Distribution and abundance of thraustochytrids in different Mediterranean coastal habitats

Lucia Bongiorni*, Fernando Dini

Dipartmento di Etologia, Ecologia ed Evoluzione, Università di Pisa, Via Alessandro Volta 4, 56126 Pisa, Italy

ABSTRACT: Habitat, seasonality and species distribution of the marine fungoid protists, thraustochytrids, were investigated in a coastal area of the Ligurian Sea (NW Mediterranean). Three habitats (the sandy surf area, the middle of a 5 m seawater column, and its underlying sandy sea bottom) were monitored monthly for 1 yr. Thraustochytrids in the surf and sea-bottom sandy samples yielded average densities of 4.4 ± 4 and $6.1 \pm 5.3 \times 10^4$ cells l^{-1}, respectively. In both sandy habitats, a similar seasonal trend was observed: the highest densities were recorded in late spring, while a significant drop in densities occurred during the autumn-winter period. In the water column, the density of thraustochytrids was much lower, $1.3 \pm 1 \times 10^2$ cells l^{-1} and did not show significant seasonal variations. A positive relationship between organic matter and thraustochytrid densities occurred in the sandy sea bottom. Among the nutrients assessed in the water column, only total phosphorus was related to variations of thraustochytrid densities. The 3 habitats analyzed showed marked differences in species number and assemblage. A higher number of species were found in the sandy sea bottom than in the sandy surf area and in the seawater column. The genus *Thraustochytrium* was the most abundant in the sandy bottom habitat. Among *Thraustochytrium* species, those characterized by sporangia with proliferation bodies were dominant. Species of the genus *Schizochytrium*, typically forming a cluster of sporangia, were dominant in the sandy surf area. Here, the possible ecological significances of these species' features are discussed.

KEY WORDS: Thraustochytrid protists · Abundance · Seasonal variations · Species assemblages · Mediterranean coasts

INTRODUCTION

Thraustochytrids, fungoid protists of the phylum Heterokonta, Kingdom Chromista (Cavalier Smith et al. 1994), are cosmopolitan, osmoheterotrophic organisms found in saline lakes, marine and estuarine waters (Porter 1990). Their wide distribution in salty environments, abundance and their primary association with decaying organic materials argue for their important ecological role as decomposers (Raghukumar 1992, Naganuma et al. 1998, Raghukumar et al. 2001). In addition, thraustochytrids are known to produce high amounts of polyunsaturated fatty acids (PUFAs), which are important nutritive factors for many marine animals

(Raghukumar 1996, Kimura et al. 1999). By virtue of their capacity to develop an ectoplasmic net and to produce lytic enzymes, thraustochytrids are capable of penetrating and absorbing nutrients from different organic substrates (Bahnweg 1979). They are thought to be effective in the degradation of refractory substrates compared to other saprophytes; thus, enhancing efficiency of the biogeochemical mineral cycling in marine environments (Bremer & Talbot 1995, Raghukumar 1996, Naganuma et al. 1998, Raghukumar et al. 2001). Recently, the carbon/cell ratio of coastal thraustochytrids has been found to be higher than that of coastal and oceanic bacteria (Kimura et al. 1999). This suggests a greater influence of these organisms on the coastal carbon cycling than previously presumed. For these reasons, thraustochytrids may assume a particular importance in shallow, coastal and estuarine environments, where particulate and dissolved organic materials originating from phytoplankton

---

*Present address: Israel Oceanographic & Limnological Research Ltd, Tel Shikmona, PO Box 8030, Haifa 31080, Israel. Email: lucia@ocean.org.il

and plant detritus are abundant. Despite this, their specific ecological role in the foregoing habitats is still not clear, and information on thraustochytrid seasonality and factors influencing their abundance is poor.

Thraustochytrid abundance has been previously investigated in areas of the North Sea (Gaertner 1969, Gaertner & Raghukumar 1980, Raghukumar & Gaertner 1980), the Baltic Sea (Schneider 1968), the west African coast of the Atlantic Ocean (Gaertner 1982), the Antarctic Sea (Bahnweg & Sparrow 1974), the reef lagoons of the Indian Lakshadweep Islands (Raghukumar 1987a), the Arabian Sea (Raghukumar et al. 1990, 2001) and the Seto Inland Sea, Japan (Naganuma et al. 1998, Kimura et al. 1999). In the Mediterranean Sea (NW, Ligurian), distribution of both thraustochytrids and ciliated protists was examined in sandy bottoms (Santangelo et al. 2000). However, there are no data as yet regarding seasonal density variations and taxonomic compositions of thraustochytrid species assemblages of the Mediterranean.

The aim of this study was to assess the distribution, seasonal variability and community structure of thraustochytrids in different coastal habitats of this 'closed', temperate sea. In addition, nutrients in the water column, chlorophyll *a* (chl *a*) and organic matter content in the sediment were assessed and related to thraustochytrid densities.

