

Biodiversity of culturable heterotrophic bacteria in the Southern Adriatic Sea Italian coastal waters*

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SUMMARY: The qualitative and quantitative composition of culturable heterotrophic bacteria in water samples from the Southern Adriatic Sea of Italy was examined. Water samples were collected monthly, for a year, at 16 stations along the coast line between Brindisi and Santa Maria di Leuca. The results obtained described the heterotrophic bacterial community over an annual cycle. Mean values of bacterial densities were 5.3×10^4 CFUml⁻¹ in Brindisi, 5.8×10^4 CFUml⁻¹ in S. Cataldo, 4.3×10^4 CFUml⁻¹ in Otranto and 6.7×10^4 CFUml⁻¹ in S. M. di Leuca. The differences in bacterial densities between the sites considered were estimated. The hydrodynamic circulation, the trophism and the geographical position of the examined sites contribute to justify the different bacterial density trends. The bacterial community consisted mainly of the genera *Aeromonas*, *Pseudomonas*, *Photobacterium* and *Flavobacterium*. The Enterobacteriaceae represented a considerable fraction of the bacterial community in the Southern Adriatic Sea. Bacilli were predominant among the Gram positive bacteria. The enzymatic versatility of the observed genera suggest their importance in organic matter turnover of this oligotrophic ecosystem.

Key words: culturable heterotrophic bacteria, Southern Adriatic Sea, biodiversity, abiotic parameters.

RESUMEN: BIODIVERSIDAD DE BACTERIAS HETERÓTROFAS CULTIVABLES EN LAS AGUAS COSTERAS ITALIANAS DEL MAR ADRIÁTICO MERIDIONAL. – Se examinó la composición cualitativa y cuantitativa de las bacterias heterótrofas cultivables en muestras de agua procedentes de aguas costeras italianas del mar Adriático meridional. Dichas muestras se recolectaron mensualmente, durante un año, en 16 estaciones a lo largo de la costa entre Brindisi y Santa Maria di Leuca. Los resultados obtenidos describen la comunidad bacteriana heterótrofa a lo largo de un ciclo anual. Los valores medios de las densidades bacterianas fueron 5.3×10^4 CFUml⁻¹ en Brindisi, 5.8×10^4 CFUml⁻¹ en S. Cataldo, 4.3×10^4 CFUml⁻¹ en Otranto y 6.7×10^4 CFUml⁻¹ en S. M. di Leuca. Se estimaron las diferencias en densidad bacteriana entre las distintas localidades. La circulación hidrodinámica, el trofismo y la posición geográfica de las localidades estudiadas contribuyen a explicar las distintas tendencias en densidad bacteriana. La comunidad bacteriana estaba constituida principalmente por los géneros *Aeromonas*, *Pseudomonas*, *Photobacterium* y *Flavobacterium*. Las Enterobacteriaceae representaban una fracción considerable de la comunidad bacteriana del mar Adriático meridional. Los bacilos eran predominantes entre las bacterias Gram positivas. La versatilidad enzimática de los géneros observados sugiere su importancia en el ciclado de la materia orgánica en este ecosistema oligotrófico.

Palabras clave: bacterias heterótrofas cultivables, mar Adriático meridional, biodiversidad, parámetros abióticos.

INTRODUCTION

A large number of studies in the past two decades show that heterotrophic bacteria not only function as

decomposers, but also channel dissolved organic substrates and inorganic nutrients into higher trophic levels through the microbial food web (Pomeroy, 1980; Azam *et al.*, 1983; Azam, 1998) hence facilitating the cycling of bioelements. The paramount importance of microbial life for the cycling of mat-

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ter in the oceans makes finding the identity of the indigenous bacteria an obvious research goal. However, heterotrophic bacteria are usually clumped together into a "black box" in conceptual and mathematical models. This anonymity is unwarranted since different marine bacteria, grown under the same conditions, exhibit different ectohydrolytic enzyme profiles, suggesting that different bacteria degrade different fractions of organic matter in the sea (Fuhrman *et al.*, 1993; Martinez *et al.*, 1996). Furthermore, the knowledge of the microbial communities composition may give important information about the different seawater habitats since bacteria respond quickly to abiotic changes in their environment. In fact, the vertical distribution of physical and chemical parameters such as temperature, salinity, oxygen content, and organic matter concentration in the marine water column may be the basis for a pronounced stratification of the prominent microbial populations (Sieburth, 1967; Rheinheimer and Gocke, 1994; Ramsing *et al.*, 1996). However, it has been recognized that bacterial populations may be considerably modified by interactions with biotic factors. The link between bacteria and their main grazers, heterotrophic nanoflagellates, is believed to have a controlling function on bacterial abundance, growth and community composition (Sanders *et al.*, 1992; Jürgens and Gude, 1994). Thus, further insight into marine bacterial diversity would be desirable.

Traditionally the diversity of marine bacteria has been assessed through identification with phenotypic tests and nutritional taxonomic approaches from collections of isolates from plating on nutrient media and enrichment. The results of such studies (Fry, 1987) have shown a relatively small number of genera being found most frequently, and these common types belonged mostly to the γ -Proteobacteria, Cytophaga-Flexibacter-Bacterioides (CFB) and gram-positive groups. The view that marine isolates are not representative of the indigenous bacteria stems from the unexplained disagreement between plate counts and the bacteria counted by epifluorescence microscopy and it has become the current dogma that marine bacteria are unculturable (Kogure *et al.*, 1979). This problem was overcome when bacterial marine diversity was first investigated by 16 S rDNA approaches (Giovannoni *et al.*, 1990). Culture independent studies of this kind are now common and have found many bacteria that are only known from their 16 S rRNA gene sequence and that do not match cultured bac-

teria. Several of these are common in marine samples (e.g. SAR 11, Giovannoni *et al.*, 1990; JAP 504, Rochelle *et al.*, 1994). At the same time, data demonstrating the dominant occurrence of culturable bacteria in the sea have been reported. With Southern California Blight isolates, Rehnstam *et al.* (1993), using 16 S rRNA probes, showed that single species of culturable bacteria can dominate the community DNA. Also Fuhrman *et al.* (1994) have reported that two culturable marine isolates each accounted for up to 20% of the bacterial community. Recent studies using various hybridization protocols (González and Moran, 1997; Pinhassi *et al.*, 1997; Hagström *et al.*, 2000) showed that bacteria able to form colonies on solid media occupy a considerably larger fraction of the marine bacterial community than the number of colony-forming units would suggest.

