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Trophic structure and microbial activity in a spawning area of *Engraulis encrasicolus*

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ABSTRACT

The abundance, biomass and size-structure of planktonic populations, and the microbial metabolic processes were studied in the Sicily Channel, one of the most important spawning areas in the Mediterranean for anchovy (*Engraulis encrasicolus*), a pelagic species of commercial interest. Results showed that prokaryotes contribute for the 83% of total carbon biomass. Microphytoplankton abundances and biomasses were dominated by autotrophic nanoflagellates and dinoflagellates (36 identified species) and contribute 11% of total biomass. The microzooplanktonic biomass showed its maximum at the surface or subsurface and its contribution was low (4%). In agreement with the general oligotrophy of the investigated area, the study highlights the prevalence of pico-sized fractions within the whole phytoplankton biomass expressed as chlorophyll content, suggesting the importance of picophytoplankton in sustaining the microbial food web. At the same time, the levels of microbial hydrolytic activities are related to productive processes recycling the organic matter and releasing nutrients (P and N), indicating also an active functioning of ecosystem at low trophic levels. Autotrophic production exceeded oxidation by respiration; at the same time, the co-variation of prokaryotic activities and eggs distribution with temperature in summer was observed. The results obtained confirmed that the area acted as a nursery for small fish and both autotrophic and heterotrophic processes supported by microorganisms were in synergy.

1. Introduction

Marine research has recently paid attention to elucidate how global change will impact marine ecosystems and resources; so attempts to link environmental biogeochemical variables and marine food webs were carried out. Three main processes, related to exploitative (fisheries), trophodynamic (species interactions) and biophysical (environmental) factors, affect marine fish productivity, as emphasised by Link et al. (2012). Among the multiple drivers that influence ecosystem dynamics, physical forces have been investigated, given the effect of rising ocean temperature on the fish distribution and productivity (Patti et al., 2010; Basilone et al., 2013). Moreover, being linked to microbial and biogeochemical activities, temperature affects the productivity of the entire ecosystem (Sarmento et al., 2010; Zaccone et al., 2015).

One of the major problems in the ecology of aquatic ecosystems is to understand how organisms use the carbon produced by primary production and how much of it passes into fish stock or is respired and returns to the atmosphere. Research carried out during the past 30 years has shown that bacteria dominate in the ocean, particularly in oligotrophic environments, where their abundance, diversity and metabolic activities play a key role within the microbial food web. Autotrophic and heterotrophic bacteria are in turn preyed by heterotrophic microbes and protozoa (nano- and micro-grazers), that are in turn grazed by larger zooplankton. In this way, through the 'microbial loop' (Azam et al., 1983), a significant amount of organic carbon is transferred to higher trophic levels of food webs driving the ecosystem functioning (Chròst and Siuda, 2006; Zoccarato et al., 2016). Further contributions to biogeochemical cycles, come from the death and lysis of bacteria and by phytoplankton excretion of organic matter with stimulation of bacterial hydrolytic enzymes; the mineralization of

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organic matter provides nutrients such as phosphorus and nitrogen fuelling primary production (Ducklow, 2000; Sarmento et al., 2010).

In the framework of an holistic approach to fisheries management it is important to deepen the relationships between trophic levels providing ecological information. Only a few studies indeed have recognized the links between environment, microbes and upper trophic levels making an ecosystem-based approach to fisheries management possible (Patti et al., 2010; Segovia et al., 2015). Some authors have focused on the role of trophic web organisms in fish larval ecology (Cuttitta et al., 2003; Link et al., 2012), but to our knowledge no study has analyzed the smallest planktonic components associated to the early life stages of fish in the environment. The role of both environmental factors (mainly primary productivity and temperature) and water circulation in egg production has recently been hypothesized for anchovy in the Sicily Channel (Basilone et al., 2013; Falcini et al., 2015). This is an oligotrophic area of the Eastern Mediterranean Sea, showing low concentrations of nutrients and chlorophyll (Thingstad et al., 2005; Van Wambeke et al., 2002; Patti et al., 2010); it is recognized as one of the most important spawning areas in the Mediterranean for a pelagic species of commercial interest such as anchovy (*Engraulis encrasicolus*), (Cuttitta et al., 2003).

As a part of a multidisciplinary research in support of fisheries management, the aims of this study were: (a) to describe the abundance, biomass and size-structure of planktonic populations, the chemical and trophic parameters and the rates of the microbial metabolic processes (primary and secondary production, total and dissolved enzymatic hydrolysis, and community respiration) in the euphotic layer of the Sicily Channel, and (b) to determine whether microbial parameters show co-variation with the main factors that affect eggs distribution.

2. Materials and methods

This study is based on information collected in the Sicily Channel (BANSIC12 survey) in July 4–23, 2012, within the RITMARE project, at six hydrological stations, located south of Capo Passero on the Iblean - Maltese platform (Fig. 1). Vertical profiles of temperature (T), salinity (S), pressure, conductivity, fluorescence and oxygen content (Ox) were measured on board of the R/V Urania of CNR by means of a CTD probe SeaBird 911plus, while water samples were obtained with Niskin bottles in the euphotic layer.

