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Shelter colour preference in the purple mud crab *Scylla tranquebarica* (Fabricius)

Gunzo Kawamura, Annita Seok Kian Yong, Deborah Crystal Roy, Leong-Seng Lim*

Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

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ABSTRACT

Cannibalism a serious problem in the grow-out of mud crabs in ponds. Studies have proven that presence of shelters decreases aggressive behaviour of *Scylla* spp. in ponds and tanks. In this study, the shelter colour preference was determined in the wild captured purple mud crab Scylla tranquebarica (Fabricius) in an outdoor round black tank. Eight out of 48 PVC pipes were painted either blue, green, red, black or white. Other eight were not painted and were provided as grey shelters. The shelters were presented in different two-colour pairs to a group of 35 crabs; eight shelters of one colour and eight of other colour were arranged alternately on the tank bottom, and the number of occupants was recorded. After emptying the shelters, the shelters rearranged for the next observation. Each colour combination was repeated five times, and total of 15 combinations were tested. The number of occupants against each colour of the different pairs were analyzed by the χ2-test and Thurstone's law of comparative judgment. The data were converted into mean z-scores and significant biases from zero were noted. Underwater visibility of each colour shelter was visually measured through a waterproof digital CCD camera. The crabs showed a strong bias for blue shelter and white shelter chosen the least under the holding condition. The underwater visibility was highest in blue and white colour shelters among the six colour shelters. The observed colour preference was probably due to colour per se and may be an innate ability, rather than due to relative underwater visibility. During the observations, the shelter occupants were tolerant of intruders; there was no aggressive competition for shelter and two to four crabs shared one shelter. It was hypothesized that the touch stimulus of shelter suppresses the aggressiveness and results in low cannibalism of *S. tranquebarica*.

1. Introduction

There are four species of mud crab, *Scylla serrata* (Forskål), *S. tranquebarica* (Fabricius), *S. paramamosain* Estampador and *S. olivacea* (Herbst) that are the focus of both commercial fisheries and aquaculture production throughout their distribution. Tropical *Scylla* spp. are strongly associated with mangrove areas throughout the Pacific and Indian oceans and form the basis of substantial fishery and aquaculture operations (Keenan, 1999). They are among the most valuable crab species in the world, with the bulk of their commercial production sent live to market (Shelley and Lovatelli, 2011). Recently, mud crab farming has gained much attention in Southeast Asian countries including Malaysia.

Mud crabs are well known for their cannibalistic behaviour, which hinders rearing them at high densities in aquaculture (Fielder and Allan, 2004; Mirera and Moksnes, 2015). Cannibalism is reported as a serious problem in the grow-out of mud crab in ponds. Cannibalism is continuous throughout the nursery and grow-out cycles and the greatest constraint to productivity in all the communal growing systems (Mann and Paterson, 2004). Cannibalism during the first crab stage and onwards is a constraint in the nursery and it could be as high as 60 % within a few days (Zainoddin, 1992). Cannibalism takes place even in the wild (Alberts-Hubatsch et al., 2014). Cannibalism continues even when enough food is provided (Mia and Alam, 2006; Pereira et al., 2017). Mortality of 60 % or more during the nursery phase of *S. serrata* and *S. paramamosain* is still reported (Islam et al., 2018). In mud crab culture, research is urgently needed on the reduction of cannibalism behaviour (Cholik, 1999).

Studies have proven that presence of shelters decreases aggressive behaviour of *Scylla* spp. in ponds and tanks. *Gracilaria* (seaweed) may be effective as crab shelter, minimizing the loss of stock due to cannibalism in ponds (Triño et al., 1999). Fielder et al. (1988) indicated that the use of crab shelters increased survival by minimizing agonistic encounters between crabs. It is well understood that the lower stocking

* Corresponding author.
E-mail addresses: prof.gunzo@gmail.com (G. Kawamura), annitay@ums.edu.my (A.S.K. Yong), deborahroy97@gmail.com (D.C. Roy), leongsen@ums.edu.my (L.-S. Lim).

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density without shelters is inferior to treatment with higher stocking density and with shelters. However, the evaluation of the presence of shelters in ponds is not conclusive yet due to escape of the mud crabs from the ponds and burrowing behaviour of *Scylla* spp., which makes harvesting difficult (Fortes, 1999; Mirera and Moksnes, 2015).