On the basis of the mentioned studies it was assessed that the major phylogenetic groups of Bacteria most commonly abundant in marine habitats are the CFB, α -Proteobacteria and γ -Proteobacteria. These groups contain many aerobic or facultative heterotrophs that are relatively easy to culture (e.g. > 60% of Proteobacteria and > 40% of CFB are culturable; Hugenholtz *et al.*, 1998). Thus, we focussed our study on culturable heterotrophic bacteria. Knowledge about culturable heterotrophic marine bacteria biodiversity in the Mediterranean Sea is still insufficient. Concerning the Adriatic Sea, Zaccone *et al.* (1998) furnished some information on the quantitative and qualitative composition of the culturable heterotrophic bacterial community of the Northern Adriatic Sea during two different seasons. However, the Southern Adriatic Sea along Italian coasts has not been thoroughly investigated. In this work the quantitative and qualitative composition of culturable heterotrophic bacteria in water samples from the Southern Adriatic Sea were examined over an annual cycle.

MATERIALS AND METHODS

Study site and sampling

The Southern Adriatic Sea extends from the Pelagosa sill (north) to the Otranto sill (south) which divides it from the Ionian Sea. This study was carried out from April 1996 to March 1997 in four transects of the Southern Adriatic Sea: Brindisi, S. Cataldo, Otranto and Santa Maria di Leuca

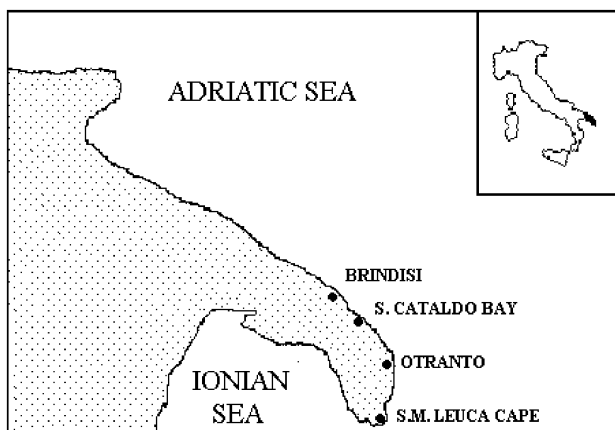


FIG. 1. – Map of the Southern Adriatic Sea of Italy showing the locations of the sampling sites.

(Fig. 1). For each examined transect four sampling points, perpendicular to the shore, were fixed. They were referred to as stations 1 (located at 0.5 kilometers from the coastal line), 2 (3 Km), 3 (5 Km) and 4 (10 Km). Water samples were taken, once a month, at 0.5 m below the water surface and at 0.5 m above the bottom (between 5 and 50 m depth depending on the bathymetry of each site). Brindisi is an industrial city (about 100.000 inhabitants) with a touristic and commercial port, S. Cataldo is a small touristic village, Otranto and S. M. di Leuca are two small touristic towns (about 5.500 inhabitants each one).

In this coastal tract of the Southern Adriatic Sea Caroppo *et al.* (1999) observed annual averages of ammonia around $0.2 \mu\text{g l}^{-1}$, nitrates mean values of $1.3 \mu\text{g l}^{-1}$, nitrites of $0.2 \mu\text{g l}^{-1}$ and phosphorus as orthophosphate from 0.01 to $0.1 \mu\text{g l}^{-1}$.

Abiotic parameters

Temperature, pH, salinity and dissolved oxygen were measured in situ using a multiparametric sounding-line “Ocean Seven 401” (Jolzonant, Italy).

Bacteriological methods

Water samples were collected with 5 L Niskin bottles. The sampling bottles were acid cleaned (10% HCl) and rinsed with distilled water before use. Samples were transferred into sterile polycarbonate flasks and processed, for enumeration and isolation of bacteria, within 4 h of sampling. In the laboratory, the number of colony-forming units (CFU) was determined by plating $100 \mu\text{l}$ of undiluted

seawater and serial dilutions of each water sample in triplicates on Bacto Marine Agar 2216 (Difco). The plates were incubated at 22°C over 7 days. At the end of the incubating period all the colonies were counted through a $\times 10$ magnifying glass. All colonies were isolated, subcultured and identified by several morphological, biochemical and cultural methods (Holt *et al.*, 1994). The standardized API 20 System (Biomerieux SA, France) was used on the isolates to study the following biochemical characteristics: hydrolysis of gelatin, nitrate reductase, presence of B-galactosidase, carbohydrate utilization: saccharose, arabinose, mannitol, fructose, glucose, maltose, starch, rhamnose, galactose, mannose, sorbitol, glycerol, urease, indole, H_2S and acetoin production, and citrate utilization. To adjust the salinity to a value more representative of sea water, a 3% NaCl solution was added to the suspension medium. The galleries were incubated for 2 days at room temperature.

Statistical analysis

The bacterial concentrations were log transformed and statistical analysis was performed by using the STATSOFT STATISTICA v. 6.0 (Statsoft, 2001).

RESULTS

Abiotic parameters

The main physico-chemical characteristics of the sampling points such as temperature, salinity, pH, and dissolved oxygen are reported in Figure 2. The lowest seasonal temperatures were reached in March both at the surface and the bottom. Summer warming up started in May with temperatures never above 19°C and the highest temperature values were observed in August at the surface, when the greatest thermal excursion between the surface and water at a depth of 50 m was observed. At the bottom the highest temperatures were recorded in Brindisi in August, in S. Cataldo in September, in Otranto and S. M. di Leuca in October. Salinity showed lowest values in winter with surface values between 36.9 and 37.7‰. The highest value of 38.6‰ was reached in S. Cataldo at the surface in November. The pH values oscillated from 7.9 to 8.41. Water samples from the different stations showed an oxygen mean percentage saturation of 109.7.

