Major constrains of the pelagic food web efficiency in the Mediterranean Sea.

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ABSTRACT

In this study, we analyzed more than 80 dilution experiments carried out at the surface in many Mediterranean sites that covered a wide range of trophic conditions, and in the meso-bathypelagic layers. Our major aim was to test the hypothesis that picoplankton, and particularly heterotrophic prokaryotes, are pivotal in sustaining not only nanoplankton but also microzooplankton energy requirements at all considered trophic states.

Our results highlighted as bacterivory was the major pathway of organic carbon in oligotrophic and meso-eutrophic environments. Microzooplankton mostly fed directly or indirectly (through nanoplankton exploitation) on picoplankton. In eutrophied conditions herbivory was the main trophic pathway, however heterotrophic picoplankton represented a not negligible source of carbon. In this condition we assessed the lowest food web efficiency, possibly because of consumers' satiation, which translated in an excess of autotrophic biomass available for export or transfer to higher trophic levels. Food web efficiency was higher in meso-eutrophic and oligotrophic conditions where the major pathway was bacterivory.

In the meso-bathypelagic layers we assessed only nanoplankton predation on heterotrophic picoplankton. Also in this case food web efficiency, nevertheless the diluted environment, was relatively high. Nanoplankton seemed able to efficiently exploit the available HP biomass.

1. INTRODUCTION

Food web efficiency is the ratio between the productivity of the highest trophic level and the productivity at the lower trophic levels (e.g. Rand and Stewart, 1998; Berglund *et al.*, 2007). The length of the food web, which characterizes different environments, influences the final amount of transferred biomass: more trophic levels less biomass (and energy) will reach top predators. In the "classic" marine pelagic food web (micro)phytoplankton are the producers, which fuel top predators through zooplankton grazing and fish predation. Since early 80's (Azam *et al.* 1983) the classic grazing food web was substituted by a more complex model that posed prokaryotes at the base of the food webs.

In the photic zone of the oligotrophic systems (i.e. open ocean) picophytoplankton (cyanobacteria and pico-eukarya), together with small autotrophic nanoplankton fix more carbon than microphytoplankton (i.e. diatoms) (e.g. Sommer *et al.*, 2002). The major consumers of picoplankton are heterotrophic nanoplankton (NP; 2-10 μm) and, directly or indirectly, microzooplankton (MZP; 10-200 μm) mainly composed by heterotrophic protists and larval stages of metazoans. Grazing of NP and MZP on smaller organisms is critical for the carbon transfer along the trophic food web and for the remineralisation of organic carbon (Sherr and Sherr, 1994). Planktonic communities are also structured by grazing pressure that controls their biomass, diversity (James and Hall, 1998, Lessard and Murrell, 1998), and primary productivity (Burkill *et al.*, 1995; Cotano *et al.*, 1998).

In the aphotic zone, despite it accounts for 70% of total seawater volume, food webs are almost unexplored (Nagata *et al.*, 2010). Deep-water communities were generally considered bottom-up controlled because prokaryotes and consumers' abundance ratio decrease from the surface with a drastic reduction of the grazing pressure. However, prokaryotes are non-random distributed because most of them are attached to sinking particles creating micro-hot spots were prey-predator interactions take place (Azam, 1998; Herndl *et al.*, 2008; Aristegui *et al.*, 2009; Nagata *et al.*, 2010). Furthermore, Aristegui *et al.* (2009) found that the prokaryotes-consumers ratio only halves in meso-bathypelagic zones from the euphotic layers' ratio, thus revaluating the significance of grazing. Recently Pachiadaki *et al.* (2014) and Rocke *et al.* (2015) have measured the grazing impact on prokaryotic bathypelagic communities and found that the removal can be more than 30% of the initial standing stock. The relevance of viral-induced mortality is still unclear: Fonda Umani *et al.* (2010) found that on average viral induced mortality of prokaryotes was 4 times less compared to grazing loss, and Parada *et al.* (2007) despite that in the bathypelagic realm virus-host ratio increased by 10-times relative to the surface, suggested that viral induced mortality is not so relevant as expected.

The assessment of the predators' grazing pressure on picoplankton is a key point in order to understand the food web efficiency, not only in the oligotrophic marine systems, but also in the most eutrophicated coastal systems (Sommer *et al.*, 2002). Recently, De Laender *et al.* (2010) using the linear inverse model approach, estimated that in microbial dominated trophic food webs bacteria are four time more important than phytoplankton in the protists' diet, while in herbivorous dominated food webs the diet of protists consist of similar amounts of bacteria and phytoplankton.

To test the hypothesis that picoplankton, and particularly heterotrophic prokaryotes, are pivotal in sustaining not only NP but also MZP energy requirements over a wide range of trophic conditions,

we compared the results of more than 80 dilution experiments (Landry and Hasset, 1982) carried out in the entire Mediterranean Sea. Part of these results were already published: Gulf of Trieste (Fonda Umani *et al.*, 2012); bathypelagic experiments during the trans-Mediterranean VECTOR cruise (Fonda Umani *et al.*, 2010); surface experiments during the same cruise (Di Poi *et al.*, 2013) and unpublished results from OBAMA cruise (see the following 2.1 Studied areas).

2. MATERIALS AND METHOIDS

2.1. Studied area

The Mediterranean Sea is considered an oligotrophic basin due to the scarce pool of nutrients and chlorophyll *a* (Krom *et al.*, 1991; Antoine *et al.*, 1995). Oligotrophy exasperates moving eastwards as remarked by major decreasing gradients of nutrient concentrations (Krom *et al.*, 1993), primary production, autotrophic biomass, export of primary production (Danovaro *et al.*, 1999; Dolan *et al.*, 1999; Turley *et al.*, 2000) and chlorophyll concentration (Williams, 1998).

On average, the most limiting nutrient is inorganic phosphorus, N:P ratio was found up to 60, while carbon and nitrogen limitations can occur and co-occur and they are influenced by depth (Sala *et al.*, 2002; Van Wambeke *et al.*, 2000, 2009). Phosphorus limits the primary production (Berland *et al.*, 1984; Thingstad and Rassoulzadegan, 1995, 1999; Thingstad *et al.*, 2005) but while phytoplankton are both N and P limited, picoplanktonic fraction is more sensitive to P limitation (Pitta *et al.*, 2005; Thingstad *et al.*, 2005; Zohary *et al.*, 2005). This depleted condition strongly affects communities that populate the Mediterranean ecosystems whose food webs are mostly microbial-dominated (Wikner and Hagström, 1988; Fogg, 1995).

Only few areas of the basin (close to river mouths, upwelling areas) are characterized by eutrophic conditions and present plankton communities where larger autotrophic and heterotrophic organisms become more representative.

Experiments were performed at 15 sites spread around the Mediterranean Sea. Specifically, from east to west: Aegean Sea (3 sites), Ionian Sea (3 sites), Otranto strait (1 site), Adriatic Sea (3 sites), Tyrrhenian Sea (1 site), Ligurian Sea (1 site), Balearic Sea (1 site), Alboran Sea (1 site) and Atlantic Ocean (1 site) (Fig. 1).

At these sites 82 dilution experiments were set up: 68 carried out at the sub-surface level (0.5 m depth) and 14 carried out in the meso-bathypelagic realm (between 670 m and 3860 m depth). Thirty four surface experiments were designed to assess MZP grazing pressure and 34 to simultaneously assess NP grazing pressure.

Experiments were performed on board during two oceanographic cruises: Trans Mediterranean campaign of the VECTOR project, from 28th of May to 28th of June 2007 on board of the R/V Urania and Universitatis (9 sites along a west to east transect); OBAMA cruise of the namesake project, from 24th of March to 06th of April 2011 on board of the R/V Urania, (5 sites between the Northern Ionian Sea and the Southern Adriatic Sea). Details of the sampling are reported in appendix Table A1 and A2. Water samples were seasonally collected at the station C1 (13.710 E, 45.701 N, depth of 17 m) in the Gulf of Trieste - Northern Adriatic Sea from autumn 1998 to summer 2005 to set up the dilution experiments that were run under *in situ* simulated conditions at the Laboratory of Marine Biology of Trieste, Italy (now Department of Biological Oceanography, BiO, OGS, Trieste, Italy). A total of 42 experiments were analysed giving a description of the lower part of the pelagic food web in a coastal area during eutrophied, meso-eutrophic and few oligotrophic conditions (for more details see Fonda Umani *et al.*, 2005, 2012).

