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Deep-sea heterotrophic nanoflagellates of the Eastern Mediterranean Sea: qualitative and quantitative aspects of their pelagic and benthic occurrence

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ABSTRACT: Due to the significant lack of information on the community structure of deep-sea nanoflagellates and other nanofauna components as a potentially important component of deep-sea matter fluxes, the aim of the present study was to check whether there is a specific deep-sea nanofauna (<20 µm) community. Studies were carried out in the deep oligotrophic basins of the Eastern Mediterranean Sea around Greece (1249 to 4260 m depth). Direct microscopic counts and quantitative cultivation techniques were used to estimate the abundance and community structure of heterotrophic flagellates and amoebae from abyssopelagial samples and deep-sea sediments. Euglenids and bodonids were the dominant groups comprising about 2/3 of the abundance and biomass of flagellate nanofauna. Our direct counts clearly demonstrate that attention also has to be paid to species which seldom appear in crude cultures. The records of hemimastigid-like forms were remarkable in this respect. Among the 35 deep-sea nanofauna taxa identified here, 8 never appeared in cultures and 18 taxa were found only in cultures. At least 9 taxa have not been previously recorded from the deep sea. *Meteora sporadica* seems to be the first record of an endemic deep-sea heterotrophic nanoprotist. The variety of functional groups recorded indicates the nanofauna as part of a complex microbial food web in the deep sea. Estimates of potential maximum growth rates of fresh isolates from deep-sea waters ranged from 1 to 6.3 d^{-1} (*Ancyromonas*, *Amastigomonas*, *Bodo*, *Caecitellus*, *Cafeteria*, *Kiitoksia*, *Massisteria*, *Percolomonas*). There was considerable clonal variability.

KEY WORDS: Protozoa · Biodiversity · Flagellates · Nanofauna · Amoebae · Growth rate · Deep-sea benthos · Deep-sea plankton

INTRODUCTION

The ability of heterotrophic nanofauna (especially flagellates) to control bacterial production by grazing, and their integral role in the fast recycling of limiting nutrients have led to the conclusion that heterotrophic flagellates are a crucial component of oceanic matter fluxes (Azam et al. 1983, Reid et al. 1991). Quantitative studies of heterotrophic flagellates during recent years in the upper layers of the water column have supported this opinion for many different parts of the world ocean (e.g. Sanders et al. 1992, Gasol & Vaqué 1993). However, the studies on heterotrophic flagellates have been concentrated nearly exclusively on the productive surface waters. Recent studies have shown that significant parts of the production of organic matter in the surface waters can reach the deep-sea floor at depths below 1000 m (e.g.

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Thiel et al. 1990, Gooday & Rathburn 1999). Aggregation due to biological (e.g. excreted polymers, fecal pellets of many different pelagic animals) as well as physical and chemical forces (cf. Alldredge & Silver 1988) may serve as vehicles for an accelerated sinking speed of many small particles, thereby bypassing remineralisation processes on the rapid transport from the surface waters down to the deep sea. These processes cause substantial sedimentation in some regions of the deep sea (see Gooday & Turley 1990, Drazen et al. 1998). In contrast, there is surprisingly little information about the abundance of heterotrophic flagellates in deep waters (e.g. Alongi 1991, Bak & Nieuwland 1997, Danovaro et al. 1998), and except for cultivation studies, there is very little known about the composition of heterotrophic flagellates with resolution down to the level of genera or species in the greatest biotope of the earth. Even from surface waters there are only few studies available (for review see Patterson & Lee 2000). The cultivation of samples from the deep-sea floor and pelagic habitats, respectively, revealed that several species of heterotrophic flagellates (at least cysts) can occur even in very deep parts of the water column (Patterson et al. 1993, Atkins et al. 1998, 2000, Patterson & Lee 2000, Hausmann et al. 2002a).

Qualitative studies from undisturbed communities of the deep-sea floor are still, to our knowledge, lacking. This is mainly due to methodological problems. Epifluorescence counts of fixed and stained bathy- and abyssobenthic samples have recorded drastic decreases of heterotrophic nanoplankton (including flagellates) in accordance with decreases in the concentration of bacteria (Bak & Nieuwland 1997, Danovaro et al. 1998). While some authors did not find flagellates in samples from the deep-sea floor (e.g. Lighthart 1969), others reported substantial densities of heterotrophic flagellates (e.g. Burnett 1977, 1979, 1981, Alongi 1987, 1991), even up to 10^5 cells cm^{-3} (Bak & Nieuwland 1997, Danovaro et al. 1998).

Due to this general, yet significant, lack of information on deep-sea flagellates and nanofauna, which is probably one of the major components of deep-sea matter fluxes, the aim of the present study was to address the following questions: Is there a particular flagellate community in the deep sea? Are deep sea communities the result of sedimentation from the above water column? Are there differences in the nanofauna community between stations with different trophic status? We concentrated our studies on naked nanoflagellates in the Eastern Mediterranean deep sea around Greece. We particularly considered tectic nanoflagellates which generally live in association with marine snow in the water column or with sediment particles in the sediment.

