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Thraustochytrids (fungoid protists): an unexplored component of marine sediment microbiota*

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SUMMARY: Thraustochytrids are poorly known fungoid protists able to decompose refractory organic substrates such as cellulose. These microorganisms probably play an important role in the microbial loop of marine sediments. This paper reports a study conducted on thraustochytrids dwelling in a sandy shore of the Eastern Ligurian Sea. One hundred twenty eight samples (1 ml each) were collected, to examine Thraustochytrid spatial distribution, following a nested ANOVA design, that incorporated 3 successively smaller spatial scales (different sampling units ranging from metres to centimetres). Samples were examined by a direct, improved, fluorochrome-count method also suitable for ciliates. Sampling was repeated 4 times in autumn-winter. The average abundance of thraustochytrids (±SE) was 42.3±2.9 ml^{-1}, with no significant decrease over time. The densities of thraustochytrids and ciliates varied significantly but at different spatial scales: the former between squares (3.3 x 3.3 m), and the latter between subplots (0.01 x 0.01m), indicating their tendency to form patches at different spatial scales. A significant correlation was found between the densities of the two protists, suggesting some interaction between them could occur. The thraustochytrid abundance supports the idea that these protists play a a important role in carbon cycling of Mediterranean sandy shores.

Key words: Thraustochytrids, Ciliates, Spatial distribution, Sandy shores, NW Mediterranean.

RESUMEN: Los Trustoquítridos (protistas fungoides), un componente inexplorado de la microbiota de los sedimentos marinos. Los Traustoquítridos son protistas fungoides poco conocidos, capaces de descomponer sustratos orgánicos refractarios como la celulosa. Dichos microorganismos desempeñan probablemente un importante papel en el bucle microbiano de los sedimentos marinos. Este trabajo informa de un estudio realizado sobre traustoquítridos de una playa arenosa del mar Ligur oriental. Se recolectaron 128 muestras de 1 ml, con el fin de estudiar su distribución espacial, siguiendo un diseño ANOVA, que incluía 3 escalas espaciales de tamaño decreciente (unidades de muestreo diferentes, que iban de metros a centímetros). Las muestras se examinaron mediante un método directo y mejorado de recuento mediante fluorocromo, que también es adecuado para ciliados. El muestreo se repitió 4 veces en otoño-invierno. La abundancia media de traustoquítridos y ciliados varió de manera significativa pero a escalas espaciales distintas: aquellos entre cuadrados (3.3 x 3.3 m), y éstos entre submuestras (0.01 x 0.01m), lo que indica su tendencia a formar manchas a distintas escalas espaciales. Se encontró una correlación significativa entre las densidades de los dos tipos de protistas, lo que sugiere que debe existir alguna interacción entre ellos. La abundancia de traustoquítridos refuerza la idea de que estos protistas desempeñan un importante papel en el ciclado del carbono de las playas arenosas del Mediterráneo.

Palabras clave: Traustoquítridos, Ciliados, distribución espacial, playas arenosas. Mediterráneo noroccidental.

INTRODUCTION

Plant detritus, phytoplankton and faecal pellets are the main sources of organic matter in marine

coastal and estuarine sandy environments. Both dissolved and particulate organic matter flushes into the sand where it is mineralised, recycled and exported to other marine systems by the interstitial microcommunity (Brown and McLachlan, 1990).

Marine fungoid protists living in sediments are

thought to play an important role in the degradation and mineralisation of organic matter. Among these the Thraustochytrids (Phylum Heterokonta, Kingdom Chromista, Cavalier-Smith *et al.,* 1994), are ubiquitous and abundant in marine environments. These microorganisms, which are able to metabolise bacteria-resistant organic macromolecules, proliferate in the later stages of the degradation process. By penetrating organic particles through an ectoplasmic net and injecting their degradative enzymes, Thraustochytrids are able to break down several complex organic substrates such as cellulose cell walls, thereby absorbing the nutriments they need (Bremer and Talbot, 1995; Raghukumar *et al.*, 1995; Raghukumar and Raghukumar, 1999; Raghukumar, 2002).

