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Colour preference and light sensitivity in trilobite larvae of mangrove horseshoe crab, *Carcinoscopius rotundicauda* (Latreille, 1802)

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The trilobite larvae of *C. rotundicauda* were tested to determine their colour preference and light sensitivity until their first moulting (25 days post hatching) under laboratory conditions. Maximum congregation size of the trilobite larvae was found in the white zone respectively where (n= 12) followed by yellow (n= 8) and orange (n= 8), which showed the larval preference for lighter zones. Morisita's index calculation showed a clumped/aggregated distribution (yellow, blue, orange and white) and uniform/hyper dispersed distribution (green, red and black) for various colours tested. Trilobite larvae showed least preference for brighter regions while tested in the experiment [black; (n=4) and red; (n=5)]. Experiments done to determine the light sensitivity of trilobite larvae showed that the larvae had more preference towards ultraviolet lights. The maximum congregation size of 38.8 and 40.7% of the larvae was encountered under ultraviolet light, when the light sources were kept horizontal and vertical, respectively. Overall, results suggested that the trilobite larvae of *C. rotundicauda,* preferred light source of shorter wavelengths (UV light) and colours of lighter zone (white, yellow, orange), which might be due to their adaptation to their natural habitat for predator avoidance, prey selection and water quality.

Keywords: Animal behaviour, Colour vision, Horseshoe crab, Light sensitivity, Mangrove, Trilobite larvae

Vision is one of the most important biological activities which help organisms to identify the shape, size and location of any object including colour[1]. All biologically relevant information (orientation or navigation, reproduction, foraging, object recognition, signal detection and discrimination, contrast enhancement and camouflage breaking) used in vision is based on the perception of light and colour. Both, these factors exist in parallel and are strictly species specific[2]. Sensitivity to light or chromatic factor in animals is adjusted constantly by adaptations to the ambient stimulus[3] and varies accordingly along the dimension, direction, intensity, wavelength and polarization[2]. Thus, the behaviour of an animal is strongly influenced by the visual information available for processing. The capacity to discriminate colours varies among species, due to different behaviours, life style and habitat, which imposes different visual tasks[4]. This ultimately affects their survival, growth, reproductive success and also the fitness in an evolutionary sense[5]. Thus, vision has a topography which depends on the structure of the environment and on the life stage of the animal[6-11]. Indeed, the advantages of visual systems are often employed by marine arthropods, especially in crustaceans for various purposes like; orientation, predator avoidance, shelter and habitat location, prey selection and con-specific identification[12]. Horseshoe crabs are one such marine arthropod which has efficiently utilized its compound eye for various life activities[13-16].

Adult horseshoe crabs *Limulus polyphemus*, have long served as an admirable model for vision research and has provided excellent information's on the physiological and behavioural mediated mechanism in vision[14]. Many behavioural studies have shown that vision plays an important role in their mating, where studies have shown that the male use vision underwater to locate and attach to females[13,17-19]. In contrast to the extensive number of studies on the visual system of adult horseshoe crabs, however, there

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is a scarcity of information on the behavioural responses of horseshoe crabs to visual stimuli in early life history stages.

Though a few reports are available for the role of the lateral and median eyes of horseshoe crabs in visual guidance, almost all these studies were basically focused on only *L. polyphemus*[20-22]. Previous laboratory observations have revealed that the newly hatched larvae of *Limulus* were positively phototactic[23,24] whereas, older larvae tend to be negatively phototactic[24-26]. Wolf and Zerrahn-Wolf[27] showed that the young *Limulus* larvae were attracted to flickering lights and the attractiveness showed a positive increase depending on the size of the field and with the frequency of flickers. When *Limulus* larvae were stimulated by 2 lights, they swim in the direction such that light intensities at the 2 lateral eyes are equal[24]. Young *Limulus* larvae were found to walk in circles under bright light, when one of their eyes was covered with black paint[25]. The lateral eyes and dorsal ocelli in *Limulus* were found effective in visual orientation, where median ocellus showed a positive photo tactic response for U.V. light[21]. Rudloe[28], observed a lunar rhythm of responsiveness to light by *Limulus* larvae under laboratory studies. A recent study has reported the visual orientation in early life history (larvae and juvenile) of *L. polyphemus*[12] and they have suggested that the visual orientation changes upon exposure to habitat and predator cues. They have also observed that the direction of the response undergoes an ontogenetic shift following metamorphosis.

Carcinoscorpius rotundicauda—is one of the four species of horseshoe crab which is found in the inshore waters of the Indo-West Pacific region. This is the only species among the four other species which is associated with the mangrove mudflat ecosystem[29]. The Singapore red data book, released in November 2008, has reported this species as vulnerable[30]. For spawning, adult *C. rotundicauda* migrates from the offshore continental shelf to intertidal sandy mud beaches and mangrove area during full and new moon phases[31]. The eggs of *C. rotundicauda* in their breeding ground hatches approximately after 5-6 weeks and the time may vary depending on the ambient temperature and other environmental factors[32]. After hatching the larvae live in the silty mudflat of the mangroves[33] before commencing their benthic life[34]. The larval and juvenile phases of the horseshoe crab life cycle is of equal interest. While, juvenile and adult horseshoe crabs are morphologically similar, the larval (post hatching) instar is morphologically more specialized and unlike than all other subsequent stages[28].

