THE HABITAT AND THE HABITAT-SELECTION BY *UMBONIUM VESTIARIUM* L.

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Umbonium vestiarium L., a Trochid gastropod is a common intertidal animal encountered on the sandy beaches of Bombay. It restricts its habitat to mid-tide zones and generally avoids sands of finer grade or sands mixed with mud. Three localities along the west coast of Bombay namely, Shivaji Park, Juhu and Versoa, where *U. vestiarium* occurs plentifully, have been examined for their physiographical and biological characteristics. On the basis of these investigations, laboratory-experiments were planned with a view to understanding the factors which influence the habitat selection by *U. vestiarium.*

U. vestiarium possesses an ability to discriminate between habitable and non-habitable sands. The sands rendered unattractive by various acid treatments, with detergents or with certain chemical compounds are not inhabited by this animal. Repellent sand, however, becomes attractive when it is inoculated with marine bacteria, flagellates or flagellate metabolites. Treatment with the tissue homogenates of *U. vestiarium* itself renders sand unattractive to *Umbonium.* Selection of the habitat is not influenced by varying day light intensities but is influenced by extreme conditions of illumination, that is total darkness and high illumination. The grade of the sand exerts some influence on the selection of the habitat; the animal prefers medium sand to finer or to coarser grade.

Introduction

A study of the interstitial life in relation to its environment has lately developed into a field of very popular interest amongst ecologists who, in that, see possibilities of examining a variety of problems pertaining to life exposed to varied and rapidly changing environmental conditions. Of the various ecological studies pursued currently, the one having bearing on the selection of the habitat by the organisms with reference to the chemistry of the substratum has received a good deal of attention. Boaden (1962) investigated the behaviour of two species of *Protodrilus* and one species of *Trilobodrilus* towards light, salinity, water-currents and grades of sands, which according to him, enabled the animals to maintain their positions on the intertidal shore. Wieser (1956) observes that most of the intertidal organisms select the grade of sand which would permit them to obtain enough food and facilitate free locomotion. Crisp (1965) brought out the importance of the surface chemistry in the settlement of the marine larvae. ZoBell (1939) and Jägersten (1940) suggested that the micro-organisms were of importance in effecting the settlement and in controlling the metamorphosis by producing a chemical substance that covered the substratum. Wilson (1937, 1953 *a*, *b*; 1954; 1955) while reporting on the metamor-

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phosis of *Ophelia bicornis* larvae concluded that the presence of favourable bacteria and the favourable grade of sand had facilitatory effect. Meadows (1964) reported that any treatment altering the primary film rendered the sand unattractive to *Corophium arenarium* and to *C. volutator*. Gray (1966) investigated the basis of highly localised occurrence of *Protodrilus symbioticus* by introducing them to the surfaces which had been modified by experimental procedures.

Investigations reported in this paper deal with the physiography and biology of the intertidal sands at Bombay. Amongst a variety of organisms observed, a Trochid gastropod, *Umbonium vestiarium* L. is abundantly found in the intertidal sands at all the three stations, viz., Shivaji Park, Juhu and Versoa.

Observations on the habitat selection of *U. vestiarium* by offering it various treated sand surfaces in the laboratory were made and the results are discussed in the light of previous investigations on the subject.

MATERIALS AND METHODS

I. *Field observations*

The sand water samples from all the three stations were collected at weekly intervals. With reference to the tidal level of the day, at each station three substations were selected at approximate low-tide, mid-tide and high tide marks along a single transect.

At each substation, the samples of the sand were collected from a spot measuring 12″ in diameter and 6″ deep. For ascertaining the physico-chemical and faunistic characteristics of the sand, the following methods were employed.

(*a*) *Physiography*—The salinity value of the sea-water samples was estimated using Knudsen's method. For the quantitative estimation of dissolved oxygen content in the sea-water and the interstitial-water the classical Winkler's method was adopted. Organic matter from the interstitial sand was estimated by Walkley and Black method described by Jackson (1958). Oxidation of chlorine was prevented by adding silver sulphate to the digestion mixture. For sand analysis, 50 gms of oven-dried sand was sieved using BST sieves of the mesh sizes 22, 44 and 60 for a linear inch, which gave diameter range of 710 μ, 355 μ and 250 μ respectively. The percentage distribution of the sand in four size ranges viz. above 710 μ, 710 to 355 μ, 355 to 250 μ and below 250 μ was determined.

(*b*) *Sand fauna*—For an estimate of the total population of bacteria, the soil dilution and a plate count method described by Johnson, *et al.* (1959) was employed. The organisms encountered in the intertidal zone are categorised into ten broad groups viz. diatoms, protozoans, coelenterates, nematodes, polychaetes, amphipods, crabs, hermit crabs, gastropods and bivalves. The detailed identification of the organisms, which needs specialised attention, has not been attempted in this work.

