A new type of plankton food web functioning in coastal waters revealed by coupling Monte Carlo Markov chain linear inverse method and ecological network analysis

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Abstract :

Plankton food webs (PFW) typology is based on different categories of functioning, according to the dominant processes and the role played by heterotrophic bacteria, small vs large phytoplankton, and small vs large zooplankton. Investigating the structure and the function of planktonic food webs in two SW Mediterranean waters (inshore and marine sites) at four seasons, using inverse (LIM-MCMC) and ecological network (ENA) analyses, we identified a new type of food web, called the “bacterial multivorous food web”. This food web adds to the conventional trophic continuum as previously reported. The “bacterial multivorous food web” present in winter showed the lowest primary production among seasons, but highest bacterial production. Several food web ratios characterized this new typology e.g. picophytoplankton net primary production to total primary production varied from 0.20 to 0.28; bacterial to primary production ratio is higher than values reported in global scale ($\cong 1$); bacterial net production to the potential protozoan prey net production was high (>0.2). In this special food web, carbon was mostly recycled, with a moderate fraction channeled to deep waters, which lead to a higher retention of carbon inside the ecosystem. This winter PFW also seemed to be the most organized, specialized, stable and mature, as related to common interpretations of ENA. The spring was characterized by herbivorous food web, with highest activity coinciding with low stability. Although less usual, the herbivorous pathway was also observed during summer, in inshore waters. The autumn food webs, which functioned as multivorous

or microbial food webs, appeared to be stable and mature. Finally, our study demonstrates the usefulness of food web models derived ratios combined with ecological network analysis indices to conduct evaluation of the structure and functioning of ecosystems and potentially to support management decisions in marine environment.

Highlights

► The seasonal change in food webs structure resulted in a shift in the properties and functioning of the system. ► New planktonic food web was identified using inverse method (LIM-MCMC) and ecological network (ENA). ► The “Bacterial multivorous food web” dominated winter and showed the lowest primary production among seasons, but highest bacterial production. ► ENA analysis showed a high carbon retention and a most organized and specialized statute of the bacterial multivorous food web.

Keywords : Food web modeling, Network analysis, Trophic structure, Coastal waters, Seasonal variations, Ecology, Bacterial multivorous food web

1. Introduction

Marine biological productivity depends on the magnitude of phytoplankton production and its trophic transfer through food webs. The structure and the functioning of the food web can in turn influence the energy flow and the carbon cycling in marine ecosystems (Vargas et al., 2007). In addition, the structure of plankton food webs can vary greatly over seasons, since seasonal change in environmental conditions (mainly the availability of nutrients, light and hydrological conditions) can affect the abundance, the size structure and the composition of plankton communities as well as the tropho-dynamics within the plankton system (Sankar and Padmavathi, 2012; Vajravelu et al., 2017; Rasconi et al., 2017). For temperate regions, there exist a conventional scheme describing the food web dynamics over seasons (Legendre and Rassoulzadegan, 1995). The increasing light during spring and the stratification of the water column, which has already been enriched by nutrients during winter mixing, lead to phytoplankton blooms dominated by micro-sized algae (i.e. diatoms $>10\ \mu\text{m}$). This high production is consumed by herbivorous zooplankton (i.e. copepods) and efficiently channeled to higher trophic levels, or exported to deeper layers (Ryther, 1969; Turner, 2001). In summer, the persistent water column stability causes a depletion of nutrients in the surface waters, causing a decrease in production and a shift in the primary producers, dominated by pico- and nano-sized algae and bacteria. This production is consumed by microbivorous zooplankton (i.e. heterotrophic nanoflagellates and microzooplankton) and transferred to higher consumers through the microbial food web (Calbet and Landry, 2004). In autumn, storms can erode water column stability and amend nutrients in the surface water, resulting in a small autumnal phytoplankton bloom that rapidly decline as light decreases. In this situation, both the microbial pathways and grazing on larger phytoplankton are important, resulting in the multivorous food web (Legendre and Rassoulzadegan, 1995). In winter, under mixed water conditions, the food web can typically function as a microbial pathway (Marquis et al., 2007). Recently, Sakka Hlaili et al. (2014), have proposed two new food webs, based on modeling data of planktonic food webs in oceanic and coastal regions, which adds to the continuum of food web dynamics by Legendre and Rassoulzadegan (1995). They identified the “phyto-microbial food web”, where microzooplankton feeding on phytoplankton is more important than grazing on bacteria, and the “poly-microbial food web”, where microzooplankton consume more or less equally the various types of food.

In the Mediterranean Sea, open waters (e.g. Aegean Sea, Levantine basin and Ligurian Sea) are basically oligotrophic supporting low phytoplankton biomass ($<0.2\ \mu\text{g Chl } a\ \text{l}^{-1}$) and

primary production ($59\text{--}150\text{ g C m}^{-2}\text{ y}^{-1}$, Siokou-Frangou et al., 2010). A large part of fixed carbon is channeled to higher trophic levels through microbial organisms, as small-size autotrophs and heterotrophs are dominant in most seasons (Siokou-Frangou et al., 2002; Zervoudaki et al., 2011; Giannakourou et al., 2014). On the contrary, the coastal waters are more productive environments ($200\text{--}400\text{ g C m}^{-2}\text{ y}^{-1}$, Nixon, 1995), mainly due to the high nutrient availability that promotes intense spring diatom-blooms. Here, phytoplankton production is efficiently transferred to higher trophic levels through the herbivorous food web (Caroppo et al., 2000; Auger et al., 2011; Alekseenko et al., 2014).

Establishing the food webs structure in the marine planktonic community is one of the most important steps to predict the ecosystem functioning and to understand the cycling of materials and flows of energy. The structure of the food web is intimately linked to the size structure of the primary producers, which itself depends on the hydrological conditions that control nutrient availability and stratification (Thingstad and Rassoulzadegan, 1999; Irigoien et al., 2005; Marquis et al., 2007). In the present study, an inverse analysis (Linear Inverse Analyses, LIM) approach was used to examine food web interactions in two coastal regions in Southern Mediterranean, the Bay and the Channel of Bizerte in Northern Tunisia. Inverse method, evolved from the physical sciences (Vézina and Platt, 1988), has been used to solve the problem of quantification of missing carbon flows through the plankton food web. Effectively, marine food webs are complex systems with various interactions that are difficult to estimate directly or entirely in field. The LIM approach has been applied to describe food web interactions in various marine ecosystems, as the Gulf of Riga, the Bay of Biscay, the Lagoon of Takapoto, the Northeast Subarctic Pacific and the Baltic Sea (Donali et al., 1999; Vézina and Savenkoff, 1999; Niquil et al., 1998, 2001; Marquis et al., 2007). In the Mediterranean Sea, different modeling analyses have been used to examine the functioning and the structure of food webs and related driving factors. Ecopath with Ecosim (EWE) approach (Coll and Libralato, 2012) is the most used approach in Mediterranean regions, including the Gulf of Gabès (South of Tunisia, Hattab et al., 2013), the Western, Central and Eastern Mediterranean Sea (Tecchio et al., 2015, Heymans et al., 2014), and for the entire Mediterranean Sea (Piroddi et al., 2015, 2017). The three dimensional modeling approach has also been used in the Northwestern Mediterranean basin (Herrmann et al., 2013; Auger et al., 2014) and the Gulf of Lion (Auger et al., 2011). The LIM have been applied in some Mediterranean waters, such as, the Lagoon of Bizerte (Grami et al., 2008) and coastal waters of the Mediterranean Sea (Olsen et al., 2001). This method was modified into the Monte Carlo Markov Chain Linear Inverse method (LIM-MCMC; Van den Meersche et al., 2009).

The LIM-MCMC, allows us to estimate the uncertainty around flows, to perform statistical tests, to compare many marine systems over different seasons and has been applied in many marine ecosystems (De Laender et al., 2010; Grami et al., 2011; Saint-Béat et al., 2014; Chaalali et al., 2015). Nevertheless, the food web structure and the trophic-interaction functioning have so far been poorly described for the southern Mediterranean regions, despite the high plankton diversity and the pronounced productivity observed there (Sakka Hlaili et al., 2008, Bel Hassen et al., 2008; Hafferssas and Seridji, 2010). Up to date, the food web of Bizerte Lagoon has been modeled during summer (Grami et al., 2008), showing an efficient transfer of primary production through the herbivory of microzooplankton. Also, the food webs of the Bay, Channel and Lagoon of Bizerte has been modelled during spring (Meddeb et al., 2018). However, these studies were based on data collected during one season only, and there exist little knowledge about the seasonal variability in food web structures and dynamics in this region and how they fit with the typology of plankton functioning. Using the LIM-MCMC method, our study aims to describe the seasonal dynamics of plankton food webs in the Bay and the Channel of Bizerte in relation to hydrological conditions. We used the ENA to characterize some functional properties of the system and to compare seasonal differences in stress levels and stability of the food webs, two attributes of primary importance in the objective of implementing an ecosystem based management of this environment.