Anchovy egg samples were obtained at each station by three replicate vertical tows of a Multi Plankton Sampler (Hydrobios, type Mini), a five-net system for the investigation along the water column, having an aperture of 0.125 m^2 and 200 μm mesh size. The samples were immediately fixed after collection and preserved in individual ultracentrifuge plastic tubes containing 70% ethanol and stored at 4 °C. Counts of anchovy eggs were then evaluated in the land-based laboratory by a binocular stereo microscope.

As far as nutrient determination is concerned, all equipment for water sampling was earlier conditioned with 10% HCl and rinsed 2–3 times with ultrapure water. Unfiltered samples were stored at −20 °C. The concentration of nitrate, orthosilicate and orthophosphate was measured by means of a Sial Autoanalyzer "QUAATRO" following classical methods (Grasshoff et al., 1999) adapted to an automated system. The accuracy for nitrate, orthophosphate and orthosilicate was 0.003, 0.005 and 0.001 μM, respectively. The limit of detection for the procedure was 0.01 μM for nitrate, 0.01 μM for orthophosphate, and 0.05 μM for orthosilicate.

Particulate organic carbon (POC) and total particulate nitrogen (TPN, i.e. both organic and inorganic particulate nitrogen) were determined on 1500 ml water samples, pre-filtered on a 250 μm net and then filtered through pre-combusted Whatman GF/F glass-fibre filters. Analyses were carried out in a Perkin-Elmer CHN-Autoanalyzer 2400, as reported by Boldrin et al. (2009).

Chlorophyll-a (CHL) was measured in samples (1.5–2.0 l) filtered through Whatman GF/F glass-fiber filters, Nuclepore polycarbonate membranes of 2.0 μm e 10.0 μm to obtain three size fractions: micro- (≥10.0 μm), nano- (≥2.0 and < 10.0 μm) and pico-phytoplankton (≥0.2 and < 2.0 μm). The filters were stored at −20 °C, extracted in a 90% acetone solution, and measured with a spectrofluorometer (Varian mod. Cary Eclipse) at 429 and 669 nm excitation and emission wavelengths, respectively, before and after acidification (Decembrini et al., 2004).

Particulate ATP (from 250 to 0.2 μm) analyses were carried out according to Holm-Hansen (1973). We used boiling TRIS-EDTA phosphate buffer to extract the nucleotide, and then measured ATP with a LUMAT LB 9507 EG&G BERTHOLD. ATP values were converted into C biomass using the conversions factor of C/ATP = 250.

Prokaryotic heterotrophic production (PHP) was estimated from the rate of [^{3}H] leucine incorporation using the micro centrifugation method according to Smith and Azam (1992). Briefly, triplicate samples and two blanks were incubated in the dark, for 1.5 h at *in situ* temperature with L-[4,5 ^{3}H] leucine (Amersham Biosciences UK Limited) (25 nM final concentration); the incorporation was stopped with the addition of 90 μl of TCA 100% to the vials. PHP was calculated according to Kirchman (1993) using *in situ* determination of leucine isotopic dilutions (ID) performed using a kinetic approach (Pollard and Moriarty, 1984).

Primary production (PP) of the three size classes (as reported for the CHL) was measured with the standard 14C label technique (Steeman Nielsen, 1952). Samples were incubated in an "on-deck" continuous-seawater-flow incubator equipped with a set of neutral density screens, in order to reproduce the irradiance intensities (PAR % E_0^+) at the sampling depth. After 4 h of exposure to light, samples were filtered as described for CHL. Filters were transferred into 20-mL vials with 10 ml of 'Aquasol' scintillation cocktail and radioactivity was assessed on a liquid scintillation counter (Beckman LS1801).

Respiration rates (R), expressed as Carbon Dioxide Production Rate (CDPR), were obtained from the amount of O_2 reduced by Electron Transport System (ETS) activity based on the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to INT-formazan (La Ferla et al., 2005).

For quantitative analysis of the plankton populations, the total prokaryotic abundance (TP) was estimated by the epifluorescence counting after 4′,6-diamidino-2-phenylindole (DAPI) staining (Porter and Feig, 1980). Cells were enumerated by a Zeiss AXIOPLAN2 Imaging epifluorescence microscope (magnification: Plan-Neofluar 1009 objective and 109 ocular; HBO 100 W lamp; filter sets: G365 exciter filter, FT395 chromatic beam splitter, LP420 barrier filter) equipped with the digital camera AXIOCAM HR (Zeiss). The images were captured and digitized on a personal computer using the AXIOVISION 3.1 software (ZEISS). The abundance of Picophytoplankton (PPP), was determined according to El Hag and Fogg (1986) using the above microscope and the specific filter sets (BP450-490, FT510 and LP515).

Water samples for Flow Cytometry (FCM) prokaryotic counts were preserved with paraformaldehyde (2% final concentration) and stored in liquid nitrogen. The samples were stained with Syto13 at a 2.5 μM final concentration (Gasol & del Giorgio, 2000; Andrade et al., 2003). FCM counts were obtained with a CyAn ADP flow cytometer (Dako, USA) equipped with a solid state laser (488 nm, 25 mW) and filter modifications (green FL1 to 515 ± 30 nm, red FL4 to 660 ± 30 nm). For calibration of side scatter and green fluorescence signals, and as an internal standard for cytometric counts and measures, fluorescent latex beads (1.58 μm diameter) were systematically added (La Ferla et al., 2015).