2.2. Dilution techniques

MZP-Dilutions experiment. Forty-eight liters of pre-screened (<200 μm) seawater collected at the surface layer was diluted with filtered (0.22 μm), particle free sea water from the same sample. Two identical bottle sets (2 L) of four dilutions each were made in the following proportions: 100% (whole sea water), 80%, 50% and 10% in three replicates each. The first set of dilutions (T_0) was immediately fixed with buffered and filtered formaldehyde solution (2% final concentration). The second set of dilution (T_{24}) was incubated at *in situ* temperature for 24 hours on the deck (or on the shore) in 600 L tanks with a circulation of sea-water. Flowing water maintained in movement the bottles that, at any rates, were turned upside each 3 - 4 hours. To estimate *in situ* phytoplankton growth rate several, but not all, incubations were conducted with and without the addition of nutrients (5 μM $NaNO_3$ and 1μM KH_2PO_4). Differences between the two estimated growth rates were not significant (Wilcoxon test *p*-value = 0.65). At the end of the incubation, the samples were fixed as the initial ones. Samples for MZP and microphytoplankton analyses were conserved in plastic bottles and at ambient temperature, while samples for nanoplankton and picoplankton analyses were conserved in black plastic bottles, stored in the dark and at 4°C, until the laboratory analysis.

NP-Dilutions experiment. Twelve liters of seawater were collected at the surface and in the meso-bathypelagic layers, pre-filtered immediately through a 200 μm mesh and then filtered through a 10 μm mesh to remove larger predators. Sets of dilutions were prepared as for MZP sets in 600 mL bottles. Sets for experiments with meso- and bathypelagic communities were incubated at *in situ* temperature for 24 hours in the dark in a portable fridge. Samples were fixed and stored as described before.

Sea water for both MZP and NP dilution experiments (as well for chl *a* assessment) was simultaneously sampled from the same Niskin bottles.

Based on the dilution method model of Landry and Hasset (1982) as modified by Landry *et al.* (1995), we computed for several classes of prey (microphytoplankton, nanoplankton, heterotrophic and autotrophic picoplankton): growth factor (μ), mortality factor (g), initial concentration of the prey (C_0),

mean concentration of the prey during the experiment [$C_m = \frac{C_0\left(e^{(\mu-g)t}-1\right)}{(\mu-g)t}$] (1),

ingestion rate [$I = g \times C_m$] (2),

potential production [$P_P = \mu \times C_m$] (3).

2.3. Microscopic analysis and cell to biomass conversion factors

Micro-plankton. Samples for microphytoplankton and MZP were processed following the Utermöhl method (1958), organisms were enumerated and measured using an inverted optical microscope. Cell numbers of ciliates were corrected multiplying them by 1.56 in order to compensate possible loss of organisms due to the fixation with formaldehyde (Stoecker *et al.*, 1994a, b).

Taxonomic assignations, standardized geometrical formulas for volume conversion and carbon conversion factor were done following Strathmann (1967) and Smayda (1978) for microphytoplankton, Putt and Stoecker (1989) for MZP (more details in Fonda Umani and Beran, 2003 and Fonda Umani *et al.*, 2005).

Nano- and picoplankton. The assessment of the picoplanktonic and nanoplanktonic fractions was performed according to the Porter and Freig protocol (1980) at the epifluorescence microscope. Aliquots of each sample were stained with a DAPI (4', 6-diamidino-2-phenylindole) solution, 1 μg mL^{-1} final concentration and placed in the dark for 15 minutes. Picoplankton was collected on 0.22 μm black polycarbonate filters (Nucleopore, 25 mm) while nanoplankton on 0.8 μm black polycarbonate filters (Nucleopore, 25 mm). The filters were immediately placed on slides between two drops of immersion non fluorescent oil and kept at -20°C in the dark. Counts were made using an epifluorescence microscope at x1000 final magnification; more than 200 cells were counted for each picoplankton and nanoplankton sample. Picoplanktonic samples were counted in triplicates. For the estimation of biomass, nanoplankton was divided into three dimensional classes: 2-3 μm, 3-5 μm and 5-10 μm as reported by Christaki *et al.* (2001).

Cell abundance data were converted in biomass by applying the following conversion factors: 20 fg C $cell^{-1}$ for heterotrophic bacteria for surface samples (Ducklow and Carlson 1992) and 10 fg C $cell^{-1}$ for the meso-bathypelagic samples (Reinthaler *et al.*, 2006). 200 fg C $cell^{-1}$ for autotrophic bacteria (Caron *et al.*, 1991). The nanoplanktonic organisms were approximated to spheres (diameter equal to the medium value of the belonging dimensional class) in order to multiply their volume for the conversion factor of 183 fg C μm^{-3} (Caron *et al.*, 1995).

2.4. Chlorophyll *a*

Chlorophyll *a* samples were collected from the same Niskin bottles sampled for the dilution experiments by filtering on board from 1 L up to 5 L of seawater through Whatman GF/F glass-fibre filters (45 mm diameter), the membranes were immediately frozen (-20°C) or stored in liquid nitrogen when available. The pigments extraction was run overnight in the dark at 4 °C with 90% acetone from the filter previously homogenized; concentrations were determined with the spectrofluorometer Perkin Elmer LS 50B (450 nm excitation and 665 nm emission wavelengths) measuring the chlorophyll *a* before and after acidification with 2 drops of HCl 1 N (Lorenzen and Jeffrey 1980). The instrument calibration was made using pure Sigma chl *a* standards and computing a linear response for the considered range.

2.5. Elaborations

The ingestion efficiencies of MZP and NP were calculated for each prey by dividing the ingestion rate by the corresponding preys' potential production estimated respectively in the MZP and NP dilution experiments. Potential production is considered a good proxy for primary production (Calbet and Landry, 2004).

The food web efficiency (FWE) was computed as the ratio of the higher trophic level production (in our truncated food web it corresponds to microzooplankton at the surface and nanoplankton in the meso-bathypelagic layers) on the total potential production of the preys (see Berglund *et al.*, 2007).

The relations between ingestion rates and available biomasses of each kind of prey were investigated for MZP and NP. The functional responses of the ingestion rates over a wide range of prey concentrations were examined against four common models:

Ivlev [$I = \alpha\left(1 - e^{-bC_0}\right)$] (4),

Mayzaud-Poulet [$I = \alpha C_0\left(1 - e^{-bC_0}\right)$] (5),

Holling Type II or Disk Equation [$I = \frac{\alpha C_0}{\beta + C_0}$] (6),

Holling Type III [$I = \frac{\alpha C_0^2}{\beta^2 + C_0^2}$] (7),

where I and C_0 are ingestion ratees and biomasses estimated in each dilution experiment, α and β are constants and represent respectively the maximum rate of ingestion and the rate at which I changes in relation with C_0. The values for α and β that minimize the residual sum-of-squares in each equation (4, 5, 6 and 7) were computed with the Nonlinear Least Squares function implemented in the *stats* package of R. Only fitting models whose parameters were significant (p-values < 0.05) were considered and compared by the analysis of variance (ANOVA) and by the maximum likelihood to the same data (with the Akaike information criterion – AIC, and the Bayesian information criterion - BIC) to evaluate the fitting quality of the models.

3. RESULTS

3.1. Surface experiments

Figure 2 shows the biomass of all primary producers and the chlorophyll *a* values assessed at the surface per each sampling event. We arbitrarily divided the increasing biomass values into three major groups: the first one with values for total autotrophic fraction < 6.44 µg C L^{-1} that we consider representative of oligotrophic conditions (mean chl *a* 0.22 mg L^{-1}); the second one that can be consider meso-eutrophic with an autotrophic total carbon < 61.93 µg C L^{-1} and mean chl *a* of 0.60 mg L^{-1} and the last one which can be considered very eutrophic (or eutrophied) with biomass largely exceeding 100 µg C L^{-1} and mean chl *a* of 2.60 mg L^{-1}. Groups presented significant differences among them (one-way Kruskal–Wallis test was highly significant, *p*-value < 0.0001).

