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## **Epibiotic community on the acorn barnacle (*Balanus amphitrite*) from a monsoon influenced tropical estuary**

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### **Abstract**

The epibiotic community (diatoms and metazoans) on the outer surface of the barnacle, *Balanus amphitrite* shell (BSh) and opercular valves, scutum and tergum (BST) was investigated on a monthly basis for one year from a tropical monsoon influenced estuary and compared with that of surrounding rock biofilm. BSh and BST were rich in *Achnanthes longipes* and *Melosira nummuloides* among the diatoms and among the invertebrates, nematodes and tardigrades were abundant. To the best of our knowledge, this is the first report of epibiosis on the acorn barnacle. Diatom abundance was maximum during the monsoon season on all the intertidal substrata viz. BSh, BST and rock. A significant correlation was found between the density of diatoms and invertebrates associated with the BSh and BST possibly due to the trophic relationship or suitability of the physical environment. The possible role of these epibionts on the barnacle settlement is currently unknown and needs further validation.

**Keywords:** Barnacle, settlement, epibionts, seasonal variation

## Introduction

Space limitation in the marine ecosystem makes the hard surfaces of organisms prone to epibiosis (Metri *et al.* 2002) that acts as a second skin, modulating the host's biotic and abiotic interaction with the environment (Wahl *et al.* 2012). In the marine environment, this is an interesting phenomenon because some groups such as sponges, ascidians and macroalgae use their surface bacteria as an antifouling strategy (Dobretsov *et al.* 2006; Armstrong *et al.* 2001); while in barnacles, they mediate the settlement of conspecific cyprids (Khandeparker *et al.* 2006). This interaction may bring benefits as well as disadvantages to the organisms involved (Fernandez-Leborans 2011). The presence of epibionts may modify the host's morphology and subsequently may alter the host's interaction with the environment (Thieltges & Buschbaum 2007).

The acorn barnacle, *Balanus amphitrite*, being a sessile animal on the rocky shore, is a dominant fouling organism all over the world and a model organism for biofouling studies (Thiyagarajan & Qian 2008; Anil *et al.* 2012; Maruzzo *et al.* 2012). This barnacle has two types of mineralized exoskeletons: a cone shaped shell and an operculum (Khalifa *et al.* 2011). The shell is calcareous in nature and is found next to a chitinous exoskeleton for protection against predators (Swift 2012). The operculum forms the door of the barnacle consisting of scutum and tergum that aid in feeding by opening the valves during high tide and closing during low tide (Swift 2012). The planktonic life cycle of the barnacle includes six naupliar stages followed by a transitional cyprid stage. The competent cyprid stage, which is simple, less developed and less organized gets transformed into a complex, well developed and organized adult, when it comes in contact with the species-specific chemical cues. These cues, being transduced into internal processes with neural and/or hormonal elements, act as morphogenetic signals that initiate a signalling cascade via external chemoreceptors which in turn brings behavioural changes, initiating larval settlement and metamorphosis (Jackson *et al.* 2002; Hadfield 2011).

In the field, barnacles are covered with epibiotic biofilm, and bacteria in the biofilm matrix have been reported to play an important role in the settlement (Khandeparker *et al.* 2003; 2006; Khandeparker & Kumar 2011; Bacchetti De Gregoris *et al.* 2012; Khandeparker *et al.* 2014). In a pilot study, Khandeparker *et al.* (2003) reported that exopolymers from the shell associated bacteria can induce barnacle larval settlement and another study proved the uniqueness of these bacteria to the barnacle shell, that helps the cyprid to recognize parentally associated biofilms (Bacchetti De Gregoris *et al.* 2012). Other than bacteria, biofilm diatoms are also known to induce the larval settlement of barnacles (Patil & Anil 2005). Further, it has been hypothesized that the nature of biofilm as well as the metabolites released by them possibly pilot the competent larvae towards the

parental population for successful settlement, recruitment, reproduction and hence survival of their population (Khandeparker *et al.* 2006). Although many studies aimed at identifying the role of the biofilm bacteria in the larval settlement, no attempt has ever been made to characterize the other components of the biofilm on the barnacle shell. Thus, in the current investigation, the epibiotic community on the outer surface of shell (BSh) as well as on the opercular valves such as scutum and tergum (BST) of *B. amphitrite* was characterized and this data was compared with that of the surrounding rocky habitat.

## **Materials and Methods**

### ***Study area***

The study area is Dona Paula Jetty (15°27.5'N, 73°48'E) in the Zuari estuary, central west coast of India. This is a monsoon influenced environment and to assess the role of monsoon on the epibiotic community structure, samples were collected on a monthly basis for a period of one year. Based on the influence of monsoon, a year can broadly be classified into pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January) seasons (Desai & Anil 2005).

### ***Organisms on the barnacle shell (BSh) and scutum and tergum (BST)***

Intact adult barnacles (N=5) having epibiosis (based on greenish colour of the shell) were collected from the intertidal zone at the same elevation level and 10-15 cm distance was maintained between any two individuals. Simultaneously, samples were also collected from the surrounding rock surface for comparison.

In the laboratory, BSh and BST of the barnacle were separated. Prior to dissection, Rostro-carinal Basal (RCB) diameter and height of the barnacle was measured. After dissection, scutum and tergum were measured using a vernier caliper by considering a square and two scalene triangles respectively. During this process, utmost care was taken to avoid contamination of the shell epibionts from the scutum and tergum. This was done to characterize the epibiotic community on the BSh and BST. Cirral part was removed initially in order to avoid the phytoplanktons which might have struck during ingestion. Subsequently the outer surface of the shell, scutum and tergum were scraped with a nylon brush, separately into a vial containing known volume of filtered seawater (0.22μ) and preserved using lugol's iodine. During the analysis, 1 ml was taken in a Sedgwick rafter counting chamber and the diatoms were enumerated to the lowest possible taxa. Since the preserved samples were analyzed immediately, the replicates were restricted to five, because enumeration and identification of diatoms is a time consuming process. The diatoms were identified by using identification keys (Desikachary 1987; Tomas 1997; Horner 2002) and the metazoan invertebrates

were identified by using available keys (Higgins & Thiel 1988). For invertebrates, the whole sample was analysed and the results were noted down.

### ***Organisms on the rock***

A nylon sieve having a 10 cm<sup>2</sup> hole was placed on the rock surface and the exposed area was scraped using a nylon brush, from five spots (in the vicinity of the barnacles) into a vial containing a known volume of filtered seawater (0.22μ). The samples collected from the rock surface were preserved in the laboratory using lugol's iodine, and 1 ml of the sample was taken in a Sedgwick rafter counting chamber and enumerated.

