Rev. Biol. Trop., 38(2A): 243-250, 1990

Marine fungi (Chytridiomycetes and Thraustochytriales) from a mangrove area at Punta Morales, Golfo de Nicoya, Costa Rica.

Annemarie Ulken[1], Roxana Víquez[2], Carmen Valiente[3], and Mitzi Campos[4]

1 Alfred Wegener Institute for Polar and Marine Research, D-2850 Bremerhaven, Am Handelshafen 12, Federal Republic of Germany
2 Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica
3 Facultad de Microbiología, Universidad de Costa Rica, Costa Rica
4 Escuela de Biología, Universidad de Costa Rica, Costa Rica

(Rec. 12-VI-1989. Acep. 25-I-1990)

Abstract. The mangrove swamp near the Biological Station at Punta Morales, Golfo de Nicoya, Costa Rica, was sampled for lower fungi during the dry season in 1987, and for a second time during the wet season in 1988. In 1987, Chytridiomycetes were found in the sediment samples, which, however, could be observed in less quantity during the wet season. In both years, the registered genera of the biflagellate organisms were *Schizochytrium, Thraustochytrium* and *Ulkenia*. In 1987 some *Labyrinthula* sp. were observed, which apparently had disappeared in 1988. In general, there were more individuals per genus during the wet season but there seemed to have been more genera during the dry season. Lower fungi seem to be important as decomposing organisms that the sediment with protein and organic nitrogenous compounds.

Key words: Fungi, mangrove, seasonality, Chytridiomycetes.

Mangrove forests have interested humans since early times and the biology of this fascinating vegetation is fairly well known (Chapman 1976, Tomlinson 1986). However, there is a remarkable lack in the knowledge of microorganisms, though the swamps are known to be the cradle of many animals that live later on in the sea. Por & Dor (1984) stated that "after all, the mangroves are still the main supplier of fish, shrimps and shellfish protein of hundreds of millions of undernourished people in the tropics".

In mangroves the leaf fall is probably the highest compared with other trees. Pool *et al.* (1975) calculated a mean litter-fall for mangrove forests in Florida and Puerto Rico of 796 g/m^2 yr. About 60% may be recycled during one year. Furthermore, algal beds, reefs and estuaries have the highest biomass among marine ecosystems (Smith 1981) like mangrove estuarine areas.

Leaf material is originally high in carbohydrates, lipids and protein (Bhosle *et al.* 1976). After an initial phase of leaching, there is an increase in protein content (Heald 1971, Fell *et al.* 1975, Untawale *et al.* 1977). This gain is probably due to fungi. Furthermore, the degradation potential of marine fungi should not be underestimated. Heiland (1988) found that about 27% degradation of chitin was caused by lower fungi in laboratory tests. Raghukumar (1986, 1987) worked on damage caused by parasitic fungi to algae at the sea coast of Goa, India. Kohlmeyer & Kohlmeyer (1979) worked on saprophytic and parasitic higher fungi in mangrove swamps. Ulken (1984) investigated several swamps for the ocurrence of lower fungi. Aleem (1980) found more fungi during the rainy season than during the dry season in

* Contribution No. 184 of the Alfred Wegener Institute for Polar and Marine Research and No. 154 from CIMAR, Universidad de Costa Rica.

Sierra Leone. All these queries had been the initiative for these investigations.

MATERIAL AND METHODS

Study area: During the dry season in 1987 and the rainy season in 1988) several stations in a mangrove swamp at Pta. Morales were sampled for lower fungi (Chytridiomycetes, Thraustochytriales and Labyrinthulales). (Fig. 1). Mangrove swamps within the Gulf of Nicoya had been investigated earlier by Gocke *et al.* (1981) regarding the oxygen consumption during the degradation of leaf litter. Jiménez & Soto (1985) investigated the higher plants in mangrove communities and Vargas (1987) the faunal elements and correlated the occurrence of the animals with physical data such as temperature, fresh water input, grain size and composition of the bottom. The largest group of animals are the Crustaceae. This may be important for our findings of labyrinthulids and thraustochytrids because many of them possess chytinolytic enzymes as found by Heiland (1988).

