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FEMS Microbiology Ecology 33 (2000) 191–196

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

Spatial distribution of protists in the presence of macroaggregates in a marine system

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Received 9 March 2000; received in revised form 27 June 2000; accepted 30 June 2000

Abstract

The spatial distribution of marine protistan communities in the presence of organic macroaggregates, formed from natural seawater, was studied in several microcosm experiments. The presence of macroaggregates had two main effects. First, the size of the communities of bacteria, flagellates and ciliates increased, as these communities were three orders of magnitude higher in the aggregates than in the microcosm water. In addition, it brought the diversification on the niches accessible to planktonic microorganisms, as three phases were formed: water, aggregates and aggregate–water interphase. Some of the detected protistan taxa were only found in the water, and therefore they can be considered as truly free-swimming protists. Others quickly colonised the aggregates, and finally, some of them showed a preference for the aggregate–water interphase. We discuss this spatial structuring of the protistan community on the basis of their feeding strategies and structural and behavioural characteristics.

Keywords: Flagellate; Ciliate; Macroaggregate; Seawater; Aggregate–water interphase

1. Introduction

Amorphous aggregates of 500 μm or larger, often called 'marine snow', have been widely found in marine waters [1–3]. Since they were first characterised, their importance as microenvironments for very active microbial metabolism is becoming clearer [4]. Bacteria are the most abundant heterotrophic components of these microenvironments, and most research has been focused on the relationship between the aggregate and the colonising bacteria [5–8]. A relatively high abundance of flagellates, ciliates and sarcodines has also been found in these particles [9–12]. However, the importance of the presence of macroaggregates in aquatic marine systems in relation to the community of protists has not been thoroughly studied.

In fact, the study of the protistan communities associated with marine snow has been constrained by the many troubles involved in the collection of natural aggregates, particularly when these are very large. Marine aggregates are usually very fragile and the most commonly used collecting devices, such as sampling bottles [13,14] or pumps [15], as well as the processes involved in the transport and storage of samples, may break them up. Due to the extreme difficulty in studying natural aggregates, several attempts have been made to obtain aggregates in the laboratory, which resemble those that were naturally formed. In order to promote the formation of macroaggregates, seawater is often supplemented with organic material of diverse origin [16,17], being questionable whether or not they represent a general model of marine snow. However, the formation of marine macroaggregates from natural seawater without the addition of allochthonous material has been successfully carried out [18–20] and, moreover, the analysis of the morphological, chemical and biological characteristics have shown that they can be considered as suitable models to study microbial processes [18,20].

The spatial heterogeneity created by the presence of macroaggregates suspended in water should be considered when describing the dynamics of the protistan communities. In the absence of these particles, all protists in the water column share similar conditions regarding physical medium or prey availability. When the aggregates appear, they are quickly colonised and transformed by bacteria [21] representing a new and distinct microenvironment

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0168-6496/00/$20.00
PII: S0168-6496(00)00061-1

that is offered for protists to grow. In this study, we aimed to characterise how the presence of suspended macroaggregates provokes a new spatial distribution of the pre-existing marine protistan community. In order to perform this task, we created microcosms (13 l in volume) which allowed us to obtain macroaggregates from natural seawater, and to determine the abundance, major taxonomic structure and some features of behaviour of the bacterivorous protists that are able to inhabit spatially heterogeneous aquatic systems.

2. Materials and methods

2.1. Field sampling

Seven experiments were carried out on seawater samples from a coastal station of the Bay of Biscay (43° 24.5′ N 3° 2.7′ W) during the summer of 1997. Seawater was collected at 2 m depth using an acid-cleaned Niskin bottle (PWS Hydro-Bios). All samples were processed in the laboratory within 6 h after collection.

Two 50-ml aliquot replications were taken and fixed with formalin (2% v/v final concentration) to carry out the bacterial counts, and two 200-ml aliquot replications were taken and fixed with alkaline Lugol (0.5% v/v final concentration)–formalin (0.2 mg ml^{-1}) [22] to perform protistan community counts.

2.2. Design of the experimental microcosms and sampling

We used a laboratory-system similar to that described by Shanks and Edmonson [18] to promote the formation of organic macroaggregates from natural seawater. The microcosms were obtained in 13-l polypropylene cylindrical tanks (34 cm diameter × 14 cm) which contained 11 l of seawater from the sampling location. The tanks were not totally filled and were opened every day in order to avoid anoxic conditions during the experiments. They were placed on a roller table which made them rotate at 2.5 rpm.