Fig. 1. Study area along the NW Mediterranean coast, Ligurian Sea, Italy. Arrowheads indicate the sampling stations

MATERIALS AND METHODS

Sampling stations. Four stations were selected along the Ligurian Sea coast, NW Mediterranean, in the vicinity of Pisa, Italy, between 43° 39′ N, 10° 13′ E and 43° 50′ N, 10° 11′ E (Fig. 1). This is part of the San Rossore National Park. The stations were sampled monthly from June 1997 to June 1998. Three of the 4 stations were located near the mouths of 3 rivers (Serchio, Morto and Arno), while the fourth station at Tirrenia was southerly located 6 km away from the southernmost Arno River. This choice was made to evaluate effects of different environments on thraustochytrid distribution. Three samples were collected at each station from different habitats: (1) sandy surf area (SSA); (2) the middle of a 5 m seawater column (SC), 200 m from the seashore; and (3) the underlying sandy sea bottom (SSB). The 2 sandy habitats differed with respect to environmental conditions. The SSA habitat was characterized by poorly sorted, medium-sized grains, thereby, according to Brown & McLachlan (1990), supporting a high interstitial flow rate, high desiccation level and usually having a low organic matter content. Following the same authors, contrasting environmental features may be assumed to characterize medium-fine-grain-size substrates of the SSB habitat. A total of 144 samples (48 from each habitat) were analyzed over the year. All mean data ± SD are reported in this paper.

Thraustochytrid sampling and analysis. Sand samples from the SSA and SSB habitats, and water samples from the SC habitat were collected using sterile, 0.5 l capacity, large bottom jars. In collecting sand samples, each jar was half filled by grazing the superficial 2 cm sandy layer inside a 2 m² area, in order to avoid the anoxic environment. Samples were carried to the laboratory in a thermal bag maintained at 7°C and analyzed about 3 h after collection. The number of thraustochytrids was estimated by baiting samples with pine pollen according to the Most Probable Number (MPN) technique modified by Gaertner (1968a). This technique underestimates the total number of thraustochytrids with respect to direct counts based on epifluorescence (Raghukumar & Schaumann 1993, Naganuma et al. 1998, Santangelo et al. 2000). However, the MPN method set at adequate dilutions and replicates yields reliable data regarding density fluctuations. Moreover, taxonomic characters of thraustochytrids, not detectable by epifluorescence, are more constantly expressed in pine pollen cultures than in other nutrient media (Gaertner 1972). The modified MPN method thus allowed the simultaneous assessment of thraustochytrid density fluctuations and species assemblages.

Sand samples were homogenized by gentle shaking and for each of them a set of 7 dilutions (0.311, 0.251,
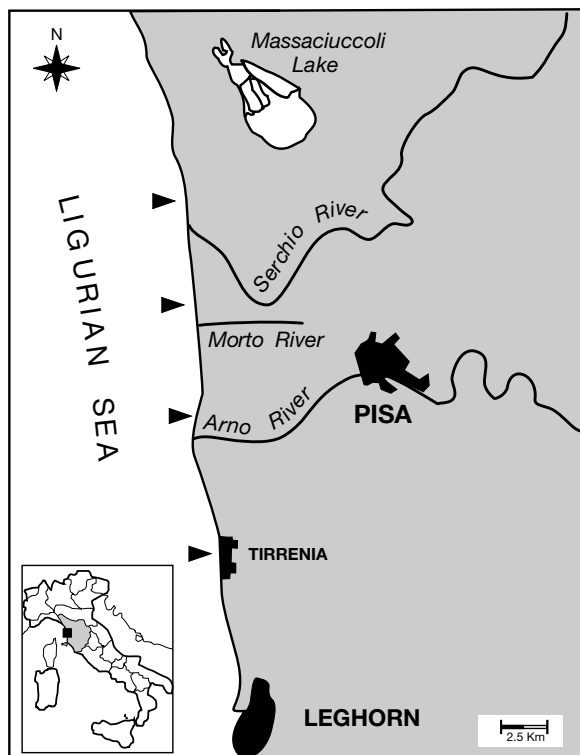
0.126, 0.061, 0.031, 0.016 and 0.011 ml) was successively set up. The desired sediment aliquots were picked up from the sample using specially designed metal spatulas. For each seawater sample, another set of 7 dilutions (20, 10, 5, 2.5, 1.25, 0.625 and 0.31 ml) was set up using graduated pipettes. In both sets of dilutions, 5 replicates were maintained for each aliquot. All the replicates were brought to a constant volume of 20 ml with sterile seawater, baited with the same quantity of pine pollen and stored at 20°C for 3 wk. The incubation time was chosen on the basis of the outcomes of preliminary counts, carried on with the same series of dilutions. The number of tubes positive for the presence of thraustochytrids increased until Week 3 of incubation. Quantitative and qualitative analyses were carried out, under a Leitz Orthoplan photomicroscope at 250 and 400× magnifications, and final densities were estimated using the Thomas' formula correction for MPN (Anonymous 1981).

Qualitative analysis aimed at species identification was carried out in each habitat on 50% of the total samples selected by random tables. Life cycles of thraustochytrid-pollen cultures were observed in a continuous flow chamber (Raghukumar 1987b) and the morphologic features were recorded. The major characteristics used for taxonomic purposes were the type of sporangia and mode of sporulation, the presence of proliferation body, morphology and size, as according to Gaertner (1972), Porter (1990) and Raghukumar (1996).