### ***Environmental variables and lab analysis***

Environmental variables of the nearby water in the low tide zone were measured because of their influence on the barnacle epibiota. Temperature was recorded by a hand held thermometer, salinity by a portable refractometer (Atago, Japan) and pH by a pH meter (InoLab, pH 7110, WTW). Samples for dissolved oxygen (DO) analysis were collected in duplicates and fixed *in situ* with Winkler reagents and later analyzed in the laboratory. Nutrient samples were collected and immediately carried to the laboratory for preservation in deep freezer (-20°C) and later analyzed by a SKALAR segmented flow autoanalyzer (Skalar San<sup>++</sup>, Netherland).

### ***Calculation of Surface area***

To calculate the surface area, the barnacle was considered as a cone and the area was calculated as per the formula given by Sun & Liu (2003). The surface area of scutum was calculated as a square whereas that of tergum was calculated as two scalene triangles as per Heron's formula (Table 1).

### ***Laboratory experiments of *Achnanthes longipes* and *Melosira nummuloides* with the BSh***

Laboratory experiments were carried out to see the effect of BSh on both the dominant diatoms *Achnanthes longipes* and *Melosira nummuloides*. The experiments included four treatments (1) Filtered Autoclaved Sea Water (FASW) (0.22μ), (2) FASW with crushed BSh (3) f/2 medium and (4) f/2 medium with crushed BSh and was repeated three times with replication (n=3). Experiments were carried out in sterile polystyrene multiwells for 14 days in 12:12hr light: dark cycles at 24°C±1. Salinity was maintained at 35 PSU for all the treatments. Barnacle shells were first brushed thoroughly to remove all the epibiotic biofilm, and then shade dried, crushed and autoclaved before use. 100 mg of crushed BSh was used in the experiment in order to fully cover the multi well homogeneously.

### ***Data analyses***

To know the influence of seasons and substrata on the abundance of diatoms, two-way ANOVA (normalized after log<sub>10</sub> transformation) was applied using SPSS statistics 17.0 software. Since the direct effect of monsoon was evident only during June, July and August, September month was excluded from the monsoonal months. Univariate and multivariate analyses were carried out using Primer 6 ecological software. Margalef's species richness (d), Shannon-Wiener diversity (H') and Peilou's evenness (J) were calculated. Non-metric multidimensional scaling (nMDS) was constructed based on the total diatom abundance per month after log (X+1) transformation and groups were formed at 90% similarity. In laboratory experiments, to know the effect of BSh on the growth of *A. longipes* and *M. nummuloides*, One-way ANOVA was applied in case of normalized data to both the diatoms and where ever the data was not normal, the nonparametric test (Kruskal-Wallis) was performed.

### **Results**

#### ***Seasonal variation in the environmental parameters***

Air and water temperature ranged from 24-30°C and 26-29°C respectively (Fig. 1). The effect of monsoonal conditions was clearly visible in the salinity and nutrients. Salinity was very low during the monsoon season, abruptly increased in September after monsoon and little variation was observed thereafter. pH varied from 7.98- 8.21 and DO from 3.58-5.64 ml/L (Fig. 1). Among the macronutrients, nitrate and silicate concentrations were more during the monsoon when compared to the premonsoon and postmonsoon seasons (Fig. 1). Phosphate concentration ranged from 0.35-1.19 µmol/L (Fig. 1).

#### ***Seasonal variation in the diatom community***

BSh, BST and rock surface harboured 25 diatom genera out of which 17 were pennates and 8 were centrales. The pennates were *Achnanthes longipes*, *Amphora* sp., *Bacillaria paradoxa*, *Caloneis* sp., *Cocconeis* sp., *Diploneis* sp., *Fragillaria* sp., *Gyrosigma* sp., *Licmophora* sp., *Navicula* spp., *Nitzschia* spp., *Pleurosigma* sp., *Pseudonitzschia* sp., *Surirella* sp., *Synedra* sp., *Thalassionema* spp., Unidentified sp. and the centrales were *Melosira nummuloides*, *Biddulphia* sp., *Chaetoceros* spp., *Coscinodiscus* spp., *Thalassiosira* spp., *Cyclotella* sp., *Dictyocha* sp., *Paralia* sp.

Abundance of diatoms was highest during the monsoon on all the substrata (Fig. 2). Amongst the substrata, BST recorded maximum abundance over BSh and rock (Fig. 2). The abundance of diatoms on different substrata was significantly different during different seasons (Table II). Shannon wiener's diversity index (H') and species evenness (J') among the substrates was found to be

maximum on BST, (1.71 in November and 0.85 in January respectively); however, species richness (d) was highest (1.19) in January on the rock (Table III). BSh showed highest (14) species diversity (S) in the month of July (Table III).

The percentage composition of diatoms (Fig. 3) revealed dominance of *A. longipes* on the rock during premonsoon, which decreased during the monsoon and the abundance was lowest during the postmonsoon season. *Navicula* spp. were abundant during the monsoon and postmonsoon seasons and were minimum during the premonsoon season. On the contrary, on the surface of BSh and BST, *M. nummuloides* was reported as the dominant diatom in the month of September. The diatom communities in the month of May and September were similar in both the BSh and BST. Apart from these, *Licmophora* sp. was present in significant numbers on the rock surface during the monsoon and was a minor contributor on BSh and BST throughout the year. From the SEM images (Plate 1), it is clear that the diatoms exhibited micropatchiness and *A. longipes* and *M. nummuloides* were the dominant diatoms”.

MDS ordination formed two groups based upon the total diatom abundance per month on all the substrates at 90% similarity (Fig. 4). In the rock community, June and July (monsoon) formed one group and the remaining months formed another group. In the BSh and BST community, a distinct group was formed in December and January (post monsoon) while the remaining months formed the other group.

#### ***Monthly variation in the metazoan community***

Metazoan community found on the rock, BSh and BST revealed seven taxa of invertebrates viz. nematoda, tardigrada, harpacticoida, uropodina, halacarida, polychaeta and foraminifera respectively. Total metazoan density on the BSh was highest in October and lowest in December and monsoon period observed significantly higher density than pre- and postmonsoon (Fig. 2). But in contrast to BSh, the metazoan density was more on the BST, being highest in June and lowest in January (Fig. 2). On the other hand, metazoan density on the rock was very low, highest being  $16 \pm 5$  ind. /10 cm<sup>2</sup> in February; but in most of the months, no metazoans were found (Fig. 2). Nematodes and tardigrades were the dominant metazoans in all the substrata, percentage being 84-100% (data not shown) of the total density and other five groups were occasionally found in less numbers.