Macroaggregates stable in size (maximum length 5–6 mm) were observed after the first 2–3 days. Bacterial, flagellate and ciliate counts were carried out in macroaggregates and microcosm water at the sixth day of the beginning of the experiments, when the colonisation of the particles by protists reaches a maximum [23], and before the aggregates collapse.

Subsampling in order to count the microbial communities in these microcosms was performed as follows: immediately before sampling, the roller table was stopped and the particles were allowed to settle. Ten-millilitre water samples were taken from the supernatant. Particles were carefully transferred from the tanks to Petri dishes filled with sterile filtered seawater by pipette with a sterile flexible rubber tube at the end. This step allowed the bacteria suspended in the interstitial water of the aggregates to be washed off. Triplicated 7-ml samples of particles without ambient water were taken placing the tip of a digital micropipette directly in the particles, and transferred to 1 ml of sterile filtered seawater. All samples were fixed as described for natural seawater.

The presence and identification of different protists in the microcosm water and aggregates was followed over the following 8 days after aggregate formation. Sampling procedure of water and particles was as we have described above, but in this case, three whole aggregates were transferred from the tanks to a 1-ml Sedgewick–Rafter chamber filled with sterile filtered seawater.

2.3. Microbial counts and observations

The counts of the free-living microbial communities (water samples) were performed in duplicate, and those of the microbial communities attached to the aggregates (particle samples) in triplicate.

Bacterial abundance was measured by acridine orange direct counting (AODC) [24]. In order to separate attached bacteria from the aggregate, the particle samples were treated with a solution of sterile tetrasodium pyrophosphate (10 mM final concentration) and incubated for 1 h at room temperature in a rotary shaker (12 rpm). Immediately before staining, the treated subsamples were sonicated (100 W, six pulses of 5 s) [25]. Appropriate volumes of fixed natural seawater, fixed microcosm water and fixed and treated particle samples were stained with acridine orange (0.01% w/v final concentration) for 2 min, and filtered on 0.2-μm pore size black polycarbonate filters. The wet filters were placed on microscope slides and mounted in low-fluorescence immersion oil. The filters were examined under a Nikon epifluorescence microscope equipped with blue light at a magnification of ×1250. Bacteria present in at least 30 randomly selected fields, with 20–30 bacteria per field, were counted.

Protistan abundance was measured by epifluorescence microscopy after diamidino-phenylindol (DAPI) staining [26]. Appropriate volumes of fixed natural seawater, microcosm water and particles were stained with DAPI (0.2 μg ml^{-1}) for 7 min, and filtered on prestained (Irgalan black, 0.2% w/v in 2% acetic acid for 24 h) polycarbonate filters. For flagellate counts 0.8-μm pore-size filters were used, and for ciliates, 3-μm pore-size filters. The wet filters were placed on microscope slides and mounted in low-fluorescence immersion oil. The filters were examined by a Nikon epifluorescence microscope equipped with UV light at a magnification of ×1250 for flagellate counts and ×200 and ×1250 for ciliate counts. At least 60 flagellates were counted for each subsample, and in the case of ciliate counts, all the filter surface was screened.

Protistan observation in the three phases created in the microcosms, water, aggregate and aggregate–water interphase, was made over live samples on 1-ml Sedgewick–

Rafter chambers. The material was observed by light microscopy at a magnification of ×200 and ×1000 (Nikon Diaphot-TMD and Optiphot, both equipped with Nomarski DIC system). The macroaggregates were left in the chamber for several minutes for the protists to settle. The observation of free-swimming protists were carried out in samples without particles. We considered the aggregate–water interphase as a microzone of about 50 μm around the aggregates because it shows abundant suspended bacterial and protistan communities. In order to differentiate those protist present in the aggregate–water interphase from those living inside the aggregate, different observation strategies were followed. The protists characterised as inhabitants of the interphase were detected by observing the edges and the water close to the aggregates. The protists living inside the aggregates were those detected after the disruption by gently shaking with a sterile tip, which was enough to break the aggregates without affecting the protistan motility. Protists were identified on the basis of their morphological and behavioural features recorded from both live and fixed samples and obtained through direct observation and microphotographs, as described in the literature [27–29].

