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Dissecting the microbial food web: structure and function in the absence of autotrophs

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ABSTRACT: The balance between organic matter producers and consumers defines the metabolism of aquatic ecosystems. Therefore, we can discriminate between net autotrophic and net heterotrophic ecosystems on the basis of the metabolic predominance of either group. Net heterotrophic systems need allochthonous inputs of organic matter. As a model of a heterotrophic ecosystem, we analyzed the structure and function of the microbial food web in littoral cave lagoons of Mallorca (Spain), where trophic food webs in the absence of light are sustained by organic matter, which enters the system through seepage. We compared the microplanktonic food web of these lagoons with that of net heterotrophic ecosystems which receive both autochthonous and allochthonous organic matter. The upper part of the water column has a similar structure and function to that of the oligotrophic open sea, but with a lower abundance and biomass of organisms, with an average of 4.7×10^5 heterotrophic bacteria (HB) ml^{-1} and 162 heterotrophic nanoflagellates (HNF) ml^{-1}. The deep zones are similar to that of the deep sea, with an average bacterial abundance of 2.9×10^5 HB ml^{-1} and 48 HNF ml^{-1} and bacterial production values as low as 0.03 µg C l^{-1} d^{-1}.

KEY WORDS: Microbial food web · Electron transport system activity · Structure · Function · Cave lagoons · Heterotrophy

INTRODUCTION

The planktonic food web is composed of autotrophs, such as phytoplankton and autotrophic bacteria, and heterotrophs, such as bacteria, Protozoa and meso- and metazooplankton, which play contrasting roles in the ecosystem as producers (autotrophs) or consumers (heterotrophs) of organic matter (e.g. Sherr & Sherr 1988, Azam et al. 1993). The balance between these 2 trophic modes determines the net metabolism of the community, i.e. whether autotrophic production exceeds respiration, or heterotrophy prevails over autotrophic processes. Although planktonic ecosystems may be out of balance at a given point such as during the wax and wane of coastal phytoplankton blooms (Sorokin 1977), oligotrophic systems are generally considered as slightly net heterotrophic over an annual cycle (Duarte & Agustí 1998). The main source of the organic matter in planktonic communities is the production by phytoplankton, which is channelled into the food web either directly through grazing, or indirectly via the bacterial utilization of dissolved organic carbon (DOC). Bacteria are also the main entry point for allochthonous DOC into planktonic food webs, and therefore play a main role as carbon sources for the community. The relative importance of bacteria as sinks (Ducklow et al. 1986, Pomeroy & Deibel 1986, Pace et al. 1990, Moloney et al. 1991) or links (Pace et al. 1984, Vézina & Platt 1988) in the carbon flow of food webs is still under discussion. However, experimental studies to date have not been able to resolve the role of bacteria as entry points of the allochthonous organic matter in structuring food web dynamics, as the bacterial carbon derived from autochthonous versus allochthonous production cannot be readily discriminated.

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Here we examine the transfer of allochthonous DOC by bacteria in the food web by focussing on an extreme environment, cave lagoons, where DOC derived from allochthonous production is the only source of carbon for the microbial food web. We focused, in particular, on differences in the structure and activity of the planktonic food web in the cave system in relation to the open coastal waters. The environmental conditions and the abundance and interactions of bacteria and heterotrophic nanoflagellates were studied in 5 cave lagoon systems on the island of Mallorca (Mediterranean Sea, Spain). In these systems, the input of organic material is largely restricted to allochthonous input. Although some chemosynthesis may occur, as suggested for other anchihaline caves (Yager 1991, Yager & Humphreys 1996), this is limited by the long residence time of the waters.

MATERIALS AND METHODS

Study area. The calcareous deposits of the Balearic Islands favor the formation of caves and other karstic structures, some being very close to the coastline and thus supplied with both seawater and freshwater. The southeast coast of Mallorca has numerous cave systems containing brackish pools with water masses of different salinity and oxygen concentrations. We selected 5 of these cave lagoon systems for the present study: Es Serral (ES), Sa Gleda (SG), Cala Varques (CV), Es Pont (EP) and Cala Falcó (CF) (Fig. 1).

The cave lagoons studied are shallow, ranging from 1.5 m water depth at EP to 7.0 m at CV. They are located inland close to the coastline, CV and CF being at 50 and 10 m distance from the seashore, respectively, ES 0.25 km, EP 0.50 km and SG 1.5 km away. The topography of CF, CV and EP is described in Trias & Mir (1977). SG has been described as the largest aquatic cave in Europe, with up to 10.5 km of submerged passages and chambers explored so far.

The studies of coastal caves mainly focus on their hydrogeology (Herman et al. 1985, Smart et al. 1988, Humphreys et al. 1999), zoology and biogeography (Stock 1981, Sket 1994, Jaume & Boxshall 1996, 1997), with only limited ecological work done thus far (Camacho 1992). Early studies on the Protozoa of cave systems only included a small list of identified organisms (Kofoid 1899). However, physico-chemical changes, meromixis and their influence on the food web structure are barely investigated. The physico-chemical aspects, such as dissolved Fe distribution (Martínez-Taberner et al. 2000) and the distribution of organisms, have only recently been studied (Palmer 1986, Humphreys 1999, Carey et al. 2001).

Sampling. The 5 cave lagoons were sampled from November 1996 to June 1999. ES and SG were studied over a seasonal cycle, and the other 3 were studied sporadically. Vertical profiles of salinity, conductivity, temperature and dissolved oxygen were measured with WTW sensors at 10 to 25 cm intervals. Water samples were obtained using a sampling tube with a double cone at the end and connected to a peristaltic pump (Miracle et al. 1992).

