Thraustochytrid and fungal component of marine detritus. I – Field studies on decomposition of the brown alga *Sargassum cinereum* J. Ag.

Veena Sathe-Pathak, S Raghukumar*, Chandrakala Raghukumar & Sumita Sharma

Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa - 403 004, India

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Thraustochytrid protists and fungi were isolated and enumerated in culture from detritus of the brown alga *Sargassum cinereum*. Both groups occurred epiphytically and endobiontically in the detritus. The thraustochytrid *Labyrinthuloides minuta* occurred in healthy as well as decomposing algal tissues, whereas *Ulkenia visurgensis* was found only in the latter. Up to $2.9 \times 10^7$ of the two thraustochytrids, $5.2 \times 10^6$ of fungi and $5.5 \times 10^{11}$ of bacteria (per g dry wt. detritus) were found, using immunofluorescence, culture techniques and direct counts respectively. Populations of all 3 groups (thraustochytrids, fungi and bacteria) were lowest in healthy algae containing higher amounts of phenolics than in detritus. Concentrations of total carbohydrates (TCH), total phenols, proteins, alginates and mannitol, as well as C/N ratios declined as decomposition progressed, concomitant with an increase in microbial biomass. Both thraustochytrids and bacteria attained peak biomass values in 21 d detritus. Maximum observed biomass of thraustochytrids corresponded to 0.07 % C and that of bacteria to 1.1 % C of detrital dry weight. The constant association of thraustochytrids with the brown algal detritus and their endobiontic habitat suggest a definite role for these protists in detrital dynamics.

Studies on the microbiology of marine macrophytic detritus have laid emphasis on bacterial biomass$^{1-2}$, biochemical transformation$^{3-4}$ and the role of bacteria in detrital food web$^{5}$. Other microbial components of such detritus comprise the unicellular, fungi-like protists, the thraustochytrids$^{6-8}$ and fungi$^{9,10}$. While the species composition and succession of fungi have been dealt with in great detail for detritus of salt marsh grass$^{10}$, mangrove$^{7,9}$ and seaweeds$^{11,12}$, estimations of fungal biomass in marine detritus are few$^{10,13}$. No information is available on thraustochytrids. Besides, algal-based detritus has received insufficient attention from the mycological point of view$^{12}$. Most studies are from subtropical and temperate waters and the role of fungi in tropical marine detrital systems has been poorly investigated.

Does marine macrophytic detritus harbour thraustochytrids and fungi to a degree which is of significance in the biochemical transformation of detritus and as a component of the detrital food web, as is known for bacteria? This question has been examined in this paper with reference to the marine brown alga *Sargassum cinereum* J. Ag. (Fucales, Phaeophyta), which is extremely abundant along the coast of Goa, and is considered to be a significant primary producer in this area$^{14}$.

Materials and Methods

**Litterbag experiments**—Five experiments were carried out in the vicinity of Dona Paula jetty at the mouth of the Zuari river in the premonsoon periods. These were: (1) 9 March 1988 - 8 April 1988; (2) 5 April 1988 - 5 May 1988; (3) 17 March 1989 - 7 April 1989; (4) 14 February 1990 - 28 March 1990 and (5) 5 April 1990 - 3 May 1990. Nylon litter bags (1 m × 0.25, 1 mm mesh), divided into 4 pouches were used. Mature algal portions were collected from plants attached to subtidal rocks and cut into 10 - 15 cm bits. About 1.5 kg wet weight of these were placed in each pouch of the bag and the pouches stapled. Up to 15 litter bags were tied to a raft at the site (ca. 8 m) at about 1 m depth. The whole operation of collecting the algae and submerging the litter bags was done within an hour to minimize death of autochthonous microflora on the algae. One to three litterbags were removed at weekly intervals. Material of experiments 1 and 2 were dried at 37°C for 72 h for biochemical analyses. Thraustochytrids and fungi were isolated and enumerated in experiments 2-4. A portion of the material from experiments 3 and 5 were fixed in 3 % formalin for biomass estimations of thraustochytrids and bacteria.
Isolation and enumeration of thraustochytrids and fungi—A general scheme is presented in Fig. 1. Surface-sterilization of algal material was carried out as given by Newell and Fell. Cornmeal and Malt-extract agar media at pH 7, containing 0.05 g streptomycin and 10000 units of penicillin per 100 ml were employed for culturing and 5 replicates were maintained. Following incubation for 1 week, average numbers of colonies of tentatively identified individual species of fungi and thraustochytrids were recorded, and numbers of individual species per g dry wt detritus estimated. Dry weight of a frond disc/stalk piece, was estimated independently (drying to constant weight at 40°C). Out of fungal species isolated from non-surface-sterilized material, only those which were also observed from surface-sterilized ones were considered. From the mean values of each such species obtained for the 2 media and 2 treatments, the highest mean values were considered. Such average values of all the species were added up to derive the total numbers.