The newly hatched larvae of horseshoe crabs encounter numerous risks during their journey from the nest, which is located supratidally and beneath the surface of the sand/mud, to the water. Once this transition is achieved the animal changes vastly both behaviourally and morphologically from the larval stage. In fact, all the early stages of horseshoe crab until their juvenile stages are important predators of the sandy intertidal community in areas adjacent to adult breeding beaches[28,35]. Information regarding the behaviour or the phototactic response of any other species of horseshoe crabs other than *L. polyphemus* in their larval form has not been well documented. In the present study the focus is to render a clear outline of the colour preference and light sensitivity in the larval stages of *C. rotundicauda,* which unlike other species of the horseshoe crabs, hatches out in mud flats. An understanding of the biology and behaviour of this species is important in developing strategies for its conservation.

Material and Methods

Animal husbandry—The present experiment was carried out with an aim to ascertain the colour and light preferences by the trilobite larvae of *C. rotundicauda*. The fertilized eggs of the Malaysian horseshoe crab (*C. rotundicauda*) were collected directly from the nest made on the breeding ground of Setiu at Terengganu (Eastern coast of Peninsular Malaysia; Lat 5°42′60″N; Long 102°42′0″E[36]. All the experiments were carried out in the marine hatchery unit of University Malaysia Terengganu. The fertilized eggs collected from the nest were kept for incubation at a constant temperature of 27±1 °C, were hatching of the trilobite larvae occurred between 42 and 45 days during the incubation[37].

Soon after hatching, actively swimming trilobite larvae were selected for the colours and light preference experiments. A total of 50 individuals were considered for each experiment and the average weight of these larvae was 0.076 g/larva. The larvae of both the groups were kept separately in two aquarium tanks of 120 L capacity provided with seawater of 20±2 ppt salinity and 27±1 °C temperature. The temperature of the aquarium tanks was controlled thermostatically. Except the experimental period, all the larvae were kept under a 15h:9h light: dark regime[28]. Larvae were fed with newly hatched *Artemia nauplii* (20-25 artemia/day/larvae) once a day, soon after the end of each day experiment. Since, previous studies have shown that the horseshoe crab larvae were more active at night than during the day time[28], all the experiments were conducted at night (11.00 hrs). Moreover, visual sensitivity of horseshoe crabs is known to increase at night due to the action of the circadian rhythm[38].

Colour preference by the trilobite larvae—Colour preference by the trilobite larvae of *C. rotundicauda* was determined using a specially designed circular fiberglass tank (diameter: 120 cm). The bottom of the circular fiberglass tank was divided into seven equal zones and each zone was painted in the respective order**;** black, yellow, red, green, blue, white and orange colours using non-hazardous biological grade epoxy paints. The inner wall of the circular fiberglass tank was also painted with similar colours corresponding to each respective zones up to the height of 0.25m.

Prior to the commencement of the experiment, the fiber glass circular tank was filled up to the height of 0.50 m with well oxygenated filtered seawater (< 22 µm). The trilobite larvae (n = 50) were then transferred slowly at the center of the tank and they were left undisturbed for an hour. The top of the tank was then covered by a dark circular plastic lid and the bottom of the lid was provided with a light source. The stimulus light was a circular fluorescent lamp (Phillips model TLE 32W/54), which delivered an intensity of 4.5×10^{15} photons $cm^{-2}s^{-1}$ at the bottom of the fiberglass tank. Reflective light intensity was measured using an International Light IL 1700 radiometer and a calibrated Ocean Optics S2000 spectrometer. All these tests were conducted in a room, where the only light came from the light stimulus. The plastic lid was removed slowly after 1 hr and the number of larvae congregated in each colour zone of the circular fiberglass tank was recorded. For larvae observed along colour zone margins, the colours surrounding greater than 50% of the organism was selected. After larvae were counted, they were returned to the rearing aquarium tank. The observation was taken very carefully to avoid any bias counting and in sufficiently large number so that the resulting data could be summarized to give valid results on the colour preference by the trilobite larvae. The present study was repeated for 25 times (until the first molting) following the similar above mentioned procedure.

Since, the colour preference choice of trilobite larvae were considered until the first moulting stage, the data collected were pooled and their percentage of preference for a particular colour was calculated using Graphpad Prism 5 software. The Standardized Morisita index (I_p), was considered the best measure of dispersion being independent of the sample size. To calculate this index, the Morisita's index of dispersion (I_d) and two critical values, the uniform index (M_u) and the clumped index (M_c) were calculated as follows[39,40]:

$$I_d = n\,[\sum x^2 - \sum x / (\sum x)^2 - \sum x\,]$$

$$M_u = \frac{\chi^{2}\,.925 - n + \sum x}{(\sum x) - 1}$$

$$M_c = \frac{\chi^{2}\,.025 - n + \sum x}{(\sum x) - 1}$$

where n is the number of observations, *x* is the number of larvae congregated in a particular colour zone, and are the values of the chi-squared with (*n*-1) degrees of freedom that have 97.5% or 2.5% of the area to the right. The Standardized Morisita index (Ip) was then calculated by one of the four following formulae:

(a) When, $I_d \geq M_c > 1$,
$Ip = 0.5 + 0.5\ [\ Id - Mc / n - Mc]$
(b) When, $M_c > I_d \geq 1$,
$Ip = 0.5\ [\ Id - 1 / Mu - 1]$
(c) When, $1 > I_d > M_u$,
$Ip = -\ 0.5\ [\ Id - 1 / Mu - 1]$
(d) When, $1 > M_u > I_d$,
$Ip = -\ 0.5 + 0.5\ [\ Id - Mu / Mu]$

The Standardized Morisita index (Ip) ranges from -1 to +1. The random patterns give a value of zero, clumped (aggregated) patterns above zero and uniform (hyper dispersed) patterns below zero.