Water samples were used to determine pH (Crison 501), alkalinity (Strickland & Parsons 1972, Golterman et al. 1978), total organic carbon (TOC-5000 A, Shimadzu), the cations Ca, Mg, Na, K, Fe and Sr (ICP-Plasma 2000, Perkin-Elmer) and suspended matter concentration

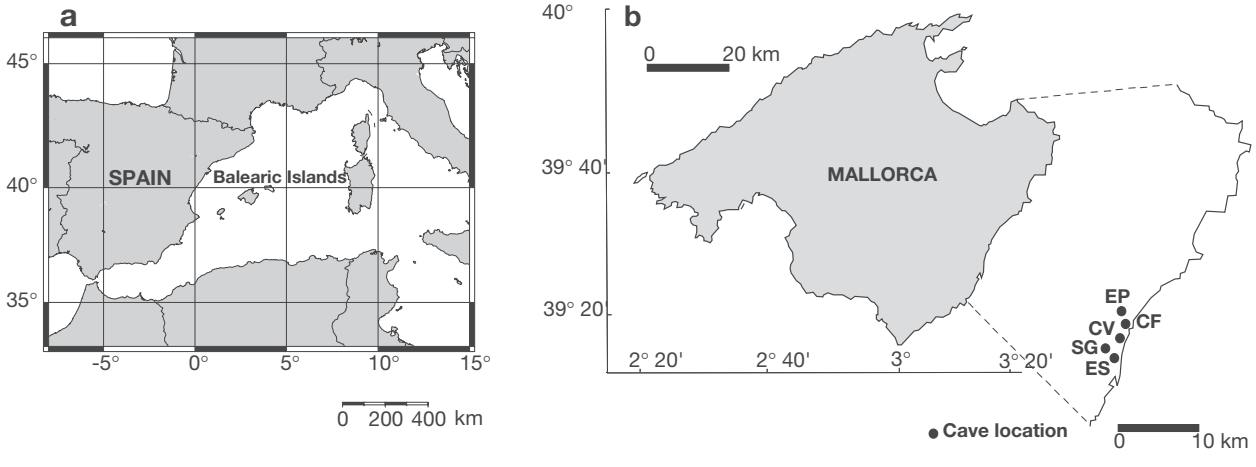

Fig. 1. Location of the 5 cave lagoon systems studied on the Mallorca coastline. (a) General location of the Balearics in the western Mediterranean Sea; (b) detailed map of Mallorca (extracted from Martínez-Taberner et al. 2000), a section of the coastline is magnified to better visualize the location of the caves (CF: Cala Falcó; CV: Cala Varques; ES: Es Serral; EP: Es Pont; SG: Sa Gleda)

(APHA-AWWA-WPCF 1989). For the suspended matter pool, organic and inorganic matter content were determined by burning organic matter at 550°C. In addition, the abundance and biomass and activity of planktonic microorganisms were determined.

Abundance and biomass of planktonic microorganisms. Heterotrophic bacteria (HB) and nanoflagellate (HNF) abundance were determined by epifluorescence microscopy (Porter & Feig 1980). A 60 ml water sample was fixed with formaldehyde (1% final conc.) and stained with DAPI (5 µg ml^{-1}, final conc.). Ten to 15 ml were filtered onto 0.2 µm black polycarbonate filters and 40 ml onto 0.4 µm black polycarbonate filters. The filters were stored frozen for enumeration by epifluorescence microscopy with an Olympus IM. The bacterial biomass was obtained by applying the formula of Norland (1993), assuming an average cellular volume of 0.048 $µm^3$ $cell^{-1}$ for coastal northwestern Mediterranean Sea bacteria (Vázquez-Domínguez 1999). The HNF biomass was obtained by measuring 20 to 100 cells per sample and estimating the average cell volume by approximation to the nearest geometric shape. The cellular volume was then multiplied by the abundance and converted to carbon biomass using 220 fg C $µm^{-3}$ (Børsheim & Bratbak 1987).

Ciliate abundance was evaluated in the samples collected at CV by sedimentation and inverted microscope enumeration (Utermöhl 1958). Water samples (1 l) were fixed with acid Lugol's solution (1% final conc.) and allowed to settle in the bottles for at least 48 h; 800 ml of the supernatant were extracted and from the remaining volume 100 ml aliquots were sedimented in chambers for 48 h. The biomass was calculated from the average cell volume (determined by approximation to the nearest geometric shape in 20 to 100 ciliates), the abundance and applying the carbon conversion factor of 0.2 pg C $µm^{-3}$ (Putt & Stoeckner 1989).

Respiratory activity measurements. The respiratory activity of plankton was determined by measuring the activity of the electron transport system (ETS) (Packard & Williams 1981). Water samples of about 5 l were filtered on Whatman GF/F glass fiber filters, which were frozen immediately in liquid N_2 and stored at –70°C until analysis. Filters were processed within 1 yr.

Parallel measurements of respiration by the decrease in dissolved oxygen concentration in BOD-flasks during the course of incubation were conducted on 2 occasions. The relationship between respiration measurements obtained by ETS activity measurement (independent variable) and by the decrease in dissolved oxygen gave different slopes of the linear regression equation for the different caves sampled (Sintes 2002), due to the different organisms and physiological state.

