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# Abstract

**Dottorando**: Di Poi Elena

**Coordinatore del collegio dei docenti**: Prof. Pierluigi Nimis

**Relatore**: Prof.ssa Serena Fonda Umani

**Titolo tesi**: MICROZOOPLANKTON GRAZING IMPACT ALONG A TROPHIC GRADIENT FROM THE ATLANTIC OCEAN TO THE WESTERN MEDITERRANEAN SEA

Microzooplankton, is a group of heterotrophic and mixotrophic planktonic organisms in the size range 10 or 20 to 200 μm, which includes many protists such as loricate (tintinnids) and naked ciliates, heterotrophic and mixotrophic dinoflagellates, foraminiferans, radiolarians, acantharians, heliozoans, as well as small metazoans such as copepod nauplii, some copepodites and some meroplanktonic larvae.

From a trophic point of view, microzooplankton plays a fundamental role as principal carrier of energy from primary producers to upper trophic levels as it is the foremost predator of nanoplankton (2 – 20 μm) and picoplankton (0.2 – 2 μm) in the "Microbial loop", as well as consumer of microphytoplankton and prey of mesozooplankton in the "Classic food web". Therefore, microzooplankton is an important link between the microbial loop and the upper trophic levels within the classic food web.

Nowadays there is a general consensus that microzooplankton is important in mesozooplankton diet, providing an essential food supply. When available, microzooplankton and especially ciliates are selectively eaten by mesozooplankton but the reported contribution of microzooplankton to mesozooplankton carbon ration is very variable.

The aim of my PhD. research is basically focused on the quantification of the carbon flux through microbial community, determining microzooplankton grazing impact on heterotrophic and autotrophic picoplankton, nanoplankton and microphytoplankton, on the identification of both predator and prey communities according to main groups, genus and species (when possible) as well as on the determination of the prey selectivity exerted by the heterotrophic communities. Furthermore, on the analysis of the synergistic and antagonistic

effects of the grazing impact of both microzooplankton and nanoflagellates on autotrophic and heterotrophic picoplankton, on the quantification of the secondary production (growth) of the predators represented by microzooplankton as well as on the evaluation of mesozooplankton grazing impact on microplanktonic community. Among the purposes, the assessment of the spatial distribution of microzooplankton, comparing both the composition and the abundance of microzooplankton collected with different sampling and conservation techniques.

All the samples analyzed in the present study, are referred to 5 sites VA (Atlantic Ocean), V4 (Alborán Sea), V3 (Balearic Sea), V1 (Ligurian Sea) and V2 (Tyrrhenian Sea) sampled at the surface layer (-5 m), on board of the N/O URANIA-CNR, during the II Leg. of the *Transmediterranean cruise* which was held from the Atlantic Ocean into the Western Mediterranean from 28 May to 11 June 2007. The campaign occurs in the frame of the V.E.C.T.O.R. project (VulnErability of Coasts and marine italian ecosystems to Climate change and their rOle in the mediterranean caRbon cycles) under the supervision of C.o.N.I.S.M.a.

The activity of the V.E.C.T.O.R. project is substantially turned to deepen our knowledge of the impact exerted by Global Climate Changes on the Mediterranean marine environment, focusing our attention on sedimentary, physical and biogeochemical processes throughout the water masses.

Among the different approaches employed in determining the grazing impacts of microzooplankton on a large variety of prey, I applied the dilution technique since the method is the most broadly used in plankton ecology. The dilution method, first established by Landry and Hassett (1982) and modify by Landry *et al*., (1995) is nowadays considered as a standard protocol that unlike the other available approaches, is extremely easy to be performed, involves minimal handling and physical disruption of the organisms and in the meantime enables the separation between consumers and prey since they belong to the analogous size classes. By means of this protocol, we are able to determine both, the specific growth and grazing rates of the predators represented by microzooplankton, as well as the specific growth and mortality rates of the prey consisting of heterotrophic and autotrophic bacteria, nanoplankton and microphytoplankton. The dilution approach relies on the reduction of encounter rates between prey and grazers. Natural assemblages are amended with varying proportions of filtered seawater (particles-free water) creating a dilution series, and grazing rate is estimated as the increase in apparent prey growth rate with dilution factor. Specifically, microzooplankton grazing rate is estimated as the slope of a regression of apparent prey growth in the various dilutions against dilution factor whereas, the growth rate of the prey is

estimated as apparent growth rate extrapolated to 100 % dilution (growth in the absence of grazers). The proceeding theoretical development involves three restrictive assumptions:

1) The growth of individual prey is not directly affected by the presence or absence of other prey. To satisfy this assumption, dissolved nutrients must remains non-limiting, or equally limiting, to growth at all dilutions during the experimental incubations; the growth of individual prey is exponential.