3. Results

3.1. Field counts

The microbial abundances in natural seawater are shown in Table 1. The bacterial abundance ranged in the seven samples from 0.6×10^6 to 2.4×10^6 bacteria ml^{-1}, and the mean value was 1.4×10^6 bacteria ml^{-1}. The heterotrophic flagellate community ranged from 0.3×10^3 to 1.3×10^3 flagellates ml^{-1}, with a mean value for the whole study of 0.7×10^3 flagellates ml^{-1}. The abundance of the ciliate community was very low. In three of the seven samples we did not detect ciliates, being the detection limit of our methodology of 0.02 ciliates ml^{-1}. Therefore, this ciliate community ranged from <0.02 to 2.7 ciliates ml^{-1} and showed a mean value of 0.7 ciliates ml^{-1}.

3.2. Microcosm data

The microcosms obtained with the rolling tanks were maintained and observed for 8–10 days, while the microbial abundance was measured after 6 days of microcosm incubation. Microscopic observations of the macroaggregates showed that they were composed of diatoms, diatom frustules, seaweed debris, faecal pellets and remains of dead organisms coming from the original seawater.

Microbial abundance in the microcosms was measured in water and in the macroaggregates, and we calculated an enrichment factor as the ratio between the number of microorganisms in 1 ml of aggregate and the number of microorganisms in 1 ml of water. The values obtained for the three main communities involved, bacteria, heterotrophic flagellates and ciliates, are presented in Table 1 and were three orders of magnitude higher in the aggregates than in the microcosm water. The mean enrichment factors were 1.9×10^3 for bacteria, 0.6×10^3 for flagellates and 2.2×10^3 for ciliates. The ciliate community in the macroaggregates averaged 2.7×10^4 ciliates per ml of particles, and was the most enriched community.

From a qualitative point of view, we recognised 27 taxa of different levels among flagellates, ciliates and sarcodines, which showed a clear spatial distribution among the macroaggregate, the aggregate–water interphase and the water. The situation of the protists in all of the habitats created in the microcosm is shown in Table 2. Some protists were always detected in the microcosm water, but never associated to the aggregates. In this group, we found the sarcodine *Amoeba radiosa*, the flagellate *Paraphysomonas* sp. and some choanoflagellates, as well as the ciliates *Cohnilembus punctatus*, *Uropedalium opisthosoma*, and species of *Strobilidium*, *Lohmanniella* and some non-identified tintinnida.

The flagellates *Ploeotia* sp., *Entosiphon* sp., and *Amastigomonas* sp. only appeared in the aggregates. The flagellate *Massisteria* sp. and the amoeba *Vannella* sp. were usual inhabitants of the aggregate and occasionally appeared on the water in the microcosms.

In the aggregate–water interphase, the most commonly found protists were the flagellates *Cafeteria* sp. and *Bico-*

Table 1
Abundance of microbial communities in natural seawater samples and in the microcosms after macroaggregates were formed

	Natural seawater	Microcosm[a]		
		Water	Aggregate	EF[b]
Bacteria ml^{-1}	1.4×10^6 (0.6×10^6–2.4×10^6)	1.1×10^6 (0.5×10^6–1.8×10^6)	1.8×10^9 (1.4×10^9–2.0×10^9)	1.9×10^3 (1.1×10^3–3.5×10^3)
Heterotrophic flagellates ml^{-1}	0.7×10^3 (0.3×10^3–1.3×10^3)	3.1×10^3 (1.7×10^3–4.7×10^3)	1.6×10^6 (0.7×10^6–3.5×10^6)	0.6×10^3 (0.2×10^3–1.0×10^3)
Ciliates ml^{-1}	0.7 (<0.02–2.7)	14.0 (3.4–37.2)	2.7×10^4 (0.1×10^4–15.0×10^4)	2.2×10^3 (43.0–1.2×10^4)

Data are given as mean value (range).
[a]Counts determined at the 6th day since the onset of the experiment.
[b]EF, enrichment factor, calculated as the ratio between the number of microorganisms in 1 ml of aggregate and the number of microorganisms in 1 ml of microcosm water.

