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ORIGINAL ARTICLE

A multidisciplinary study of the Cape Peloro brackish area (Messina, Italy): characterisation of trophic conditions, microbial abundances and activities

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Conflicts of interest
The authors have declared no conflicts of interest.

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Abstract

In the framework of the VECTOR DIVCOST Project, a 2-year investigation was started in 2006, with the aim of testing the sensibility of microbial parameters to environmental changes and of assessing whether they can provide information about functional changes in the carbon cycle. The investigation was performed in the surface waters of two small brackish ponds (Ganzirri and Faro), located in the Cape Peloro transitional area (Sicily, Italy). The seasonal changes in both the microbial compartment [bacterioplankton, vibrios, exoenzymatic hydrolysis of proteins and polysaccharides, bacterial secondary production (HBP) and community respiration] and the trophic state of suspended matter [total suspended matter (TSM), particulate organic carbon (POC), particulate organic nitrogen (PON), C/N] were analysed in relation to the hydrological characteristics [temperature, salinity, oxygen, fluorescence, NH_4, NO_2, NO_3, PO_4]. Despite marked differences in the nutritional input and the diversification in both carbon budget and trophic level, the two ponds show similar trends in many of the investigated factors, hardly influenced by seasonal variations. Temporally coupled trends were observed for some parameters (enzyme activities, vibrios abundances, respiratory activity), whereas others (POC, PON, heterotrophic bacterial production, bacterioplankton) showed a seasonal shift between the two lakes. The different behaviour found for the some biotic parameters suggests that their response to environmental conditions may be modulated differently between the two lakes, which, despite their spatial proximity and reciprocal connection, do not always show contemporaneous functional processes.

Problem

Transitional areas are among the most geochemically and biologically active of the biosphere and play an important role in the global biogeochemical cycles. These ecosystems are characterised by typical hydrological and geomorphologic features, such as shallow depth, confined circulation, weak hydro-dynamism, marked space–time variations in salinity and temperature, *etc.* Moreover they are usually affected by nutrient enrichment from terrestrial runoff, which, concomitantly with extreme climatic conditions, such as rapid temperature increase, high pressure, lack of wind, *etc.*, may lead to occasional and sometimes dramatic anoxia crises.

The rapid changes in the environmental features which characterise these areas, make them particularly suitable for ecological investigations, concerning the study of the ecosystem functioning overall.

Notwithstanding their peculiarities and biodiversity, to date there has been little research concerning microbial community and biogeochemical processes in these areas. In this context, a 2-year investigation was started in

2006, in the framework of the VECTOR DIVCOST Project. This study was devoted to the analysis of the microbial community, in term of abundances (bacterioplankton, vibrios) and activities (ectoenzymatic hydrolysis of proteins and polysaccharides, bacterial secondary production and community respiration), which was performed contextually with suspended matter quality [total suspended matter (TSM), particulate organic carbon (POC), particulate organic nitrogen (PON), C/N], and physical and chemical parameters (temperature, dissolved oxygen, fluorescence, NH_4, NO_2, NO_3, PO_4).

The aim of this study was to test the sensibility of microbial parameters to the climatic changes and assess whether they can provide information about the functional changes in the carbon cycle, in order to hypothesize their possible utilization as environmental indices. Moreover, our study may contribute to knowledge of all the factors involved in microbial biogeochemical activities and their reciprocal interactions, also with the goal to provide a helpful tool for planning proper management strategies for the transitional ecosystems.

The carbon flux in aquatic environments is ruled by the microorganisms (Hoppe 1991) that provide, through the production, decomposition, and respiration processes, turnover of organic matter, regulating the carbon flux through the trophic web (Azam *et al.* 1983; Cho & Azam 1988; Chrost 1990; Fuhrman 1992; Azam *et al.* 1993).

Many studies show that the elemental composition of particulate organic matter changes in relation to the different incidences of autotrophic and heterotrophic biomasses and detritus, reflecting the trophic status of the ecosystem (Fabiano *et al.* 1999; Ferrari *et al.* 2003; Bates *et al.* 2005). Integrated analyses of the microbial compartment, together with particulate organic matter stoichiometry, may provide ecological information on the efficiency of microbial metabolism involved in the transformation of organic matter.

Study Area

The Cape Peloro (Messina, Italy) transitional area is located in the north-easternmost part of Sicily, between the Tyrrhenian and the Ionian Sea. It is constituted of two brackish neighbouring basins denominated Lake Ganzirri and Lake Faro, with noticeable differences in geomorphologic, hydrographic and trophic features (Fig. 1).