2) The clearance rate of individual consumer is assumed to be constant at all dilutions.

3) The probability of a prey being consumed is a direct function of the rate of encounter of consumers with prey cells. This implies that consumers are not food-satiated at natural prey densities and that the number of prey ingested by given consumer is linearly related to prey density. The change in the density of a prey C, over a period of time t (24h) can be represented appropriately by the following exponential equation, based on Landry and Hassett protocol (1982) (Landry, 1993):

$$C_t = C_0 \, e^{(k-g)t}$$

or

$$(1/t)\ln(C_t/ C_0) = k-g$$

Where:

$C_0$ = the concentration of the prey (or total biomass) at the beginning of the experiment, $C_t$ = the concentration of the prey (or total biomass) at the end of the incubation (time t), k = the instantaneous coefficient of population growth, g = the instantaneous coefficient of grazing mortality, t = the incubation time (24h).

From the first postulate, the instantaneous coefficient of population growth *k* is not influenced by the dilution series, it remains constant during incubation; the instantaneous coefficient of grazing mortality *g* in accordance with the third postulate is proportional to the consumers and prey density. Since *k* is constant and *g* is proportional to the dilution series, the equation with two unknown *k* and *g* may be graphically solved from regression of apparent growth against dilution factor. The growth rate of pico-, nano- and microphytoplankton, and microzooplankton grazing rate can be calculate from Model I regressions of apparent growth against dilution factor, based on Landry and Hassett (1982) (Landry, 1993). The apparent growth rate coefficient $(1/t)\ln(C_t/ C_0)$ is on the ordinate axis whereas the dilution rates are on abscissa axis. The Y-axis intercept, where g = 0 is the prey growth rate (k), while the negative slope of this relationship is the grazing coefficient (g).

When parameters such as the concentration of the prey at the beginning of the experiment ($C_0$), the instantaneous coefficient of population growth (k) the grazing coefficient (g) are provided, then, we are able to calculate the production (P) and the ingestion rate (I) as

well as further parameters. The real production (Pr), the potential production (Pp), the potential production removed by grazing (PP%), and the initial production removed by grazing (SP%) were useful data for a more accurate data analysis.

Sampling procedures were executed by means of a SBE sampler carousel equipped by a 24 Niskin bottles rosette, each furnished with 12 L Niskin bottles fitted-out by silicon elastic ribbon and red silicon O rings. An amount of ca. 100 L was collected from the surface, at each selected station. In order to eliminate any possible mesozooplanktonic grazer, immediately after collection, natural assemblages were gently poured through a nylon sieve with a 200 µm mesh size into 5 polypropylene carbuoys whereas, an amount from the same natural assemblage was filtered onto 0.22 µm pore size millipore filters by means of a peristaltic pump. This filtration step eliminated all but some very small bacteria, mostly vibrios. Measured volumes of seawater from the carbuoys were poured into polycarbonate incubation bottles and successively, diluted with the ones filtered onto 0.22 µm, according to four dilution levels (100% whole water, 80%, 50%, 20%) and 3 replicates each dilution level where performed. The experiments involved 4 parameters: microzoo-, microphyto-, nano- and picoplankton.

The initial samples ($C_0$) were filled with the same procedure mentioned for incubation bottles, immediately conserved in 2% buffered formaldehyde and stored in a cold room (5°C) maintained in the dark. For picoplankton fraction only, the preservative was pre-filtered onto 0.2 µm in order to eliminate any possible impurity.

As nutrient limitation during summer was anticipated in the Mediterranean basin, nutrient were added equally to each incubation bottle; the nutrient addition was: 5 µM $NaNO_3$ and 1 µM $KH_2PO_4$ respectively. Incubations were carried out on the main deck, in a flowing seawater incubator maintaining *in situ* conditions of temperature and light. All dilution bottles were constantly monitored during incubation time; after 24 hours, ($C_t$) samples were conserved as the initial ones. Nanoplanktonic organisms ($C_0$ and $C_{24}$ samples) were preserved in 1% buffered glutaraldheyde contrarily to the other protozoans, since glutaraldheyde is commonly used as preservative for nanoplankton.