Light preference by the trilobite larvae—The experiment on the preference of infrared (IR) and/or ultraviolet (UV) lights by the trilobite larvae was conducted in a specially designed rectangular glass tank (120×50×50 cm). The inside of the rectangular tank was divided into 3 equal sections by a 50×50 cm glass plate, fixed vertically with silicon glue and painted black. This glass plate helped in avoiding the mixing of IR and UV light inside the rectangular tank. All sides of the rectangular tank were painted with mat black paint on all internal surfaces in order to minimize reflections. Two small circular (5 mm diameter) windows were left free of paint on the opposite sides of the tank, to allow penetration of the IR and UV light. The third section was devoid of any light and was considered as a control to determine the aggregation of larvae in the absence of light.

In the present study, two different positions of the light sources were examined to minimize the sampling error. In the first experiment, the lights were introduced horizontally by fixing the lights on the side of the tank and the thickness of the aquarium tank was 1 cm. For infrared light source, an infra-red bulb (1000W halogen bulb, Phillips) and for ultraviolet light source, an ultraviolet tube (150W Xenon arc lamp) was used. In the Infra-red light source, an IR filter unit was attached. The light was first passed through a chamber where the running water absorbed most of the heat radiance beyond the IR part of the spectrum. The light was then passed through IR filters (2 glass filter and 1 gelatin filter) which only permitted a minimal amount of light of wavelength shorter than ≈ 780 nm to pass the aquarium[41]. The UV stimulus ≈ 480 nm was passed through a band pass filter (CWL 480 nm)[42,43]. The light rays emitted from each source were passed through a PVC pipe (diam.: 190 mm, length: 10 cm). Anterior ends of both the PVC pipes were closed by end caps, which were provided with small apertures (5 mm diam.). This small aperture helped in introducing only a narrow beam of the light, preventing the scattering of light inside the rectangular tank. The top of the rectangular tank was covered by a plastic sheet and the experiment was conducted in a dark room, where all external light sources were excluded. Similarly, in the second set of experiments, the same light assemblies were fixed at the top of the rectangular tank to introduce light vertically. The light source was positioned exactly 1cm above the water level inside the tank, so as to maintain an uniform pattern of light distribution as in the first experiment.

At first, the experimental tank was filled with filtered well aerated seawater (< 22 µm) up to the height of 25 cm. A total of 50 larvae was then slowly introduced into the tank for the experiment. The larvae were introduced into the tank from the opposite side of the light source. Soon, after the release of larvae the light sources were switched on for next 30 min, followed by recording the number of larvae congregated in each light source. Larvae which entered the respective partitions (IR, UV and control) only were recorded. The larvae congregated away from the light source where no influence of any light observed (50 cm) was considered as zero zone assemblage. After, recording the number of larval congregations in each zone, they were transferred to the rearing aquarium tanks. The same procedure was repeated for 25 times (until the first molting) to collect maximum data on congregation size of the larvae with respect to infrared and ultraviolet lights.

Statistical analysis—The trilobite larvae are free swimming animals and they move spontaneously and independently in an environment. Since the same larvae were followed during the 25 day experiment, the data obtained were not treated as independent. Therefore, to determine whether there was a significant effect on colour preference and light choice in the trilobite larvae, a Repeated measures analysis of variance (RM-ANOVA) was conducted using ‘IBM SPSS Statistics 20’ software. To study the specific pattern of movement of the trilobite larvae with respect to preference of particular colour during the 25 days of the experiment under controlled conditions, cluster analysis of ward’s with the help of Square Euclidean Distance SPSS software was used to show whether the congregation size was natural or random. The cluster was also used to demonstrate the homogeneity in groups congregated in different colour zones. Data were analyzed using Graphpad Prism 5 software to confirm the preference of lights by the trilobite larvae.

Results

Colour preference by the trilobite larvae—Average congregation size of trilobite larvae during the course of the experiment in different colour zones of the fiberglass tank are presented in Fig. 1.

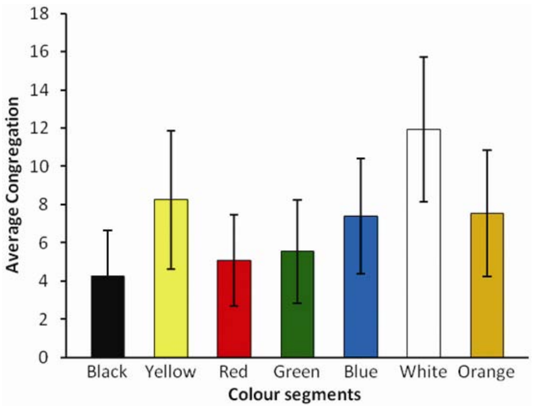

Fig. 1—Average congregation size of trilobite larvae in different colour zones

The maximum average congregation size of the trilobites was in white (n= 12) followed by yellow (n= 8) and orange (n= 8). The minimum average congregation was observed in the dark zones; black (n=4) and red (n=5). The maximum percent congregation size was calculated in the white zone (23.84%), whereas, the minimum (8.48%) in the black color zone (Table 1). The congregation size at the 25% percentile, in white zone was 9 individual whereas at the 75% percentile it was 14, which were the maximum among all groups (Table 1). On the contrary, in the black zone, it was only 3 at the 25% percentile and 6 at the 75% percentile (Table 1). Similarly, at 95% confidence limit, the maximum range was between 10.37 and 13.47 for white zone that was again the highest as compared to black zone (3.24-5.23) with lowest values (Table 1). The analysis of repeated measures of ANOVA for larval congregations under different colours results showed that Mauchly's test of sphericity has not been violated [$\chi2(20)=28.842$, $P = 0.094$]. Hence, the "Mauchly's test of sphericity output was corrected using an ANOVA with repeated measures with sphericity assumed correction. The mean larval congregation under different colours were found statistically significant under this [$F(6, 144)=15.067$, $P < 0.05$];

The Morisita's index values range from -1 to +1. The Morisita index value for each colour tested was -1.0065 for black, yellow (0.9931), red (-1.0045), green (-1.0025), blue (0.997), orange (0.996) and white (0.977) (Table 1). The result showed that three colours were having values less that 0 (black, red and green), which showed a uniform (hyper dispersed) pattern of larval congregation. However, other colours tested showed (blue, orange, yellow and white) a value above 0 and thus forming a clumped distribution. This revealed that the larvae's were congregated more in these zones. To test the possibility of the maximum congregation size of trilobite larvae in different colour zones and similarity in their congregation size pattern, the data collected for all groups were superimposed on the Dendrogram. The results clearly showed six clear clusters superimposing on each other (Fig. 2). The least preferred cluster was between red and green colour whereas the maximum at white zone and yellow colour.