Total biomass was made up by micro-zooplankton (MZP), micro-phytoplankton (MPP), nano-plankton (NP), heterotrophic picoplankton (HP) and autotrophic picoplankton (AP). In oligotrophic conditions total biomass was mostly composed by NP and HP, on average 27.4% and 46.8% respectively. In meso-eutrophic conditions mean total biomass was almost equally composed by MPP for 28.8%, HP for 33.7% and AP for 21.1%. MPP dominated in eutrophied conditions where it reached 91.1% of the total biomass.

Considering only preys' biomass for microzooplanktonic consumers (HP, AP, NP, MPP), in oligotrophic and meso-eutrophic conditions NP and picoplankton constituted on average almost 80% of total biomass and picoplankton alone more than 60%. MPP represented only a small fraction and mainly because of the presence of small organisms other than diatoms. In eutrophied conditions, MPP accounted from 78 to 98% of total preys' biomass and it was mainly constituted by diatoms.

When the biomass of the preys increased the equitability (computed with Jaccard index) of MZP major taxa decreased and few species became dominant: in 4 cases they were non-loricate ciliates, in 2 cases Tintinnids *(Stenosemella ventricos*a and *S. nivali*s) and in 1 case another species of protists.

The overview of MZP ingestion rates per each dilution experiment highlights as the daily amount of carbon ingested increased according to the trophic level (Fig. 3). In oligotrophic conditions NP and HP mainly supported MZP, whose ingestion rates ranged from 0.77 to 16.72 µg C L^{-1} d^{-1} and from 1.19 and 23.86 µg C L^{-1} d^{-1}, respectively. In meso-eutrophic situations picoplankton suffered the highest mortality rates with an average of 29.12 µg C L^{-1} d^{-1} for HP and 8.31 µg C L^{-1} d^{-1} for AP. MZP ingestion on NP was detected in 7 cases out of 11 and ranged from 0.79 to 4.68 µg C L^{-1} d^{-1} while MPP ingestion occurred within a range from 3.38 to 36.93 µg C L^{-1} d^{-1}. In eutrophied conditions grazing rates on MPP were the highest ones ranging from 59.15 to 182.11 µg C L^{-1} d^{-1} followed by ingestion rates on HP (1.47 - 66.90 µg C L^{-1} d^{-1}), NP (0.58 - 7.43 µg C L^{-1} d^{-1}) and AP (0.14 - 5.59 µg C L^{-1} d^{-1}.)

NP potential ingestion rates (Fig. 4) increased with prey availability from oligotrophic to meso-eutrophic conditions while values decreased in eutrophied conditions. HP represented always the most exploited preys with mean ingestion rates of 5.30, 23.41 and 14.80 µg C L^{-1} d^{-1} respectively;

ingestion rates for AP on average ranged from 1.87 µg C L^{-1} d^{-1} in oligotrophic state to 9.69 µg C L^{-1} d^{-1} in meso-eutrophic and 0.36 µg C L^{-1} d^{-1} in eutrophied conditions.

Total MZP ingestion efficiencies (as the ratio between Ingestion (I) and Potential Production (PP) on total preys) for each dilution experiments are reported in Figure 5a. In the graph we reported also the bisector, which indicates a 1:1 ratio. In oligotrophic and meso-eutrophic conditions the ratio was very close to the balance between I and PP. In eutrophied conditions there is a prevalence of PP over I, with the exception of two points that correspond to February 2001 and August 2000 experiments. These experiments were carried out at the end of a diatom bloom.

Total NP ingestion efficiency is reported in Figure 5b with the indication of the bisector. As a general rule PP overcomes I rates or the ratio was very close to 1, with the relevant exceptions of four meso-eutrophic points and one in oligotrophic conditions.

Among the functional response models tested to describe how MZP ingestion rates increase with the availability of prey biomasses only Holling Type III and Ivlev models gave significant fittings with the available dataset and only for HP, MPP and NP. Figure 6a shown Type III functional responses indicating a possible lower threshold and a likely upper saturation threshold for HP, MPP and NP; only for MPP the Ivlev model suggested solely a saturation threshold. Comparing Type III and Ivlev fitting models for MPP, no clear differences emerged (ANOVA not significant, AIC respectively 199.8 and 199.3, BIC respectively 203.3 and 202.7). The two significant fitting models for MZP grazing on MPP made us confident to suggest an upper mean threshold value of 196.5 µg C L^{-1} d^{-1} (Type III α = 184 µg C L^{-1} d^{-1}, Ivlev α = 209 µg C L^{-1} d^{-1}).

For NP we detected significant functional response only for HP described by Holling Type III and Ivlev models (Fig. 6b); the comparison between them raised no significant differences with ANOVA while Type III reached slightly better scores for AIC (189.5 versus 191.5 of Ivlev) and for BIC (193.7 versus 195.7 of Ivlev) criterions.

3.2. Meso-bathypelagic experiments

Figure 7a reports HP biomasses estimated in the dilution experiments carried out in the meso- and bathypelagic layers where HP represented the only available prey for NP. Biomasses generally varied from 0.14 to 0.97 µg C L^{-1} with the exception of two mesopelagic stations with relatively high values of 6.45 µg C L^{-1} and 7.24 µg C L^{-1}. The mean biomass for NP was 0.37 µg C L^{-1} with a standard deviation of ±0.31 µg C L^{-1}, and it did not increase were high prey biomass were encountered. NP ingestion rates ranged between 0.05 and 3.2 µg C L^{-1} d^{-1} with the exception of two mesopelagic values (13.29 and 16.74 µg C L^{-1} d^{-1}) that correspond to the exceptionally high HP biomass.

NP ingestion efficiency was generally low (Fig. 7b), and particularly at low PP values. At high PP ingestion exceeded PP in two mesopelagic experiments, the two characterized by high stock biomass; while in the most bathypelagic experiments (VIERA) PP largely overcame ingestion.

NP ingestion rates increased accordingly to HP biomass increase (Fig. 7c) and only Holling Type III functional response significantly fitted the scatterplot.

3.3. C-flux models

Mean values of biomasses and ingestion rates of all considered preys and predators were used to produce models of trophic carbon pathways for the three trophic conditions described at the surface and in the meso-bathypelagic zones (Fig. 8). In eutrophied conditions, MZP grazed mostly on MPP (mean ingestion 119.60 μg C L^{-1} d^{-1}, mean MPP biomass 326.94 μg C L^{-1}) and on HP (mean ingestion 18.24 μg C L^{-1} d^{-1}, mean biomass 17.78 μg C L^{-1}) while NP fed almost uniquely on HP (mean ingestion 14.80 μg C L^{-1} d^{-1}). In this case we can hypothesise that the excess of MPP, not grazed at the surface, can be exploited by larger consumers (mesozooplankton) or exported toward the bottom in a mean amount of 23.15 μg C L^{-1} d^{-1}; it has to be kept in mind that ingestion rates of MZP and NP were the maximum potential rates for these consumers since in the natural contest they are actively grazed by higher trophic level consumers. In meso-eutrophic conditions, MZP principally grazed on HP (mean ingestion 32.35 μg C L^{-1} d^{-1}, mean biomass 22.50 μg C L^{-1}), while on MPP and AP ingestion rates were lower (13.24 μg C L^{-1} d^{-1} and 9.23 μg C L^{-1} d^{-1}, on mean biomasses of 19.27 μg C L^{-1} and 14.09 μg C L^{-1}, respectively). NP intensely exploited HP (23.41 μg C L^{-1} d^{-1}) and the contribution of AP was also significant (9.69 μg C L^{-1} d^{-1}). In oligotrophy, MZP grazed mostly on HP (8.98 μg C L^{-1} d^{-1} on mean biomass of 8.43 μg C L^{-1}) and secondarily on NP (4.98 μg C L^{-1} d^{-1} on a mean biomass of 4.94 μg C L^{-1}). NP grazed more on HP (5.30 μg C L^{-1} d^{-1}) than on AP (1.87 μg C L^{-1} d^{-1}).

In the meso - and bathypelagic layers, NP could graze only on HP with mean ingestion rate of 3.09 μg C L^{-1} d^{-1} on a mean biomass of 1.33 μg C L^{-1}.

As average, in the surface experiments food web efficiency as the ratio between production at the higher level and production of all preys increased from oligotrophic to meso-eutrophic scenarios, respectively 0.03 and 0.10, and decreased in eutrophied conditions (0.01). In the meso-bathypelagic domain the food web efficiency computed considering NP as top predators was 0.13.