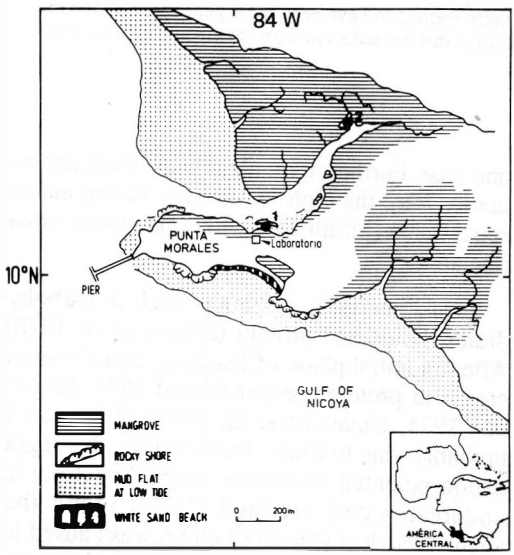

Fig. 1: Mangrove swamp at Pta. Morales

Sampling: The area studied was the estuary of River Morales which enters the Gulf of Nicoya (Fig.1). The mud was sampled from the surface with a small grab. It was collected in sterilized small containers of about 50 ml. In the laboratory, they were divided into subsamples with calibrated spoons (Gaertner 1968) and stored in vials (Beckman) with sterilized sea water. The sediment was dried and heated at 550°C for one night for combustion of the organic matter. The sample then was weighed again and the difference is assumed to be the carbon content. Aliquots of the dry sediment were sieved through different sieves of the following mesh sizes: 5, 10, 18, 25, 35, 45, 60, 80, 120, 170, and 230 µm in order to estimate the grain sizes. In 1987, five stations were visited four times (two at high and two at low tide). During 1988, only the station nearest to the *rampa* was reinvestigated, two times at high and two times at low tide and a place at the inner end of the River Morales was also sampled. Thus, for comparison of fungal occurrence between the rainy and the dry season, only the values at the *rampa* were taken into account. In order to test fungal life in an anaerobic environment we plated out several of our pure fungal cultures originating from different mangrove swamps onto agar dishes and stored them in an anaerobic vessel "Gas Pak" in a laboratory at 25°C.

A cluster analysis was performed on the fungi present in the samples of 1987 after Ward's method in Clifford and Stephenson (1975).

The growth of the fungi, which sometimes live in anaerobic conditions, is influenced by light; for this reason, the photon fluence rates were measured at the soil surface during low tide with a quantameter equipped with a quantum sensor (Biggs *et al.* 1971).

RESULTS

Physical data appear in Tables 1 and 2.

The estimated number of chytrids and thraustochytrids and related organisms are given in Table 3. During the dry season, several chytrids were found in spite of low or no input of fresh water, and some forms similar to *Dermocystidium* were recognized. By far the most frequently occurring organisms belonged to *Schizochytrium* and *Thraustochytrium*. These organisms prefer fairly high salinities. There was no sample without thraustochytrids and labyrinthulids, and we observed *Labyrinthula* sp. in several samples (Figs. 2, 3); it was also found in the slime layer of a tough *Rhizophora mangle* leaf, which had fallen into the water. At stations 2 and 4, *Labyrinthula* sp.

TABLE 1

Physical data in 1987

Station No.	Date	Hour	Salinity S °/oo	pH Value	Temp °C
1++	Feb. 23	4.45 pm	29	-----	
2	Feb. 23	5.10 pm	26	-----	
3	Feb. 23	6.00 pm	31	---- *	
4	Feb. 23**	6.27 pm	-----	---- *	
5	Feb.23	6.05 pm	31	---- *	
1	Mar. 6	4.15 pm	28	7.85	32
2	Mar. 6	4.20 pm	29	8.08	32
3	Mar. 6	4.27 pm	31	8.14	32
4	Mar. 6	4.30 pm	31	8.2	31
5	Mar. 6	4.40 pm	31	8.2	32
1	Mar. 7	7.45 am	31	8.2	32
2	Mar. 7	7.55 am	31	8.17	28
3	Mar. 7	8.00 am	28	8.2	29.5
4	Mar. 7	8.07 am	30	8.27	29.5
5	Mar. 7	8.30 am	29	8.25	29

++ all samples had been stored in a refrigerator until 26 Febr. 1987.
* not measured.
** Stat. 4 was sampled later than Stat. 5 due to lack of oil in the tank.

TABLE 2

Physical data of two stations in 1988

Station No.	Date	Hour	Salinity S °/oo	pH Value	Temp. °C	Redox Potential eH
2	July 11	2.15 pm	12	7.9	29	0.59
1	July 11	2.50 pm	18	7.9	28	0.64
2	July 12	6.30 am	26	7.4	28	0.14
1	July 12	7.30 am	26	8.1	27	-0.20

was up to about 9,000 infective units per litre of the muddy sample.