Thraustochytrids were also enumerated by using the pine pollen/baiting/MPN method. Homogenates of 1, 0.5, 0.25, 0.1 and 0.05 ml of the non-surface-sterilized detritus were added to 5 ml of sterile seawater in 20 ml flat-bottomed screw caps and bailed. Most probable numbers (MPN) of thraustochytrids per ml of homogenate were estimated. Numbers of thraustochytrids per g detritus were calculated based on the dry wt of material present in 1 ml of suspension. Representative cultures of distinct morphological types of thraustochytrids were examined under a continuous flow chamber and identified up to species level. *Labyrinthisuloides minuta* was recognized by characteristic finger-like projections at the periphery of colonies and their morphology. Since many obligate marine fungi may not appear in culture plates, seawater incubation and moist-chamber incubation were also carried out.

Biomass estimations—Biomass of the two most commonly isolated thraustochytrids, *Labyrinthisuloides minuta* and *Ulkenia visurgensis* was estimated using an immunofluorescence counting technique (IF). Intravenous injections (1 ml each) of whole cells with a protein concentration of 480 μg/ml in the case of *L. minuta* and 650 μg/ml in the case of *U. visurgensis* were given to New Zealand white rabbits on days 0, 2, 4, 6, 8, 10 and 17. Animals were bled on day 26, antisera purified and the presence of antibodies against the two species confirmed by double immunodiffusion. Dilutions of 1:2 and 1:4 of the fluorescein-isothiocyanate (FITC)-conjugated antisera yielded best fluorescence against the two respective thraustochytrids. Preparations were examined with an epifluorescence (Olympus BH2 RFL) microscope using a 495 nm excitation filter, an autofluorescence suppression filter EY 455 and a

**Fig. 1—Flow chart of procedures used for isolation of thraustochytrids and fungi**

- **Algal parts**
  - Frond: 0.5 mm discs
  - Stalk: 2 mm bits

- **Surface sterilized**
  - Homogenised
    - 20 no./15 ml Water
      - Plating 0.1 ml 
      - Baiting & MPN
  - Not homogenised
    - Seawater incubation

- **Not surface sterilized**
  - Homogenised
    - 20 no./15 ml Water
      - Plating 0.1 ml
      - Baiting & MPN
  - Not homogenised
    - Moist chamber incubation
515 nm barrier filter. Control thecoschizyrid species, namely the two species against each other, _Thraustochytrium motivum_ Goldstein (NIOCC 84), _Corallochytrium limacisporum_ Raghukumar (NIOCC 73), _Thraustochytrium striatum_ Schneider (NIOCC 65), a mold _Aspergillus_ sp., the filamentous cyanobacterium _Mastigocoleus_ sp. and bacteria and epiphytic algae on the detritus itself revealed no cross-reactivity with the antiserum. Formalin- preserved samples of frond detritus were stained with the appropriate dilutions of the FITC-conjugated antiserum and examined under the microscope. Number of cells and mean size of each species in 5 sets of 10 random microscope fields each of the detritus (total 50 fields) were counted. Mean numbers (of the 5 sets) per unit area were extrapolated to the area of a 5 mm diam frond disc, multiplied by a factor of 2 to include both sides of the disc, and the number of cells per g detritus calculated based on the weight of one disc. The biovolume of the cells (as spheres) was calculated, Since cells of the two thecoschizyrids in detritus were globose in shape. The conversion factor of 21.97 pg per cell of 51 μm was used to convert the total biovolume to biomass carbon. Number of bacteria in the samples were enumerated using the acridine orange direct count (AODC) method and the values converted to bacterial carbon based on the conversion factor of 20 fg of C per bacterial cell.

