine in water, a stock solution was prepared with ethanol (ratio of benzocaine to ethanol 1:9) to obtain a concentration of 100 mg ml⁻¹. Aliquots of the stock solutions were used to achieve the desired experimental concentrations. MS-222 with its inherent solubility in water was added directly to the tanks to obtain the desired test concentrations. Preliminary trials indicated that the highest concentration of ethanol used in each trial did not have any noticeable analgesic effect on *H. kuda* for at least 20 min. A known volume of 2-phenoxyethanol (PE) was initially mixed with a little seawater in a reagent bottle (200 ml) and then stirred to disperse the chemical to form small droplets before adding to anaesthetic induction tanks.

2.3. Induction and recovery stages of anaesthesia

Since the characteristics that define different stages of anaesthesia induction and recovery in seahorses have not been reported previously, a pilot study was conducted for describing these stages by carefully observing the behavioural responses in *H. kuda*. Clove oil (50 mg L⁻¹) was added to the anaesthetic induction tanks ($37.5 \times 22.5 \times 22.5$ cm) that were filled with fresh, aerated seawater (capacity, 15 L). Water temperature, dissolved oxygen and pH values in the tanks were 29 ± 0.2 °C, 6.1 ± 0.5 mg L⁻¹ and 7.9 ± 0.1 , respectively. Water in each tank was stirred with a glass rod and aerated to facilitate complete mixing. Adult *H. kuda* were netted from the rearing tanks and transferred to the

anaesthetic induction tank for studying the stages of anaesthesia. Changes in the physiological status of the anaesthetized fish were assessed in three consecutive stages as described by Summerfelt and Smith (1990), with slight modifications based on the species-specific behavioural response of *H. kuda*. Different anaesthesia induction stages (IS) and recovery stages (RS) were defined by checking opercular movements, equilibrium and absence of response to tactile stimulus.

For practical purposes, four IS (IS1—sedation, IS2—partial loss of equilibrium, IS3—cessation of swimming and loss of reflex reactivity, IS4—modulatory collapse) were considered (Table 1). When the seahorses reached IS3, they were immediately netted from the experimental tank, and placed on a wet surface for a period of 90 seconds. Our previous studies (Pers. obs.) indicated that the duration of this time period was sufficient to take length and weight measurements or fixing of a tag. Then the fish was transferred to a recovery tank (capacity, 100 L) filled with fresh, aerated sea water for recording stages of anaesthesia recovery. Three RS (RS1—start of the operculum and fin movement, RS2—inability to balance head, tapping head on tank bottom, RS3—normal) were described. Experiments were repeated in triplicate to verify findings. An induction time of 3 min or less with complete recovery in 5 min suggested by Marking and Meyer (1985) was used to establish a new set of anaesthesia induction (IS3) and recovery (RS3) times for different anaesthetics presented in this study (Table 1).

Table 1: Stages of anaesthetic induction and recovery in *Hippocampus kuda* (IS—induction stage; RS—recovery stage).

2.4. Anaesthetisation procedure

The efficacy of four anaesthetic agents in adult *H. kuda* was assessed by testing several doses of each anaesthetic. Choice of minimum and maximum doses of each anaesthetic was based on previously published information for teleosts (Weber et al., 2009; Gomes et al., 2001). The following doses of each agent were evaluated: MS-222 and clove oil (25, 50, 75, 100, 125 and 150 mg L⁻¹), benzocaine (75, 100, 125, 150, 175 and 200 mg L⁻¹) and PE (250, 500, 750, 1000, 1250 and 1500 µl L⁻¹). Anaesthetic experiments performed were similar to the pilot study and three seahorses were exposed to each anaesthetic concentration. Fish behaviour at each concentration was monitored individually throughout the induction and recovery stages. The induction and recovery times for four anaesthetic agents were measured under similar set of environmental conditions using an electronic stopwatch. When a particular seahorse reached stage IS3, it was immediately netted from the anaesthetic induction tank and kept on a wet surface for 90 seconds—a time deemed sufficient to

carry out routine aquaculture procedures (e.g. length and weight measurements or fixing a tag). After this, it was transferred to recovery tanks for recording recovery stages.

