Journal of the Marine Biological Association of the United Kingdom, 2010, 90(5), 941–946.
doi:10.1017/S0025315410000032

Influence of substratum colour on the recruitment of macrofouling communities

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Settlement of the fouling community is generally influenced by the physical, chemical and biological properties of the settling surface. The colour of the substratum may also exert an influence on larval settlement. In the present study, the influence of surface colour on the recruitment of fouling communities was investigated by suspending acrylic panels (red, green, blue, white and yellow) in coastal waters. Results showed that the colour of the substratum plays an important role in the recruitment of sessile species. Recruitment was comparatively high on red and blue coloured surfaces. Barnacle and tubeworm recruitment showed significant variation (Tukey test, $P < 0.05$*) between different coloured surfaces. The significant conclusion of this study is that the colour of the artificial substrata should be taken into consideration when interpreting results from short-term biofouling studies.*

Keywords: biofouling, fouling community, artificial substrata, barnacle, substrata colour, ascidians, tubeworms

Submitted 27 December 2008; accepted 19 October 2009; first published online 20 April 2010

INTRODUCTION

Many marine invertebrates with planktonic larval stages exhibit selectivity during settlement on a surface (Keough, 1998; Jeffery, 2002). Substratum selection represents a critical behaviour during settlement because the right choice can have a significant impact on their fitness (Herbert & Hawkins, 2006; Pardo *et al.*, 2007). Settlement of marine larvae is generally influenced by a wide range of factors like physical, chemical and biological cues (Pawlik, 1992; Bers & Wahl, 2004; Faimali *et al.*, 2004). Physical cues such as light, gravity, surface orientation and colour may be important for the recruitment of benthic larval forms on a surface (James & Underwood, 1994; Rittschof *et al.*, 1998; Taylor *et al.*, 1998; Su *et al.*, 2007).

Shaded and dark coloured surfaces attract more marine larvae than well-illuminated and light coloured surfaces (Dahlem *et al.*, 1984). While shade is the partial darkness due to the interception of light rays, colour is defined as a visual attribute of objects that results from the light they emit or transmit or reflect. The influence of the colour of a substratum on the recruitment of a fouling community was initially observed by Visscher (1928). According to Bakus (1988), test panel colour seems to be more important for settling larvae than panel position. Similarly, Swain *et al.* (2006) emphasized the importance of considering colour of the paint along with other factors when undertaking short-term testing of antifouling coatings.

Understanding the role of the physical and chemical properties of a substratum on the settlement of a fouling community is fundamental in terms of optimizing the performance of coatings designed to reduce biofouling. Extensive research has been carried out on the physical, chemical and biological characteristics of surfaces that promote or prevent fouling (Yule & Walker, 1984; Holmes *et al.*, 1997; Callow & Callow, 2000). However, compared to the other factors affecting the settlement of benthic organisms on hard substrata, colour has received relatively little attention and only a few experimental studies have been done on this aspect. Studies on the reaction of the larval forms of the fouling organisms to colour will be of commercial value in determining the colours of antifouling paints for testing (Daniel, 1963). Hence, in the present study, an attempt has been made to observe the recruitment of the fouling community on different coloured substrata. The main objective of this study was to investigate whether differences in surface colour influence the recruitment of a macrofouling community.

MATERIALS AND METHODS

The study was carried out at Kudankulam (8°9′N and 77°39′E) on the east coast of India. Kudankulam is located in the distal end of the Gulf of Mannar (Figure 1). Seasons at Kudankulam can be classified into pre-monsoon (June–September), monsoon (October–January) and post-monsoon (February–May). The wind direction is north–north-easterly from June to December and changes to westerly during the rest of the period. The seabed is sandy with soft substrata and few rocky reefs.

Acrylic sheets were used as test panels (10 × 10 × 0.3 cm) for the present study. Commercially available red, green, blue, white and yellow coloured acrylic sheets were used for panel preparation. The panels were fixed onto a wooden raft in vertical position in such a way so as to keep a 10 cm distance between