Lake Ganzirri is a brackish coastal pond; it covers a 34-ha area (maximum depth: 7 m; water volume: 106 m^3). Its

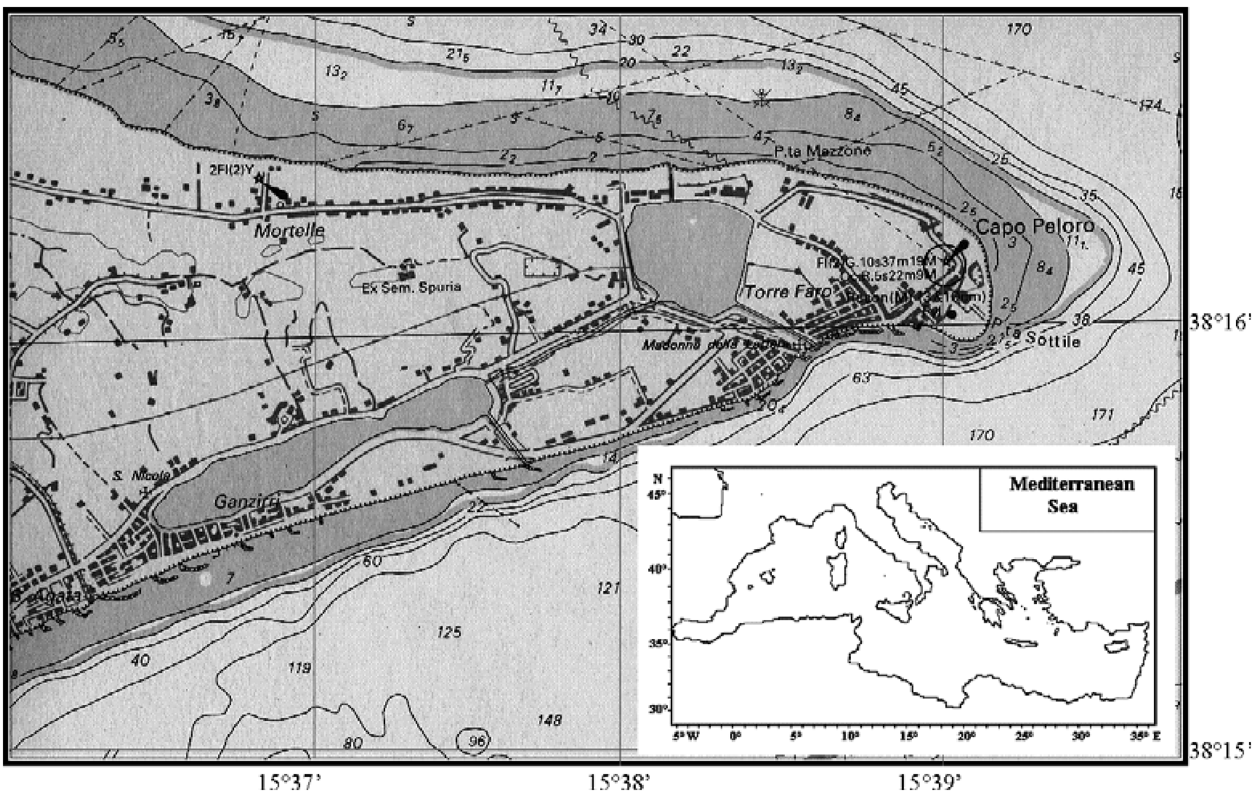

Fig. 1. Map of the study site (Cape Peloro transitional ecosystem).

length and width are 1670 m and 282 m, respectively. It communicates with the Straits of Messina and Lake Faro by means of narrow shallow channels (Vanucci *et al.* 2005). Lake Ganzirri is colonised by macroalgae and frequently suffers dystrophic crisis. Sometimes the blooms spread over the lake, causing temporary dystrophic events and marked reduction of the dissolved oxygen (Giacobbe *et al.* 1996).

Lake Faro is a small and relatively deep meromictic coastal pond. It covers a 26-ha area and has a nearly circular shape. Its depth in the central part reaches 28 m. It communicates with the Tyrrhenian Sea and with the Straits of Messina through artificial shallow channels. Lake Faro, with its particular shape, represents a rare example of a meromictic basin and is an interesting subject of study (Acosta Pomar *et al.* 1988; Brugnano *et al.* 2006; Saccà *et al.* 2008). It is characterised by massive development of coloured phototrophy sulphur bacteria capable of carrying out photosynthesis even in the absence of oxygen at depth (Trüper and Genovese 1968).