MATERIALS AND METHODS

Study area. The study areas were in the deep oligotrophic basins of the Eastern Mediterranean Sea around Greece (Fig. 1). Sampling was carried out from RV 'Meteor' (Cruise METEOR 40/3, from 28 December 1997 to 18 January 1998, cf. Hieke et al. 1999). Sediment samples were taken from 3 different stations in the Eastern Mediterranean Sea: the Sporades Basin (39° 16.6′ N, 23° 42.5′ E, sampling point 838/97, 1249 m depth; 39° 15.5′ N, 23° 42.5′ E, sampling point 839/97, 1230 m depth; 39° 15.0′ N, 23° 42.5′ E, sampling point 002/98; 1226 m depth), the Ierapetra Basin (34° 26.0′ N, 26° 4.0′ E, sampling point 015/98, 4157 m depth; 34° 25.0′ N, 26° 4.0′ E, sampling point 020/98, 4260 m depth; 34° 24.5′ N, 26° 4.5′ E, sampling point 024/98, 4261 m depth; 34° 24.9′ N, 26° 5.8′ E, sampling point 044/98, 4265 m depth) and the Cretan Sea (35° 49.0′ N, 25° 16.0′ E, sampling point 048/98, 1840 m depth; 35° 50.5′ N, 25° 16.0′ E, sampling point 061/98, 1840 m depth; 35° 51.6′ N, 25° 16.0′ E, sampling point 062/98, 1841 m depth). In addition, plankton samples were taken at these stations.

The water temperature of the deep-sea sediment was 13.0°C (13.2°C in surface waters), 13.8 to 14.0°C (18.0°C in surface waters) and 14.2°C (16.0°C in surface waters) in the Sporades Basin, the Ierapetra Basin and the Cretan Sea, respectively. These extraordinarily high temperatures are typical for the deep sea of the Mediterranean due to the warm climate and the fact that exchange processes with the cold Atlantic deep-sea regions are very much reduced by the high sill at Gibraltar. Salinity in surface waters varied between 37.1 and 39 PSU, and was relatively constant below 500 m depth at 39 PSU (cf. Hieke et al. 1999). Oxygen generally penetrates up to at least 5 cm into the deep-sea sediments of the Mediterranean (Boetius et al.

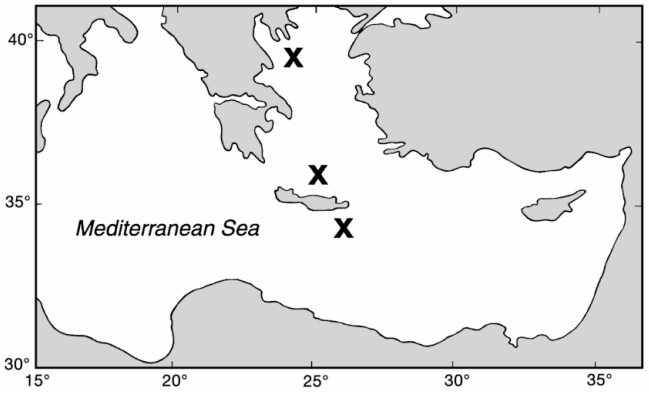

Fig 1. Study area in the Eastern Mediterranean Sea. Sampling stations in the Sporades Basin, the Cretan Sea, and the Ierapetra Basin are indicated by crosses (for coordinates see text)

1996, Hieke et al. 1999). There is a decreasing trophic gradient from the Sporades Basin towards the other 2 stations (Kröncke et al. unpubl.).

Sampling procedures. In principle, we used standard sampling techniques as have been used earlier for protozoological studies of the deep sea (e.g. Patterson et al. 1993, Atkins et al. 1998). Plankton samples were taken from the surface down to 4200 m depth (e.g. at 0, 10, 30, 50, 100, 200, 500, 1000, and 2000 m etc. depending on the water depth at the respective station) by a Neil Brown CTD system (courtesy of C. Begler and the Institut für Meereskunde Kiel). Sampling started at the deepest depth so that the open sampler was washed for several minutes or even hours in depths with lowest concentrations of flagellates. Once on deck, samples were immediately filled into sterile 50 ml tissue culture flasks (Sarstedt) for either cultivation, centrifugation, or direct observation. Abundance estimates were given as mean values for samples from the surface layer (0 to 200 m), from the mesopelagic layer (200 to 1000 m) and from the deep-sea layer (1000 to 4000 m).

Benthos samples were taken by means of a multiple corer system and a box corer. Only cores with undisturbed sediment and overlying water were used for sampling. The top and bottom of corers are closed on sampling, so that the contamination by organisms or cysts from other water depths should be negligible. Once on deck, subsampling of the cores was immediately carried out. After carefully pipetting off the supernatant, water samples were taken from the upper 5 mm from the box cores by means of a sterile syringe or a heat-sterilized sampling spoon. The centre of the cores from the multiple core sampler was subsampled by carefully pushing a sterile cut-off plastic syringe (diameter 13 mm) into the sediment and generating a negative pressure by simultaneously pulling out the stamp. The upper 0.5 cm of the cores, equivalent to a volume of 0.66 cm^3 (2 cm^2), were diluted 1:3 with filtered (<0.2 µm) biotope water of ambient temperature and immediately counted. On one occasion (Sporades Basin, sampling point 838/97), the vertical distribution of protozoans was analyzed in 3 different layers (0 to 1 cm, 3 to 4 cm, 8 to 9 cm) from 9 cm long cores taken by means of sterile cut-off syringes from 3 replicate multicorers.

Since there were only minor changes in the temperatures of deep and surface waters (see above), temperature stress should have been low during lifting of benthos samples. All samplers were lifted at a speed of ca. 1 m s^{-1}.

Care was taken to reduce the possibility of contamination of cultures on board to a minimum. In all cases, sterile material was used (sterile pipettes, sterile isolations etc.) and transfers between cultures were done close to the flame of a burner. All checks for contamination due to handling on board and exposing sterile medium and enrichments revealed negative results.