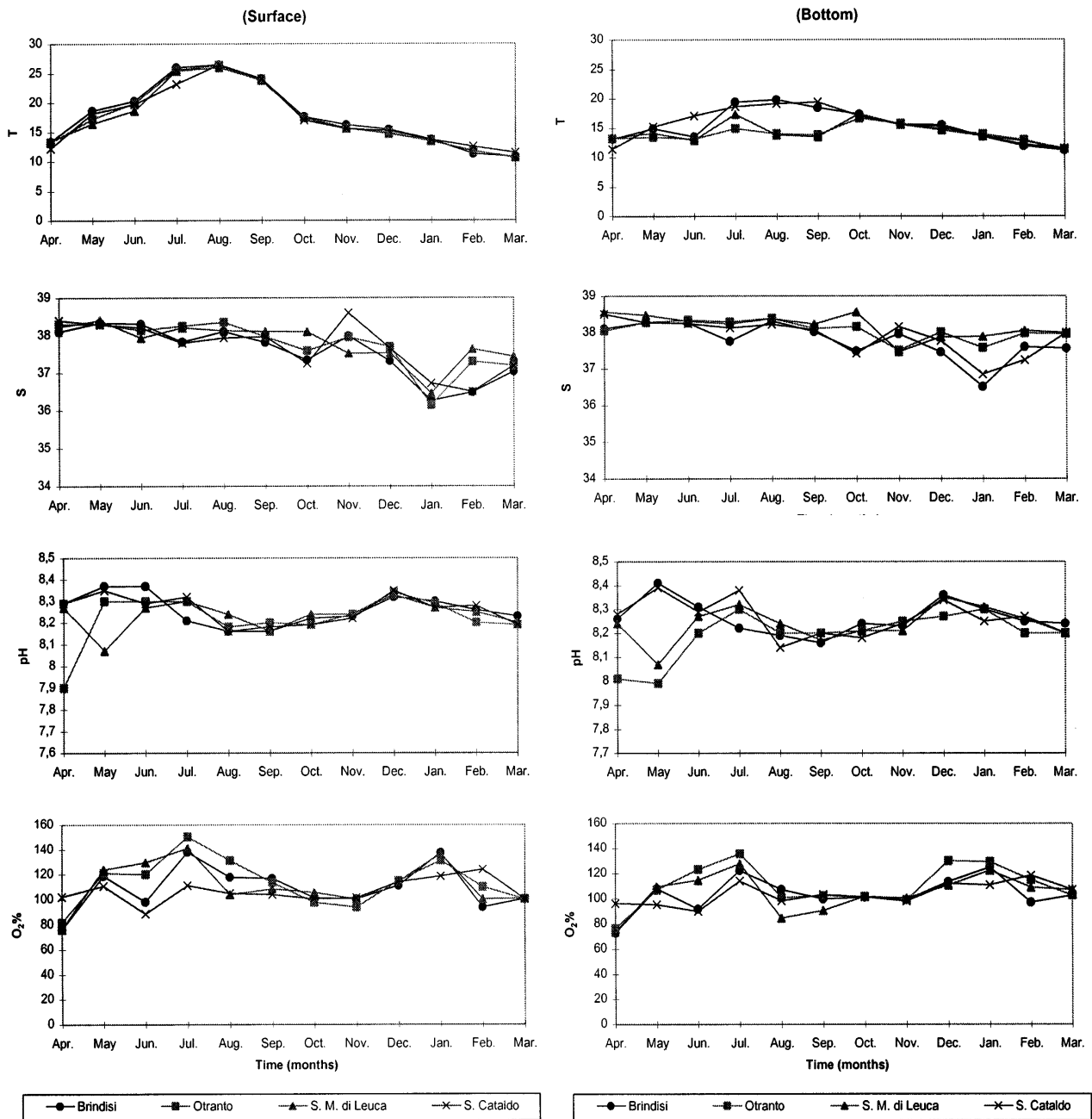


FIG. 2. – Yearly values of: Temperature (T, °C), Salinity (S, ‰), pH, dissolved oxygen (O₂ %), measured at the various sampling sites.

Heterotrophic bacterial counts

Culturable heterotrophic bacterial densities were usually higher at the bottom than at the surface in all the stations examined. In Brindisi the mean bacterial densities were 4.0×10^4 CFU ml⁻¹ in the surface sample and 6.6×10^4 CFU ml⁻¹ in the deep sample, in S. Cataldo 4.6×10^4 and 7.0×10^4 CFU ml⁻¹, in S. M. di Leuca 6.4×10^4 and 7.0×10^4 CFU ml⁻¹. Only at Otranto were the mean values of heterotrophic bacterial counts comparable in the sur-

face and depth samples (4.3×10^4 CFU ml⁻¹). The seasonal changes in culturable heterotrophic bacterial densities are shown in Figures 3a, b, c, and d. In Brindisi (Fig. 3a) the trend of the bacterial abundances showed several peaks and the lowest values both at the surface and the bottom were observed in October. No relationship between the bacterial abundance and the temperature was observed ($R = 0.094$, $p < 0.01$, $n = 96$). In S. Cataldo (Fig. 3b) the bacterial counts displayed higher values during the spring-summer period either at the surface or at the