Table 2
Protistan taxa detected in the three phases of the experimental microcosm: aggregate, aggregate-water interphase and water

		Habitat in the microcosm		
		Aggregate	Interphase	Water
Flagellates				
O. *Euglenida*	*Ploeotia* sp. Dujardin 1841	+		
	Entosiphon sp. Stein 1878	+		
O. *Dinoflagellida*	*Oxyrrhis* sp. Dujardin 1841		+	+
O. *Bicosoecida*	*Cafeteria* sp. Fenchel and Patterson 1988		+	
	Bicosoeca maris Picken 1941		+	
	Pseudobodo tremulans Griessmann 1913		+	+
O. *Kinetoplastida*	*Bodo designis* Skuja 1948	+	+	
	Rhynchomonas nasuta Klebs 1893	+	+	
O. *Choanoflagellida*	*Desmarella* sp. Kent 1878			+
	Naked choanoflagellates			+
	Salpingoeca sp. James-Clark 1866		+	+
	Loricate and thecate choanoflagellates		+	+
Apusomonads	*Amastigomonas* sp. de Saedeleer 1931	+		
Cercomonads	*Massisteria* sp. Larsen and Patterson 1990	+		+
Chrisophytes	*Paraphysomonas* sp. de Saedeleer 1929			+
Ciliates				
O. *Hypotrichida*	*Euplotes vannus* Müller 1786	+	+	
	Aspidisca steini Buddenbrock 1920	+	+	
O. *Scuticociliatida*	*Uronema marinum* Dujardin 1841		+	+
	Cohnilembus punctatus Kahl 1931			+
	Uropedalium opisthosoma Kahl 1931			+
O. *Oligotrichida*	*Strobilidium* sp. Schewiakoff 1893			+
	Lohmanniella sp. Leegard 1915			+
	Tintinnids			+
Sarcodines				
Subcl. *Gymnamoebia*	*Vannella* sp. Bovee 1965	+		+
	Amoeba radiosa Bory de St. Vincent 1822			+
Cl. *Heliozoea*	Heliozoa			+
Cl. *Polycystinea*	Radiolaria			+

O., order; Subcl., subclass; Cl., class.

soeca maris, which never appeared either inside the aggregate or in the microcosm water. However, some of the protists found in this interphase appeared in some of the other habitats: the aggregates, as the flagellates *Bodo designis* and *Rynchomonas nasuta* and the ciliates *Euplotes vannus* and *Aspidisca steini*, or the microcosm water as the flagellates *Oxyrrhis* sp., *Pseudobodo tremulans, Salpingoeca* sp. and the ciliate *Uronema marinum*.

4. Discussion

The formation of aggregates in seawater creates spatial heterogeneity in the system. The change from an 'only water' situation, detected in the natural seawater samples, to a new 'water plus aggregates' situation obtained in the microcosms, generates the presence of a solid support with a convoluted structure composed by diatom frustules, seaweed debris, faecal pellets, remains of dead organisms and other materials coming from the seawater. They were highly enriched in utilisable organic matter which can support an intense microbial activity. A consequence of this enrichment is the establishment of a dense community of attached bacteria: as it can be observed in Table 1, the bacterial community in the aggregates was three orders of magnitude higher than the free-living bacterial community observed in the microcosm water. But the bacterial community is not the only coloniser of the aggregates. When the communities of flagellate and ciliated protists are considered, this degree of enrichment of the aggregates in respect of the water is maintained. It is even possible to detect the influence of these aggregates densely colonised by flagellates and ciliates in the surrounding water.

The presence of aggregates allows a diversification of the niches accessible to planktonic microorganisms. The aqueous phase is characterised by the presence of low densities of usually small, mostly inactive [30] and freely dispersed bacterial prey. The aggregates represent a solid support in which high densities of bacteria can be found, either firmly attached to the aggregates or embedded in their polymeric matrix [3,31] and their role as colonisers implies high ecto- and extracellular enzymatic activities [21,32,33] which are necessary to utilise the components of the aggregate. As a consequence, strong gradients of chemical compounds may appear. In some cases, this may create hostile conditions for microorganisms, but it also implies the liberation of utilisable dissolved organic matter (UDOM) as well as the release of large, actively

growing bacteria from the surface of the aggregates [34–36]. In this context, a third microenvironment should be taken into account: the aggregate–water interphase, which would support a dense community of bacteria sharing characteristics both of the free-living and aggregate-associated bacteria: they are mostly active, large and freely dispersed.

