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# ECOLOGICAL OBSERVATIONS AND GC-MS ANALYSIS OF METHANOLIC EXTRACT OF SACOGLOSSAN *ELYSIA BANGTAWAENSIS* (SWENNEN)

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## ABSTRACT
*Elysia bangtawaensis* Swennen (sacoglossan) was reported from intertidal mangrove habitat from the polyhaline zone of Mandovi estuary, Goa. The detailed ecological survey of the mangrove region of Goa revealed that the density of *E. bangtawaensis* ranged from 0 (July, August) to 400 (January) individuals $m^{-2}$ and were found to be mainly depends on phytoplankton population, their exposure to the sunlight (no. of low tides in day time) and moderately on other parameters such as salinity, pH and temperature (air and water). Moreover, GC-MS analysis of methanolic extract of *E. bangtawaensis* showed major constituents of fatty acids such as myristic acid, oleic acid, stearic acid, arachidic acid etc. The presence of such bioactive compounds (identified as fatty acid) justifies the use of whole animal in pharmaceutical application.

## INTRODUCTION

Sacoglossan gastropods are a vast group of herbivorous molluscs that feed mainly on green siphonaceous algae (Marín and Ros, 2004). They are primarily associated, with a few exceptions, with siphonalean green algae (Jensen, 1980) and are often well camouflaged, as much of their color being derived from the chloroplasts of algae on which they feed (Marin and Ros, 1993). Earlier studies showed that some sacoglossans maintain the chloroplasts in their digestive cells which photosynthetically active for weeks or months and become "solar powered" (Muniain *et al.*, 2001; Hyman, 1967). The Plakobranchidae (Elysiidae) forms a largest family in the Sacoglossa with *Elysia* as the most specious genus. They are distributed in tropical mangrove swamp of Australia, Thailand (Swennen, 1997) and India (Jagtap *et al.*, 2009). The population of *Elysia* was mainly confined to the protected, shadowed water pools, and algal and seagrass patches associated with the mid and lower intertidal polyhaline zones of the estuary.

Genus *Elysia* have been intensively investigated as a source of numerous secondary metabolites. They derive biologically active natural products, such as diterpenoids, sesquiterpenoids (Gavagnin *et al.*, 2000) and depsipeptides (Horgen *et al.*, 2000; Becerro *et al.*, 2001) from their algal preys (Becerro *et al.*, 2001) which show significant biological and pharmacological activities. The peptide derivatives Kahalalides have been isolated from the sacoglossans *E. rufescens* (Rao et *al.*, 2008) and *E. ornata* (Horgen et *al.*, 2000) which showed antimalarial (El-Sayed *et al.*, 1996) anticancer (Hamann *et al.*, 1996) antipsoriatic (Izquierdo and Miguel, 2004) antituberculosis (El-Sayed *et al.*, 2000) and antifungal activity (Donia and Hamann, 2003). Although numerous secondary metabolites and variety of marine natural products have been isolated from sacoglossans (Faulkner, 1992; Avila, 1995), their ecological roles still remains uninvestigated. However, such study has not been carried out on *E. bangtawaensis* so far. Hence, present study was aimed to monitor the ecological conditions and to analyse number of compounds (fatty acid) present in methanolic extract of *E. bangtawaensis*.

## MATERIALS AND METHODS

Monthly observations were carried out (November 2007 – October 2008) during ebb tide to monitored *Elysia* population and their ambience at Capao Island (15°32'46.1"N and 73°54'27.2"E) in Mondovi estuary, Goa, west coast of India (Fig. 1). Using quadrat (size $25cm^2$) method, ten quadrats were randomly placed to evaluate density (individuals $m^{-2}$) of *E. bangtawaensis* population. Data were pooled and expressed on average basis. The relevant ecological parameters such as temperature (air, water and sediment), salinity and pH from ambient environment were measured using standard method and expressed on average basis (Parson *et al.*, 1984). Number of low tides was counted using standard tide table. Surface sediments and overlying water from the vicinity of the *Elysia* population were also collected and preserved in Lugol's solutions for phytoplankton counting. One millilitre of concentrated phytoplankton sample was analysed using Segwick rafter counting chamber and the counts were averaged and expressed as the number of cells $l^{-1}$ and given in percentage of each season.

Fresh organisms after collection were washed with distilled water to remove attached debris which was then extracted with 95% methanol by maceration. The extract was filtered through muslin cloth, and concentrated by using vaccum evaporator (BUCHI Rotavapor R-200) at 40ºC. The crude MeOH extract was partitioned between *n*-hexane and ethyl acetate which was then concentrated under vacuum. The constituent of fractions were analysed by GC-MS analysis.