Quantitative studies. For quantitative estimates of benthic nanoflagellates, 2 methods were used: live-counting (LC) of untreated samples and cultivation of defined aliquots of the diluted sample (liquid aliquot method; LAM). LC was performed directly after sampling in modified microchambers of the Sedgewick-Rafter type (area 10 × 45 mm, height 0.2 mm) filled by a calibrated micropipette (generally 5 µl of diluted sample; for more details see Arndt et al. 2000). Sedgewick-Rafter chambers (1 ml) were used for inspections of plankton samples. Inspections and counting was done using a ZEISS Axioskop 50 (phase contrast Neofluars and Long-Distance-Apochromates); in some cases differential interference contrast was applied. Generally, 1 µl of the sediment was added to the chamber and suspended in several (<20) µl sterile seawater. The chamber was covered by a large cover slip. Per sample, 3 to 4 subsamples were counted. Several times only a few (10 to 20) protists were observed per sample, so that direct counts showed a relatively large scatter of data, although they may serve as a first estimate of deep-sea protist life not affected by the influence of cultivation methods.

The alternative method, quantifying eukaryotic cells in fluorescently stained fixed samples, had to be rejected since no methodological tests of the reliability of this method exists, especially not for deep-sea conditions. All critical comparisons of live and fixed samples of heterotrophic flagellates have found significant discrepancies between parallel counts of fixed and living samples (e.g. Massana & Güde 1991, Sonntag et al. 2000). There are several reasons for a possible overestimation or underestimation of heterotrophic nanofauna using fluorochrome counts of unspecifically fixed samples (see Arndt et al. 2000). Unfortunately, the analysis of fixed samples of heterotrophic nanofauna is generally applied relatively — if not completely — uncritically in nanofauna studies. In many cases it is even hard to clearly differentiate between the different eukaryotic cell types, especially in fixed benthic samples (e.g. Dietrich & Arndt 2000, Arndt et al. 2000). It is also quite impossible to differentiate between flagellate species. The LC technique has its problems as well, since — as in fixed counts — organisms might be disrupted during sampling. However, the revealed counts of flagellates give a minimum abundance estimate of the different taxa that are actually present in trophic forms at the time of sampling. Future methodological studies must solve the problem, e.g. using specific fixations of samples already in the depth of sampling, in addition to staining with specific molecular probes.

To estimate the abundance of seldom occurring protists, deep-sea samples were investigated using LAM (for more details see Butler & Rogerson 1995, Garstecki & Arndt 2000). Two cubic centimetres of the sediment were suspended in 8 ml autoclaved artificial seawater. Aliquots of this sediment suspension were added to sterile culture flasks or wells by means of sterilized automatic pipettes. The volume of the aliquots was adjusted to be small enough (checked in preliminary experiments) for there only to be a minimal chance of inoculing a species twice in the same vessel (cf. Darbyshire et al. 1996). To check for the occurrence of the more seldom protists, 50 or 100 µl of the sediment suspension were added to 5–10 × 50 ml tissue culture flasks which were filled with sterile artificial seawater medium and supplied either by a sterilized wheat grain or by dried and sterilized bakers yeast. To check for the occurrence of more common protists, 10 or 50 µl of the sediment suspension were added to each well of sterile multiwell plates (Sarstedt). Culture flasks and plates were incubated either at 13°C (deep-sea samples) or 20°C (surface samples). Inspections were carried out on board under an inverted microscope ZEISS Axiovert S100 (phase contrast LD-Achromates 20× to 63×) at least twice a week. The number of culture vessels containing a certain species allowed a calculation of the abundance of organisms/cysts according to Darbyshire at al. (1996).

Vertical profiles of the number of planktonic nanofauna appearing in cultures were investigated by means of the LAM as described above. Approximately 20 portions of 30 ml biotope water taken from rosette CTD samplers at each of the different depths were incubated in 50 ml tissue culture flasks immediately after sampling, either at 13°C (samples from below thermocline, >200 m depth) or 20°C (samples from above thermocline), respectively. Each flask was supplied with a sterilized wheat grain. The flasks were inspected by means of an inverted microscope (ZEISS Axiovert 100). Species of each flask were recorded and the values of the different replicates were used to estimate the abundance of organisms/cysts according to Darbyshire et al. (1996). Additional semiquantitative studies were done using direct counts of 60 ml subsamples that were concentrated by centrifugation (3000 rpm [1700 *g*]) and investigated in Sedgewick-Rafter type chambers under a microscope.

Qualitative studies. In addition to the samples and cultures used for the quantitative estimates, ca. 8 cm^3 of the upper 0 to 5 mm layer of the sediment taken by box corers was distributed in portions of ca. 1 cm^3 into each of 6 sterile plastic petri dishes (diameter 9 cm, Greiner). The dishes were filled with ca. 15 ml of biotope water. Sterilized dried bakers yeast was added to 3 of the 6 petri dishes in order to facilitate bacterial growth. Controls (6 petri dishes) were carried out to evaluate the possibility of contaminations from the procedures carried out on board. Sterilized seawater was taken instead of the sample. No contamination was observed in the course of 3 wk. All samples were stored at 20°C. Inspections of the culture dishes were carried out daily (during the first 2 wk on board) or weekly. In addition, rarely occurring planktonic flagellates were recorded at the deepest station (Ierapetra Basin) from 10 sterilized 0.5 l glass bottles to which plankton samples and 5 sterilized wheat grains were added. Subsamples of the glass bottles were investigated in Sedgewick-Rafter chambers under the upright microscope twice a week.

Taxonomic identification. Identifications were made on the basis of live observations. Both the inverted and upright microscopes were equipped with a video camera. For documentation and further analyses, species were documented by S-VHS-video-recording (Panasonic) on board. Problematic species were determined on microscopic slides using a 100× ZEISS-Neofluar (oil) or a water immersion objective 100× ZEISS-Apochromate, both equipped with phase and interference contrast. Often original species descriptions had to be consulted (e.g. Larsen & Patterson 1990, Vørs 1992, Patterson et al. 1993, Patterson & Simpson 1996, Tong et al. 1998, Lee & Patterson 2000). Video documentation helped in the verification of several species by other specialists.