Further investigations were exhorted into physical shelter quality such as type, configuration and size, reduction of cannibalism through improved shelters, and behavioural tendencies of mud crabs (Genodepa, 1999; Tan, 1999; Fielder and Allan, 2004). Many studies on shelter types and substrate complexity have been done for various decapod crustaceans (reviewed in Romano and Zeng, 2017). Colour is one of the important physical traits of shelters. Tank colour has been shown to affect physiology and behaviour of many crustaceans (Hacker and Madin, 1991; Yasharian et al., 2005; Rabbani and Zeng, 2005; Laohavisuti and Ruangdej, 2014).

In previous crustaceans studies on the shelter materials including colour, the interpretation of the results was difficult since the materials and configurations were not identical and the experiments were not designed to detect the effect of colour (Johnston et al., 2006; Mamun et al., 2010; Mirera and Moksnes, 2013; Oniam et al., 2015; Fatihah et al., 2017). In the other experiments, black, grey, white and transparent shelters were used; not true colours but with different brightness (Juarez et al., 1987; Mariappan and Balasundaram, 2003).

This study was conducted to determine the shelter colour preference of *S. tranquebarica*, a species with colour vision (Kawamura et al., n.d.). The relative underwater visibility of colour shelters was also determined to examine a possibility of colour shelter choice based on the colour contrast of shelters against the tank wall. It is proposed that application of preferred colour shelters in aquaculture would ensure the wellbeing of farmed *S. tranquebarica*.

2. Materials and methods

2.1. Ethics

All the experimental trials were conducted in accordance with the Researcher's Guidelines On Code Of Practice For The Care And Use Of Animals For Scientific Purposes Universiti Malaysia Sabah.

2.2. Crab source and holding condition

Forty eight wild captured live adult mud crab *S. tranquebarica* of both sex were obtained in a local market, Kota Kinabalu, Sabah. They were kept for acclimatization in an outdoor holding tank made of black hard solder rubber with cement floor (370 cm diameter × 50 cm height, with seawater 30 cm deep) placed in the roofed Shrimp Hatchery, Borneo Marine Research Institute, Universiti Malaysia Sabah (Fig. 1). The holding tank was divided into two zones; A-zone the shelter testing zone and B-zone as the holding zone. The half of the tank bottom, B-zone, was covered with sand and dead coral fragments in 10 cm deep as bottom substrate. Tank water was aerated throughout the holding. Four bio-filtration units filled with dead coral pebbles were haphazardly arranged in the tank (Fig. 1). They were fed with the marsh clam *Polymesoda expansa* (Mousson) ad libitum at 08:00 and 13:00 h every day in B-zone. Clam meat is the best food for *S. tranquebarica* (Marasigan, 1999).

During the first 4 days of the acclimation period, mortality was high in the holding tank due to chelipeds autotomy and unknown factor probably the after effect of stress caused by packing during a long distance transport. The shelter choice experiment was commenced on the 6th day of acclimation when mortality subsided. The number of *S. tranquebarica* survived was 35 (14 males, 8.1 − 10.6 cm in external carapace width; 21 females, 8.1 − 10.4 cm). Water temperature ranged 25.6 − 27.6 °C; salinity 31.24 − 31.42 ppt; pH 7.18 − 7.64; dissolved oxygen 4.6 − 5.2 mg / l (pH/ORD/EC/DO tester, Hanna Instruments, HI 9828, Washington, USA) during the holding.

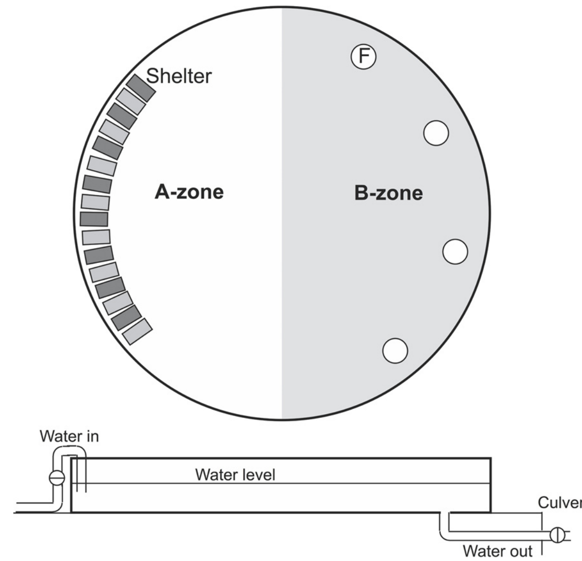

Fig. 1. Illustration showing the arrangement of colour shelters and the configuration of the experimental holding tank placed under the roofed hatchery. Bottom of B-zone was covered with sand and dead coral fragments as bottom substrate. F, biofiltration unit.