2. Materials and Methods

2.1. Study areas

The Bay of Bizerte is an important fishing region located in the south-western Mediterranean basin (Fig. 1), with depth varying from 16 to 20 m. The Bay is an open embayment facing the Western Mediterranean basin, which is largely influenced by surface Modified Atlantic Water (MAW) (Millot, 1987, 1999). Throughout the year, this water crosses the Straits of Gibraltar and runs along the North African coasts and reaches the northern Tunisian coasts before branching into two veins, one towards the eastern basin and one towards the Tyrrhenian Sea. The MAW caused a mixed layer of 40–50 m with salinity increasing from Gibraltar (36 psu) to the Algera-Provençal basin (38psu). Consequently, the hydrological structure of the water column in the Bay seems to vary little among seasons. The Bay is located in the semi-humid bioclimatic zone, with significant periods of rainfall (especially in winter) causing an important inputs of terrestrial nutrients. Nutrients are commonly abundant in the surface layers throughout the year (0.1–0.7 P μ M; 1–3.5 N μ M; 0.5–2.25 Si μ M) (Sahraoui et al., 2009). Seasonal dynamics has previously been shown for phytoplankton, with high chlorophyll biomass (2–4 μ g l⁻¹) during spring and summer, and

lower values in the other seasons ($<1.5 \mu\text{g l}^{-1}$). This coincided with the predominance of diatoms ($10^5 \text{ cells l}^{-1}$) in spring and summer, and of nanoflagellates during the rest of the year (Sahraoui et al., 2009, Meddeb et al., 2018). Diatoms are also present in autumn and winter and may contribute up to 20–35% to the phytoplankton assemblage (Sahraoui et al., 2009). The Channel of Bizerte, 7 km long, 300 m wide and 12 m deep, connects the Lagoon of Bizerte to the Bay, and is therefore influenced by marine inflow from the Bay, particularly during summers.

The Lagoon is the most important Tunisian site of fisheries and bivalve culture. The tidal regime in the region is dominated by a semi-diurnal cycle with a low amplitude ($<15 \text{ cm}$), but the current velocities can reach 1 m s^{-1} in the middle of the Channel (Harzallah, 2003; Béjaoui et al., 2008). The Lagoon receives a large amount of freshwater from several rivers and from the big lake of Ichkeul due to strong rains during winter and spring, which also influences the Channel during these seasons (Ben Ismail et al., 2012; Zaaboub et al., 2015). In addition, by receiving high nutrient inputs and effluents from land from several anthropogenic activities ($1\text{--}11.5 \text{ P } \mu\text{M}$; $3.5\text{--}55 \text{ N } \mu\text{M}$; $2\text{--}11 \text{ Si } \mu\text{M}$, Bouchouicha et al., 2012), the Channel is considered as a more eutrophied system than the Bay. Pronounced diatom blooms ($10^6 \text{ cells l}^{-1}$) were previously observed in the Channel during summer. Diatoms are also present in spring together with nanoflagellates. These latter predominated during other seasons (Bouchouicha et al., 2012).

2.2. Sampling and hydrological structure of the water column

Data for the study were obtained from several surveys conducted during four seasons (spring, summer, and autumn 2012, and winter 2013) at a marine station located in the Bay (station B, $37^{\circ}16'54'' \text{ N}$, $9^{\circ}53'42'' \text{ E}$) and an inshore station in the Channel (station C, $37^{\circ}15'04'' \text{ N}$, $9^{\circ}52'34'' \text{ E}$) (Fig.1). During each season, the sampling was carried out at four depths of each station (0.5, 2.5, 5/6 and 8/16 m) using an acid-washed 2.5-L plastic water sampler (Hydro-Bios). Sampled water was filtered through a $200 \mu\text{m}$ mesh screen to remove meso- and macrozooplankton (except water used for DOC determination) and stored in polyethylene containers until analyses. Metazooplankton, for two size classes ($200\text{--}700 \mu\text{m}$ and $> 700 \mu\text{m}$), were sampled using two vertical net tows with $200 \mu\text{m}$ and $700 \mu\text{m}$ screen mesh. Vertical profiles of salinity and temperature were measured *in situ* with a microprocessor conductivity meter (LF 196) (Fig. 2).

Nutrient concentrations [NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} and Si (OH)_4] were measured at the four depth of each station (0.5, 2.5, 5/6 and 8/16 m), following the spectrophotometric method (Parsons et al., 1984).

2.3. Linear Inverse Method-Monte Carlo Markov Chain analysis (LIM-MCMC)

To have a complete quantification of the processes in the food web, we used the inverse analysis method to estimate the unknown flows from the known ones. The LIM-MCMC method (Van Den Meersche et al., 2009), derived from the inverse analysis method of Vézina and Platt (1988), was adopted to reconstruct trophic carbon flows through the eight planktonic food webs. This approach is based on four steps: (i) building an *a priori* model including all possible flows between the considered compartments or between the compartments and the outside; (ii) setting mass-balance between flows, for each compartment, as equalities; (iii) setting two groups of inequalities, the first ones based on *in situ* calculated flows, and the second ones consists on a number of biological constraints taken from the literature, to reduce the range of possible values for each flow; and (iv) calculating a large sample of possible solutions for unknown flows.

2.3.1. *A priori* model

The *a priori* model describes all possible carbon flows among the trophic compartments. For all seasons, the model (Fig. 3) includes nine compartments: heterotrophic bacteria (bac), picophytoplankton (pic, $<2\ \mu\text{m}$), nanophytoplankton (nan, $2\text{--}10\ \mu\text{m}$), microphytoplankton (mic, $10\text{--}200\ \mu\text{m}$), protozooplankton (pro = heterotrophic nanoflagellates, dinoflagellates and ciliates, $<200\ \mu\text{m}$), metazooplankton $200\text{--}700\ \mu\text{m}$ (met1), metazooplankton $>700\ \mu\text{m}$ (met2), dissolved organic carbon (doc) and detritus (det). Forty-three flows were considered in the *a priori* model of each season (Fig. 3, Table 3).

The gross primary production (gpp) of the three phytoplankton size fractions (pic, nan, and mic) is the only source of carbon input to the network. Carbon loss is driven by respiration of all living compartments and sinking of all compartments except protozooplankton, picophytoplankton and bacteria. We assumed that the very small size of pic and bac did not allow them to generate sinking flux. Detritus dissolution, phytoplankton exudation and zooplankton excretion (i.e. by pro, met1 and met2) contributed to the doc pool. Doc was only used by bacteria, since other potential consumers of doc (such as choanoflagellates) were absent in our samples. All living compartments, except bac and pic, contributed to the detritus pool through mortality, production of feces by metazooplankton (i.e. met1 and met2) and zooplankton sloppy feeding. Protozooplankton grazed on bac, pic and nan, but also on mic, since they have been reported as potential grazers on large preys in several coastal areas, including our study sites (Meddeb et al., 2018). Metazooplankton, known as inefficient grazers on particles $< 5\ \mu\text{m}$ (Fortier et al., 1994), consumed nano- and microphytoplankton, but not picophytoplankton. Protozooplankton and detritus represented

another food source for the two size classes of metazooplankton (met1 and met2), while large metazoans (met2) consumed smaller ones (met1).

2.3.2. Equalities and inequalities

Establishing the set of linear equalities is an essential step to obtain the mass balances of the network. If the mass of the compartment is stable during the period considered, the sum of the flows entering into this compartment should equal to the sum of the flows leaving it. The mass balance equations for all compartments are given in Table 1.

Imposing linear inequalities for each unknown flow helps to reduce the range of possible solutions. Two ranges of inequalities were adopted, as previously considered in Meddeb et al. (2018). Firstly, a bound of an upper and lower values for each flow were considered by the calculation of a confidence interval around the field data, i.e. using the minimum and maximum of the average value of each flows estimated *in situ* (Table 2). More details are given in Meddeb et al. (2018).

A second group of constraints was adopted to constrain the unknown flows. For all seasons, the inequalities used for the Channel and the Bay models were described in several previous works (Pace et al., 1984; Vézina and Platt, 1988; Vézina and Pace, 1994; Vézina and Savenkoff, 1999; Steinberg et al., 2000; Vézina et al., 2000; Vézina and Pahlow, 2003). They constrain the respiration of bacteria, phytoplankton, protozooplankton and metazooplankton; the doc production by phytoplankton, bacteria and zooplankton; the growth efficiency of bacteria and zooplankton; the assimilation efficiency of zooplankton; the detritus production by zooplankton and the dissolution of detritus (Table 4).