II. *Laboratory observations on* Umbonium vestiarium

For the laboratory experiments on the substrate selection, freshly collected specimens of *U. vestiarium* from Shivaji Park sand were used. They were kept in sterilized sea-water prior to their use as the experimental animals. The suitability of the animal for every experiment was ascertained on the basis of its ability to burrow into the sand, which it normally did within 10–15 min. The treated sand and the natural sand, the latter being used as a control, were offered in an enamel tray measuring 12″ × 10″ × 2″ for simple choice experiments. The test-organisms were allowed to select the substratum for a period of two hours (Figs. 1, 2) at the end of which, their number in each of the substrates was counted. Fifty animals were used for each experiment.

The significance of the choice ratios obtained in different experiments was assessed by the X^2 test (Fisher and Yates 1953). For a given treatment, the organisms failing to select either treated or control sand were not considered (Meadows 1964; Gray 1966). The choice of the animals with respect to the illumination and sand grain sizes was expressed in terms of an 'Attractive index' as described by Gray (1966). The treatments of the sand are categorised into different groups depending upon their ability to render the sand attractive or unattractive. The results are described under each of these categories.

RESULTS

1. *Physiography of the habitat*

The salient features of the habitat of *U. vestiarium* are given below.*

(*a*) *Salinity*—The salinity values of the sea-water of Bombay during July to December, the monsoon and the non-monsoon periods, range from 14‰ to 35.5‰ (Table I). The salinity in the interstitial sands varies from station to station, the influx of fresh water by way of sewage, in addition to the rain water, being the causative factor. At Shivaji Park, where industrial waste water is discharged and small sewage outlets open, salinity varies between 3.5‰ to 32.00‰. At Versoa, variation is not much (20.5‰ to 36.0‰). The salinity values of the interstitial sands at the three stations are given in Tables II–IV.

(*b*) *Temperature*—The sea-water temperature at Bombay varies from 24.4°C to 30.0°C during July to December. The variations closely follow the changes in atmospheric temperature.

The temperature of the interstitial sand at each station closely follows the sea-water temperature (Tables II–IV). The interstitial temperature is generally higher than the sea-water by a degree or a half. During the warmer days, difference in interstitial temperatures during morning and noon hours, may be as high as 7°C.

(*c*) *Dissolved oxygen*—Dissolved oxygen content in the sea-water varied from 0.6 to 6.3 ml/1. The oxygen content in the interstitial sand water is appreciably lower than that in the sea-water. The values varied between 0.4 to 3.5 ml/1. At Versoa, the oxygen values of the interstitial sand were much lower than at the other two stations (Tables II–IV).

(*d*) *Organic matter*—Table V summarises organic matter content of the interstitial sands in the intertidal zones at the three stations. The intertidal sand at Shivaji

*The detailed observations will be published elsewhere.

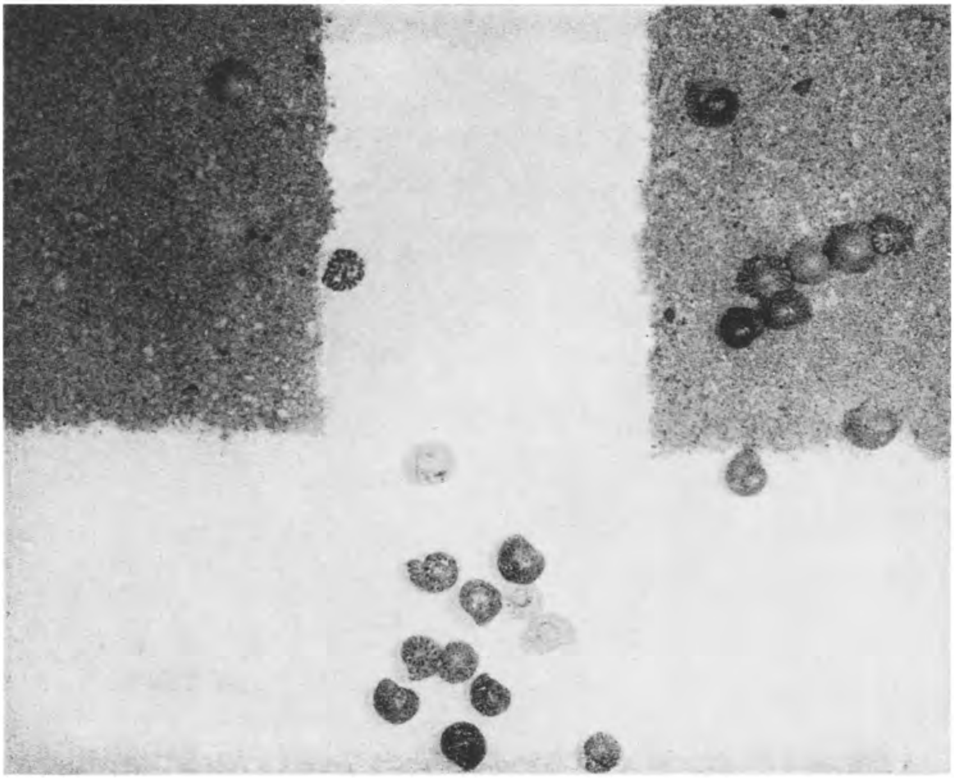


FIG. 1. Typical experiment (reconstructed) showing *Umbonium vestiarium* selecting inhabitable sandy substratum.