Total extracellular enzymatic activity (EEA) rates were immediately measured after sample collection, using fluorogenic substrates [the methylumbelliferyl (MUF)-derived compounds MUF-phosphate and MUF-β-Glucopyranoside (Sigma Aldrich) for alkaline phosphatase (AP) and β-glucosidase (GLU) activities, respectively, and the methylcoumarine (MCA)-derived compound L-leucine–MCA (Sigma Aldrich) for leucine aminopeptidase (LAP) activity], according to a multi-

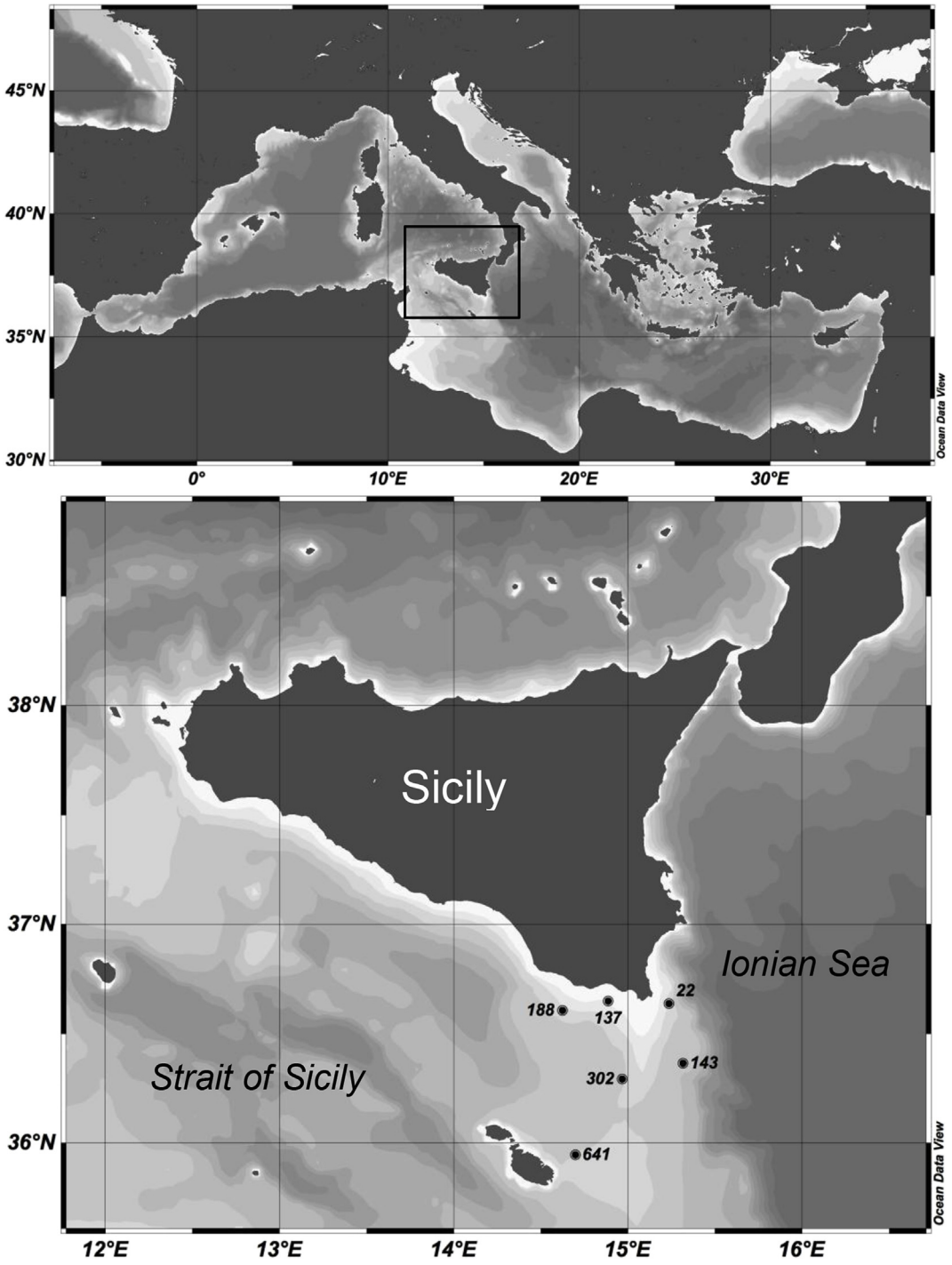


Fig. 1. Location of the sampling stations.

concentration method (Hoppe, 1993). Five increasing concentrations of each fluorogenic substrate were added to 10 ml water samples in triplicate; sterile prefiltered seawater was used as the blank. Incubation was carried out at *in situ* temperature for 3 h. The fluorescence was measured with a Turner TD 700 fluorimeter, equipped with 380–440 and 365–445 nm filters (excitation-emission wavelengths) for Leu-MCA and MUF substrates, respectively. The increase of fluorescence was converted into the hydrolysis velocity using a standard curve of MUF and MCA (Zaccone et al., 2012). Free enzyme activities, were measured as described above, after sample filtration through a low binding protein membrane filter (0.2-μm pore-size filter, Millipore) (Baltar et al., 2010).