#### ***Correlation between the microalga and metazoans***

Due to the possibility of trophic relationship existing between microalgae and several metazoan invertebrates, correlation was drawn between them and a positive relationship was observed (( $r=0.67$

( $p \leq 0.05$ ) and  $r = 0.70$  ( $p \leq 0.05$ ) on the BSh and BST respectively. But no significant relationship was found between the two components found on the rock substratum (Fig. 5).

### ***Interaction of the epizooic diatoms Achnanthes longipes and Melosira nummuloides with the BSh***

It was observed that growth of both *Achnanthes longipes* and *Melosira nummuloides* was significantly higher, irrespective of the media used (FASW and f/2 medium) when supplemented with crushed BSh (Fig. 6). In case of *Achnanthes longipes*, all the treatments were significantly different from day 2 to day 12 (one way ANOVA,  $p \leq 0.001$ ) whereas in *Melosira nummuloides*, significant difference was observed from day 4 to day 14 (Kruskal-wallis,  $p \leq 0.05$ ).

### **Discussion**

Epibiosis on the acorn barnacle in the current investigation is the first report to the best of our knowledge. The barnacle shell (BSh) and scutum and tergum (BST) although are quite similar chemically (Khalifa *et al.* 2011), differ from one another in terms of their motility. The former is stationary while the latter is highly motile when the barnacle is immersed in water in order to capture food particles from the water and becomes inactive during low tide during the emersion period. While both the structures are prone to diatom attachment, the density was more on the BST than on the BSh, which could be due to their motile nature that possibly makes the diatoms to gain access to nutrients. Moreover, different diatom assemblage was found on the rock (epilithic diatom) when compared to the barnacle (epizooic diatom) and this can be attributed to the inert nature of the rock. Earlier, it has been reported that the community structure of benthic organisms depends upon the mineralogical/ chemical composition of the substrate (Bavestrello *et al.* 2000) and possibly this is also applicable to the diatom communities. A recent study on the diatoms on the rocks (epilithon) and on the macroalgae (epiphyton) attached to rocks reported that the remarkable difference between these two assemblages and their species composition largely depends upon the particular substrata (Almeida & Beltrones 2012). It has also been earlier reported that the density of most of the diatoms is more on the living substrata such as macroalgae and marine angiosperms than on non-living substrata (Hopkins 1964; Main & McIntire 1974). Our study highlights the rich diversity associated with the epizooic assemblages than the epilithic assemblages which contrasts to the earlier study (Almeida & Beltrones 2012) that describes more diversity on epilithic assemblages over epiphytic one. Rocky substratum, being inert, possibly does not interact chemically or biologically with the epibionts and remains stable irrespective of environmental conditions (Bergey 2008). In contrast, the host barnacle, although stable, biologically interacts with epizooic diatoms from the time of recruitment and similar interactions are known from the studies on macroalgae (Huang & Boney

1983). Moreover, the diatoms also produce some allelochemical compounds such as aldehydes, flavonoids, alkaloids, phenols and even cyanogen bromide that can suppress the growth or be lethal to other members of the phytoplankton altering the structure of diatom assemblages (Ianora & Miralto 2010; Scholz & Liebezeit 2012; Vanellander *et al.* 2012). Hence it is possible that the distinct diatom assemblages interact among themselves in a complex manner as well as with the exudates from the barnacle (including SIPC) and shell associated invertebrates.

Microscale patchiness of bacteria in the marine environment is previously known (Khandeparker *et al.* 2006) and in this current investigation, dominant diatoms such as *A. longipes* and *M. nummuloides* also exhibited micropatchiness on the outer surface of BSh and BST. To the best of our knowledge, this is the first report of these diatoms found epibiotically on the acorn barnacle. *A. longipes*, being a chain forming pennate diatom, has been previously reported in the epilithic or epiphytic community in the intertidal habitat of estuaries, forming dense patches in biofilms (Round 1971; Lange-Bertalot & Krammer 1989; Lewis *et al.* 2002; Mikhail & Gergis 2008). It produces highly organized extracellular adhesive biocomposites for cell motility and gets attached permanently to submerged structures with the help of stalks (Wang *et al.* 1997). The stalk helps the diatom to remain vertical for access to more nutrients and light outside of the biofilm matrix (Lewis *et al.* 2002; Molino & Wetherbee 2008). On the other hand, *M. nummuloides* is a centric chain-forming diatom having planktonic, benthic as well as epiphytic mode of life in the intertidal environment (Lebour 1930; Rendall & Wilkinson 1986; Mitbavkar & Anil 2008). This species tolerates wide ranges of salinity making it suitably adapted to estuarine conditions as observed in this study. Moreover, this species has been reported to uptake organic amino acids under the conditions of low or absence of inorganic nitrogen and meet their requirement due to the presence of effective transport systems in the membrane; but it cannot utilize other organic substrates such as sugars, organic acids or sugar alcohols (Hellebust & Guillard 1967; Hellebust 1970; McLean *et al.* 1981).

The high growth rate of *M. nummuloides* as well as *A. longipes* in the presence of barnacle shell could be attributed to three possibilities. Firstly, introduction of crushed shell increased the surface area, thus increasing the attachment. Secondly, the shell protein may act as a nitrogen source for the diatoms. Thirdly, barnacle shell might contain some biochemical cues for diatom settlement as the shell SIPC protein is known to induce the settlement of barnacle cyprids (Matsumurad *et al.*, 1998; Khandeparker and Anil, 2011; Pagett *et al.*, 2012). An earlier study indicated that *M. nummuloides* forms long and healthy chains in the presence of few pieces of *Enteromorpha* sp. while in its absence, it forms short and broken chains (Dalziel & Perkins 1982), suggesting the presence of some positive factor responsible for the growth of *M. nummuloides* ((Dalziel & Perkins 1982). In this



scenario, the presence of *M. nummuloides* as well as *A. longipes* epibiotically on the barnacle surface could have relevance to the settlement of *B. amphitrite* but needs further validation.

Apart from diatoms, many metazoan invertebrates like nematodes, tardigrades, harpacticoids, halacarid mites, urpodina mites, polychaetes and foraminiferans were also present on the shell of *B. amphitrite*. Out of them, nematodes and tardigrades were dominant and this can be attributed to their trophic link or to the suitability of the physical environment. Diatoms have been observed under the microscope in the gut of nematodes (personal observation) describing their link in the food chain while the same is not known in the case of tardigrades. Moreover grazing of diatoms by invertebrates is one additional factor that shapes the diatom community structure (Totti *et al.* 2007). Hence it will be more challenging to rear these invertebrates in the laboratory and test whether their presence on the barnacle shell has any relevance to the settlement process of barnacle cyprids.