Park is generally richer in organic matter than at Juhu or Versoa. At all the three stations, the low-tide zones have maximum amount of organic matter, there being, however, only slight differences between low-tide and mid-tide zones. At Versoa, where the organic matter is generally poor, the differences in the values at the three tidal zones are negligible. The organic matter content during the monsoon, at all the three tidal zones in the three stations, has been found to be less than in the summer months (Table VI).

(*e*) *Sand-grain analysis*—After initial examination, the sand is divided into two arbitrary grades. The grains below 355 μ diameter are designated as 'fine' and those above 355 μ diameter are designated as 'coarse' grains.

At two stations, Shivaji Park and Juhu, the intertidal sand, in general, is composed of fine grains. However, the ratio between fine and coarse grains varies considerably from time to time. At Versoa, the composition of the sand grains differs from the other two stations, in that there is a graded increase in the coarse grains from low-tide to high-tide levels. At high tide the percentage of coarse grains is characteristically high.

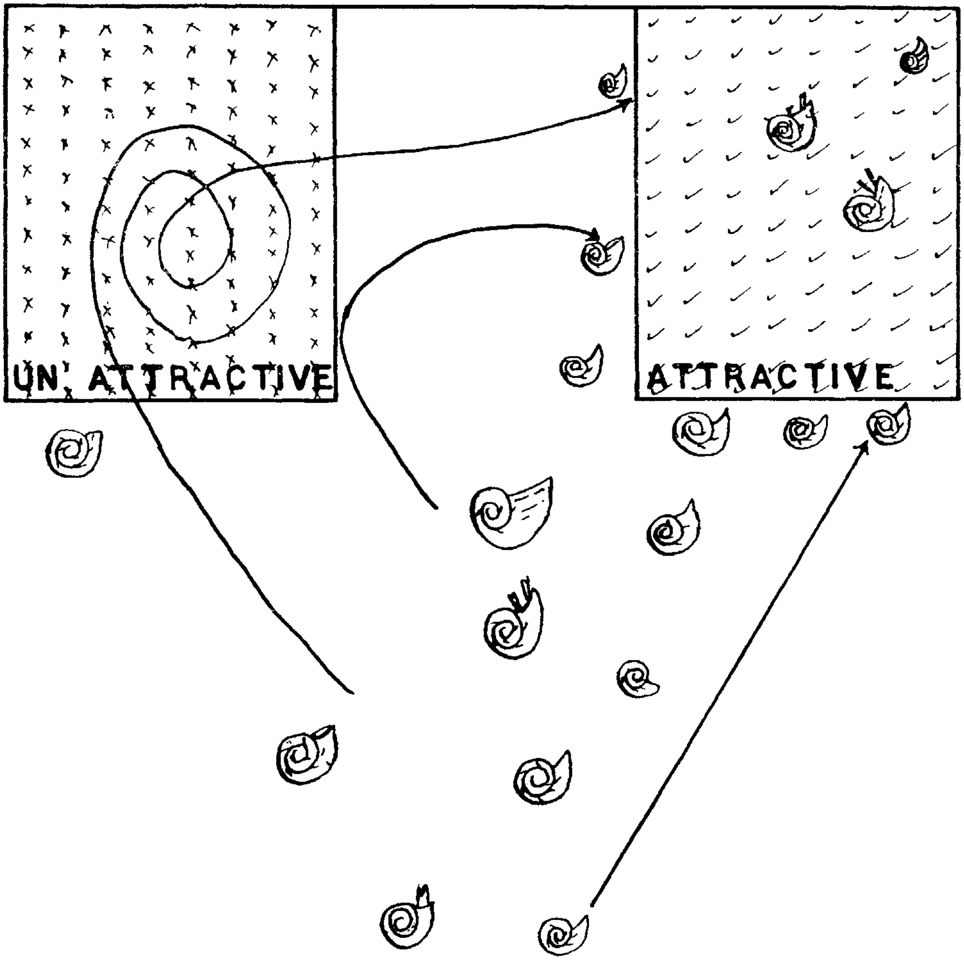


FIG. 2. Schematic illustration of the behavioural pattern of *Umbonium vestiarium* towards attractive and unattractive sands.

(*f*) *Fauna of sandy beach*—Fig. 3 summarises the abundance of variety of animal life incident at intertidal zones at the three stations. At Shivaji Park, the animal life at low -and mid-tide zones is generally rich. Almost all the groups, if not species, noted at low-tide are also found in more or less equal abundance in the mid-tide zone. Thus, there is a certain amount of overlapping between two tidal zones. At high-tide zone, where the desiccation is considerable, occurrence of ciliates, nematodes, polychaete worms and amphipods at all three stations is noted with interest.