Contemporary to the dilution experiments, microzooplankton growth (or secondary production µg C $L^{-1}d^{-1}$) was also investigated. On board, 6 L ca. of pre-filtered water were necessary to perform the experiment. The whole water (100%) was first, poured into 3 polycarbonate incubation bottles with the equal addition of nutrients; then, samples were immediately incubated at the *in situ* conditions for at least 48 hours. At the end of the incubation time, samples were preserved in 2% buffered formaldehyde and stored in a cold room maintained in the dark (5°C).

For quali-quantitatives analysis of the consumers' community, distribution experiments were performed along the cruise track, at each selected station. 5 L of surface water were collected without sustaining any filtration procedure. In the meantime, 300 mL of whole water were poured into glass bottles and fixed in 2% Lugol's solution whereas the remaining amount was immediately filtered onto 10 µm pore size sieve by inverse filtration. The concentrated samples were preserved in 2% buffered formaldehyde and stored in the dark as the previous ones. Samples preserved in Lugol's solution were collected in all but in the Balearic station (V3). Lugol's solution and formalin are the most commonly used fixatives for ciliate assemblages. In the present study, a double preservation was accomplished with the purpose of a comparison between the two fixatives.

During the *Transmediterranean cruise*, both dilution and mesozooplankton grazing experiments were run simultaneously, with the exception of three occasions: the Atlantic Ocean (VA), the Alborán station and the Balearic one (V3) where, the massive presence of gelatinous plankton (e.g. cnidarians, jellies) did not permit the collection of mesozooplankton community from the nets. Thus, the two easternmost locations of the western basin represented by the Ligurian station (V1) and the South Tyrrhenian site (V2) were the only two stations being sampled.

Mesozooplankton was collected by means of oblique tows, and a 200 µm mesh plankton WP2 net. Immediately after capture, mesozooplankton was diluted into a 5 L glass incubator and finally transferred at the laboratory on board, where a number of dominant species were selected by a previous overview of individuals at the stereomicroscope.

To assess the grazing rate of mesozooplankton on microplankton, 3 polycarbonate bottles of whole water 100% were simultaneously performed. In each bottle, a number of selected species were equally inoculated into incubation bottles. Incubations were carried out on board at the simulate *in situ* conditions for 24h. The 100% bottles from the dilution series also served as initial ($C_0$) and control ($C_{24}$) samples, for the ones containing mesozooplankton. After 24h, samples were preserved in 2% buffered formaldehyde and stored in a cold room (5°C) maintained in the dark.

The quali-quantitative analysis was carried out at the University of Trieste, in the laboratories of the Department of Life Science, under the supervision of the Prof. Serena Fonda Umani. During my PhD thesis, a number of 750 samples were analysed using both epifluorescence and inverted microscopes.

Picoplankton samples were preserved in pre-filtered formaldehyde at 2% final concentration, and filtered onto black 0.2 µm polycarbonate membrane filters laid over pre-wetted 0.45 µm nitrocellulose backing filters by means of a filtration apparatus. Heterotrophic

cells were stained for at least 15 minutes in DAPI (4'6 – diamidino – 2 – phenylindole) at 1µg $mL^{-1}$ final concentration, while the autotrophic share was filtered separately. For both heterotrophic and autotrophic picoplankton, sample volumes were adjusted per dilution: 10 mL for 20%, 6 mL for 50%, 4 mL for 80% and 3 mL for 100% whole water for the heterotrophic fraction; 20 mL for 20 and 50% dilutions, 15 mL for 80% and 10 mL for 100% whole water for autotrophic cells respectively. Heterotrophic bacteria were filtered in 9 replicates whereas 3 replicates were filtered for the autotrophic ones. Filters were finally maintained at -20°C until processed. Picoplankton enumeration was conducted using an epifluorescence microscope equipped by a 100x oil objective. Cell numbers of hetero- and autotrophic picoplankton were converted to carbon biomass using specific conversion factors available in literature. An amount of 480 samples were analysed.