4. DISCUSSION

Our results highlighted that picoplankton, and particularly HP were grazed by both NP and MZP in the surface experiments in all trophic conditions. We are aware that results of MZP dilution experiments include the effect of viral lysis (Parada, 2007; Fonda Umani *et al.*, 2010; Di Pol *et al.*, 2013) and the mortality due to NP predation (e.g. Stoecker *et al.*, 2013). To partially solve this latter problem we performed parallel experiments to estimate the predation of NP alone. We can expect three different models of interaction: i) only NP graze on picoplankton, therefore the ingestion rates calculated in NP experiments are the same obtained in the MZP experiments; ii) MZP grazing on NP reduces the ingestion calculated for NP alone; iii) MZP directly feed on picoplankton, and consequently ingestion rates obtained for MZP experiments are higher than for NP experiments (Fonda Umani and Beran, 2003). In most of the cases we detected higher ingestion rates in MZP experiments in respect to NP experiments. In particular, in eutrophied conditions we observed a direct impact of MZP on HP in >80% of experiments. At any rate, MZP always relied on picoplankton biomass through grazing on NP that is the majority of the cases in meso-eutrophic conditions.

The contribution of picoplankton in the MZP diet, aspect that is seldom investigated, was noticeable particularly in meso-eutrophic and oligotrophic conditions where HP resulted the most affected stock. Ingestion rates on HP were higher than on MPP, NP and AP; solely in few experiments NP and AP contributions to MZP diet were higher than the HP one. In eutrophied condition, the highest MZP mean ingestion rates were detected on MPP that represented more than 80% of the MZP mean daily diet, although the grazing pressure affecting HP stock was not negligible since they cover almost 14% of MZP diet.

Ingestion efficiency of the consumers can be expressed as the ratio between ingestion and potential production rates of preys. This comparison, with some precautions as suggested by Cáceres *et al.* (2013), could be a proxy for the carbon balance of the system thus suggesting the carrying capacity for higher trophic levels. MZP ingestion efficiency in oligotrophic and meso-eutrophic conditions was close to the 1:1 ratio, indicating a good balance between production and consumption. In eutrophied conditions PP in most of the cases overcome the ingestion, thus justifying the possible export. The cases in which I exceeded PP corresponded to the end of diatom blooms therefore indicating the role of top down control in removing the previously produced biomass.

Although composed essentially of picoplankton, the NP potential daily diet was targeted on HP - the prokaryotic fraction offering the highest available biomass - especially in oligotrophic and meso-eutrophic conditions where their contribution exceeded 80%.

NP ingestion efficiency was equal or lower to 1 indicating a general prevalence of production over ingestion. The few recorded cases of ingestion exceeding potential production were no more observed in the parallel MZP experiments, suggesting that the potential high ingestion rates of NP were reduced by MZP grazing over NP.

In the meso- and bathypelagic layers biomasses of MZP, NP and HP were lower than at the surface and values fell within ranges proposed by Nagata *et al.* (2010), and references therein. At two mesopelagic stations, HP biomass was comparable with those found at the surface in oligotrophic conditions, although the biomass of NP did not parallel this increase. The higher biomasses might

suggest an input from the above euphotic layers, which enhanced HP production before our sampling (Hansell and Ducklow, 2003). In these two cases, the low NP biomass that did not match the prey increase might point out to a delay or slowness in the NP growth. On the other hand HP potential production had already dropped at lower levels, indicating the end of the possible previous input. Recently Pernice *et al.* (2014) found that over all oceans the ratio of eukaryotes (thus NP)-prokaryotes biomasses was constantly lower in the mesopelagic rather than in bathypelagic layers.

Despite the mean biomass of HP in meso- and bathypelagic layers was 6 to 16% of the surface biomass, the ingestion rates was from 13 to 58% of the surface ones, suggesting a strong feeding adaptation of NP in high diluted-prey conditions as reported by Cho *et al.* (2000) in the East China Sea and recently by Pachiadaki *et al.* (2014) for the eastern Mediterranean Sea and by Rocke *et al.* (2015) for the North Atlantic Deep Water and the Antarctic Intermediate Water. All of them used fluorescently-labelled prokaryote tracing techniques that have been shown to produce comparable but lower results in respect to those obtained in dilution experiments (Vaqué *et al.*, 1994)

Surface food web efficiency reflected the ingestion efficiencies estimated for each experiments being higher in oligotrophic and meso-eutrophic conditions where we observed a fully exploitation of prey potential production. Conversely, in the eutrophied conditions a large part of the new produced biomass was inefficiently exploited, thus leaving resources for upper level consumers or for export toward the bottom. Our results confirmed the hypothesis of Sommer *et al.* (2002) on the decrease of food web efficiency in eutrophied conditions.

In the meso-bathypelagic layers we could assess food web efficiency only considering nanoplankton as top predator. Also in this case food web efficiency, nevertheless the diluted environment, was relatively high. Nanoplankton seemed able to efficiently exploit all available HP biomass.

Lastly, testing several functional response models to describe the feeding behaviour of consumers we highlighted as generally grazing activity of MZP (at the surface) and the potential grazing activity of NP (at the surface and in the meso-bathypelagic layers) correlated with the Holling Type III model. Furthermore only MZP on MPP and NP on HP correlated with the Ivlev model. In the sigmoidal curves the low threshold correspond to low ingestion rates that have not paired slight biomass increases. These conditions were detected mainly in oligotrophy and in meso-bathypelagic environments; they might be explained with the dilution of available preys that reduce the prey-consumer encountering rates (Wikner and Hagström, 1991; Pastor, 2008) and that can induce predators to use other food sources (Strom *et al.*, 2000). Our dataset lacks of ingestion rates on bacterial when their biomass is very low so we did not suggest any low threshold for the consumers. The high threshold instead occurs only in eutrophied conditions for MPP, and in all trophic conditions for the other preys. The observed satiation threshold can be interpreted as the result of the individual inability to handle higher prey availability as suggested also by a modelling-approach study of Gentleman and Neuheimer (2008). A possible explanation is a delay in the match of consumers' growth with prey increases. Also these findings need to be tested with larger datasets that include more data from ecosystems characterized by high production and ingestion rates.

5. CONCLUSION

We are aware of the limit of dilution experiments because they cannot fully represent natural conditions, however they can be used to compare different trophic situations when, as in our case, they are set up following the same protocol.

Thus here we proposed some constrains based on the trophic condition of the environment that oriented the carbon flux in those ecosystems:

- Bacterivory was the major pathway of organic carbon in oligotrophic and meso-eutrophic surface conditions.
- In eutrophied conditions herbivory was the main trophic pathway. However picoplankton, principally HP, represented a not negligible source of carbon.
- Food web efficiency was higher in meso-eutrophic and oligotrophic conditions where the major pathway was bacterivory.
- Low food web efficiency was registered when herbivory was the dominant pathway possibly because of satiation effect, which translate in an excess of autotrophic biomass.
- In the meso- and bathypelagic layers, NP ingestion rates on HP diminished but not at the same order of magnitude as their biomasses, thus determining a high efficiency of this truncated food web.

ACKNOWLEDGMENTS

We are grateful to all colleagues and SFU students who took part in the dilution experiments, to Giovanni Bacaro for his support with the statistical analysis and to the crews of research vessels Urania and Universitatis. This study was supported by the PERSEUS Project, which provided the Ph. D. fellowship to L Z. Thank are due also to three reviewers who helped in improving an early version of the manuscript.

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635 **7.** Appendix A

636 **Table A1 - Sampling sites for surface experiments (at 0.5 m depth).** Experiments were
637 carried out separately for MZP and NP. Coordinates are reported according to Decimal
638 Degrees (DD) system.