Bacterial production measurements. Bacterial production was determined via the uptake of [^{3}H]-leucine ([^{3}H]-Leu) (Smith & Azam 1992). Four replicate samples and 2 controls (with 5% TCA final conc.) of 1.2 ml each were incubated in Eppendorf tubes with [^{3}H]-Leu (40 nM final conc.) in the dark at *in situ* temperature for 4 h. TCA (5% final conc.) was added to terminate the incubations and the Eppendorf vials were centrifuged (12 000 × *g* for 10 min), the supernatant discarded and the pellet resuspended in 5% (v/v) TCA; the latter step was repeated 3 times. Finally, the pellet was resuspended in 0.5 ml scintillation cocktail and allowed to sit for 24 h; thereafter, the radioactivity was determined in a Beckman scintillation counter. The uptake of [^{3}H]-Leu was calculated and converted to bacterial carbon production according to Sommaruga & Psenner (1995).

Determining the grazing activity of Protozoa. Bacterivory of protists was evaluated by the rate of disappearance of fluorescent particles (Pace et al. 1990, Vaqué et al. 1992). Duplicate 1 l samples were transferred into polycarbonate bottles with a known concentration of tracers (DTAF-stained *Escherichia coli* minicells, provided by E. Vázquez). Samples were mixed and incubated at 19°C in the dark and 60 ml aliquots were taken at 0, 24 and 48 h and fixed with glutaraldehyde (1% final conc.). Ten and 40 ml subsamples were DAPI-stained and filtered on 0.2 and 0.4 µm polycarbonate filters, respectively, for enumeration of bacteria and DTAF-stained particles, and HNF. The grazing rate (g, d^{-1}) and the net growth rate (µ, d^{-1}) were calculated according to the formulas of Salat & Marrasé (1994):

$$g = \frac{-1}{t \times \ln\left(F_t / F_0\right)} \quad (1)$$

$$\mu = \frac{1}{t \times \ln\left(N_t / N_0\right)} \quad (2)$$

where F_t and F_0 are the number of tracers at times t and 0, respectively, N_t and N_0 are the number of natural bacteria at times t and 0. The number of grazed bacteria (G, HB ml^{-1} d^{-1}) was calculated from the trace particles consumed and the bacterial abundance:

$$G = \frac{-g}{\mu \times (N_t - N_0)} \quad (3)$$

Total grazed bacteria (TG) was then calculated as:

$$TG = F_0 - F_t + G \quad (4)$$

The net growth rates of nanoflagellates (µ, d^{-1}) were calculated as those for bacteria, from the increase in abundance of flagellates over time assuming exponential growth.

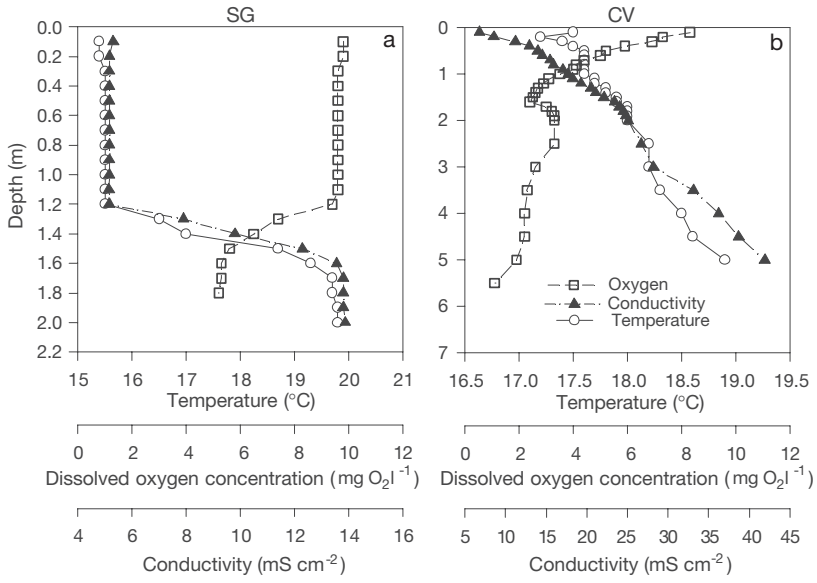

Fig. 2. Physico-chemical differences in the structure of the water column in the cave lagoons. (a) Presence of an intense halocline and oxycline in SG in February. (b) Progressive clines in CV in February. See Fig. 1 for cave abbreviations

RESULTS

Seasonal dynamics in the cave systems

Physico-chemical characteristics. All cave lagoons studied showed conductivity and salinity gradients with values increasing with depth reaching near marine conductivity values at near-bottom waters. ES, SG and EP had intense clines, exemplified for SG in Fig. 2a, which caused meromixis and a layering of the water column in 3 zones: an upper layer where conductivity increased only slowly (mixolimnion or epilimnion), a chemocline where conductivity increased sharply, and a lower layer where conductivity values approached marine values. In contrast, CV and CF did not exhibit such sharp clines and conductivity increased steadily from the surface to near-bottom waters of the cave lagoons as shown for CV in Fig. 2b.

Salinity varied little in the layers below the chemocline in all caves over the different seasons (maximum variation 2.2), while in surface waters it varied by up to 6.0.

Vertical profiles of the other variables also exhibited a vertical structure. Temperature showed a profile similar to that of conductivity, and dissolved oxygen decreased from the surface to the deeper waters of the cave lagoons. Temperature varied between 15 and 20°C, with larger fluctuations in the surface waters, while deeper cave waters maintained almost constant temperature, ranging from 19 to 20°C throughout the different seasons. Dissolved oxygen concentrations decreased with depth (Fig. 2, Fig. 3a), reaching low values towards the bottom of the caves (minimum 21% saturation). Generally, even the upper water column was slightly undersaturated in oxygen for all seasons; only EP had slightly supersaturated oxygen concentrations at the surface in February (Fig. 3b). Concentrations of iron were below the detection limit of the method (<11.64 nM) in most of the lagoons. A remarkable exception was EP in the winter, with up to 137.88 nM in the mixolimnion (Table 1). TOC concentration ranged from <21 to 1655 µM for all lagoons. Highest concentrations were obtained in CV in the summer and in SG in the winter at or above the chemocline (Table 1), although sometimes TOC concentrations increased again towards the deeper waters of the cave lagoons.