The establishment of these ecological niches provokes a spatial structuring of the protistan community in our microcosms, which can be seen in Table 2. All the detected protists came from seawater samples where macroaggregates were not detected, and once formed, these planktonic protists chose one or more microenvironments.

Those protists living in the aqueous phase should be good swimmers and adapted to profit from relatively low densities of bacterial prey by filtration or raptorial feeding. Among flagellates, we observed organisms of the genera *Desmarella* and other non-identified naked choanoflagellates, as well as species of the genera *Paraphysomonas*. We also detected the scuticociliates *Cohnilembus punctatus*, *Uropedalium opisthosoma*, several non-identified tintinnids and species of *Strobilidium* and *Lohmanniella*. With regard to sarcodines, several Heliozoa and Radiolaria were observed, as well as *Amoeba radiosa*, a floating ameboid form with pseudopodia looking for new habitats. None of them were found either in aggregates or in the aggregate–water interphase in the seven microcosm experiments carried out, and therefore they can be considered as truly free-swimming protists.

The flagellate *Massisteria* sp. and the amoeba *Vannella* sp. present similar strategies: the forms found in the microcosm water were those free-swimming non-feeding morphological types, which allowed their dispersion through the aquatic system, while in the aggregates we found their trophic morphologies adapted to feed on bacteria attached to solid supports.

The three taxa found exclusively in aggregates should be able to move on solid surfaces and have structures to separate the attached bacterial prey from the aggregate. The euglenida *Ploeotia* sp. and *Entosiphon* sp. moved through the aggregates by gliding and fed on attached bacteria by using their ingestion apparatus. The apusomonad *Amastigomonas* moved by gliding too, while fed upon attached bacteria by sweeping the solid substrate with its proboscis.

A high diversity of protists was found in the aggregate–water interphase. The flagellates *Cafeteria* sp. and *Bicosoeca maris* were found only in this interphase. Both showed similar feeding mechanisms: they were permanently attached to the aggregate, and used their anterior flagellum to feed on the bacteria suspended near the particles. Therefore, they obtained full profit from the characteristics of this interphase.

The remaining taxa shared other microenvironment in addition to the interphase. Some of them were also found in the aggregates, as the hypotrich ciliates *Aspidisca steini* and *Euplotes vannus* and the flagellates *Bodo designis* and *Rynchomonas nasuta*. All of them have often been described [29,37–39] as particle-associated protists, because they are poor swimmers, but they easily move over and inside the aggregates. Both ciliates create strong currents pulling the bacteria towards their ingestion areas, whereas the bodonids use the anterior flagellum (*Bodo designis*) or the proboscis (*Rynchomonas nasuta*) to loosen the attached bacteria and make their capture easier. Hence they are able to benefit not only from the bacteria truly attached to the aggregates, but also from those free-floating around them.

The flagellates *P. tremulans* and species of *Salpingoeca* have been previously described as being associated to marine snow [29,40]. However, in this study, we have seen them as free living with transient attachment to the surface of the aggregates. The attachment of flagellates provokes higher filtration rates than when they are free-swimming [41,42], which may be a mechanism to feed more efficiently, while the free-swimming forms may be a way to explore new food sources.

More surprising was the presence of some protists, which are known to be good swimmers, very near to the aggregate. The flagellate *Oxyrrhis* sp. and the ciliate *U. marinum* were both found in the microcosm water and in the interphase aggregate–water. It seems that in the presence of aggregates, these protists move towards them and stay near the aggregate surface. These protists might be able to detect chemical changes around the aggregates induced by the activity of the bacterial colonisers, swim towards those zones where high densities of prey are available, and remain there.

In summary, we have observed that the spatial heterogeneity created by the presence of aggregates in a marine system has relevant effects on the protistan communities. The aggregates are microenvironments heavily enriched in these communities, but even more important is that the different protists, according to their feeding, structure and behaviour characteristics, are able to choose the most convenient microenvironment. Some protistan taxa, in spite of the presence of abundant particles, remain suspended in the water; others tend to leave this aqueous phase, approach, and in some cases colonise, the inside and surface of the aggregate. Finally, the preference of diverse protists for the aggregate–water interphase is of note, which may be explained when considering the abundance of large and active bacterial prey easily available by different feeding mechanisms.