Sandy samples were analyzed to determine porosity values (Buchanan 1984), amounts of total organic matter (Giere 1993) and chl *a* contents (Plante-Cuny 1974). Temperatures were measured during each sampling and additional seawater samples were collected and analyzed for total nitrogen, phosphorus, nitrite, nitrate, ammonia and phosphate, in accordance with standard analytical procedures (IRSA-CNR 1984, Genchi 1990, Ribera d'Alcalà et al. 1990a,b, Saggiomo et al. 1990).

Parametric statistical tests were performed on the natural logarithms of thraustochytrid data. Nutrient concentrations and transformed thraustochytrid densities were analyzed by multivariate regression procedures (REG procedure, SAS 1999). Two different approaches (the forward and the backward model-selection techniques) were employed. The forward selection technique calculates the contribution (*F*) of each single independent variable entering in the model comprising all the parameters tested. The variables that show the largest *F* are added to the model one by one, until no significant variables remain. Con-versely, the backward elimination technique gives the *F* of each variable for a model including all the independent variables. The variables that show the smallest contribution to the model are deleted one by one, until all the variables present produce a significant *F*.

RESULTS

Variation in thraustochytrid densities

Thraustochytrids were found regularly throughout the year in all the habitats and stations sampled. The SSA and SSB habitats yielded an annual thraustochytrid average density of $4.4 \pm 4 \times 10^4$ cells l^{-1} sediment (n = 48) and $6.1 \pm 5.3 \times 10^4$ cells l^{-1} sediment (n = 48), respectively, whereas $1.3 \pm 1 \times 10^2$ cells l^{-1} water (n = 48) were found in the SC habitat. According to previous estimations (Kimura et al. 1999), these densities corresponded to biovolumes of 2.3×10^7, 3.2×10^7 and 6.8×10^4 μm^3, and biomasses of 10.00, 7.27 and 0.021 µg C l^{-1}, respectively.

When habitats SSB and SSA were compared, the SSB habitat showed significantly higher thraustochytrid densities (Wilcoxon test, p < 0.001, Siegel 1956). Significant higher (Wilcoxon test, p < 0.001) values of porosity, total organic matter and chl *a* were found in the SSB (Table 1). A positive correlation (Pearson, n = 42, r = 0.34, p < 0.05) between total organic matter and thraustochytrid densities occurred in this habitat (Fig. 2). An exception to this relation was represented by 6 samples yielding the highest thraustochytrid densities (14.2 to 20.0×10^4 cells l^{-1}) and corresponding to low values of organic matter (0.9 to 1.8 w/w %). Such samples occurred at the Arno River station during September 1997 and June 1998, and in all 4 sampling stations during May 1998. During these periods, no evident drops of nutritive sources were detected either in the sediment or in the water columns. No relation between the total organic matter and thraustochytrid densities was found in the SSA habitat. Moreover, no correlations were found between thraustochytrid densities, porosity and chl *a* contents in both sandy habitats.

Table 1. Quantitative estimations (means ± SD) of thraustochytrid abundance, sediment porosity, total organic matter and chlorophyll *a* (chl *a*) concentration occurring in the sandy surf area (SSA) and sandy sea bottom (SSB) habitats. Significantly different (Wilcoxon test, p <0.001) when letters are different

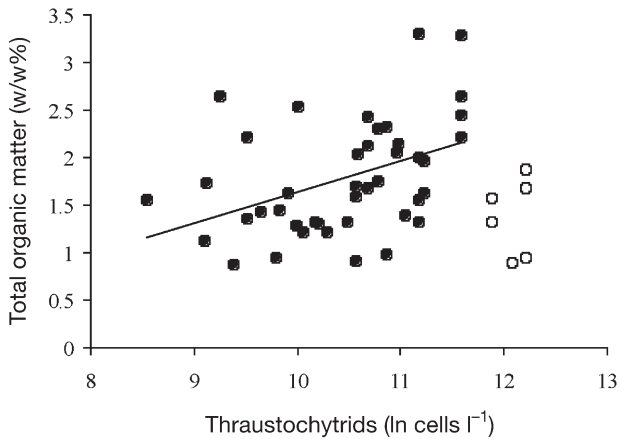| Habitat	Thraustochytrids ($\times 10^4$ cells l^{-1})	Porosity (w/w %)	Total organic matter (w/w %)	Chl *a* (µg l^{-1})
SSA	4.4 (±4)[a]	19.80 (±1.25)[a]	0.92 (±0.31)[a]	0.02 (±0.05)[a]
SSB	6.1 (±5.3)[b]	23.84 (±2.13)[b]	1.73 (±0.59)[b]	0.83 (±0.7)[b]

Fig. 2. Regression analyses of thraustochytrid densities and total organic matter in the sandy sea bottom (SSB) habitat (r = 0.34, n = 42, p < 0.05). (○) 6 samples in which thraustochytrid blooms corresponded to low level of organic matter

Among all the nutrients measured in the water column during the entire year (nitrite, nitrate, ammonia, phosphate, total phosphorus and nitrogen), only total phosphorus ranging from 0.29 to 2.55 μM l^{-1} was significantly related to thraustochytrid densities (R^2 = 0.082, F = 4.1, p < 0.05, forward selection technique and backward elimination technique, see 'Materials and methods' (Table 2). Nitrate (0.06 to 9.32 μM l^{-1}) showed the higher contribution to the model comprising all the independent variables. A summary of the analyses is shown in Table 2.