The comparison between fungi found during the dry and wet seasons (Table 4) clearly shows higher numbers during the wet season. However, during the dry season, there seem to have been more genera. During the rainy season in 1988, *Labyrinthula* sp. had disappeared completely. We did not find it again in any of the samples. In Fig. 2, protruding ectoplasmatic net elements of *Labyrinthula* can be seen, beginning from a cluster of spindle cells (arrow). The gliding movement of these organisms within the net elements is shown in Fig. 3. As we were not successful in isolating them from adhering bacteria, we did not perform a cell wall analysis, which is necessary for taxonomy.

Cluster analysis

For the samples of 1987 (Fig. 4) all stations show little affinity. There are only few resemblances between single stations (less than 40%). Stations 1, 4 and 5 belong to one group and Stations 2 and 3 to another.

Fungal growth in anaerobic conditions

After four weeks, the experiment on anaerobiosis was stopped because no growth of any

TABLE 3

Number (x10²) of infective untis of lower fungi per litre of moist sediment, at Pta. Morales, during the dry season (Feb./March) 1987

	Name	Stat. 1	Stat. 2	Stat. 3	Stat. 4	Stat. 5
Feb.23, l.t.	Chy:	5.4	-.-	2.4	10.3	-.-
	L:	5.4	91.3	-.-	91.3	45.7
	S:	137.0	122.3	157.2	274.0	274.0
	T:	365.3	274.0	456.6	220.0	274.0
	U:	41.2	31.0	157.1	122.3	91.3
Febr.23, h.t.	Chy:	10.3	-.-	-.-	-.-	134.0
	D:	-.-	-.-	10.8	-.-	10.8
	L:	5.4	24.6	19.5	-.-	-.-
	S:	180.0	122.3	5.3	228.3	91.3
	T:	228.3	319.6	319.6	122.3	137.0
	U:	45.7	229.3	10.3	91.3	45.7
March 6, h.t.	D:	-.-	-.-	-.-	24.5	2.4
	L:	5.4	91.3	45.7	-.-	45.7
	S:	137.0	319.6	228.3	137.0	228.3
	T:	228.3	319.6	182.6	228.3	410.7
	U:	45.7	97.8	122.3	16.1	45.7
March 7, l.t.	D:	91.3	-.-	-.-	10.3	24.5
	L:	24.5	2.4	45.7	-.-	-.-
	S:	24.5	-.-	274.0	182.7	182.7
	T:	365.3	182.6	274.0	137.0	228.3
	U:	24.5	-.-	122.3	24.5	137.0

abbr.: Chy = Chytridiomycetes D = *Dermocystidium*
S = *Schizochytrium;* T = *Thraustochytrium*
L = *Labyrinthula;* U = *Ulkenia*
h. t. = high tide: l. t. = low tide; Stat. = station

of the cultured organisms was observed. However, upon transferring the vessels from the dark to a laboratory subject to natural day-night rhythm for several more weeks a surprising growth occurred. The striking facts of these experiments were that anaerobiosis does not kill these fungi and they need light for growth.

Light measurements

The light (photon fluence rate) entering the upper layer of the swamp at low tide on July 12th, 1988 at 08.38 h was: 820 mnol m^{-2} sec^{-1}.

Sediment analysis

The organic carbon and general analysis of the sediments at the five stations of 1987 (all around Station 1 in Fig.1) are shown in Table 5 (mean size). The first sampling trip runs from numbers 1 to 5, sampling in the morning, and the second from 6 to 10, afternoon sampling trip. Mean values are given in Table 5.

DISCUSSION

The occurrence of thraustochytrids and labyrinthulids as degrading organisms in mangrove swamps (Ulken 1984) was confirmed in the swamp near Pta. Morales, Costa Rica. This is noticeable, especially because most animals of the meiofauna are Crustaceae (Vargas 1987) and these fungi have chitinolytic activities. A very rough estimation of the biomass resulted in only about 1 microgram per litre of wet sediment (Edler 1979). However, these cells are very rich in energy and they are known to be a depot of aminoacids and vitamins (Bahnweg