It is well known that the monsoon alters the structure of microalgal community by altering hydrodynamics particularly increasing turbidity and decreasing salinity (Devassy & Goes 1988; Krishnakumari *et al.* 2002; Madhu *et al.* 2010; Patil & Anil 2011). Earlier studies on the glass and fibreglass substrata in the subtidal environment suggests that during monsoon the density of biofilm diatoms is minimum due to low light (cloud and turbidity), low salinity, precipitation, land run-off and wind speed (Patil & Anil 2005). But the maximum diatom abundance found in the current investigation on the intertidal rock, BSh and BST substrata during monsoon could be due to increased nutrient concentration in the water column. Alternatively, heavy precipitation during monsoon probably keeps the biofilm diatoms in better shape by preventing them from desiccation.

The sudden change in the diatom community during the monsoon is clearly evident because of prevailing monsoonal conditions. The presence of *Navicula* spp. during the monsoon and postmonsoon could be due to their adhesion strength and growth forms (Hudon and Legendre, 1987). *Navicula* and *Nitzschia* are very mobile and hence have low adhesion strength (Hudon and Legendre, 1987), making them easily resuspended in high energy environments during monsoon. After the monsoon, change in the diatom community may be possibly due to drastic changes observed in salinity and nutrients. In a community, diatoms undergo ecological succession and both *A. longipes* and *Licmophora* sp. appear when a community becomes mature (Hudon and Legendre, 1987). These matured forms were clearly visible on the rock surface during the premonsoon and a changing community was observed during the monsoon, probably generated by the runoff of freshwater.

Barnacle settlement is very crucial for rocky shore food web dynamics and has been a topic of interest in monsoon influenced habitats (Desai & Anil 2005; Gaonkar & Anil 2013). Exopolymers, secreted by the biofilm bacteria and diatoms have been known to induce cyprid settlement carrying a

plethora of inductive chemical cues (Patil & Anil 2005; Khandeparker *et al.* 2006; Bacchetti De Gregoris *et al.* 2012). Moreover, exopolymers of *Achnanthes* sp. have been reported to induce the larval settlement of polychaete *Hydroides elegans* and bryozoan *Bugula neritina* (Lam *et al.* 2003; Dahms *et al.* 2004) but no data are available on the role of *M. nummuloides* and *A. longipes* on the larval settlement of *B. amphitrite*. The current study reports some interesting facts, previously unknown, that BSh harbours diverse diatoms and invertebrates other than bacteria and possibly interact in a complex manner. The role of each of these components and their metabolites in the settlement of the cyprids of *B. amphitrite* remains unexplored and needs further validation.

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### **Legends to Tables**

**Table I.** Surface area (Mean  $\pm$  SD in cm<sup>2</sup>) of the Barnacle shell (BSh) and Scutum and Tergum (BST)

**Table II.** Two way ANOVA on the influence of seasons and substrates on the diatom abundance ( $p \leq 0.01^*$ ,  $p \leq 0.001^{**}$ )

**Table III.** Monthly variation in univariate diversity indices of the diatoms on different substrata (S-Total species, d-Species richness, J'-Pielou's evenness, H'-Shannon Wiener's diversity index).

### **Legends to Figures**

**Fig. 1.** Monthly variation in the environmental parameters of the study area.

**Fig.2.** Monthly variation in the diatom abundance (cells/dm<sup>2</sup>) and metazoan abundance (ind./10cm<sup>2</sup>) on different substrata (BSh, BST and Rock). Vertical lines indicate standard deviation from the mean.

**Fig. 3.** Monthly variation in the percentage composition (%) of the diatoms on different substrata (BSh, BST and Rock).

**Fig. 4.** MDS ordination (after log (X+1) transformation at 90% similarity) based on the total diatom abundance.

**Fig. 5.** Correlation between the diatoms and the metazoan abundance on different substrata (BSh, BST and Rock).

**Fig.6.** Growth response of the diatoms *Achnanthes longipes* and *Melosira nummuloides* in FASW (Filtered autoclaved sea water), FASW+ BSh, f/2 medium and f/2 medium+BSh for a period of 14 days. Vertical lines indicate standard error from the mean (n=9).

### **Legends to Plate**

**Plate 1.** a. *Balanus amphitrite*, b. Barnacle shell after removal of the biofilm, c. Patch of *Achnanthes longipes* on the barnacle shell, d. Magnified image of *Achnanthes longipes*, e. Patch of *Melosira nummuloides*, f. Magnified image of *Melosira nummuloides*.

Table I. Surface area (Mean  $\pm$  SD in cm<sup>2</sup>) of the Barnacle shell (BSh) and Scutum and Tergum (BST)

Months	BSh	BST
May	2.05 $\pm$ 0.40	0.17 $\pm$ 0.01
Jun	1.58 $\pm$ 0.38	0.12 $\pm$ 0.03
July	2.15 $\pm$ 0.61	0.16 $\pm$ 0.03
August	2.03 $\pm$ 0.34	0.14 $\pm$ 0.03
September	2.66 $\pm$ 0.49	0.27 $\pm$ 0.03
October	1.42 $\pm$ 0.11	0.18 $\pm$ 0.05
November	2.75 $\pm$ 0.31	0.23 $\pm$ 0.04
December	2.72 $\pm$ 0.84	0.21 $\pm$ 0.03
January	2.73 $\pm$ 0.53	0.26 $\pm$ 0.12
February	2.73 $\pm$ 0.52	0.25 $\pm$ 0.11
March	2.41 $\pm$ 0.50	0.32 $\pm$ 0.09
April	2.77 $\pm$ 0.44	0.28 $\pm$ 0.03

Table II. Two way ANOVA on the influence of seasons and substrates on diatom abundance ( $p \leq 0.01^*$ ,  $p \leq 0.001^{**}$ )

	Sum sqrs	df	Mean sqr	F	p
Season	0.379	2	0.190	5.5	0.008*
Substrate	5.042	2	2.521	73.2	0.000**
Season $\times$ Substrate	2.228	4	0.557	16.1	0.000**

Table III. Monthly variation in univariate diversity indices of diatoms on different substrata