No significant differences in thraustochytrid densities occurred between the stations in either the SSB or SC habitats. However, thraustochytrid density differed between the stations in the SSA habitat (Friedman analysis of variance, Siegel 1956, p < 0.05) decreasing from Arno River, Tirrenia, Serchio River and Morto River stations in this order (cf. Fig. 3a).

On the whole, strong seasonal differences in thraustochytrid densities occurred in all the stations of the SSA and SSB habitats (Kruskal-Wallis analysis of variance, Siegel 1956, p < 0.05 for SSA, and p < 0.001 for SSB), (Fig. 3). Similar temporal patterns were observed in all stations of both sandy habitats. Low and constant densities were assessed in autumn and winter when the temperature was 15 ± 3.8 and 13 ± 1°C, respectively. The highest densities of thraustochytrids occurred in spring when the temperature reached 21.7 ± 0.7°C. However, no linear correlation between thraustochytrid densities and temperature was found, since at the highest average temperature (24.3 ± 1°C) occurring in summer, thraustochytrid densities were lower than in spring. On the contrary, thraustochytrids in the SC habitat did not show any seasonal variation and kept low throughout the year (1.3 ± 1 × 10^2 cells l^{-1}). Peaks of thraustochytrids occurred in the water column during the autumn months: October 1997 at the Tirrenia station, 3 × 10^2 cells l^{-1}, and November 1997 at the Arno and Serchio River stations, 4.5 and 4 × 10^2 cells l^{-1}, respectively.

Species assemblages

Eleven species belonging to 5 different genera were identified in 72 samples randomly selected from those collected throughout the year. The most frequent species in the 3 habitats were: *Schizochytrium octosporum* (39.2%), *Thraustochytrium motivum* (25.6%) and *Thraustochytrium multirudimentale* (10.4%). The SSA, SC and SSB habitats were characterized by 4, 5 and 8 species, respectively. The list of species identified and their frequencies in each habitat are given in Fig. 4. Communities of SSB and SC showed a closer similarity (Sorensen's index, S = 0.6) compared to SSA (S = 0.3).

Table 2. Summary of the backward elimination and forward selection analyses steps (SAS procedure). Dependent variable is thraustochytrid density in the water column, expressed as ln (cells l^{-1}), independent variables are ammonia (N-NH$_4$), nitrite (N-NO$_2$), nitrate (N-NO$_3$), total nitrogen (N), phosphate (P-PO$_4$) and total phosphorus (P), expressed as μM l^{-1}. For each variable n = 48

	Independent variable	Partial R^2 of the removed variables	F	p
Backward elimination				
Step 0	N-NH$_4$		2.19	0.146
	N-NO$_2$[a]	0.001	0.01	0.942
	N-NO$_3$		3.36	0.074
	N		0.01	0.916
	P-PO$_4$		2.33	0.134
	P		1.52	0.224
Step 1	N-NH$_4$		2.28	0.138
	N-NO$_3$		3.65	0.063
	N[a]	0.003	0.02	0.901
	P-PO$_4$		2.38	0.130
	P		1.66	0.205
Step 2	N-NH$_4$[a]	0.043	2.32	0.134
	N-NO$_3$		3.84	0.056
	P-PO$_4$		2.43	0.126
	P		2.39	0.129
Step 3	N-NO$_3$[a]	0.055	2.90	0.095
	P-PO$_4$		3.30	0.076
	P		4.51	0.039
Step 4	P-PO$_4$[a]	0.031	1.58	0.214
	P		5.67	0.021
Step 5	P		4.10	0.048
Forward selection				
Step 1	P	0.082	4.10	0.048
[a]Removed variable at each analysis step				

The SSA shared only 2 species with the SSB and SC habitats. Different thraustochytrid genera dominated the 2 sandy habitats. *Schizochytrium*, comprising the *S. octosporum* and *S. aggregatum* species, was the most abundant genus (83%) in the SSA habitat. Between these 2 species, *S. octosporum*, distinguishable in the pine-pollen cultures as it produced smaller clusters of cells and a lower number of zoospores, was the more abundant (67%). However, the *Thraustochytrium* genus, represented by the species *T. motivum*, *T. multirudimentale*, *T. roseum* and *T. striatum*, dominated the SSB habitat (68%). The first 2 species, characterized by one or more proliferation bodies, respectively, were the most frequent (56%).

Based on sporangial development, and other life cycle features, thraustochytrids can be divided into 4 groups. Forms characterized by: (1) single globose sporangia leaving *in situ* proliferation bodies after sporulation (e.g. *Thraustochytrium motivum* and *T. multirudimentale*); (2) single globose sporangia yet lacking proliferation bodies (e.g. *T. aggregatum*, *T. roseum*, *T. striatum*, *Ulkenia minuta* and *U. visurgensis*); (3) sporangia developing from clusters of cells, formed by repeated binary divisions of the initial thallus (e.g. *Schizochytrium aggregatum* and *S. octosporum*); and (4) typical flagellum lacking spores, produced by simple mitotic divisions of the initial thallus, that move by the aid of ectoplasmic filaments (e.g. *Diplophrys marina* and *Labyrinthuloides* sp.). Differences in the distribution of the foregoing forms were observed in the 3 habitats (Fig. 5). Representatives of all 4 groups occurred in both the SSB and SC habitats, where those comprising Group 1 turned out to be the most abundant. The SSA habitat was lacking thraustochytrids of Group 4 and was mostly colonized by representatives of Group 3.