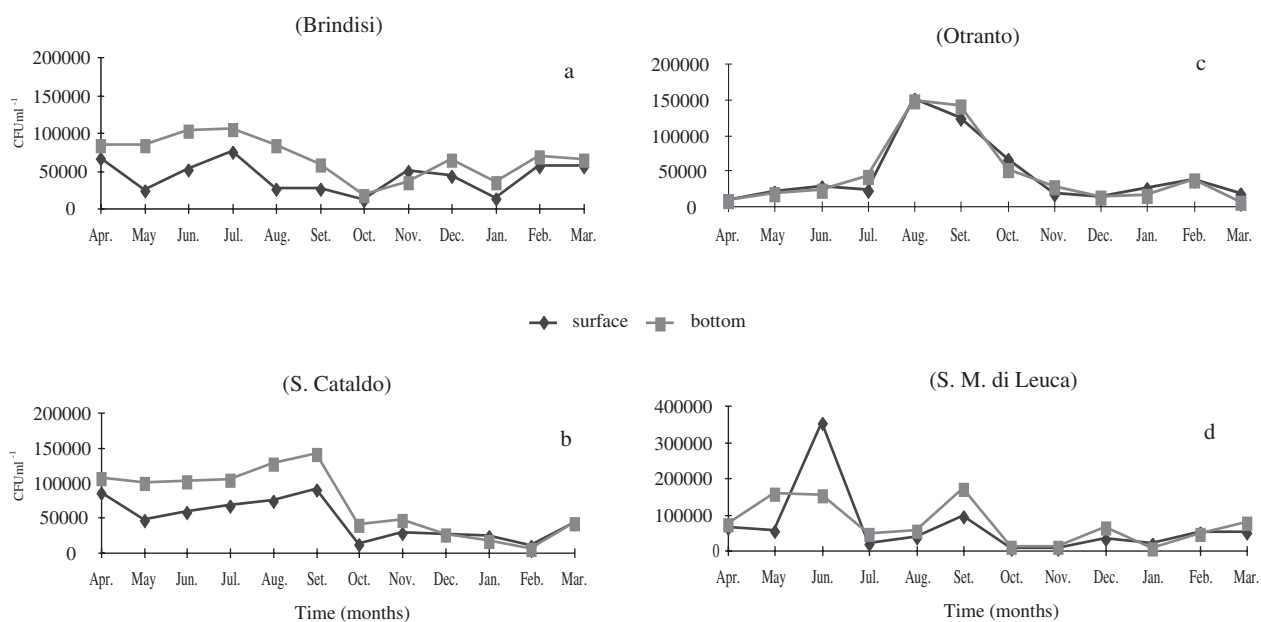


FIG. 3. – Culturable heterotrophic bacterial abundance over an annual cycle in Brindisi (a), S. Cataldo (b), Otranto (c) and Santa Maria di Leuca (d), at the surface and bottom levels.

bottom, whereas the lowest densities were observed in October and February. At this site a significantly positive relationship between the bacterial abundance and the temperature ($R = 0.639$, $p < 0.01$, $n = 96$) was found. In Otranto (Fig. 3c) the highest concentrations of the culturable heterotrophic bacteria were monitored in the August-September period both at the surface and the bottom. Also, in this site as well as in S. Cataldo, a significantly positive relationship between the bacterial density and the temperature was observed ($R = 0.545$, $p < 0.01$, $n = 96$). In S. M. di Leuca (Fig. 3d) the pattern of the microbial abundances was more diversified compared with those of the other sites: several peaks and declines occurred. No relationship was

observed between the bacterial abundance and the temperature ($R = 0.009$, $p < 0.01$, $n = 96$).

Bacterial biodiversity

It was possible to identify up to the genus level about 86% of the isolates grown on Marine agar. In all the sampling sites (Figs. 4, 5, 6, 7, 8) the Gram-negative bacteria prevailed over the Gram-positive ones. The genus *Aeromonas* dominated in all the four transects examined either at the surface or at the bottom with a mean percentage of isolation at the surface of 23, 13, 19.4 and 13.6% in Brindisi, S. Cataldo, Otranto and S. M. di Leuca respectively, and 15.5, 15, 18.2 and 15.2% at the bottom. Apart from

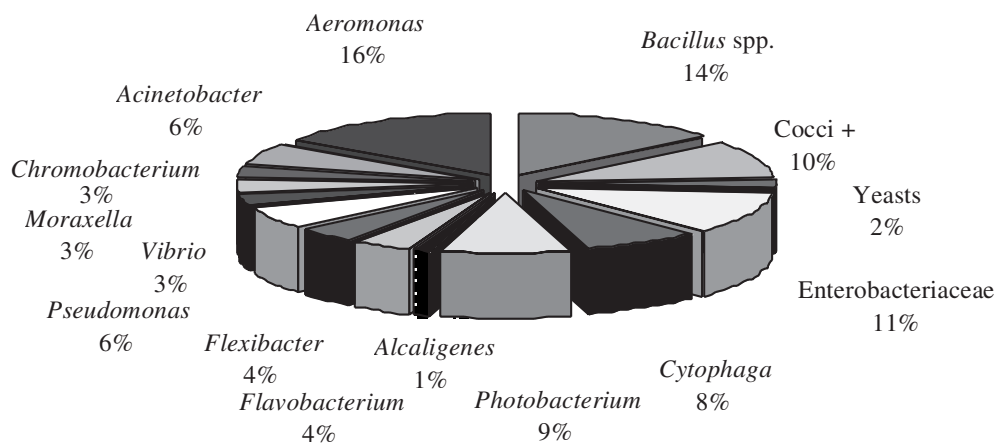


FIG. 4. – Qualitative analysis of culturable heterotrophic bacterial community over an annual cycle in the Southern Adriatic Sea.