Acknowledgements

This study was supported by grants to A.L. and J.I. from the University of the Basque Country (EB 033/95), and to J.I. from the Spanish Government (MEC PB97-0635).

References

[1] Silver, M.W. and Alldredge, A.L. (1981) Bathypelagic marine snow: deep sea algal and detrital community. J. Mar. Res. 39, 501–530.

[2] Fowler, S.W. and Knauer, G.A. (1986) Role of large particles in the transport of elements and organic compounds through the oceanic water column. Prog. Oceanogr. 16, 147–194.

[3] Iriberri, J. and Herndl, G.J. (1995) Formation and microbial utilization of amorphous aggregates in the sea: ecological significance. Microbiología SEM 11, 309–322.

[4] Alldredge, A.L. and Silver, M.W. (1988) Characteristics, dynamics and significance of marine snow. Prog. Oceanogr. 20, 41–82.

[5] Alldredge, A.L. and Youngbluth, M.J. (1985) The significance of macroscopic aggregates (marine snow) as sites for heterotrophic bacterial production in the mesopelagic zone of the subtropical Atlantic. Deep-Sea Res. 32, 1445–1456.

[6] Alldredge, A.L., Cole, J.J. and Caron, D.A. (1986) Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnol. Oceanogr. 31, 68–78.

[7] Herndl, G.J. (1988) Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. II. Microbial density and activity in marine snow and its implication to overall pelagic processes. Mar. Ecol. Prog. Ser. 38, 265–275.

[8] Azam, F., Martinez, J. and Smith, D.C. (1993) Bacteria-organic matter coupling on marine aggregates. In: Trends in Microbial Ecology (Guerrero, R. and Pedrós-Alió, C., Eds.) pp. 410–414. Spanish Society for Microbiology, Barcelona.

[9] Silver, M.H., Shanks, A.L. and Trevil, J.D. (1978) Marine snow: microplankton habitat and source of small-scale patchiness in pelagic populations. Science 201, 371–373.

[10] Prézelin, B.B. and Alldredge, A.L. (1983) Primary production of marine snow during and after an upwelling event. Limnol. Oceanogr. 28, 1156–1167.

[11] Silver, M.H., Gowing, M.M., Brownlee, D.C. and Corliss, J.O. (1984) Ciliated protozoa associated with oceanic sinking detritus. Nature 309, 246–248.

[12] Caron, D.A., Davis, P.G., Madin, L.P. and Sieburth, J.McN. (1986) Enrichment of microbial populations in macroaggregates (marine snow) from surface waters of the North Atlantic. J. Mar. Res. 44, 543–565.

[13] Calvert, S.E. and McCartney, M.J. (1979) The effect of incomplete recovery of large particles from water samplers on the chemical composition of oceanic particulate matter. Limnol. Oceanogr. 24, 532–536.

[14] Gibbs, R.J. and Konwar, L.N. (1983) Sampling of mineral flocs using Niskin bottles. Environ. Sci. Technol. 16, 298–299.

[15] Gibbs, R.J. (1981) Flocs breakage by pumps. J. Sediment. Petrol. 51, 670–672.

[16] Krank, K. and Milligan, T. (1980) Macroflocs: production of marine snow in the laboratory. Mar. Ecol. Prog. Ser. 3, 19–24.

[17] Biddanda, B.A. (1985) Mycrobial synthesis of macroparticulate matter. Mar. Ecol. Prog. Ser. 20, 241–251.

[18] Shanks, A.L. and Edmonson, E.W. (1989) Laboratory-made artificial marine snow: a biological model of the real thing. Mar. Biol. 101, 463–470.

[19] Alldredge, A.L., Gotschalk, C., Passow, U. and Riebesell, U. (1995) Mass aggregation of diatom blooms: insights from mesocosm study. Deep-Sea Res. 42, 9–27.

[20] Unanue, M.A., Azúa, I., Arrieta, J.M., Herndl, G.J. and Iriberri, J. (1998) Laboratory-made particles as a useful approach to analyse microbial processes in marine macroaggregates. FEMS Microbiol. Ecol. 26, 325–334.