The recent findings in coastal waters and sediments of quite high thraustochytrid densities and biomass, and a C/N ratio three times higher than that of bacteria (Kimura *et al.*, 1999) have led to the suggestion that these organisms play a far more important role in promoting carbon turnover than previously supposed (Naganuma *et al.,* 1998; Kimura *et al.,* 1999, 2001; Santangelo *et al.*, 2000). In addition, thraustochytrids have been found to produce high levels of polyunsaturated fatty acids (PUFAs; Nakahara *et al.*, 1996; Bowles *et al.,* 1999), which are essential dietary components for marine animals. Marine animals are unable to synthesize the required amounts of PUFAs and must obtain them through their food chain. Although some bacteria and protozoa do produce polyunsaturated fatty acids (De Long and Yayanos, 1986; Yzawa *et al.*, 1988; Zhukova and Kharlamenko, 1999), they are known to be produced mainly by photosynthetic organisms (Zhukova and Kharlamenko, 1999). Thus, Thraustochytrids may represent one of the main sources of PUFAs in the microbial loop (Kimura *et al.*, 1999).

Despite the seeming importance of such a role, to date, few studies have reported on thraustochytrid abundance in marine sandy sediments. This lack of knowledge is particularly acute for the Mediterranean Sea. In this paper we report data on thraustochytrid distribution in a Western Mediterranean coastal area. In particular we looked at the spatial distribution of both thraustochytrids and ciliates following a nested ANOVA sampling design incorporating three different spatial scales (from metres to centimetres). The exploration of different spatial scales in a sampling design, seldom applied to protists, could help to shed light on their spatial distribution. This is especially true when the organisms in question tend to patch. In addition, the analysis of spatial scales is an important step towards understanding the ecological processes controlling population dynamics (Underwood and Chapman, 1996; Olobarria and Chapman, 2001).

MATERIALS AND METHODS

Sampling was carried out during the 1998-1999 autumn-winter in Eastern Ligurian Sea (NW Mediterranean) sandy shores. The sampling area, into which three different rivers flow (Fig. 1), is located in northern Tuscany (43°40'N, 10°13'E - 43°50'N, 10°11'E). The sampling was based on a nested ANOVA design by which different spatial scales were included (Underwood, 1997). Samples were processed by an enhanced direct-count, fluorochrome staining method (Santangelo *et al.*, 2000). This method allowed us to quickly process a large number of replicates, while simultaneously enumerating both thraustochytrids and ciliates from the same sample. We were thus able to investigate whether any associations existed between these two important components of the interstitial microfauna. Protists were fixed directly in sandy samples with glutaraldehyde, then separated from the sediment by
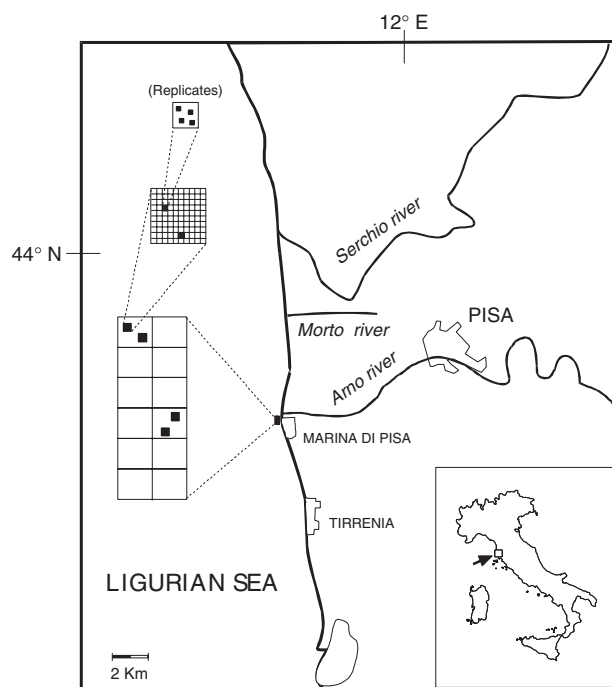

FIG. 1. – Map of the studied area, showing the sampling site (black rectangle) and the nested arrangement of the sampling units.

