Indian Journal of Marine Sciences
Vol. 27, Sept. & Dec. 1998, pp. 489-491

Settlement pattern of the larvae of *Hydroides elegans* (Polychaeta) in the presence of petroleum hydrocarbon compounds

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Polyaromatic hydrocarbons (PAHs) have been recognised as the main toxic components of crude oil polluting the marine environment. The larvae of invertebrates, including polychaetes, are more susceptible to toxicants in the environment. The effect of different concentrations of water accommodated hydrocarbons on survival and settlement of *Hydroides elegans* (Haswell) was studied in the laboratory. A parallel study of the settlement pattern in the field was conducted using terra-cotta tiles. The dissolved / dispersed hydrocarbon concentration in the chosen area of Cochin harbour was monitored during the study period. The presence of PAHs in high concentrations was observed to have an adverse effect on the settlement of the larvae of *Hydroides elegans*.

Larvae of marine benthic organisms are an important ontogenic link which decides the pattern of survival, abundance and distribution of adults. Usually they are highly sensitive microscopic meroplanktonic organisms with an ephemeral existence. The process of attachment is the cardinal factor which controls survival of the adults. It has been proved that these larvae have the ability to explore the substratum and delineate the physical and chemical characteristics of the environment[1,2] Those adults having larvae with such abilities have successfully colonized the subtidal areas. Thorson[3] has discussed at length the relevance of planktonic larvae with reference to the distribution of benthic adults. Sessile hard bottom-dwelling invertebrates normally produce large numbers of meroplanktonic larvae developed from telolecithal eggs. Such larvae form excellent experimental material for various methods of testing including behaviour at the point of settlement. The fact that they remain planktonic for a short duration helps in preparing effective experimental protocols to analyse pre-settlement behaviour. *Hydroides elegans* is a ubiquitous and dominant species in the fouling community found abundantly in the Cochin Harbour. Its larvae can be subjected to toxicological experiments in the laboratory with relative ease. The present investigation involves release of larvae of a polychaete *Hydroides elegans* into polluted experimental medium and analyses of subsequent settlement behaviour.

Six-day old trochophore larvae of a polychaete *Hydroides elegans* (Haswell) were used as test organisms in the laboratory and one week and above old settled larvae were monitored in the field study in Cochin harbour (lat. 9° 55′ N; long. 76° 15′ E). *Hydroides elegans* larvae were reared to metamorphic competence in six days using the method of Hadfield *et al.*[4] They were fed with a culture of phytoplankton *Isochrysis galbana* for a period of six days.

Water accommodated fraction (WAF) of Bombay High Crude (BHC) was prepared by stirring BHC and filtered seawater (30 ppt) in the ratio of 1:10, for 14 hours. The fluorescence of the extract of WAF in n-hexane (HPLC grade) was recorded using a Hitachi F-3010 fluorescence spectrophotometer (Ex-310nm Em-360nm) and the strength of the WAF calculated in µg / l (ppb) from a standard graph for BHC.

The LC_{50} of five-day old *Hydroides elegans* larvae was used as a convenient preliminary screening method to determine the approximate sublethal and lethal concentrations. The Hadfield *et al.*[4] method was used as the guideline for designing the experiment. Required concentrations (25, 50, 75, 100 ppb) of dissolved / dispersed hydrocarbons were prepared from the WAF. About 25 ml of the required concentration was taken in a petridish. A glass slide, immersed in seawater for 3 days for the formation of a biofilm was introduced into the petridish and 1 ml of the seawater containing six-day old larvae was introduced into the petridish. *Isochrysis galbana* (1 ml) was also added and the petridish covered. Tests for each concentration were done in· triplicate. The experiment was repeated on five occasions.

The average number of larvae per ml was calculated by counting under a stereo microscope. The total number of larvae which had settled was computed as a percentage of the total number of larvae introduced and compared with that of total number settled in control dishes.

For field study terra-cotta tiles were hung at approximately 1 m depth in a jetty area which is a chronically polluted area in Cochin harbour with regard to petroleum hydrocarbons. The dissolved and dispersed petroleum hydrocarbon content of both surface and subsurface levels were monitored regularly using the standardised method recommended by the IOC[5].The settlement of *Hydroides elegans* on tiles were counted on a fortnightly basis and expressed as no./25 cm^2. A correlation between the presence of petroleum hydrocarbons in water and the individual settlement was attempted.

Larval settlement in the presence of high concentrations of polyaromatic hydrocarbons (PAHs) on the substratum was studied by using four sets of tiles, prepared in the following way and hung at the above station at 1 m depth: 1) control tile with no coatings, 2) tile coated with binder (5 g of rosin), 3) tile coated with binder (5 g of rosin) and 5 ml of BHC, (PAH-0.001575 gm / ml), and 4) tile half coated with binder and other half with binder and 5 ml of BHC. Settlements on the tiles were compared seven days after immersion.

