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EFFECTS OF SUBSTRATE COLOUR PREFERENCE ON GROWTH OF THE SHRIMP *LITOPENAEUS VANNAMEI* (BOONE, 1931) (DECAPODA, PENAEOIDEA)

BY

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ABSTRACT

We tested the substrate colour preference and its effect on the specific growth rate (SGR), food intake and feeding efficiency (FE) of the shrimp *Litopenaeus vannamei* (Boone, 1931). Preference was tested in 50 cm diameter circular tanks divided into 4 lateral compartments, each filled with 2 cm depth of fine-grain, coloured sand (yellow, blue, green and red). The respective walls of each tank were also kept with the same colour, using coloured paper. Tanks were filled with 10 l of water (salinity of 4) and illuminated with fluorescent light ($n = 35$). Tanks with natural sand (without a specific colour) were used as controls ($n = 6$). Thirty-five shrimps were observed individually for three days at 7, 10, 13 and 16 h, for 20 min each time. Visit frequency to each compartment was registered every 2 min. Shrimps did not show any preference for a specific colour substrate for the first two days, however, there was higher preference for the yellow and red substrates on the third day. Control shrimps did not show preference for any compartment. To test the effects of substrate colour on feeding rate and growth, 25 shrimps were isolated for 60 days in a 15-l aquarium covered with coloured paper on the walls and filled with 2 cm of coloured substrate (yellow, blue, green, red and natural sand, $n = 5$ for each colour). Shrimps were fed daily and uneaten food removed and recorded. Shrimps were weighed every 10 days. Feeding rate was higher in the red environment than in blue or green environments, and FE was also higher for red than for blue shrimps group. SGR was higher in red and yellow environments. These results suggest that yellow and red substrate enhance the shrimps' visual perception and food detection, and thus supports the finding that these substrate colours can improve FE and SGR of cultivated shrimps.

RESUMO

Testamos a preferência por coloração do substrato e seus efeitos sobre a taxa de crescimento específico (SRG), ingestão alimentar e eficiência alimentar (FE) do camarão *Litopenaeus vannamei*

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(Boone, 1931). A preferência foi testada em tanques circulares de 50 cm de diâmetro com 4 compartimentos preenchidos com 2 cm de areia fina colorida (azul, amarelo, verde e vermelho). A parede de cada compartimento foi forrada com papel da mesma cor do substrato. Os tanques foram cheios com 10 l de água (salinidade de 4) e iluminado com luz fluorescente, $n = 35$. Sedimento natural foi usado para o controle ($n = 6$). Os 35 camarões foram individualmente observados quanto à frequência nos compartimentos por 3 dias, às 7, 10, 13 e 16 h, durante 20 min em cada período. A frequência de visitação foi registrada a cada 2 min. Não houve preferência pelos compartimentos nos primeiros dois dias, porém no dia 3, os camarões permaneceram tempo maior nas cores amarelo e vermelho. No teste controle não houve preferência entre os compartimentos. No teste de crescimento e ingestão, 25 camarões foram isolados em aquários de 15-l forrados com papel colorido e preenchidos com 2 cm de areia colorida (azul, amarelo, verde, vermelho e areia natural, $n = 5$ para cada cor) por 60 dias. Os camarões foram alimentados diariamente e o restante de comida foi retirado e contabilizado. Os camarões foram pesados a cada 10 dias. Houve maior ingestão alimentar no substrato vermelho do que no ambiente verde e azul, e a FE foi maior para o grupo no vermelho do que no azul. A SRG foi maior no substrato vermelho e amarelo. Estes resultados indicam que os ambientes com coloração vermelha e amarela melhoram a acuidade visual e a detecção de alimento por *L. vannamei* e, portanto, embasam o fato de que ambientes coloridos podem melhorar a FE e a SRG dos camarões em cultivo.