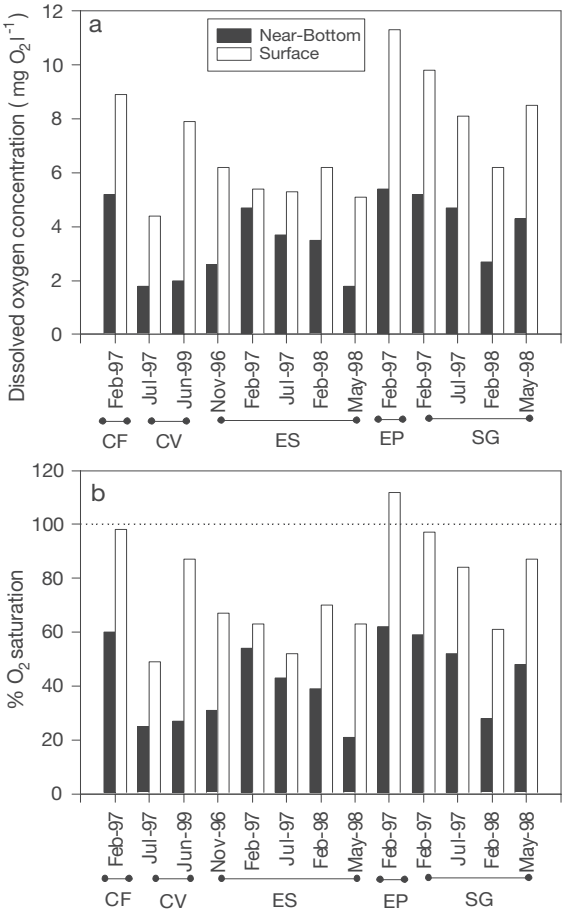

Fig. 3. Surface and near-bottom (a) dissolved oxygen concentrations and (b) saturation level in the different caves and seasons. The caves are arranged in increasing distance to the coastline. See Fig. 1 for cave abbreviations

Total suspended matter concentrations ranged from 0.82 to 29.30 mg l^{-1}, with a contribution of organic matter from 3.6 to 25.3%. TOC and the suspended organic matter concentrations showed a similar vertical distribution although they were not correlated ($r^2 = 0.04$).

Microbiological characteristics. Bacterial abundance peaked in the surface water of SG in the winter, while in the chemocline and the monimolimnion bacterial abundance increased from winter towards the summer (Fig. 4b, Table 1). ES exhibited, however, a higher bacterial abundance in May than in the other months (Fig. 4a). A higher bacterial abundance was usually found in the surface waters of the cave lagoons, decreasing rapidly below the chemocline, as exemplified for SG in Fig. 5c. Occasionally, highest bacterial abundance was observed at the chemocline (Fig. 5a) as well as near the bottom (in ES) (Fig. 5a,b).

HNF abundance peaked in the surface waters and generally showed a vertical distribution similar to that of bacteria, as exemplified for CV in Figs. 6 & 7. Ciliates were present at very low concentrations in CV; the highest abundance was found in the surface waters, decreasing to non-detectable levels deeper in the water column of this cave lagoon (Fig. 6).

Respiration rates assessed via ETS measurements. ETS values were usually lower in the caves along the coastline than further inland (Table 1). The vertical profile of ETS activity showed 2 different patterns: one with highest values at the surface waters and also near the bottom (in ES) (Fig. 5b) and almost non-detectable levels at intermediate depths and at the chemocline. The other pattern showed highest

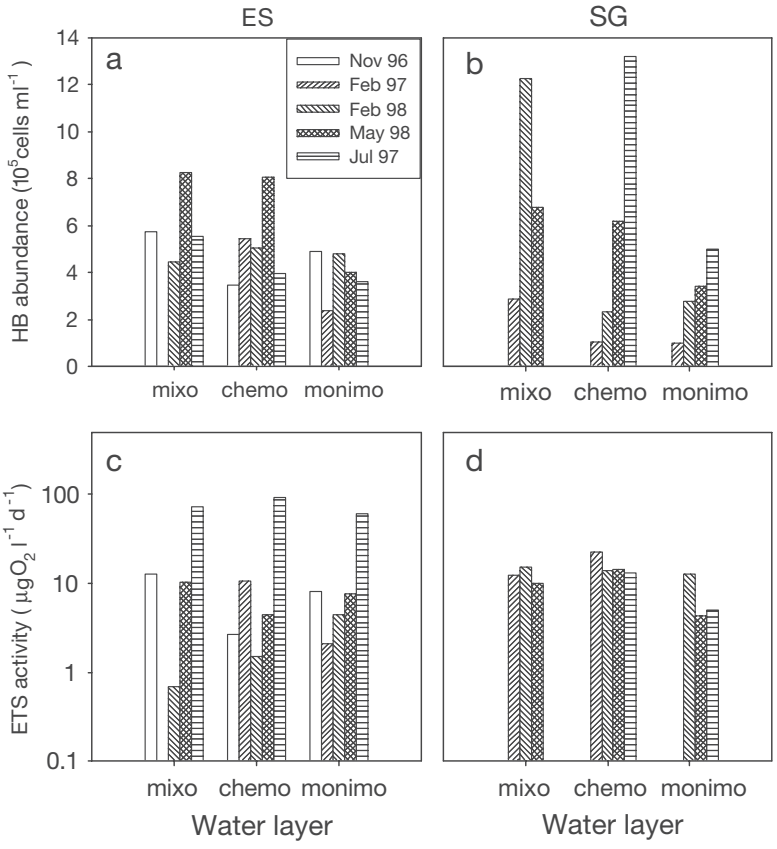

Fig. 4. Seasonal dynamics in (a,b) heterotrophic bacterial (HB) abundance and (c,d) electron transport system (ETS) activity in the different water layers of cave lagoon (a,c) ES and (b,d) SG. Mixo: mixolimnion; chemo: chemocline; monimo: monimolimnion. See Fig. 1 for cave abbreviations

