Distribution and abundance of thraustochytrids in different Mediterranean coastal habitats

Lucia Bongiorni*, Fernando Dini

Dipartimento 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 ± 5.3 × 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 ± 1 × 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 Het-erokonta, 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...
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 $a$ ($chla$) and organic matter content in the sediment were assessed and related to thraustochytrid densities.

**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 & Schumann 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).

Quantitative 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, Saggiono 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. Conversely, 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 ± 4 × 10^4 cells l^-1 sediment (n = 48) and 6.1 ± 5.3 × 10^4 cells l^-1 sediment (n = 48), respectively, whereas 1.3 ± 1 × 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>
<thead>
<tr>
<th>Habitat</th>
<th>Thraustochytrids (× 10^4 cells l^-1)</th>
<th>Porosity (w/w %)</th>
<th>Total organic matter (w/w %)</th>
<th>Chl a (µg l^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA</td>
<td>4.4 (±4)^a</td>
<td>19.80 (±1.25)^a</td>
<td>0.92 (±0.31)^a</td>
<td>0.02 (±0.05)^a</td>
</tr>
<tr>
<td>SSB</td>
<td>6.1 (±5.3)^b</td>
<td>23.84 (±2.13)^b</td>
<td>1.73 (±0.59)^b</td>
<td>0.83 (±0.7)^b</td>
</tr>
</tbody>
</table>

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.
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 ($1.3 \times 10^2$ cells l⁻¹). Peaks of thraustochytrids occurred in the water column during the autumn months: October 1997 at the Tirrenia station, $3 \times 10^2$ cells l⁻¹, and November 1997 at the Arno and Serchio River stations, $4.5 \times 10^2$ 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-NH₄), nitrite (N-NO₂), nitrate (N-NO₃), total nitrogen (N), phosphate (P-PO₄) and total phosphorus (P), expressed as µM l⁻¹. For each variable $n = 48$

<table>
<thead>
<tr>
<th>Backward elimination</th>
<th>Partial $R^2$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 0</td>
<td>N-NH₄</td>
<td>2.19</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>N-NO₂</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>N-NO₃</td>
<td>3.36</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.01</td>
<td>0.916</td>
</tr>
<tr>
<td></td>
<td>P-PO₄</td>
<td>2.33</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.52</td>
<td>0.224</td>
</tr>
<tr>
<td>Step 1</td>
<td>N-NH₄</td>
<td>2.28</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td>N-NO₃</td>
<td>3.65</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>N⁺</td>
<td>0.003</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>P-PO₄</td>
<td>2.38</td>
<td>0.130</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.66</td>
<td>0.205</td>
</tr>
<tr>
<td>Step 2</td>
<td>N-NH₄</td>
<td>0.043</td>
<td>2.32</td>
</tr>
<tr>
<td></td>
<td>N-NO₃</td>
<td>3.84</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>P-PO₄</td>
<td>2.43</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2.39</td>
<td>0.129</td>
</tr>
<tr>
<td>Step 3</td>
<td>N-NO₂</td>
<td>0.055</td>
<td>2.90</td>
</tr>
<tr>
<td></td>
<td>P-PO₄</td>
<td>3.30</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>4.51</td>
<td>0.039</td>
</tr>
<tr>
<td>Step 4</td>
<td>P-PO₄</td>
<td>0.031</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>5.67</td>
<td>0.021</td>
</tr>
<tr>
<td>Step 5</td>
<td>P</td>
<td>4.10</td>
<td>0.048</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Forward selection</th>
<th>Partial $R^2$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>P</td>
<td>0.082</td>
<td>4.10</td>
</tr>
</tbody>
</table>

*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 (63%) 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 $6.5 \times 10^{-5}$ 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 $8.3 \times 10^{-2}$, $4.7 \times 10^{4}$ and $3.6 \times 10^{-1}$ times that of the above organisms, respectively, suggesting an important 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, Børshsem & 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 $20 \times 10^4$ cells l$^{-1}$) and exceed those detected in North Sea bottom sediment ($1.8$ to $7.3 \times 10^4$ 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

10–1 times that of the above $\times 10^4$ cells l$^{-1}$), and exceed those detected in North Sea bottom sediment ($1.8$ to $7.3 \times 10^4$ 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.
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 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 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 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.
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.

**Acknowledgements.** The authors are grateful to the managers, particularly to V. Giaconi, collaborators and technicians of the ARPAT (Azienda Regionale per la Protezione Ambientale della Toscana), for the technical assistance with the fieldwork and the nutrients analysis. The ‘Ente Parco Regionale di Migliarino-San Rossore-Massaciuccoli’, Tenuta di San Rossore, Pisa, kindly offered the opportunity to sample in the park area. We wish to thank A. M. Ulken and V. Cuomo for initial guidance on thraustochytrid isolation and identification, and S. Raghukumar for his helpful suggestions and guidance on thraustochytrid taxonomy. Thanks to F. Kopel for help with the SAS analysis, D. Nyberg and G. Santangelo for their suggestions on the manuscript, and F. Erra for the figures. The doctoral programme of L.B. was supported by ENEA (Ente per le Nuove tecnologie, l’Energia e l’Ambiente, Roma) and F.D. had an EU, Regione Toscana, INTERREG III Toscana-Corsica-Sardegna grant.

**LITERATURE CITED**


Instituti di Ricerca sulle Acque 59:301


Submitted: July 16, 2001; Accepted: July 18, 2002

Proofs received from author(s): October 7, 2002

Editorial responsibility: David Caron, Los Angeles, California, USA