INTRODUCTION

Colour perception is a helpful feature for animals to be able discriminating details in their environment. Many animals exhibit a set of photoreceptors in their retinas that seems to match well the photic characteristics of their natural habitats (Lythgoe, 1979; Chiao et al., 2000), which impart advantages for navigation, prey recognition and capture, defence, mating and communication (Wheeler, 1982; Cronin, 1986; Cronin & Jinks, 2001; Nunes & Andreatta, 2010). Colour vision is critically important for animals that inhabit places where partial or full spectrum of sunlight can be found (Brown, 1957; Caldwell & Dingle, 1975; Pitcher, 1993). Many species of crustaceans have the ability of true colour vision (Marshall et al., 1996), with rhabdoms containing more than ten different visual pigments, allowing them to discriminate from short ultraviolet to far red ranges (Cronin, 1985; Marshall & Oberwinkler, 1999), a clear evolutionary advantage in habitats where light is made up of many colours.

Since shrimp exhibit morphological and neural structures to see colours (Marshall et al., 1996; Marshall & Oberwinkler, 1999), this ability may modulate the animals' physiological and behavioural processes, affecting their survival and fitness. Some studies have demonstrated colour influence on biological processes of crustaceans and other aquatic animals. For instance, in the prawn *Palaemon serratus* (Pennant, 1777) food assimilation is affected by different colours of light (Van Wormhoudt & Ceccaldi, 1976), and the mantis shrimp *Gonodactylus smithii* Pocock, 1893 can detect and respond to conspecific colour signals (Chiao et al., 2000). Other studies investigating the effects of colours suggest that blue and green light have the same effect on maturation of the shrimp *Penaeus* (*Fenneropenaeus*) *indicus* H. Milne Edwards, 1837 (Emmerson et al., 1983) and green and natural illumination produce a larger spawning volume (Primavera & Caballero, 1992). However, these studies only focused on blue, green and white colours.

In spite of this, related effects are not similar for all species, and even the inverse could be demonstrated, indicating a species-specific effect. Hence, this illustrates the necessity of determining colour preferences for each species; a reasonable approach could be using preference tests, wherein the animal can choose among some offered parameters (Dawkins, 1998; Galef & Whiskin, 2004). Preference tests have been widely used to indicate the best environmental conditions that an animal might choose in a specific situation. To make a choice, one presupposes that the animal is able to discriminate between pleasant and unpleasant conditions, and the chosen condition may be the one that best fits the animal's preference (Gonyou, 1994).

For benthic invertebrates, such as shrimp, substrate has been recognized as an influential driving factor for survival (Dall et al., 1990). Substrate is not only their main foraging location, but also their source of shelter, since shrimp burrow into the substrate to avoid predation. Substrate characteristics can affect shrimps physiology and behaviour. Therefore, substrate colour preference tests can be a useful way to better understand how shrimps recognize their environment and which colour of the substrate may represent a better habitat.

Under rearing conditions, different substrate characteristics may affect the performance of shrimps, influencing for example predator perception and avoidance, or food perception and feeding rate (Dall et al., 1990). Thus, optimal substrate colour may improve growth and productivity of intensively cultivated species. Feeding rate and growth can be regarded as valuable variables for estimating general performance and well-being of animals under cultured conditions, but these variables are also the most important for economical profitability of an aquaculture operation. Thus, the substrate colour that improves fitness may allow the shrimp to spend more energy on growth than in unsuitable conditions. Despite the possible positive effects of certain substrate colours on food intake and growth, this area has received surprisingly little attention among crustacean researchers.

Therefore, the aim of this study was to test the substrate colour preference of the shrimp *Litopenaeus vannamei* (Boone, 1931) in a four-chambered test tank and, subsequently, to test the effects of the preferred substrates on growth and feeding rate. The four substrate colours used in the experiments were chosen from the two ends of the visible spectrum of light (blue and red), while green and yellow were chosen to represent intermediate colours. It was hypothesized that preference or avoidance of certain substrate colours would affect shrimps performance when used for rearing.

MATERIALS AND METHODS

The shrimp *Litopenaeus vannamei*, originating from the national producer Aquarium-Aquicultura do Brasil LTDA, were held at the laboratory of the Departamento de Ciências Animais, Universidade Federal Rural do Semiárido in a plastic stock tank (water volume 1000 l), supplied with well filtered water (27°C) with a salinity of 4, exposed to 12L:12D photoperiod (ca. 50 lux at the water surface) and fed with commercial shrimp food (Fri Aqua 35PB, 35% protein, FriRibe®, Ceará, Brazil).