Material and Methods

Water samples were seasonally collected from the surface layer in a central station of Lake Faro and of Lake Ganzirri, for a 2-year period starting in September 2006.

Temperature, salinity, fluorescence and oxygen measurements were taken using an oceanographic multiparametric sensor (SBE 19 Plus).

For the dissolved oxygen analysis, water samples were fixed immediately after collection, and then analysed using Winkler's method (Carpenter 1965).

Samples for nutrient determinations (NH_4, NO_2, NO_3, PO_4) were filtered using GF/F glass-fibre filters and kept frozen (−20 °C). Analytical determinations were performed according to Strickland & Parsons (1972), and NH_4 was measured according to Aminot & Chaussepied's method (1983). All nutrient concentrations were determined using a Varian Mod. Cary 50 spectrophotometer.

Total suspended matter (TSM) was evaluated by a gravimetric method using a Mettler AT261 electronic microbalance (accuracy ± 1.0 μg). Particle material was collected by filtering variable volumes of water on pre-combusted (480 °C for 4 h) pre-weighted glass fibre filters (Whatman GF/F), which was then oven-dried at 60 °C for 24 h.

For estimation of particulate organic carbon and nitrogen (POC and PON), 500-ml water samples were concentrated on precombusted Whatman GF/F glass-fibre filters and processed at 980 °C in a Perkin-Elmer CHN-Autoanalyzer 2400, using acetanilide as standard (Iseki *et al.* 1987).

Bacterioplankton (BA) abundance was determined using DAPI (Porter and Porter & Feig 1980) and image analysis (Zeiss AXIOPLAN 2 Imaging microscope). Bacterioplankton biomass (BB) was estimated by cell counting and volumetric measurements according to La Ferla *et al.* (2004).

Vibrios abundance was determined on TCBS agar added with 1.5% of NaCl and incubated at 35 °C for 24 h (Zaccone *et al.* 1992).

Microbial ectoenzymatic activity measurements were performed to estimate the potential activity rates of leucine aminopeptidase (LAP) and β-glucosidase (β-GLU), two enzymes involved respectively in protein and polysaccharide decomposition mediated by the microbial community. The enzymatic assay relies on the hydrolysis of specific fluorogenic substrates, L-leucine-7 amido-4-methyl coumarin hydrochloride (Leu-MCA) and 4-methylumbelliferyl- β-D-glucoside, respectively a derivative of methylcoumarin (MCA) and a derivative of methylumbelliferone (MUF), following the method reported by Caruso *et al.* (2005). Increasing amounts (from 20 to 400 μmol) of substrates were added to 10-ml subvolumes of water and spectrofluorometer measurements were performed at the initial time and after incubation at '*in situ*' temperature for 2 h. Through calibration with the standard curves obtained with known amounts of MCA and MUF, LAP and β-GLU values were expressed in term of maximum velocity of hydrolysis (V_{max}, in nmol of substrate hydrolysed per litre and per hour, nM·h^{-1}) and converted into nanograms of C mobilised assuming that 1 nmol of substrate hydrolysed released 72 ng of C.

Net heterotrophic bacterial production (HBP) (Ducklow & Carlson 1992) was estimated from the rate of [^{3}H] leucine incorporation using the micro centrifugation method according to Smith & Azam (1992). Triplicate 1.0-ml samples and two blanks were incubated in the dark, for 1 h at *in situ* ±1 °C temperatures with L-[4,53H]leucine (Amershan Biosciences UK Limited) (25 nM final concentration). HBP was calculated according to Kirchman (1993) using leucine isotopic dilution factor (ID) *in situ* determined according to Pollard & Moriarty (1984). Bacterial turnover rate (BTR) was defined as the amount of days necessary to produce one bacterial standing stock BB (BTR = BB l^{-1}·HBP l^{-1}·day^{-1}).

The respiration rates and the consequent metabolic production of CO_2 (R) were measured by the study of the electron transport system activity (ETS); the assay is based on the conversion of tetrazolium salt in formazan (Packard & Williams 1981). The results are reported as V_{max} and have been converted into C by using a respiratory quotient of 1.

Results

The obtained values of biotic and abiotic parameters are reported in Fig. 2.