Intertidal sands at Juhu and Versoa are poor in animal life, the mid-and high-tide zones being very sparsely inhabited. In mid-tide zones, however, *Umbonium vestiarium* occurs plentifully and as many as 500 animals per square foot area are usually seen buried in the superficial sands at Versoa.

TABLE I

Mean sea-water temperature, salinity, rainfall, atmospheric temperature and whole sunshine hours per day

Period	Mean salinity (‰)	Total rainfall (mm)	Temperature (°C) Sea-water temperature	Temperature (°C) Atmospheric temperature	Average whole sunshine (hr/day)
July					
1st week	28.0	288.1	29.0	26.8	1.2
2nd week	27.0	46.6	29.0	28.4	4.3
3rd week	27.0	114.8	29.0	27.7	1.2
4th week	21.0	70.3	28.0	27.7	3.4
August					
1st week	22.0	74.7	29.0	27.1	1.7
2nd week	22.0	38.2	28.0	27.2	1.7
3rd week	20.0	17.8	28.0	27.6	4.7
4th week	21.0	88.7	30.0	27.6	5.5
September					
1st week	20.0	279.3	28.0	24.4	0.02
2nd week	25.0	54.8	28.0	24.3	3.6
3rd week	26.0	40.6	29.0	23.7	3.0
4th week	32.5	1.9	27.0	24.5	6.9
October					
1st week	33.5	00.0	28.0	27.7	8.1
2nd week	33.5	00.0	28.0	28.8	9.0
3rd week	33.5	00.0	28.0	29.5	7.5
4th week	33.0	00.0	28.0	28.8	9.7
November					
1st week	34.0	00.0	28.0	28.6	9.9
2nd week	33.0	00.0	28.0	28.8	8.9
3rd week	32.0	00.0	29.5	28.7	9.0
4th week	33.0	00.0	29.0	29.0	7.4
December					
1st week	27.0	00.2	29.0	28.4	6.5
2nd week	33.0	00.0	28.0	28.1	7.1
3rd week	33.5	00.0	27.0	—	—
4th week	33.0	00.0	—	—	—

Table VII gives the distribution of bacterial flora at three sub-stations. The distribution pattern with reference to tidal levels and also with reference to monsoon and non-monsoon periods is irregular. The major genera encountered are named under this Table.

During the course of this work 62 species of diatoms were recorded. A detailed report on diatoms and other micro-organisms will be published elsewhere.

TABLE II

Mean temperature, salinity and dissolved oxygen content in open sea and interstital water samples collected at Shivaji park

Temperature (°C)		Salinity (°/oo)		Dissolved Oxygen (ml/L)	
Sea water	Interstitial water	Sea water	Interstitial water	Sea water	Interstitial water
26.5	27.0	19.0	19.0	3.8	2.1
30.0	30.0	16.0	16.0	0.6	0.4
27.0	27.0	21.0	20.0	2.6	1.2
28.5	28.0	14.5	3.5	2.9	1.2
28.5	28.0	19.5	5.0	4.5	2.1
29.0	29.0	26.0	17.5	3.4	0.9
26.0	26.0	32.1	11.0	5.1	2.0
24.0	25.0	30.5	6.5	1.7	0.7
29.0	28.5	27.0	27.0	1.3	0.54
23.0	25.0	33.5	32.0	6.0	2.9

TABLE III

Temperature, salinity and dissolved oxygen content in open sea and interstitial water samples collected at Juhu

Temperature (°C)		Salinity (°/oo)		Dissolved Oxygen (ml/L)	
Sea water	Interstitial water	Sea water	Interstitial water	Sea water	Interstitial water
26.5	27.0	26.0	26.0	3.5	2.1
27.5	27.5	25.0	23.0	3.4	2.3
30.0	30.0	23.5	20.5	5.6	0.9
28.0	28.0	26.5	10.5	5.2	3.3
26.0	26.5	14.0	30.0	—	—
30.0	30.0	32.5	10.0	3.1	2.7
27.0	27.0	30.5	30.0	2.8	2.2
28.5	29.0	19.5	19.5	3.1	1.7
29.0	29.0	32.0	30.5	3.2	1.2
25.0	25.0	34.0	24.0	2.2	1.1

II. *Substrate selection by* Umbonium vestiarium

(*a*) *Influence of light on choice*—Preliminary experiments carried out on the surface selection by *U. vestiarium* indicated that this animal selects its sand substrate within ten minutes under normal experimental conditions. Selection of its habitat under laboratory conditions is not influenced by varying ambient day light intensities. The animal, however, responds differently under conditions of extreme illuminations viz. total darkness and high level of illumination. The animal prefers the latter condition to the former (Table VIII).