DISCUSSION

This work represents the first report regarding seasonality and taxonomic composition of thraustochytrids in a Mediterranean area. Our results point out the ubiquity of these protists in the coastal locations examined. Thraustochytrid abundance in sandy habitats resulted in 2.8, 2.8 and 6.5×10^{-5} times that yielded by ciliates, flagellates and aerobic-heterotrophic bacteria, respectively, sampled in the same area (Santangelo & Lucchesi 1995). The total biomass of thraustochytrids resulted 8.3×10^{-2}, 4.7×10^4 and 3.6×10^{-1} times that of the above organisms, respectively, suggesting an impor-

tant contribution of thraustochytrids to the microbial loop and carbon cycling of the Mediterranean sandy shores. The organisms' total biomasses were calculated on the estimated carbon content reported by Kimura et al. (1999) for thraustochytrids, Pelegrí et al. (1999) for ciliates, Børsheim & Bratbak (1987) for flagellates and Fukuda et al. (1998) for aerobic-heterotrophic bacteria.

Thraustochytrid densities that we found in the sandy bottom habitat are the highest reported so far (0.5 to 20×10^4 cells l^{-1}) and exceed those detected in North Sea bottom sediment (1.8 to 7.3×10^4 cells l^{-1}) by Raghukumar & Gaertner (1980), employing the same MPN technique we used. Average densities of the same order of magnitude ($4.2 \pm 3.5 \times 10^4$ cells l^{-1}), were found in the same Mediterranean location by Santangelo et al. (2000) using a direct count technique.

The density range recorded in this study in the water column was similar to those reported from the Zuary estuary in the Arabian Sea (Raghukumar et al. 1990) and various parts of the North Sea (Raghukumar & Gaertner 1980). However, Schneider (1968), using the MPN technique, found 1 order of magnitude higher
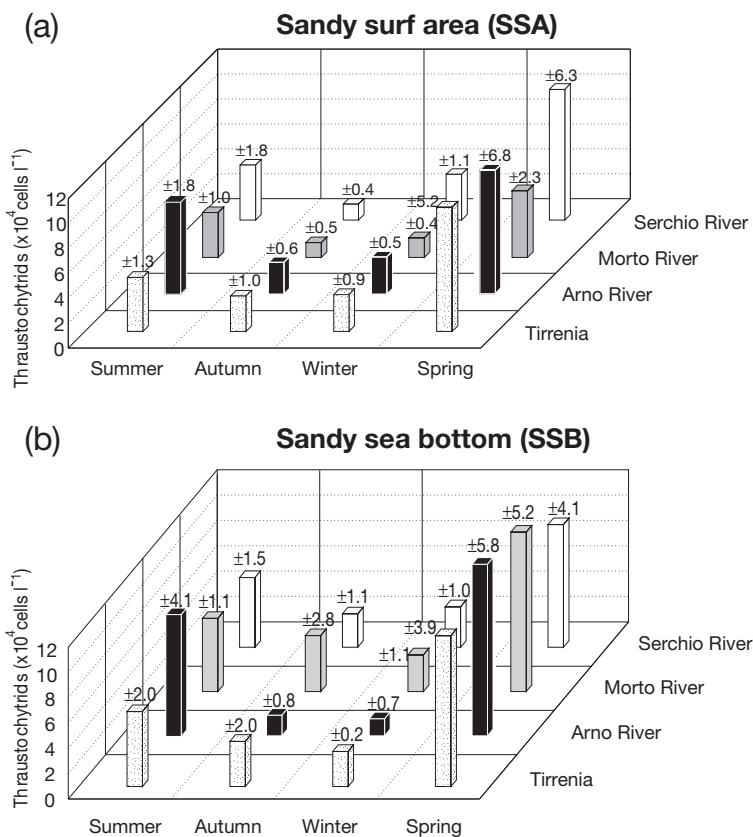