The classification was generally done according to the system provided by Hausmann & Hülsmann (1996) and Patterson (1999). It has to be considered that most of the heterotrophic nanoflagellates species have only been described on the basis of light microscopic studies; only a few species have been studied by electron microscopy. Molecular characterisations are just starting (e.g. Atkins et al. 2000, Lopez-Garcia et al. 2001) and GenBank data are available only for very few species. Therefore, the species list provided in the present contribution should be considered as a 'minimum' list of the species present in the deep-sea samples.

Growth potential estimates. The changes in abundance of several surface-associated flagellate species were estimated from culture vessels (tissue culture flask or multiwell plates) exposed at either ambient temperatures (13°C) or standard temperature used for comparative studies in literature (20°C) on board. Generally 100 to 200 specimens were counted per inspection. Maximum potential growth rates (r_{max}) per day were calculated assuming exponential growth according to the equation:

$$r_{max} = (\ln N_{t_2} - \ln N_{t_1})/(t_2 - t_1)$$

where N is the abundance of organisms at the beginning (t_1) and at the end (t_2) of the interval of observation.

RESULTS

Qualitative investigations

A total of 35 flagellate taxa were identified from the deep sea at ≥1000 m. To our knowledge, 5 species and 4 other taxa have not been previously reported from the deep sea (Table 1). Bodonids, euglenids and protistan groups of uncertain systematic position (Protista *incertae sedis*) contributed the major part of taxa found. Eight taxa identified from the deep sea never appeared in cultures; among these taxa were hemimastigids. Another 18 taxa were found only in cultures, and 8 taxa appeared in both direct counts and cultures.

Table 1 contains a list of flagellate species which occurred in sediment and plankton samples at depths ≤1000 m. Species names are given when all characters of specimens fitted well to the original descriptions or to recent redescriptions (e.g. Larsen & Patterson 1990, Vørs 1992, Tong et al. 1998). It was often impossible to extract specimens of rare species for a more detailed observation on board. Most specimens observed in cultures resembled well described and common species distributed worldwide. Several additional organisms were found which belong to species not yet described. However, since we were not able to cultivate most of these species, we could not describe them accurately. Among these forms were representatives of thaumatomonads, euglenozoans and representatives of Protista *incertae sedis*. One small extraordinary species (diameter ca. 3.5 µm) possessed 2 axial flagella-like cell appendages and 2 characteristic lateral arms which conduct almost continual rowing movements. This species was named *Meteora sporadica* (Hausmann et al. 2002b).

Quantitative investigations

Direct counts

The abundances at the Sporades Basin were highest, ca. 5 times higher than those from the more oligotrophic and deeper stations in the Ierapetra Basin and the Cretan Sea (Table 2). Due to low abundances and the low numbers counted within 1 h of sampling, there was a great scatter of abundance data. Several times it was not possible to register flagellates at a sampling point. Maximum abundances reached only about 3000 flagellates per cm^3 of sediment.

The composition of taxa could only be estimated for the Sporades Basin which contained the highest abundances. Euglenozoans were the prevailing nanobenthos organisms in the Sporades Basin (Fig. 2). In samples of the Cretan Sea, only *Percolomonas* and *Bodo* appeared in direct counts. Cercomonads were the only living flagellates found in direct counts of the Ierapetra Basin. No amoebae could be detected by LC.

Most probable number estimates

Since previous investigations of crude cultures revealed no information on the relative contribution of the different taxa in deep-sea samples, and only a few species appeared in our live counts, attempts were made to apply LAM for deep-sea studies. We cultivated aliquots of the sediment samples from the Sporades Basin. The following species appeared in LAM cultures: *Bodo* sp., *Rhynchomonas nasuta*, *Ancyromonas sigmoides*, cf. *Kiitoksia ystava*, *Massisteria marina*, *Cercomonas* sp., *Percolomonas cosmopolitus*, and *Amastigomonas mutabilis*. Two LAM abundance estimates were available for the Sporades Basin for the 0 to 5 mm surface layer with 124 and 183 flagellates (or their cysts) per cm^3 sediment. The corresponding live counts (550 and 934 flagellates per cm^3, respectively) were about 5 times higher. From flagellates appearing in LAM cultures, only *Bodo*, *Percolomonas*, and *Cercomonas* could be registered in direct counts, indicating the limitation of the cultivation method. The preliminary study of the vertical distribution of flagellates in the sediment of the Sporades Basin revealed 133 (±94) flagellates per cm^3 for the upper sediment layer (0 to 1 cm) and 16.7 (±40.9) flagellates per cm^3 sediment in the 3 to 4 cm layer (n = 6). No flagellates appeared in the 6 cultures of the 8 to 9 cm layer.

Gymnamoebae, which are difficult to estimate by direct counting, were regularly found in cultures and should be considered as typical components of deep-sea nanobenthos. However, abundances were relatively low (at Sporades Basin ca. 1/3 of LAM estimates of abundance for flagellates). LAM cultures revealed 1 vexilliferid-like amoebae and 2 species of fan-shaped (*Vannella*-like) amoebae. Small (diameter 4 µm) and medium sized (diameter 10 to 20 µm) forms were recorded. Naked amoebae were only observed in samples from the surface layer of the sediment, where abundances reached up to 50 amoebae (or their cysts) per cm^3 sediment.