Cruise	Station	Sampling date (dd/mm/yyyy)	Long (E)	Lat (N)	Depth (m)	Location
OBAMA	CF_16	02/04/2011	18.181	39.286	1035	Northern Ionian Sea
OBAMA	MS_03A	01/04/2011	18.320	39.330	775	Northern Ionian Sea
OBAMA	O_36	26/03/2011	17.267	41.380	1132	Southern Adriatic Sea
OBAMA	O_37B	27/03/2011	17.336	41.430	1108	Southern Adriatic Sea
VECTOR	V1	06/06/2007	8.000	43.500	2490	Ligurian Sea
VECTOR	V10	22/06/2007	28.323	35.953	3870	Aegean Sea
VECTOR	V2	08/06/2007	12.999	39.500	3570	Tyrrhenian Sea
VECTOR	V3	05/06/2007	6.073	39.314	2850	Sardino-Balearic Sea
VECTOR	V4	01/06/2007	-0.998	36.502	2640	Alboran Sea
VECTOR	V6	14/06/2007	18.000	38.495	3000	Ionian Sea
VECTOR	V7	16/06/2007	20.875	35.135	3200	Aegean Sea
VECTOR	VA	30/05/2007	-8.332	35.000	2776	Atlantic Ocean
VECTOR	VIERA	24/06/2007	26.087	34.412	4760	Aegean Sea
Survey	C1	17/11/1998	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	08/02/1999	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	12/05/1999	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	18/08/1999	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	15/12/1999	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	07/02/2000	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	15/05/2000	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	07/08/2000	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	20/11/2000	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	12/02/2001	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	07/05/2001	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	07/08/2001	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	15/11/2001	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	13/02/2002	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	14/05/2002	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	07/08/2002	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	12/03/2003	13.710	45.701	17	Northern Adriatic Sea

Survey	C1	29/09/2003	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	10/03/2004	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	05/04/2004	13.710	45.701	17	Northern Adriatic Sea
Survey	C1	09/08/2005	13.710	45.701	17	Northern Adriatic Sea

639

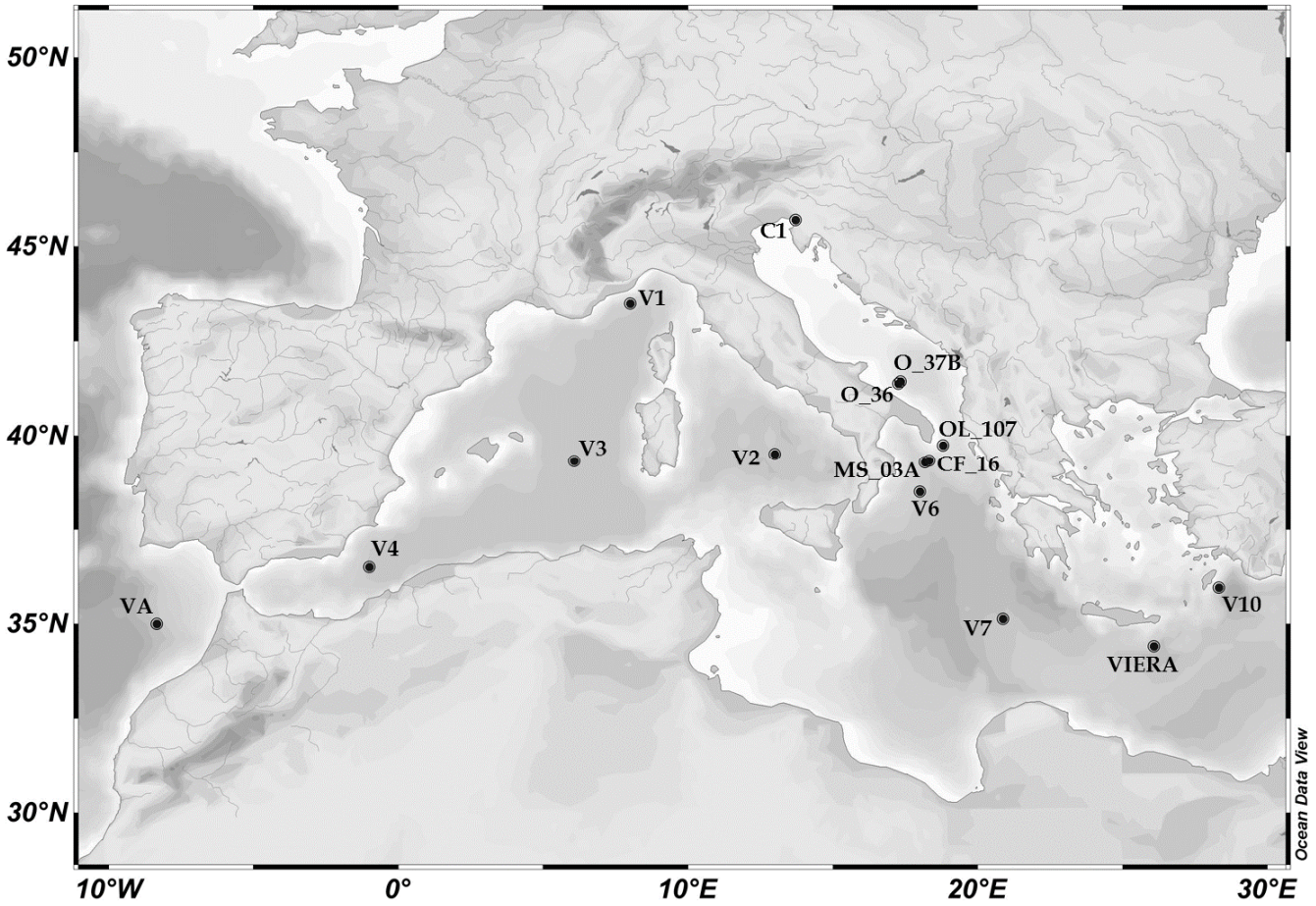
640 **Table A2 - Sampling sites for meso- and bathypelagic experiments (at +5 m from bottom**
641 **depth).** Experiments were carried out only on NP. Coordinates are reported according to
642 Decimal Degrees (DD) system.

Cruise	Station	Sampling date (dd/mm/yyyy)	Long (E)	Lat (N)	Depth (m)	Location
OBAMA	CF_16	26/03/2011	18.181	39.286	1035	Northern Ionian Sea
OBAMA	MS_03A	27/03/2011	18.320	39.330	775	Northern Ionian Sea
OBAMA	O_36	02/04/2011	17.267	41.380	1132	Southern Adriatic Sea
OBAMA	O_37B	01/04/2011	17.336	41.430	1108	Southern Adriatic Sea
OBAMA	OL_107	03/04/2011	18.810	39.741	674	Otranto Channel
VECTOR	V1	30/05/2007	8.000	43.500	2490	Ligurian Sea
VECTOR	V10	01/06/2007	28.323	35.953	3870	Aegean Sea
VECTOR	V2	05/06/2007	12.999	39.500	3570	Tyrrhenian Sea
VECTOR	V3	06/06/2007	6.073	39.314	2850	Sardino-Balearic Sea
VECTOR	V4	08/06/2007	-0.998	36.502	2640	Alboran Sea
VECTOR	V6	14/06/2007	18.000	38.495	3000	Ionian Sea
VECTOR	V7	16/06/2007	20.875	35.135	3200	Aegean Sea
VECTOR	VA	24/06/2007	-8.332	35.000	2776	Atlantic Ocean
VECTOR	VIERA	22/06/2007	26.087	34.412	4760	Aegean Sea

Figure captions:

- Figure 1: Map of the Mediterranean Sea. The sampling sites are located by the blue dots.
- Figure 2: Primary producers' biomass and chlorophyll *a* distribution in sampling events.
- Figure 3: Ingestion rates of MZP in the dilution experiments.
- Figure 4: Ingestion rates of NP in the dilution experiments.
- Figure 5: comparison of total Ingestion rates versus preys total Potential Production for MZP-targeted dilution experiments (a) and for NP-targeted dilution experiments (b). Solid lines represent graph bisector and thus the 1:1 ratio between I and PP. Dashed lines represent the linear regression for the plotted points, equation and r^2 are reported.
- Figure 6: Comparison of ingestion rates of MZP with MPP, NP, HP and AP biomasses (a). Comparison of ingestion rates of NP with HP and AP biomasses (b). Reported curves describe functional responses models that provided a significant fitting.
- Figure 7: a) HP and NP biomasses for all dilution experiments carried out in the meso- and bathypelagic layers. b) Comparison of Ingestion rates with Potential Production among meso- and bathypelagic dilution experiments. c) Ingestion rates over prey biomasses with fitting curve describing the functional response for Holling Type III model.
- Figure 8: carbon flux models with mean Ingestion rates (µg C L^{-1}) of MZP and mean potential Ingestion rates (µg C L^{-1} d^{-1}) of NP (dashed lines) on considered prey stocks computed at the surface in eutrophied, meso-eutrophic and oligotrophic conditions and in the meso-bathypelagic layers only for NP. In the graph the relative mean biomass (µg C L^{-1} d^{-1}) for all classes of organisms are reported.