2.3. Shelter materials

In the preliminary observation in the holding tank, *S. tranquebarica* passed beside a transparent shelter (a PET bottle, 20 cm long, 15 cm internal diameter, with both sides opened) without touching and never occupied it, therefore, transparent shelter was not employed in the shelter colour choice experiment.

Forty eight polyvinyl chloride (PVC) pipes (20 cm long, 11 cm inner diameter, grey in colour) were used. Use of grey PVC pipe shelters has been practiced for *Scylla* spp. in the Shrimp Hatchery, Borneo Marine Research Institute. The whole surface of forty pipes (entire pipe) was painted either blue, green, blue, black or white (Nippon Paint, Q-Lac oil). Eight pipes were not painted and were used as grey shelters. After the paints were dried, the colour shelters were washed in running seawater for 5 d.

The light reflectance spectra of the blue, green, red, grey, black and white shelters were recorded under natural light with a spectroradiometer (HSR-8100, Maki Manufacturing Co., Ltd., Hamamatsu, Japan) in the wavelength range of 350–700 nm (Fig. 2).

2.4. Experimental protocol for shelter choice

The colour shelter choice experiment was conducted under the natural light at 07:00 to 17:00 when the minimal luminance was over 50.5 cd / m^2 which was higher than the colour vision threshold of *S. tranquebarica* (4.4 cd / m^2) (Kawamura et al., In press). Background light at the tank water surface was measured with a luminance meter (Konica Minolta LS-150, Tokyo, Japan) based on the intensity of light reflected from magnesium oxide powder with light reflectance coefficient of 0.991 (Acros Organics, New Jersey, USA) in a glass petri dish (Kawamura et al., 2018).

A group of 35 *S. tranquebarica* was tested for shelter choice by presenting a set of 16 shelters, in pairs of two different colours at a time. A set was consisted of eight shelters of one colour and eight shelters of different colour, which were arranged alternately in the A-zone of the tank (Fig. 1). The crabs were allowed to choose and occupy shelters for one hour, and then the shelters were emptied and the number of crabs in each shelter was recorded. Before each subsequent trial, half of the tank water was drained and replaced with new seawater, and the crabs positioned near the shelters in the A-zone were gently driven with a

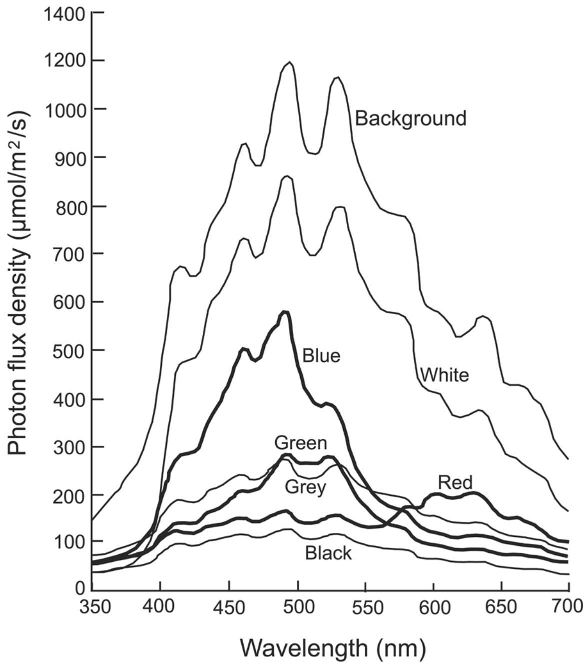

Fig. 2. Light reflectance spectra for blue (with peak wavelength at 501 nm), green (525 nm), red (634 nm), grey, black and white shelters used in the experiment, and spectral light intensity of natural background light. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

plastic besom towards the B-zone to avoid choice of the closest shelter and ensure the choice by shelter colour. This procedure was repeated five times for each colour pair. The pairing of two colours was made at random using a die and all the 15 possible colour pairs were tested. After the last trials of the day, the shelters were left overnight in the tank. During the shelter choice observations for 15 d, two individuals died due to unsuccessful molting and one molted shell was collected. The lost individuals were not taken into account in data analyses.

2.5. Underwater visibility of colour shelters

The relative visibility of the colour shelters was visually judged through a waterproof CCD camera (OLYMPUS TG-5, Shanghai, China) in the holding tank. Six shelters of different colours were arranged in perpendicular to the black tank wall in the A-zone, and photographed at around noontime with the camera at 25 cm intervals in the same water condition with that during the shelter choice experiment. The camera was hand held in front of the shelters.