Light preference by the trilobite larvae— The next attempt to determine the phototaxis preference of trilobite larvae revealed a positive response and was found to attract more towards ultraviolet light (Table 2). The analysis of repeated measures of ANOVA for larval congregations under UV and infra-red lights along both horizontal [$\chi2(5)=9.712$, $P = 0.094$] and vertical position [$\chi2(5)=6.033$, $P = 0.303$] showed that Mauchly's test of sphericity has not been violated. Hence, the Mauchly's test of sphericity output was corrected using sphericity assumed correction. The mean larval congregation under different lights were found statistically

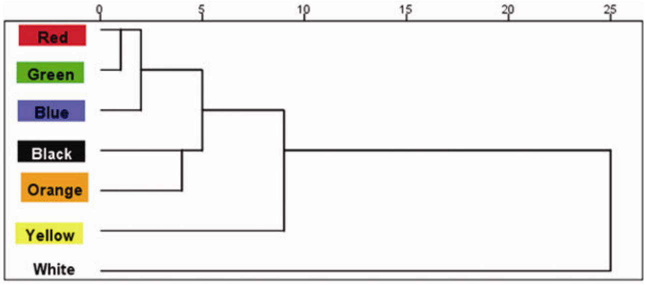

Fig. 2—Dendrogram with six clusters superimposing on each other

Table 1—Colour preference of the trilobite larvae

Parameters	Colours						
	Black	Yellow	Red	Green	Blue	White	Orange
Minimum	0	3	1	1	2	4	3
Maximum	9	18	10	10	13	19	17
Average congregation	4	8	5	6	7	12	8
Percent congregation	8.48	16.48	10.16	11.12	14.8	23.84	15.12
25% Percentile	3	5	3	3	4	9	5
75% Percentile	6	9	7	8	10	14	10
Lower 95% CI of mean	3.24	6.73	4.08	4.46	6.17	10.37	6.21
Upper 95% CI of mean	5.23	9.74	6.07	6.65	8.62	13.47	8.90
Morista Index	-1.006	0.993	-1.005	-1.003	0.997	0.977	0.996

CI= Confidence limit

Table 2—Light preference of the trilobite larvae with respect to different positions of the light sources

Parameters	Horizontal Position			Vertical position		
	Infrared light	Ultraviolet light	Control (no light)	Infrared light	Ultraviolet light	Control (no light)
Minimum	4	14	6	3	15	2
Maximum	16	25	17	13	30	20
Average congregation	8	19	10	9	20	9
Percent congregation	15.2	38.08	20.4	16.8	40.7	17.4

significant for both horizontal [F(3, 72)=44.378, $P < 0.0005$] and vertical light source [F(3, 72) = 35.146, $P < 0.0005$]. However, not much difference was observed between the groups (vertical and horizontal light position). About 53-57% larvae were seen aggregating near to infrared and UV lights when the light sources were used either horizontally or vertically in the experimental tank (T2). When compared to the congregation size between the infrared and ultraviolet lights, maximum percentage of the larvae were counted under ultraviolet light *i.e.* 38.8 and 40.7% horizontally and vertically, respectively. The percent congregation size under ultraviolet light was 15.2 and 16.8% for horizontal and vertical positions of the light sources, respectively (Table 2). Not much significance was observed for congregation of larvae, when the infrared light source was compared with the control (no light source). This revealed that larvae's were positive phototaxis to low intensity light (ultraviolet), while larvae were negative phototaxis to high intensity light (Infrared).

Discussion

Larval instar stages of horseshoe crabs are morphologically more specialized than all other subsequent stages (juveniles and adults)[28]. This stage is confronted with the necessity of moving successfully from a nest buried several centimeters beneath the sand or mud to the water surface. Once this transition is achieved, the animal will not re-enter the upper beach environment until it is a sexually active adult, where they undergo atleast 16-17 times moulting[44]. During this time it will be vastly changed, both behaviourally and morphologically, from the larval stage[28]. Behavioural studies on the adult horseshoe crabs have shown that their visual systems have a major role in shaping them to adapt well with the environment[13,17]. Horseshoe crab uses their compound lateral eyes for visual orientation. The attempt to determine the spectral and light sensitivity of newly hatched larvae until their first moulting stage have showed the visual system of horseshoe crab might be functional from the early stages of its development. Results, showed that the larvae preferred white and yellow zone the most, where the difference in colour preference between white and yellow was only 7% and also they were attracted more towards the ultraviolet than infrared light. Previous studies have shown that trilobite larvae of *L. polyphemus* (7-10 days post hatched), possess numerous ommatidia, which are capable of object recognition and directional vision[45]. They also possess rudimentary photoreceptors which are capable of perceiving light[45]. These rudimentary photoreceptors present in the lateral eye of trilobite larvae might be able to distinguish colours of different wavelength as evident from the present study. As the larvae undergo developmental process, the photosensitivity of their lateral eyes is believed to increase, as the number of ommatidia increases from an average of 17 in the late stages of trilobite larvae to 30 at the first juvenile stage and 1000 in adults[12,45,46].