Fig. 3. Seasonal variations of thraustochytrid densities in the sampling stations of (a) the sandy surf area (SSA) and (b) the sandy sea bottom (SSB) habitats
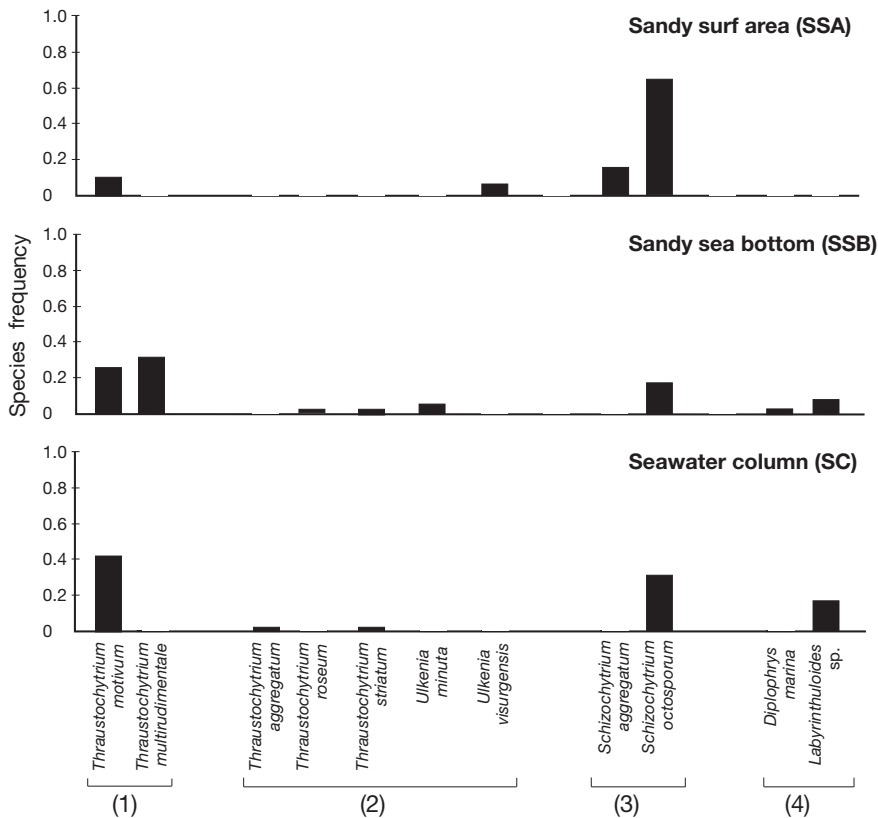
Fig. 4. Frequencies of the single thraustochytrid species in the 3 monitored habitats

densities in the coastal waters of the Baltic Sea and recently, far higher densities were found by Naganuma et al. (1998) and Kimura et al. (1999) from the coastal waters of Japan when employing a direct count method.

The 2 Mediterranean sandy habitats (SSA and SSB) showed clear cut seasonal variations in thraustochytrid densities (cf. Fig. 3). In contrast, no seasonal variations were found by Raghukumar & Gaertner (1980) in North Sea sediments. These authors speculated that this could have been due to a non-limited level of organic matter in sediments. The well-defined and similar seasonal pattern we observed in both sandy habitats, despite the qualitative and/or quantitative differences in physical-chemical features, is evidence of the major role played by the temperature in regulating thraustochytrid proliferation in sediments at Mediterranean latitudes. Our data suggest an optimal growth temperature ranging 21 to 22°C, as thraustochytrid densities did not increase in summer when the maximum temperature values were reached (25°C). This is in agreement with the observations of Ulken (1966) and Jones & Harrison (1976) that thraustochytrids have optimum growth temperatures of 20 to 25°C.

Despite similar seasonal trends, significant differences in thraustochytrid total density and species composition

were found between the SSA and SSB habitats. If temperature is not a discriminating factor, other environmental factors could have accounted for the inter-habitat differences. Tides and backwashing waves continuously stir intertidal sediment, causing fast changes in nutrient concentrations and in its overall physical-chemical conditions (Brown & McLachlan 1990). In accordance with this, the total organic matter content and the thraustochytrid abundance assessed in the intertidal SSA habitat were lower than those in the SSB habitat, 0.5 and 0.7 times, respectively (cf. Table 1). The positive correlation found between the thraustochytrid densities and the total organic matter in the sandy bottom habitat is in agreement with a recent study (L. Bongiorni, L. P. Pignataro & G. Santangelo unpubl.), and supports the idea that total organic matter in Mediterranean sandy environments represents an important food source for these organisms. Further detailed studies will, however, be needed to detect which of the biochemical components of organic matter is responsible for the above relationship. Such studies could also give an explanation for the occurrence of high thraustochytrid densities we observed in the presence of low levels of total organic matter.

The particulate and/or dissolved matter from phytoplankton or degradation of phytodetritus seems to play a role in supporting thraustochytrid proliferation in coastal areas (Naganuma et al. 1998). According to this, the average concentration of chl *a* we found in the SSB habitat (the most abundant in thraustochytrids) was 40-fold higher than in the SSA habitat. In addition, higher levels of chl *a* and phytoplankton densities (data obtained from the ARPAT-agency coastal monitoring program) were found in the water column of the Arno River station, which yielded the highest thraustochytrid densities. However, no correlation between thraustochytrids and chl *a* concentrations or phytoplankton cells was detected in this study, neither in the sediment nor in the water column. Such relations were also not found by Raghukumar & Gaertner (1980) in coastal waters of the North Sea and by Raghukumar et al. (2001) in oceanic waters of the Arabian Sea. Both papers report high numbers of thraustochytrids at the end or after phytoplankton blooms. These observations as well as the occurrence of a correlation between thraustochytrids and particulate organic carbon (POC), (Bahnweg & Sparrow 1974, Raghukumar et al. 2001)
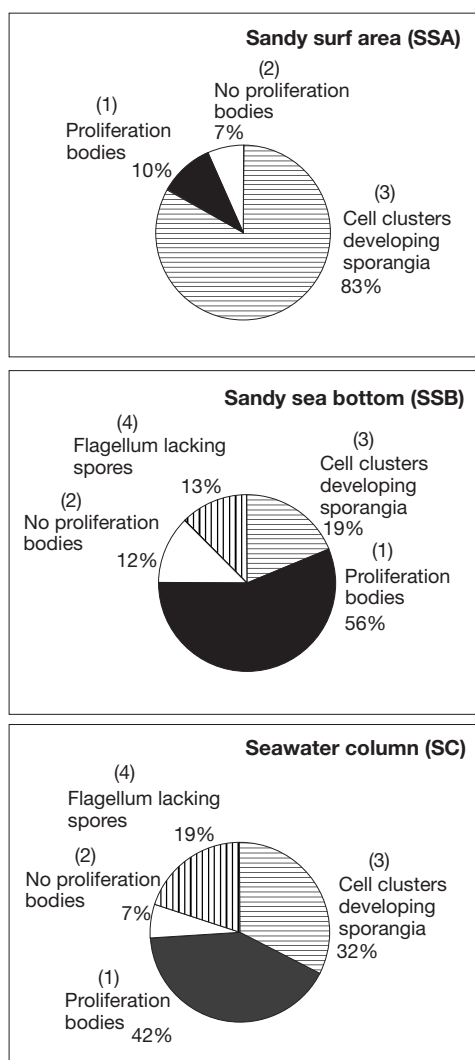
Fig. 5. Percentage of the thraustochytrid groups of species sharing similar life-cycle characteristics. Forms distinguished for (1) producing or (2) lacking proliferation bodies, (3) sporangia developing from cluster of cells and (4) lacking bi-flagellate zoospores