2.6. Data analyses

Quantitative analysis of the number of *S. tranquebarica* occupying the shelters was performed using the χ^2-test and Thurstone's law of comparative judgment (Thurstone, 1927). The numbers of crab occupying shelters were summed over the five trials for each colour of a colour pair, and then transformed into proportions, and the z-scores were obtained from the table of cumulative probabilities of the standard normal distribution. The relative preference of *S. tranquebarica* for different colours was deduced from the mean z-score of each shelter colour. A negative z-score is below the mean, a positive z-score is above the mean, and a higher z-score shows a higher preference for a colour (Kawamura et al., 2010). Thus, z-scores imply the magnitude of relative preference. For statistical comparison of the mean z-sores, 95 % confidence interval was calculated for each mean z-score.

Table 1
Sumary of number of crabs occupied colour shelters presented as colour pairs in the test tank.

	Number of crabs by colour pair (left colour:top colour)				
	Grey	White	Green	Blue	Red
Black	31:26	31:7***	23:27	31:49*	21:27
Grey		30:6***	21:39**	19:36**	18:27
White			22:24	28:38	22:26
Green				21:38*	30:16*
Blue					40:24*

Notes: *, ** and *** denote significances at $\alpha = 0.05$, $\alpha = 0.01$ and $\alpha = 0.005$, respectivey (χ2-test).

3. Results

3.1. General behaviour of crab

In the tank, *S, tranquebarica* were distributed over the bottom and several individuals burrowed themselves in the substrate in the B-zone. They were sedentary but often exhibited aggressive contacts and chasings. After setting shelters, some of them immediately approached to the shelters. They accepted and occupied all colour shelters without hesitation. They showed no aggressive competition for shelter and shelter occupants were tolerant of intruders; neither of evicting an intruder by an occupant or evicting an occupant by an intruder, and two to four crabs shared one shelter. Several pairs of male and female in courtship were observed in the shelters; a male cradling a female but released her on emptying the shelter.

3.2. Colour shelter choice

The results of the colour shelter choice are summarized in Table 1. Of all the 15 possible colour pairs, significant biases were seen between eight colour pairs. Of the five pairings of blue shelter with other colours, significant bias toward the blue shelter was evident in four pairings. The black − grey, black − green, black − red, grey − red, white − green, white − blue, and white − red pairs indicated no preference for either of them.

The mean z-score was significantly highest for blue shelter (Fig. 3), indicating the highest preference of *S. tranquebarica* for blue. The z-scores for green and black were about the same (0.112 and 0.094 respectively), and also for red (−0.038) and grey (−0.036). The white shelter had least z-score (−0.444) indicating the least preference for white.

3.3. Underwater visibility of colour shelters

The photographs of six colour shelters taken in the holding tank are shown in Fig. 4. The images of colour shelters faded with distance. The black shelter was not seen, and colour of red, grey and green shelters was not discriminated at distance 150 cm. The blue, red, grey and green shelters were not detectable at 175 cm. While the blue and white shelters were still visible, the other colour shelters were totally undetectable at 200 cm. Thus, the blue and white shelters were most visible among the six colour shelters in the holding tank condition.

4. Discussion

This study clearly demonstrated for the first time that *S. tranquebarica* detected and preferred different colours of shelter under the experimental condition. The crab accepted all colours without hesitation but preferentially occupied blue shelter, and did not clearly distinguish green from black. The crab preferred white shelter the least. The observed relative shelter colour preference cannot be explained by the brightness of the shelters. The level of spectral reflectance was