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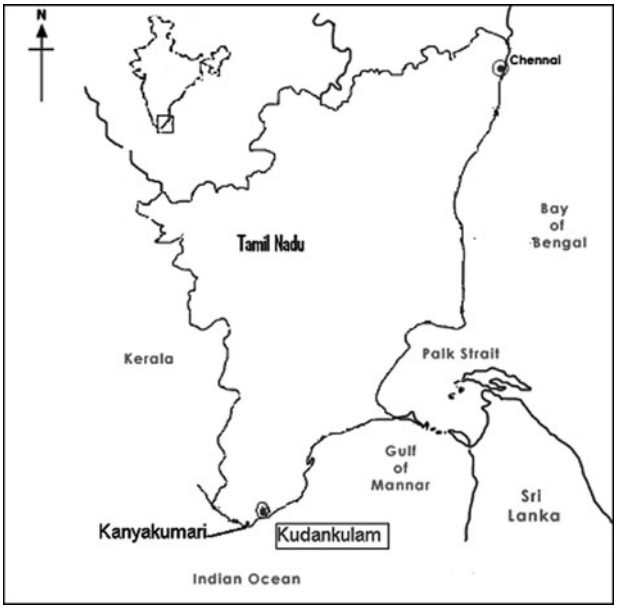

Fig. 1. Map showing the study area.

panels. The raft was submerged in the coastal waters at 1 m depth from the mean sea level on a short-term basis (15 days' duration). For the evaluation of temporal variability, the investigation was carried out over a period of five months from February to June 2005. The panels (in replicate, N = 6, same raft) were retrieved after 15 days of submersion and preserved in 5% neutral formalin for further analysis. The test panels were analysed for the total biomass (dry weight), species composition and abundance of the fouling community. The total biomass of the fouling community was estimated after carefully scraping the fouling organisms into a Petri plate and weighing them in a balance. The dry weight of the entire fouling assemblage from the panels was noted by drying them at 60°C to constant weight and expressed as $g.dm^{-2}$.

Barnacles and tubeworms were counted manually after separating them into different species or groups. The abundance of colonial fouling organisms was estimated using the random point sampling technique (Nandakumar, 1998) and expressed as percentage of coverage on test panels. For this purpose, a quadrat of the same size as the panel was marked with points at 1 cm intervals on a transparency sheet. This sheet was laid over the panel and the area covered by colonial forms within 10 randomly chosen 1 cm^2 quadrats on the test panel was recorded. The results were presented for a submersion period of 15 days on a monthly basis (i.e. mean ± SD of $6 \times 2 = 12$ panels for each month) in order to analyse the temporal variability (relation to submersion month).

To test the variability of fouling community recruitment between different coloured substrata, the data were analysed by two-way ANOVA using MS-Excel. Colour and month were considered as two independent factors for the ANOVA. The *post-hoc* Tukey test was also carried out between different colour treatments.

RESULTS

Results showed that the fouling community recruitment was considerably high on red and blue coloured panels. The total biomass of the fouling community showed significant variations between different coloured panels and also between months (two-way ANOVA; Table 1). The biomass (dry weight) values varied between 0.201 ± 0.03 and 2.9 ± 0.25 $g.dm^{-2}$ on red coloured panels. The biomass values ranged between 0.375 ± 0.06 and 3.37 ± 0.21 $g.dm^{-2}$ on blue and 0.104 ± 0.008 and 1.52 ± 0.07 $g.dm^{-2}$ on green coloured panels. On white coloured panels, the total biomass of the fouling community varied between 0.226 ± 0.036 and 1.36 ± 0.11 $g.dm^{-2}$. Yellow coloured panels showed a biomass value between 0.194 ± 0.006 and 1.21 ± 0.11 $g.dm^{-2}$ during the study (Figure 2). Biomass values were high during the month of May on all panels except the green ones. Green coloured panels showed the maximum value during June. Generally, the total biomass values were very low during February on all the panels. The *post-hoc* Tukey test indicated that the biomass of the fouling community on blue coloured panels significantly varied from yellow and green ones (Table 2). The biomass of the fouling community that settled on red coloured panels also varied significantly from yellow and green coloured panels. Comparisons of other colour treatments did not show significant variations (Table 2).

Barnacles, tubeworms, colonial ascidians and errant forms like polychaetes and amphipods were the common foulers that recruited on the panels. Of these, the recruitment patterns of barnacles, tubeworms and ascidians were considered in detail. Among the barnacles, *Amphibalanus amphitrite* (=*Balanus amphitrite*) was the dominant species that recruited on the panels. Barnacle recruitment was high on red and blue coloured panels and low on white and yellow panels. The abundance of *Amphibalanus amphitrite* on red coloured panels varied between 32 ± 3 (February) and 378 ± 20 $no.dm^{-2}$ (May). On blue coloured panels, the number of barnacles varied from 10.5 ± 2.1 (March) to

Table 1. Two-way ANOVA (analysis of variance) of fouling community recruitment on different coloured acrylic panels (colour and month as factors).