2.3.4. Solutions

The last step of the inverse analysis was the calculation of unknown flows. The estimations of each unknown flow were obtained by the LIM-MCMC method, based on the mirror technique defined by Van Den Meersche et al. (2009). A jump value of $10 \text{ mgC m}^{-2} \text{ d}^{-1}$ and 300000 iterations were adopted to run the models in order to optimize the coverage of all the possible solutions (i.e. correct polytope exploration). A Matlab[®] translation by Alain Vézina and Lauriane Campo of the R-CRAN project package LIM-Solve was used (Van den Meersche et al., 2009). More details on the method are available in Van Den Meersche et al. (2009) and Niquil et al. (2012).

2.4. Data

Data used in the eight models were collected during seasonal samplings at the Bay and the Channel. These data permitted to determine 14 carbon flows for each model. In each sites, sampled water has served for the determination of doc and particulate organic carbon (POC)

concentrations as well as the plankton abundance and diversity. See Meddeb et al. (2018) for more details on the preservation of samples and analyses. Briefly, Samples for doc measurement were filtered on sterilized 0.2 μm polycarbonate filters before being analyzed using a Shimadzu TOC-5000 carbon analyser (Knap et al., 1993). The carbon concentration of doc was obtained considering that 1 μM of doc is equal to 12 mg C m^{-3} (Grami et al., 2008; Meddeb et al., 2018). POC was analysed on a CHN elemental analyser (Perkin–Elmer 2400) after filtration of samples through pre-combusted (at 450°C, during 2 h) GF/F Whatman filters (21 mm) (Knap et al., 1996).

Heterotrophic bacteria and picophytoplankton (pic= prokaryotes and eukaryotes) were enumerated under a CETI Topic-T epifluorescence microscope on samples filtered through 0.22 μm black polycarbonate filters (Nuclepore) laid over 0.45 μm nitrocellulose backing filters (Millipore) (Parsons et al., 1984; Mac Isaac and Stockner, 1993). The mean cell volume of bac and pic was converted into cell carbon using specific conversion factors, 0.35 $\text{pg C } \mu\text{m}^{-3}$ for bacteria (Bjørnsen, 1986), 0.22 $\text{pg C } \mu\text{m}^{-3}$ for prokaryotic pic (Søndergaard et al., 1991) and 0.22 pg C cell^{-1} for eukaryotic pic (Mullin et al., 1966). Phytoplankton (nan and mic) and protozooplankton were counted and identified under an inverted microscope (Lund et al., 1958) on 100 ml settled samples fixed with 3% acid Lugol solution and 5% alkaline Lugol solution, respectively. The mean cell volume of each taxon of phytoplankton and protozooplankton was determined by applying standard geometric shape, as proposed by Hillebrand et al. (1999). Then, the mean cell volumes were converted to cell carbon using specific formula or factors (see Table 1, Meddeb et al., 2018).

Metazooplankton samples were preserved in borate-buffered formalin (5% final concentration) for identification and counts of organisms under a dissecting stereomicroscope (Leica). For each metazoan organism, the length and the width were converted into carbon content using conversion factors corresponding to each taxonomic group, as detailed in Meddeb et al. (2018). For each plankton group (bac, pic, nan, mic, pro, met1 and met2), the carbon biomass was estimated by multiplying their carbon content by their corresponding abundances. The detrital organic carbon (det) was estimated as total POC, determined from water column, minus the total carbon biomass of all plankton communities. Finally, the areal carbon stocks (mg C m^{-2}) for each living and non-living compartments were calculated by vertically integrating the carbon biomass calculated for each sampling depth. More details of calculations are given in Meddeb et al. (2018).

The gross production rate of size-fractionated phytoplankton (pic, nan and mic) and bacteria as well as consumption rate of these living components by protozooplankton were seasonally assessed at each sampling depth of each site by *in situ* dilution experiments (Landry and Hassett, 1982). This method is relatively simple to set up, it minimizes the manipulation and disruption of the micrograzer population and is an appropriate technique for determining total microzooplankton grazing activity (Sakka Hlaili et al., 2008; Grattepanche et al., 2011). Details for experimental procedure and calculation are provided in Meddeb et al. (2018) and Meddeb et al. (submitted).

Dilution experiments could be described as mixing free-particle sea water (filtered on 0.2 µm) with pre-filtered water on 200 µm mesh net (to remove meso- and macrozooplankton grazing effect) to give four dilution factors (100 %, 75 %, 50 % and 25 %). Each dilution mixture was then distributed into three clean 2L polycarbonate bottles, incubated *in situ* at the sampling depths fortnight. The estimated rates k and g were used to calculate gross production rates for phytoplankton and bacteria (P , mg C m⁻³ d⁻¹) and their consumption rates (G , mg C m⁻³ d⁻¹) as suggested by Moigis (2000) and in more recent works (Sakka Hlaili et al., 2008, Grattepanche et al., 2011):

$$P = k \times C_0 [e^{(k-g)t} - 1] / (k - g) \times t$$

$$G = g \times C_0 [e^{(k-g)t} - 1] / (k - g) \times t$$

where C_0 (mg C m⁻³) is the initial biomass of phytoplankton or bacteria, k is the growth rates of bacteria and size-fractionated phytoplankton (d⁻¹), g is the grazing rates by protozooplankton (d⁻¹) and t (d) is the incubation time (1 d).

The areal rates (mg C m⁻² d⁻¹) of production (P) and consumption (G) were determined by integrating the values obtained from the four sampling depths.

The consumption rates of phytoplankton (nan and mic) by metazooplankton (met1 and met2) were estimated by gut content experiments (Slaughter et al., 2006), which were conducted seasonally at each sites. The description of the experiment procedure and the calculation were detailed in Meddeb et al. (submitted).

Metazooplankton vertically sampled from bottom to surface in each site allowed the calculation of phytoplankton grazing by two size fractions of metazoa (200-700 µm and >700 µm). Subsamples (0.5L or 1L) were filtered (47 mm GF/F), homogenized (90% acetone) and filtered through 25 mm GF/F filters to remove pulp. The filtrates containing extracted gut

pigment were measured before and after acidification with 10% hydrochloric acid using the spectrophotometric method of Lorenzen (1967).

Gut pigment content (GP, mg pigment m⁻³) was calculated using the following equation:

$$GP = (GP_{sub} \times v) / (F \times V_{net})$$

where, GP_{sub} is the gut pigment concentration (mg pigment m⁻³) from the subsample, v (m³) is the volume of the subsample, F is the fraction of subsample processed for gut pigment content, and V_{net} (m³) is the total volume of water filtered during each net tow.

Metazooplankton grazing rate (GM, mg C m⁻³ d⁻¹) was calculated as:

$$GM = GP \times WC \times C:Chl\ a$$

where GP (mg pigment m⁻³) is the concentration of gut pigment, C:Chl *a* is the depth-averaged C:Chl *a* ratio determined for >2 µm phytoplankton (i.e. nano- and microphytoplankton) at each station, and WC (d⁻¹) is the portion of water cleared per day.

Finally, GM was multiplied by the vertical depth of the net tow at each site to estimate the areal rate of consumption of metazooplankton (GM_C, mg C m⁻² d⁻¹).

Sediment traps were used to measure sinking rates of particles and faecal pellets, during each season and at each site, as described in Meddeb et al. (2018).

2.5. Ecological Network Analysis

Ecological network analysis (Ulanowicz, 1997) was used to describe and to characterize the structural properties and function of plankton food webs resulted from the LIM-MCMC analysis. The MATLAB[®] algorithms, written by Carole Lebreton and Markus Schartau (GKSS Research Center, Geesthacht Germany), were used to calculate the indices that characterize food webs in term of activity, organization, specialization, recycling, maturity and stability (Table 5). The ENA indices used in this work as trophic functioning indicators were the total system throughput (TST), the ascendancy (A), the development capacity (DC), the relative ascendancy (A/DC), the average path length (APL) the Finn cycling index (FCI) and the detritivory/herbivory ratio (D/H). The definition and the formulae calculation of these indices are presented in Table 5.

2.6. Cliff's δ test for comparing network indices between seasons

The statistical comparison between the four seasons, concerning the set of indices calculated under Matlab (TST, DC, A, A/DC, APL, FCI and D/H) was performed with Cliff's δ test, which is usually used when having large sample sizes (here 300000 values for each flow) (Tecchio et al., 2016). For each ENA index, six pairwise comparisons were performed (comparing seasons) using the effsize package under R (version 3.4.3, www.r-project.org).

Then, the following values were used to define small, medium, and large effects according to Romano (2006). No p values can be calculated based on delta.