Generally, attraction towards the colour is encountered for a particular need of the animal and these animals may change their preference for colours at different life cycles[47-49]. This phenomenon is clearly observed in horseshoe crabs during their different developmental stages. Adult males have shown to identify dark colour carapace of females for mating as such they preferred darker colours more as compared to other colours[50,51]. Another possible reason for the dark colour preference of the adult horseshoe crabs is because of the fact that they totally become benthic and remains in the euphotic zone of the sea[32]. Meanwhile, the juvenile stages of horseshoe crabs appear to avoid objects with high visual contrast *i.e.*, black and grey contrasts[12,52,53]. The young juveniles are pelagic in nature and for their active feeding they remain in the photic zone of the sub-tidal sea for the first few years[54,55] and later move offshore and live in the bottom of the sea at deeper zone. This could be one of the reasons for difference in colour preference than the adults. Another possibility for the avoidance of dark colour by the juvenile horseshoe crabs is that animals might consider the darker object as their potential predators. This phenomenon of predator avoidance response has already been reported in other marine arthropods[56]. In the present study with *C. rotundicauda*, the larvae were found to prefer lighter zones (white, yellow and orange) than darker zones (black and red). Previously, Rudloe[28] have suggested that the newly hatched trilobite larvae with their lunar and circadian activity rhythms, which are synchronized with the tide level in the water column may use a positive phototaxis. They avoid dark areas as an adaptation to emerge from the nests and disperse towards the surface[12]. The present results suggest that the avoidance of darker zones by the newly hatched trilobite larvae is more likely to be an endogenous rhythm. Since larval forms of horseshoe crabs are known to live in the pelagic environment[57], the choice of white zone and other lighter colours might refer to their habitat preference and feed availability[58,59]. While colour is very useful in shallow-water environments, it is an unreliable cue deeper in water due to the spectral modification of light as it travels through water of various depths or of varying optical quality[60].

Similar to horseshoe crabs, a number of other arthropods have also reported to show difference in colour preference at various stages of their life cycles. A significant difference has been observed by Reza and Parween[49] for the preference of colour among larvae and adults of red flour beetle (*Tribolium castaneum*). The second instar larvae have been found to be attracted more towards yellow and pink colours. On the contrary, the fourth instar larvae of the same species were reported to avoid red or pink colour. The adults of *T. castaneum* were attracted more towards black colour. Similar observations were made on anthomyiid flies where a shift of colour choice from pre-reproductive adult was reported by Jenkins and Roques[61]. The immature anthomyiid flies were attracted towards yellow colour whereas the mature flies towards purple colour.

Usually shallow water living species have much broader spectral band than the deep living life forms[62]. For, reasons mainly concerned with light availability, many marine animals specialize in the blue region of the spectrum, where they consist of numerous visual pigments with more blue sensitive cones even to shorter wavelengths[62]. The spectral sensitivity of many marine organisms for the purpose of selective feeding also suggests the innate preference for the particular colour. Bowmaker[63] reported that visual pigments of penguin showed sensitivity to blue-green part of the colour spectrum which is present in the Antarctic waters. Similarly, naïve moth shows a narrow peak around 450 nm wavelengths suggesting an innate preference for the blue colour[64]. Studies on the photoreceptors in the compound eyes (lateral and ventral eyes) of adult horseshoe crabs have reported a spectral sensitivity range between 520 and 545 nm[65-67]. However, such information are lacking in the early stages of horseshoe crabs. The present study on larval forms of *C. rotundicauda* has showed their preference of colours in the range of 450-620 nm in the visible spectrum, with white zone (combination of all wavelengths) being the ideal one. Though the habitat, food and life stages could be considered as a possible factor for the variation in the spectral sensitivity between the adult and early larval stages, however, the decline in the range of recognition by adults to a very narrow spectrum lying only within 25 nm is rather interesting and hence further detail investigations are warranted.

Exploitation of visual cues in ultraviolet ranges as communication signals are of considerable significance in invertebrate behaviour[68]. The role of ultraviolet lights for various life activities have already been demonstrated successfully in various organisms including butterflies[69], jumping spiders[70], birds[71,72] and reptiles[73]. The perception of ultraviolet lights also plays an important role in predator–prey interactions of many animals[68,74] and also in the selection of hunting sites[75]. Previous attempts using electrophysiological and behavioural studies have shown that these animals are most responsive to the short wavelength range (360-380 nm) of the sun's emission spectrum[76]. Electrophysiological observations have confirmed the presence of a UV detecting photoreceptor cells in the median ocellus of the horseshoe crab's eye with a maximal sensitivity at 360 nm[43,77-80]. Many invertebrates possess UV receptors, where they utilize them to detect the plane-polarized light[81,82].

Earlier observations had revealed that the stimulation of the median ocelli in the horseshoe crabs by UV light from the night time sky enhances the sensitivity of the lateral eyes to visible light[50,83,84]. The sensitivity of horseshoe crab eyes has also been found to increase at night by the action of the centrally located circadian clock[38,50], where the sensitivity of the eyes can increase up to 10^6 compared to natural daylight[18,85]. Barlow[50] demonstrated that the ultraviolet lights from full moon were used by the male horseshoe crabs for their dim light vision at night to effectively identify female crabs during their mating. Thus it could be summarized that the eyes of horseshoe crabs have clear visions both at day and night[18]. Though not much information's are available for the UV light preference in the early stages of the horseshoe crabs, the present study have shown a clear sign that the larval forms of horseshoe crabs also rely well on UV lights for their activities. Nearly 38-41% of the experimental larvae showed a photo taxis effect of UV light, irrespective of the position of the light source. However, further detail investigations are required to unravel, how UV lights are utilized by the larvae for its nocturnal activities. At night, circadian rhythm activities in the brain of *Limulus* are reported to activate and transmits neural activities to the lateral and median eyes[86].