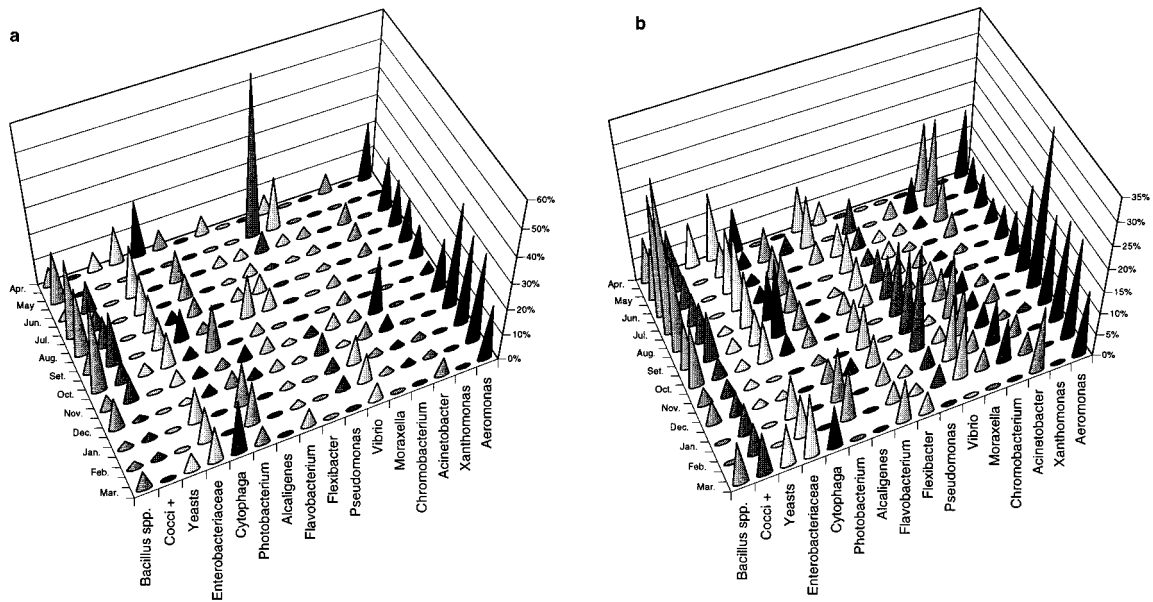


FIG. 5. – Annual trend of culturable heterotrophic bacterial diversity in Brindisi at surface (a) and bottom (b).

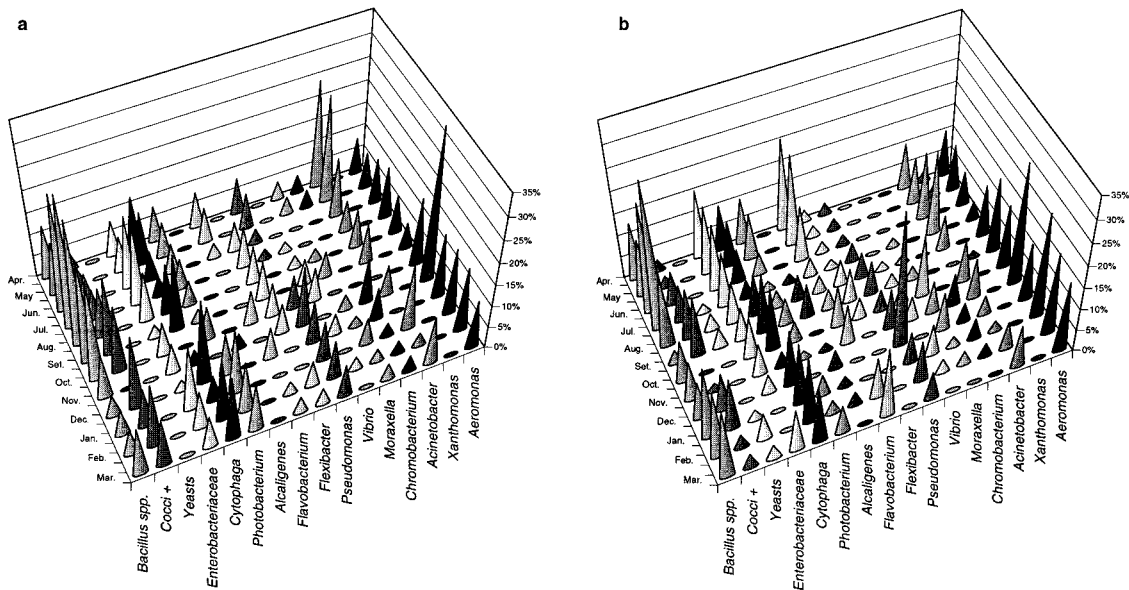


FIG. 6. – Annual trend of culturable heterotrophic bacterial diversity in S. Cataldo at surface (a) and bottom (b).

Aeromonas, the culturable bacterial community of the Southern Adriatic Sea consisted mainly of the genera *Pseudomonas*, *Photobacterium*, *Cytophaga*, *Acinetobacter* and *Flavobacterium* (Fig. 4). The family Enterobacteriaceae represented a considerable fraction of the bacterial community too. In Brindisi in fact the mean isolation percentage was 11.9% at the surface and 9.7% at the bottom. In S. Cataldo the Enterobacteriaceae were found throughout the year except in January and February at the bottom with a mean isolation percentage of about 9.5%. In Otranto this family showed a mean isolation percentage of 13.4% and in S. M. di Leuca of 10.4%. Results con-

cerning the main culturable bacterial genera isolated in the four transects of the Southern Adriatic Sea are summarized in Table 1. *Pseudomonas* was present with a mean value of 8% in Brindisi, of 6.6% in S. Cataldo and 7.6% in S. M. di Leuca. In Otranto *Pseudomonas* was more abundant at the bottom (10%) than at the surface (4%). *Photobacterium* was isolated with a mean isolation percentage of 7.5% in Brindisi, 6.5% in S. Cataldo, 8.9% in Otranto and 9.9% in S. M. di Leuca. In this last transect the presence of *Photobacterium* was comparable to *Aeromonas* at the surface (mean isolation percentage of 13.6%). *Cytophaga*, *Moraxella*, *Acinetobacter*,