	Biomass			***Amphibalanus amphitrite***		**Sabellariids**		**Ascidians**	
	Df	**SS**	**F**	**SS**	**F**	**SS**	**F**	**SS**	**F**
Colour	4	12.27988	220.54*	497540.1	224.91*	365.68	113.13*	1032.3888	21.22*
Month	4	22.97851	412.68*	361231.1	163.29*	129.48	39.35*	10750.5088	221.03*
Colour*month	16	7.160011	32.14*	381109.7	43.07*	71.42	5.42*	2864.7552	14.72*
Error	25	0.348001		13825.92		20.565		303.98	
Total	49	42.7664		1253707		587.145		14951.6328	

*, significant at 5% level.

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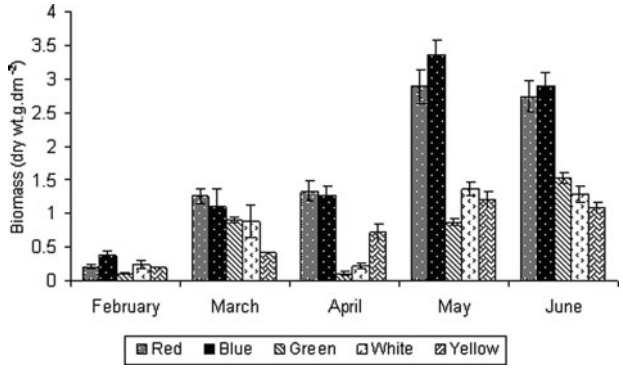

Fig. 2. Total biomass of the fouling community recruited on different coloured acrylic panels (mean ± SD, N = 12).

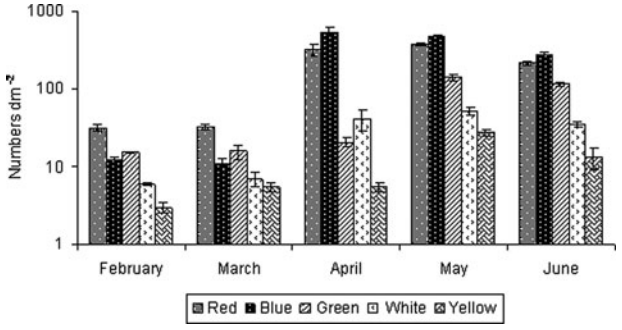

Fig. 3. Recruitment pattern of *Amphibalanus amphitrite* (=*Balanus amphitrite*) on different coloured acrylic panels (mean ± SD, N = 12).

532 ± 95 no.dm^{-2} (April). On green panels, barnacle abundance ranged between 15 ± 0.4 (February) and 142 ± 13 no.dm^{-2} (May). The density of barnacles on white and yellow coloured panels showed a maximum of 53 ± 6 and 28 ± 3 no.dm^{-2} respectively (Figure 3) during May. Barnacle recruitment was high during April and May and low during February. Statistical analysis of barnacle recruitment indicated a significant variation among the different coloured surfaces and also between months (Table 1). The Tukey test showed that the recruitment of barnacles on red coloured panels significantly differed from white and yellow coloured panels (Table 3). Barnacle recruitment on blue coloured panels also varied significantly from that on green, white and yellow coloured panels.

The tubeworms recruited on the panels mainly belonged to the family Sabellariidae. The abundance of sabellariids was high on red and low on yellow coloured panels. On red coloured panels, the number of recruits varied between 7 ± 1 (March) and 14 ± 1.4 no.dm^{-2} (May). The abundance of sabellariids on yellow coloured panels ranged from 2 ± 0.3 (February) to 8 ± 0.7 no.dm^{-2} (May). The number of sabellariids recruited on white coloured panels varied from 2 ± 0.2 (February) to 8 ± 0.7 no.dm^{-2} (April) and on blue coloured panels, the number of individuals varied from 9 ± 0.8 to 12 ± 0.9 no.dm^{-2} (March). On green coloured panels, a maximum of 8 ± 1 no.dm^{-2} was observed during May and a minimum of 3 ± 0.5 no.dm^{-2} in February (Figure 4). Two-way ANOVA showed a significant variation in tubeworm recruitment between different coloured panels and also between panel submersion months (Table 1). The *post-hoc* Tukey test indicated that tubeworm recruitment on red colour panels varied significantly from green, white and yellow coloured ones (Table 4). Similarly, sabellariid recruitment on blue coloured panels showed significant variation from green, white and yellow coloured panels.