3. Results

3.1. Hydrodynamic status

Water temperatures, which were similar among stations and over depths, showed a seasonal cycle, ranging between a minimum in winter (12–13.7 °C) and a maximum in summer (25.7–27.1 °C). In the Bay, the salinity showed little variation within and between depths and seasons (36.9–37.4 psu). The water column of the Channel was vertically homogenous regarding the salinity during summer, autumn and winter (36.5–37.5 psu). In contrast, there was a haline stratification of the inshore water during spring due to low saline surface water coming from the Bizerte Lagoon. During this season, there was an exceptional decrease of salinity in the Lagoon (14–24 psu) caused by important river runoff associated with a large amount of rain during the winter 2012 (213 mm, INM, 2013). Further, high levels of nutrients were recorded in the Channel (Fig. 4). In both stations, pronounced concentrations of NO_2^- , NO_3^+ , and NH_4^+ were detected in spring, summer and autumn (10–24 μM) compared to the winter (3–4 μM) (Fig. 4A). PO_4^{3-} (0.14–0.99 μM) and Si(OH)_4 (0.84–2.86 μM) were available throughout the year in both stations (Fig. 4 B, C).

3.2. Primary and bacterial production

The LIM-MCMC analysis allowed the estimation of 44 carbon flows for each study site during each season (Table 3).

The primary production was relatively high during spring (966–727 $\text{mg C m}^{-2} \text{ d}^{-1}$), moderate in summer and autumn (394–571 $\text{mg C m}^{-2} \text{ d}^{-1}$) and low in winter (160–256 $\text{mg C m}^{-2} \text{ d}^{-1}$) (Fig. 5). During most seasons and in both stations, microphytoplankton (mic) contributed significantly to total production (Bay: 48–84%; Channel: 60–82%), except in the inshore station during autumn, when the proportion of picophytoplankton production was high 60%. During the rest of the year, the picophytoplankton contribution ranged from 10 to 29%. Nanophytoplankton production had low proportion during spring-summer (8–29%) and even lower (0.29–3.03%) in autumn-winter in all stations.

In both sites, bacterial production was low from spring to autumn (48–117 $\text{mg C m}^{-2} \text{ d}^{-1}$) and significantly increased in winter (135–274 $\text{mg C m}^{-2} \text{ d}^{-1}$). Bacterial production was very low relative to primary production (5 to 15 times less) in most cases, but in winter, it almost equaled the phytoplankton production at inshore and marine stations.

3.3. Carbon outflows

Respiration constituted the major carbon outflow from the eight model systems, with higher proportion during winter (66–84% of gross primary production) compared to the other seasons (45–53%) (Fig. 6). Carbon sinking contributed from 26–31% and 14–29 % of gross primary production, respectively, in the inshore and marine stations (Fig. 6A, B). Added to these two carbon loss pathways, the remaining carbon could be available for trophic transfer *via* the predation on metazooplankton. This trophic transfer was high during spring, summer and autumn (21–31% of gross primary production), but did not exceed 2% in winter at both stations (Fig. 6A, B).

3.4. Carbon flows to grazers

In the eight plankton food webs, the diet of protozooplankton was mainly composed of autotrophs during spring (90–97%), summer (86–88%) and autumn (86–93%), but equally shared between bacteria and phytoplankton in winter (i.e. mic and pic) (Fig.7A). Microphytoplankton formed the main prey of protozoa in both stations during spring (73–77% of total diet) and summer (36–47% of total diet). During autumn, microalgae remained the major food source in the marine station (66% of total diet), but in the inshore food web, picophytoplankton supplied 67% of carbon demand. The proportion of nanophytoplankton to protozoan diet was low in spring and summer (3–22 %) and even lower during autumn and winter (<2%).

In both sites, metazooplankton was mainly carnivorous, since protozooplankton and small metazoan (met1) formed their major food source in all seasons (40–62% of total diet). Herbivory of metazooplankton was low (<15% of total diet), but increased slightly (20–25% of total diet) in some cases (i.e. in spring and winter, in the inshore and marine stations). Detritus formed almost half of the food for the mesozooplankton during summer. The detritus contributed little to the metazoan diet in spring and autumn (26–45%) and even lower in winter (15–20%) (Fig. 7B).

3.5. Structural and functional proprieties of food webs

The total system throughput (TST) varied proportionally to the total input in the plankton food web system. This index, related to the total activity of the system, was high in spring (2934–3818 mg C m⁻² d⁻¹), moderate in summer and autumn (1733–1743 and 1710–2384 mg C m⁻² d⁻¹) and low in winter (879–1562 mg C m⁻² d⁻¹) (Fig. 8). The two food webs in spring exhibited the highest development capacity (DC) (Channel: 11.106 mg C m⁻² d⁻¹; Bay: 8489 mg C m⁻² d⁻¹, Fig. 8, Table 6) compared to the other seasons (2634–6951 mg C m⁻² d⁻¹) (Fig. 8). The ascendancy showed similar pattern as DC, with higher values in spring (4838–6054 mg C m⁻² d⁻¹) in comparison to the rest of the year (1566–3963 mg C m⁻² d⁻¹, Fig. 8,

Table 6). The A/DC ratio, which quantifies the degree of organization (information) of the system, reached higher values in winter (59.4–59.6%, Fig. 8) and decreased from autumn (57–58%) to spring (54.5–56%) and summer (53.4–53.9%) (Fig. 8). The highest values of APL, that quantifies the capacity of retention of the system, were detected in winter (4.48–5.09) in both sites, while the lowest ones were recorded during spring in both sites (2.95–3.03) and during summer in the Channel (3.20). Similarly, the Finn cycling index was highest in winter (41–46%). During autumn (for both sites) and summer (for the channel), this index reached higher values (16–17%) than in spring (for both sites) and summer (for the Bay) (13–14%) (Fig. 8, Table 6). Finally, the detritivory/herbivory ratio (D/H) showed significant increases during summer (0.59–0.66, Fig. 8, Table 6) in both sites and moderate (0.22–0.48, Fig. 8, Table 6) to lower values (0.05–0.12, Fig. 8, Table 6) were observed respectively during the spring, autumn and winter in all study sites.

4. Discussion

4.1. Hydrodynamics and nutritional status over seasons

The Bay of Bizerte is characterized by marine water mainly originated from the surface Modified Atlantic Water (MAW) (Millot, 1987, 1999), which flow into the Bay throughout the year. Even in winter, the freshwater discharges from the rivers surrounding the Lagoon of Bizerte only partly reaches the Bay. These conditions can lead to little variation of hydrological structure throughout the year. Consequently, nutrients were available throughout the year inside the Bay. In contrast, the Channel was influenced both by the marine water from the Bay (mostly in summer) and by lagoonal water (mostly in winter). Hence, the spring situation within the Channel was characterized by a strong halocline resulting from low saline surface water coming from the Lagoon of Bizerte, which was caused by an important river runoff during late winter (Fig.2A). In summer, the direction of the current was reversed, with an inflow of marine water from the Bay resulting in well mixed water (Fig. 2A). In autumn vertical mixing became more important, resulting in a well-mixed water column rich in nutrients (Fig. 3). This hydrological situation was maintained in winter with lower nutrient levels (particularly for nitrogenous nutrients).

4.2. Description of the new type of planktonic food web

To identify the dominant trophic pathway in marine environment, Sakka Hlaili et al. (2014) have proposed a specific ratio: the ratio of picophytoplankton net production to total phytoplankton net production (R7 ratio). This ratio was proposed by the authors to discriminate between herbivorous ($R7 \leq 0.1$), multivorous ($R7: 0.1–0.6$), and microbial food webs ($R7 \geq 0.6$). Based on this ratio, the spring food webs encountered in the Channel ($R7 =$

0.1) acted almost as a multivorous structure with a tendency towards herbivorous one, and the Bay ($R7 = 0.07$) functioned as herbivorous pathways. During the summer food web functioned as herbivorous pathway in the Channel ($R7 = 0.1$), and as multivorous in the Bay ($R7 = 0.27$). The situation in autumn was different between the sites (Fig. 9A, B). In the Bay, the food web did not change much in structure from that in summer and continued to function as a multivorous pathway ($R7 = 0.21$), while, it functioned approximately as a microbial pathway in the Channel ($R7 = 0.7$). More explanations in the evolution of food webs in both sites during spring, summer and autumn were reported in the supplement annex.