Quantitative data on the responses of invertebrate larvae to environmental stimuli (photo response, light intensity, wave length, colour preference, dark and light adaptation) is helpful in understanding the complex interaction of factors influencing the larval distribution and movement[87]. However, such quantitative data on response of many arthropod larvae remain poorly understood including horseshoe crabs. The information such as how a population of organism is structured in terms of species composition, their abundance, their interaction with environments and population dynamics are invaluable to develop conservation plans for natural areas or recovery plans for threatened or endangered species like horseshoe crabs. It is thus anticipated that the basic biological and behavioural response of the trilobite larvae generated from the present study could be beneficial for further strengthening the conservation plans for this valuable marine ancestor.

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References

1 Ross W D & Mingollab E, Recent progress in modeling neural mechanisms of form and colours vision, Image, *Vision Comput*, 16 (1998) 447.
2 Skorupski P & Chittka L, Is colour cognitive?, *Opt Laser Technol,* 38 (2009) 323.
3 Webster M A & Mollon J D, Adaptation and the colours statistics of natural images, *Vision Res*, 37 (1997) 3283.
4 Arikawa K, Spectral organization of the eye of a butterfly, Papilio, *J Comp Physiol*, 189(A) (2003) 791.
5 Atema J, Review of sexual selection and chemical communication in the lobster, *Homarus americanus, Can J Fish Aquat Sci,* 43 (1986) 2283.
6 Hughes A, The topography of vision in mammals of contrasting lifestyle: Comparative optics and retinal organization, in *Handbook of sensory physiology*, edited by Autrum H (Springer, Berlin) 1977, 613.
7 Zeil J, Nalbach G & Nalbach H O, Eyes, eye stalks and the visual world of semi-terrestrial crabs, *J Comp Physiol*, 159(A) (1986) 801.
8 Zeil J, Nalbach G & Nalbach H O, Spatial vision in a flat world: Optical and neural adaptations in arthropods, in *Neurobiology of Sensory Systems*, edited by R N Singh & Strausfeld Nicholas James (Plenum, New York) 1989, 123.
9 Collin S P & Pettigrew J D, Retinal topography in reef teleosts. I. Some species with well-developed area but poorly developed streaks, *Brain Behav Evol*, 31 (1988) 269.
10 Collin S P & Pettigrew J D, Retinal topography in reef teleosts. II. Some species with prominent horizontal visual streaks and high density, *Brain Behav Evol*, 31 (1988) 283.
11 Land M F, Variations in the structure and design of compound eyes, in *Facets of vision*, edited by Stavenga D G & Hardie R C (Springer, Berlin, Heidelberg, New York) 1989, 90.
12 Medina J M & Tankersley R A, Orientation of larval and juvenile horseshoe crabs *Limulus polyphemus* to visual cues: Effects of chemical odors, *Curr Zool*, 56 (5) (2010) 618.
13 Barlow R B, Ireland L C & Kass L, Vision has a role in Limulus mating behavior, *Nature*, 296 (1982) 65.
14 Barlow R B, Hitt J M & Dodge F A, *Limulus* vision in the marine environment, *Biol Bull*, 200 (2001) 169.
15 Walls E A, Berkson J & Smith S A, The horseshoe crab, *Limulus polyphemus*: 200 million years of existence, 100 years of study, *Rev Fish Sci,* 10 (1) (2002) 39.
16 Guo M, Dodge F & Barlow R, Circadian rhythms in the locomotor activity of juvenile horseshoe crabs, *Invest Ophthalmol Vis Sci*, 46 (2005) E-Abstract 5663.
17 Powers M K, Barlow Jr R B & Kass L, Visual performance of horseshoe crabs day and night, *Visual Neurosci*, 7 (1991) 179.
18 Herzog E D, Powers M K & Barlow R B, *Limulus* vision in the ocean day and night: Effects of image size and contrast, *Visual Neurosci*, 13 (1996) 31.
19 Passaglia C, Dodge F A, Herzog E, Jackson S & Barlow R B, Deciphering a neural code for vision, *Proc Nat Acad Sci USA*, 94 (1997) 12649.
20 von Campenhausen C, The ability of Limulus to see visual patterns, *J Exp Biol*, 46 (1967) 557.
21 Lal A B & Chapman R M, Phototaxis in Limulus under natural conditions: Evidence for reception of near-ultraviolet light in the median dorsal ocellus, *J Exp Biol*, 58 (1973) 213.

22 Passaglia C L, McSweeney M E, Stewart K M, Kim E, Mole E J, Powers M K & Barlow R B, Visual performance of horseshoe crabs: Role of underwater lighting, *Biol Bull*, 193 (1997) 205.

23 Loeb J, Uber kilnstliche Umwandlung positiv heliotropischer Tiere in negative heliotropischeund umgekehrt, *Pflügers Archiv*, *Eur J Physiol*, 53 (1893) 81.

24 Northrop J H & Loeb J, The photochemical basis of animal heliotropism, *J Gen Physiol*, 5 (1922) 581.

25 Cole W H, Circus movements of *Limulus* and the tropism theory, *J Gen Phytiol*, 5 (1922) 417.

26 Cole W H, The effect of laboratory age upon the phototropic reactions of *Limulus*, *J Gen Physiol*, 6 (1923) 295.

27 Wolf E & Zerrahn-Wolf G, Reactions *of Limulus* to illuminated fields of different areas and flicker frequency, *J Gen Physiol*, 20 (1937) 767.