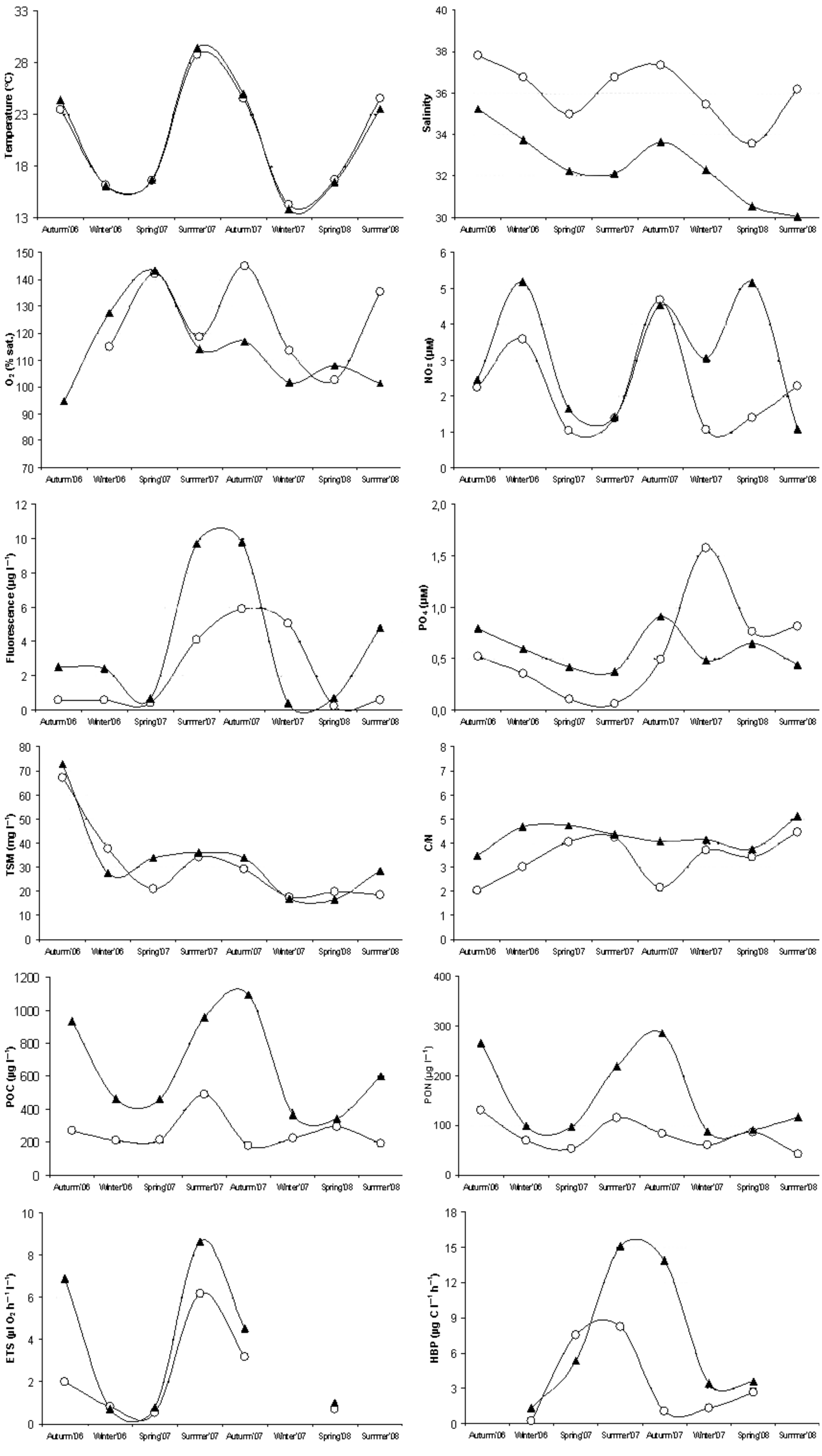

Fig. 2. Seasonal patterns of investigated parameters. ▲, Lake Ganzirri; ○, Lake Faro.