GC-MS analysis of the samples was carried out using Shimadzu Make QP-2010 with non-polar 60 M RTX 5MS Column. Helium was used as the carrier gas, the temperature of programming was set with initial oven temperature at 400ºC and held for 3 min, and the final temperature of the oven was 480ºC with rate at 10ºC min. sup $^{-1}$. A 2 $\mu$L sample was injected with splitless mode. Mass spectras were recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min. The chemical components from the methanolic extracts of *E. bangtawaensis* were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Significant variations of ecological parameters were investigated by correlation matrix. Difference were considered statistically significant when $p<0.05$.

## RESULTS

In postmonsoon (November, 2007-January, 2008) *E. bangtawaensis* population was found to be higher (50 – 400 individuals m$^{-2}$) with average body dimension 30-40 mm long and 2.5-3 mm wide. The animal was observed as dark green in colour when an average phytoplankton population (59.09%) was found to be predominent (Fig. 2). Air temperature, water temperature, pH, Salinity showed a range of 30-34ºC, 25-27.2ºC, 6.72-6.77, 12-21 PSU respectively, when the number of low tides showed increasing trend during day time in post monsoon (Table 1).

During premonsoon *Elysia bangtwaensis* showed colour change *i.e.* green to yellowish colour, and decrease in body length (10-20 mm) when phytoplankton was 34.66% (Fig. 2). Similarly, air temperature, water temperature, pH and salinity was observed in range of 31-32ºC, 29.5-30ºC, 7.5-7.4 and 28.4-29.8 PSU respectively. Whereas, density of organism

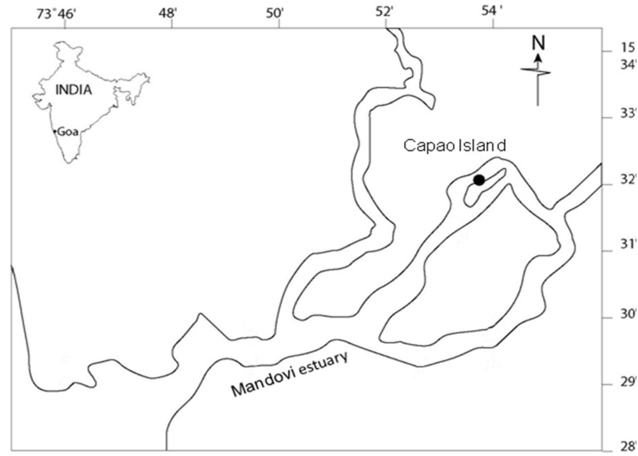


**Figure1: Geographical position of sampling station**

(15-150 individual m$^{-2}$) and number of low tides (in day time) decreased from February to May as compared to post monsoon season (Table 1).

However, in monsoon season (June, 2008–September, 2008) density of *Elysia* population (0-20 individuals m$^{-2}$) was almost negligible when average phytoplankton population (Fig. 2), air temperature and number of low tides in day time was decreased. Water temperature, pH and salinity were found in range of 25.5-26ºC, < 6, 0.08-0.1 respectively (Table 1).

Number of low tides in day time showed more significant positive correlation with population density (individuals m$^{-2}$) ($r = 0.7552$) at $p<0.01$, $n = 11$ whereas, number of low tides in night time showed negative correlation ($r = -0.8974$) at $p<0.001$, $n = 11$ (Fig. 3).

GC-MS analysis of methanolic extract of *E. bangtawaensis* is given in Table 2. On comparison of the mass spectra of the constituents with the NIST library, peaks were characterized and identified on the basis of retention time (RT) in minutes. The various fatty acids obtained in hexane and ethyl acetate fraction showed medicinal and pharmaceutical properties.

## DISCUSSION

Seasonal changes in physico – chemical properties in the estuarine and costal water in the Indian subcontinent are dominated by annual monsoon cycle (Gamito *et al.*, 2005). In present study, it has been observed that *E. bangtawaensis* populations depends upon light intensity (day to day tide