2.5. Post-treatment survival

After anaesthetic treatment, seahorses were transferred to post-treatment tanks (capacity, 60 L) for seven days to assess behavioural changes and mortality. Seahorses that were exposed to the same concentration of anaesthetic were kept in the same post-treatment tank. During the post-treatment period, seahorses were fed twice a day *ad libitum* with live mysids, and 50% of the tank water was exchanged on a daily basis.

2.6. Statistical analysis

A Kruskal–Wallis test was used to assess the difference in induction and recovery times of different concentrations of the same anaesthetic agent (Zar, 1999). 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 of significance, represented as $P < 0.05$. All results were processed and analysed with the Graph Pad Prism 5 statistical software (San Diego, California, U.S.A).

3. Results

3.1. Induction and recovery stages of anaesthesia

Significant differences ($P < 0.05$) in the induction and recovery stages at different concentration levels of the four anaesthetic agents were identified. Induction times generally decreased significantly with increasing doses for all of the anaesthetic agents evaluated. Induction time (IS3) ranged from 359 ± 36 s (100 mg L^{-1}) to 92 ± 9 s (150 mg L^{-1}) for MS-222, from 1165 ± 49 s (75 mg L^{-1}) to 150 ± 16 s (200 mg L^{-1}) for benzocaine, 457 ± 89 s (25 mg L^{-1}) to 36 ± 7 s (150 mg L^{-1}) for clove oil and 1154 ± 69 s ($500 \text{ } \mu\text{l L}^{-1}$) to 61 ± 13 s ($1500 \text{ } \mu\text{l L}^{-1}$) for 2-phenoxyethanol (PE) (Table 2). Recovery times increased with increasing concentrations of anaesthetic agents ($P < 0.05$). Recovery time ranged from 180 ± 15 (100 mg L^{-1}) to 358 ± 24 s (150 mg L^{-1}) for MS-222, from 41 ± 6 (75 mg L^{-1}) to 678 ± 44 s (200 mg L^{-1}) for benzocaine, 238 ± 33 (25 mg L^{-1}) to 927 ± 50 s (150 mg L^{-1}) for clove oil and 170 ± 21 ($500 \text{ } \mu\text{l L}^{-1}$) to 426 ± 45 s ($1500 \text{ } \mu\text{l L}^{-1}$) for PE (Table 2).

Table 2: Induction and recovery times for *Hippocampus kuda* anaesthetized with various concentrations of four anaesthetic agents (a) MS-222 (b) Benzocaine (c) Clove oil and (d) 2-phenoxyethanol (PE). Data are presented as mean±s.d.

The minimum optimal doses of different anaesthetic agents evaluated were: 125 mg L⁻¹ for MS-222, 175 mg L⁻¹ for benzocaine, 50 mg L⁻¹ for clove oil and 1,000 µl L⁻¹ for PE. MS-222 and PE produced induction and recovery times of less than 3 and 5 min, respectively. Both benzocaine and clove oil met the induction criterion but the recovery time limits exceed by about 20 and 30% respectively. The minimum effective concentration of clove oil induced relatively quicker anaesthesia (115±28 s) and longer recovery (385±37 s) in *Hippocampus kuda*.

3.2. Comparative efficacy of anaesthetics

A significant correlation was observed between anaesthetic concentration and induction time for all tested anaesthetic agents ($P < 0.05$), whereas scatter plots yielded an inverse exponential relationship (Fig. 1). The regression equations of times to reach IS3 and concentrations (C) of four anaesthetic agents in *H. kuda* were $IS3 = 2748e^{-0.023C}$ for MS-222; $IS3 = 5419e^{-0.018C}$ for benzocaine; $IS3 = 420e^{-0.018C}$ for clove oil and $IS3 = 6162e^{-0.003C}$ for PE. Similarly, a significant correlation ($P < 0.05$) was observed between anaesthetic concentration and times to reach RS3 for all anaesthetic agents, whereas scatter plots showed exponential relationship (Fig. 1). The regression equations established for recovery time and concentrations were $RS3 = 68e^{0.010C}$ for MS 222, $RS3 = 6e^{0.023C}$ for benzocaine, $RS3 = 206e^{0.010C}$ for clove oil and $RS3 = 102e^{0.001C}$ for PE.

Fig. 1. Induction (IS3) and recovery (RS3) times (s) in relation to anaesthetic concentrations for *Hippocampus kuda* (n=3 for each trial).