Table 1. Chemical and biological parameters in cave lagoons (mean and range). Temp: temperature (°C); Cond: conductivity (mS cm^{-2}); Alk: alkalinity (meq l^{-1}); Fe: inorganic iron concentration (nM); TOC: total organic carbon (µM); ETS: electron transport system activity (µg O_2 l^{-1} d^{-1}); BA: bacterial abundance (10^5 heterotrophic bacteria ml^{-1}); BB: bacterial biomass (µg C l^{-1}); HNFA: heterotrophic nanoflagellate abundance (HNF ml^{-1}); HNFB: heterotrophic nanoflagellate biomass (µg C l^{-1}) in the different caves (CF: Cala Falcó; CV: Cala Varques; ES: Es Serral; EP: Es Pont; SG: Sa Gleda). Sea: seawater sample for comparison was collected outside at Cala Falcó; nd: not determined

		Temp	Cond	Alk	Fe	TOC	ETS	BA	BB	HNFA	HNFB
Increasing distance to the sea ↓	Sea		51.70	2.57	<11.64	39		5.26	7.53		
	CF	19.6	18.90	5.16	<11.64	91		3.32	4.76		
		17.7–20.0	13.70–20.50	5.07–5.25		56–128	nd–10.83	3.29–3.36	4.72–4.81		
	CV	17.8	25.88	5.47				3.23	4.63	121	75.31
		14.4–19.7	6.80–46.20	2.54–7.50	<11.64–28.65	<21 –1655	nd–20.04	0.69–9.85	0.99–14.11	5–474	68.50–82.38
	ES	19.4	24.07	5.24				3.03	4.34	116	143.78
		15.9–19.7	17.54–26.00	4.91–5.97	<11.64–11.64	<21–814	nd–126.25	0.34–7.99	0.49–11.45	24–325	109.08–209.52
	EP	17.6	9.30	4.49	70.37	42	13.08	2.71	3.89		
		15.7–19.9	3.12–18.82	2.99–5.28	30.44–137.88	37–50	1.20–24.96	0.74–6.40	1.06–9.17		
	SG	17.3	10.90	5.48	<11.64			4.52	6.48	93	539.64
		15.0–19.9	5.20–32.90	5.05–5.92		<21–1159	nd–22.23	0.98–12.25	1.40–17.55	64–116	15.13–4461.93

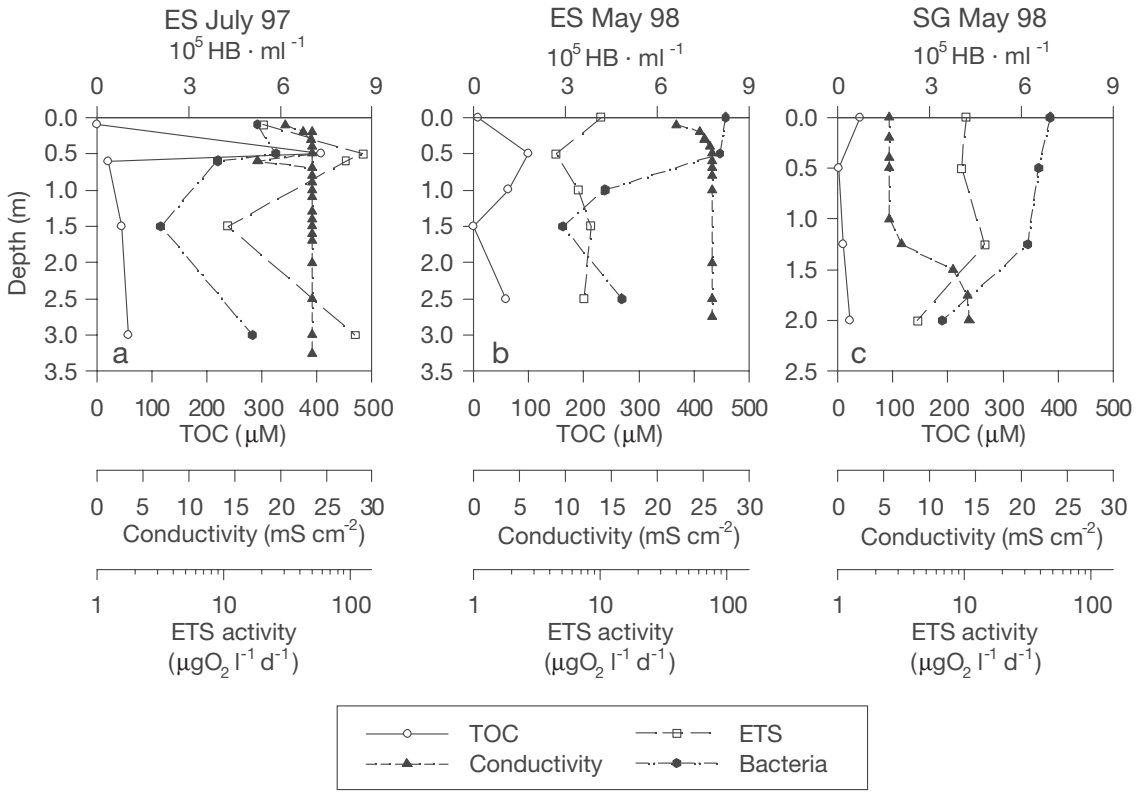

Fig. 5. Vertical distribution of heterotrophic bacterial (HB) abundance, electron transport system (ETS) activity and total organic carbon (TOC) in relation to the water column structure. (a) ES in July 97; (b) ES in May 98; (c) SG in May 98. For water column characterization, the dynamics of the conductivity are also shown. See Fig. 1 for cave abbreviations