suggest a more probable association between thraustochytrids and senescent or dead phytoplankton, rather than living cells.

At present, thraustochytrid species categorization based on morphological and life history distinctiveness seems to be only partially supported by the molecular data dealing with 18S rDNA (Honda et al. 1999, Leander & Porter 2001). Therefore, the classical taxonomy used in the present investigation, as the primary criterion of species status, may have to be reconsidered. However, we carried out a comparative analysis of thraustochytrid colonization in different habitats with respect to the adaptability of some morphological and life cycle characteristics.

Marked differences in group species dominance and community structure were detected between the different sandy habitats. The sandy sea bottom harbored a richer species assemblage with respect to the surf area (cf. Fig. 4). The sandy bottom was mostly colonized by *Thraustochytrium* species, characterized by one or more proliferation bodies (Group 1). These are parts of the sporangia that do not cleave into zoospores and remain *in situ* after sporulation, providing an advanced stage in the development of a new vegetative cell. Our findings support Raghukumar's (1996) idea that such structures may provide higher proliferation capacity to thraustochytrids. This could be an advantage when nutritive and environmentally persistent substrates are available. On the other hand, the sandy surf area was mainly colonized by species of the genus *Schizochytrium* (Group 3) characterized by clusters of sporangia. This genus has been usually reported to colonize sandy environments characterized by low levels of organic matter and high ionic content (Gaertner 1968b, Booth 1971). Our findings confirm the ability of the *Schizochytrium* species to adapt and proliferate in environments, where the physical, chemical and biological conditions are limiting for other species. We can thereby surmise that the occurrence of clusters rather than singles sporangia in the *Schizochytrium* genus, could benefit a higher capacity of exploitation of substrates characterized by low organic matter content. On the other hand, the considerable mass of zoospores released by these types of sporangial clusters, could facilitate species dispersion when frequent and drastic fluctuations of the chemical and physical conditions occur.

LITERATURE CITED

Anonymous (1981) Standards methods for the examination of water and wastewater. American Public Health Association, Washington, DC

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*.* Ver Inst Meeresforsch Bremerh 17:245–268

Bahnweg G, Sparrow FK Jr (1974) Occurrence, distribution and kinds of zoosporic fungi in subantartic and Antarctic waters. Veroeff Inst Meeresforsch Bremerhav Suppl 5: 149–157

Booth T (1971) Distribution of certain soil inhabiting chytrid and chytridiaceous species related to some physical and chemical factors. Can J Bot 49:1743–1755

Børsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Prog Ser 36:171–175

Bremer GB, Talbot G (1995) Cellulolytic enzyme activity in the marine protist *Schizochytrium aggregatum*. Bot Mar 38:37–41

Brown AC, McLachlan A (1990) Ecology of sandy shore. Elsevier Science Publishers, Amsterdam

Buchanan JB (1984) Sediment analysis. In: Holme NA, McIntyre AD (eds) Methods for the study of marine benthos. IPH Handbook 16. Blackwell Scientific Publications, London, p 41–65

Cavalier Smith T, Alsopp M, Chao EE (1994) Thraustochytrids are chromist not fungi: signature sequences of heterokonta. Phil Trans R Soc Lond B 346:387–397

Fukuda R, Ogawa H, Nagata T, Koike I (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Appl Environ Microbiol 64:3352–3358

Gaertner A (1968a) Eine Methode des quantitativen Nachweises niederer mit Pollen koederbarer Pilze im Meerwasser und im Sediment. Veroeff Inst Meeresforsch Bremerhav Suppl 3:75–92

Gaertner A (1968b) Niedere mit Pollen köderbare marine Pilze diesseits und jenseits des isländ. Färöer Rückens im Oberflächenwasser und im Sediment. Veroeff Inst Meeresforsch Bremerhav 11:65–82

Gaertner A (1969) Marine niedere Pilze in Nordsee und Nordatlantik. Ber Dtsch Bot Ges 82:287–306

Gaertner A (1972) Characters used in the classification of thraustochytriaceous fungi. Veroeff Inst Meeresforsch Bremerhav 13:183–194