3.3. Post-treatment survival

H. kuda reared in post-treatment tanks showed no signs of stress or disease symptoms. No mortality occurred during a post-treatment period of 7 days. Seahorses exhibited normal feeding and physiological behaviour.

4. Discussion

Strong commercial demand for seahorses coupled with concerns over the long-term viability of exploiting natural populations have stimulated a renewed interest in breeding and rearing of seahorses. Seahorse aquaculture has expanded considerably in terms of the number and size of operations and number of species cultured (Koldewey and Martin-Smith, 2010). Use of anti-stress agents form an integral component of modern day aquaculture practices. A number of considerations such as availability, cost-effectiveness, ease of use, nature of the study and user safety should be considered before choosing an anaesthetic for aquaculture (Gilderhus and Marking, 1987; Mylonas et al., 2005). Because stress responses vary widely between species, it is often necessary to screen dosages of different anaesthetic agents for each cultured species (Ross and Ross, 1999).

Although the importance of anaesthesia in fisheries research and aquaculture is well established, their administration and efficacy in seahorse husbandry has received scant attention. Although some published papers have cite the use of MS-222 (Boisseau, 1967; Bull, 2002; Jones and Lin, 2007; Olivotto et al., 2008), AQUI-S (Isoeugenol) (Woods, 2000; Woods and Martin-Smith, 2004; Koldewey, 2005; Morgan and Bull, 2005; Mattle and Wilson, 2009), benzocaine (Wilson et al., 2006; Martins et al., 2010) and clove oil (Castro et al., 2008; Otero-Ferrer et al., 2010) in a few seahorse species, but the protocols of their administration were seldom addressed. This study demonstrated that all the four anaesthetic agents are efficient in anaesthetizing adult *Hippocampus kuda* based on induction and recovery times.

A general trend found for all anaesthetics evaluated was that induction times decreased significantly with the increase in anaesthetic concentration ($P < 0.05$). These results are in good agreement with previous studies that suggested that induction time decreases inversely according to the concentration of anaesthetic agent in teleost fishes (Mattson and Ripley, 1989; Hseu, et al., 1998; Mylonas, et al., 2005; Gullian and Villanueva, 2009; Weber, et al., 2009; Heo and Shin, 2010). On the other hand, longer recovery times with increasing dose of anaesthetics were observed in adult *H. kuda*. Prolonged recovery with increased anaesthetic dosage has been reported in seven species of tropical reef teleosts (Cunha and Rosa, 2006), sockeye salmon (Woody et al., 2002) and cobia, *Rachycentron canadum* (Gullian and Villanueva, 2009). However, experiments by Mylonas et al. (2005) documented recovery times decreasing with increase in anaesthetic concentration in European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) anaesthetized with clove oil and 2-phenoxyethanol (PE). Decreased recovery times with increasing doses of PE and metomidate and dose-independent recovery time in juveniles of Senegalese sole (*Solea senegalensis*)

anaesthetized with clove oil and MS-222 has been reported by Weber et al. (2009). The explanation put forward by these authors for such an unusual trend is that with the highest doses, the fish is not in contact with the anaesthetic for long, which would allow faster recovery. Other factors such as differences in the physiological responses of fish to the anaesthetic agents also influence this trend (Weber et al., 2009). This has been exemplified by Keene et al. (1998) who documented slower recovery times with MS-222 compared to clove oil based on different effects on the cardio-respiratory system in rainbow trout. Further studies determining the anaesthetic induction and recovery times in seahorse species combined with physiological responses to a wide range of anaesthetic concentrations are necessary for improved understanding their interdependency.

In the present study, the onset of induction (IS3) and recovery of anaesthesia (RS3) varied significantly with the concentrations of the anaesthetic ($P < 0.05$). The efficacy criteria to be acceptable for routine aquaculture practices is that the induction time is 3 minutes or less, with complete recovery in 5 minutes (Marking and Meyer, 1985). All the anaesthetics evaluated here met the first criterion, while recovery times were prolonged in *H. kuda* anaesthetized with clove oil and benzocaine than with MS-222 and PE (Table 2). Relatively quicker induction in *H. kuda* anaesthetized with clove oil may be due to its high lipophilic nature which allows it to adhere easily and penetrate the gill epithelium for adsorption in tissues (Mylonas et al., 2005). On the other hand, delayed recovery might be due to its persistent nature on the gill surface, which effectively increases the recovery time (King et al., 2005; Sladky et al., 2001). Quicker induction (89 ± 62 s) than recovery (119 ± 78 s) in adult *H. reidi* anaesthetised with a single concentration of clove oil (0.05%) has been reported by Castro et al. (2008). Although induction time (115 ± 28 s) obtained with minimum effective concentration of clove oil (50 mg L^{-1}) in adult *H. kuda* was comparable, the recovery times (375 ± 22 s) however were 3 times higher than those reported for *H. reidi* (Castro et al., 2008). Such difference in the respective recovery times might be related to species, size, physiological status and environmental conditions (Burka et al., 1997; Ross and Ross, 1999). The dynamics of recovery times in anaesthetized fish seems to be more complex. For example, in Senegalese sole (*Solea senegalensis*), recovery times were weight-dependent for metomidate and dose-dependent for clove oil and MS-222 (Weber et al., 2009).