*Biochemical analyses*—Triplicate analyses were made for proteins, reducing sugars, total carbohydrates (TCH) and mannitol, total phenols and alginates. Ash content was determined as loss of weight after heating at 450°-500°C for 5 h. Total carbon and nitrogen were analysed using a Heraeus CHN.O rapid analyser.

Results are expressed as percentages of dry wt detritus and ash-free dry weight (AFDW). In addition, percentage dry weight values of various parameters were normalised to ash content of healthy algae (0 d detritus) (see Discussion) as follows:

\[
\text{\% Ash content of detritus on day '0' } \times \frac{\text{\% dry wt value of parameter on day 'x'}}{\text{\% Ash content of detritus on day 'x'}}
\]

**Results**

Surface-sterilized as well as non-surface-sterilized algal detritus yielded thecoschizyrids upon plating (Figs 2,3). While the former treatment mostly yielded only _Labyrinthuloides minuta_, both this species and _Ulkenia visurgensis_ were isolated from non-surface-sterilized detritus. Staining of formalin-preserved detrital samples with lactophenol-cotton blue for microscopic observations revealed the typical, spindle-shaped cells of _L. minuta_.

**Density of thecoschizyrids, fungi and bacteria and population fluctuations with age of detritus**—There were no clear differences between the plating and baiting methods in terms of number of thecoschizyrids obtained (Figs 2,3). The plating technique, however, did not yield thecoschizyrids from all samples, whereas the baiting technique was more consistent. Therefore, fluctuations in populations of thecoschizyrids were followed from data obtained using the baiting and MPN as well as IF technique (Figs 2,3,5,6).

Cells of both _Labyrinthuloides minuta_ and _Ulkenia visurgensis_, as observed by IF, were globose
to subglobose and ranged from 3.5 to 6.5 \( \mu \text{m} \) in size. Although these thraustochytrids could be viewed by epifluorescence microscopy, they could not be detected by light microscopy because of (a) the thickness of detrital material and (b) the chromophilic nature of the detrital surface which prevented differential staining of the protists.

Thraustochytrids, fungi and bacteria were generally fewer in healthy algae and increased in numbers with age of both frond and stalk detritus (Figs 2-7). The baiting and MPN technique yielded the highest numbers of thraustochytrids from 7 and 14 d old detritus of fronds and stalks respectively in the case of Expt. 3 (Fig. 2) and from 28 day old ones in Expt. 4 (Fig. 3). Direct IF counts gave maximum numbers in 21 day old frond detritus (Figs 5, 6). Bacteria also reached peak numbers by day 21 (Fig. 7). A decline in thraustochytrid and bacterial population was generally observed thereafter (Figs 2, 3, 5-7). Highest numbers of fungi generally occurred between 14 and 28 days (Fig. 4) of decomposition.

Lower numbers of thraustochytrids and fungi were observed in Expts. 4 and 5 carried out in 1990 than in Expts. 2 and 3 of 1988 and 1989 (Figs. 2-6).

Species composition and succession—Healthy algac harboured only *Labyrinthuloides minuta* among thraustochytrids (Figs 2, 3, 5, 6). Other species, including *Ulkenia visurgensis* were isolated only after the 7th day. After this period, both species were observed in almost equal abundance in decomposing algae (Figs 2, 3, 5, 6).

Only terrestrial taxa of fungi were recovered from healthy and decomposing algae using the plating technique (Table 1). The most frequent among these were *Phoma* sp., yeasts and non-sporulating forms. The only obligate marine species was *Lindia thalassiae* which was isolated using the moist chamber technique.

