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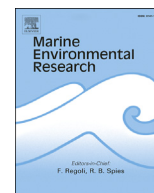

Seasonal changes on microbial metabolism and biomass in the euphotic layer of Sicilian Channel

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ABSTRACT

As a part of a wider project on fisheries ecology, several biological and environmental parameters were monitored during two oceanographic cruises (BANSIC 2012 and NOVESAR 2013) in the Sicily Channel, which connects the Western and Eastern Mediterranean basins.

The prokaryotic abundances and biomass as well as hydrolysis rates on organic matter were investigated in the euphotic layer of a retention area for fish larval stages including anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) with the aim to investigate the different biogeochemical signatures in two seasonal conditions. The environmental parameters, particulate organic carbon and nitrogen together with heterotrophic production were also measured.

Results showed significant increases for most of the studied parameters with increasing temperature during summer. This had effects on the Carbon cycle and recycling of nutrients; in fact total prokaryotic abundance and biomass, as well as carbon hydrolyzed by two enzymes (Leucine aminopeptidase and β-glucosidase), increased significantly during summer. Conversely Alkaline phosphatase activity, Chlorophyll concentration and Oxygen increased during winter. The same environmental parameters affected also the presence of fish eggs. Moreover high percentages of free enzymes (i.e., enzymes not associated with cells) were measured, accounting for percentages variable from 12 to 95 % of the total enzymatic activity, with values generally higher in summer than in winter.

In this oligotrophic environment, the prokaryotic biomass was supported by the C hydrolyzed by enzymatic activities. The ratio between the hydrolyzed C and prokaryotic biomass was higher in winter than in summer, indicating that alkaline phosphatase activity contribute to an efficient incorporation of C into biomass in winter.

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1. Introduction

Phytoplankton and prokaryotes (bacteria and archaea) are at the base of organic carbon cycle since their metabolism, both productive and degradative, is the link connecting the biotic and abiotic compartments of the oceans. The heterotrophic prokaryotes, by their biomass and metabolism transfer matter and energy to higher trophic levels, transform particulate organic matter (POM) into dissolved organic matter (DOM) and mineralize DOM to CO_2 and inorganic nutrients (Cho and Azam, 1988).

In this study we focused on the hydrolysis of organic polymers into small molecules (<600 Da) that can be uptaken into bacterial cells through the periplasmic membrane, producing living biomass. The specificity of enzymes affects the fate of many of both autochthonous or allochthonous organic compounds in the marine environment, determining how much organic carbon may be utilized in the water column and how much sinks to the deep layers or buries in the sediment. The enzymatic reactions are regulated by the availability of substrates and reaction products and are influenced by several environmental factors (temperature, pH, inhibitors etc.) (Hoppe, 1993).

As a part of a wide project in support of fisheries management, aimed at providing ecological information and at improving an ecosystem-based approach to fisheries management of European anchovy and sardine, several biological and environmental

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parameters were monitored during two oceanographic cruises (BANSIC 2012 for anchovy and NOVESAR 2013 in the case of sardine) in the Strait of Sicily, which connects the Western and Eastern Mediterranean basins.

The Sicily Channel is a well-known spawning area of different commercial fish species, including *Engraulis encrasicolus* and *Sardina pilchardus* (Basilone et al., 2013; Cuttitta et al., 2006; Garcia Lafuente et al., 2002). Distinct environmental conditions of both abiotic and biotic nature are recognized as factors influencing the early stages of development of these fish species. In particular, the role of both environmental factors (mainly primary productivity and temperature) and circulation of water masses on egg production has recently been hypothesized for anchovy and used to assess the potential fishery yields in different areas of the Sicily Channel (Garcia Lafuente et al., 2002; Basilone et al., 2013; Falcini et al., 2015). The anchovy species, in particular, are very sensitive to the physical environment particularly in the early stage and their survival is affected by temperature (Guisande et al., 2004; Basilone et al., 2006).

Fisheries science traditionally focuses on the ecology and biology of target species. However, the entire ecosystem should be taken into account to achieve an effective assessment of the fitness of any fisheries and to establish an accurate conservation strategy. Evidence of this is provided by the prominence given to ecosystem-based management in the European Commission's current review of the Common Fisheries Policy (CFP). Some Authors have focused on the role of trophic web organisms on fish larval ecology (Cuttitta et al., 2003, 2006; Link et al., 2010), but to our knowledge no study has analyzed the smallest planktonic component associated to the early life stages of fish in the environment.