Few reports citing the use of MS-222, benzocaine and clove oil in seahorse species are available (Wilson et al., 2006; Castro et al., 2008; Olivetto et al., 2008; Martins et al., 2010; Otero-Ferrer et al., 2010). For recording length and weight measurements, juveniles of *H. reidi* were lightly anaesthetized at MS-222 concentration of 50 mg L^{-1} (Olivetto et al., 2008). Wilson et al. (2006)

used benzocaine at 10 mg L^{-1} for recording weight in *H. abdominalis* juveniles, whereas adults of *H. reidi* were anaesthetized in a benzocaine solution (50 mg L^{-1}) before sacrificing them for histological analysis by Martins et al. (2010). Castro et al. (2008) anaesthetised adult *H. reidi* with clove oil (0.05%) for assessment of diet composition. Also, 25 mg L^{-1} clove oil was found effective and safe in anaesthetizing *H. hippocampus* juveniles for taking biometric measures (Otero-Ferrer et al., 2010).

Unfortunately, the aforementioned studies do not provide details on the anaesthetic induction and recovery times of the various anaesthetic agents. Castro et al. (2008) measured induction and recovery times in anaesthetized adult *H. reidi* with clove oil, but at a single concentration (0.05%). Our study differed from those conducted previously on the use and efficacy of anaesthetic agents in seahorse species. For the first time, we defined the stages of anaesthetic induction and recovery in seahorse species and the lowest effective concentrations of different anaesthetic agents were determined based on the anaesthetic induction and recovery times using a wide range of doses. Therefore, we strongly believe that the minimum effective concentrations of different anaesthetic agents provided here seem to be closer to practical scenarios.

There are many reports on the effective concentrations of MS-222, benzocaine, clove oil and PE required for anaesthetization of teleosts. A concentration of 75 mg L^{-1} of MS-222 was effective in anaesthetizing *Gadus morhua* (Mattson and Ripley, 1989) and Senegalese sole (Weber et al., 2009), respectively. Gomes et al. (2001) documented that benzocaine applied at doses of $100\text{-}150 \text{ mg L}^{-1}$ resulted in quick induction, total immobilization and fast recovery in juveniles of tambaqui (*Colossoma macropomum*). PE at doses of 600 mg L^{-1} and 0.12% produced optimal anaesthetic inductions in Senegalese sole (*Solea senegalensis*) (Weber et al., 2009) and in goldfish (*Carassius auratus*) (Yasui et al., 2009), respectively. Cunha and Rosa (2006) recommended 20 mg L^{-1} clove oil dosage for seven tropical reef fishes. Also, Weber et al. (2009) reported 30 mg L^{-1} clove oil was effective in anesthetizing *S. senegalensis*. In contrast, higher concentrations were required to anaesthetise the *H. kuda*. One of the possible reasons for requirement of higher doses of anaesthetics in seahorses could be related to modified form of general teleost gill pattern (random tufted gills) and narrow opercular opening (small cavity volume) which may entail lower water volumes splashed onto the gills (inadequate irrigation) and existence of slow rate of inhale and exhale water across the gills (Gert Roos, personal communication). It has been reported that entry and excretion of anaesthetics in fish takes place primarily via the gills, whereas internal organs are only slightly

involved (Ross and Ross, 1999; Hoskonen and Pirhonen, 2006). Further research on structure of gills influencing the anaesthetic dosage in different teleost species, however is required.