Month	Substratum	S	d	J'	H'
M	Rock	8	0.71	0.63	1.32
	BSh	9	0.78	0.41	0.91
	BST	11	0.80	0.32	0.76
J	Rock	12	0.90	0.49	1.21
	BSh	11	0.83	0.47	1.13
	BST	8	0.55	0.71	1.47
J	Rock	9	0.71	0.71	1.57
	BSh	14	1.11	0.55	1.45
	BST	12	0.86	0.59	1.47
A	Rock	10	0.93	0.59	1.36
	BSh	10	0.79	0.58	1.34
	BST	7	0.47	0.59	1.15
S	Rock	10	1.04	0.36	0.84
	BSh	10	0.82	0.43	1.00
	BST	5	0.34	0.46	0.74
O	Rock	9	0.80	0.42	0.92
	BSh	10	0.76	0.66	1.53
	BST	11	0.77	0.58	1.40
N	Rock	9	0.78	0.45	0.99
	BSh	10	0.87	0.53	1.23
	BST	11	0.86	0.71	1.71
D	Rock	5	0.52	0.54	0.87
	BSh	7	0.68	0.67	1.30
	BST	5	0.43	0.80	1.28
J	Rock	11	1.19	0.65	1.57
	BSh	7	0.70	0.65	1.26
	BST	3	0.20	0.85	0.93
F	Rock	7	0.64	0.61	1.19
	BSh	10	0.86	0.50	1.16
	BST	5	0.35	0.63	1.02
M	Rock	8	0.81	0.51	1.06
	BSh	11	0.97	0.69	1.64
	BST	7	0.56	0.57	1.10
A	Rock	4	0.37	0.25	0.35
	BSh	12	1.00	0.56	1.39
	BST	7	0.48	0.52	1.01

S- total species, d-species richness, J'- species evenness, H- Shannon wiener's diversity index