663 Fig: 1



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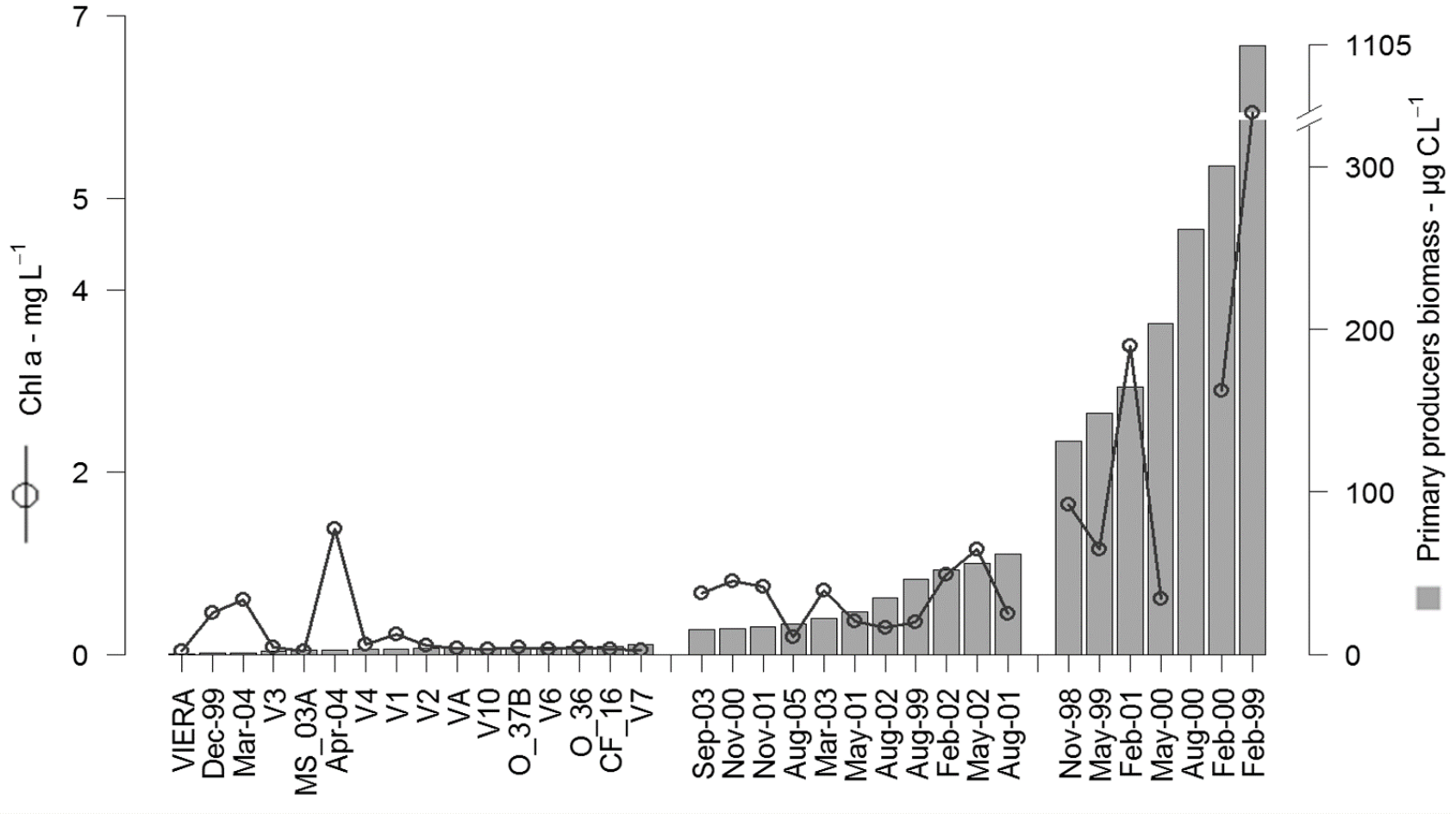
666 Fig. 2:



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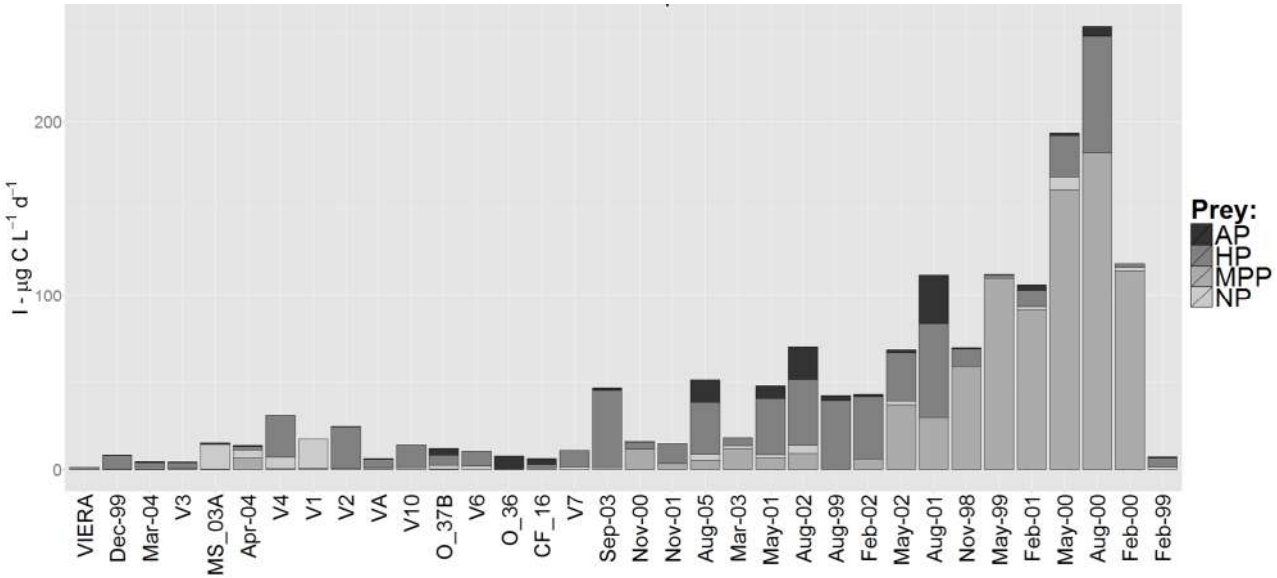

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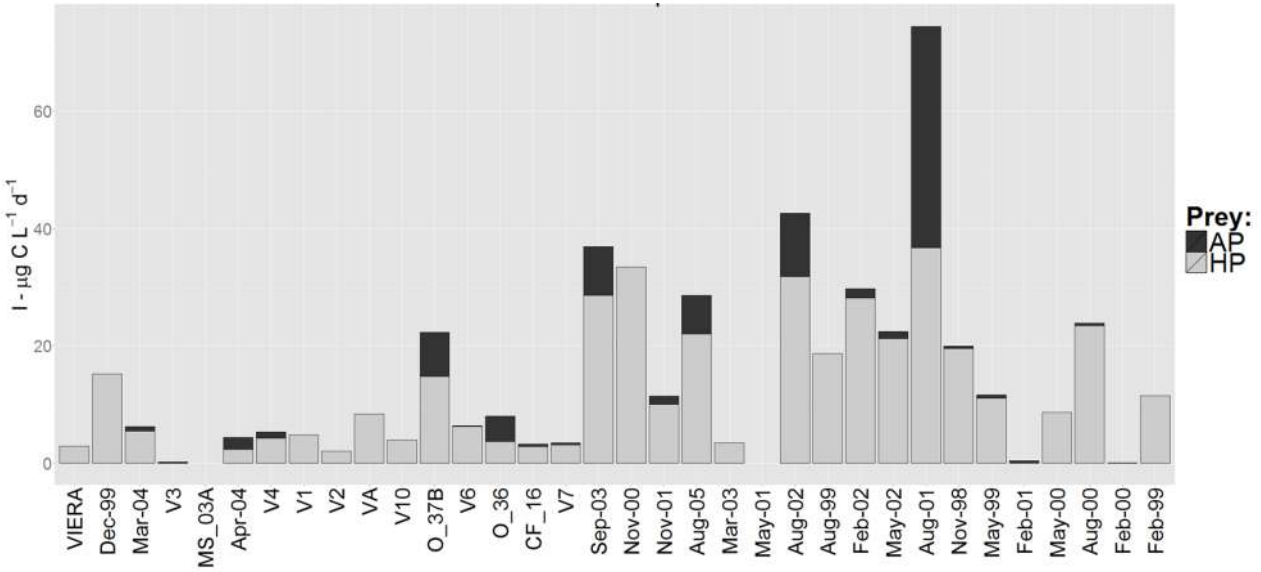