Behavioural response of seahorses to anaesthetic agents is markedly different from those in other teleosts. For example, it is difficult to identify the state of death in seahorses as they do not fall or lie horizontally on the bottom of the tank. Instead, *H. kuda* stand vertically on the bottom of tank and appear to be alive (pers. obs.). This peculiar behaviour of seahorses is often misleading and might lead to overdosing the fish as experienced during our pilot study. This calls for careful observation when anaesthetising seahorses.

Lastly, the different induction and recovery times in the same sized adult yellow seahorses anaesthetized with four anaesthetic agents under same environmental conditions could be related properties of each of the anaesthetic agent evaluated, more particularly to the mode of action and their pharmacokinetics. Further studies on different life stages, gender, reproductive state and sizes, followed by assessments of the effects of anaesthetics on haematological profile and respiration rate, will considerably advance our understanding of anaesthesia in husbandry of the yellow seahorse, *H. kuda*.

5. Conclusion

Although all four anaesthetic agents evaluated were effective and presented a good margin of safety, MS-222 and clove oil proved to be most effective in the yellow seahorse, *Hippocampus kuda*. Due to the investigation of different anaesthetic induction and recovery stages, as well as the identification of the lowest effective concentrations of each anaesthetic agent, the findings of the present study has potential significance with regards to seahorse husbandry, stress, survival, revenue and concerns over residues.

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Table 1. Stages of anaesthetic induction and recovery in *Hippocampus kuda* (IS—induction stage; RS—recovery stage).

Stage	Descriptor	Behavioural response of the seahorse
IS1	Sedation	Slight fall in opercular rate; slow swimming; partial loss of reaction to external stimuli
IS2	Partial loss of equilibrium	Striking the water column; erratic swimming; increased opercular rate; reaction only to strong tactile and vibration stimuli
IS3	Cessation of swimming and loss of reflex reactivity	Complete cessation of swimming and standing on the tank bottom; slow decrease in opercular rate; no reaction to stimuli
IS4	Modulatory collapse	Cessation of opercular movement (over dose and or longer immersion in anaesthetic solution); Subsequent death
RS1	Regaining of operculum and fin movement	Restarts operculum movement, starts erratic movement of body.
RS2	Inability to balance head, tapping head on tank bottom	Unable to balance the head, often keeping the head down and trying to get up with tail force; mouth tapping on the bottom of tank
RS3	Normal	Ability to swim normally and regular opercular rate; reactive to the external stimuli.

Table 2. Induction and recovery times for adults of *Hippocampus kuda* anaesthetised with various concentrations of four anaesthetic agents (a) MS-222 (b) Benzocaine (c) Clove oil and (d) 2-phenoxyethanol (PE). Data are presented as mean±s.d.

(a)

Stages	Concentrations (mg L ⁻¹)					
	25	50	75	100	125	150
IS1	266±73	94±16	59±12	32±8	17±5	6±2
IS2	-	227±26	186±12	125±19	34±6	21±4
IS3	-	-	-	359±36	115±16	92±9
RS1	-	-	-	18±3	32±11	44±13
RS2	-	-	-	54±8	94±23	98±21
RS3	-	-	-	180±15	246±36	358±24

(b)

Stages	Concentrations (mg L ⁻¹)					
	75	100	125	150	175	200
IS1	41±10	42±9	28±5	25±6	19±4	11±4
IS 2	253±16	178±19	142±44	69±12	46±8	35±12
IS 3	1165±49	999±82	629±1.04	354±15	175±19	150±16
RS 1	5±2	10±5	15±5	45±7	69±13	216±10
RS2	11±3	19±6	30±8	119±12	181±13	235±26
RS3	41±6	50±8	122±14	236±27	354±55	678±44

(c)

Stages	Concentrations (mg L ⁻¹)					
	25	50	75	100	125	150
IS1	31±7	21±3	21±4	12±2	8±3	6±1
IS2	47±16	28±3	30±7	15±2	12±3	13±2
IS3	457±89	115±28	81±15	76±17	45±8	36±7
RS1	31±6	51±18	108±21	229±30	305±59	369±39
RS2	52±5	70±27	251±63	210±39	420±66	545±55
RS3	238±33	385±37	485±66	563±49	734±96	927±50

(d)