Biomass of thraustochytrids and bacteria—Thraustochytrid biomass contributed a minimum of 0.00031 % of C to the dry weight of 0 day detritus and showed a maximum of 0.065 % in 21 day old detritus of Expt. 3 (Fig. 5). Their biomass was much lower in Expt. 5, where only values of 0.00006 to 0.00087 % C were recorded for 0 and 21 day old detritus respectively (Fig. 6). Bacterial

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**Fig. 3**—Numbers of thraustochytrids in detritus of different ages of *Sargassum cinerereum* fronds and stalks obtained using plating and baiting techniques (Expt. 4)

**Fig. 4**—Numbers of fungi in detritus of different ages of *Sargassum cinerereum* fronds and stalk obtained using plating technique.
biomass values in this experiment corresponded to 0.02 to 1.1 % C in 0 and 21 day old detritus respectively (Fig. 7). Fungal hyphae could not be detected microscopically in any stage of detritus.

Biochemical changes in detritus—Decomposing algal fronds rapidly lost up to 41.75 % of weight by 7 d and up to 50 % by 14 d (Fig. 8). This was accompanied by a considerable loss of total carbohydrates (TCH), reducing sugars, phenols and alginates (Figs 8,9). Subsequently, between 14 and 21 d, there was an increase in ash content compared to the original value of about 30 %. A decline in proteins and mannitol and a further loss of TCH and alginate took place in terms of total dry weight, ash-free dry weight and ash content normalised to original values. After 21 days, ash content increased further up to 58%. Although proteins, TCH and reducing sugars increased in relation to ash-free dry weight during this time, they declined in terms of ash content normalised to original values. Alginates continued to decrease.

Carbon to nitrogen ratios steadily decreased and starting from values of 15 to 19, reached values of 5 to 7 by 28 d.

Discussion

Endobiontism—This study demonstrated that thraustochytrids and fungi are constantly associated with healthy as well as decomposing Sargassum cinereum. The consistent isolation of Labyrinthuloides minuta in culture after surface-sterilization of detritus (Figs 2,3) and the microscopic confirmation of its presence within decomposing algal tissues suggest its predominantly endobiotic mode of life. Thraustochytrids and labyrinthulid protists are known to be capable of subsisting in this manner. Such thraustochytrids may occupy a niche equivalent to that of endobiotic fungi in solid substrata by penetrating and utilising the nutrients within. On the contrary, Ulkenia visurgensis, which was isolated predominantly as an epibiont, may penetrate organic particles by means of ectoplasmic net elements to draw nutrients.
from within, as is common among thraustochytrids.

In order to ensure that fungal colonies in culture plates arose from hyphae actively growing within the detritus and not from surface spore contamination, surface-sterilization of the algal material was resorted to. The results indicate that fungi were active as endobionts in detritus. Besides, the isolation of terrestrial species of fungi (geofungi) from such detritus (Table 1) suggests a definite role for them in this habitat, supporting the view of Miller and Whitney.

Population fluctuations and biochemical changes—Changes in biochemical parameters have been expressed in literature as—a) percentage of dry weight detritus; b) percentage of ash-free dry weight and c) absolute values remaining in the litter bag. Both a and b indicate only relative changes depending upon fluctuations in other parameters, while c expresses total values present in the litterbag. In order to deduce actual changes in a given detrital particle, it would be meaningful to express it in terms of another parameter which would remain constant. As a preliminary exercise, it was assumed that there is no substantial addition from external sources or a depletion in ash content, and values expressed in terms of ash content at a given age normalised to original ash content.

The lower numbers of thraustochytrids, fungi and bacteria in healthy algae, despite higher amounts of nutrients (Figs 2-9) might be attributed to antimicrobial defence mechanisms such as phenols, which are prevalent in marine algae. The ability of Labyrintheuloides minuta to grow in such algae suggests its capability to withstand to some extent the defence mechanisms of the algae and to exist as a weak parasite, similar to fungi in epidermal cells of terrestrial plants.