Ascidians belonging to the genus *Didemnum* were the dominant colonial forms recruited on the test panels. The colonial ascidians' recruitment on red coloured panels varied from 12 ± 1.7 (June) to 80.5 ± 3.5% (February). On blue coloured panels, ascidian coverage ranged between 11.15 ± 1.3 (June) and 68 ± 4.8% (February). On white coloured panels, a maximum coverage of 49 ± 5.6% was observed during February and a minimum of 13 ± 2.8% was observed in June. The recruitment of ascidians on yellow coloured panels varied between 9 ± 2 (June) and 38 ± 2.8% (February). Ascidian coverage on green coloured panels ranged from 7 ± 0.9 (June) to 38.5 ± 4.9% (February). In general, ascidian recruitment was high on red and blue coloured panels and low on yellow and green coloured panels. Peak recruitment was observed during February and it was very low during June on all the panels (Figure 5). Two-way ANOVA revealed a significant variation in ascidian recruitment between different coloured panels and also among the months (Table 1). However, the *post-hoc* Tukey test did not show any significant variation between colour treatments (Table 5).

DISCUSSION

In the present study, the variations found in the recruitment of the fouling organisms between different coloured panels

Table 2. *Post-hoc* Tukey test of total fouling biomass on different coloured panels.

Group 1	Group 2	Mean difference	SE	q
Blue	Red	0.114	0.260	0.437
	White	1.006	0.260	3.866
	Yellow	1.081	0.260	4.153*
	Green	1.104	0.260	4.241*
Red	White	0.892	0.260	3.429
	Yellow	0.967	0.260	3.716*
	Green	0.990	0.260	3.804*
White	Yellow	0.075	0.260	0.287
	Green	0.098	0.260	0.375
Yellow	Green	0.023	0.260	0.088

*, $P < 0.05$.

Table 3. Comparison of *Amphibalanus amphitrite* (=*Balanus amphitrite*) recruitment on different coloured panels (*post-hoc* Tukey test).

Group 1	Group 2	Mean difference	SE	q
Red	Blue	−65.300	40.992	1.593
	Green	135.400	40.992	3.303
	White	169.000	40.992	4.123*
	Yellow	186.700	40.992	4.555*
Blue	Green	200.700	40.992	4.896*
	White	234.300	40.992	5.716*
	Yellow	252.000	40.992	6.147*
Green	White	33.600	40.992	0.820
	Yellow	51.300	40.992	1.251
White	Yellow	17.700	40.992	0.432

*, $P < 0.05$.

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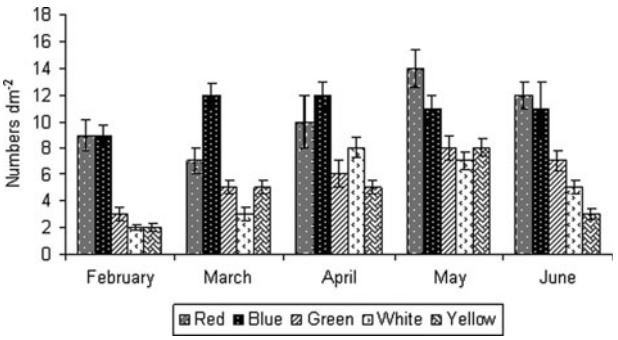

Fig. 4. Recruitment pattern of tubeworms (sabellariids) on different coloured acrylic panels (mean ± SD, N = 12).

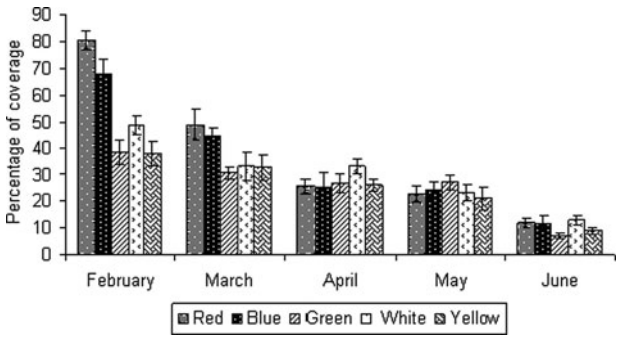

Fig. 5. Recruitment of colonial ascidians (*Didemnum* spp.) on different coloured acrylic panels (mean ± SD, N = 12).

indicated that surface colour has some influence on recruitment. Fouling community recruitment on blue and red coloured panels was significantly different from green, white or yellow coloured ones. However, no significant effect was observed between blue and red or white, green and yellow coloured panels. The reaction of larval forms to colour may be due to a complex of factors involving the quantities of radiant energy absorbed or reflected (Daniel, 1963). The higher recruitment of invertebrates on red, blue and black surfaces may be due to the preference of larvae for darker, deep colour and less reflective substratum (Su *et al.*, 2007). For example, the barnacle, *Amphibalanus amphitrite* preferentially recruited on red or blue coloured panels. It is possible that they may prefer to settle on surfaces that do not reflect much light (e.g. dark colours).