Nanoplankton was preserved in 1% glutaraldheyde; sample volume was 100 mL for 20 and 50% dilutions, 80 mL for 80%, and 60 mL for 100% whole water. Samples were filtered onto black 0.8 µm polycarbonate filters positioned on 1.2 µm nitrocellulose backing filters. Cells were stained and stored as described for picoplankton. Nanoplanktonic cells (3 replicates) were analyzed at the epifluorescence microscope as previous mentioned. Autotrophic nanoflagellates were indistinct from the heterotrophic component, mainly due to the loss in Chl. *a* fluorescence, thus, the fraction was only separated in 3 dimensional sizes: < 3 µm, 3-5 µm e >5 µm. Cells number was then converted into carbon biomass by means of a specific conversion factor applied for nanoplankton of oligotrophic systems. An amount of 120 samples were analyzed.

Microphyto – and microzooplankton samples were preserved in 2% formaldehyde and stored in a cold room maintained in the dark (5°C) until processed. Volume of sedimentation cylinder used for this purpose, according to Uthermöhl (1958), was 100 mL. Original samples (2 L) after being still for at least 48 h (in order to allow the pre-sedimentation of cells), were then concentrated to 200 mL ca. and previous homogenization, 100 mL was settled by sedimentation for al least 72 hours. Sample analysis was performed in 3 replicates ($C_0$, $C_{24}$) and conducted at the inverted microscope using a 32x objective. Half sedimentation chamber was observed for the autotrophic fraction whereas, the whole chamber was analyzed for the heterotrophic one. Microplankton individuals were converted into cell $L^{-1}$ and carbon biomass was assessed by previous determination of biovolumes by means of standard geometrical formulae, and measuring the species' linear dimensions which were compared to standard geometrical shapes; thus, the consequent biovolumes were finally converted in carbon content using specific conversion factors available in literature. 120 samples were analysed for this purpose.

Volume of sedimentation cylinder used for distribution analysis, ranged from 50 mL to 100 mL, depending on the cell concentration. 9 samples were settled by sedimentation and counted at the inverted microscope; counts were executed on the whole chamber and microplankton individuals were then converted into cells $L^{-1}$.

In order to assess microzooplankton secondary production, composition and carbon biomass ($\mu CL^{-1}$) at the beginning ($C_0$) and at the end ($C_{48}$) of the experiment were compared; 3 replicates of $C_{48}$ samples (100% whole water) were processed as the initial ones ($C_0$), following the Uthermöhl (1958) method and conducing sample analysis at the inverted microscope. 15 samples were processed in this investigation.

The grazing activity exerted by mesozooplankton on microplankton fraction, was quantified evaluating the abundance as well as the biomass of the prey at the beginning and at the end of the experiment. The 100% bottles from the dilution series served as initial ($C_0$) and control ($C_{24}$) samples, for the 100% bottles ($C_{24}$) containing mesozooplankton. An amount of 100 mL from the pre-concentrate samples (3 replicates) were used for microscopic analysis using sedimentation chambers (Uthermöhl, 1958) and the inverted microscope; 6 samples were processed for this purpose. Half sedimentation chamber was analyzed for microphytoplankton whereas the entire chamber was observed for the microzooplankton fraction. All replicates containing the dominant copepods ($C_{24}$), were filtered onto 200 µm by inverse filtration then, mesozooplankton was counted at the stereomicroscope in order to verify the abundance of the consumers selected at the beginning of the experiment. Individuals were converted in cells $L^{-1}$ and finally into carbon biomass by means of specific conversion factors available in literature.

The water masses along the II Leg. of the "*transmediterranean cruise*" performed in the western basin were characterized by the typical summer oligotrophy and P-limitation. The marked oligotrophy found in my study, was also a response to the anomalous winter conditions occurred in the previous months, where higher average temperatures, scarce mixing of the water column, few precipitations and terrestrial inputs were recorded all over the basin. The exhaustion of nutrient from the surface layer, the strong stratification, the scanty concentration of primary production and Chl *a* during sampling, implied a strong decrement in the autotrophic component within the trophic web.

As expected, microzooplankton resulted impoverished, mainly resource-limited, following a decreasing longitudinal gradient from the Atlantic station ($2.08 \times 10^2$ ind. $L^{-1}$; 0.8 µgC $L^{-1}$) to Southern Tyrrhenian ( $7.6 \times 10^1$ ind. $L^{-1}$; 0,2 µgC $L^{-1}$). Most of the predators were characterized by forms of medium (30-50 µm) and small size (<30 µm) such as aloricate ciliates mainly of the genus *Strombidium* and by nanoflagellates (< 20 µm), followed by

dinoflagellates such as the genus *Gymnodinium* and the species *Gyrodinium fusiforme*, that justified the narrow biomass of the community during the summer period. Tintinnid ciliates, with their suddenly decline in both abundance and biomass, from the Atlantic site into the Western Mediterranean, showed a constant low concentration within the western basin that never exceeded 2.0 x $10^1$ ind. $L^{-1}$ but, in the meantime they accounted for a significant part of the biomass along with the micrometazoans within microzooplankton assemblage.