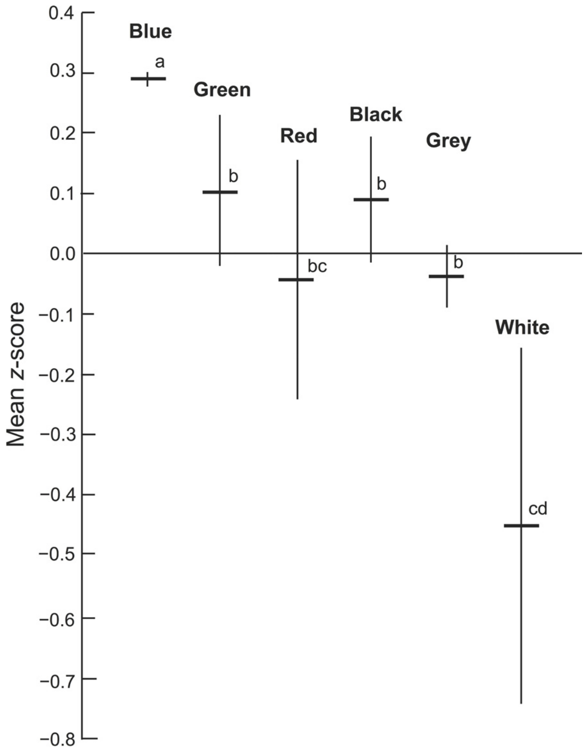

Fig. 3. Mean z-scores for the occupation of *Scylla tranquebarica* in different colour shelters. Blue shelter is preferred the best and white shelter is preferred the least. Note: vertical bar; 95 % confidence interval. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

much higher for white shelter than for green one, and much higher for green shelter than for black one.

S. tranquebarica has colour vision (Kawamura et al., in press), but they could distinguish between colours as different colours but as different levels of underwater visibility. The underwater visibilities measured in the tank showed that the blue and white shelters were much more visible compared with green, red, grey and black shelters. The mean z-scores obtained in the shelter colour choice test showed that *S. tranquebarica* preferred the blue shelter best and the white shelter least. Thus the possibility of colour shelter preference based on the level of underwater visibility is ruled out. The observed colour preference was probably due to colour *per se*. Kawamura et al. (1996) examined the effect of colour of fish aggregation devices on the fish aggregations in the sea and reported that more attractive colours, blue and green, were the less visible ones and most visible colour, white, was least attractive, thus, and concluded that fish were attracted on the basis of hue rather than contrast or visibility.

Colour preference seems to be species specific in crustaceans. The hermit crab *Litorina* sp. tends to choose lighter colour shells (Rellinger et al., 2004), but in *Calcinus tubularis* (L.) dark colour crabs chose dark shells and light colour crabs chose light colour shells (Pessani and Tirelli, 2006). The giant freshwater prawn *Macrobrachium rosenbergii* prefers blue food colour over yellow, red, grey and black food (Kawamura et al., 2016; Yong et al., 2018), while the whiteleg shrimp *Litopenaeus vannamei* (L.) prefers yellow food over blue, green, red and black food regardless of background colour (Kawamura et al., 2017a). Colour vision of these two species has been determined behaviourally (Kawamura et al., 2017a). Background colour preference changes with growth. Post larvae of *M. rosenbergii* prefer black background (Kawamura et al., 2017b), while adults showed no difference in occupancy among black, red, and orange polyethylene cylinders (dos Santos et al., 2015d).

It is interesting to know whether the colour preference in the purple

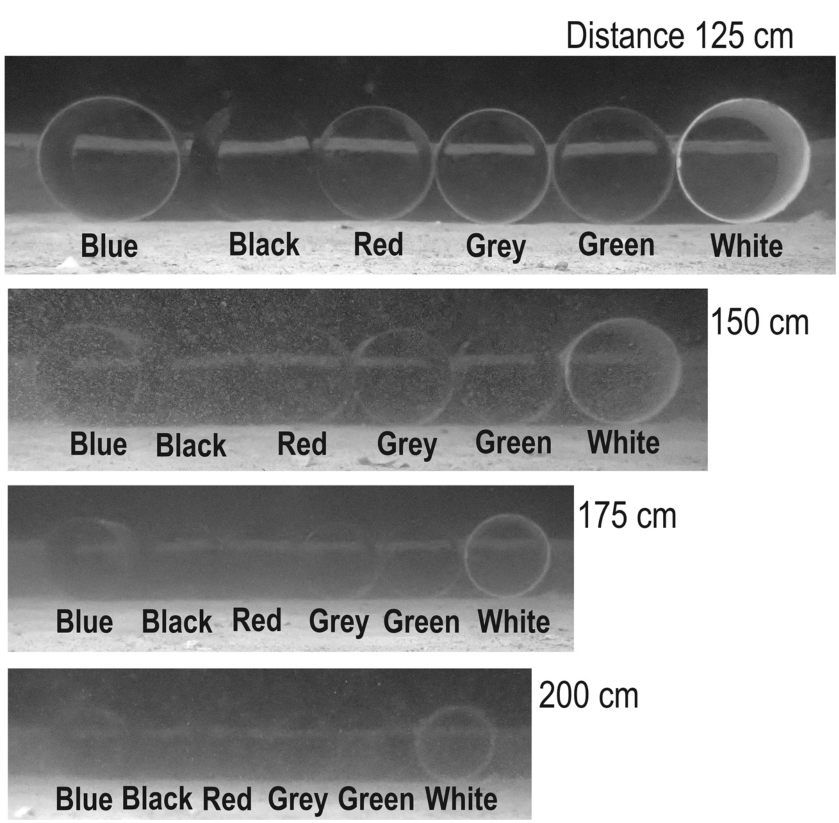

Fig. 4. Underwater photographs of colour shelters, taken at different distances. White and blue shelters were most visible among the six colour shelters. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