[21] Unanue, M.A., Azúa, I., Arrieta, J.M., Labirua-Iturburu, A., Egea, L. and Iriberri, J. (1998) Bacterial colonization and ectoenzymatic activity in phytoplankton-derived model particles: cleavage of peptides and uptake of amino acids. Microb. Ecol. 35, 136–146.

[22] Sherr, B.F., Sherr, E.B. and Rassoulzadegan, F. (1988) Rates of digestion of bacteria by marine phagotrophic protozoa: temperature dependence. Appl. Environ. Microbiol. 54, 1091–1095.

[23] Artolozaga, I., Santamaría, E., López, A., Ayo, B. and Iriberri, J. (1997) Succession of bacterivorous protists on laboratory-made marine snow. J. Plankton Res. 19, 1429–1440.

[24] Hobbie, J.E., Daley, R.J. and Jasper, S. (1977) Use of Nucleopore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Microbiol. 33, 1225–1228.

[25] Velji, M.I. and Albright, L.J. (1986) Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments. Can. J. Microbiol. 32, 121–126.

[26] Porter, K.G. and Feig, S. (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25, 943–948.

[27] Patterson, D.J. and Fenchel, T. (1990) *Massisteria marina* Larsen and Patterson 1990, a widespread and abundant bacterivorous protist associated with marine detritus. Mar. Ecol. Prog. Ser. 62, 11–19.

[28] Carey, P.G. (1992) Marine Interstitial Ciliates. An Illustrated Key. Natural History Museum Publications, Chapman and Hall, London.

[29] Patterson, D.J., Nygaard, K., Steinberg, G. and Turley, C. (1993) Heterotrophic flagellates and other protists associated with oceanic detritus throughout the water column in the Mid North Atlantic. J. Mar. Biol. Ass. 73, 67–95.

[30] Rodriguez, G.G., Phipps, D., Ishiguro, K. and Ridgway, H.F. (1992) Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Appl. Environ. Microbiol. 58, 1801–1808.

[31] Heissenberger, A., Leppard, G.G. and Herndl, G.J. (1996) Ultrastructure of marine snow. II. Microbiological considerations. Mar. Ecol. Prog. Ser. 135, 299–308.

[32] Smith, D.C., Simon, M., Alldredge, A.L. and Azam, F. (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle disolution. Nature 359, 139–142.

[33] Agis, M., Unanue, M., Iriberri, J. and Herndl, G.J. (1998) Bacterial colonization and ectoenzymatic activity in phytoplankton-derived model particles. Part II. Cleavage and uptake of carbohydrates. Microb. Ecol. 36, 66–74.

[34] Jacobsen, T.R. and Azam, F. (1984) Role of bacteria in copepod fecal pellet decomposition: colonization, growth rates and mineralization. Bull. Mar. Sci. 35, 495–502.

[35] Kjelleberg, S., Hermansson, M. and Mardén, P. (1987) The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. Annu. Rev. Microbiol. 41, 25–49.

[36] Unanue, M.A., Ayo, B., Azúa, I., Barcina, I. and Iriberri, J. (1992) Temporal variability of attached and free-living bacteria in coastal waters. Microb. Ecol. 23, 27–39.

[37] Lee, J.J., Hutner, H. and Bovee, E.C. (1985) An Illustrated guide to the Protozoa. Society of Protozoologists, Lawrence, Kansas.

[38] Caron, D.A. (1987) Grazing on attached bacteria by heterotrophic microflagellates. Microb. Ecol. 13, 203–218.

[39] Albright, L.J., Sherr, E.B., Sherr, B.F. and Fallon, R.D. (1987) Grazing of ciliated protozoa on free and particle-attached bacteria. Mar. Ecol. Prog. Ser. 38, 125–129.

[40] Caron, D.A. (1991) Heterotrophic flagellates associated with sedimenting detritus. In: The Biology of Free-living Heterotrophic Flagellates (Patterson, D.J. and Larsen, J., Eds). Systematics Association, Oxford.

[41] Lighthill, J. (1976) Flagellar hydrodynamics. Soc. Ind. Appl. Math. Rev. 18, 161–230.

[42] Fenchel, T. (1982) Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. Mar. Ecol. Prog. Ser. 8, 211–223.