During winter, the intensification of vertical mixing and the low light condition in both sites were accompanied with a sharp decrease of primary production, which reached the lowest rates registered throughout the year ($160\text{--}256 \text{ mg C m}^{-2} \text{ d}^{-1}$, Fig. 9). These levels were very low compared to the one found in coastal areas. Instead, the bacterial production increased (especially in the Bay) and almost equalled the primary production (bacterial to primary production ratio, $PB:PP = 0.85\text{--}1.07$). This differed from that observed for the other seasons ($PB:PP$ ratio $0.06\text{--}0.21$), which were in the range of values found in other coastal areas, such as the Arcachon Bay (0.17 , Tortajada et al., 2012: spring), the Bay of Biscay (0.23 , Marquis et al., 2007, 2011: spring), and the lagoon of Bizerte ($0.23\text{--}0.26$, Grami et al., 2008: summer). Bacteria supplied 50% of the C demand of protozooplankton, which were mainly composed of aloricate ciliates and HNF in both sites during winter ($50\text{--}70\%$, Meddeb et al., submitted). Consequently, the microbivorous activity of protozooplankton increased relatively to their herbivory (Fig. 9 B), while metazooplankton consumed more microphytoplankton than during the other seasons. For these reasons, we were cautious to use only the $R7$ ratio to identify the dominant food web during winter, because bacteria seemed to play a stronger role in the winter food web than during the other seasons. In this case, another ratio was calculated, the $R5$ proposed by Sakka Hlaili et al. (2014) to determine the bacterial contribution to the production of potential microzooplankton food. The $R5$ is defined as the ratio between the net bacterial production to the net production of potential protozoan food (i.e. sum of net production of bacteria, phytoplankton, detritus and doc). $R5$ values showed that, in all sites, the bacteria contributed largely to the production of potential microzooplankton food during winter ($0.26\text{--}0.29$) than the other seasons ($0.03\text{--}0.1$). The $R7$ ratio calculated for winter (0.20 and 0.28 for the Channel and the Bay, respectively) suggested that the winter structure acted as a multivorous food web, but with the use of the $R5$ ratio, we preferred to call it “bacterial multivorous food web”. In this food web, bacteria and

microphytoplankton were the main actors and together fuelled the system. This newly defined food web type, which seems to be highly unusual, was not reported in the meta-analysis by Sakka Hlaili et al. (2014), who summarized and defined planktonic food webs from various regions. We propose that the bacterial multivorous food web should be added to the typology proposed by Legendre and Rassoulzadegan, (1995) and Sakka Hlaili et al. (2014) (Fig. 10).

For the determination of this “bacterial multivorous food web”, we here propose to researchers and managers, new criteria that they could use to identify quantitatively the trophic pathways that dominate the multivorous food web. The first criterion was the R7 ratio (i.e, picophytoplankton to total phytoplankton production), which separated the three main types of food web: herbivorous < 0.1 , multivorous $0.1-0.6$, and microbivorous > 0.6 . The second criterion was the R5 ratio (i.e. net bacterial production to the net production of potential microzooplankton food), which is used into the multivorous interval and discriminates between the multivorous (low R5 < 0.1) and bacterial multivorous food web (high R5 > 0.2). By using these two ratios in succession, researchers and managers can identify the newly defined food web type, “bacterial multivorous food web”.

Despite the impact of many factors on the seasonal evolution of planktonic food webs functioning, it is possible to identify an overall pattern of evolution. The accumulation of nitrates in the water column during winter allows the development of a spring bloom of phytoplankton when light and temperature conditions become more favorable. Thereafter, the mesozooplankton species develop rapidly and the ‘herbivorous’ food web is set up. This carbon pathway cannot persist in time (Legendre and Rassoulzadegan, 1995). The grazing activity of heterotrophic organisms enriches the pool of carbon and dissolved nitrogen (DOC and DON) and ammonium (excretion). DON promotes remineralization of ammonium by bacteria and their associated predators (protozoa) and a microbial food web settles while the classical herbivorous food web persists, leading to the formation of a ‘multivorous food web’ (Legendre and Rassoulzadegan 1995). This scenario was observed in the offshore site from the summer to the autumn (Fig. 10B). Phytoplankton communities are dominated by diatoms more suitable for non-limiting conditions of nitrates as was previously observed in other systems (Reynolds 2000, Alves de Souza et al., 2008). Smaller producers dominate when nitrates decrease, which was observed in our inshore station during autumn. Other conditions may favor proliferation of small producers leading to their dominance in relation the total primary production. This leads to the increase of the grazing activity of microzooplankton on the small cells and thus causes the installation of a microbial food web (Fig. 10C). In winter,

the fall of temperature and light was not favorable to the development of phytoplankton and therefore caused the decrease of the primary production. In this season, nitrogen was still quite low, and bacteria fed on these nutrients and did not use the remineralization process. This led to the increase of the bacterial production which became equal to the primary production accentuating the installation of the “bacterial multivorous food web” (Fig. 10G).

4.3. Ecosystem indicators and emergent properties

Ecological ratios (R7 and R5) are useful to discriminate between types of planktonic food webs and to identify “bacterial multivorous food web”. In complement, the calculation of Ecological Network Analysis indices are effective tools for characterizing the functioning of the food webs and especially of the new one (Ulanowicz 1986, 1997, Ulanowicz et al., 2009).

For both sites, all ENA indicators have obviously varied over seasons parallel to the change of trophic type (Fig. 8). The ENA variation showed also that there is a seasonal pattern of ecosystem properties, which coincides with specific food web, as illustrated by Fig 11. The highest ecosystem activity, indicated by TST, was observed in spring (2933–3818 $\text{mg C m}^{-2} \text{ d}^{-1}$), when algal blooms occurred and primary production dominating by microphytoplankton (Table 3) resulting to the herbivorous food web. In the opposite, the winter “bacterial multivorous web” showed the lowest activity. The ascendancy (A) and the development capacity (DC) are strongly related to the TST (Table 5, Ulanowicz, 2004) and showed the same pattern of variation. The relative ascendancy (A/DC) is an essential indicator of the degree of ecosystem organization and specialization (Ulanowicz, 1997; Patrício et al., 2004). The A/DC decreased from spring to summer and then rose in autumn/winter, which perfectly followed the seasonal change in the activity of the observed trophic structures over seasons. In other words, the increase of the amount of carbon circulating (i.e. TST) seemed to favor the direct pathways. Consequently, the carbon flows through few particular flows (bac-pro, mic-pro, pro-met; Fig. 10G) leading to an increase in carbon transfer efficiency and a better organization and specialization (Fig. 11). Other emergent properties of ecosystems (i.e. stability, maturity, resilience...) should also vary among seasons. For example, Legendre and Rassoulzadegan (1995) stated that an herbivorous food web could be considered as a system of low stability, since it is of transient nature and thus inherently unstable. In contrast, the multivorous and microbial food webs have higher stability and thus are longer lasting and will dominate the pelagic environment in most oceans. Our result are coherent with this proposition. Effectively, the FCI and APL indexes

have increased from spring (FCI: 13–14%; APL: 2.95–3.20) to summer/autumn (FCI: 16–17%; APL: 3.27–3.33) and then showed the highest value in winter (FCI: 41–46%; APL: 4.48–5.09). Moreover, during the same succession, the A/DC values have slightly increased, and the maximum value reached is slightly inferior to 60%, hence. With this situation, the maximum possible specialization was not reached, letting a reserve of redundancy, important for the robustness of the food web as underlined by Ulanowicz et al. (2009). This indicates a concomitant increase of ecosystem stability and maturity, when the trophic typology has evolved from the herbivorous web to multivorous and microbial webs and reached the “bacterial multivorous web”. This new trophic pathway based on a high heterotrophic bacterial production had the strongest carbon recycling, which lead to a higher retention of carbon inside the ecosystem and then appears to be the most stable and mature.

The D/H ratio is often considered as another index that could bring an important information to complete the description of the food web functioning. This ratio has effectively showed a seasonal shift in the food source used by consumers inducing an alternation between a system based on primary production pathway in spring, autumn and winter (lower D/H: 0.05–0.48, Fig. 9) and a system based on detrital energy pathways in summer (higher D/H: 0.59–0.66, Fig. 9). In our modelling work, the local information was limited on the detrital pathway toward mesozooplankton. So, detritivory flows were not enough constrained when calculating the unknown flows, which made us uncomfortable on linking the new typology of functioning and D/H values. Therefore, more experiments should be done to help limiting detritivory unknown flows before investigating the link between plankton food web typology and D/H index.

An important emergent property of each food web, highly related to the internal organization described with the ENA indices, is the distribution of carbon as transfer from the planktonic system, to non-planktonic pelagic consumers or down to the benthic consumers. The fate of biogenic carbon is dependent on the amount of primary production, the size of primary producers and the structure of the food web (Legendre and Rassoulzadegan, 1995; Vézina and Savenkoff, 1999; Legendre and Rivkin, 2002). Herbivorous food webs, based on large producers with high production rate, are considered as having the highest capacity to channel C to higher pelagic and benthic consumers, whereas in microbial food webs, most organic carbon is expected to be lost through respiration and trophic transfer will be low (Legendre and Rassoulzadegan, 1995, Rivkin et al., 1996; Legendre and Le Fèvre, 1995). Interestingly, a specific situation has been found in winter during which the “bacterial multivorous web” was acting that differs from others seasons (Fig. 6). In spring, summer and

autumn, the respiration was around 50% of gpp and a substantial fraction of primary production could reach higher pelagic consumers (21–31% of gross primary production, gpp). Meanwhile, another significant part of the production sank down to the benthic food web (20–30% of gpp), mainly due to the downward flow of metazoan faecal pellets (50–72% of total sinking material). These important proportions of sinking was in accordance to the assumptions made above for the herbivorous and multivorous food webs found in the Channel (during spring/summer) and the Bay (during spring/summer/autumn), but was unexpected for the Channel in autumn. During this season, the microbial pathway was observed and picophytoplankton dominated the primary production, which typically results in carbon leaving the plankton food web mainly through respiration (Legendre and Rassoulzadegan, 1995; Rivkin et al., 1996; Legendre and Le Fèvre, 1995). In our case, it seems that the capacity of the food web to channel carbon to higher trophic levels does not depend so much on the size of the producers, but rather on the amount of primary production, as stated previously (Rivkin et al., 1996, Marquis et al., 2007, 2011). The pronounced capacity of spring, summer and autumn food webs in channeling carbon to pelagic and benthic consumers may result from their high activity, as shown by a high value of TST, but also from the low recycling (FCI) and the low retention of carbon associated with low path length (APL) in the system (Fig. 11).