mud crab is innate or learned by association. In food colour preference in fish, Protasov (1970) suggested that the selection of the baits according to colour is not inherent and that the ability develops during the early stages of growth. *S. tranquebarica* forage on crustaceans, molluscans and fishes (Nesakumari and Thirunavukkarasu, 2014) both day and night (Ikhwanuddin et al., 2012). Zoea and megalopa larvae of *Scylla* spp. are zooplankton feeder (Williams et al., 1999; Baylon, 2009). If colour preference is developed during the larval stages, *S. tranquebarica* might learn preference for transparent prey given that most oceanic zooplankton are transparent (Johnsen and Widder, 1998). Kawamura et al. (2010) examined the food colour preference of five marine fishes of the family Sparidae in four different holding conditions and reported that food colour preference for Antarctic krill dyed yellow over those dyed blue, green, red and black, and stated that this food colour preference is innate ability. An innate colour preference has been reported for many other animals especially insects and birds (reviewed in Lunau and Maier, 1995)

Shelters not only provide protection from predation but also likely to suppresses the aggressiveness of *S. tranquebarica* in the shelters. During our observations, the shelter occupants were tolerant of intruders; there was no aggressive competition for shelter and two to four crabs shared one shelter. Nakata and Goshima (2003) reported the aggressive competition for shelter occupation in two crayfish species *Cambaroides japonicus* (De Haan) and *Pacifastacus leniusculus* (Dana). The aggressive shelter occupation behaviour was also reported for the freshwater prawn *Macrobrachium nobilii* (Henderson and Matthai) (Mariappan and Balasundaram, 2003) and the European lobster *Homarus gammarus* (L.) (Aspaas et al., 2016). However, aggressiveness of *S. serrata* was reported to reduce in the presence of shelters (Mann et al., 2007; Mirera and Moksnes, 2013). Genodepa (1999) observed during harvest that each hole contained five to six *S. serrata* and stated that this situation perhaps further increased the incidence of cannibalism. On the other hand, Kawamura et al. (2017b) stated that the mechanical stimulus of the shelter likely suppresses the stress-induced aggressiveness of the giant freshwater prawn *M. rosenbergii*; postlarvae occupying the plastic netting shelters were sedentary and exhibited no aggressive contact in the shelter while they did frequent aggressive contacts out of shelters. Thus, shelters might functioned as a suppresser for the aggressiveness of the post larvae. Therefore, we hypothesize that the touch stimulus of shelter suppresses the aggressiveness and results in low cannibalism of *S. tranquebarica* in confined conditions.

In Asian countries, for fattening or soft-shelled crab production purpose, commercial individual farming of mud crabs is practiced to eliminate contact with one another (Islam et al., 2018). In this farming system, the crabs are confined in small containers; outdoor floating plastic basket or indoor plastic boxes, each contains a single mud crab. Captivity with restricted movement in a small container gives stress to the captive animals of a variety of species (reviewed in Morgan and Tromborg, 2007; McGaw and Nancollas, 2018 and long-term captivity does not mitigate the stress response (de Assis et al., 2015d). Stress causes appetite reduction and reduced food intake in crustaceans and fish (Bonga, 1997; Teegarden and Bale, 2008; Conde-Sieira et al., 2008; Elwood et al., 2009; Wale et al., 2013; Conde-Sieira et al., 2018). Islam et al. (2018) reported that the weight gain was significantly lower in *S. tranquebarica* reared individually in indoor compartments compared with those communally reared in an outdoor tank. The poor weight gain might be due to the captivity stress. Provision of blue, which is favourite shelter colour for *S. tranquebarica*, compartments would mitigate the captivity stress of the crab. According to Conte (2004) and Kawamura et al. (2017c), the internal background colour of culture tanks and the reflective nature of light often act as stressors that affect fish, and fish growth can be improved by altering the background colour in the tank.

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Declaration of Competing Interest

None.

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