TABLE 1. – Multifactorial nested ANOVA calculated for thraustochytrid densities. Nested factors: Square (S), Plot (P), Subplot; orthogonal random factor: Time (T) with 4 levels. Factor Square was significant.

Source of variation	df	SS	F	p	F vs	variance components	% variance components
Time	3	76.9245	1.78	0.2903	Square (T)	1.052	9.108
Square (T)	4	43.2578	5.73	**0.0178**	Plot[S(T)]	2.23	19.31
Plot [T(S)]	8	7.5547	0.90	0.5407	Subplot {T[S(P)]}	0.108	0.935
Subplot {T[S(P)]}	16	8.4141	1.04	0.4213	Residual	0.085	0.736
Residual	96	8.0755				8.075	69.91
Total	127						
Cochran Test		Not sig.					
Transformation		None					

TABLE 2. – Multifactorial nested ANOVA calculated for ciliate densities. Nested factors: Square (S), Plot (P), Subplot; orthogonal random factor: Time (T) with 4 levels. Factors Time and Subplot were significant.

Source of variation	Df	SS	F	p	F vs	variance components	% variance components
Time	3	105.7292	13.19	**0.0153**	Square (T)	3.053	35.46
Square (T)	4	8.0156	1.70	0.2414	Plot[S(T)]	0.207	2.40
Plot [T(S)]	8	4.7031	0.52	0.8227	Subplot {T[S(P)]}	0.537	6.24
Subplot {T[S(P)]}	16	9.0000	2.63	**0.0019**	Residual	1.396	16.21
Residual	96	3.4167				3.416	39.68
Total	127						
Cochran Test		Not sig.					
Transformation		None					

centrifugation on silica-gel gradient (percoll), concentrated on black polycarbonate filters and stained with three different fluorochromes: acriflavin for thraustochytrids, DAPI and FITC for ciliates (Santangelo *et al.*, 2000).

The ANOVA sampling design was carried out as follows: a rectangular 19.6 x 6.6 m shallow sandy area at Marina di Pisa, south of the Arno river mouth, was divided into twelve 3.3 x 3.3 squares. Two squares were selected randomly from within each area; each square yielded two 1 x 1 m random plots, and each plot, two 0.1 x 0.1 m subplots, and finally four 1 ml - replicates were collected from each subplot (Fig.1). Three successively smaller spatial scales (square, plot, subplot) were thus explored. Sampling was repeated at 4 different, randomly selected times during the autumn-winter period. Overall, 128, 1 ml replicated samples (corresponding to 2.175±0.09 g) were collected.

Total organic matter and porosity of the sand were measured in two 20-ml replicated samples collected from each square during each sampling session, for a total of 16 samples. Porosity, expressed as weight % of sand water content, was measured as difference between weight of wet and dry sand samples (110°C for 24 hrs; Buchanan, 1984). Weight lost % of dry samples by combustion (540°C for 2 hrs) provided the TOM, total organic matter content (Giere, 1993). The biomasses of both protists were determined by applying the previously estimated volume/biomass ratios as conversion factors (Kimura *et al.*, 1999; Putt and Stoecker, 1989).

RESULTS

The average densities found were 42.3 ± 2.9 thraustochytrids ml^{-1} and 41.5 ± 2.3 ciliates ml^{-1} (±SE), corresponding to an average biomass of 6.930 and 130,000 pgC ml^{-1} respectively.

Multifactorial ANOVAs revealed some differences between the trends in thraustochytrid and ciliate abundances over time (Tables 1, 2; Fig. 2 A, B). Time was not a significant factor for thraustochytrids, showing constant low densities during the autumn-winter period. Time was instead significant for ciliates, whose density decreased remarkably from autumn to winter.