Despite the narrow biomass of the community, microzooplankton growth was performed for most of the taxa involved; it means that in most of the sites micro-grazers, found their suitable prey. The secondary production however was scarce, it never exceeded 0.63 µgC $L^{-1}$ $d^{-1}$.

Microzooplankton grazing was highly selective, depended on the composition of the prey as well as on the structure of the predators community. When it occurred, microzooplankton exerted a top down control on its prey (g>k) as already displayed in typical non productive systems such as the Mediterranean in the frame of this study.

During summer 2007, autotrophic fraction was extremely scarce compared to the heterotrophic assemblage; it was always < 1.5 µgC $L^{-1}$ due to the nutrients depletion of the surface layers. Smaller size cells, in these conditions, benefited of their higher ratio between cellular surface and volume to provide a better nutrient uptake compared to the larger fractions.

The extreme scarce microphytoplankton was present with on average 2 x $10^3$ ind. $L^{-1}$ and it was mostly characterized by small phytoflagellates as well as by armoured dinoflagellates < 20 µm and coccolithophorids; this fractions provided low carbon contents to the micro-grazers; ingestions were always < 0.38 µgC $L^{-1}$ $d^{-1}$. Autotrophic prokaryotes that account for ~ 5 x $10^6$ cells $L^{-1}$ displayed similar undetectable low ingestions within the western basin.

Among different dimensional sizes, the highest carbon content which fuelled toward the upper trophic levels was due to heterotrophic prokaryotes with on average 11.4 µgC $L^{-1}$, whereas nanoplankton carbon amount was halved 5.3 µgC $L^{-1}$; these categories were the most grazed within the microbial loop. Heterotrophic bacteria which accounted for ~ 5 x $10^8$ cells $L^{-1}$ displayed sometimes very high ingestion rates as it occurred in the Alborán and in the Tyrrhenian sites with 27.14 and 24.56 µgC $L^{-1}$ $d^{-1}$ respectively. High ingestions were also detected for the nano-sized prey in the Ligurian Sea with 17.88 µgC $L^{-1}$ $d^{-1}$, since all nano-sized classes were here subjected to grazing (<3 µm, 3-5 µm, >5 µm). Beside the rest, total nanoplankton was present with on average 6.3 x $10^5$ cells $L^{-1}$. Thus, heterotrophic prokaryotes and nanoplankton constituted the main carbon resources for microzooplankton

during the extreme oligotrophic period found during summer 2007. The results of the present study demonstrate that most of the carbon fluxes in Western Mediterranean passes through microzooplankton, which is able to control the smaller-sized producers and consumers.

The double estimation of the grazing activity exerted on prokaryotes by microzooplankton (MZP) on the one hand and by heteronanoflagellates (HNF) on the other hand (performed with separate dilution experiments) provided a better understanding of the dynamics of the system under study and the establishment of the complexity of the food web. The opening of the black box, allowed me to identify several potential grazing models. In the Atlantic Ocean, MZP ingestion on prokaryotes was lower in respect to the one of HNF; in this way, MZP only reduced the loss of the prokaryotes biomass by means of the contemporary grazing activity exerted on HNF. In the Alborán Sea, in the Balearics as well as in Southern Tyrrhenian, MZP exerted a direct uptake of bacteria, since MZP ingestion on prokaryotes was higher compared to the one of HNF in the first case; in the second case, MZP did not feed on HNF that in its turn did not feed on heterotrophic prey. More over, in the Tyrrhenian Sea, MZP did not feed on HNF; thus, both predations were summed. Finally, in the Ligurian Sea, only HNF fed on prokaryotes; thus, the potential grazing performed by HNF on heterotrophic picoplankton, was inhibited by the simultaneous grazing impact of MZP on HNF fraction.