Benthic nanofauna in the pelagial

Except for *Meteora sporadica*, all flagellate taxa which we were able to identify in the deep sea (see Table 1) are known from marine surface waters and littoral sites. Therefore, we would like to evaluate the possibility that benthic nanofauna originates from sed-

Table 1. List of heterotrophic flagellates recorded from different depths in the Aegean Sea (Mediterranean) during December 29 and January 16, 1998. D = species recorded by direct counts of living samples, C = species appearing in cultures (1 atm) from the respective depth. Last column: deep-sea records in literature from depths below 1000 m: 1 = North Atlantic sediment (Patterson et al. 1993), 2 = North Atlantic plankton (Patterson et al. 1993); 3 = Mediterranean sediments (Hausmann et al. 2002a), 4 and 5 = North Atlantic sediments (Turley et al. 1988, Turley & Carstens 1991), 6 = Eastern Pacific Ocean sediments (Atkins et al. 2000)

Species	Deep-sea benthic records			Deep-sea pelagic records	Epi-pelagic records of the same species		Deep-sea records by other authors
	Sporades Basin	Ierapetra Basin	Cretan Sea	Aegean Sea	Aegean Sea	Aegean Sea	World-wide
	1225–1249 m	4160–4260 m	1840 m	1000–3000 m	0–200 m	200–1000 m	
Euglenozoans							
Bodo saltans Ehrenberg 1832	D						2,3
Bodo saliens Larsen & Patterson 1990	D,C			C	C	C	3,6
Bodo designis Skuja 1948	D,C		C		C	C	1,2,3
Bodo curvifilus Griessmann 1913				C			1,2,4
cf. *Pseudocryptobia sorokini* (Zhukov 1975)	C						
Bodo spp.	C		D				1,2,3,5
Rhynchomonas nasuta (Stokes 1888) Klebs 1892	C		C				1,2,3,6
cf. *Heteronema* sp.	D						
Petalomonas pusilla Skuja 1948	C						
Petalomonas minuta Hollande 1942	D						1
Petalomonas sp.	D,C						
cf. *Ploeotia* sp.	D				C		1
Heteroloboseans							
Percolomonas cosmopolitus (Ruinen 1938) Fenchel & Patterson 1986	C		D		C		3
Cryptomonads							
Goniomonas amphinema Larsen & Patterson 1990	C				C		2,3
Stramenopiles							
Paraphysomonas sp.	C				C		2,3,4,6
Cafeteria roenbergensis Fenchel & Patterson 1988	C			C	C	C	1,2
Pseudobodo tremulans Griessmann 1913	C				C	C	1
Caecitellus parvulus (Griessmann 1913) Patterson et al. 1993				C	C		1,2,6
Alveolates (not investigated in detail)							
Pronoctiluca pelagica Fabre-Domergue 1889				D			
cf. *Gyrodinium*	D						3
Thaumatomonads							
Protaspis simplex Vørs 1992	C						
Protaspis sp. 1 (12 µm)	C						
Protaspis sp. 2 (10 µm)		C	C		C		1
Apusomonads							
Amastigomonas mutabilis (Griessmann 1913) Molina & Nerad 1991	C						1,2
Amastigomonas debruynei De Saedeleer 1931					C	C	2
Ancyromonads							
Ancyromonas sigmoides Kent 1980	C						1,2,6
Cercomonads							
Cercomonas spp.	D,C	C			C		3,4,6
Massisteria marina Patterson & Fenchel 1990	C						2,3,6
Kathablepharids							
Leucocryptos marina (Braarud 1935) Butcher 1967	D				D,C		1,3
Choanoflagellates							
Salpingoeca sp.	C				D,C		1
Protista *incertae sedis*							
Allantion tachyploon Sandon 1924	C				C		
cf. *Kiitoksia ystava* Vørs 1992	C		C		C		
Meteora sporadica Hausmann, Weitere, Wolf & Arndt 2002	C						
cf. *Spironema* sp.	D						
cf. *Hemimastix* sp.	D						

Table 2. Mean abundance (ind. cm^{-3}) and biovolume (1000 $\mu m^3/cm^3$) (means ± SD) of heterotrophic flagellates at 3 different stations in the Eastern Mediterranean Sea around Greece. Data are given for the investigation of live samples and biovolume estimates from living material (direct counts) per cm^3 of sediment in the upper 0.5 cm sediment layer

	Abundance		n	Biovolume	
Sporades Basin	870	560	7	70.8	87.6
Ierapetra Basin	24	88	14	0.15	0.56
Cretan Sea	140	240	7	1.87	3.53

imentation through the water column. The abundance of tectic nanofauna occurring in plankton samples was investigated using the LAM technique. Highest abundances were recorded from the Sporades Basin (Table 3). We found that most of the species from the deep-sea sediment also occurred in the water column above (Fig. 3, Table 1). There was no clear trend regarding the vertical distribution of the abundance of tectic nanofauna in the water column. Besides flagellates, the other important component of benthic and pelagic deep-sea nanofauna were gymnamoebae, which increased their relative contribution to tectic nanofauna in cultures from surface waters (15%) towards the deep sea (60%; Fig. 3). Amoebid, vexilliferid and vannellid-like forms seemed to prevail.

Growth potential of deep-sea nanofauna

Little is known about the ecology of several of the isolated flagellate species from the Aegean Sea unknown. Thus, isolates from deep-sea waters (>1000 m depth), as well as from surface waters, were investigated regarding their potential growth rates under presumably unlimited food conditions in organically enriched cultures at 1 atm (Table 4). Experiments were done within the first days after sampling from the deep sea, so that only a few generations had passed and selective isolation of especially adapted organisms should be minimal. Growth rates of flagellates were very high and ranged up to 6.3 d^{-1} for *Percolomonas*. Naked amoebae (not included in Table 4) grew nearly as fast as the flagellates. Growth rates were 1.64 d^{-1} (±0.55, n = 6) , 1.6 d^{-1}, and 1.94 d^{-1} (n = 1) for a vexilliferid (cell dimensions: 25 × 10 × 3 µm), a large (18 × 10 × 2.5 µm) and a small (4 × 4 × 2.5 µm) vannellid-like amoeba, respectively.