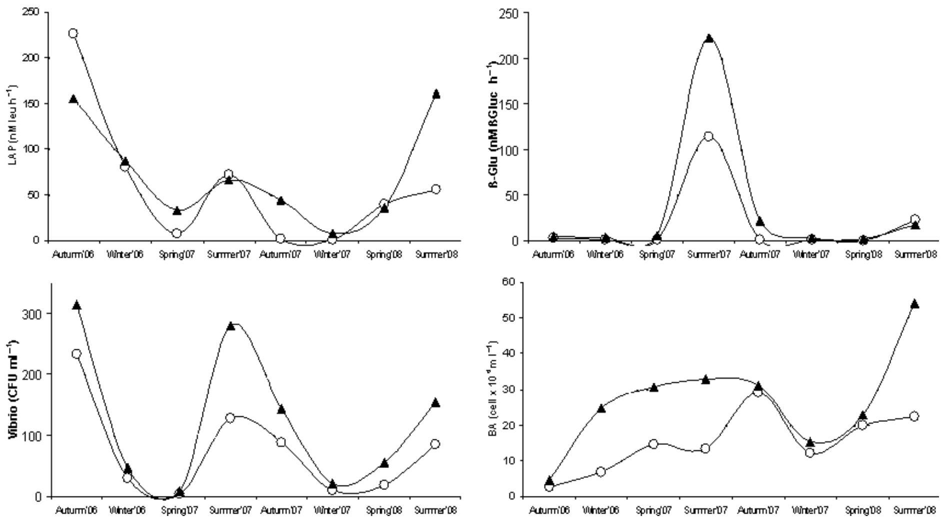

Fig. 2. (*Continued*.)

Temperature values ranged from 13.80 to 29.43 °C and from 14.23 to 28.79 °C in Lake Ganzirri and Lake Faro, respectively, showing typical seasonal trends in both ponds.

Salinity values were higher in Lake Faro than in Lake Ganzirri, ranging from 33.56 to 37.83 and from 30.56 to 35.24, respectively. The seasonal trend is characterized by lower values in spring in both ponds.

In both lakes, during the investigated period, dissolved oxygen expressed in terms of saturation values were above the 100% level, with maximum values in spring (145%) and minimum values in summer (118%).

Lake Ganzirri showed concentrations of NH_4, NO_2, NO_3 and PO_4 (average values 1.61, 0.19, 3.54 and 0.58 μM, respectively) similar to those found in Lake Faro (average values 1.71, 0.22, 3.08 and 0.57 μM, respectively). During all investigated periods the nitrate incidence was never lower than 1.0 μM in Lake Ganzirri and 1.5 μM in Lake Faro. The ammonia (data not shown) increased in the autumn and winter periods (>2.4 μM) in both the lakes and phosphates were lower during the spring–summer period.

The highest TSM content (90.20 $mg \cdot l^{-1}$) was found in Lake Ganzirri in September 2006. In general, lower values were recorded in the winter–spring period and higher values in the summer–autumn period in both ponds.

Higher POC incidences were recorded in Lake Ganzirri (range 273.00–1245.50 $\mu g\ C \cdot l^{-1}$) than in Lake Faro (range: 179.49–491.18 $\mu gC \cdot l^{-1}$); similarly were found for PON, which was significantly correlated with POC in both investigated lakes (Ganzirri, r = 0.95, $P < 0.01$; Faro, r = 0.58, $P < 0.01$). The seasonal distribution in Lake Ganzirri was characterised by higher values in the warm period (summer–autumn), and lower values in the cold period.

The C/N ratio, ranging between 3.48 and 4.73, indicated a significant predominance of the heterotrophic biomasses in the particulate matter. Highly significant correlations were in fact found between POC and ETS (r = 0.67, $P < 0.01$), as well as between POC and HBP (r = 0.66, $P < 0.01$), and vibrios abundance (r = 0.507, $P < 0.01$).

There was a very wide range of BA (0.46–5.42 × 10^7 $cells \cdot ml^{-1}$ in Lake Ganzirri and 0.26–2.91 × 10^7 $cells \cdot ml^{-1}$ in Lake Faro) with no apparent seasonal pattern. Substantial differences were observed between Lake Ganzirri and Lake Faro; in the latter, the values registered were always lower. In Lake Ganzirri the prokaryotic biomass, modulated by both cell abundances and biovolumes, was in the range 74.28–1341.49 $\mu gC \cdot l^{-1}$, with the highest value in summer 2008.

Vibrios abundance showed the highest peaks in summer and a coincident trend in both lakes during 2006 and 2007. A comparable peak was found in autumn 2006, when high fish mortality occurred in Lake Ganzirri. The minimum values were observed in winter and spring 2007, coinciding with a temperature decrease. In general, mean values were lower in Faro than in Lake Ganzirri, as observed for other parameter trends (Fig. 2).