activities at the surface and the chemocline (in SG). One irregularity was the high ETS activity at 50 to 60 cm depth in the summer, associated with high suspended matter concentrations in ES (Fig. 5a). ETS activity also showed a high seasonal variability in all cave lagoons, peaking in summer in ES (Fig. 4c) and in SG during the winter (Fig. 4d).

Spatial dynamics in microbiological parameters in CV

Dynamics in bacterial production. Data on bacterial production are scarce as it was measured only once in the summer in CV. The vertical profile of the bacterial production in CV is related to the meromixis of this lagoon, with higher production rates above the chemocline and decreasing drastically below this layer. Bacterial production above the chemocline ranged from 29.97 to 42.23 µg C l^{-1} d^{-1} and dropped at the chemocline to 2.89 µg C l^{-1} d^{-1}, while production ranged from 0.37 to 0.03 µg C l^{-1} d^{-1} below the chemocline (Fig. 7).

Bacterivory. Bacterivory was evaluated only once in the summer in CV. It was fairly constant down to 1.5 m depth, ranging between 2.9 and 4.5 × 10^5 HB ml^{-1} d^{-1}, and then sharply decreased at the chemocline to 1.2 × 10^5 HB ml^{-1} d^{-1} (Fig. 6).

Bacterial and protozoan net growth rates determined from the bacterivory experiments. The growth rate of HB during summer (Fig. 8) showed a similar distribution as bacterial production (Fig. 7), but with a slight decrease at the mixolimnion at 50 cm depth. A sharp decrease occurred at the chemocline, where even negative growth rates were obtained, despite the high bacterial abundance (see 'Discussion').

Growth rates of HNF showed a similar vertical distribution to their rates of bacterivory, also decreasing at 50 cm depth (Fig. 8). At the chemocline, the decrease was sharp, reaching –0.5 d^{-1}.

DISCUSSION

Characterization of the cave lagoons

Coastal cave lagoons are characterized by salinity gradients with highest salinities in near-bottom waters associated with the intrusion of marine waters and salinity minima near the surface due to freshwater influence. This causes a meromixis of the water

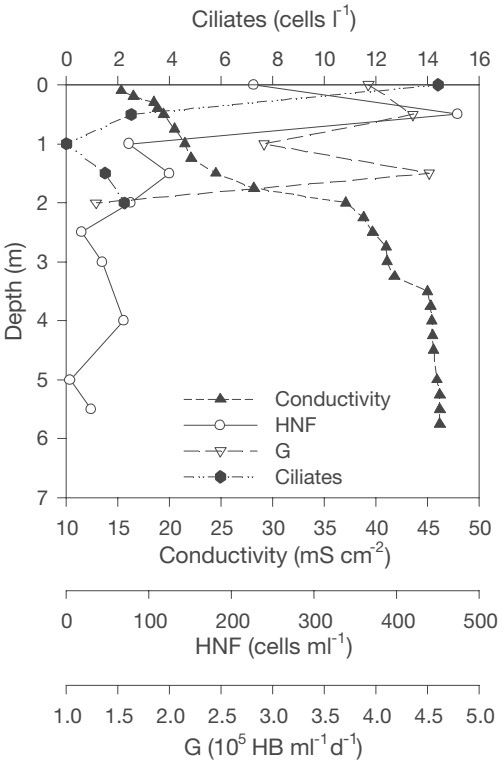

Fig. 6. Vertical profile of heterotrophic nanoflagellate (HNF) and ciliate abundance, and grazed bacteria (G) in cave lagoon CV (Cala Varques) in June. Conductivity is also shown

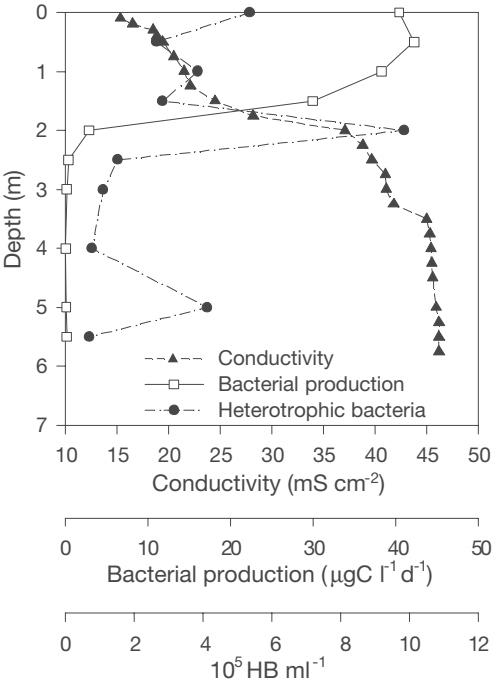

Fig. 7. Vertical profiles of bacterial production and heterotrophic bacterial (HB) abundance in CV (Cala Varques) in June. For water column characterization, the dynamics of the conductivity are also shown