(*b*) *Treatments destroying attractiveness of sand*—The sand was cleaned with equal volumes of concentrated sulphuric acid or concentrated nitric acid for 10 min

TABLE IV

Temperature, salinity and dissolved oxygen content in open sea and interstitial water samples collected at Versoa

Temperature (°C)		Salinity (°/₀₀)		Dissolved oxygen (ml/L)	
Sea water	Interstitial water	Sea water	Interstitial water	Sea water	Interstitial water
27.0	27.0	16.5	21.0	3.6	1.8
26.5	26.5	26.5	—	1.1	0.5
27.0	27.0	21.5	20.5	2.6	1.9
28.0	28.0	15.5	26.0	3.5	1.4
27.5	27.5	26.5	29.0	—	—
25.0	23.0	35.0	29.0	6.3	1.5
29.0	27.0	34.5	24.5	2.5	1.8
26.0	25.0	35.5	35.5	3.3	1.7
27.0	28.5	33.5	28.5	—	—
25.0	25.0	33.5	36.0	3.0	1.0

TABLE V

Organic matter content of the interstitial sand in the intertidal zones at Shivaji Park, Juhu and Versoa Beach

Zone	Shivaji Park Organic matter (%)			Juhu Organic matter (%)			Versoa Organic matter (%)		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Low tide	0.37	0.57	0.25	0.22	0.31	0.12	0.12	0.20	0.07
Mid tide	0.34	0.52	0.23	0.19	0.44	0.13	0.13	0.23	0.003
High tide	0.27	0.48	0.18	0.16	0.37	0.07	0.07	0.30	0.007

TABLE VI

Organic matter content during monsoon and post-monsoon months at Shivaji Park, Juhu and Versoa Beach

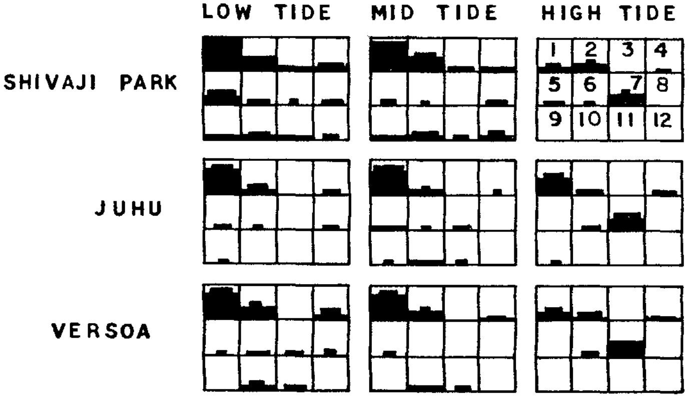
Zone	Shivaji Park Average organic matter (%)		Juhu Average organic matter (%)		Versoa Average organic matter (%)	
	Monsoon	Post-monsoon	Monsoon	Post-monsoon	Monsoon	Post-monsoon
Low tide	0.31	0.41	0.16	0.27	0.09	0.14
Mid tide	0.33	0.34	0.17	0.22	0.06	0.16
High tide	0.18	0.32	0.10	0.20	0.07	0.14

TABLE VII

Distribution pattern of bacteria in intertidal zone at Shivaji Park

Date	No. of bacteria/g sand (in thousands)		
	Low tide	Mid tide	High tide
29-7-1969	0.9	7.0	1.9
7-9-1969	7.7	102.5	7.9
13-8-1969	4.5	1.8	1.6
28-8-1969	4.8	4.0	0.9
5-9-1969	24.5	78.3	1.1
11-9-1969	5.7	5.8	25.7
17-9-1969	3.4	2.2	6.5
25-9-1969	1.3	0.5	190.3
17-10-1969	4.5	1.5	6.0
23-10-1969	132.2	38.0	25.0
12-11-1969	2.4	1.0	0.8
27-11-1969	16.3	5.2	2.1
3-12-1969	40.0	0.5	4.8
10-12-1969	10.0	23.1	17.7

Genera encountered : *Bacillus* sp., *Nocordia* sp., *Clathrochloris* sp., Coccobacilli, Streptomyces, Yeast and *Desulphovibrio* sp.



1. Diatoms
2. Protozoa
3. Coelentrate
4. Nematodes
5. Polychaetes
6. Amphipods
7. Crabs
8. Hermit Crabs
9. Gastropods
10. *Umbonium* sp
11. Bivalve
12. *Solen* sp

FIG. 3. Distribution (variety and abundance) of intertidal fauna at Shivaji Park, Juhu and Versoa Beach, Bombay.

and subsequently thoroughly washed free of acid. The sand was then washed six times with sea-water. The sand samples were also treated independently with 1 per cent solution of detergents Dedanol N, Teepol and 40E. The results are summarised in Table IX. The attractiveness of sand was reduced to almost nil in all the cases examined.