The cypris larvae of barnacles are positively phototropic during the early stages and are most sensitive to green light (Visscher & Luce, 1928). But at the time of attachment they are negative to light and tend to move to darker areas (McDougall, 1943). Yule & Walker (1984) also reported that the tenacity of cyprids was much greater on darker coloured surfaces. The Tukey test revealed that the magnitude of ascidian recruitment between different coloured panels was not significant. This indicates that the factors responsible for settlement and recruitment vary between species, as they may have different requirements and respond to specific cues (Crisp, 1974; Raimondi, 1988).

Similar to barnacles, tubeworm recruitment was also high on red and blue coloured panels. The preference of some benthic groups for darker coloured surfaces has previously been demonstrated in a number of studies. Swain *et al.* (2006) observed higher settlement rate of the seaweed *Ulva* sp. and tubeworm *Spirorbis* sp. on black surfaces. Saucedo *et al.* (2005) observed that the larvae of the pearl oyster *Pinctada mazatlanica* (Hanley) recruited more on the red/black colour combination collector than that on green coloured ones. Su *et al.* (2007) also reported that red and blue colour plastic sheets attracted significantly more larvae of the pearl oyster *Pinctada martensii* than green and yellow coloured sheets. Furthermore, Daniel (1963), while studying the factors influencing the recruitment of fouling organisms at the Madras coast (east coast of India), observed that red and black coloured surfaces attracted cyprids in larger numbers than green and grey coloured panels.

The results also showed considerable temporal variability in fouling community recruitment during the study period. Temporal variability in fouling community abundance has been mainly linked with larval availability (Pineda, 1994), wind-driven currents (Hawkins & Hartnoll, 1982) and internal waves (Shanks, 1983). Since the panels were submerged mainly during the post-monsoon season, it is not clear whether the same results could be obtained if the experiments were carried out during pre-monsoon and monsoon seasons. But the number of replicates both within each submersion period and over a temporal scale in the present study provides sufficient data to explain the variability of fouling community recruitment among different coloured surfaces.

In general, the present study demonstrates that the recruitment of fouling communities on artificial substrata is greatly affected by substratum colour. Furthermore, it should be noted that marine invertebrate larvae contacting a substratum during exploration of potential habitats for settling are

Table 4. Comparison of sabellariid recruitment on different coloured panels (*post-hoc* Tukey test).

Group 1	Group 2	Mean difference	SE	q
Blue	Red	0.600	0.986	0.609
	Green	5.200	0.986	5.274*
	White	6.000	0.986	6.086*
	Yellow	6.400	0.986	6.492*
Red	Green	4.600	0.986	4.666*
	White	5.400	0.986	5.477*
	Yellow	5.800	0.986	5.883*
Green	White	0.800	0.986	0.811
	Yellow	1.200	0.986	1.217
White	Yellow	0.400	0.986	0.406

*, $P < 0.05$.

Table 5. Comparison of ascidian recruitment on different coloured panels (*post-hoc* Tukey test).

Group 1	Group 2	Mean difference	SE	q
Red	Blue	3.260	8.189	0.398
	Green	12.060	8.189	1.473
	White	7.760	8.189	0.948
	Yellow	12.660	8.189	1.546
Blue	Green	8.800	8.189	1.075
	White	4.500	8.189	0.550
	Yellow	9.400	8.189	1.148
Green	White	−4.300	8.189	0.525
	Yellow	0.600	8.189	0.073
White	Yellow	4.900	8.189	0.598

https://doi.org/10.1017/S0025315410000032 Published online by Cambridge University Press

exposed to chemical and physical cues derived from surface-associated microorganisms (O'Connor & Richardson, 1996). It should also be noted that the link between the colour of the surface and viable cell numbers (biofilm community) in a surface has been established by Pitts *et al.* (1998). Hence, more studies related to the formation of biofilms on different coloured surfaces may improve our understanding in this field. The present study showed that the colour of the substrata should be taken into consideration along with other factors when interpreting results from short-term biofouling studies (ecological studies to test various hypotheses or antifouling trials). For practical reasons, the colour of the substratum used for biofouling studies should be kept as similar as possible. The results also suggested that light coloured surfaces would be preferable for marine applications. Further studies on the development of micro- and macrofouling communities on different coloured surfaces from other regions are also necessary to ascertain the influence of surface colour on biofouling.

ACKNOWLEDGEMENTS

We thank the Ministry of Earth Sciences, Government of India for providing financial assistance. We also thank two anonymous referees for their valuable comments.

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https://doi.org/10.1017/S0025315410000032 Published online by Cambridge University Press