**Table 1: Ecological parameters and density status of *E. bangtawaensis* and associated habitat**

| Months | Air temp. (°C) | Water temp. (°C) | pH | Salinity (‰) | No. of low tides Day | Night | Density of *E. bangtawaensis* (individuals m$^{-2}$) |
|---|---|---|---|---|---|---|---|
| Nov., 2007 | 30 | 26 | 6.72 | 12 | 19 | 18 | 100 |
| Dec., 2007 | 34 | 27.2 | 6.77 | 15.02 | 25 | 12 | 230 |
| Jan., 2008 | 32 | 25 | 6.77 | 21 | 34 | 10 | 400 |
| Feb., 2008 | 31.7 | 29.8 | 7.52 | 29.52 | 31 | 12 | 150 |
| Mar., 2008 | 32.1 | 30 | 7.59 | 29.8 | 30 | 18 | 130 |
| Apr., 2008 | 31 | 29.5 | 7.52 | 28.44 | 28 | 25 | 50 |
| May, 2008 | 32 | 30 | 7.46 | 29.57 | 29 | 31 | 15 |
| Jun., 2008 | 27 | 26 | 6.09 | 0.09 | 23 | 31 | 5 |
| Jul., 2008 | 26.1 | 25.5 | 6 | 0.08 | 20 | 31 | 0 |
| Aug., 2008 | 27 | 26 | 6.55 | 0.1 | 22 | 32 | 0 |
| Sept., 2008 | 27 | 26 | 6.77 | 0.11 | 23 | 30 | 20 |
| Oct., 2008 | 33 | 26.3 | 6.75 | 16 | 28 | 29 | 50 |