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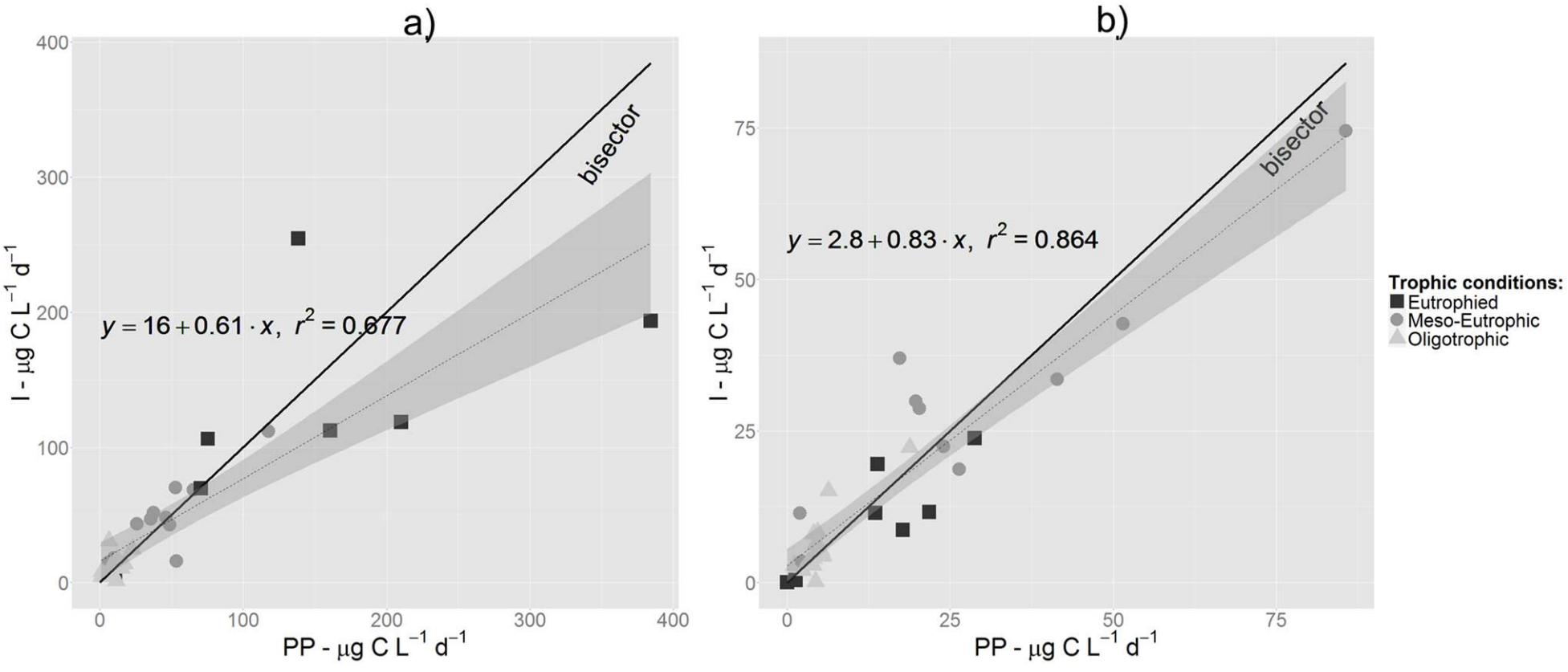

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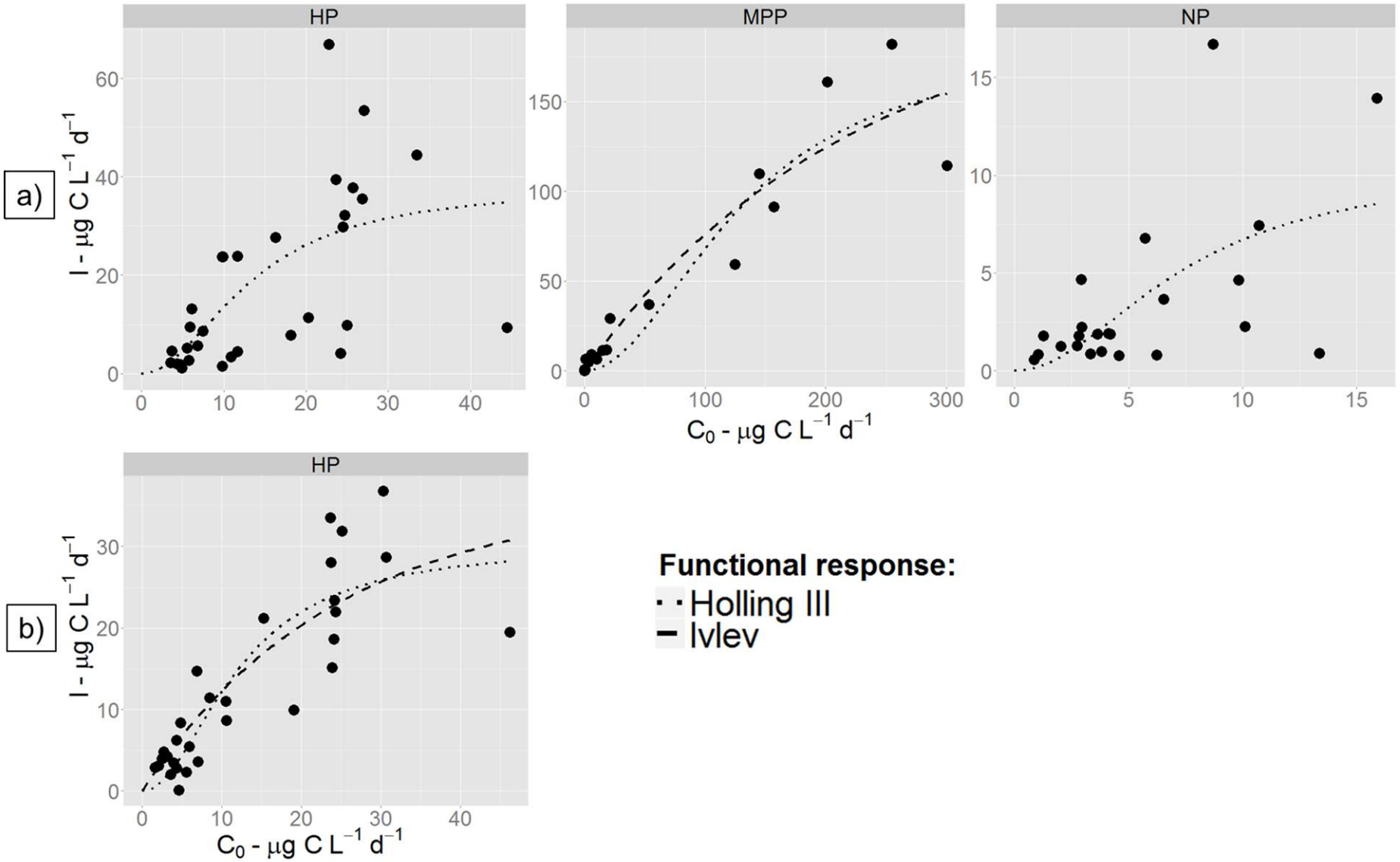