When the “bacterial multivorous food web” was observed, during winter, most produced carbon was lost by respiration (66–84% of gpp), leaving a little amount available for higher pelagic consumers (2–2.32% of gpp, Fig. 6). Obviously, this was a consequence of the functioning of the “bacterial multivorous web”, which was characterized by a strong recycling activity and long internal food chains, as supported by the highest values of FCI and APL (Fig. 11). The loss by sinking during winter (14–31% of gpp) did not differ that much from other seasons, but the sedimentation was mainly due to sinking of phytoplankton (59–75% of total sinking material). Thus, the winter food web exported carbon to the benthic system, just as the herbivorous pathway, and in the same time showed high cycling activity, as a microbial pathway. This reinforces our suggestion about a new type of functioning during winter, the so-called “bacterial multivorous food web”. This understanding of emergent properties determining the functioning of the system and in particular the fate of carbon is primordial as it impacts directly ecosystem services, such as shellfish farming or fishing which are the main activities in both study sites. This is of first importance for environment managers.

4.4. Ecological indicators, ecosystems health monitoring and management outlook

The present results highlight the importance of selecting the right indicators and that this directly links with management needs. ENA indices have been proposed as ecosystem health indicators (Niquil et al 2014) and proved to be sensitive to different impacts on marine ecosystems (Baird et al., 2009; Tecchio et al., 2016; Pezy et al., 2017; de la Vega et al., 2018a, b). In line with these former studies, our results showed significant implications of these approaches for the understanding of the ecosystem dynamics and for adaptive management. Some types of food webs can be considered as indicators of trophic status (Fath et al., 2019, Safi et al., 2019) and ecosystem services (Schückel et al., 2018). Thus, the present study appears as a relevant track for managers to develop indicators of health status of ecosystems. Based on the present results, we propose to researchers and managers new criteria (R7 and R5) to consider, in complement to ENA, and that they could use to identify quantitatively the newly defined food web type, “bacterial multivorous food web” which we suggest to be added to the existing typologies of planktonic food webs proposed by Legendre and Rassoulzadegan (1995) and modified by Sakka Hlaili et al. (2014).

Results of ENA analyses contribute to a better knowledge of the impacts of seasonal variations and anthropological pressures on food webs. They also allow defining recommendations for environmental managers and industry in terms of monitoring the effects on marine ecosystems by the limitation of nutrient and pollutant loads in coastal zone and ensuring restoration and increasing ecosystem resilience. Effectively, some ecological indicators, such as TST, A/DC, APL and FCI are already used to examine the impact of anthropization on marine ecosystems. Previous studies have considered TST as an indicator of eutrophication (Niquil et al., 2012; Grami et al., 2008; Scharler and Baird, 2005). Our results reported also very high TST values in spring, related to the high productivity of the ecosystems during this season, and especially to that of the autotrophic organisms (i.e. phytoplankton). The increase in TST could be interpreted as eutrophication and affiliated to the presence of a higher biodiversity of sources of carbon in the ecosystem and also to the proximity of the study stations to sources of nutrient discharges (urban, agricultural and shellfish farming input). Effectively, eutrophication events has been always reported for the Bay and the Channel sites (Sakka Hlaili et al., 2007). The relative ascendancy (A/DC), considered as a proxy for maturity, is supposed to decrease with an increase of stress (Ulanowicz, 1997). In our study, we found that A/DC followed trends predicted by this theory. The observed pattern mean that the least disturbed systems (autumn, winter) appeared to be less vulnerable to disturbance, more tightly organized, resistant and more efficient in processing energy (high A/DC) than perturbed systems (spring, summer). In addition, relative

indices (e.g. A/DC) appeared to be less affected by equilibrium process than the absolute indices (e.g. A). This confirms that the relative information indices appear more suitable for monitoring the effects of anthropogenic impact (as pollution, eutrophication...) on an ecosystem level than the absolute indices (Ulanowicz et al., 2009) and are preferred by many authors (Baird et al., 1991; Baird and Ulanowicz, 1993; Christensen 1995; Monaco and Ulanowicz, 1997; Rybarczyk and Elkaïm 2003; Rybarczyk et al., 2003; Patricio et al., 2004) because it excludes throughputs effects and allows comparison between ecosystems (Heymans et al., 2002).

It was also stated that stressed ecosystems show high FCI (Finn, 1976; Heymans, 2002; Richey et al., 1978; Ulanowicz, 1996; Mann et al., 1989). For example, Baird and Ulanowicz (1993) have shown that FCI index was higher in the most polluted estuary and lower in the less stressed systems, assessing that this index can be viewed as an indicator of pollution. Regarding findings based on chemically polluted and eutrophied ecosystems, FCI values were high in comparison to more pristine systems (Baird et al., 2004; Patricio et al., 2004; Scharler and Baird, 2005) and the structure of cycling changed dramatically between the unimpacted and the eutrophic systems (Patricio et al., 2004), consequently, FCI can be considered as a good candidate index for comparing stress states of many ecosystems. In the Bay and the Channel of Bizerte, chemical contamination by different pollutants has been widely described (Derouiche et al., 2004; Boufahja, 2010; Pringault et al., 2016). Effectively, the high FCI values of the winter (0.41–0.46) and those lower but still high compared to literature values, in other seasons (0.13–0.17), revealed that both systems could be considered as stressed over all the year. Furthermore, for both sites and over all seasons the APL responded to the environmental perturbation to the same gradient as FCI, thus suggesting that either index would be sufficient for evaluating the ecological status of studied ecosystems.

The differences observed in the functioning of the studied sites (the Channel and the Bay of Bizerte) indicate that those ecosystems, strongly impacted by human activities, must be monitored based on several representative sites, chosen regarding their main habitat type and environmental status. In addition, further comparative studies for the same sites should be made in different stations and seasons in term of food web monitoring development perspective. Monitoring programs covering the majority of ecosystem compartments and for the different habitats should be applied. Presently, there are major gaps in knowledge and data, in particular for higher trophic levels such as fish, birds, mammals ... and for lower trophic levels such as meiofauna or benthic diatoms. For this purpose, we advise managers to study and to integrate information about complete trophic systems by recommending a field

monitoring that covers plankton as well as fish and benthos compartments. Moreover, some other trophic strategies are important to be considered and studied for trophic transfer in planktonic environment (e.g. mixotrophy, parasitism, saprophitism). Integrating all these processes into a single food-web model gathering all carbon flows will allow a complete understanding of the ecosystem functioning. Such an approach is essential for applying a holistic ecosystem management strategy. Finally, we propose the use of well-studied areas as reference sites, selected according to available data (research projects) and existing monitoring programs (sampling takes place regularly, e.g. at least annual basis) for comparison.

5. Conclusion

Based on series of physico-chemical and plankton measurements along a seasonal gradient and using the LIM-MCMC approach, this study presents the dynamics and functioning of plankton food webs in inshore and marine waters of SW Mediterranean Sea during all seasons. These waters were characterized by specific environmental and hydrological conditions that were reflected in the food web structures, which showed seasonal dynamics that diverged from the conventional scheme proposed by Legendre and Rassoulzadegan (1995). The herbivorous food web dominated during spring, and showed the highest gross primary production that was largely dominated by microphytoplankton. This coincided with high availability of nutrients and light and stratification of the water column, caused by an increased freshwater inflow in the inshore waters. More surprisingly, the herbivorous food web was also identified during summer, as nutrient and light conditions remained favorable for microphytoplankton production. The multivorous pathway was dominant in the marine site during summer and autumn, when microalgal production decreased and picophytoplankton became more productive. In contrast, the autumn food web in inshore site functioned as microbial pathway, as primary production was mainly dominated by picophytoplankton. The microphytoplankton exhibited the lowest production in autumn, probably due to the decrease of light. The winter situation corresponded to a new type of pathway, the “bacterial multivorous food web” which we suggest can be added to the existing typologies of plankton food webs. In this newly defined food web, bacterial production equalled that of primary production, hence bacteria and microphytoplankton were the main actors that together fuelled the system.