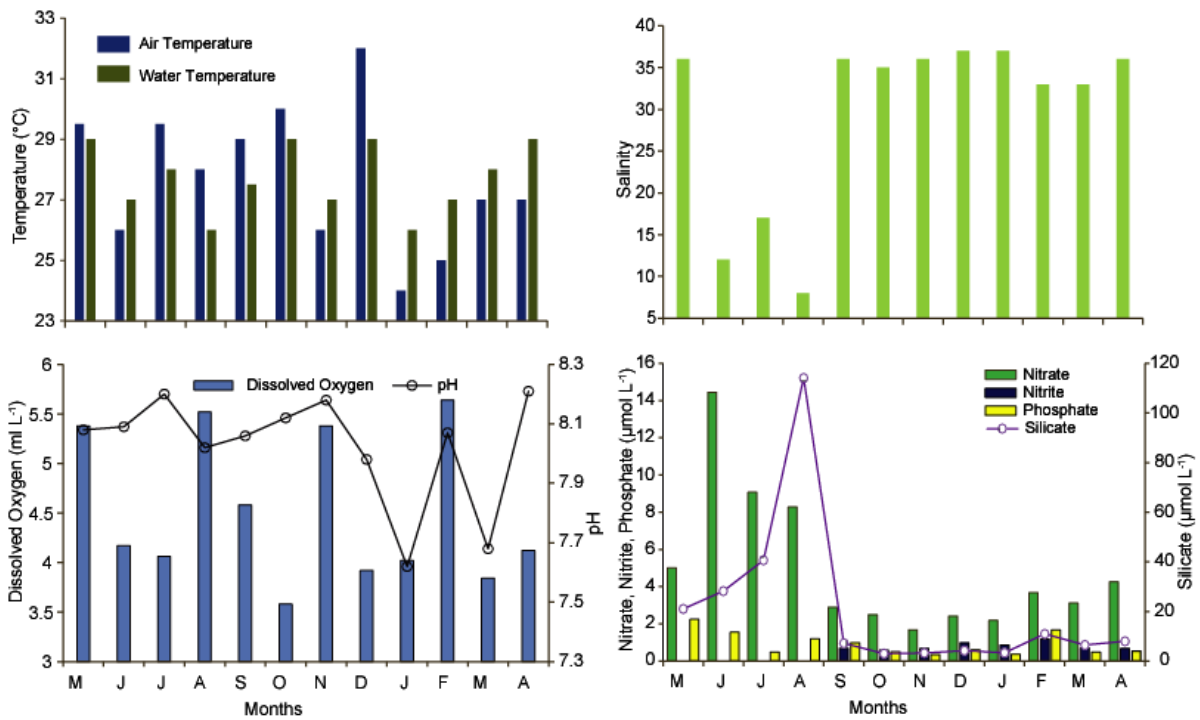


Fig. 1

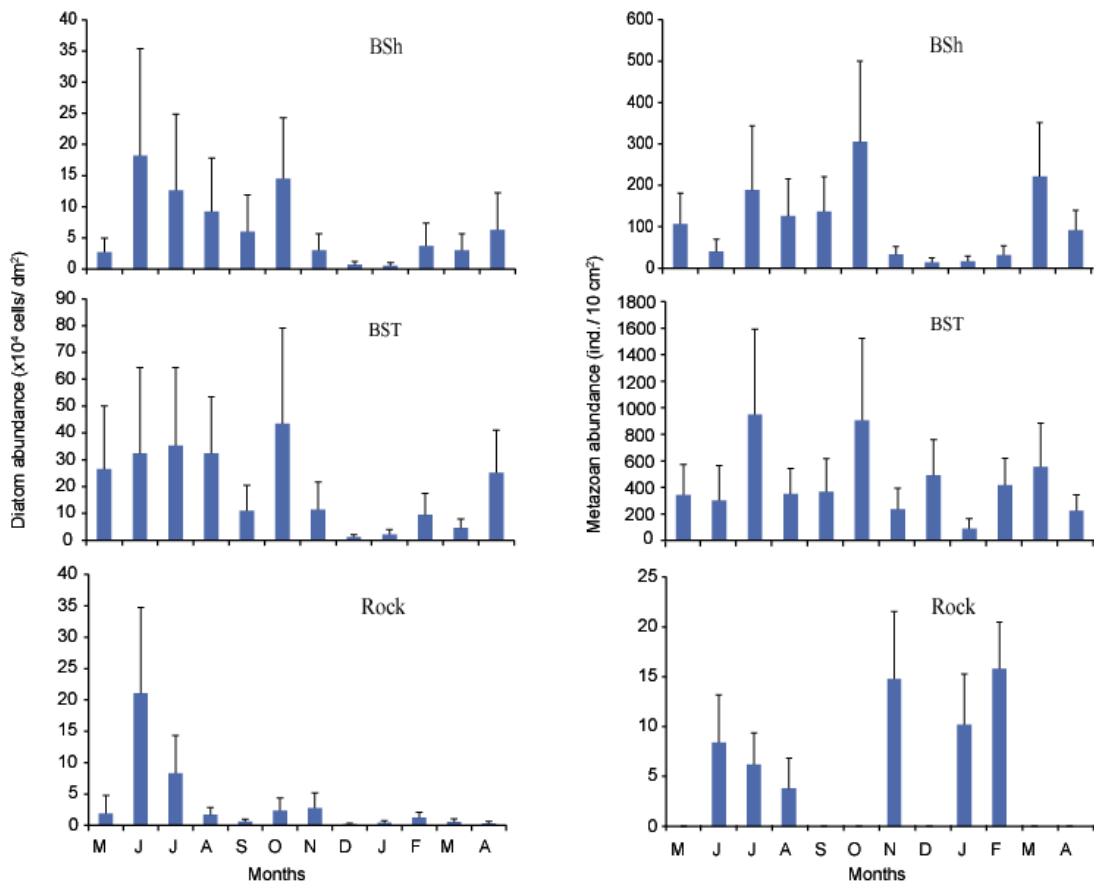


Fig. 2