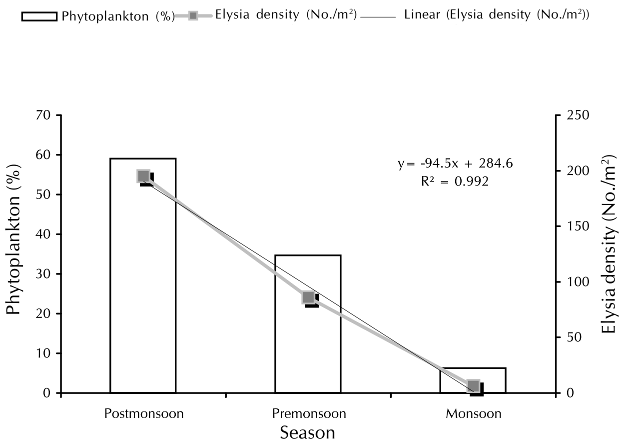


**Figure 2: Seasonal changes in phytoplankton percentage and *E. bangtawaensis* density**

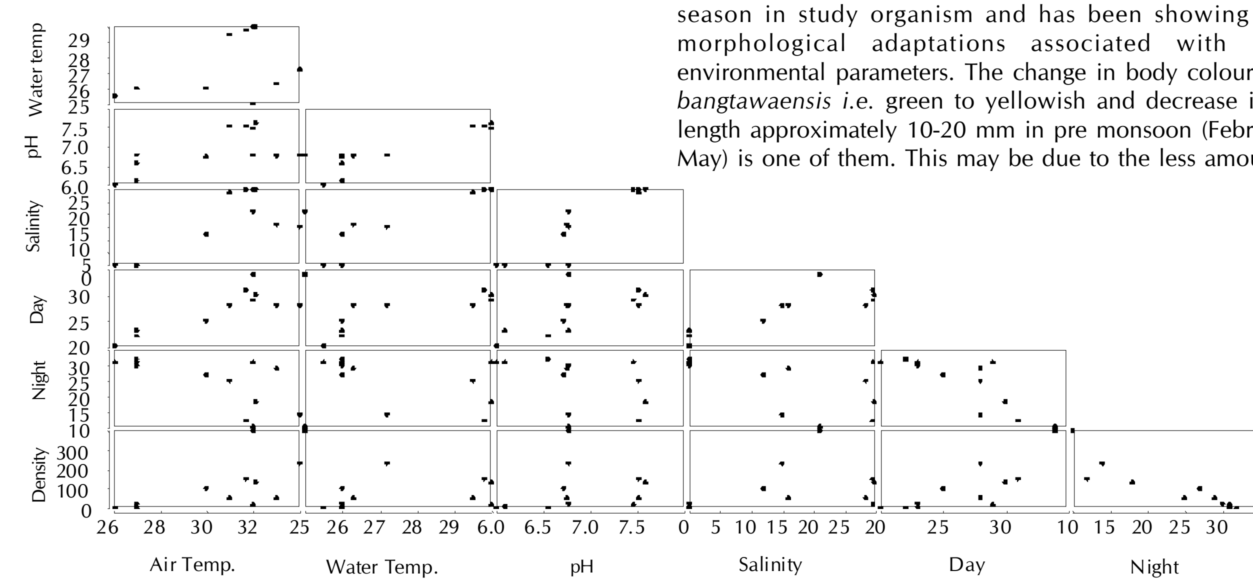


**Figure 3: Box plot diagram showing correlation between density of *E. bangtawaensis* and ecological parameters**

**Table 2: Fatty acids and fatty acid esters from methanolic extracts of *E. bangtawaensis***

| Molecular formula | Systematic name |
|---|---|
| $C_{14}H_{28}O_2$ | Tetradecanoic acid |
| $C_{18}H_{34}O_2$ | 9-Octadecenoic acid |
| $C_{18}H_{36}O_2$ | Octadeconoic acid |
| $C_{20}H_{34}O_2$ | 8,11,14-Eicosatrienoic acid |
| $C_{20}H_{40}O_2$ | Eicosanoic acid |
| $C_{15}H_{30}O_2$ | Methyl tetradecanoate |
| $C_{17}H_{34}O_2$ | Hexadecanoic acid, methyl ester |
| $C_{18}H_{36}O_2$ | Hexadecanoic acid, 14 methyl, methyl ester |
| $C_{19}H_{38}O_2$ | Octadecanoic acid, methyl ester |
| $C_{19}H_{36}O_2$ | 8- Octadecenoic acid, methyl ester |
| $C_{21}H_{34}O_2$ | 5,8,11,14-Eicosatetraenoic acid, methyl ester |
| $C_{21}H_{38}O_2$ | 11,13, Eicosadicanoic acid, methyi ester |

environmental conditions change unpredictably in time, variation in life–history traits can be an adaptive response. Individual coloration was changed considerably according to season in study organism and has been showing both morphological adaptations associated with some environmental parameters. The change in body colour of *E. bangtawaensis i.e.* green to yellowish and decrease in the length approximately 10-20 mm in pre monsoon (February–May) is one of them. This may be due to the less amount of

intervals), and phytoplankton abundance in mangrove swamps. It was found that density of *E. bangtawaensis* was higher during postmonsoon when phytoplankton (59.09%) population was predominant (Fig. 2) and directly related to the air temperature and light intensity throughout the year (Fig. 3). Moreover, estuarine water is turbid in nature which is more true in mangrove swamp, when more accretion and erosion occurs during high tide as well as in monsoon season. The turbid water does not allow much light to penetrate up to the algal bed and *Elysia* patches. So naturally whatever light these animal and algal bed gets for their photosynthesis is during low tide exposure only. Earlier studies suggested that, photosynthetic efficiency of plastids in ascoglossan varies with light intensity (Goetzfried, 1977; Clark *et al.*, 1981) and temperature (Stirts and Clark, 1980). Accordingly, it has been observed that maximum number of population of *E. bangtawaensis* found only during January where more number of low tides was during day times and air temperature was high (30-34ºC). Increase in climatic factor, mainly light and temperature may lead to a higher feeding rate and higher metabolic activity of sacoglossan (Cavas *et al.*, 2005). When plastid present in *E. bangtawaensis* when avaialbility of phytoplankton is less as compared to postmonsoon. They feed exclusively on algae (Hyman, 1967) by puncturing siphonoceous algal cells and sucking out the cell contents and only chloroplast which is engulfed phagocytotically (Rumpho, 2000). This intracellular association of algal chloroplast showed unique symbiotic relationship which might give them green colour for adaptation.

In this case study, we are not yet confirmed about specific food source of *E. bangtawaensis*. But observed that it showed the high density in intertidal zone during postmonsoon season when number of low tide during the day time (January) and phytoplankton population was maximum (Fig. 2 and 3). A sign of versatility of the food was observed. *E. bangtawaensis* were found to be associated with mixed microalgae dominated by *Amphora* sp., *Coscinodiscus* sp., *Navicula* sp., *Melocera* sp., *Nitzschia* sp. and *Pinnularia* sp.

However, GC-MS analysis showed major constituents of fatty acids in methanolic extract of *E. bangtawaensis* such as Palmitic acid, Myristic acid, Oleic acid, Stearic acid, Arachidic acid etc.

(Table 2). However, Palmitic acid and Myristic acid are most prevalent fatty acid which is commonly occurring in marine benthic diatom (Orcutt and Patterson, 1975). Whereas, marine microalgae also showed the presence of Oleic acid in appreciable quantity (Shammel, 1993; Aliya *et al.*, 1995). Fatty acid composition of photosynthetic diatom of the surface sediments are dominated by 14:0, 16:0 and 18:0, which are ubiquitous biological markers of phytoplankton in the marine environment (Carrie *et al.*, 1998). The C14, C16, C20 acid comprises the bulk of diatom fatty acids (Arredondo-Vega *et al.*, 2004). However, lipid has been recognized as essential component in animal nutrition as well as aquaculture feed. Therefore, deposition of lipid (Fatty acid) which found as a major constituent in methanolic extract of *E. bangtawaensis* might be obtained from their food. These compounds help to defend sacoglossans against predators. The invented chemical defenses are thought to provide ecological advantages and may function as a driving force in the evolution of this group (Cimino and Ghiselin, 1998). Certain food versatility is somewhat striking, gives the close relationship between *Elysia* and diatoms. It is likely that the yellow color was due to degradation of pigments in aging chloroplasts and less food availability in monsoon. In order to understand the survival of population in particular environment, the detailed study of life cycle, biology of its food and its predator and many physical aspects of its environment has to be carried out.

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