Nanoplankton (NA), microphytoplankton (PHYTO) and microzooplankton (MICROZOO), were fixed with Lugol's iodine solution and examined by an inverted microscope (Axiovert 200M Zeiss) equipped with phase contrast at a magnification of 200 × , 400 × and 630 × . Counting was performed following the Utermöhl method (Utermöhl, 1958). For MICROZOO different volumes were filtered and resuspended at the final volume of 200 ml before the fixation, samples were stored at 4°, and examined by an inverted microscope (Olympus IX51). The cell biovolumes were measured with an eyepiece scale, approximating species shapes to geometrical models (Hillebrand et al., 1999). The carbon content of NA and PHYTO was calculated from mean cell biovolumes using the formula introduced by Menden-Deuer and Lessard

(2000), while Putt and Stoecker (1989) was adopted for aloricate ciliates, Verity and Lagdon (1984) for tintinnids and Michaels et al. (1995), and Beers and Stewart (1970) for the other protists and micro metazoans.

Statistical analysis of the data set was performed using the PAST software version 2.17. To evaluate the associations between microbial and physico-chemical parameters, Pearson's correlation coefficients and Canonical Correspondence Analysis (CCA) were computed. The resulting ordination axes were linear combinations of the environmental variables that explain microbial variability.

3. Results and discussion

Hydrological parameters in the study area showed that temperature ranged from 15.0 to 27.0 °C and salinity from 37.26 to 38.67. A marked thermocline was observed at about 20 m depth. The minimum value of salinity was observed at 25 m depth. The oxygen content ranged from 6.29 to 8.21 mg L^{-1}, reaching high values at DCM layer. The euphotic layer of Sicily Channel was characterized by high dynamisms, and by the presence of Atlantic waters (MAW, Modified Atlantic Water, highlighted by the minimum in the salinity profiles), flowing eastward along the Sicilian coast. The MAW was warmer, less saline and poorer in nutrient than the Ionian Surface Water (ISW) present in the same area (Patti et al., 2010; Placenti et al., 2013).

Anchovy eggs mainly occurred in the 0–10 and 0–25 m depth intervals, with peaks at 137 station (Table 1) confirming previous studies on the distribution of recruitment areas for this species in the Mediterranean (Basilone et al., 2013). Nutrients were generally low (ranges 0.15–1.87 μM and 0.29–1.59 μM for nitrates and silicates, respectively) even more the phosphates showing values near to the detection limit (0.01–0.06 μM). Low nutrient concentrations in the area were probably due to the seasonal trophic phase and the high N/P ratios observed (10.8–89.9), indicated that inorganic P was consumed by phytoplankton in the surface layer in this oligotrophic area (Placenti et al., 2013).

3.1. Biomass distribution

The phytoplankton biomass, as CHL concentration, showed low mean values (range 0.08–0.15 μg CL^{-1}) which were homogeneously distributed among the stations. The vertical distribution exhibited the lowest values at the surface layer and a Deep Chlorophyll Maximum (DCM), located between 56 m and 89 m, was observed; the CHL maximum concentration was recorded at the deepest DCM layer (Fig. 2). The low CHL concentration and the vertical distribution are typical characteristics of the seasonal summer stratified conditions.

Table 1
Eggs density and physical and chemical parameters (range) at the different stations. The layer indicates the depth of eggs recruitment.

Stations	layer (m)	Eggs n°	Salinity	Temperature °C	Oxygen mg/l
302	0–10	1	38.1–38.2	24.9–26.4	6.36–6.67
641	0–10	5	38.0–38.1	25.4–25.5	6.49–6.53
	20–30	1	37.2–37.5	17.5–18.5	7.34–7.79
143	0–25	5	37.5–38.1	17.9–26.7	6.29–7.89
188	0–25	2	38.0–38.4	18.1–26.9	6.37–8.15
137	0–15	24	38.3–38.4	26. - 26.1	6.37–6.44
	15–25	3	38.1–38.3	19.1–26.0	6.63–8.21
	25–60	1	38.1–38.5	15.0–19.1	7.22–8.21
22	0–10	4	38.2–38.3	26.2–26.3	6.38–6.43
	10–20	3	37.9–38.3	19.6–26.2	6.41–7.67