28 Rudloe A, Locomoter and light responses of larvae of the horseshoe crabs, *Limulus Polyphemus* (L.), *Biol Bull*, 157 (1979) 494.

29 Cartwright-Taylor L, Lee J & Hsu C C, Population structure and breeding pattern of the mangrove horseshoe crab *Carcinoscorpius rotundicauda* in Singapore, *Aquatic Biol*, 8 (2009) 61.

30 Davison G W H, Ng P K L & Ho H C, The Singapore Red Data Book. Threatened Plants and Animals of Singapore, (Nature Society, Singapore) 2008, 285.

31 Zaleha K, Kamaruzzaman B Y, John B A & Ong M C, Cd, Cu and Pb Concentration Levels in Horseshoe Crab Nesting Grounds of Pahang Coast, Malaysia, *J Biol Sci*, 10 (2010) 790.

32 Chatterji A, *The Horseshoe crab — A living fossil* (A Project Swarajya Publication, India) (1994) 157.

33 Itow T, Mishra J K & Ahmed A T A, Horseshoe crabs (King crabs) in the Bay of Bengal, South Asia, *Bull Fac Edu Shizuoka Univ Nat Sci*, 54 (2004) 13.

34 Botton M L, Tankersley R A & Loveland R E, Developmental ecology of the American horseshoe crab *Limulus polyphemus*, *Curr Zool*, 56 (2010) 550.

35 Rudloe A, *Limulus polyphemus*, A review of the ecologically significant literature. in *Biomedical application of the Horseshoe crab (Limulidae)*, edited by Cohen E. (Alan R. Liss, INC, New York) 1979, 27.

36 Srijaya T C, Pradeep P J, Faizah S & Chatterji A, A study on the energy source in the developing embryo of the mangrove horseshoe crab *Carcinoscorpius rotundicauda* (Latreille), *Invertebr Reprod Dev*, I (2011) 1.

37 Srijaya T C, Pradeep P J, Mithun S, Hassan A & Chatterji A, A new record on the morphometric variations in the populations of Horseshoe crab (*Carcinoscorpius rotundicauda*) obtained from two different ecological habitats of Peninsular Malaysia, *Our Nature*, 8 (2010) 204.

38 Barlow R B, Circadian rhythms in the *Limulus* visual system, *J Neuroscience*, 3 (1983) 856.

39 Brower J E, Zar J H & von Ende C N, *Field and laboratory methods for general ecology*, (WCB/McGraw-Hill, Dubuque, Iowa) 1998, 288.

40 Sapkota I P, Tigabu M & Oden P C, Spatial distribution, advanced regeneration and stand structure of Nepalese Sal (*Shorea robusta*) forests subject to disturbances of different intensities, *Forest Ecol Manag*, 257 (2009) 1966.

41 Attramadal Y G, Fossa J H & Nilsson H L, Changes in behavior and eye-morphology of *Boreomyszs Megalops* G.O. Sars (Crusstacea: Mysidaecea) following exposure to short periods of artificial and natural daylight, *J Exp Mar Biol Ecol,* 85 (1985) 135.

42 Nolte J & Brown J E, Electrophysiological properties of cells in the median ocellus of *Limulus, J Gen Physiol*, 59 (1972 a) 167.

43 Nolte J & Brown J E, Ultraviolet-induced sensitivity to visible light in ultraviolet receptors of Limulus, *J Gen Physiol*, 59 (2) (1972 b) 186.

44 Shuster C N Jr, Observations on the natural history of the American horseshoe crab, *Limulus polyphemus*, *Woods Hole Oceanographic Inst Contr,* 564 (1950) 18.

45 Harzsch S, Vilpoux K, Blackburn D C, Platchetzki D, Brown N L, Melzer R, Kempler K E & Battelle B A, Evolution of arthropod visual systems: Development of the eyes and central visual pathways in the horseshoe crab *Limulus polyphemus* Linnaeus, 1758 (Chelicerata, Xiphosura), *Dev Dynam*, 235 (2006) 2641.

46 Meadors S, McGuiness C, Dodge F A & Barlow R B, Growth, visual field, and resolution in the juvenile *Limulus* lateral eye, *Biol Bull*, 201 (2001) 272.

47 Kring J B, Red spheres and yellow panels combined to attract apple maggot flies, *J Econ Entomol*, 63 (1970) 466.

48 Jenkins M J & Roques A, Attractiveness of colour traps to *Strobilomyia* spp. (Diptera: *Anthomyiidae*), *Envir Entomol*, 22 (1993) 297.

49 Reza A M S & Parween S, Differential preference of coloured surface in *Tribolium castaneum* (Herbst), *Invertebrate Surv J*, 3 (2006) 84.

50 Barlow R B, Vision in Limulus mating behavior, *Biol Bull*, 161 (1981) 339.

51 Ehlinger G S & Tankersley R A, Survival and Development of Horseshoe Crab (*Limulus polyphemus*) Embryos and larvae in hyper saline conditions, *Biol Bull*, 206 (2004) 87.

52 Errigo M, McGuiness C, Meadors S, Mittmann B, Dodge F & Barlow R, Visually guided behavior of juvenile horseshoe crabs, *Biol Bull*, 201 (2001) 271.

53 Ridings C, Borst D, Smith K, Dodge F & Barlow R, Visual behavior of juvenile *Limulus* in their natural habitat and in captivity, *Biol Bull*, 203 (2002) 224.

54 Shuster C N Jr, Distribution of the American horseshoe crab *Limulus polyphemus* (L.), *Prog Clin Biol Res*, 29 (1979) 3.

55 John B A, Sihin S, kamaruzzaman B Y & Zaleha K, Sediment profiling of the nesting grounds of horseshoe crabs at east Peninsular Malaysia, *Int J Biol*, 4(2) (2012) 159.