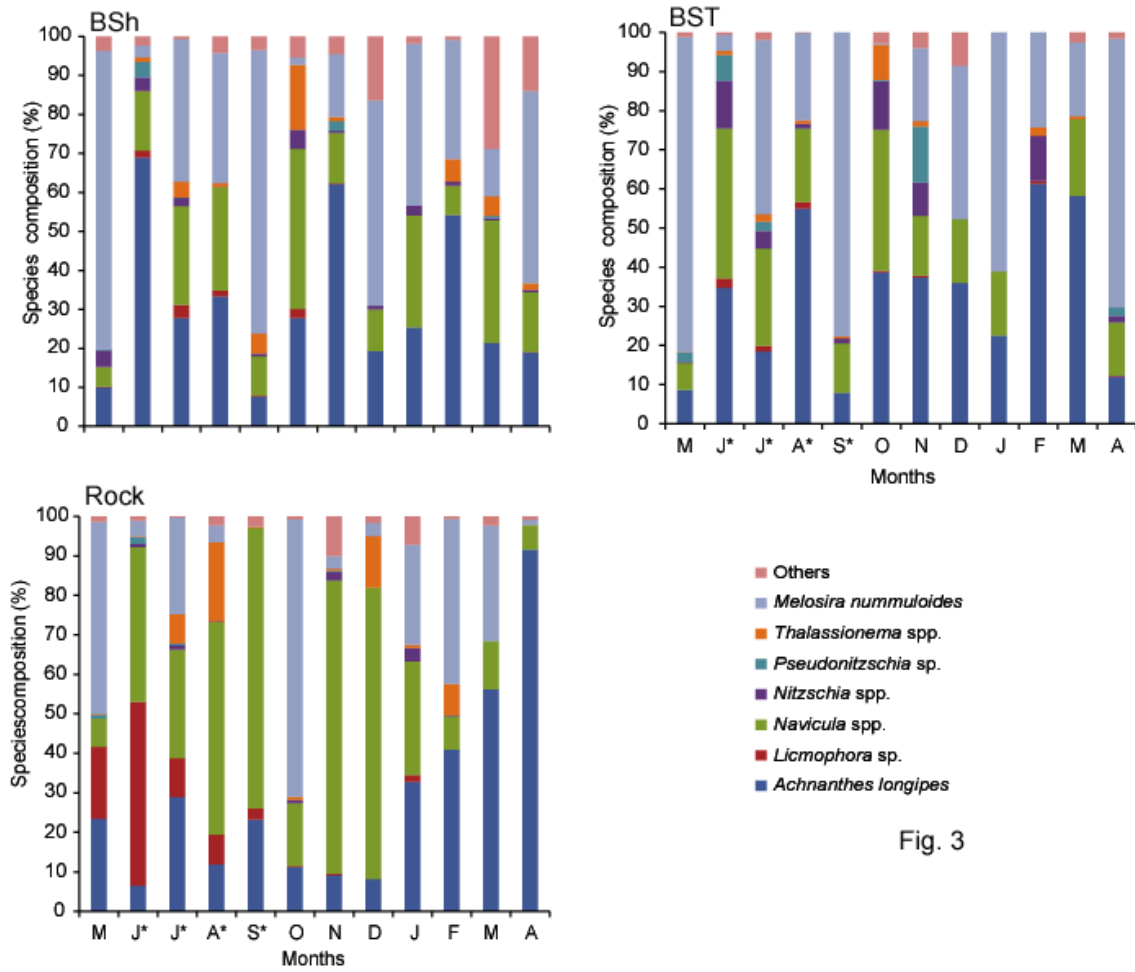


Fig. 3

Fig. 3

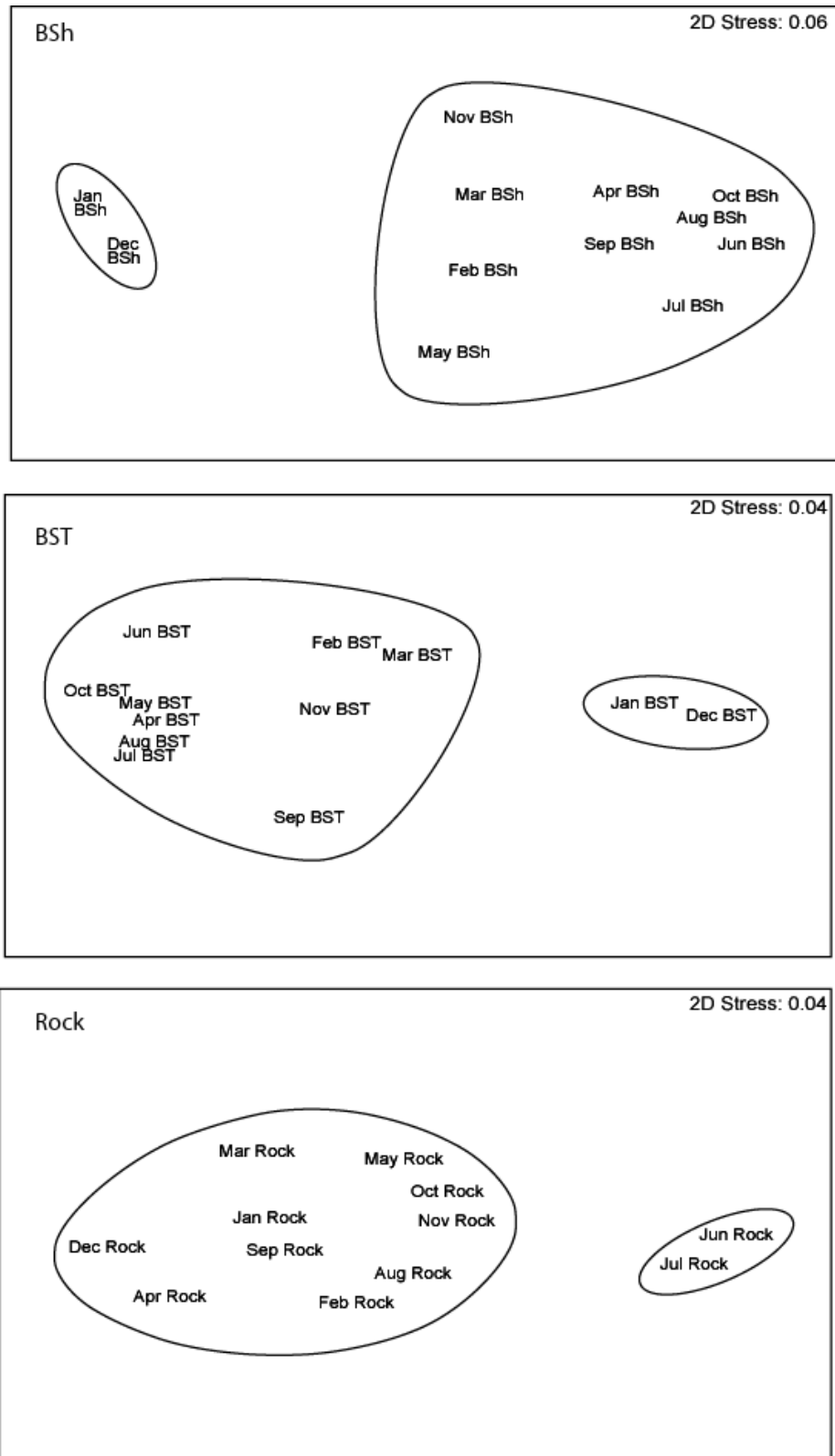


Fig. 4

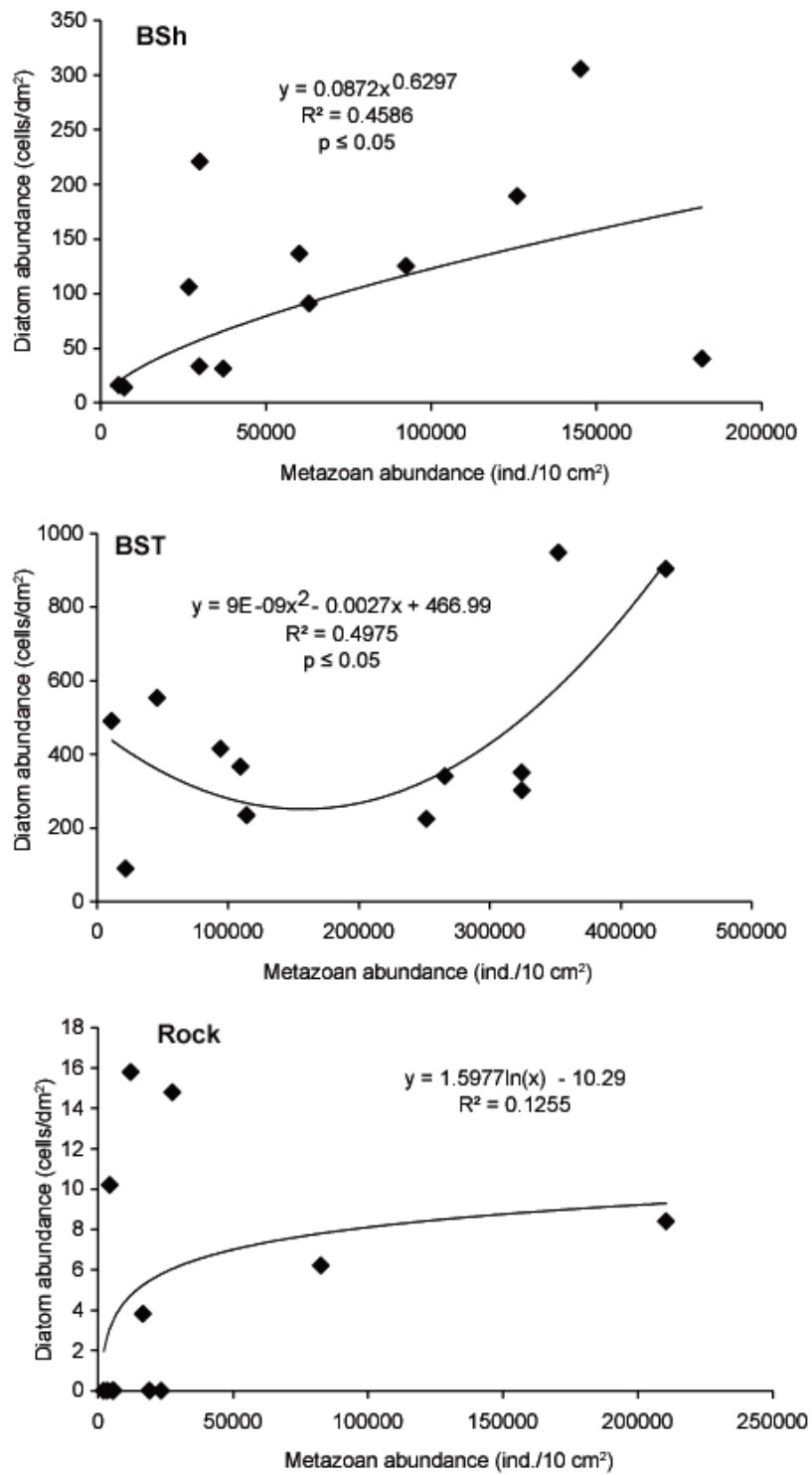