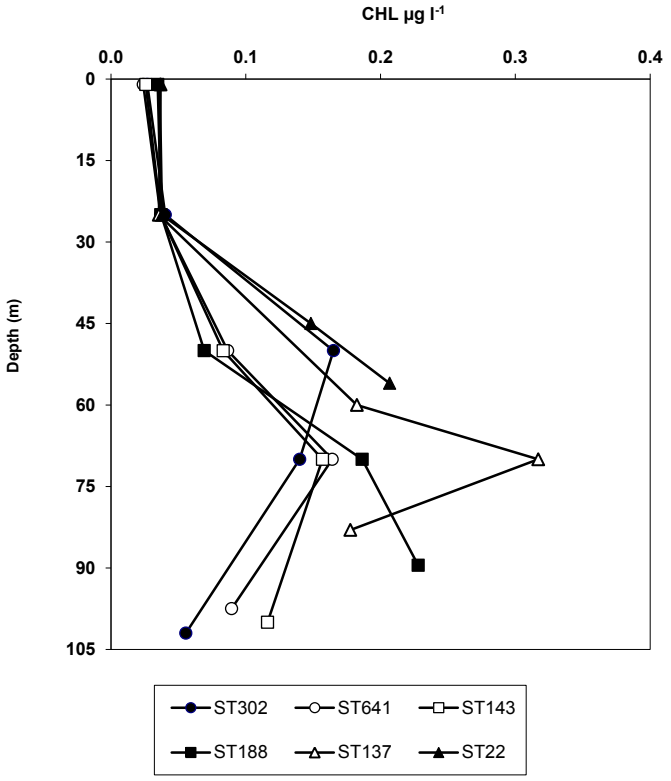


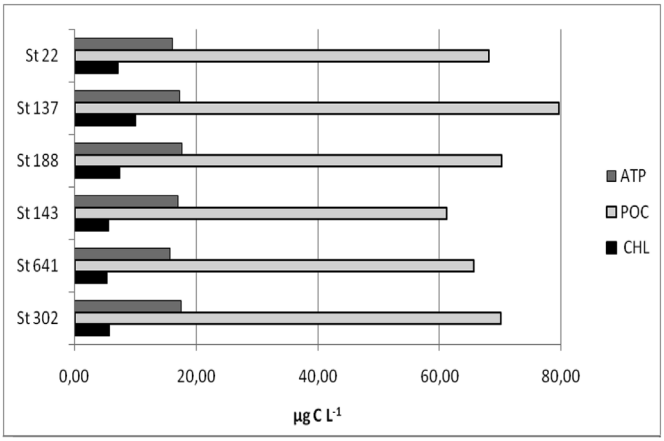
Fig. 2. Depth profiles of CHL in the different stations.

In agreement with CHL, peaks of POC and TPN were recorded at the DCM layer or below the thermocline (correlation coefficients r = 0.46 and r = 0.53, P < 0.01, respectively). POC concentration was generally low, ranging between 34.95 μg C L^{-1} and 112.60 μg C L^{-1}, as well as the TPN (range: 5.95–16.12 μg C L^{-1}); similar concentrations were reported in previous works in Mediterranean Sea (Boldrin et al., 2009; Zaccone et al., 2012).

The living part of Carbon (ATP biomass), ranged from 22 to 28% of total C (Fig. 3a). The autotrophic biomass (derived by CHL), contributed to POC in percentages ranging from 8 to 13%. However, in this seasonal condition, the CHL biomass showed percentages ranging from 32 to 58% of total ATP. Similar percentages were previously observed in the Ionian Sea by La Ferla et al. (2005).

The size spectrum of CHL showed a phytoplankton community dominated by the pico-fraction (2.0–0.2 μm) that produced 67% of the total biomass. Small cells are dominant also with the nano-fraction (2–10 μm) that represented the 23% of total CHL. The larger cells of the micro-fraction (> 10 μm), achieved only the 10% of the total phytoplanktonic biomass. These findings confirmed the predominance of the pico-phytoplankton over the other phototrophic cells as already assessed in the Southern Adriatic Sea in a two-year study (Cerino et al., 2012) and in the Ionian Sea in autumn (Zaccone et al., 2004). In the Aegean Sea, most of the autotrophic biomass and PP was attributed to small-size cells (Siokou- Frangou et al., 2002). As an average on the whole Mediterranean basin, picoplankton accounts for 59% of the total chl-*a* and 65% of the primary production (Magazzù and Decembrini, 1995). During the study period phytoplankton likely was in a declining phase, underlining the important the role of the small sized fractions in sustaining the food web in this oligotrophic area.

The ATP-derived biomass showed the pico-, nano- and micro-size fractions were more homogeneously distributed than the analogous CHL size fractions, contributing on average to 33, 30 and 37% of the total ATP-derived biomass, respectively. The total ATP values were significantly related to total CHL and to the respective size-fractions (P < 0.01).