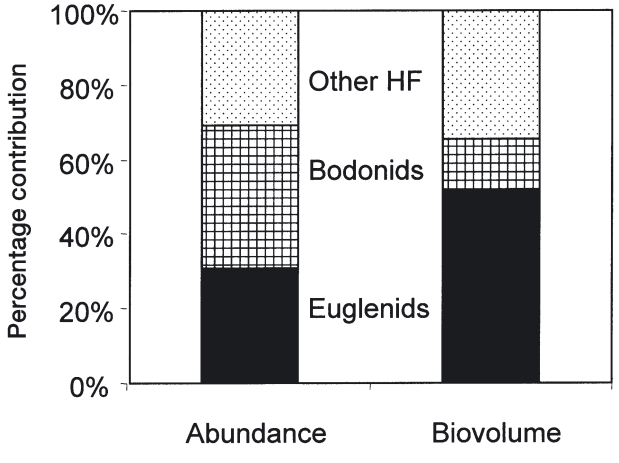

Fig 2. Relative contribution of different taxonomic groups to the abundance and biovolume of the deep-sea benthic flagellate community in Sporades Basin in the upper 0.5 cm sediment layer at a depth of 1225 m (results of live counts). HF: heterotrophic flagellates

DISCUSSION

Qualitative composition of deep-sea nanofauna

There are very few studies on the taxonomic composition of protozoans in the deep sea (e.g. Patterson et al. 1993, Atkins et al. 2000, Hausmann et al. 2002a). The 35 flagellate taxa reported during our present study seems to be the highest number identified in samples from depths ≥1000 m. At least 9 of the 35 taxa have not been previously recorded in the deep sea. Among the 35 taxa identified from depths greater than 1000 m, 8 never appeared in cultures and were only found in direct counts. This demonstrates that special attention has to be paid to species which seldom appear in crude cultures. A total of 18 taxa were found only in cultures. Most of these taxa belong to the most widespread species (Patterson & Lee 2000), suggesting that these species might have a wide range of tolerance with regard to several environmental parameters, such as temperature and pressure (cf. Atkins et al. 1998).

Most of the current estimates of flagellate diversity in the literature rely on cultivations which certainly favour only specific species. Lim et al. (1999) found that the species *Paraphysomonas imperforata* was very common in cultures from marine plankton but appeared only rarely in direct counts from the same sites. Our direct counts immediately after the collection of samples revealed that most organisms disintegrate after only a few minutes. Thus delicate forms and seldom occurring species are even more difficult to consider by cultivation techniques or by the fixation of samples. Several morphotypes observed could not be assigned to any described taxon. Most of these taxa probably belonged to new species. In addition, it has to be considered that our species list may include both

Table 3. Abundances of planktonic flagellates and naked amoebae (ind. l^{-1}) obtained by the liquid aliquote method from CTD samples taken from samples at the 3 stations in the Eastern Mediterranean. Data were summarized for the epipelagic region (0 to 200 m), the mesopelagic region (200 to 1000 m) and for the bathy- and abyssopelagic region (>1000 m). (Range of values; number of samples)

	0–200 m	200–1000 m	1000–4000 m
Flagellates (ind. l^{-1})			
Sporades Basin	97 (0–200; 14)	6.6 (0–53; 11)	14.3 (0–57; 6)
Ierapetra Basin	15 (0–95;19)	14.1 (0–54; 6)	8.0 (0–67; 24)
Cretan Sea	15 (0–50; 20)	21.9 (0–75; 8)	0 (0–0; 12)
Gymnamoebae (ind. l^{-1})			
Sporades Basin	39.2 (0–71; 14)	14.2 (0–45; 11)	13.8 (0–29; 6)
Ierapetra Basin	2.7 (0–36; 19)	0.7 (0–2; 6)	8.1 (0–33; 24)
Cretan Sea	0 (0–0; 20)	0 (0–0; 8)	0 (0–0; 12)

active cells as well as records of cysts. Species recorded in cultures may have originated from population growth either of living cells or cysts hatched in the course of cultivation. Several of these cysts might have sunk down from the surface waters and would never have been active in the deep sea. On the other hand, our experiments showed that 8 out of 26 taxa recorded from cultures also appeared in live counts. This indicates that a considerable part of the most common species (e.g. *Bodo saltans*, *B. designis*, *Petalomonas minuta*, cf. Table 1) which might have hatched from cysts are active members of the deep-sea nanofauna community.

The low abundances, the possible losses of species during the lifting of samples and the limited morphological features available using light microscopy clearly indicate that the list of species presented here is a 'minimum' list.

Quantitative estimates

The nanofauna abundance estimated in our study ranged between 24 and 870 cells per cm^3. These values lay between the extremes reported in literature. While some authors did not find flagellates in samples from the deep-sea floor (e.g. Lighthart 1969), others reported abundances up to 10^5 cells cm^{-3} (Bak & Nieuwland 1997, Danovaro et al. 1998). One problem lies in the methodological difficulties associated with deep-sea studies, as already mentioned in the 'Materials and methods' section. There are problems with both methods: counts of fixed and fluorochrome stained samples (e.g. fixation artefacts, problems with identification of nanofauna), and counts of living samples (e.g. limitations at low abundances and with counting of replicate samples). The reliability of both methods has not been proven for deep-sea conditions. Organisms may disrupt during lifting of the samples from the deep sea, which is a problem of both methods.