Enzyme activity levels showed high spatial and seasonal variability, although similar activity patterns were found in both the studied lakes. On the whole, higher enzyme activity rates were measured in Ganzirri compared to Lake Faro (Fig. 2), both for LAP (ranging from 7.78 to 161.44 $nM \cdot h^{-1}$ and from 1.059 to 226.76 $nM \cdot h^{-1}$, respectively) and β-GLU (ranging from 1.49 to 222.4 $nM \cdot h^{-1}$ and from 0.42 to 114 $nM \cdot h^{-1}$, respectively). High LAP activity rates were measured during autumn 2006; another peak of proteolytic activity, although slightly lower, was also observed in summer 2007, followed by a decline in winter months. During the successive spring–summer period (2008) LAP increased again. During the warmest months, in Lake Ganzirri, LAP values were, on average, twice those detected during the coldest months, whereas in Lake Faro they were on average three times higher than those observed during the coldest months. The observed trends in LAP activity data reflected those of temperature values and total suspended matter content, although statistical relationships calculated between LAP activity values and these parameters did not reach the level of significance (Fig. 2).

Low rates of β-GLU activity (Fig. 2) were always detected during the autumn–spring period, with values lower than 2.61 and 4.60 $nM \cdot h^{-1}$ in Lake Faro and Lake Ganzirri, respectively; the highest activity rates were detected during both summer samplings. In Lake Ganzirri, these peaks in β-GLU activity were associated with the highest levels of BA. During the warmest months, β-GLU values in Lake Ganzirri were on average 20 times higher than those measured during the coldest months, whereas in Lake Faro, the values were 50 times higher than those detected during the coldest months.

The calculation of the LAP/β-GLU ratio provided some information on the quality of the hydrolysed organic matter. In Lake Faro, the LAP/β-GLU ratio showed values lower than 0.75 from spring 2007 to winter 2007, suggesting during this period a greater importance of polysaccharides within the organic matter. Conversely, during autumn–winter 2006 and in spring 2008, the most labile protein fraction of the organic matter was prevalent, as suggested by values of LAP/β-GLU ratio ranging from 6.26 to 7.78.

HBP seasonal values in Lake Ganzirri varied from 0.64 $\mu gC \cdot l^{-1} \cdot h^{-1}$ in winter 2006 to 21.30 $\mu gC \cdot l^{-1} \cdot h^{-1}$ in summer 2007, higher than those usually reported for coastal lagoons. In Lake Faro, HBP values were about one order of magnitude lower than in Lake Ganzirri.

Respiratory activity (ETS) determined in the two ponds ranged from 0.53 to 6.18 $\mu l\ O_2 \cdot h^{-1} \cdot l^{-1}$ in Lake Faro and from 0.73 to 8.65 $\mu l\ O_2 \cdot h^{-1} \cdot l^{-1}$ in Lake Ganzirri. In the two analysed ponds the seasonal trends were similar, with lower values in winter and spring, and higher values in summer and autumn. The levels of ETS activity in Lake Ganzirri were generally higher than in Lake Faro.

Discussion

From the overall data, seasonal variability was generally high for all the examined parameters; however, as reported in previous studies, strong fluctuations in both abiotic and biotic parameters are typical for transitional areas (Caruso *et al.* 2005, 2006).

Nitrogen and phosphate levels recorded in Lake Ganzirri and Lake Faro fell within a range of values usually found in other studied transitional ecosystems (Leonardi *et al.* 2005); they were not limiting elements for phytoplankton production, as suggested by the overproduction of O_2, which always exceeded the 100% saturation level.

Despite marked differences in the nutritional input and the diversification in both carbon budget and trophic level, ranging from oligotrophy (Faro) to mesotrophy (Ganzirri) (Caruso *et al.* 2006), the two ponds show similar trends in many of the investigated factors, hardly influenced by the seasonal variations.

During warm periods there were increases in both microbial metabolism and vibrios abundance, as well as in POC and PON contents. Positive correlations were observed between temperature and microbial activities (HBP, β-GLU, ETS), and particulate organic matter (POC, PON) in both the lakes. Vibrios abundance was significantly correlated with temperature in both lakes ($r = 0.86$ in Faro and $r = 0.56$ in Ganzirri, $P < 0.01$), indicating a direct stimulation of warm season on bacterial growth (Zaccone *et al.* 1992; Crisafi 1998). The correlations observed throughout the study among LAP, β-GLU and vibrios abundance (Ganzirri $r = 0.78$, $P < 0.01$ and 0.35, $P < 0.05$, respectively; Faro: $r = 0.59$ and 0.79, $P < 0.01$, respectively), underline the role of vibrios in organic matter cycling; similarly, in Lake Ganzirri, BA was positively related to β-GLU (0.34, $P < 0.05$) and HBP (0.66, $P < 0.01$).