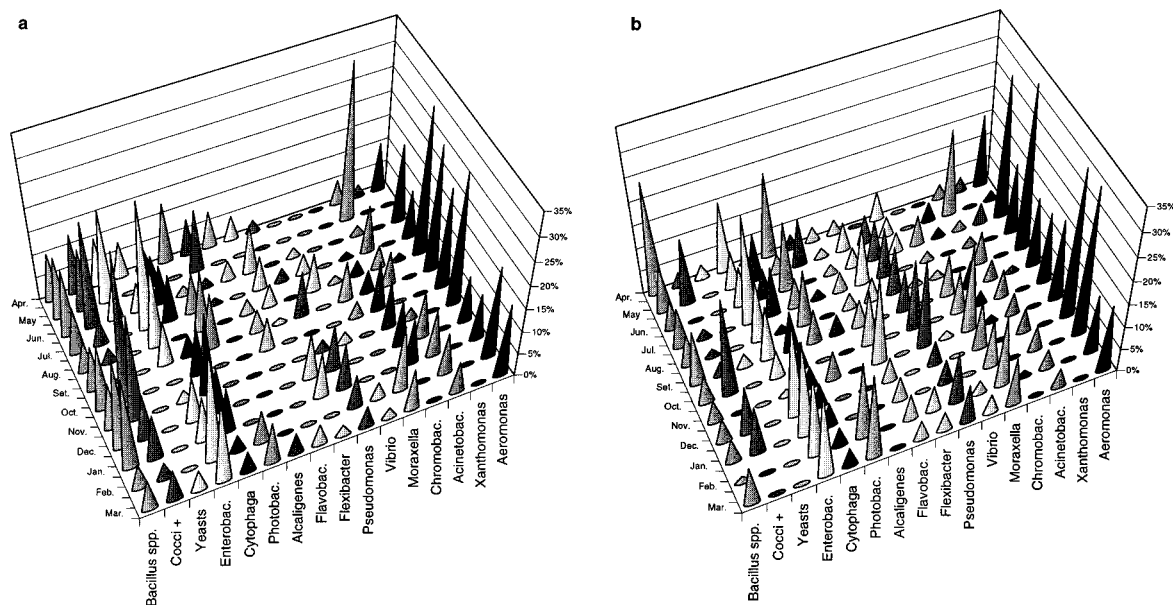


FIG. 7. – Annual trend of culturable heterotrophic bacterial diversity in Otranto at surface (a) and bottom (b).

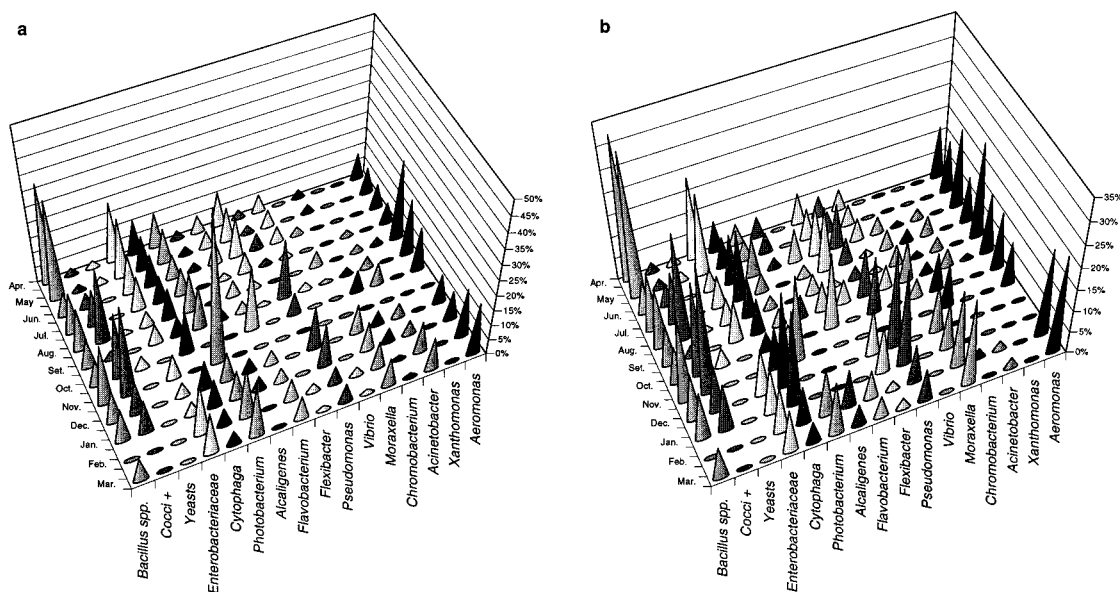


FIG. 8. – Annual trend of culturable heterotrophic bacterial diversity in Santa Maria di Leuca at surface (a) and bottom (b).

and *Flavobacterium* were identified with a total isolation percentage less than 6.5% in Brindisi and Otranto whereas in S. Cataldo the presence of *Cytophaga* (10.6%) and *Acinetobacter* (9.1%) was remarkable. Also in S. M. di Leuca the occurrence of *Cytophaga* (8.9%) was conspicuous. *Vibrio* was evidenced with a low mean isolation percentage and was more abundant in the January-February period in Brindisi both at the surface and the bottom. In S. Cataldo the highest isolation percentage occurred in October at the surface (8%) and in January at the bottom (7%). In Otranto the mean isolation percentage of *Vibrio* was 2% and the highest concentration was

found at the surface in August (8%). In S. M. di Leuca *Vibrio* showed a mean isolation percentage of 2.6% and was absent during the October-January period. In all the transects considered, among the Gram-positive bacteria, bacilli were predominant. Yeasts were occasionally isolated. From the analysis of the seasonal succession of the heterotrophic bacterial biodiversity it is evident that in Brindisi bacterial diversity was strongly reduced in May at the surface. During the other months no substantial differences in the bacterial community composition were observed. In S. Cataldo the bacterial community diversity was uniform throughout the year both at the surface and

TABLE 1. – Mean isolation percentages of the main bacterial genera present in the four transects of the Southern Adriatic Sea.