All different substrate used for the experiments (coloured sand) were obtained from local pet stores and natural sand was collected at the experimental university farm, where the experiments were developed. The coloured sand was classified by its chromaticity parameters, using the RGB (Red, Green, Blue) system, since this factor has absolute character and quantitative consistence (Motoki et al., 2006). Thus measured, the chromaticity parameters for the sand used for this study are: blue 99.4 ± 44.3, green 83.4 ± 47.1, red 123.6 ± 57.9, yellow 130.4 ± 44.4 and natural sand 126.0 ± 37.2.

Substrate colour preference trial

For the substrate colour preference tests, circular grey plastic tanks (50 cm diameter) were divided into four lateral compartments of similar size (approximately 350 cm^2) with a hole in the central region to allow the shrimps to freely move among compartments. The tank was supplied with 10 l water, and one air stone provided oxygen in the middle of each tank. The laboratory was illuminated by fluorescent tubes kept at a 12L:12D photoperiod. Different sand colours (blue, green, yellow and red) were randomly chosen for each compartment that was filled with 2 cm depth of the fine-grain sand. The respective walls of the tank were also made the same colour using coloured plastic paper fixed on the inside of the tank wall. Light intensity was set around 50 lux at the surface of the water.

Substrate colour preference of individuals (1 shrimp/tank) was observed for a period of three days. Each shrimp was placed in the experimental tank one day before the beginning of observations. During this period, shrimp visit frequency in each compartment was observed to check for a possible preference among environmental colours. Visit frequency was directly observed by checking the compartment location of the shrimp at 2-min intervals for 20-min periods at 7, 10, 13 and 16 h, making a total of 40 observations per day. Food was not offered during the experimental days in order to prevent any stimulus that could propel the shrimp to choose one specific compartment due to any other driving force than environmental colour. A total of 35 shrimps was individually observed.

As a control trial, 6 shrimps were individually observed in the same experimental tank, where all four compartments were filled with natural fine sand and the walls were kept white. These shrimps were observed for three days, four periods per day, as described above. During this period, shrimp visit frequency in each compartment was observed to check for a possible preference between natural sand compartments. Each individual shrimp was tested only once. All shrimp observed were weighed at the end of three experimental days (mean 5.22 ± 1.27 g).

For statistical analyses, a non-parametric Friedman ANOVA was used to analyze multiple group visit frequency. A Friedman test was used because shrimp preference for one compartment over of the others violates assumptions of independence. In cases where the Friedman test was significant ($p < 0.05$), the non-parametric Student-Newman-Keuls post-hoc test was used to determine significant differences among compartments (Zar, 1999).

Growth trial

The sides of 25 aquaria (40 cm × 20 cm × 25 cm; water volume 15 l) were covered with blue, red, yellow, green or white plastic paper and filled with 2 cm of fine-grain sand of different colours (blue, red yellow, green and natural), illuminated by white fluorescent tubes set at around 50 lux (5 aquaria of each colour), with a photoperiod of 12L:12D. The aquaria were supplied with filtered and aerated 27°C water (pH 7.2, salinity 4). Air was also provided in each aquarium through air stones connected to aquarium air pumps.

Shrimps were reared individually in the aquaria for 60 days (7 December 2009-4 February 2010) after being allowed to acclimatise to the aquarium conditions for 7 days. Shrimps were fed by hand twice per day (at morning and evening) with commercial dry food (Fri Aqua 35% protein, FriRibe®). Food (around 10% of shrimp weight) was placed on a small plastic plate and remaining food was siphoned out and counted 60 min after delivery. As the mass of one pellet was calculated from 10 samples of 50 pellets, it was possible to estimate the amount of food eaten in each aquarium by subtracting the number of uneaten pellets from the fed amount. The wet mass (to 0.1 g) of every individual was measured at the beginning of the experiment and then every 15 days thereafter.

Specific growth rate ($\mathrm{SGR} = 100 \times (\ln W_2 - \ln W_1)/t$, where W_1 and W_2 are the mass (g) at the beginning and end of the experimental period, respectively, and t is the time in days), food intake and feeding efficiency (FE = mass gain/food intake) were calculated. A one-way ANOVA was used (after testing for the homogeneity of variances and normality) to compare the means of measured and calculated parameters of the five different substrate colours. Post-hoc comparisons between sample means were performed using the Student-Newman-Keuls test and $p = 0.05$ was used as the level of significance.