Microbial metabolism was particularly intense in Lake Ganzirri, in relation to the availability of both autochthonous and allochthonous nutritional sources. As microbial ectoenzyme activity is specifically, functionally and biochemically tightly coupled with the presence of high-molecular-weight substrates that are predominating constituents of organic matter, the activity of some enzymes may respond to their concentrations and thus may depend on the trophic conditions of lakes (Chrost & Siuda 2006). Our LAP and β-GLU activity values confirmed this assumption. Both the enzyme activities were significantly, inversely, related to oxygen content in Lake Ganzirri ($r = -0.40$ and -0.41, $P < 0.05$, for LAP and β-GLU, respectively) but only LAP in Lake Faro ($r = -0.43$, $P < 0.05$), confirming that the oxygen was consumed by the active microbial community living in their waters.

C/N values, always lower than 5, suggested the predominance of heterotrophic biomasses in this ecosystem, as confirmed by the significant correlation found between POC and ETS, HBP, and vibrios abundance.

Respiratory activity appeared a key parameter linking the different ecosystemic components (autotrophic and heterotrophic compartments as well as the pool of organic matter). In Lake Ganzirri, R was positively correlated with the fluorescence, vibrios density, the microbial ectoenzymatic activities, HBP and the pool of matter (TSM, POC, PON).

The seasonal range of POC and PON was comparable with that reported by Bertoni *et al.* (2004), but markedly higher than those recorded in other Sicilian transitional areas (Sarà *et al.* 1999; Leonardi *et al.* 2000, 2006).

The enzymatic values recorded in our study fell within a range similar to other coastal ecosystems as well as to lagoons and estuarine waters (Hoppe *et al.* 2002; Williams & Jochem 2006), but they were higher than those detected in marine environments (Caruso & Zaccone 2000; La Ferla *et al.* 2001; Zaccone & Caruso 2002). LAP activity provided not only a C but also an N source to sustain microbial metabolism.

In both ponds the ETS data comprised the range of values already determined in the Mediterranean Sea. In winter and summer, the values were comparable to those from pelagic areas (La Ferla *et al.* 1996), whereas in summer and autumn they were comparable with those from estuarine environments (La Ferla *et al.* 1996, 2001).

BA values are comparable to those reported by Araùjo & Leal Godinho (2008) in a tropical fluvial-lagunar system (2.7–5.1 cells $\times 10^7$ ml^{-1}) and lower than those reported by Gocke *et al.* (2004) in a study in the coastal lagoon of the Colombia (0.6–9.1 cells $\times 10^7$ ml^{-1}). BB values were similar to those found in a coastal lagoon of the Colombia by Gocke *et al.* (2004) (ranging from 77 to 1542 μgC·l^{-1}) and also to those reported in coastal lakes of Southeastern Brazil by Farjalla *et al.* (2001) (ranging between 480 and 1432 μgC·l^{-1}). Our BB values were higher than those measured in a tropical lake by Erikson *et al.* (1999), between 240 and 930 μgC·l^{-1}.

An estimate of C flux through the microbial community was performed. Heterotrophic bacteria derive their carbon and energy sources from biologically available dissolved and particulate organic carbon sources. Figure 3 shows the percentage of POC potentially mobilised *per*