		Enterobacteriaceae	Cytophaga	Photobacterium	Flavobacterium	Pseudomonas	Vibrio	Moraxella	Acinetobacter	Aeromonas
Apr.	Brindisi	15	16	4	9	0	4	0	11	18
	S. Cataldo	12	11	9	14	5	0	2	17	9
	Otranto	11	3	16	5	1	3	0	4	13
	S. M. di Leuca	19	9	8	3	4	5	0	0	10
May	Brindisi	0	0	0	0	60	20	0	0	0
	S. Cataldo	8	12	8	8	8	0	4	24	8
	Otranto	4	8	2	1	3	2	0	26	1
	S. M. di Leuca	19	9	8	3	4	5	0	0	10
Jun.	Brindisi	17	1	10	4	6	3	3	9	14
	S. Cataldo	16	7	6	8	3	0	0	14	9
	Otranto	7	7	8	3	4	3	3	2	23
	S. M. di Leuca	9	11	8	8	8	5	3	1	13
Jul.	Brindisi	17	2	9	1	3	3	2	6	11
	S. Cataldo	16	4	5	2	3	1	1	13	8
	Otranto	25	10	8	2	7	2	1	6	13
	S. M. di Leuca	9	13	7	3	4	2	2	3	8
Aug.	Brindisi	14	8	7	3	4	2	3	3	12
	S. Cataldo	11	11	5	2	3	2	2	8	10
	Otranto	16	1	13	3	5	5	2	6	33
	S. M. di Leuca	6	10	8	5	2	1	5	3	24
Set.	Brindisi	10	15	5	11	5	2	5	0	11
	S. Cataldo	5	16	5	2	4	4	4	4	11
	Otranto	11	7	10	5	9	0	9	6	21
	S. M. di Leuca	8	7	9	5	15	4	5	4	16
Oct.	Brindisi	12	5	11	6	8	1	6	1	12
	S. Cataldo	9	7	6	9	6	6	7	5	15
	Otranto	3	8	0	10	8	0	6	3	18
	S. M. di Leuca	0	9	2	19	10	0	2	4	14
Nov.	Brindisi	3	2	3	8	11	4	9	2	27
	S. Cataldo	7	4	1	5	11	3	5	8	31
	Otranto	2	11	3	10	11	2	10	4	23
	S. M. di Leuca	4	5	35	0	0	0	0	0	5
Dec.	Brindisi	2	2	6	6	16	3	9	1	30
	S. Cataldo	3	16	5	4	20	2	6	2	14
	Otranto	16	8	0	3	8	0	5	8	21
	S. M. di Leuca	8	16	5	2	18	0	10	2	5
Jan.	Brindisi	12	0	15	4	5	13	4	4	28
	S. Cataldo	8	10	10	0	8	4	4	9	19
	Otranto	16	8	0	3	8	0	5	8	21
	S. M. di Leuca	8	16	5	2	18	0	10	2	5
Feb.	Brindisi	12	0	15	4	5	13	4	4	28
	S. Cataldo	4	15	7	6	9	3	3	5	17
	Otranto	22	4	12	3	9	3	15	7	16
	S. M. di Leuca	14	7	14	7	7	1	13	6	18
Mar.	Brindisi	15	16	4	9	0	4	0	11	18
	S. Cataldo	9	14	9	10	7	0	2	10	14
	Otranto	18	4	14	4	6	3	10	5	15
	S. M. di Leuca	12	5	15	6	7	1	15	7	20

at the bottom. In Otranto the bacterial diversity was unchanged throughout the year at the bottom whereas at the surface it decreased in May and increased in March. In S. M. di Leuca the bacterial community diversity declined drastically in November both at the surface and the bottom.

DISCUSSION

The present investigation contributes to the knowledge of culturable heterotrophic bacteria in the waters of the Southern Adriatic Sea along Italian Apulian coasts by the quantitative and qualitative

description of the variations over an annual cycle. The Southern Adriatic Sea is characterized by high hydrodynamic circulation related to rapid water exchange due to the overlapping of water masses having different thermoaline features and flowing in both directions, from the Northern Adriatic Sea and from the Ionian Sea in the Otranto Strait (Russo and Artegiani, 1996). The outgoing flow towards the Ionian Sea is made up of Adriatic Surface Waters (ASW) and overlapping Adriatic Deep Waters (ADW) present all along the Italian west coast. Inlet flow, instead, is composed of Ionian Surface Waters (ISW) and Levantine Intermediate Waters (LIW) found in the basin mainly on the Greek and Alban-

ian eastern coasts (Orlic *et al.*, 1992). In summer, the gap between these two water types is the greatest, which gives rise to a very strong out-flowing current from the Adriatic mainly in the Otranto Channel (Franco *et al.*, 1982). In winter the strongest current comes in along the eastern coast. In spring and autumn the strength of the incoming current equals that of the outgoing one. The overall circulation in the lower Adriatic Sea as well as the resulting trophic resources availability seem to affect the bacterial abundances in those transects of the Southern Adriatic Sea not exposed to allochthonous inputs (S. Cataldo, Otranto and S. M di Leuca). Particularly, in S. Cataldo and Otranto the minimum values in temperature coincided with the minimum values in bacterial densities and a positive relationship between the water temperature and the culturable heterotrophic bacterial counts were observed. Thus, the bacterial densities seemed limited by temperature during non-summer seasons. Nevertheless temperature is not the only causation in reducing bacterial densities in the winter period in these two transects of the Southern Adriatic Sea. Pomeroy and Wiebe (2001) provided indirect evidence that the temperature and substrate concentration are potential limiting factors that interact in temperate ocean surface waters and estuarine waters. Limiting resources for bacteria are, typically, labile carbon substrates as well as inorganic and organic N and P (Pace and Funke, 1991). Bacteria are strong competitors with phytoplankton for inorganic nutrients (Jürgens and Gude, 1990; Suttle *et al.*, 1990). In such a competition, in winter, phytoplankton prevailed; in fact a vernal phytoplanktonic bloom was evidenced in the Southern Adriatic Sea (Caroppo *et al.*, 1999).

In summer, the temperature was favourable to the bacterial growth as well as the substrate supply increase. In this season the effects of the hydrological features determined the very low dissolved inorganic nutrient concentration which leads to low phytoplankton biomass (Caroppo *et al.*, 1999; Gacic *et al.*, 1996). The lack of nutrients could favour a trophic web consisting of small cells as producers, as pico- and nanoplankton are more efficient in the utilization of the resources in oligotrophic systems (Thingstad and Sakshaug, 1990). In these conditions, regenerative dissolved organic phosphorus (DOP) and nitrogen (DON) may represent an important source of nutrients available for the culturable bacteria. In fact, the presence of DOP has been documented in this area typically in this period of the year (Civitarese *et al.*, 1998).