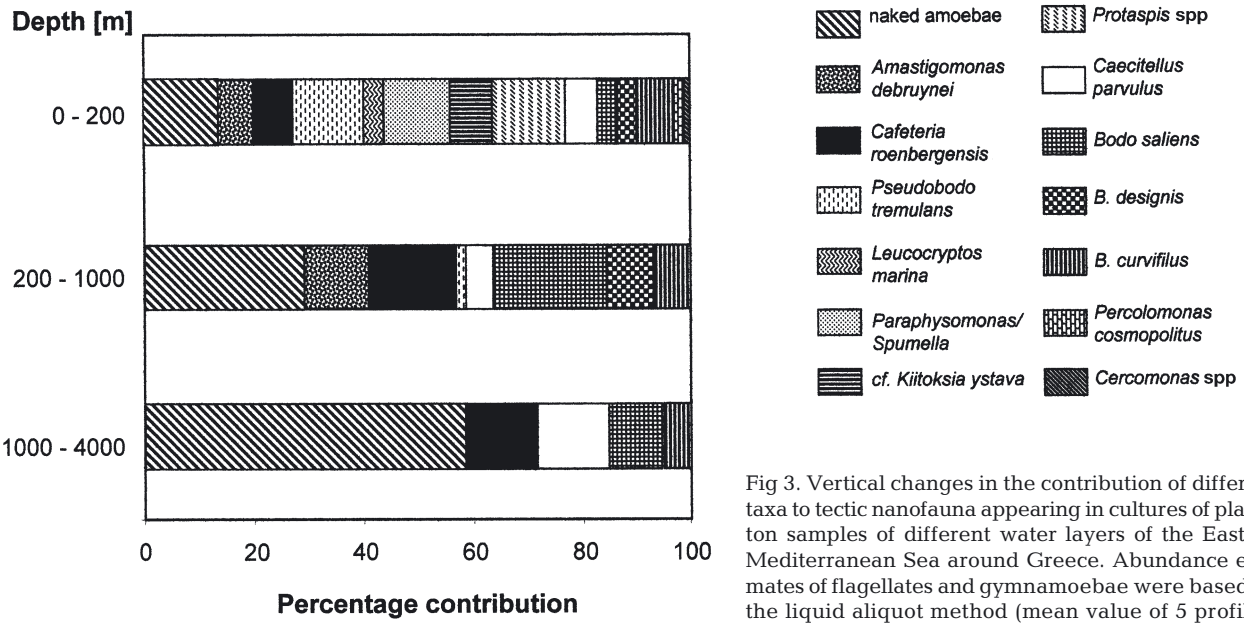

Fig 3. Vertical changes in the contribution of different taxa to tectic nanofauna appearing in cultures of plankton samples of different water layers of the Eastern Mediterranean Sea around Greece. Abundance estimates of flagellates and gymnamoebae were based on the liquid aliquot method (mean value of 5 profiles)

Table 4. Potential growth rates of heterotrophic flagellates isolated from the Eastern Mediterranean Sea during December 29, 1997 and January 16, 1998. Growth rates are separated for those species isolated from either deep sediments (most species) or deep pelagic waters (*Caecitellus*, *Cafeteria*) (the depth of the origin of the isolated organisms is given in brackets behind the species name) and those which were isolated from surface waters (0 to 200 m) but which have also been reported from the deep sea at the same sites. Up to 5 different isolates (A to E) for each species were investigated

	Biovolume (μm^3)	Experimental temperature (°C)	Growth rate estimates (d^{-1}) A	B	C	D	E	Mean	SD	n
Strains isolated from the deep sea										
Rhynchomonas nasuta (1249 m)	31	20	1.17	2.11	1.17	1.23	0.68	1.27	0.52	5
Bodo sp. (1249 m)	35	20	0.97	1.31	2.04	1.05		1.34	0.49	4
Percolomonas cosmopolitus (1249 m)	24	20	2.92	3.53	6.31	3.55		4.08	1.52	4
Ancyromonas sigmoides (1249 m)	31	20	1.21	1.91				1.56		2
Massisteria marina (1249 m)	17	20	3.01	5.58	3.26	5.43	2.14	3.88	1.54	5
Amastigomonas mutabilis (1249 m)	94	20	1.02					1.02		1
cf. *Kiitoksia ystava* (1249 m)	5	20	1.72					1.72		1
Caecitellus parvulus (1500 m)	18	13	2.96					2.96		1
Cafeteria roenbergensis (1500 m, 1000 m)	33	13	2.62	1.37	1.4	1.19		1.65	0.66	4
Strains isolated from surface waters										
cf. *Kiitoksia ystava*	5	13	4.2					4.20		1
Metopion fluens	220	13	1.11					1.11		1
Pseudobodo tremulans	42	13	2.23					2.23		1
Bodo saliens	38	13	2.4					2.40		1
Bodo curvifilus	35	13	1.37					1.37		1
Bodo designis	48	13	2.19	1.52				1.86		2
Protaspis sp.	226	13	1.65	1.49				1.57		2
Amastigomonas debruynei	25	13	2.08	1.18	5.03			2.76	2.01	3
Amastigomonas debruynei	25	20	4.47	5.3	5.32	5.25		5.09	0.41	4

We had to choose live-counts since this is the only method at the moment which allows a taxonomic resolution of benthic nanofauna samples. We consider our direct live counts as minimum estimates.

A comparison of our live counts from the different regions investigated during this study revealed a considerable decrease in the abundance and biovolume of heterotrophic flagellates from the Sporades Basin to the Cretan Sea and the Ierapetra Basin (Table 2). The total organic carbon content of the sediment was ca. 71% in the Sporades Basin and was only ca. 53% at the 2 other stations (Kröncke et al. unpubl.). Despite its depth, the organic content in the Ierapetra Basin was similar to that of the Cretan Sea, a phenomenon that has been described as benthic hot spots in the Mediterranean trenches (Boetius et al. 1996). Nevertheless, there is at least a tendency of lower abundances and biovolumes of nanofauna at the deepest station. This might be explained by relatively high abundances of oweniid polychaetes (*Myriochele fragilis*, Fiege et al. 2000) in the Ierapetra Basin. Polychaetes may have exerted a significant pressure by grazing or competition on nanofauna while effectively consuming detritus particles with its microbial components. The decrease in the abundance of nanofauna with an increase in depth is in accordance with observations by Duineveld et al. (2000) from the Cretan Sea.