The seasonal change in food webs structure resulted in a shift in the system properties and functioning of the ecosystem summarized in Figure 11. Comparing the ENA indices

among different seasons showed that the spring trophic pathway (herbivorous food web) was the most active with pronounced total system throughput (TST). In opposite, they showed lower stability in comparison to the systems encountered during summer (multivorous pathway) and autumn (microbial and multivorous pathways), with relatively high cycling activity and path length. The strongest carbon recycling coincided with the “bacterial multivorous food webs” observed in winter. This pathway was the most stable and mature, with pronounced capacity to retain carbon inside the system, leading to low export. The winter ecosystem also seemed to be most organized and specialized, as indicated by the high relative ascendancy (A/DC), with a probable more direct carbon pathways (Fig. 11).

Finally, this study propose appropriate indicators and evaluation tools to be used by managers and researchers for the assessment of the structure, functioning and emergent properties of ecosystems in natural and in disturbed conditions. The suggestion is to combine the use of R5 and R7 ratios to identify the newly defined food web type “bacterial multivorous food web” and ENA indices, to distinguish different states of the food web functioning across natural evolution and anthropogenic pressures.

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Figures

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Fig. 1. Location of the two study stations in the Channel (inshore station; C) and the Bay of Bizerte (marine station; B).

Fig. 2. Temperature and salinity profiles of the water column in the Channel (inshore station) and the Bay (marine station) over the seasons.

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Fig. 3. *A priori* model used to build the eight planktonic food web systems. Heterotrophic bacteria = bac, picophytoplankton $< 2 \mu\text{m}$ = pic, nanophytoplankton $2\text{--}10 \mu\text{m}$ = nan, microphytoplankton $20\text{--}200 \mu\text{m}$ = mic, protozooplankton $< 200 \mu\text{m}$ = pro, metazooplankton $200\text{--}700 \mu\text{m}$ = met1, metazooplankton $> 700 \mu\text{m}$ = met2, dissolved organic carbon = doc and detritus = det.

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Fig. 4. Average concentrations of nutrients (μM) in the Channel (inshore station) and the Bay (marine station) over the seasons (average $\pm$ SD, $n = 3$).

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Fig. 5. Production ($\text{mg C m}^{-2} \text{ d}^{-1}$) of the three phytoplankton size-classes (mic, nan and pic) and the heterotrophic bacteria (bac) in the inshore and marine stations over the seasons. The two bars refer to autotrophic vs heterotrophic production.

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Fig. 6. Three pathways of carbon output from the planktonic food web system (as % of gross primary production) in the inshore and marine stations over the seasons.

Fig. 7. Composition of diets for the protozooplankton (A) and the metazooplankton (B) in the inshore and marine stations over the seasons.

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Fig. 8. Synthetic diagram illustrating the seasonal variation of ecological indicators related to the dynamics of food web typology.

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Fig. 9. Synthetic diagram illustrating the seasonal dynamics of food web topology related to the hydrological condition and planktonic features.

Fig. 11. Seasonal variation of trophic food webs typology in parallel with some ecological network analysis indices.

Tables

Table 1

Mass equilibrium defining linear equations used for the inverse analysis. Flow names in the LIM-MCMC were composed of the three-letter code of the compartment of origin followed by the three-letter code of the destination compartment. Abbreviations: gpp = gross primary production, res = respiration, los = loss for the considered system.

Compartments	Mass balance equation	Equations common to the eight models
pic	Mass balance for picophytoplankton	$gpp\text{-}pic - (pic\text{-}res + pic\text{-}doc + pic\text{-}pro) = 0$
nan	Mass balance for nanophytoplankton	$gpp\text{-}nan - (nan\text{-}res + nan\text{-}doc + nan\text{-}det + nan\text{-}pro + nan\text{-}met1 + nan\text{-}met2 + nan\text{-}los) = 0$
mic	Mass balance for microphytoplankton	$gpp\text{-}mic - (mic\text{-}res + mic\text{-}doc + mic\text{-}det + mic\text{-}pro + mic\text{-}met1 + mic\text{-}met2 + mic\text{-}los) = 0$
pro	Mass balance for protozooplankton	$(pic\text{-}pro + nan\text{-}pro + mic\text{-}pro + bac\text{-}pro) - (pro\text{-}res + pro\text{-}doc + pro\text{-}det + pro\text{-}met1 + pro\text{-}los) = 0$
met1	Mass balance for metazooplankton 200-700 μm	$(nan\text{-}met1 + mic\text{-}met1 + pro\text{-}met1 + det\text{-}met1) - (met1\text{-}res + met1\text{-}doc + met1\text{-}det + met1\text{-}met2 + met1\text{-}los) = 0$
met2	Mass balance for metazooplankton > 700 μm	$(nan\text{-}met2 + mic\text{-}met2 + pro\text{-}met2 + met1\text{-}met2 + det\text{-}met2) - (met2\text{-}res + met2\text{-}doc + met2\text{-}det + met2\text{-}los) = 0$
bac	Mass balance for bacteria	$doc\text{-}bac - (bac\text{-}res + bac\text{-}doc + bac\text{-}pro) = 0$
doc	Mass balance for dissolved organic carbon	$(pic\text{-}doc + nan\text{-}doc + mic\text{-}doc + pro\text{-}doc + met1\text{-}doc + met2\text{-}doc + bac\text{-}doc + det\text{-}doc) - (doc\text{-}bac + doc\text{-}los) = 0$
det	Mass balance for detritus	$(nan\text{-}det + mic\text{-}det + pro\text{-}det + met1\text{-}det + met2\text{-}det) - (det\text{-}doc + det\text{-}met1 + det\text{-}met2 + det\text{-}los) = 0$

pic = picophytoplankton, nan = nanophytoplankton, mic = microphytoplankton, pro = protozooplankton, met1 = metazooplankton1, met2 = metazooplankton2, bac = bacteria, doc = dissolved organic carbon, det = detritus

Table 2

Minimum and maximum values of flows (derived from *in situ* measurement for each seasons) used as inequalities for the eight networks.

Process integrated as inequalities	Flux	Spring		Summer		Autumn		Winter	
		Minimum - Maximum							
		Channel model	Bay model	Channel model	Bay model	Channel model	Bay model	Channel model	Bay model
pic gross primary production	gpp-pic	54-77	46-69	36-53	92-128	202-268	10-68	28-38	62-82
nan gross primary production	gpp-nan	88-121	48-75	102-135	92-122	4-6	1-2	4-6	2-3
mic gross primary production	gpp-mic	687-904	522-698	208-276	172-227	133-175	326-562	75-140	139-206
pic grazing by protozooplankton	pic-pro	17-22	31-46	33-36	41-93	168-204	68-81	18-28	37-66
nan grazing by protozooplankton	nan-pro	73-90	31-41	37-51	20-25	2-3	1-1.5	2-3	1.62-2.27
mic grazing by protozooplankton	mic-pro	425-468	210-478	80-110	76-82	48-65	190-352	46-49	67-84
doc uptake by bacteria	doc-bac	90-117	20-59	46-61	40-92	42-85	63-67	90-135	244-274
bacterial grazing by protozoa	bac-pro	58-61	13-16	29-32	18-37	42-47	24-53	67-68	137-159
mic sinking	mic-los	86-521	29-55	12-19	36-44	31-35	6-7	39-57	24-54
nan sinking	nan-los	1.5-44	1-1.5	0.02-0.03	0.4-0.6	2-3	0.3-0.5	0.20-0.31	0.71-0.91
mic and nan grazing by met1	mic + nan-met1	36-49	17-26	32-48	5-8	9-11	19-29	7-8	18-22
mic and nan grazing by met2	mic + nan-met2	66-85	0.12-0.16	0.10-0.20	14-21	0.05-0.08	7-19	14-18	40-52
met1 + met2 sinking	met1 + met2-los	16*	21*	0.65*	0.62*	4.24*	0.91*	0.47*	0.48*
det sinking	det-los	2361**	657**	6849**	1611**	4126**	7083**	1238**	684**

All flows are considered as intervals between the minimum and maximum values estimated *in situ*, with the following exceptions:

* Flows considered as minimum

** Flows considered as maximum

Table 3

Flows description and values ($\text{mg C m}^{-2} \text{d}^{-1}$) estimated by the LIM-MCMC for the eight plankton food webs.