TABLE VIII

Choice experiments with natural sand illuminated with varying intensities of light

Experiment No.	Illumination	% of animals in sand	Attractiveness index*	Remarks
1	Direct sunlight	53.1	1.14	In.
	Indoor light	46.9		
2	Total darkness	00.00	0.00	VS
	Indoor light	100.00		

In, insignificant; *VS*, very significant

$$\text{*Attractiveness index} = \frac{\text{No. of animals in varying illumination}}{\text{No. of animals in indoor light}}$$

TABLE IX

Destruction of attractiveness of natural sand

Treatment	% animals in treated sand	% animals in natural sand	X^2	Remarks
Conc. H_2SO_4	6	94	30.72	VS
Conc. HNO_3	7.9	91.1	28.94	VS
Dedanol -N	15.3	84.7	6.24	S
Teepol	8.0	92.0	29.50	VS
Detergent E-40	0.0	100.0	12.00	VS

S, significant *VS*, very significant

(*c*) *Treatments effecting alteration of sand surface*—An alteration of the sand surface was brought about by treating it with 25 per cent formalin : 75 per cent sea-water and 25 per cent ethyl alcohol : 75 per cent sea-water for one hour. Alteration of the surface film was also effected by subjecting the sand to air drying for 48 hr. This treatment does not remove appreciable amounts of nutritive organic material from the sand surfaces but the nature of the film is certainly altered (Meadows 1964).

The results of these experiments are summarised in Table X. All these treatments effected partial loss of the attractiveness of the sand and, therefore, are different from those recorded in Table IX where the sand lost its attractiveness completely.

(*d*) *Treatments effecting change in primary film of the sand*—Experimental work carried out by Meadows (1964) has suggested that the primary film on the sand comprised of bacteria can be altered by treating the sand with distilled water

TABLE X

Treatment effecting partial loss of attractiveness of sand

Treatment	% animals in treated sand	% animals in natural sand	X^2	Remarks
Formalin-treated sand	63.0	37.0	3.13	In.
Alcohol-treated sand	63.6	36.4	1.33	In.
Air-dried sand	64.0	36.0	3.92	In.
Sterilized sand	43.9	56.1	0.61	In.

In, insignificant

and with non-electrolytes such as sucrose and glycerol. Gray (1966) reported almost 90 per cent loss of bacteria when the sand was shaken with sterile sea-water. The alterations are also brought about by electrolytes such as magnesium chloride, sodium chloride and sodium sulphate, the desorption of bacteria in these cases being comparatively poor.

In order to ascertain the role played by the bacteria in rendering the sand attractive or otherwise, sand samples were treated with the solutions of the more common ions in sea-water, made up to ionic strength of 0.7, equivalent to the ionic strength of sea-water. Solutions of non-electrolytes were prepared at a concentration of 1.0 M, i.e., at same osmotic pressure as sea-water (Harvey 1955).

The different biochemical actions of anaerobic and aerobic micro-organisms will affect the nature of films on the surfaces of sand grains (Meadows 1964). With a view to ascertaining the ability of *U. vestiarium* to discriminate between two surfaces the following experiment was carried out. *U. vestiarium* was offered a freshly collected sand and sand previously stored anaerobically under liquid paraffin in a closed flask. Both sands were offered to the test organisms in the usual way. The results are summarised in Table XI.

TABLE XI

Treatments effecting change in the primary film of the sand

Treatment	% animals in treated sand	% animals in natural sand	X^2	Remarks
Soaked in distilled water for 1 hour	59.2	40.8	1.65	In.
Soaked in distilled water for 7 days	44.7	55.3	0.53	In.
Sucrose	52.8	47.2	0.08	In.
Glycerol	54.0	46.0	0.32	In.
Sodium sulphate	43.0	57.0	1.00	In.
Sodium chloride	30.4	69.6	7.04	S
Calcium chloride	22.9	77.1	14.08	VS
Potassium chloride	54.3	45.7	0.35	In.
Magnesium chloride	49.0	51.0	0.02	In.
Magnesium sulphate	53.0	47.0	0.18	In.
Anaerobic bacteria	61.7	38.3	5.48	S

In, insignificant; *S*, significant; *VS*, very significant

It is observed from this Table that treatments with distilled water and the non-electrolytes designed to dissociate bacteria from the sand, did not render the sand unattractive to the animal. The attractiveness of the sand treated with distilled water and non-electrolytes was comparable to the attractiveness of the sand treated with electrolyte solutions, thereby indicating that bacteria have an insignificant role to play in rendering the sand attractive. However, attractiveness of the repellent sand inoculated with anaerobic bacteria was highly significant.

(*e*) *Reconstruction of the attractiveness of the sand*—Gray (1966) has stated that the attractiveness of the inhospitable sand can be reconstituted by treating it with the favourable species of bacteria. With a view to ascertaining the same, the following experiments were carried out. Acid treated repellent sand was inoculated with cultures of sand bacterium *Bacillus* sp., with laboratory grown cultures of a dinoflagellate *Dunaliella* sp. and with the dinoflagellate metabolites. In another experiment, attractive sand was treated with the tissue-homogenate of *U. vestiarium* with a view to assessing its acceptability to the same animal. The results of the above-mentioned treatments are summarised in Table XII. The attractiveness of the sand was fully reconstituted with the inoculation of bacteria, flagellates and with flagellate metabolites. The sand treated with the tissue homogenate was found to be very significantly repellent to the animal.

TABLE XII

Reconstitution of attractiveness

Treatment	% animals in treated sand	% animals in natural sand	X^2	Remarks
Bacterial culture	50.0	50.0	0.00	VS
Dunaliella sp. culture	38.0	62.0	2.27	S
Dunaliella sp. metabolite	51.1	49.9	0.02	VS
Tissue homogenates of *U. vestiarium*	6.8	93.2	32.82	In.