Thraustochytrids varied significantly at the square level (between different 3.3 m^2 areas). Moreover, as the highest component of the variance was residual (69.9%), the higher variability occurred between different replicates (1 ml-samples). Ciliate density instead varied significantly at the subplot
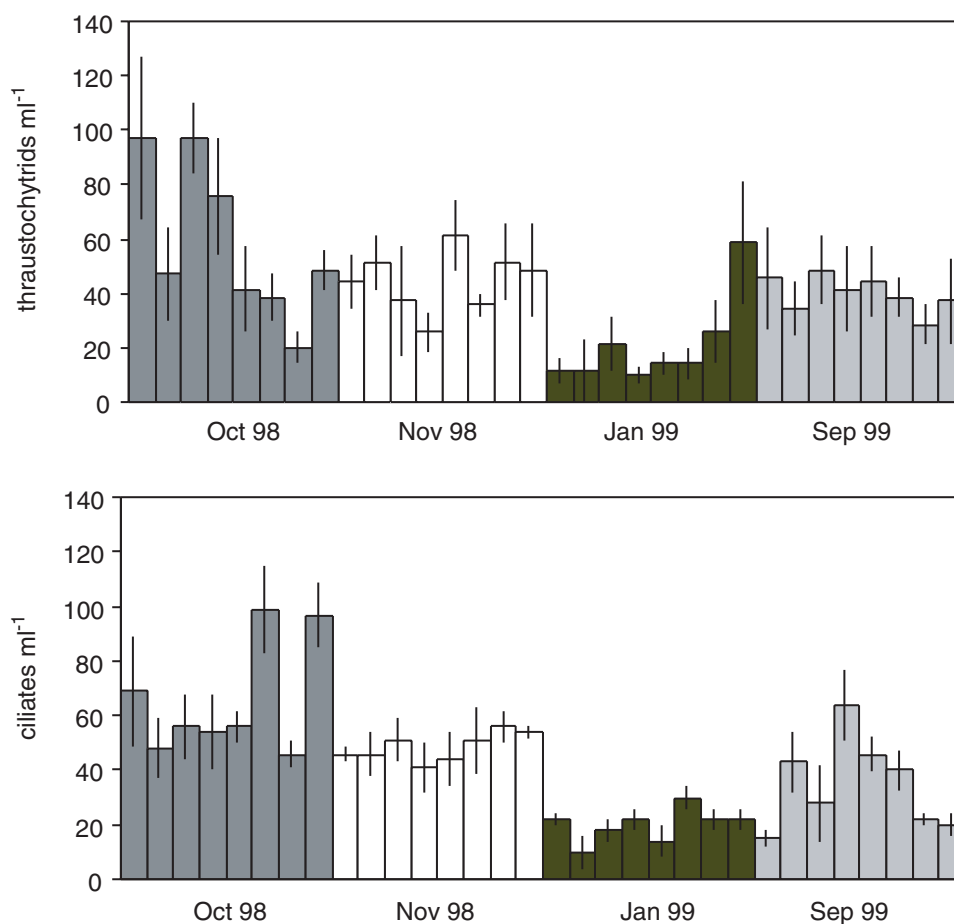
Fig. 2. – Thraustochytrid (A) and ciliate (B) densities (mean ± SE) in the four autumn-winter samplings (4 subplots, 4 replicates each).
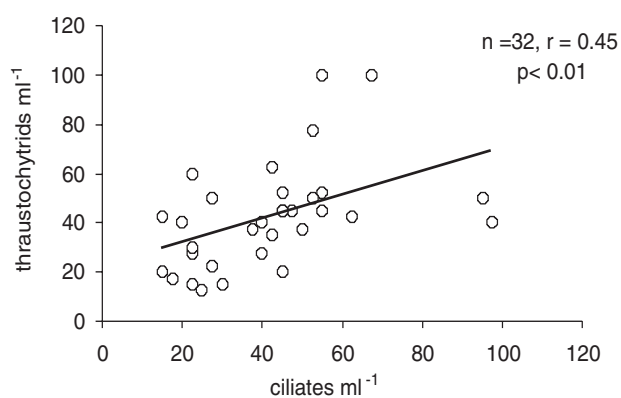

Fig. 3. – Relationship between the abundances of ciliates and thraustochytrids, y= 0.48 x + 22.

level (between 10 cm² areas), while residual variance was lower (39.68%). A highly significant linear correlation was found between thraustochytrid and ciliate abundances (r = 0.456, p <0.01, n = 32, Fig. 3).