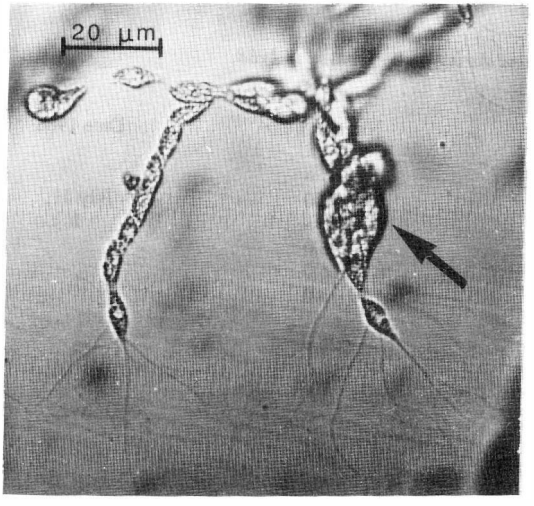

Fig. 2: Aggregation of *Labyrinthula* cells (arrow) and protruding ectoplasmatic net elements.

1979 a, b). The finding of *Labyrinthula* should be mentioned especially. This organism is known to cause severe damage to several marine plants like eel grass at the coast of North America (Muehlstein *et al.* 1988) and to diatoms at the coast of Goa (Raghukumar 1987). There was a great change of frequency of this organism between the two years of investigation for which no explanation is at hand. Though we found it as an inhabitant of mucus on dead mangrove leaves it seems to be inactive in the destruction of leaves or other parts of the mangrove plants.

The results of Aleem´s investigation (1980) on the fungal flora in a mangrove swamp of Sierra Leone could be confirmed by the observation of lower fungi in Costa Rica. During the rainy season there are more infective units in the mud; however, there seem to be more genera during the dry season.

The occurrence of marine fungi was correlated with the grain size of the sediment. At a higher grain size a higher quantity of infective units was found. This observation confirms previous results (Ulken 1986).

Olive (1975) and Perkins (1974), and also Moss (1986), made new reflections regarding the position of thraustochytrids and labyrinthulids in the system of the living organisms. According to ultrastructural studies, the position is still unsolved and many more investigations regarding the biochemical and ribosomal RNA studies are necessary to solve these questions.

The influence of light on normally or sometimes anaerobic living organisms within the upper layer of sediments should be investigated in more detail. Mani & Swamy (1981) found differences in permeability in fungal cells when exposed to light. The measured photon fluence rate seems to be sufficient to initiate growth of some thraustochytrids and chytrids which could be shown by some experiments in the laboratory (Ehlken & Ulken, unpublished). Early in the morning of July 12th, at Station 1, there were anaerobic conditions with a redoxpotential of -0.20 (Table 2).

ACKNOWLEDGEMENTS

The authors thank M. M. Murillo (CIMAR), and Edgar Salgado at the Facultad de

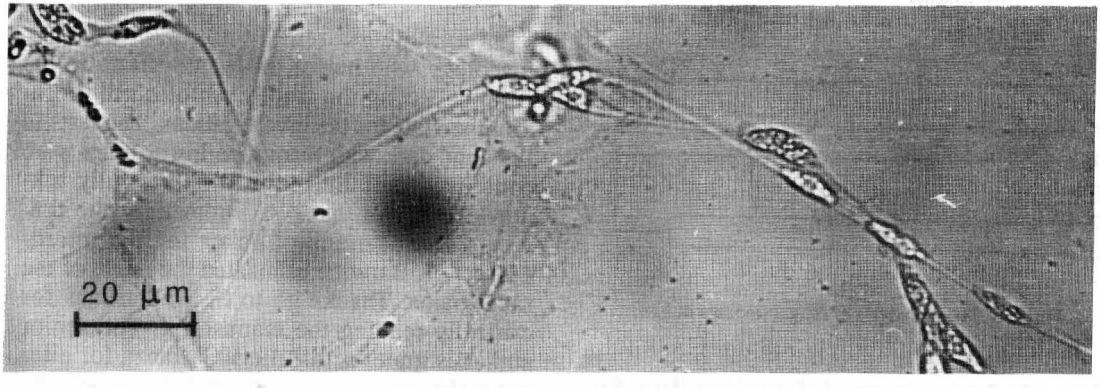

Fig. 3: Spindle cells of *Labyrinthula* sp. gliding through the ectoplasmatic net elements.

TABLE 4

Fungi found during the dry season in 1987 and during the wet season in 1988.