RESULTS

Substrate colour preference trials

Litopenaeus vannamei from the control preference tests (compartments with natural sand) showed equal distribution among the compartments on the three days of testing (Friedman ANOVA, day 1: $\chi^2 = 1.62$, $p = 0.65$; day 2: $\chi^2 = 3.78$, $p = 0.28$; day 3: $\chi^2 = 2.10$, $p = 0.55$) and the shrimps showed no preference for any specific compartment.

During the first and second day of observation in the substrate colour preference experiment (compartments with colour sand) shrimps showed no preference for any of the compartments (Friedman, day 1: $\chi^2 = 6.55$, $p = 0.08$; day 2: $\chi^2 = 5.68$, $p = 0.13$; fig. 1a and b). During the third day, shrimps had significantly higher visit frequency to yellow and red sand compartments, while blue and green sand compartments were avoided (Friedman, $\chi^2 = 10.34$, $p = 0.018$; fig. 1c).

Growth trial

Shrimp weight increased from an average (± S.D.) of 3.75 ± 0.4 g to 6.22 ± 0.2 g during the 60-day experiment. At the end of the experiment there were significant differences among treatments both in final shrimp biomass (ANOVA, $F = 6.84$, $p = 0.02$) and specific growth rate (ANOVA, $F = 7.06$, $p = 0.002$). Post-hoc comparisons indicated that the shrimps reared in red and yellow sand tanks were significantly bigger than the shrimp in blue and green sand tanks, but not different from shrimp reared in natural sand (control) (fig. 2a). Mean food intake was significantly higher in the red sand tanks than in the blue and green sand tanks (ANOVA, $F = 4.86$, $p = 0.01$) but not different from yellow and natural sand tanks. Feeding efficiency was lower in blue and green sand tanks compared to red sand tanks (ANOVA, $F = 6.85$, $p = 0.002$), while yellow and natural sand tanks did not differ from red sand tank (fig. 2b).

DISCUSSION

In this study, the shrimp *Litopenaeus vannamei* preferred yellow and red substrate colours (fig. 1) and showed higher food intake and growth rates when reared on these colour substrates than in blue and green substrate tanks (fig. 2), the colours avoided during the preference trials. Therefore, food intake and weight gain were positively correlated to preferred substrate colour. This result suggests that substrate colour should be taken into account when developing an economically sound culture for this shrimp species.

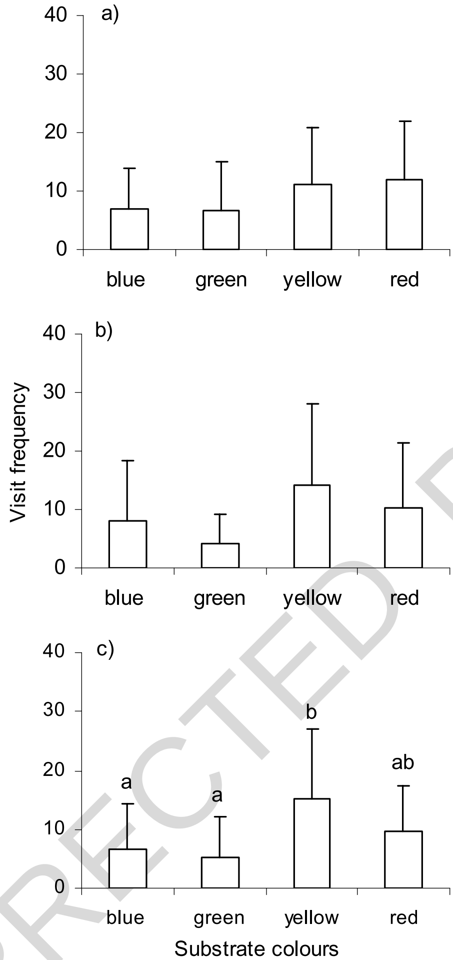

Fig. 1. Preference of the shrimp *Litopenaeus vannamei* (Boone, 1931) for blue, green, yellow and red substrate colours ($n = 35$). Bars represent the mean (± S.D.) visit frequency of 20-min observation periods at 7, 10, 13 and 16 h over 3 consecutive days: a, day 1; b, day 2; and, c, day 3. Statistical difference of shrimps visit frequency in each compartment is indicated by different lower case letters (Friedman, $p = 0.016$).