In, insignificant; *S*, significant; *VS*, very significant

TABLE XIII

Treatment of sand with toxic compounds

Treatment	% animals in treated sand	% animals in natural sand	X^2	Remarks
8-hydroxyquinoline	33.3	66.7	3.67	In.
p-nitrophenol	56.3	43.7	0.7	In.
Dieldrin	16.3	83.7	19.55	VS
Aldrin	10.2	89.8	30.08	VS
Copper sulphate	15.0	85.0	13.37	VS

In, insignificant; *VS*, very significant (*f*) *Toxic sand*—Two hundred ml of fresh sand was thoroughly mixed with one p.c. (W/V) of chemical compounds for one hour. The sand was washed six times in sea-water prior to its use. The toxic concentration of the sand after washing was not determined. Three compounds viz., dieldrin, aldrin and copper sulphate were found to be highly toxic, whereas two compounds viz., 8-hydroxyquinoline and *p*-nitrophenol were ineffective. Insoluble compounds like pentachlorophenol, 2, 4, 5-trichlorophenol, tributylin tin oxide (TBTO) and soluble compounds like nicotine and nicotinic acid were found to be highly toxic.

The results are presented in Table XIII.

DISCUSSION

Earlier works on the substrate selection by the marine organisms deal with the larval settlement and the subsequent metamorphosis of the larvae in the sand. Observations were also made on the habitat selection by the adults. The organisms used for this work were *Scolocolepis fulginosa* (Claparade), *Protodrilus rubropharyngeus* J., *P. symbioticus* G., *Corophium volutator* (Pallas), *C. arenarium* Crawford and *Nassarius obsoletus* Say. Most of the workers observed that the organisms experimented on by them showed complete lack of response towards the acid-treated sand. *U. vestiarium* behaved in a similar manner towards the sand rendered unattractive by the acid treatment. This behaviour of *U. vestiarium* suggests that it has an ability to discriminate between inhabitable and uninhabitable substrates and therefore proves its usefulness as a test animal for the substrate selection experiments.

Gray (1966) reported that the detergents like CTAB (90 μg/l) which would kill 99.9 per cent of *Staphylococcus aureus* did not destroy the attractiveness of the sand completely. The treatments of sands with detergents Dedanol-N, Teepol and 40 E brought about complete loss of attractiveness so far as *U. vestiarium* was concerned. The loss of attractiveness in case of this group of detergents was, therefore, comparable with that of acid treatments. The treatments with formalin and ethyl alcohol which fix both the primary film and its constituent micro-organisms maintain the sand attractive to *U. vestiarium*. Meadows (1964) observed that these treatments rendered sand unattractive to *Corophium volutator* and *C. arenarium*. Gray (1966) also pointed out that *Protodrilus symbioticus* did not accept the sand treated with the fixatives.

The air-dried sand has been reported to be unattractive to *Cumella vulgaris* Hart (Wieser 1956) and to *Corophium arenarium* (Meadows 1964). *U. vestiarium* also found the dried sand partially unattractive. Wieser attributed this loss of attractiveness to the 'almost total depletion of nutritive material'. Meadows, however, felt that the removal of nutritive material from the surface of dried sand was unlikely and that the micro-organisms would only be killed. He suggested that in avoiding the dried sand, the animals were responding to an altered film and not to the lack of food.

Various treatments such as sterilization, soaking in distilled water and in non-electrolytes intended to remove bacteria from the sand surface did not render the sand unattractive to *U. vestiarium*. Both Gray (1966) and Meadows (1964) reported similar observations with respect to *Protodrilus symbioticus* and *Corophium* spp.

respectively. All these experiments therefore suggest that the loss of surface bacteria in itself does not result in a particular sand becoming unattractive.

Meadows (1964) found that sand treated with magnesium chloride and calcium chloride was unattractive to *Corophium arenarium* although these treatments are known to retain the bacteria on sand. To *U. vestiarium,* calcium chloride and sodium chloride treated sands were unattractive but the sand treated with magnesium chloride remained attractive. These discrepancies in the behaviour of animals *vis-a-vis* that of the treatments, "cannot be explained due to complete lack of physicochemical data on the absorption of inorganic and organic substrates and of bacteria, on to the surface in sea-water" (Meadows 1964).

The reconstitution of the attractiveness of the sand is achieved for *U. vestiarium* when the repellent sand is inoculated with marine *Bacillus* sp. cultures. The attractiveness here is comparable with that of the natural sand. Wilson (1954) observed that diatoms did not render acid treated sand attractive. Gray (1966) reported that to *P. symbioticus* the sterile sand treated with sand bacteria was more attractive than the natural sand. The presence of bacteria on the sand, therefore, is not without significance.