Name	Number x 10^2	Date 1987	Number x 10^2	Date 1988
Chy:	10.3	Febr. 23,	73.0	July 11,
L:	5.4	h. t.	-.-	h. t.
S:	180.0		146.0	
T:	228.3		366.0	
U:	-.-		73.0	
sum:	424.0		658.0	
Chy:	5.4	Febr. 23,	-.-	July 12,
L:	5.4	l. t.	-.-	l. t.
S:	137.0		45.7	
T:	365.3		365.3	
U:	41.2		-.-	
sum:	554.3		411.0	
Chy:	-.-	March 6,	10.1	July 28,
L:	5.4	h. t.	-.-	h. t.
S:	137.0		10.0	
T:	228.3		438.1	
sum:	370.7		458.2	
Chy:	-.-	March 7,	-.-	July 29,
L:	24.5	l. t.	-.-	l. t.
S:	24.5		73.0	
T:	365.3		657.0	
U:	24.5		-.-	
sum:	438.8		730.0	

abbr.: Chy= Chytridiomycetes L= *Labyrinthula*
S= *Schizochytrium;* T= *Thraustochytrium*
U= *Ulkenia*
h. t.= high tide; l. t. = low tide

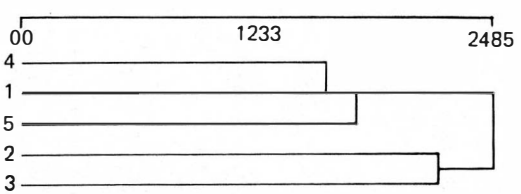

Fig. 4: Cluster analysis of fungal findings in 1987

Micro-biología, Universidad de Costa Rica, for laboratory facilities in San José and at Pta. Morales, and the students of course II in marine mycology who helped with enthusiasm in the field, collecting the muddy samples and preparing the vials for the investigations. The tests in light and dark with mangrove lower fungi were done by Renate Ehlken who is gratefully acknowledged. We thank A. Bremer for the drawings and Marianne Lücker for the micrographs. E. Barwich calculated the mean of the sand grains. Thanks go to S. Wellershaus for critically reading the manuscript. The senior author is grateful to Julieta Carranza for allowing the use of facilities at the Escuela de Biología. A travel grant from the DAAD is gratefully acknowledged.

TABLE 5
Analysis of grain sizes in 1987

Sample No.	1. Field trip	Organic matter %	2. Field trip	Organic matter %
Morning trip				
1	12	6.34	12	8.56
2	d.s.	11.63	8	18.89
3	48	11.99	30	11.85
4	55	15.20	19	17.87
5	9	13.31	50	4.92
Afternoon trip				
6	18	6.96	18	9.32
7	27	12.41	15	13.26
8	10	11.89	5	12.81
9	20	15.76	5	16.25
10	20	12.50	11	9.34

Explanation: In sample No. 2, first field trip, there seem to be two different sediments (d.s.) mixed in one sample.

REFERENCES

Aleem, A. A. 1980. Distribution and ecology of marine fungi in Sierra Leone, (Tropical West Africa). Bot. Mar. 23: 679-688.

Bahnweg, G. 1979. Studies on the physiology of Thraustochytriales. I. Growth requirements and nitrogen nutrition of *Thraustochytrium* spp., *Schizochytrium* sp., *Japonochytrium* sp., *Ulkenia* spp. and *Labyrinthuloides* spp. Veröff. Inst. Meeresforsch. Bremerh. 17: 245-268.

Bahnweg, G. 1979. Studies on the physiology of Thraustochytriales. II. Carbon nutrition of *Thraustochytrium* spp., *Schizochytrium* sp., *Japonochytrium* sp., *Ulkenia* spp. and *Labyrinthuloides* spp. Veröff. Inst. Meeresforsch. Bremerh. 17: 269-273.

Bhosle, N. B., V. K. Dhargalkar & S. G. P. Matondkar 1976. Biochemical composition of mangrove leaves from Goa. Indian J. Mar. Sci. 5: 239-241.

Biggs, W. W., A. G. Edison, J. D. Eastin, K. W. Brown, W. J. Meanville & M. D. Clegg. 1971. Photosynthesis light sensor and meter. Ecology 52: 125-131.

Chapman, V. J. 1976. Mangrove Vegetation. J. Cramer, FL-9490 Vaduz.

Clifford, H. T. & W. Stephenson 1975. An introduction to numerical classification. Academic Press, New York. 224 p.

Edler, L. 1979. Recommendations on Methods for Marine Biological studies in the Baltic Sea. Phytoplankton and chlorophyll. The Baltic Marine Biologists 5: 1-38.

Fell, J. W., R. C. Cefalu, I. M. Master & A. S. Tallman 1975. Microbial activities in the mangrove *(Rhizophora mangle)* leaf detrital system, p. 661-679. *In* G. E. Walsh, S. C. Snedaker & H. J. Teas (eds.). Proceedings International Symposium on the Biology and Management of Mangroves, Honolulu, 1974, Univ. of Florida, Gainesville.