The quantitative estimates of nanofauna give at least an initial idea about the relative contribution of taxonomic groups in deep-sea environments. Most taxonomic groups of heterotrophic nanofauna that were found during the present study are typical for sediments in general (for review see Arndt et al. 2000). Euglenids and bodonids formed the highest portion of the heterotrophic flagellate community. The records of hemimastigid-like forms, which have seldom been reported even from surface waters or soils (Foissner & Foissner 1993), were remarkable and deserve further studies to check whether this group is typical for deep-sea environments.

Among the flagellate taxa found in the deep sea, bacterivorous forms dominated by far. However, the occurrence of some large euglenids indicates that potential herbivores among heterotrophic flagellates may also be able to directly utilize sedimented algae. In addition, the occurrence of at least one potential predatory form (*Leucocryptos*) gives evidence for the trophic diversity even among the smallest deep-sea animals (see also species list from the same area from Hausmann et al. 2002a).

Since the abundance of nanofauna in the oligotrophic deep sea was very low, we additionally had to search for enrichment techniques. We tested the applicability of LAM which had been successfully used

for the quantification of naked amoebae (Butler & Rogerson 1995, Smirnov et al. 1998). The results from the Sporades Basin indicated, however, that the abundances calculated from LAM records were about 5 times lower than abundance estimates from live counts. From flagellates appearing in LAM cultures, only *Bodo*, *Percolomonas*, and *Cercomonas* could be recorded in direct counts, indicating the limitation of the cultivation method.

Growth rates of deep-sea nanofauna

The growth rates determined for the deep-sea nanofauna isolates indicate that several species survive the transport to the surface. Growth rates obtained for *Rhynchomonas nasuta* and *Caecitellus parvulus* are close to values reported by Atkins et al. (1998). These authors did not find clear barophilic species. Our own recent experiments indicated that several benthic flagellate species found in the deep sea survived exposure to high pressure (>500 atm) without a reduction of growth rates (Arndt et al. unpubl.). Our present experiments were done within the first days after sampling from the deep sea, so that only a few generations had passed in our experiments and selective isolation of especially adapted organisms should have been minimal. For most of the representatives of the Protista *incertae sedis*, growth rates have not previously been reported in literature, though they should significantly contribute to benthic nanofauna in littoral sediments, too. Some growth rates of heterotrophic flagellates (*Percolomonas*: 6.3 d^{-1}) were among the highest ever reported from literature (e.g. Fenchel 1982, Eccleston-Parry & Leadbeater 1994). *Percolomonas* is an example of a flagellate which was also found in direct counts (1840 m depth). Despite the uncertainty whether the high growth rates observed in our experiments at 1 atm couls also be reached under the deep-sea pressure, the nanofauna should have by far the highest reproductive potential among the deep-sea fauna, being able to respond to short-term food pulses or food patches.

Origin of deep-sea nanoflagellates

The probably endemic forms existing in the deep sea as well as the occurrence of living nanofauna with relatively high abundances point to the existence of a specific deep-sea nanofauna community. On the other hand, sedimenting oceanic detritus may be occupied by a diverse protozoan community (e.g. Caron et al. 1982). In the present study, we found about half of the benthic species also occurring in the water column above (Table 1). The significant abundance of benthic nanofauna in the water column below 1000 m depth (Fig. 3, Table 3) indicated that tectic flagellates probably occurring on sinking detritus may contribute substantially to the benthic deep-sea nanofauna. The abundance of forms known to be aggregate dwellers decreased with depth in accordance with the decrease in organic matter suspended in the water column. The chlorophyll content of CTD samples decreased from 0.05 to 0.3 µg chl l^{-1} in the surface waters (0 to 200 m) to less than 0.01 µg chl l^{-1} in deeper layers during the present investigation (Begler et al. 1999). In addition to flagellates, the other important components of benthic and pelagic deep-sea nanofauna were gymnamoebae, which increased their relative contribution to aggregate dwelling nanofauna in cultures from surface waters to the deep-sea plankton (Fig. 3). Except for a few studies, amoebae have, until now, generally been neglected in plankton studies, though they may be at least occasionally abundant in plankton samples (e.g. Caron & Swanberg 1990, Arndt 1993, Murzov & Caron 1995). This seems to be mainly a methodological problem, since most amoebae are difficult to study in fixed samples.

The transport in the form of cysts might play an important role in order to outlast periods of very low food concentration in the extremely oligotrophic deep-sea regions. Cysts could also play an important role in exchange processes with the upper layers (Della Tommasa et al. 2000). It can be argued that the settlement of water column protists (active cells as well as resting stages) is probably a recruitment strategy for some deep-sea protozoans.

To our knowledge, these are the first quantitative estimates of nanofauna community structure in the deep sea. Though the number and statistic treatment of samples was rather limited, as is usual for deep-sea investigations, the relatively large variety of taxonomic groups that have up to now been reported from studies of the deep sea (Table 1), as well as the fact that several functional groups may occur, indicate that the deep sea nanofauna is a diverse community with several trophic interactions on this level of organism size. Thus nanofauna is expected to contribute to a complex deep-sea food web that probably plays an important role in the stability and resilience of the deep-sea ecosystem. Though small organisms are expected to contribute the major part to deep-sea zoobenthos biomass (Pfannkuche & Soltwedel 1998), the contribution of nanofauna seems to be relatively low. However, the high growth potential might especially enable nanofauna organisms to exploit locally or temporally limited pulses of food supply. Our live counts indicated that cultivation techniques always ignore a significant part of the community.

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