Fig. 5

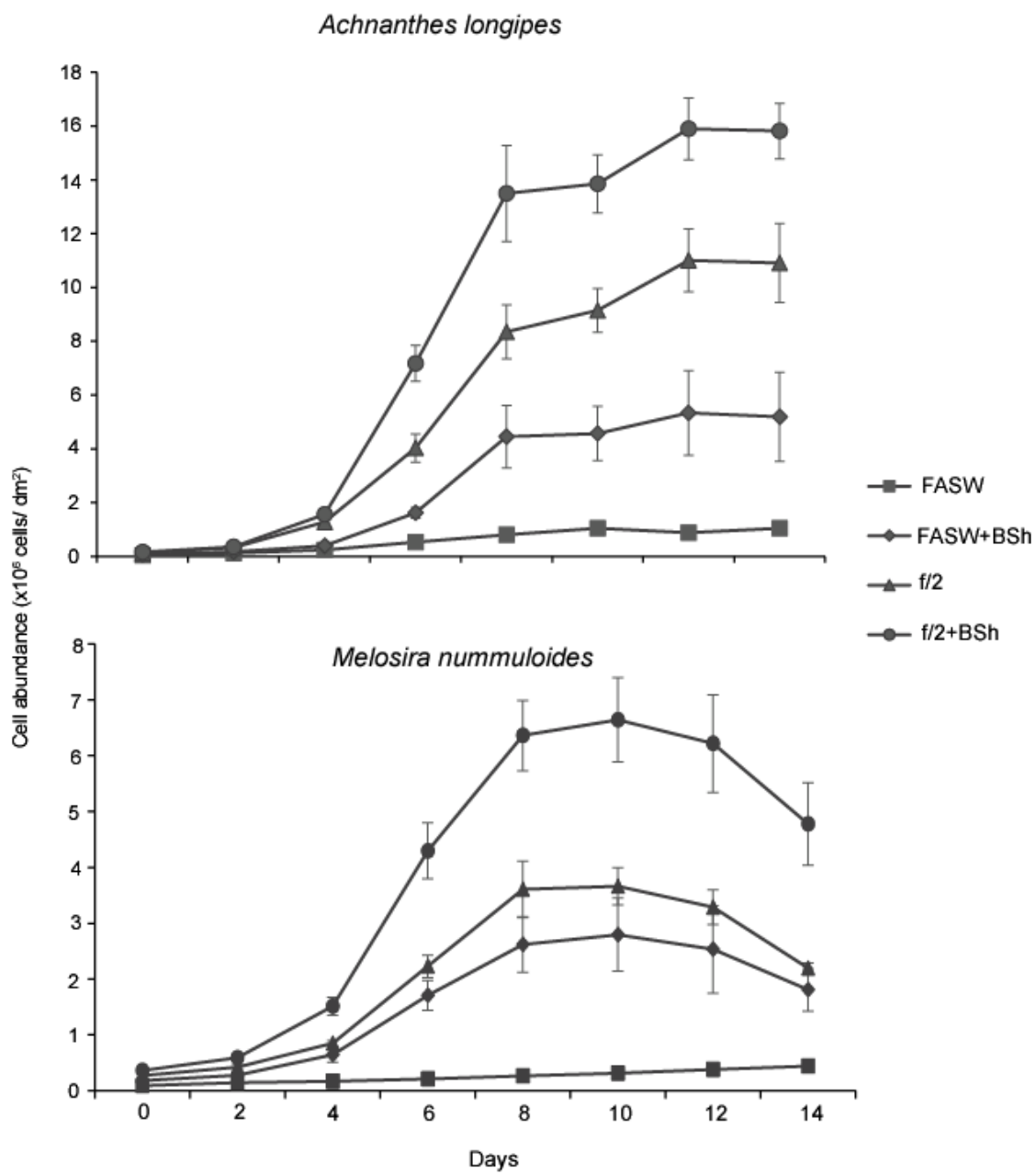


Fig. 6

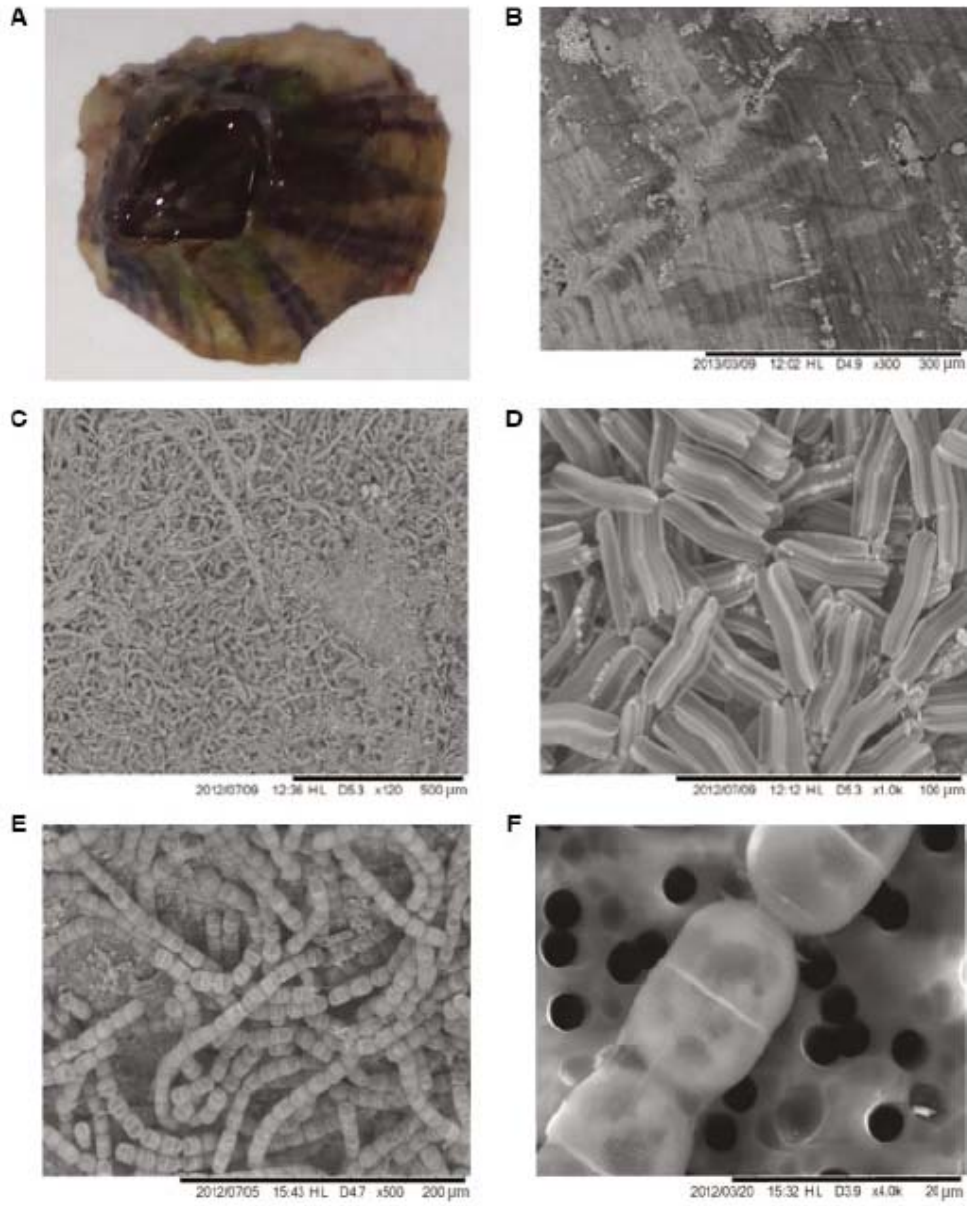


Plate: 1