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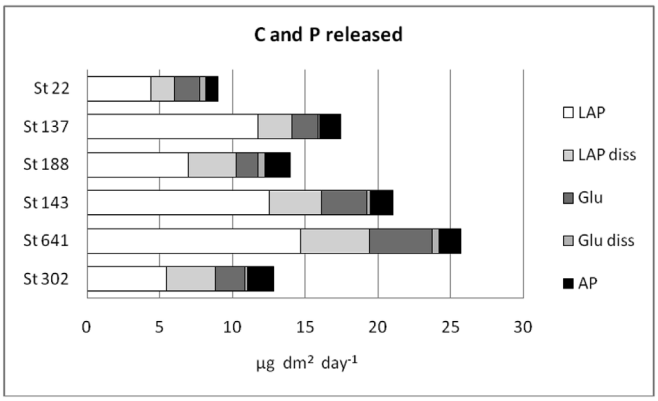

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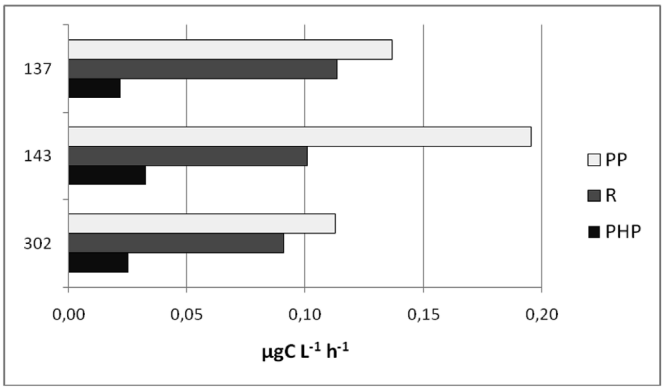

c

Fig. 3. a) Carbon contribution of POC, total ATP and CHL measured at each station. b) Carbon released by enzymatic activity both total and dissolved (LAP and GLU) and Phosphorus released by Alkaline Phosphatase -AP (Depth integrated and normalized rates per day measured at each station). c) Carbon produced by Primary Production (PP) and Prokaryotic Heterotrophic Production (PHP); Carbon released by microbial Respiration (R) at 3 stations.

The total prokaryotic abundance (TP) varied in the range of 6.4 - 18.7 $\times 10^8$ cell L^{-1}; picophytoplankton abundance (PPP) (range 1.2 - 79.5 $\times 10^6$ cell L^{-1}) was mainly represented by coccoid cyanobacteria, belonging to the genus *Synechococcus*. The abundance of TP and PPP increased between the thermocline and the DCM (between 25 m and 60 m). TP was significantly related to POC and PTN ($r = 0.52$ and $r = 0.42$, $P < 0.01$ respectively). The prokaryotic abundances determined by FCM resulted lower than TP ranging between 0.83 and 6.87 $\times 10^8$ cell L^{-1}, presumably due to the weak fluorescence signal by small prokaryotic cells. However, significant correlations between FCM and both POC and PTN were observed ($r = 0.59$ and $r = 0.50$, $P < 0.01$).

Total nanoplankton abundance (NA) (range 124 - 194 $\times 10^3$ cell L^{-1}) and biomass (range 0.11–1.71 µg C L^{-1}), were represented by the dinoflagellates *Amphidinium carterae* and *Heterocapsa niei*. The coccolithophorid *Emiliania huxleyi,* autotrophic and heterotrophic flagellates of uncertain taxonomic identification were also abundant. NA values were also related to temperature ($r = 0.51$ $P < 0.01$). The microphytoplankton abundance (PHYTO) and biomass varied between 3.0 and 9.1 $\times 10^4$ cell L^{-1} and between 1.42 and 6.44 µg C L^{-1}, respectively. The PHYTO community appeared dominated, in terms of both abundance and biomass, by phytoflagellates and dinoflagellates. These latter were represented by more taxa (36 identified species) compared to those belonging to diatoms (24 species) and coccolithophorides.

Microzooplankton (MICROZOO) populations were numerically dominated by eggs, nauplia and metazoan larval stages that prevailed in terms of biomass in almost all samples. Heterotrophic dinoflagellates, because of their high abundances, were a relevant group, while aloricates ciliates and tintinnids, despite the large number of taxa identified in this study, did not significantly contribute to the total biomass. In other Mediterranean areas aloricates, ciliates, heterotrophic dinoflagellates and micrometazoans are reported to dominate the microzooplankton community in oligotrophic waters; moreover, grazers mainly preyed on nanoplankton and heterotrophic prokaryotes, which represent the preferred prey under in oligotrophic condition (Zoccarato et al., 2016). In this way microzooplankton is recognized to channel organic carbon from the microbial loop toward the upper trophic levels of classic trophic web (Azam et al., 1983; Calbet and Saiz, 2005).

Comparing the different fractions of plankton, as calculated by integrated data, the major role of picoplankton to the global Carbon budget was evident, reaching over than 83%. PHYTO contributed for the 11%, while MICROZOO and NA represented only low fractions (4% and 1%, respectively). The importance of heterotrophic picoplankton as fundamental source for the Carbon budget was confirmed in the examined summer period. High bacterial percentages (59–69% of total heterotrophs) in the Aegean Sea were observed by Siokou-Frangou et al. (2002).

3.2. Microbial activities

As regards the enzymatic activities, total LAP activity - as an indicator of protein hydrolysis and total GLU activity as indicator of polysaccharides hydrolysis - contributed significantly to the recycling of particulate and dissolved organic matter. Depth integrated data showed high proteolytic activity (range 4.4–14.6 µg C dm^2 per day); the maximum values were observed at station 641 and 143; the minimum was observed at st. 22 (Fig. 3b). LAP was negatively related to POC and PTN ($r = -0.47$ and $r = -0.73$ respectively, $P < 0.01$).