S. M. di Leuca, because of its geographical position, at the extreme point of the Apulia, is influenced by both the incoming ASW from the Northern Adriatic Sea and the incoming LIW from the Ionian Sea both furnishing resources. The ASW, coming from a basin heavily supplied by rivers, features low salinity and high levels of dissolved nutrients, as well as high suspended matter concentration (Fonda Umani *et al.*, 1992; Franco and Michelato, 1992). The LIW is relatively rich in nutrients (Civitarese *et al.*, 1998). Thus, in this site the continuous loading of nutrients stimulates the bacterial growth, masking the effect of the other environmental factors, including temperature. This situation could explain the absence of a significant relationship between the culturable bacteria density and the temperature in S. M. di Leuca.

The situation recorded in Brindisi was quite different. Brindisi is an industrial city with a touristic and commercial port which is set in a bay receiving considerable amounts of allochthonous inputs (industrial and agricultural wastes). These furnish trophic resources throughout the year and stimulate bacterial growth independently from the environmental factors. This situation could explain the scarce modifications in the bacterial abundances observed throughout the sampling year at this transect and the absence of a significative relationship between the bacterial density and the temperature.

In recent years, preserving the world's biodiversity has become a priority in environmental studies. Because of bacterial importance in the marine environment it is vital to understand the full extent of bacterial diversity and the roles of the most abundant species. The use of both cultivation-based and cultivation-independent approaches has provided new perspectives on the nature of bacterioplankton communities in the sea over the past decade (Giovannoni *et al.*, 1990; Suzuki *et al.*, 1997). Major groups that contain cultivable members include the gamma proteobacteria (i.e. *Vibrio* spp., *Pseudoalteromonas* spp. and *Pseudomonas* spp.), the Flexibacter, Bacteroides and Cytophaga phylum (i.e. *Cytophaga* spp. and *Flavobacterium* spp.), the Cyanobacteria (*Synechococcus* spp. and *Prochlorococcus* spp.) and the α -proteobacteria. In our study some genera representative of these groups were isolated. *Aeromonas* spp. prevailed among the Gram-negative bacteria identified. Aeromonads are among the most abundant bacteria found in aquatic environments. They also belong to the flora of fish, amphibia and other marine organisms (Shotts *et al.*, 1972; Kueh and

Chan, 1985). Bacterial infections caused by motile members of the genus *Aeromonas* are among the most common and troublesome diseases in fish. These Aeromonads are facultative, which means they are capable of utilizing nutrients present in water and surviving for long periods in the absence of host fish (Camus *et al.*, 1998). Enterobacteriaceae were quite abundant during the whole sampling period. A similar pattern was seen for *Cytophaga*, *Pseudomonas* and *Photobacterium*. The enzymatic versatility of these genera is well known and has been suggested as an explanation of their importance in particle turnover (De Long *et al.*, 1993). As naturally occurring bacteria, *Cytophaga* spp. are capable of the biodegradation of natural polymers such as cellulase, pectin, keratin, agar and chitin as well as the degradation and purification of organic compounds such as phenol, o-cresol and orcinol. The isolation of *Cytophaga* in our work is in good agreement with other studies that report frequent isolation of this genus in marine samples. Members of the *Cytophaga-Flavobacterium* group usually formed a large bacterial group in marine water. The relative abundance ranged from 2% in the Baltic Sea to 72% in the Antarctic Ocean (Glöckner *et al.*, 1999). *Pseudomonas* represents a fraction of the total microbial population characterized by high metabolic versatility: this genus plays an important role in the mineralization of organic matter and in nitrogen cycling and it is known for its capacity to degrade a considerable quantity of synthetic organic compounds. *Pseudomonas* is often encountered in sea water, sediments, phytoplankton and zooplankton (Nair and Simidu, 1987). *Photobacterium* are usually found in marine environments, hence the requirement of sodium for growth. They may be free-living in the water, or found associated with intestinal contents of marine animals and with the light organs of fish (Baumann and Baumann, 1984).

The presence of *Acinetobacter*, *Chromobacterium*, *Flexibacter*, *Moraxella* and *Vibrio* was also remarkable. Members of the genus *Acinetobacter* are usually isolated in water. They are capable of degrading aromatic compounds and their metabolic versatility is comparable to that of the Pseudomonads (Abd-El-Haleen *et al.*, 2002). *Chromobacterium* is ubiquitous in nature and found in waters from polar regions to the tropics. *Flexibacter* is abundant near the shore, on seaweeds and on decaying sea animals. *Moraxella* plays an important role in nitrogen cycling as well as *Vibrio* (Stolp, 1988). *Vibrio* occurs in saline aquatic environments, both free in

the water and bound to animate and inanimate surfaces. These microorganisms are readily culturable from seawater, marine animals and seaweeds. Several studies on the role of *Vibrio* spp. in the marine environment have shown their importance in biodegradation, nutrient regeneration and biogeochemical cycling (Ducklow, 1983; Jørgensen, 1983; Colwell, 1994). In conclusion, in the Southern Adriatic Sea, the remarkable bacterial density and diversity observed throughout the year together with the high metabolic versatility of the identified genera shows the ability of the bacterial compartment to utilize the scarce trophic resources of such an oligotrophic ecosystem to their best: the different bacterial genera act synergically to accomplish complete degradation of the trophic substrates.

Finally, on the basis of the results obtained it is difficult to explain the reduction of bacterial diversity observed in May in Brindisi and Otranto and in November in S. M. di Leuca, as well as the increase observed in March in Otranto. It is well known that consumption or lysis by predators and viruses is considered a key control of the dynamics of bacterial communities. Probably such a phenomenon could justify the variations in bacterial diversity observed. Thus, further studies will be carried out to establish the eventual role of grazing and/or other factors, until now not well defined, on controlling bacterial distribution and diversity.

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