Other studies on colour effects on shrimps show that natural light colour improves growth and food intake, followed by green and yellow, while a blue environment reduces performance of Chinese shrimp (*Fenneropenaeus chinensis* (Osbeck, 1765)) (cf. Wang et al., 2003). Although studies on the effects of colour on shrimp are rare, this area is relatively well-documented for fish. Some studies have shown effects of red light or tank colour on fish. Ruchin (2004) found a negative effect of red on the growth rate of three different fish species, and Luchiari & Pirhonen (2008) recorded a lower growth rate of rainbow trout in a

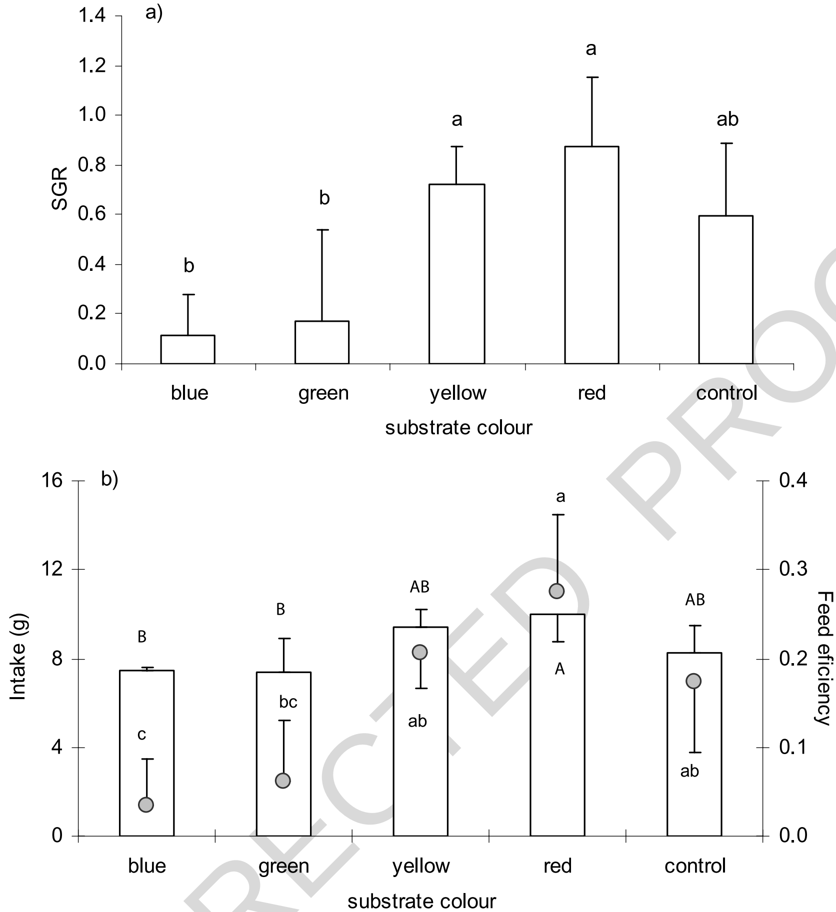

Fig. 2. Growth parameters of the shrimp *Litopenaeus vannamei* (Boone, 1931) after 60 days under different colours conditions. Background and substrate colours used were blue, green, yellow, red and natural sand ($n = 5$ for each colour). a, Bars represent the mean (± S.D.) specific growth rate (SGR); values denoted by different letters indicate statistical differences among colours (ANOVA, $p < 0.05$); b, bars correspond to mean (± S.D.) intake and circles denote mean (± S.D.) feed efficiency; different capital letters means statistical differences among intake in colours (ANOVA, $p < 0.05$) and different small case letters means statistical differences among feed efficiency in colours (ANOVA, $p < 0.05$).

red than in a green environment. On the contrary, Luchiari et al. (2009) showed the advantageous effects of a red environment for pikeperch in term of food intake and growth, even though this species showed no preference for colour in a preference test. Thus, the present results of the benefits of a yellow/red substrate for the shrimp *L. vannamei* reinforce the need for investigation of the colour preference and effects for different species of interest for cultivations. Undoubtedly, the cited species are sensitive to different colours, which was the result of adaptations to their natural environment.