Stages	Concentrations (µl L ⁻¹)					
	250	500	750	1000	1250	1500
IS1	62±12	22±4	15±3	13±4	11±2	7±2
IS2	324±52	86±6	59±5	44±7	34±5	30±6
IS3	-	1154±69	796±55	176±22	93±11	61±13
RS1	-	5±1	14±3	43±10	92±21	127±12
RS2	-	16±4	61±12	94±23	186±20	190±15
RS3	-	170±21	208±22	271±37	382±47	426±45

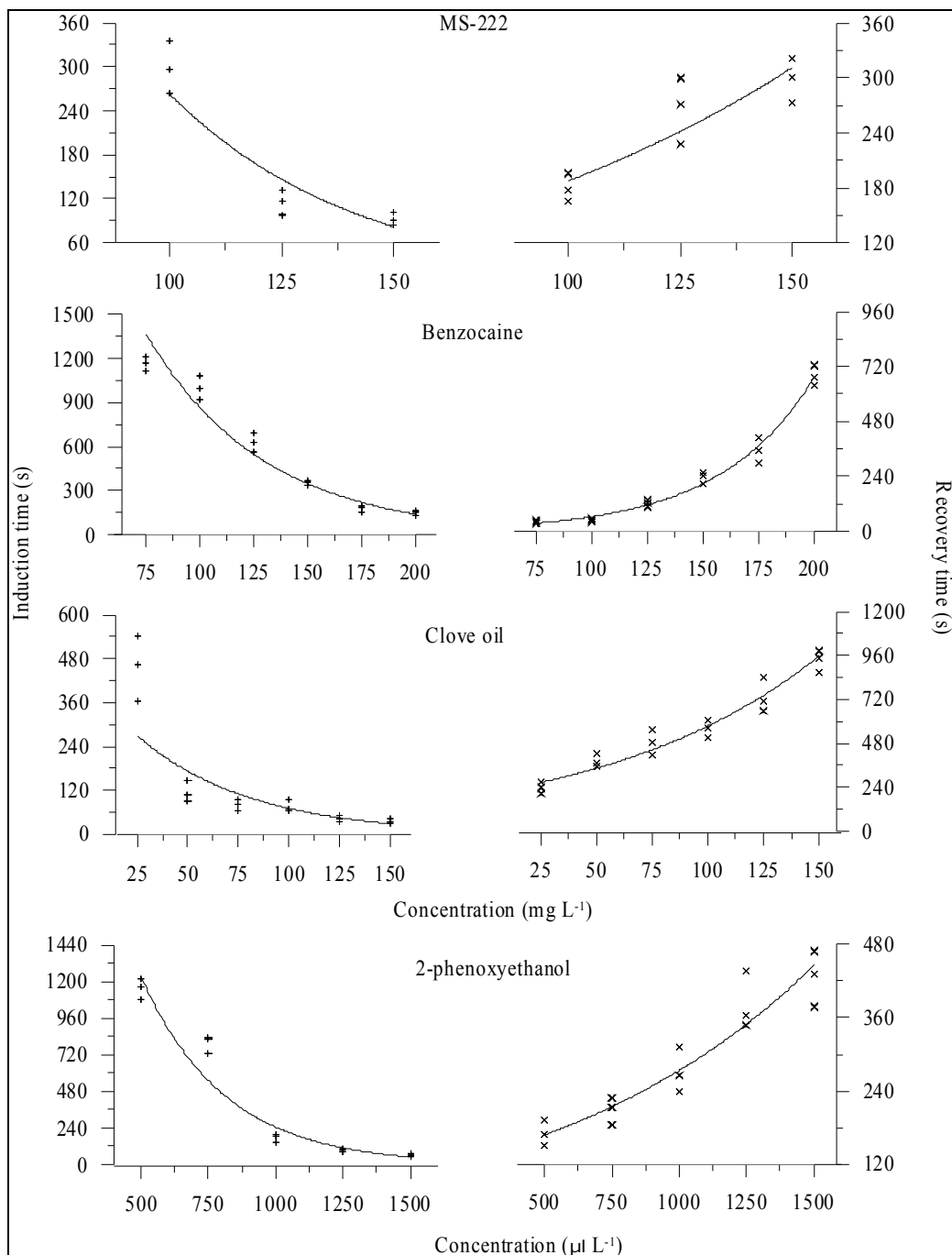


Fig. 1. Induction time—anaesthetic concentration and recovery time—anaesthetic concentration, relationships for *Hippocampus kuda* following exposure to four anaesthetic agents.