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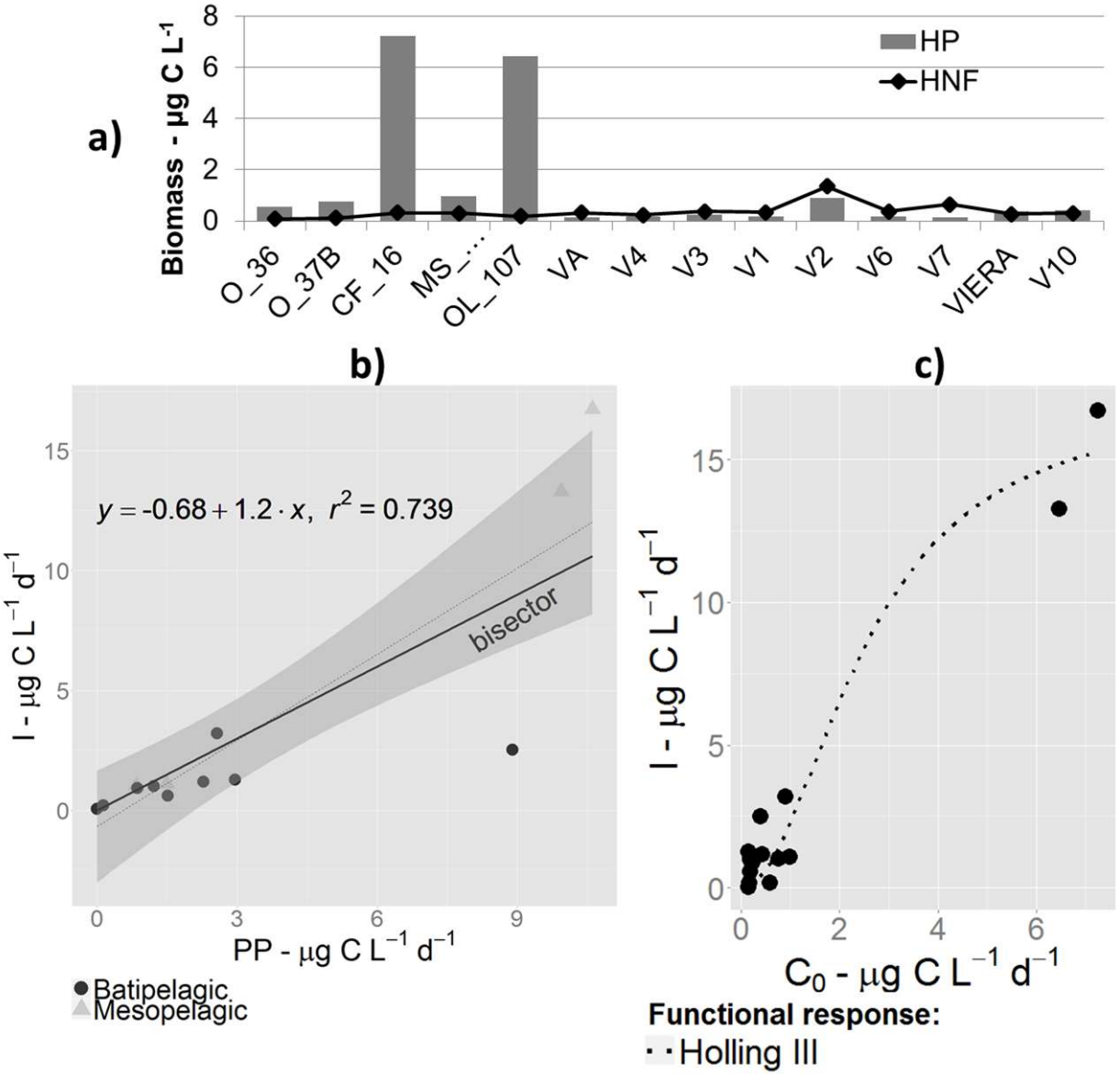
679 Fig. 7:



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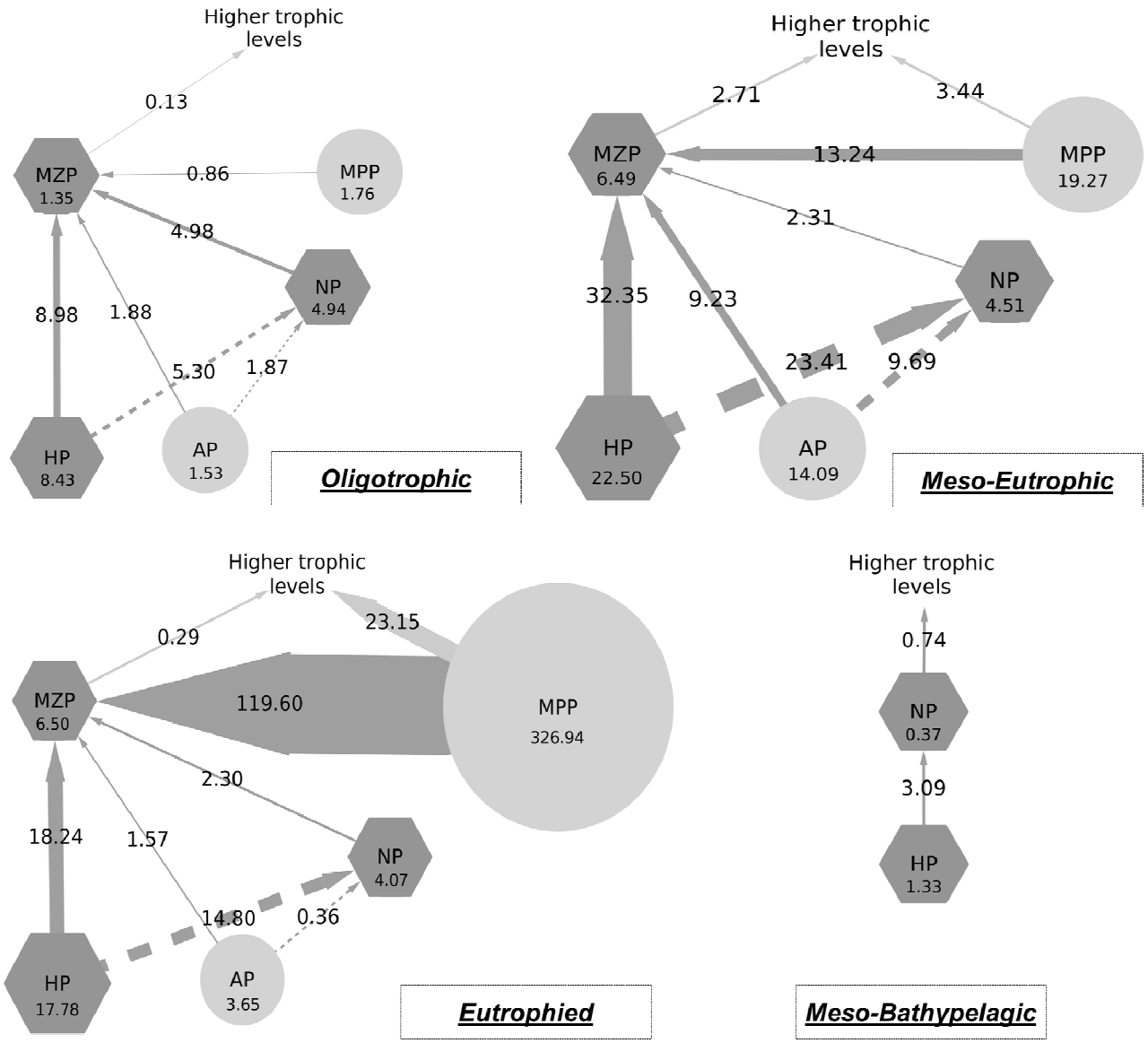
681

682 Fig. 8:


Higher trophic levels
0.13
MZP 1.35
0.86
MPP 1.76
4.98
NP 4.94
8.98
1.88
5.30
1.87
HP 8.43
AP 1.53
Oligotrophic
Higher trophic levels
2.71
3.44
MZP 6.49
13.24
MPP 19.27
2.31
NP 4.51
32.35
9.23
23.41
9.69
HP 22.50
AP 14.09
Meso-Eutrophic
Higher trophic levels
0.29
23.15
MZP 6.50
119.60
MPP 326.94
2.30
18.24
1.57
NP 4.07
14.80
0.36
HP 17.78
AP 3.65
Eutrophied
Higher trophic levels
0.74
NP 0.37
3.09
HP 1.33
Meso-Bathypelagic

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