Zheng & Zhang (1985) showed that white shrimp (*Fenneropenaeus penicillatus* (Alcock, 1905)) are sensitive to colours near 490 and 570 nm of the visible spectrum, seen as green and yellow colours. This species lives on muddy bottoms in shallow water to a maximum of 45 m (Boyd, 1997), and may have developed photo-receptor cells able to capture photons most common in this environment. In fact, coastal waters and waters with abundant plankton and organic matter absorb blue light to a great extent and alter the transmitted light from blue-green to green-red (McFarland, 1986; Johnson et al., 2002).

According to Loew & Lythgoe (1978), animals' retinas almost always contain cell pigments well matched to the ambient light in which they are found, in addition to some cone cells containing pigments that are offset to shorter wavelengths from the maximum water transmission. Thus, the results of this study for *L. vannamei* suggest that background colours of yellow and red may allow better visual perception and improve life quality and fitness. Even though types of pigments found in *L. vannamei* retinas are not yet documented, it is very likely that pigments capable of medium and long wavelengths would be found.

According to Cronin & Jinks (2001), filter pigments in decapod eyes may have yellow, orange or red bases developed from pigments in the larval retina that change only in proportion during the growing stages. Loew & Lythgoe (1978) and McFarland & Munz (1975) stated that vision is much improved in places where background matches the main pigment colour present in the eye, because visual sensitivity increases when visual pigments absorb a higher amount of photons. This condition may have been achieved in red and yellow substrate conditions.

The better an animal can see in a certain environment, the better it is able to forage. If the food cannot be seen against the background, it will be difficult to find and may result in poor food intake and growth. If this is the case, shrimps kept in blue and green environments could not easily find food (dark brown pellets) for the period it was available, resulting in a low feeding rate and smaller shrimps.

In relation to food intake, *L. vannamei* ate around 4.5% of its body weight on yellow, red and control substrates, versus around 1% on blue and green substrates, with the highest food ingestion in the red substrate group (9.9 ± 1.2 g). Similar results were found by Yasharian et al. (2005) rearing the caridean prawn *Macrobrachium rosenbergii* (De Man, 1879) in a red environment. In addition, since there was a significant positive correlation in FE with weight gain and a clear increase in shrimp weight and SGR during the experiment, it appears that the shrimps studied here grew well. The increase in growth performance of *L. vannamei* under red substrate conditions may be related to the colours that most mimic the natural environment in which the species evolved (brown, grey and orange) and may be more productive than substrate colours that are rarely found in nature (blue and green). This appears to be the trend that this study follows. Additionally, the natural environment where *L. vannamei* is found is typically turbid with low visibility and high amounts of substrate (Visscher & Duerr, 1991), where no light of wavelength below 500 nm can penetrate. Thus, the perception and preference for yellow and red colours better match the light conditions present in natural environment, which may improve FI and growth in such places.

Under the controlled conditions in this study, growth rate was intermediate between the blue-green substrate and the red-yellow substrate conditions. It must be noted here that the growth rate was not significantly different from the control and red-yellow groups, although the growth rate increased significantly from blue-green to red-yellow substrate conditions.

In conclusion, the best rearing conditions for *L. vannamei* should be yellow and red substrates, as suggested by our results, in addition to dim light conditions, according to Rodriguez & Naylor (1972). Support for the original hypothesis was provided as substrate colour preference of *L. vannamei* shrimp matched the results of the improved feeding efficiency and growth. Therefore, the use of substrate that match red and yellow is recommended, while green or blue substrates colours should be avoided to improve growth in culture systems of penaeidean shrimps. This result may allow commercial shrimp farmers to better choose their substrate (mainly using the RGB system) in order to apply substrates that match yellow and red colours. Thus, it will probably increase growth and decrease production cost.

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