Since thraustochytrid and bacterial numbers increased only slowly during the first 7 d, it is likely that the loss of nutrients during this period were more due to leaching than to microbial utilisation. This would correspond to the first, leaching phase of decomposition.

The steep decline in phenolics by 7 d might have led to the second phase, wherein thraustochytrid and bacterial populations increased steadily to reach maximal levels by 14 to 21 days (Figs 2,3,5 – 7). Cundell et al. implicated loss of phenols in buildup of microbial biomass in mangrove detritus. Mannitol and alginates, which were steadily lost till 21 d might have been utilised by the thraustochytrids and bacteria, both of which

<table>
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<th>Fungal species</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
<th>Expt. 4</th>
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<tr>
<td></td>
<td>Frond</td>
<td>Stalk</td>
<td>Frond</td>
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<tr>
<td><em>Acremonium spp</em></td>
<td>17500</td>
<td>45000</td>
<td>12700</td>
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<td>(14)</td>
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<td>(0)</td>
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<td><em>Alternaria sp.</em></td>
<td>5000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus spp.</em></td>
<td>45000</td>
<td>6500</td>
<td>0</td>
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<tr>
<td></td>
<td>(28)</td>
<td>(21)</td>
<td>(14,28)</td>
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<td></td>
<td>(14)</td>
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<tr>
<td><em>Nigrospora oryzae</em></td>
<td>5000</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>(14)</td>
<td></td>
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<tr>
<td>Non-sporulating forms</td>
<td>0</td>
<td>52000</td>
<td>38200</td>
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<tr>
<td></td>
<td>(7)</td>
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<td>(0)</td>
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<tr>
<td><em>Paeclomycetes sp.</em></td>
<td>25000</td>
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<tr>
<td></td>
<td>(28)</td>
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<tr>
<td><em>Phoma sp.</em></td>
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<td>0</td>
<td>13600</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(0,14)</td>
</tr>
<tr>
<td>Yeasts</td>
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<td>13600</td>
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<td></td>
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<tr>
<td><em>Lindra thalassiae</em></td>
<td>–</td>
<td>–</td>
<td>+</td>
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</table>

*(Isolated only by the moist-chamber incubation method)*
are known to use these or their breakdown products for growth. The decline in microbial populations after 14 to 28 d might correspond to the refractory phase of detritus. TCH and reducing sugars decreased in terms of values normalized to original ash content (Fig. 8), indicating that they were being continually lost in detritus. However, they increased as % AFDW, suggesting that other organic compounds such as alginates were being used even more rapidly.

The decrease in C/N ratios (Figs 8,9) is consistent with other studies. Microorganisms may increase the nitrogen content of detritus, thereby reducing its C/N ratio. The present, as well as other studies have shown that increases in N do not necessarily reflect those in protein. Protein values decreased with decomposition (Figs 8,9), probably owing to: 1) complexing of proteins, amino acids and microbial enzymes with carbohydrates and phenolics, giving rise to recalcitrant nitrogen; 2) rapid leaching out of proteins and 3) utilisation of proteins as a major carbon source by microorganisms.

Microbial biomass—Total bacterial and thraustochytrid biomass in Expt. 5, where both were estimated ranged only from 0.02 to 1.11 % C in detritus (Figs 5-7), falling within the ranges of microbial biomass reported for macrophytic detritus. Carbon and nitrogen contribution of even the relatively high biomass of the bacteria to the nutrition of metazoan detritivores has generally been considered insignificant by several workers.

The importance of thraustochytrids, whose biomass was much less than that of the bacteria may not be in terms of their C and N contribution to detritus. However, they may supply essential nu-
tritents such as fatty acids, sterols, vitamins and other growth factors to detritivores. Findlay et al. have reported high amounts of certain polyunsaturated fatty acids (PUFAs) in thraustochytrids. These are known to play a vital role in the nutrition of several crustaceans. In addition, the endobiontic habitat of the thraustochytrids and their constant association with the brown algal detritus suggests a definite functional role for these protists in organic matter degradation. These aspects need to be examined in future.

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References