From the comparative analysis of microzooplankton, assessed by means of different sampling methodologies and fixatives I can assert, that no statistical differences were found within the 3 replicates (2 L bottles) derived from the dilution experiment, whereas statistical difference were displayed among the different samples (2 L, 5 L, 300 mL samples) treated with distinct methodologies and fixatives. The best representation in terms of species richness were obtained by means of higher sampling volumes, the 5 L conserved in 2% buffered formaldehyde; whereas, the 300 mL preserved in 2% Lugol's solution showed higher abundances and provided a better distinction of the naked ciliates but in the meantime, it caused a loss of tintinnid ciliates. These concentrations, were probably overestimated by the small volume applied to the analysis (300 mL) whereas higher volumes (5 L) appeared underestimated compared to the 3 replicates (2 L) from the dilution experiments which, after all better represented the diversity of the predators community. Thus, in my research, each methodology had its advantages and drawbacks to be keep in mind. In fact, nowadays despite the recognized pivotal role assumed by microzooplankton within the trophic webs, there is not yet a commonly accepted method to sample (net vs bottle), nor a common consensus on the volume to observe and the best fixative (and relative concentration) to use. On my

experience, it seems opportune to conduct the analysis applying more than one method and collecting this frail fraction preferably in different volumes, when possible.

In the only two sites V1 and V2 where mesozooplankton grazing on microphyto- and microzooplankton was assessed, the transfer of the biomasses < 200 µm towards upper trophic levels (copepods) was very scarce: 0.76 µgC $L^{-1}d^{-1}$ at V1 and 0.06 µgC $L^{-1}d^{-1}$ at V2 respectively. In the Ligurian Sea, the omnivorous calanoid *Centropages* spp. grazed on both microphyto- and microzooplankton. Mesozooplankton and microzooplankton opted for the portioning of the resources available. In V1, for instance microzooplankton fed on diatoms whereas mesozooplankton fed on dinoflagellates > 20 µm as well as on the coccolithophorids. In Southern Tyrrhenian, the carnivorous cyclopoid *Corycaeus* spp. fed exclusively on microzooplankton with the only exclusion of aloricate ciliates. No export of autotrophic assemblages was detected in the Ligurian Sea whereas a minimum export of 2.84 µgC $L^{-1}d^{-1}$ was verified in Southern Tyrrhenian.

In such oligotrophic conditions, micro-grazers consumed prevalently heterotrophic bacteria and in a minor part heteronanoflagellates which in turn, fuelled the upper trophic levels through predation of mesozooplankton on microzooplankton. The relative dominance of heterotrophic biomass corresponds to the expectations of lower carbon export especially in systems such as the Mediterranean in which, primary production is dominated by small autotrophs during summer.

Thus, the Mediterranean basin, that in summer oligotrophic conditions such those found during the II Leg. of the *Transmediterranean cruise*, seems to be characterized by a microbial dominated food web, made up by considerable small heterotrophs and narrow small phototrophs, displays a scenario of little energy transfer toward upper trophic levels.

Although the “microbial loop” coexists with the “microbial food web” forming the so called “Mistivourous food web” the majority of the heterotrophic biomass focus on the non recently photosynthesized matter and the energy derived from DOC (Dissolved Organic Carbon), likely more refractory, mostly fuels throughout the smaller dimensional sizes, (DOC – bacteria – nanoplankton – microzooplankton and recycled again into DOC constituting the so called “microbial loop”) characterized by intense metabolisms and fast turnover time. As a consequence, the system under study, records a higher heterotrophic biomass of smaller size in respect to the primary producers. In these circumstances, the organic matter derived both from the dissolved organic substrates and in a minor part from autotrophic uptake, is intensely respired in the upper layers by micro-heterotrophs and returned to the atmosphere more than it could be fixed. The intense respiration of the whole system, causes a scarce production compared to respiration needs. Consequently in summer conditions, we can

consider the western Mediterranean pelagic system basically heterotrophic and based on DOC regenerations within the microbial loop.

Results obtained from these analysis, will bring a significant contribute to the studies on the carbon fluxes within the Mediterranean pelagic ecosystem and jointly to the results obtained from other experimentations performed during the Transmed such as for instance those on the sedimentary processes and physics, on biogeochemical cycles as well as on the biodiversity of the basin etc. will deepen our knowledge on the active roles played by the Mediterranean basin in the Global Carbon cycle which is among the final aims of the *Transmediterranean cruise* in the frame of the V.E.C.T.O.R. project. The prevalence of one trophic web over the other (Microbial loop vs. Classical food web) one is of great implication in the time of turn over of $CO_2$ of the basin that can act as a "source" or a "sink" of $C0_2$".