Wilson (1955), Scheltema (1961) and Gray (1966) have emphasised that it is the quality of the bacteria that matters in rendering the sand attractive. Gray (1966) named various species of bacteria which helped in reviving the attractiveness of the sand. Our study has brought out that in addition to *Bacillus* sp., the anaerobic bacteria also impart attractiveness to the repellent sand to *U. vestiarium.*

In our studies, we noted that the acid-treated sand became significantly attractive to *U. vestiarium* when it was inoculated with *Dunaliella* sp. culture or with Erd Schrieber culture medium freed from *Dunaliella* cells. It is likely that in the latter case the sand owed its attractiveness to the metabolites of *Dunaliella* cells. Wilson (1954) observed that Erd Schrieber culture medium alone failed to revive attractiveness of the acid-treated sand.

Knight-Jones (1954) reported that the residual layers of organic nature, left by barnacles attracted settling cyprids. The natural sand treated with tissue homogenate of *Umbonium* lost its attractiveness to *U. vestiarium.* A further investigation on the nature of this organic factor in the tissue-homogenate may prove interesting.

For *U. vestiarium* both the acid-treated sand and the natural sand, offered in complete absence of light are unattractive. However, if it is forced to choose between highly illuminated repellent sand and poorly illuminated natural sand, it prefers the former to the latter. It is, therefore, seen that the attractive or unattractive factor of the sand is not only influenced by the intrinsic factor of the sand but also by an extrinsic factor such as light. Our experiments with some toxic compounds have shown that the industrial effluents in the sea-water are likely to affect attractiveness of the sand at Bombay.

Grain size and shape are the another but less important factors that influence the settlement of certain sand-dwelling species (Wilson 1953 *b*). Bhatt (1959) in his exhaustive and informative work on intertidal organisms of Bombay has pointed out that this gastropod avoids sand mixed with mud. That the grade does exert some influence has been observed in *U. vestiarium* since it prefers medium grain sand to fine or coarse grades.

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REFERENCES

Bhatt, Y. M. (1959). A study of the intertidal organisms of Bombay. Ph. D .Thesis, University of Bombay.

Boaden, P. J. S. (1962). Colonization of graded sand by an interstitial fauna. *Cah. Biol., mar., T*-**3**, 245.

Crisp, D. J. (1956) Surface chemistry, a factor in the settlement of marinei nvertebrate larvae. *Botanica gothoburgensia,* **Bd. 3**, 51–65.

Fisher, R. A., and Yates, F. (1953). Statistical Tables for Biological Agricultural and Medical Research. Oliver and Boyd (U.K.).

Gray, J. S. (1966). The attractive factor of intertidal sands to *Protodrilus symbioticus. J. mar. biol. Ass. U. K.*, **46**, 627–645.

Harvey, H. W. (1955). The Chemistry and fertility of sea-water, Cambridge University Press (U.K.).

Jackson, M. L. (1958). Methods in Soil Chemical Analysis. Prentice-Hall (India) Pvt. Ltd.

Jägersten, G. (1940). Die Abhangigkeit der metamorphose von substrate des Biotops bei *Protodrilus* and other branches. *Ark. Zool., Bd.*, **32,** 1–22.

Johnson, L. F. Curl, E. A., Bond, J. H., and Fribourg, H. A. (1959). Methods for studying soil microflora plant disease and relationship. Burgess publishing Co., Minneapolis (USA).

Knight-Jones E. W. (1954). Laboratory experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. *J. expt. Biol.*, **30**, 584–598.

Lawson, G. W. (1966). The littoral ecology of West Africa, Oceanography and Marine Biology, *A. Review*, **4**, Edt. Barnes.

Meadows, P. S. (1964). Experiments on substrate selection by *Corophium* species: Films and bacteria on sand particles. *J. expl. Biol.*, **41**, 499–511.

Scheltema, R. S. (1961). Metamorphosis of the veliger larvae of *Nassarius obsoletus* Gastropod in response to bottom sediment. *Biol. Bull.*, **120**, 92–109.

Wieser, W. (1956). Factors influencing the choice of substratum in *Cumella vulgaris* Hart. *Limnol. Oceanogr.*, **1**, 274–285.

Wilson, D. P. (1937). The influence of the nature of the substratum on the metamorphosis of *Notomostus* larvae., *J. mar. biol. Ass. U.K.* **22**, 227–243.

———(1953 *a*). The settlement of *Ophelia bicornis* S. larvae. The 1951 experiments. *J. mar. biol. Ass. U.K.*, **31**, 413–438.

———(1953 *b*). The settlement of *Ophelia bicronis* S. larvae, The 1952 experiments. *J. mar. biol. Ass., U. K.*, **32**, 209–233.

———(1954). The attractive factor in the settlement of *Ophelia bicronis S. J. mar. biol. Ass., U. K.*, **33**, 361–380.

———(1955). The role of micro-organisms in the settlement of *Ophelia bicronis* S. *J. mar. biol. Ass., U. K.*, **34**, 531–543.

ZoBell, C. E. (1939). The role of bacteria in fouling of submerged surfaces. *Biol. Bull,* **77**, 302.