The LC_{50} for penultimate settlement phase and survival of larvae was found to be 225 ppb of water accommodated hydrocarbons within 1% level of significance. Settlement was observed in concentrations above 225 ppb up to 1000 ppb but the settled larvae did not survive beyond 24 h. At sublethal concentrations all the settled larvae remained alive and active when fed regularly. The settlement of larvae, which occurred even under lethal concentrations[6] of hydrocarbons (HCs) shows that HCs have no direct effect on the mucoid adhesive secretion at the time of attachment of the larvae. It is also possible that HCs trigger premature settlement or interfere with the general metabolism of the larvae leading to death of the larvae. It is beyond the scope of the present study to pinpoint the exact mechanism by which HCs cause the death of settled larvae. However, if it is assumed that the manner in which HCs affect life and activity of larvae is similar to the general response of invertebrate larvae to chemical cues[3], then mortality could be attributed to interference with the general metabolism of the larvae. If HCs interfere with the enzyme cascade reaction or the production of a second messenger, it would lead to depolarization of cell membranes which in turn would render the nervous system inactive. Prolonged narcotization would lead to non co-ordination of metabolism and consequently death[7].

The results of the sublethal toxicity laboratory study on penultimate settlement phase (Table 1) reveals a declension in the number of settlement as the concentration of dissolved / dispersed HCs in the medium increased. This could be due to either or both of the following factors:

i) The activation of genetically programmed sequence of events leading to metamorphosis is through the binding signal molecules on specialized receptor cells of larvae[7] . This process may be disrupted by petroleum hydrocarbons present either in the medium or the substratum.

ii) It was noticed that the presence of a biofilm was essential for the settlement of the larvae. It is possible that petroleum hydrocarbons at higher concentration have a toxic effect on the biofilm.Further studies involving the response of biofilms to petroleum hydrocarbons is necessary to establish this.

A negative correlation between the presence of dissolved / dispersed HCs and settlement of larvae is evident from the results of field study (Table 2). This can be taken as a trend, though definite conclusions cannot be made about the same as several other factors such as light intensity, temperature fluctuations, dissolved oxygen content , availability of food, turbidity, currents, conditions on the substratum etc. contribute to the nature of

Table 1—Percentage of settlement of *Hydroides elegans* larvae in the presence of various concentrations of water accmmodated hydrocarbons

Concentration of water accommodated petroleum hydrocarbons (ppb)	Percentage of *H.elegans* larval settlement ± SD
0	90.8* ± 2.8
25	64.8* ± 7.4
50	57.1* ± 5.0
75	53.9* ± 6.0
100	51.9* ± 4.8

Significance level: * $p< 0.001$

Table 2 - *Hydroides elegans* larval settlement and concentration of dissolved hydrocarbons in the ambient medium during peak period

Date of collection	Av. no. of larvae settled/25 cm^2	Av. surface conc. of dissolved HCs (ppb)	Av. subsurface conc. of dissolved HCs (ppb)
12 Dec.' 95	15 ± 5.26	137.2	45.7,
24 Dec.' 95	15 ± 6.29	153.3	109.5
14 Jan. '96	12 ± 4.23	420.6	155.9
08 April '96	2 ± 1.81	713.6	225.7

settlement. The experiment was not continued after 7 days as sporadic growth of sponges was noticed on the substrata coated with PAH.

It may be difficult to correlate the results of the laboratory study with that of the field studies as the conditions encountered by the settling larvae in the laboratory are totally different from those in the field. However, it has been noticed that in the laboratory, the larvae did not survive beyond 24 h in the 225 ppb -1000 ppb range of water accommodated petroleum hydrocarbon fraction, whereas survival and settlement of larvae was found to be possible even above 225 ppb in the field. A major difference between the water accommodated petroleum hydrocarbons used in the laboratory study and that encountered in the field is that the water accommodated fraction used in the laboratory study was freshly prepared while the dissolved / dispersed hydrocarbons found in the field were `aged' or weathered, the aging process having removed many of the toxic components. Some evidence of this can also be gathered from the fact that freshly spilled oil appears to have been acutely toxic, causing large scale mortality of settled *Hydroides elegans* after the oil spill on 15 March 1996 in Cochin harbour[8] . Evidence for the non-settlement of the larvae on substratum with high concentrations of PAHs was obtained from an analysis of the results of field study (Table 3). The larvae might have either avoided the PAH coated area on the panels or might have failed in their attempt to settle due to a physical factor induced by the presence of PAHs. Settlement is effected by the microcilia of larvae, followed by secretion of the mucoid adhesive disc[9]. On a PAH - coated surface, the larval microcilia may have been unable to get a firm hold and hence this factor could have been the deterrent.

Table 3 - Effect of high concentration of PAHs on substratum on settlemtent of *Hydroides elegans* larvae

Type of substratum treatment	Number of *H.elegans* larvae settled/25 cm^2
Control	7
Binder	5
Binder + BHC	Nil
One half coated with binder	5
The other half coated with binder + BHC	Nil

An oil spill occurred in Cochin harbour on 15 March 1996. Water analysis on 17 March 1996 showed the presence of 14.1 mg / l oil in subsurface waters at the monitoring station. About 99.4% mortality was noticed among the settled *H.elegans*. Death was due to both smothering by the heavier fractions of the slick as well as the toxic effect of petroleum hydrocarbons in the medium, as some tiles with dead colonies were not coated with the water-in-oil emulsion as the others were.

The presence of dissolved/dispersed hydrocarbons in the water column and on the substrata adversely affect the settlement of the larvae of *Hydroides elegans*. It was proved through laboratory studies that failure to successfully settle and subsequent mortality of settled larvae is controlled by increase in concentrations of dissolved/dispersed hydrocarbons. It was also proved that freshly spilled/added hydrocarbons are more lethal or detrimental to settlement..

Financial assistance, from CSIR, New Delhi to one author (MJA) is gratefully acknowledged.

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