Gaertner, A. 1968. Eine Methode des quantitativen Nachweises niederer, mit Pollen köderbarer Pilze im Meerwasser und im Sediment. Veröff. Inst. Meeresforsch. Bremerh. Sonderbd. 3: 75-89.

Gocke, K., M. Vitola & G. Rochas. 1981. Oxygen consumption patterns in a mangrove swamp on the Pacific coast of Costa Rica. Rev. Biol. Trop. 29: 143-154.

Heald, E. 1971. The production of organic detritus in a south Florida estuary. Univ. Miami Sea Grant Tech. Bull. 6: 110 p.

Heiland, R. 1988. Untersuchungen zum Chitinabbau von Niederen Pilzen. Diplomarbeit, Universität Bremen, 1-58.

Jiménez, J. A. & R. Soto 1985. Patrones regionales en la estructura y composición florística de los manglares de la costa Pacífica de Costa Rica. Rev. Biol. Trop. 33: 25-37.

Kohlmeyer, J. & E. Kohlmeyer 1979. Marine Mycology: The Higher Fungi. Academic Press, New York.

Mani, K. & R. N. Swamy 1981. Light-induced Changes in Permeability in Two Groups of Fungi. Exp. Mycol. 151: 292-294.

Moss, S. T. 1986. Biology and Phylogeny of the Labyrinthulales and Thraustochytriales, p. 105-129. *In*

S.T. Moss (ed.). The Biology of Marine Fungi. Cambridge University Press.

Muehlstein, L. K., D. Porter & F. T. Short 1988. *Labyrinthula* sp., a marine slime mold producing the symptoms of wasting disease in eelgrass, *Zostera marina*. Mar. Biol. 99: 465-472.

Olive, L. S. 1975. The Mycetozoans. New York, Academic Press.

Perkins, F. O. 1974. Phylogenetic considerations of the problematic thraustochytriaceous - labyrinthulid-*Dermocystidium* complex based on observations of fine structure. Veröff. Inst. Meeresforsch. Bremerh., Suppl. 5: 45-63.

Pool, D. J., A. E. Lugo & S. C. Snedaker. 1975. Litter production in mangrove forests of south Florida and Puerto Rico, p. 213-237. *In* G. E. Walsh, S. C. Snedaker & H. J. Teas (eds.). Proceedings International Symposium on the Biology and Management of Mangroves, Honolulu, 1984, Univ. of Florida, Gainesville.

Por, F. D. & I. Dor 1984. Hydrobiology of the Mangal. The Ecosystems of the Mangrove Forests. Junk, The Hague 260p.

Raghukumar, C. 1986. *Chytridium polysiphonia* - a fungal pathogen on the red alga, *Centroceras clavalatum* (C. Agardh) Montagne, from Goa. Indian J. Mar. Sci. 15: 42-44.

Raghukumar, C. 1986. Thraustochytrid fungi associated with marine green algae, *Cladophora* and *Rhizoclonium*. Bot. Mar. 29: 289-297.

Raghukumar, C. 1986. Thraustochytrid fungi associated with marine algae. Indian J. Mar. Sci. 15: 121-122.

Raghukumar, C. 1987. Fungal parasites of marine algae from Mandapam (South India). Dis. Aquat. Org. 3: 137-145.

Raghukumar, C. 1987. Fungal parasites of the green algae *Chaetomorpha media*. Dis. Aquat. Org. 3: 147-150.

Smith, S. V. 1981. Marine macrophytes as global carbon sink. Science 211: 338-340.

Tomlinson, P. B. 1986. The Botany of Mangroves. Cambridge University Press, London.

Ulken, A. 1984. The fungi of the mangal ecosystem, p. 27-33.*In* F.D. Por & I. Dor (eds.). Hydrobiology of the Mangal. Junk The Hague.

Ulken, A. 1986. Estimation of thraustochytrid propagules in two mangrove swamps. Bot. Mar. 29: 85-89.

Untawale, A. G., N. B. Bhosle, V. K. Dhargalkar, S. G. Matondor & S. Bukhari. 1977. Biochemical changes in mangrove foliage during growth and decomposition. Ind. J. Mar. Sci. 6: 104-106.

Vargas, J.A. 1987. The benthic community of an intertidal mud flat in the Gulf of Nicoya, Costa Rica. Description of the community. Rev. Biol. Trop. 35: 299-316.