The glucosidase, showed lower activity than protease (range 1.5–4.3 µg C dm^2 per day); the LAP/GLU ratio always > 1 was observed as well as in other temperate zones, suggesting the availability of fresh material, of proteic nature, in the euphotic layer, below the thermocline (Zaccone et al., 2004, 2012). The polysaccharides, belonging to semilabile organic matter, are degraded by heterotrophic bacterioplankton when the input of labile compounds is not sufficient to sustain bacterial needs (Hoppe, 1993; Kirchman et al., 2001).

AP values were generally low, releasing from 0.8 to 1.8 µg P dm^2 per day (Fig. 3b). This enzyme plays a key role in the mineralization of organic phosphates and it is found in autotrophic, heterotrophic prokaryotes and phytoplankton (Hoppe, 1993). The interactions between bacteria and organic matter by hydrolytic processes are very important in aquatic ecosystems, since they are involved in the mineralization processes, releasing monomers and elements, to support the new

autotrophic (PP) and heterotrophic production (Chrost and Siuda, 2006). In this study the significant relationship between AP and PHP ($r = 0.71$, $P < 0.01$) indicated the supply of key elements for bacterial growth as inorganic phosphorus; the availability of P is a crucial factor for bacterial production, to compensate P limitation in the environment (Zaccone and Caruso, 2002; Ivancic et al., 2010). In the east Mediterranean, while phytoplankton was generally N and P limited, bacterial growth was mainly P limited; this limitation could modify the pattern of microbial food web and influence the carbon flow (Thingstad et al., 2005). In other marine environments, however, bacterial remineralization of soluble phosphates may exceed the immediate needs of the bacteria, thus making the excess P directly available to drive primary production of phytoplankton, and consequently increased biomass of their predators (White et al., 2012).

Total enzymatic activity rates were comparable to those obtained in the oligotrophic zones of the Ionian and Mediterranean seas, while this is among the few reports of the dissolved fraction available in the Mediterranean Sea (Zaccone et al., 2015).

The contribution of free enzymes (i.e., enzymes not associated to cells or particles) to total activity ranged from 19.6 to 62% for dissolved LAP; high percentages were observed at st.137 and 188 (62 and 48%). The highest dissolved GLU was observed at st. 188 with a percentage of 30% on the total (Fig. 3b). Dissolved LAP and GLU were related to the respective total activities ($r = 0.59$ and $r = 0.43$, $P < 0.05$). In North Adriatic Sea free enzymes activity was scarce in surface and higher at 10 m and at bottom (Ivancic et al., 2010). However little is still known about the ecological role of these dissolved fractions (Baltar et al., 2010); free enzymes may be present in the waters as consequence of decay of cells, viral lysis or protist grazing; they may be still active in the sea for long time and far from the producing cells, contributing significantly to total activities (Arnosti, 2011).

The PHP distribution showed peaks at 25 m (0.06 $\mu g\ C\ l^{-1}\ h^{-1}$) and a decreasing trend with depth. PHP was related to TP and PPP ($r = 0.80$ and $r = 0.56$, $P < 0.01$ respectively), indicating that an active prokaryotic community was present.

The microbial processes of PP and R, are shown in Fig. 3c; the PP/R ratio was always higher than 1, assessing the prevalence of productive processes at the examined stations. In other areas of Mediterranean, the ratio of primary production/respiration was higher than 1 in winter and spring, suggesting that pelagic ecosystem was autotrophic, whereas in summer and in autumn the ratio was lower than 1, suggesting a shift towards net heterotrophic status (Fonda-Umani et al., 2012).

The contribution of fraction lower than 2 μm to total PP was also important (range 62–79%), so the most part of C was fixed by low size cells and a large part of the fixed carbon was channelled through the microbial food web. The observed oligotrophy is not in contrast with this finding, since in ecosystems dominated by small size organisms, C assimilation rates (PP) are high and cells have short turnover times (La Ferla et al., 2005). The integrated PHP/PP ratios were on average between 0.17 and 0.23 at the examined stations and these values were consistent to those generally found in oligotrophic environments (Ducklow, 2000). Similar ratios (0.23–0.32) were also found in other oligotrophic regions during the stratified period (Siokou-Frangou et al., 2002). In agreement with their hypothesis, the observed PHP/PP ratios suggest that a high proportion of primary production is left for the higher trophic levels in the Sicily Channel.