Gaertner A (1982) Lower marine fungi from the Northwest African upwelling areas and from the Atlantic of Portugal. Meteor Forsch Ergebn D 34:9–30

Gaertner A, Raghukumar S (1980) Ecology of the thraustochytrids (lower marine fungi) in the Falden Ground and other parts of the North Sea I. Meteor Forsch Ergebn A 22: 165–185

Genchi G (1990) Metodi nell'ecologia del plancton marino: azoto e fosforo totali. Nova Thalassia 11:171–179

Giere O (1993) Meiobenthology. Springer-Verlag, Berlin

Honda D, Yokochi T, Nakahara T, Raghukumar S, Nakagiri A, Schaumann K, Higashihara T (1999) Molecular phylogeny of thraustochytrids based on the sequencing of 18S ribosomal RNA gene. J Eukaryot Microbiol 46:637–647

IRSA-CNR (1984) Metodi di analisi per acque di mare. Quaderni dell'Istituto di Ricerca sulle Acque 59:301

Jones EBG, Harrison JL (1976) Physiology of marine phycomycetes. In: Jones EBG (ed) Recent advances in aquatic mycology. Elek Science, London, p 261–276

Kimura H, Fukuba T, Naganuma T (1999) Biomass of thraustochytrid protoctists in coastal water. Mar Ecol Prog Ser 189:27–33

Leander CA, Porter D (2001) The Labyrinthulomycota is comprised of three distinct lineages. Mycologia 93:459–464

Naganuma T, Takasugi H, Kimura H (1998) Abundance of thraustochytrids in coastal plankton. Mar Ecol Prog Ser 162:105–110

Pelegrí SP, Dolan J, Rassoulzadegan F (1999) Use of high temperature catalytic oxidation (HTCO) to measure carbon content of microorganisms. Aquat Microb Ecol 16: 273–280

Plante-Cuny MR (1974) Evaluation par spectrofotometrie des teneurs en chlorophylle-a fonctionelle et en phaepigments des substrats meubbles marins. ORSTOM, Nosy-Bè

Porter D (1990) Labyrinthulomycota. In: Margulis L, Corliss JO, Melkonian M, Chapman D (eds) Handbook of Protoctista. Jones and Bartlett, Boston, p 388–398

Raghukumar S (1987a) Occurrence of the thraustochytrid, *Corallochytrium limacisporium* gen. et sp. nov. in the coral reef lagoons of the Lakshadweep Island in the Arabian Sea. Bot Mar 30:83–89

Raghukumar S (1987b) A device for continuous microscopic observation of aquatic microorganisms. Indian J Mar Sci 16:132–133

Raghukumar S (1992) Bacterivory: a novel dual role for thraustochytrid fungi in the sea. Mar Biol 113:165–169

Raghukumar S (1996) Morphology, taxonomy, and ecology of thraustochytrids and labyrinthulids, the marine counterparts of zoosporic fungi. In: Dayal R (ed) Advances in zoosporic fungi. Publications PVT LTD, New Delhi, p 35–60

Raghukumar S, Gaertner A (1980) Ecology of the thraustochytrids (lower marine fungi) in the Falden Ground and other parts of the North Sea II. Veroeff Inst Meeresforsch Bremerhav 18:289–308

Raghukumar S, Schaumann K (1993) An epifluorescence microscopy method for direct detection and enumeration of the fungi-like marine protists, the traustochytrids. Limnol Oceanogr 38:182–187

Raghukumar S, Raghukumar C, Rajendran A (1990) Abundance of thraustochytrid fungi in the Arabian Sea. Estuar Coast Shelf Sci 31:351–358

Raghukumar S, Ramaiah N, Raghukumar C (2001) Dynamics of thraustochytrid protists in the water column of the Arabian Sea. Aquat Microb Ecol 24:175–186

Ribera d'Alcalà M, Catalano G, Saggiamo V (1990a) Metodi nell'ecologia del plancton marino: Nitriti. Nova Thalassia 11:133–138

Ribera d'Alcalà M, Catalano G, Saggiamo V (1990b) Metodi nell'ecologia del plancton marino: Nitrati. Nova Thalassia 11:139–150

Saggiomo V, Catalano G, Ribera d'Alcalà M (1990) Metodi nell'ecologia del plancton marino: Ortofosfati. Nova Thalassia 11:123–132

Santangelo G, Lucchesi P (1995) Spatial distribution pattern of ciliated protozoa in a Mediterranean interstitial environment. Aquat Microb Ecol 9:47–54

Santangelo G, Bongiorni L, Pignataro L (2000) Abundance of traustochytrids and ciliated protozoans in a Mediterranean sandy shore determined by an improved, direct method. Aquat Microb Ecol 23:55–61

SAS (1999) SAS OnlineDoc®, Version 8. Cary, NC

Schneider J (1968) Über niedere Phycomyceten der westlichen Ostsee. Veroeff Inst Meeresforsch Bremerhav Suppl 3:93–102

Siegel S (1956) Non parametric statistics for the behavioral sciences. McGraw-Hill, New York

Ulken A (1966) Untersuchungen über marine Pilze im äquatorialen Atlantik vor der Küste Brasiliens. Veroeff Inst Meeresforsch Bremerhav 10:107–116