Isolation, culturing and larval rearing of adult Barnacles - a Universal biofouler from Visakhapatnam coast on Artificial Panel (WIC) System.

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The most dominant species that has successful settlement on the panel system was observed is *Amphibalanus amphitrite*. Adults were cultured in the laboratory for releasing nauplii and were reared under laboratory conditions and used in settlement assay. The results of this study indicated that the Barnacle *Amphibalanus amphitrite* is the most common biofouling organism present on all the three types of materials used in the experiment, in the coastal region of Visakhapatnam harbor.

[Keywords] Biofouling, barnacles, nauplii, cypris, artificial panel (W = wood, I = iron, C = concrete) system.

Introduction

The term ‘fouling’ (or ‘biofouling’) refers to the colonization of a substrate by an opportunistic organism (or multiple species of colonizing organisms), and has been extensively studied and qualified by numerous researchers 1,2,3,4.

Previous research has identified discrete stages in the substrate colonization process, with the initial stages of fouling occurring almost instantaneously on contact with the (typically) marine aquatic environment 3,4. The rapid formation of this initial biological ‘film’ over a surface is often quickly followed by colonization by larger, more conspicuous and persistent organisms 3,4. Fouling of ship hulls and niche areas represents a significant cost for the maritime industry. Higher maintenance costs, increased fuel requirements due to greater levels of hull drag, lost productivity due to more frequent dry-docking for removal of fouling organisms, and the cost of compliance with environmental regulations create a significant burden for maritime operators, whether private or public 6,7,4. It is well known that marine fouling organisms often cause technical and economic problems by settling on artificial surfaces submerged in seawaters in coastal waters of Visakhapatnam harbor.

Materials and Methods

Visakhapatnam harbour is located at 17°41′38.25″N to 17°42′31.3″N and 83°16′48.1″E to 83°19′49.3″E, along the east coast of India. It consists of an inner harbor and an outer harbor. There are 24 berths and 4 moorings to accommodate 28 ships at a time. Visakhapatnam harbor, a semi-enclosed water body is one of the largest and busiest ports of India along the east coast of India. Since the beginning of 1950s, many large scale basic and other major industries were set up by the government and the private sector in its vicinity. The harbor is land-locked and this has resulted in limited natural flushing processes enhancing pollution stress on the harbor 17,12. (Fig. 1.)

Wood Iron Concrete (WIC) panel system of size (15x15) cm was placed in water tied with nylon threads along the berths of Visakhapatnam Harbor. After 3 months, the WIC panels were collected in sterile polythene covers and brought to laboratory for further investigations. The artificial iron panels were
washed with sterile physiological saline to remove vegetative cells and the panel system is cleaned with brush to remove unwanted flora and the panel system is placed in 20 l drum with aerated sea water for further analysis.

Artemia nauplii and algae Chaetoceros gracilis were used as substitute feed for the brood stock of Balanus amphitrite. Laboratory temperature was maintained at 23±1°C instead of 25°C and raw sea water of salinity ranging between 30-33‰ was used instead of 5‰. Artemia cysts were kept in a “V”-shaped container (inverted bottle) along with seawater filtered through glass microfiber filter paper (GF/C, 1μm) and provided with vigorous aeration keeping in mind that no cyst should settle down.

Adults of barnacle Balanus amphitrite were collected from the field by using Artificial panel system (WIC) plates. WIC plates (15x15 cm) immersed at Fishing harbor Visakhapatnam, tying with thread at mid tide zone. During the immersion period (February-April), the plates were observed every week to check the growth of fouler. After three months of exposure period, plates with adult B. Amphitrite were brought to the laboratory and washed with fresh water to clean the debris. Dead shells and barnacles under stress with loose operculum were scraped out. Culture of adult barnacles was carried out by following methods of 14, 15. Collected healthy barnacles were maintained as brood stock in a 20 l drum with aerated sea water and on a mixed diet of Artemiasalina naupli (200 individuals/barnacle/day) and Chaetocerosgracilis (2 x 10^5 cells/ml). The laboratory temperature was maintained at 28±1°C. Culture water was renewed every day.