TOM (total organic matter) in the sediment ranged between 0.72-1.17% of samples dry weight (g/g). The nested ANOVA revealed significant variability in this parameter at the square level (3.3 x 3.3 m), the same level at which thraustochytrids showed their maximum variability (Table 3). Although no correlation was revealed between TOM and thraustochytrid or ciliate densities, a positive linear correlation was found between TOM and total protists (thraustochytrids + ciliates) abundance (r=0.49, p=0.05, n=16). Porosity ranged between 19.2-21.3%. No significant correlation was found between this parameter and the densities of either protists, probably due to the narrow range of porosity values found in the sampled area.

DISCUSSION

The thraustochitrid densities reported in this study indicate these organisms are relatively abundant in the Mediterranean sandy shore examined.

TABLE 3. – Multifactorial ANOVA calculated for total organic matter in sand. Nested factors: Square; orthogonal random factor: Time (T) with 3 levels. Factor Square was significant at p< 0.05.

Source of variation	Df	SS	F	p	F vs	variance components	% variance components
Time	3	0.0271	1.03	0.4671	Square (T)	0.0002	1.31
Square (T)	4	0.0262	6.65	**0.0117**	Residual	0.0111	73.03
Residual	8	0.0039				0.0039	25.66
Total	15						
Cochran Test		Not sig.					
Transformation		None					

Although thraustochytrid biomass was about 20 times less than that of ciliates, it was still several orders of magnitude higher than that estimated for flagellates and bacteria previously found in the same area (Lucchesi and Santangelo, 1995). These findings agree with those obtained by a different, indirect, enumeration method based on pine pollen cultures by Bongiorni and Dini (2002). These authors found similar, clear-cut seasonal trends, with low, stable thraustochytrid densities during the autumn-winter period. All these results, obtained employing different counting methods, strengthen the idea that temperature could be one of the factors promoting thraustochytrid proliferation at these latitudes. Contrary to thraustochytrids, ciliates showed a significant negative trend over the autumn-winter period, confirming previous findings (Santangelo and Lucchesi, 1995).

Spatial analyses based on nested ANOVA sampling designs, like that in this study, can provide some clues about the spatial scales at which microorganisms tend to patch. Thraustochytrid density varied significantly between different squares (3.3x3.3 m). Nevertheless, a large part of the variability was due to residuals, i.e. the differences between 1 ml-replicates collected at a few centimetres distance. These results suggest a tendency of these protists to form broad as well as small patches. This finding could be explained as the result of two different factors at work. As the total content of organic matter in sand samples (TOM) varied significantly at the same spatial scale at which thraustochytrid densities varied (i.e. square), TOM could act on thraustochytrids at a relatively larger scale. On the other hand, as asexual reproduction commonly occurs in the life cycle of many thraustochytrids and results in the formation of cell aggregates, it may instead be the factor responsible for thraustochytrid distribution at the smaller spatial scale examined (between 1 ml-replicates).

Ciliate densities varied significantly between sub-plots (10 cm^2) indicating a clear-cut tendency of these protozoa to patch at this scale. Moreover, the low residual variance found indicates limited variability between replicates and suggests that the ciliates did not patch at the smallest scale during the period examined. Although we cannot exclude that patching could occur during the winter at a spatial level below the scales examined, it seems more likely that the increased hydrodynamic disturbances and the low temperatures characterizing the winter kept the ciliates from patching (Santangelo and Lucchesi, 1995).

The significant correlation found between thraustochytrids and ciliates suggests that some interaction between them could occur. As ciliates feed on thraustochytrids under laboratory conditions (Raghukumar and Balasubramian, 1991; Bongiorni, unpublished data), trophic interactions between them could also occur in nature. Moreover, acriflavin does not stain small cells or zoospores lacking the polysaccharide cell wall (Raghukumar and Shaumann, 1993). Since these cells probably represent easier, more digestible prey than mature cells for a wide size-range of ciliates, a bias to this relationship may have been introduced.

The non-negligible thraustochytrid abundance found supports the idea that these protists have a considerable impact on marine sediment carbon cycling, and that they may also represent a valuable source of nutrients and polyunsaturated fatty acids (PUFAs) in the microbial loop of Mediterranean sandy shores.

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