3.3. Statistical analysis

Statistical analysis performed among environmental variables (T, S, Ox), microbiological parameters and egg abundances by two dimensional CCA – using scaling type 2 - yielded the outputs shown in Fig. 4. The length of the vectors (environmental variables) indicates the degree of correlation with the axes; the position of parameters with respect to the lines indicates the extent to which the microbial fraction and activities were influenced by the environmental parameter represented by

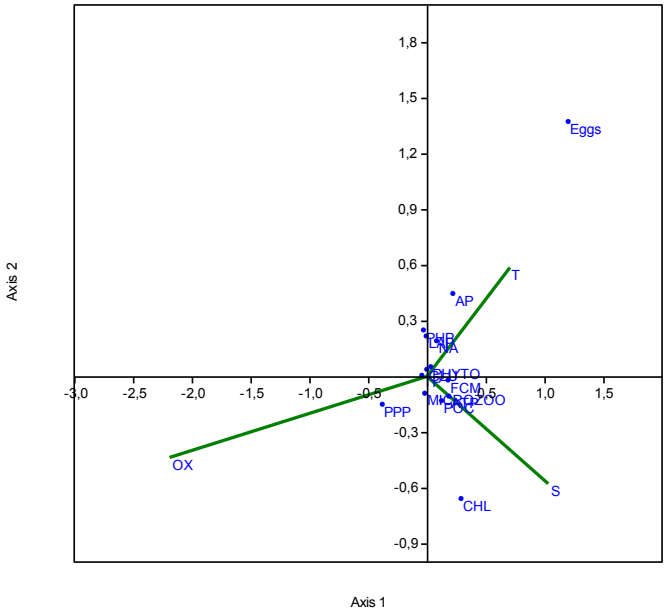

Fig. 4. Two dimensional canonical correspondence analysis (CCA) between quantitative environmental variables (Temperature, Salinity, Oxygen) and microbiological parameters (see the test for abbreviations).

lines. The cumulative percentage variance showed that the first and second canonical axes accounted for the 94.9% and 5.1% of this variance, respectively. Eggs distribution was affected by T variation during summer as well as AP, ATP, CHL, POC, FCM, NA, PHYTO which were positively influenced by the axis 1 (the same of T). The axis 2 affected positively the anchovy eggs distribution (together with T) and PHP, AP, LAP, GLU, TP, NA, PHYTO. PHP and AP were also related to T ($r = 0.45$ and $r = 0.56$, $P > 0.01$), confirming what observed by CCA.

Temperature variation was an important factor driving the biological parameters during summer, being correlated to microbial processes; high rates of metabolic activities and abundance of prokaryotes were generally recorded in coincidence with high temperature (Zaccone et al., 2015). In fact, increasing T favours microbial growth, but cells require also substrate and nutrients, supplied by microbial activities. These finding let as to suppose that rising T could cause an increase in heterotrophy (La Ferla et al., 2005). Light changes in the stock of biomass or activities of compartment of microbial food web in the euphotic zone of ocean could have major impacts on the carbon cycle operated by microbes (Sarmento et al., 2010).

Low nutrient concentrations, particularly regarding the phosphorus and reduced/limited level of CHL, POC, TPN values were found in the present study. In agreement with these findings, the rates of PP, PHP, AP, LAP, GLU (both total and free enzymes) activities confirmed the general oligotrophy of the Sicily Channel.

Temperature variation, can affect predators and fisheries and in turn affect the food web. The association of T with eggs and larvae distribution has already been pointed out; there is an optimum temperature range for the deposition of anchovy, and the formation of stable warm waters creates a favourable spawning habitat. Indeed eggs deposition in the Sicily Channel was also found to be related to fluorescence (CHL) and variation in primary production (Basilone et al., 2013) and these environmental conditions are also favourable for the survival and growth of fish larvae, which may feed on the small fraction of phytoplankton. In the Cadiz Gulf, the oceanographic conditions (upwelling, east current, and stratification) led to an aggregation of plankton and anchovy larvae in the central area, where an optimal range of temperature and chlorophyll, as an indirect food proxy for anchovy larval development, were registered (Teodósio et al., 2017). The formation of the DCM in summer and the zooplankton biomass

associated to the season offers an important food source for the larvae. Additionally, the inputs of continental waters in certain areas cause fertilization of surface waters and some species, as anchovy, takes advantage of this. These strategies, together with the high ecological efficiency of oligotrophic systems, contribute to the relatively high yield of Mediterranean fisheries. (Sabates et al., 2007). Hence, the Sicily Channel is area is of great interest as a nursery area for small fish and both autotrophic and heterotrophic processes supported by microorganism are in synergy.

4. Conclusions

This is the first attempt to link the prokaryotic activities with fish eggs productivity. The co-variation of these parameters observed in summer suggested a relationship between them; however, the cause-effect factor is still not clear and deeper studies are needed to shed light on this feature. Different environmental variables can influence each one of microbial size component and the processes associated with global changes could be accelerated or compensated. Anchovy egg occurrence was preferentially controlled by temperature, water column stability and fluorescence of spawning waters (Basilone et al., 2013).

In conclusion this study has shown the general oligotrophy of the investigated area, where the microbial trophic web seems to be developed; most of the carbon is fixed by pico-sized populations as also observed by Siokou-Frangou et al. (2002). At the same time, the levels of microbial hydrolytic activities are related to productive processes recycling the organic matter and releasing nutrients (P, N). In this context, because of the complexity of marine ecosystems, microorganisms may strongly interact with larger organisms in an array of complex, direct and indirect interdependencies; as a consequence, changes in autotrophic and heterotrophic components of microbial community may affect not only marine biogeochemical cycles but also higher food chain components. How and how much the microbial web sustains fish reproduction and larval survival in the Sicily Channel need a more comprehensive analysis and will be focused in further research.

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