The brood stocks were subjected to regular exposure to air to stimulate the release of nauplii. Excluding one side, remaining all the sides of culture tank was covered with black cloth. Once hatched, the larvae were attracted to the uncovered side by using a point light source. The aggregated larvae were collected by using pasteur pipette and transferred to 1 l culture beaker with filtered seawater (GF/C, 1μm). Black tape was stickered to the outside of the larval culture beaker at the water level to prevent the positively phototactica-nauplii to concentrate at the meniscus. The larval density was maintained as 2 larvae/ml of filtered sea water and on a diet of Chaetocerosgracilis with a cell concentration of 2 x 10^5 cells/ml. Laboratory temperature was maintained at 23±1°C. Cyprids were collected on 5th-6th day and used in settlement assay.

**Results and Discussion**

Two species of adult barnacle from Visakhapatnam harbor were identified to species level, namely Balanus balanus, Balanus amphitrite on test panels, all the three materials showed fouling activity. Wooden panel showed maximum fouling activity compared to the iron and the concrete panels. (Fig 3) The adult barnacles were identified to species level based on their morphology and served as the adult reference collection for subsequent comparison.

Temperature, salinity and pH of the sampling site were recorded as 31°C, 35 ppt and 7.8 respectively. The same hydrographic parameters were maintained in the laboratory during the larval rearing.

Visakhapatnam sea coast has a natural harbor of high activity and is unexplored in the study of biofouling (macrofouling). Hence there is need to study the locally associated biofouling organisms so that this could become the reference in future research. Intensive field work was carried out to generate continuous data on biofouling and their settlement pattern. It contributed substantially to the fouling information at Visakhapatnam harbor, one of the busiest harbor along the east coast of India. In view of increasing naval activities, the environmental status is changing rapidly and in fact, this is affecting species diversity endemic to the harbor. The study was undertaken to collect information on changes in...
species richness and its occurrence in relation to the type of panels used (wood, iron and concrete). (Fig. 2)

In recent years, barnacles have come under detailed investigation because of the following reasons: i) they usually dominate a fouling community, irrespective of the geographical region and season, ii) they secure permanent attachment on many kinds of substrata, and iii) their larval stage, the Cypris, possesses the remarkable capacity to settle under different environments such as in fast-moving waters, coastal waters, offshore areas, backwaters and polluted ports. In order to develop a successful antifouling system, an accurate knowledge of the organisms involved and the processes that control their settlement are required.

The observations showed that the species *Amphitrite* were evenly distributed on all the three substrates provided as test panels, it is evident that barnacles are the major fouling organisms settling on the hard surfaces throughout the world (Fig 3). Temporal and spatial fluctuations in the recruitment of barnacles and also other fouling organisms on various substrata have been studied over the decades. The populations of marine invertebrates in a given area are mainly controlled by the availability of larvae in the water column and the lifecycle of barnacles consists of six planktonic naupliar stages and a pre-settling cyprid stage.

Temperature and nutritional conditions are the major factors reported to affect the breeding and molting processes in barnacles. The adult barnacle species identified in this study belong to the genera, namely *Amphibalanus*, which is commonly found in tropical and subtropical mangrove habitats. This current study is the first report of identification of the fouling barnacle species in Visakhapatnam region. *Amphibalanus amphitrite (=Balanus amphitrite)* is used as a model organism for ecological studies and antifouling assays due to its worldwide distribution and year-round breeding activity.

Cyprid attachment and metamorphosis on hard surfaces play a key role in the barnacle life cycle and determine the distribution and abundance of marine habitats. Hence, the larvae are reared in the laboratory conditions to study the antifouling settlement assay. Several studies were conducted on the reproduction, larval development and metamorphosis of *A. Amphitrite* in the seas worldwide.

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Fig. 2 — (a) WIC Panel System (b) Wooden panel (c) Concrete panel and (d) Iron panel before immersion into sea water, each panel is measuring 15 cm.

Fig. 3 — (a) WIC panel immersed in sea water (b) barnacles present on wooden panel (c) Concrete panel (d) iron panel after 3 months.

Fig. 4 — (a) Collected healthy barnacles were maintained as brood stock in a 20 l drum with aerated sea water and on a mixed diet of *Artemiasalina* naupli.
Conclusion
Visakhapatnam sea coast is a natural harbor of high activity and is unexplored in the study of biofouling activity. Barnacles are a type of arthropod belonging to the infraclass Cirripedia in the subphylum Crustacea, two different species namely *Balanus amphitrite* and *Balanus balanusan* were isolated from the artificial panel system and proved that the barnacles are the universal biofoulers and to study the antifouling settlement assay, the larvae of the Barnacles are reared exclusively in the laboratory for further study.

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Reference