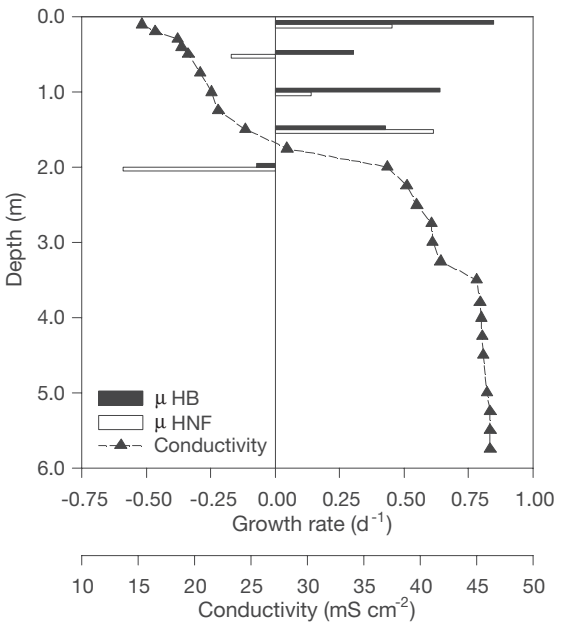

Fig. 8. Heterotrophic bacterial (HB) and nanoflagellate (HNF) growth rates in CV (Cala Varques) in June. Conductivity is also shown

column (Margalef 1983) with an upper layer where salinity increases slowly, the mixolimnion, and a chemocline with a sharp increase in salinity, and a lower zone with salinity values close to typical marine values (33.5 in CV). The structure of the water column derived from the salinity gradient allows the distinction of 2 classes of caves (Martínez-Taberner et al. 2000): one with intense haloclines such as SG, EP and ES (Fig. 2a), and others with smooth and progressive clines, such as CV and CF (Fig. 2b). Vertical profiles of the other parameters are determined by this vertical profile in salinity. Temperature generally shows a profile similar to that of salinity and dissolved oxygen a gradient with surface maxima. Iron, if detectable, is highest at the surface, probably associated with input from freshwater sources.

Significant levels of dissolved Fe were detectable only in EP (Table 1), in the range of 5 to 759 nM, where some iron bacteria have been reported (Halbach et al. 2001), although growth based on Fe at circumneutral pH is believed to occur only at concentrations higher than 10 µM (Emerson 2000). In this cave lagoon, higher levels of ETS activity and bacterial abundance occur despite the lower TOC concentrations. Chemoautotrophy could also be due to filamentous sulfur bacteria, as suggested for other anchihaline cave systems (Pohlman et al. 1997); however, this has not been tested in the present study and requires further investigation.

TOC concentrations varied over a remarkable range in these systems (Table 1), while in the open Mediter-

ranean Sea DOC concentrations only vary over a narrow range (50 to 80 µM) (Copin-Montegut & Avril 1993). In the open water column, TOC commonly closely resembles DOC concentrations (G. J. Herndl pers. comm.); however, in these cave systems turbulence is generally low, leading to a larger heterogeneity of the TOC concentrations in the water column (see Fig. 5). The occasionally extremely low TOC concentrations of <21 µM particularly in the deep waters of the cave systems which resembled, in terms of salinity and temperature, closely open Mediterranean waters might indicate adsorption and/or biological removal of TOC from infiltrating surface Mediterranean Sea water through the porous karst. Unfortunately, no data are available on the infiltration rate and the residence time of infiltrating Mediterranean Sea water in the karst.

Water column structure and the microbial community

Microbial food web structure and processes were also influenced by the physical structure of the water column. Microorganisms were generally more abundant and active in the layers above the chemocline, where a higher protozoan biomass occurred mainly near the surface, as observed also for other stratified systems (Fenchel et al. 1990). Bacterial biomass increased at the chemocline but, concomitantly, bacterial production decreased. This decline in bacterial production is reflected in the negative net growth rate, and low ETS activity was also measured at the chemocline. Taken together, these results indicate that a rather inactive senescent bacterial community accumulated at the chemocline. In the monimolimnion, both bacteria and protozoan abundance were low, although some increase in bacterial numbers was detectable near the bottom, probably associated with resuspension of sediment and/or outwelling of nutrients from the sediment.

Although we have not measured the grazing activity of protozoans below the chemocline, the bacterial abundance in the monimolimnion was usually lower than10^5 ml^{-1}, which is below the minimum density of food particles required to support protozoans according to Fenchel (1980, 1982a,b); others, however, have found lower thresholds (e.g. Cho et al. 2000). In these layers of the water column, HNF abundance varied between 4 and 69 cells ml^{-1}, values similar to those obtained for the deep Atlantic (Patterson et al. 1993) and mesopelagic waters of the NW Mediterranean Sea (Tanaka & Rassoulzadegan 2002). The abundance of ciliates in the mixolimnion and chemocline was in the range given for deep NW Mediterranean waters (Tanaka & Rassoulzadegan 2002).

If we apply the qualitative model of Gasol (1994) to determine the control of HNF (Fig. 9), we obtain a lower abundance of HNF than theoretically expected based on the bacterial abundance detected, indicating top-down control of the HNF by ciliates or, more likely, other predators such as crustaceans. Although these cave lagoons are oligotrophic systems, there is indication that predators control HNF abundance, a situation commonly reported for eutrophic systems. This apparent discrepancy might indicate that Gasol's (1994) model is not applicable to these particular environments as data sets from such environments were not included in the original model. Another explanation of the observed pattern might be that a large fraction of the DAPI-stained particles was inactive or dead cells, not grazed or grazed only at a lower efficiency by HNF than highly active cells (Sherr et al. 1992, López-Amorós et al. 1998). In addition, the protozoans observed in these layers could complement their diet with detritus or colloidal DOC (Sherr 1988).