The Sicily Channel has been classified as an oligotrophic area of the Eastern Mediterranean Sea (Malanotte-Rizzoli et al., 1997; Van Wambeke et al., 2002), due to low levels of chlorophyll (Patti et al., 2010) and POC (Leonardi et al., 2014). The general surface circulation pattern of this area, characterized by a high dynamism, is locally controlled by the motion of the Modified Atlantic Water (MAW), less saline, warmer and nutrient poor, in contrast to SMW (Surface Mediterranean Water), which is more saline, cooler and richer in nutrients. The MAW bifurcates in the Atlantic Ionian Stream (AIS), a meandering surface current flowing towards the Ionian Sea, and the Atlantic Tunisian Current (ATC). AIS and ATC are considered a quasi-permanent feature of the area (Béranger et al., 2004; Falcini et al., 2015).

In oligotrophic environments, a shift from the classical to the microbial food web has been observed, with cyanobacteria playing a key role as primary producers and both auto- and heterotrophic microbes driving ecosystem functioning (Van Wambeke et al., 2002; Zaccone et al., 2003, 2010, 2012). Moreover, organic matter recycling by prokaryotes metabolism has also been associated to both seasonal cycle of the organic matter and dynamics of circulatory patterns (Azzaro et al., 2012).

In the Mediterranean Sea, several papers underlined the relationship among microbial parameters and physical chemical conditions of waters (Zaccone et al., 2010, 2012) as well as water masses (Zaccone et al., 2003); it is also well known that subtle changes in key environmental variables can drastically modify the abundance, distribution, and availability of fish populations and their spawning products (Patti et al., 2004; Selvin Pitchaikani and Lipton, 2012; Basilone et al., 2013; Falcini et al., 2015).

The specific objectives of present study were to investigate the biogeochemical signatures in the euphotic layer of a coastal area of the Sicily Channel and to assess the variability of prokaryotic abundances and biomass as well as their metabolic activities in two seasonal conditions. Moreover for the first time the contribution of the free enzyme activity was evaluated in an oligotrophic area of Mediterranean Sea.

2. Materials and methods

2.1. Study area and sampling

In the frame of the RITMARE project, two oceanographic cruises were performed in the Sicily Channel (Fig. 1) on board of the R/V Urania of CNR, in summer 2012 (BANSIC, July 4–23, 2012) and winter 2013 (NOVESAR, January 14–28, 2013).

Vertical profiles of temperature (T), pressure, conductivity, fluorescence and oxygen content (Ox) were performed in three stations by means of a CTD probe SeaBird 911plus. The values of Chlorophyll (Chl) concentrations were estimated from fluorescence probe measurements.

Water samples were collected in the epipelagic layer (from 5 to 100 m depth) and processed for specific measurements by treatment on board or stored for subsequent laboratory analyses.

2.2. Egg and larval sampling

Egg and larval samples were obtained at each station by three-replicate vertical tows of a Multi Plankton Sampler (Hydrobios, mod. Mini), a five-net system for the investigation along the water column, having an aperture of 0.125 m^2 and 200 μ mesh size. The samples were immediately fixed after collection and preserved in individual ultracentrifuge plastic tubes containing 70% ethanol and saved at 4 °C. Counts of anchovy and sardine eggs and larvae were then evaluated in the land-based laboratory by a binocular stereo microscope and standardised to numbers per cubic metre. The geographical locations of each station are reported in Fig. 1.

2.3. Microbiological variables

Total prokaryotic abundance (PA) was determined after sample fixation with formaldehyde (2% final concentration) and DAPI staining (Porter and Feig, 1980) with a Zeiss AXIOPLAN 2 Imaging microscope equipped with the Axiocam digital camera. The AXIOVISION 3.1 image analysis software was used to measure bacterial cell sizes and morphotypes. Prokaryotic biomass (PB) was estimated from the cell counts and volumetric measurements according to La Ferla et al. (2012, 2014). Picophytoplankton abundance (PPPA) and biomass (PPPB) were also determined by fluorescence microscopy, according to Porter and Feig (1980). Details of the method are reported in Cerino et al. (2012).

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-concentration method (Hoppe, 1983). Aliquots of water samples (10 ml) were added with increasing amounts (5 concentrations) of each specific fluorogenic substrate and incubated in the dark at "*in situ*" temperature for 3 h. The increase of fluorescence over time (measured with a Turner TD 700 fluorimeter) was converted into the velocity of substrate hydrolysis using a standard curve of MUF and MCA. The maximum velocity of the reaction (V*max*) was calculated through a Lineweaver-Burke linear transformation and reported as nanomoles per litre and per hour (nmol L^{-1} h^{-1}).

The dissolved enzymatic fractions (defined as enzymes not associated with cells or surfaces) were measured as described above, after filtration of sample through a low binding protein membrane filter (0.2-μm pore-size filter, Millipore) (Baltar et al., 2010).

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