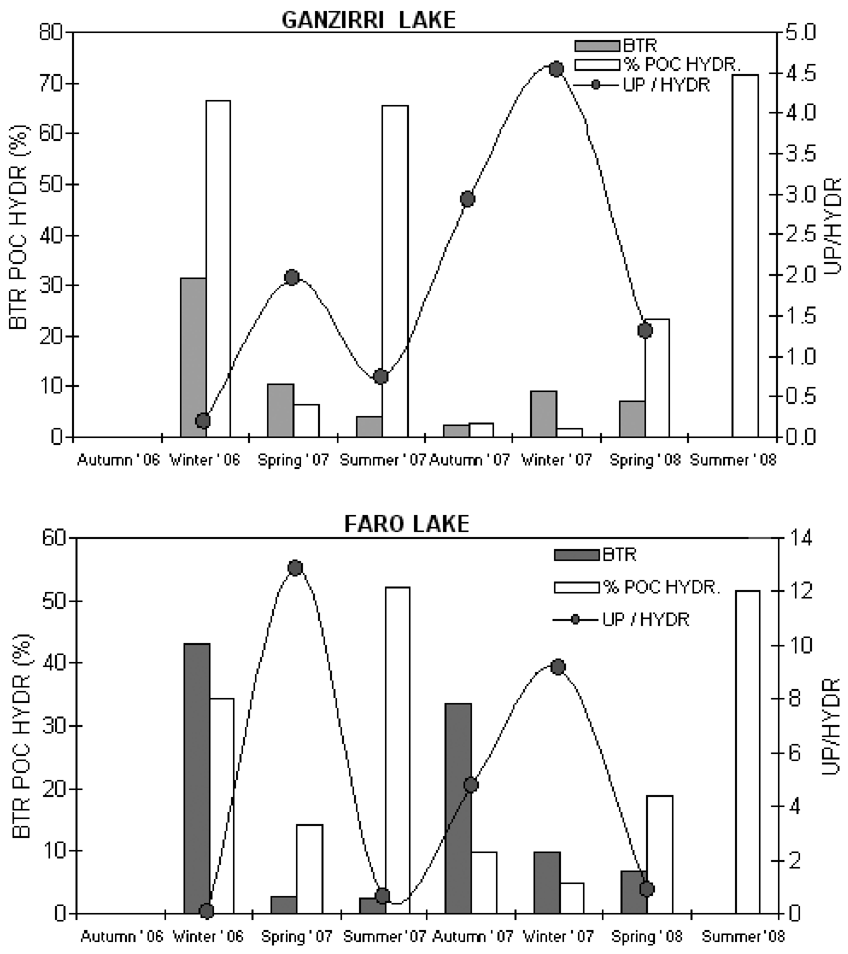

Fig. 3. Uptake to Hydrolysis (UP/HYDR) ratio, Bacterial Turnover rates (BTR), percentage of mobilised POC by hydrolysis (% POC/HYDR), calculated in Lake Ganzirri and Lake Faro.

day by enzyme hydrolysis (LAP+β-GLU) (%POC-HYDR). High percentages of POC were hydrolysed during summer 2007 and 2008, due to the high activity rates measured during those periods (Ganzirri: 65.44–71.41% of POC; Faro: 51.43–52.09% of POC); however, high percentages of POC were also hydrolysed in both the lakes (66.66% and 34.34% in Ganzirri and Faro, respectively) during winter 2006, due to the low amount of POC available in the presence of still enhanced enzyme activity levels. Analysing the amounts of C potentially released by LAP+ β-GLU activities (HYDR, hydrolysis) and incorporated into BB (UP, uptake), together with bacterial turnover rates (BTR), a different behaviour of Lake Ganzirri and Lake Faro was observed. In the studied environments, the bacterial C uptake exceeded the amount of monomeric C produced by hydrolysis, namely the C uptake: the C hydrolysis ratio was greater than 1 (Lake Ganzirri: spring 2007, autumn 2007, winter 2008, spring 2008; Lake Faro: spring 2007, autumn 2007, winter 2008), indicating that considerable amounts of dissolved monomers, other than those produced by enzymatic hydrolysis (*i.e.* exudates), flowed into BB. Coinciding with these periods of imbalance between HYDR and UP, lower BTR were observed. A marked reduction of BTR from 30–40 days to 2–3 days coupled with small changes in BB would suggest the development of active bacterial control mechanisms, such as viral lysis and/or grazing processes. Consequently, important amounts of bacterial DOC can flow to the surrounding environments, supplying, within the microbial loop, an additional source of biologically available carbon for bacterial cell production.

Conclusion

This study provides evidence of the importance of a multidisciplinary approach to understand ecosystem microbial processes in the transitional ecosystems, underlying the active role of microbes and the significance of heterotrophic processes, such as decomposition and bacterial production, in carbon turnover. Integrated investigation of microbial activities together with particulate organic matter stoichiometry provided ecological information about the functional changes in the carbon cycle in the Cape Peloro ecosystem, which may be useful indices of the actual trophic conditions, as already asserted by Chrost & Siuda (2006). The determination of organic matter decomposition allowed quantification of the amounts of C potentially flowing through the microbial community, while the enzymatic assays led us to define to what extent the microbial community is actively involved and efficient in organic matter processing.

In particular, our study demonstrated that microbial production, enzymatic degradation, and oxidation of organic matter in the studied area varied with seasons, as well as vibrios abundance.

Temporally coupled trends were observed for some parameters (enzyme activity, vibrios abundance, respiratory activity), while others (POC, PON, HBP) varied with a seasonal shift between the two lakes. Although the activity levels were generally lower in Lake Faro than in Lake Ganzirri, the similarities found in the enzyme patterns led us to suppose that microbial metabolism is driven by similar environmental forcing factors (such as temperature and trophic availability). Conversely, the seasonal shift found for some biotic parameters suggests that their response to environmental conditions may be modulated differently between the two lakes, which, despite their spatial proximity and reciprocal connection, do not always show contemporaneous functional processes; further, more detailed, studies are indicated.

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