We found 2 patterns in the vertical distribution of ETS: one with maxima at the surface and in near-bottom waters, and the other with maxima near the surface and above the chemocline corresponding to the peak abundance in microorganisms and TOC concentrations. However, no direct correlation between ETS activity and TOC was obtained, probably due to the fact that TOC comprises primarily refractory material.

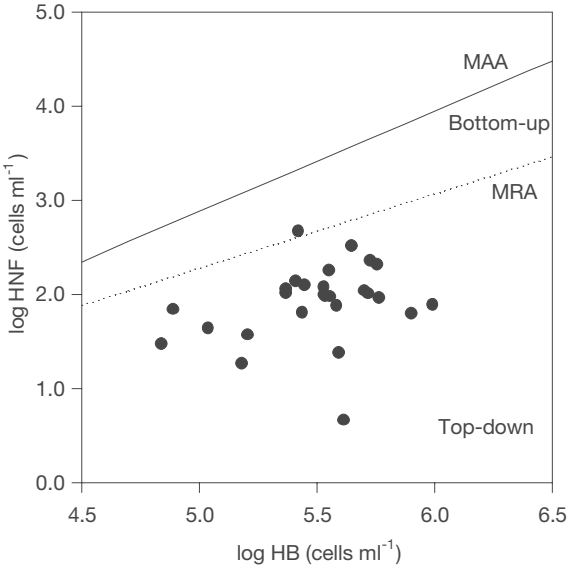

Fig. 9. Application of the Gasol (1994) model to determine whether heterotrophic nanoflagellates (HNF) are top-down or bottom-up controlled. Abundance of heterotrophic bacteria (HB) and HNF in the cave lagoons is plotted. MAA: maximum attainable HNF abundance; MRA: mean realized HNF abundance

Occasionally, dynamics in bacterial biomass covaried with ETS activity (Fig. 5a,c). More commonly, however, ETS activity was not related to the dynamics in bacterial abundance and biomass (Fig. 5b), indicating that ETS activity is more an indicator of activity than of biomass (e.g. Packard 1985, Arístegui & Montero 1995).

Comparison between microbial communities in cave lagoons and other net heterotrophic aquatic systems

Systems similar to that of the cave lagoons are the deep sea and the groundwater, because they rely on allochthonously produced organic matter (Smith & Kaufmann 1999). The deep sea microbial communities, however, are exposed to complex and variable effects due to hydrostatic pressure (Jannasch & Wilsen 1982, Patching & Eardly 1997).

The ETS activity obtained in cave lagoons was low and comparable to that of oligotrophic marine areas, e.g. the tropical Pacific (Packard et al. 1975, King et al. 1978) and aphotic deep layers (Packard & Williams 1981, Savenkoff et al. 1993). They were also similar to the values we obtained for other oligotrophic coastal areas of the Balearics (Sintes 2002). The activity of the microbial food web, i.e. bacterial production and bacterivory, in cave lagoons is similar to oligotrophic areas elsewhere (e.g. Eguchi & Ishida 1990, Ribes et al. 1999) and to the oligotrophic Balearic coasts (Sintes 2002), while bacterial biomass was substantially lower in the caves.

Daily carbon fluxes from bacteria to predators represent 100% of the bacterial biomass, with twice as much bacterial production in the mixolimnion, reflecting situations characteristic for the oligotrophic open sea (Sintes 2002). At the chemocline, daily carbon flux is about 1% of the standing stock, but 6 times higher than production. As stated above, HNF seem to be top-down controlled, which would imply a bottom-up control of bacteria by resources. However, the measured daily carbon fluxes suggest that other predators rather than HNF can control the bacterial community, at least in the upper part of the water column. The absence of a correlation between microbial biomass and TOC, contrary to observations on aquifers and deep marine sediments (Kieft et al. 1995, Cragg et al. 1998), implies that either the availability of organic compounds for bacterial utilization or the availability of electron acceptors, mainly oxygen, limit bacterial production, as suggested for sapropels (Cragg et al. 1998).

Compared to other systems (e.g. the deep sea), bacterial biomass of the monimolimnion is similar to that reported for the 100 to 1000 m depth layer (Nagata et al. 2001, Tanaka & Rassoulzadegan 2004) and pristine aquifers (Griebler et al. 2002), while surface bacterial abundance was similar to groundwater and non-contaminated wells (Alfreider et al. 1997, Griebler et al. 2002). Bacterial production in the monimolimnion is slightly higher than the values reported for the 1000 m depth horizon in the ocean, while our surface values are similar to those reported for oceanic surface waters (Hara et al. 1996, Nagata et al. 2000).

In conclusion, the microbial food web structure based on allochthonous organic matter differs little from that of oligotrophic marine areas. The activities at the surface, where input of organic matter for potential bacterial utilization is higher than in the deeper waters of the cave lagoons, are close to that observed in the open Mediterranean, or even in coastal sites with high water exchange with the open sea. Conversely, the biomass of bacteria and flagellates is significantly lower in most cases. The water column below the chemocline in the cave lagoons is characterized by lower microbial activities (Fig. 7) and biomass, although near-bottom waters can show elevated levels of biomass and activity, probably associated with input of organic matter or outwelling of nutrients from the sediment. It seems that the availability of substrate limits bacterial abundance while HNF are top-down controlled. This presents a major difference to oligotrophic surface waters, where HNF are mostly bottom-up controlled.

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