56 Diaz H, Orihuela B, Forward R B Jr & Rittschof D, Orientation of *blue crab Callinectes sapidus* (Rathbun) megalopae: Responses to visual and chemical cues, *J Exp Mar Biol* Ecol, 233 (1999) 25.

57 Botton M L & Loveland R E, Abundance and dispersal potential of horseshoe crab (*Limulus polyphemus*) larvae in the Delaware Estuary, *Estuaries*, 26(6) (2003) 1472.

58 Rudloe A, Aspects of the biology of juvenile horseshoe crabs, *Limulus polyphemus*, *Bull Mar Sci*, 31(1) (1981) 125.

59 Shuster C N Jr, A pictorial review of the natural history and ecology of the horseshoe crab *Limulus polyphemus*, with reference to other Limulidae, in *The physiology and biology of horseshoe crabs: studies on normal and environmentally*

stressed animals, edited by Bonaventura J, Bonaventura C & Tesh S. (Alan R. Liss, Inc., New York) 1982, 1.

60 Cronin T W, Shashar N, Caldwell R L, Marshall J, Cheroske A G & Chiou T, Polarization vision and its role in biological signaling, *Integr Comp Biol*, 43 (2003) 549.

61 Jenkins M J & Roques A, Attractiveness of colored traps to *Strobilomyia* spp. (Diptera: Anthomyiidae), *Env Entomol*, 22(2) (1993) 297.

62 Loew E R & Lythgoe J N, The ecology of colour vision, *Endeavour*, 14 (1985) 170.

63 Bowmaker J K, Microspectrophotometry of vertebrate photoreceptors, *Vision Res*, 24 (1984) 1641.

64 Kelber A, Why do Manduca sexta feed from white flowers? Innate and learnt colour preferences in a hawkmoth, *Naturwissenschaften,* 95 (2008) 569.

65 Hubbard R & Wald G, Visual pigment of the horseshoe crab, *Limulus polyphemus, Nature (London)*, 186 (1960) 212.

66 Wald G & Krainin J M, The median eye of *Limulus:* an ultraviolet receptor, *Proc Nat Acad Sci USA*, 50 (1963) 1011.

67 Wasserman G S, *Limulus* receptor action spectra *Vision Res*, 9 (1969) 611.

68 Bhaskara M R, Brijesh C M, Ahmed S & Borges R M, Perception of ultraviolet light by crab spiders and its role in selection of hunting sites, *J Comp Physiol A*, 195 (2009) 409.

69 Papke R S, Kemp D J & Rutowski R L, Multimodal signalling: Structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L. (Pieridae), *Anim Behav*, 73(1) (2007) 47.

70 Lim M L M, Lib J & Li D, Effect of UV-reflecting markings on female mate-choice decisions in *Cosmophasis umbratica*, a jumping spider from Singapore, *Behav Ecol*, 19(1) (2008) 61.

71 Cuthill I C, Partridge J C, Bennett A T D, Church S C, Hart N S & Hunt S, Ultraviolet vision in birds, *Adv Stud Behav*, 29 (2000) 159.

72 Hausmann F, Arnold K E, Marshall N J & Owens I P F, Ultraviolet signals in birds are special, *Proc R Soc L Biol Sci*, 270 (2002) 61.

73 LeBas N R & Marshall N J, The role of colour in signalling and male choice in the agamid lizard *Ctenophorus ornatus*, *Proc R Soc L Biol Sci*, 267 (2000) 445.

74 Honkavaara J, Koivula M, Korpimäki E, Siitari H & Viitala J, Ultraviolet vision and foraging in terrestrial vertebrates, *Oikos* 98(3) (2002) 505.

75 Lim M L M & Li D, Extreme ultraviolet sexual dimorphism in jumping spiders (Araneae: *Salticidae*), *Biol J Linn Soc*, 89 (2005) 397.

76 Herzog E D & Barlow R B, Ultraviolet-light from the nighttime sky enhances retinal sensitivity of Limulus, *Biol Bull*, 81 (1991) 321.

77 Nolte J, Brown J E & Smith T G Jr, A hyperpolarizing component of the receptor potential in the median ocellus of *Limulus. Science (Washington)*, 162 (1968) 677.

78 Nolte J & Brown J E, Spectral sensitivities of single photoreceptors in the median ocellus of *Limulus*, *J Gen Physiol*, 54 (1969) 636.

79 Nolte J & Brown J, The spectral sensitivities of single receptor cells in the lateral, median and ventral eyes of normal and white-eyed Limulus, *J Gen Physiol*, 55(6) (1970) 787.

80 Waterman T H, Polarisation sensitivity, in *Handbook of sensory physiology,* edited by Autrum J (Springer, New York) (1981) 281.

81 Wehner R, Neurobiology of polarization vision, *Trends Neurosci* 12 (1989) 353.

82 Herzog E D & Barlow R B Jr, Ultraviolet light from the nighttime sky enhances retinal sensitivity of Limulus, *Biol Bull*, 181 (1991) 321.

83 Westerman L A, Barlow R B, Ultraviolet responses of the Limulus median ocellus, *Biol Bull*, 161 (1981) 352.

84 Powers M K & Barlow R B, Behavioral correlates of circadian rhythms in the *Limulus* visual system, *Biol Bull*, 169 (1985) 578.

85 Eisele L E, Kass L & Barlow R B Jr, Circadian clock generate optic nerve activity in the excised Limulus brain, *Biol Bull*, 163 (1982) 382.

86 Young C M & Chia F, Ontogeny of phototaxis during the larval development of the sedentary polychaete, *Serpula vermicularis* (L.), *Biol Bull*, 162 (1982) 457.