Description	Symbol	Inferred value ($\text{mg C m}^{-2} \text{d}^{-1}$)							
		Spring		Summer		Autumn		Winter	
		Channel model	Bay model	Channel model	Bay model	Channel model	Bay model	Channel model	Bay model
Microphytoplankton gross primary production	gpp-mic	795	609	242	199	154	453	123	180
Nanophytoplankton gross primary production	gpp-nan	65	61	118	107	5	2	5	3
Picophytoplankton gross primary production	gpp-pic	105	56	44	109	236	117	33	73
Microphytoplankton respiration	mic-res	40	32	12	10	8	35	6	9
Microphytoplankton dissolved organic carbon excretion	mic-doc	69	54	21	17	13	49	11	16
Microphytoplankton detritus production	mic-det	35	143	77	41	34	76	0.2	0.3
Microphytoplankton grazing by protozooplankton	mic-pro	444	326	95	79	56	250	46	74
Microphytoplankton grazing by metazooplankton1	mic-met1	33	15	20	3	10	24	6	18
Microphytoplankton grazing by metazooplankton2	mic-met2	66	0.1	0.06	9	0.04	13	13	40
Microphytoplankton sinking	mic-los	109	40	15	40	33	6	40	24
Nanophytoplankton respiration	nan-res	4	4	6	6	0.3	0.1	0.2	0.1
Nanophytoplankton dissolved organic carbon excretion	nan-doc	6	6	10	9	0.5	0.2	0.4	0.3
Nanophytoplankton detritus production	nan-det	9	7	37	57	0.1	0.02	0.2	0.01
Nanophytoplankton grazing by protozooplankton	nan-pro	19	36	44	23	2	1	3	2
Nanophytoplankton grazing by metazooplankton1	nan-met1	9	7	20	3	0.1	0.02	1	0.01
Nanophytoplankton grazing by metazooplankton2	nan-met2	9	0.1	0.06	9	0.03	0.03	1	0.01
Nanophytoplankton sinking	nan-los	9	1	0.03	0.5	2	0.4	0.2	1
Picophytoplankton respiration	pic-res	6	4	3	7	12	6	2	4
Picophytoplankton dissolved organic carbon excretion	pic-doc	9	6	4	10	20	10	3	6
Picophytoplankton grazing by protozooplankton	pic-pro	90	46	36	92	208	101	28	62
Protozooplankton respiration	pro-res	181	127	63	67	89	113	39	89
Protozooplankton dissolved organic carbon excretion	pro-doc	95	66	33	35	47	59	38	89
Protozooplankton detritus production	pro-det	94	64	31	33	46	58	20	28
Protozooplankton consumption by met1	pro-met1	158	99	43	50	76	91	42	69
Protozooplankton consumption by metazooplankton2	pro-met2	84	66	35	34	46	58	5	0.1
Metazooplankton1 respiration	met1-res	92	80	54	45	43	63	19	36
Metazooplankton1 dissolved organic carbon excretion	met1-doc	47	41	28	23	22	33	18	36
Metazooplankton1 detritus production	met1-det	45	39	26	22	21	31	10	11
Metazooplankton1 consumption by metazooplankton2	met1-met2	67	60	42	33	33	48	19	28
Metazooplankton1 sinking	met1-los	38	31	21	18	16	25	1	0.02
Metazooplankton2 respiration	met2-res	96	68	45	43	37	57	13	22
Metazooplankton2 dissolved organic carbon excretion	met2-doc	48	35	23	22	19	29	12	22
Metazooplankton2 detritus production	met2-det	46	32	21	21	18	27	6	7
Metazooplankton2 sinking	met2-los	96	69	45	44	38	58	11	17
Bacteria respiration	bac-res	26	13	14	20	19	16	27	55
Bacteria dissolved organic carbon production	bac-doc	32	21	17	30	23	22	40	82
Bacteria grazing by protozooplankton	bac-pro	58	14	29	26	42	28	67	137
Dissolved organic carbon uptake by bacteria	doc-bac	117	48	60	77	85	65	135	274
Dissolved organic carbon output	doc-los	236	223	103	96	81	167	0.5	0.01
Detritus dissolution	det-doc	46	43	26	25	21	31	13	22
Detritus consumption by metazooplankton1	det-met1	89	130	87	84	50	85	17	24
Detritus consumption by metazooplankton2	det-met2	61	78	56	44	53	33	5	0.1
Detritus sinking	det-los	34	34	23	20	16	25	1	0.01

Table 4
Constraints (inequalities) used for the eight networks based on literature.

Process	Concerned compartment	Bound	Description	Equation	Reference
Respiration	pic, nan, mic	Lower	Autotroph respiration (r) is higher than 5% of gross primary production (gpp)	$5\% gpp < r$	Vézina and Platt (1988)
		Upper	Autotroph respiration (r) is no $>$ 30% of gross primary production (gpp)	$r < 30\% gpp$	
		Lower	Bacteria respiration (r') is higher than 20% of total uptake of doc ($udoc$)	$20\% udoc < r'$	Vézina and Savenkoff (1999)
Dissolved organic carbon production	pic, nan, mic	Lower	Zooplankton respiration (r'') is at least 20% of their total ingestion (ΣIng)	$20\% \Sigma Ing < r''$	Vézina and Savenkoff (1999)
		Upper	Autotroph doc excretion (e) is higher than 10% of their net primary production (npp)	$10\% npp < e$	Vézina and Savenkoff (1999)
		Upper	Autotroph doc excretion (e) do not exceed 55% of their net primary production (npp)	$e < 55\% npp$	
Growth efficiency	pro, met1, met2	Lower	Zooplankton excretion (ex) of doc is at least 10% of their total ingestion (ΣIng)	$10\% \Sigma Ing < ex$	Vézina and Pace (1994)
		Upper	Zooplankton excretion (ex) doesn't exceed their respiration (r'')	$ex < r''$	Vézina and Platt (1988)
		Upper	Bacteria doc release ($rdoc$) is lower than their respiration (r')	$rdoc < r'$	Vézina and Pace (1994)
Assimilation efficiency	pro, met1, met2	Lower	The growth efficiency of zooplankton is at least 25% of their carbon uptake	$25\% \Sigma Ing < Ing - (r'' + ex + det)$	Vézina and Pahlow (2003)
		Upper	The growth efficiency of zooplankton is no $>$ 50% of their carbon uptake	$Ing - (r'' + ex + det) < 50\% \Sigma Ing$	Vézina and Pahlow (2003)
		Lower	Growth efficiency of bacteria is at least 5% of their total ingestion	$5\% udoc < udoc - (r' + rdoc)$	Vézina and Pahlow (2003)
Detritus production	pro, met1, met2	Upper	Growth efficiency of bacteria is no $>$ 50% of their total ingestion	$udoc - (r' + rdoc) < 50\% udoc$	Vézina and Pahlow (2003)
		Lower	Assimilation efficiency of zooplankton is at least 50% of their total ingestion	$50\% \Sigma Ing < Ing - det$	Vézina et al. (2000)
		Upper	Assimilation efficiency of zooplankton is no $>$ 90% of their total ingestion	$Ing - det < 90\% \Sigma Ing$	
Detritus dissolution	det	Lower	Zooplankton det production is at least 5% of their det production and respiration combined	$5\% (zoo-det + zoo-res) < zoo-det$	Steinberg et al. (2000)
		Upper	Zooplankton det production is no $>$ 42% of their det production and respiration combined	$Zoo-det < 42\% (zoo-det + zoo-res)$	
		Upper	Det dissolution does not exceed 10% of net primary production	$det-doc < 10\% npp$	Pace et al. (1984)

Table 5

Ecological indicators used for the Channel and the Bay models. Nomenclature of symbols used in formula calculation of network index equations: i : flow out of the system from i , $i = 1, \dots, n$; j : flow into the system to j , $j = 1, \dots, n$; T_i : Total outflow from compartment i ; T_j : Total inflow for compartment j ; and T_{ij} : Flow from compartment i to compartment j . $TSTc$ is the total flow that is recycled, Z : equals the sum of all exogenous inputs.

Ecological indicator	Definition	Formula calculation	Unit	Reference
Total System Throughput (TST)	The sum of all the flows circulating through all compartments and a measure of the total system activity.	$TST = \sum_{i=1}^n \sum_{j=1}^n T_{ij}$	$\text{mg C m}^{-2} \text{ d}^{-1}$	Kay et al. (1989)
Ascendency (A)	Ascendency represents the organized part of the ecosystem and merges the quantification of the system activity and the degree of specialization.	$A = \sum_j (T_j) * \log\left(\frac{T_j * TST}{T_j * T_1}\right)$	$\text{mg C m}^{-2} \text{ d}^{-1} \times \text{bits}$	Ulanowicz and Wulff (1991) and Ulanowicz (2000)
Development Capacity (DC)	Represents the maximum possible value of Ascendency that an ecosystem may reach.	$DC = -TST * \sum_j \frac{T_j}{TST} * \log\left(\frac{T_j}{TST}\right)$	$\text{mg C m}^{-2} \text{ d}^{-1} \times \text{bits}$	Ulanowicz and Wulff (1991) and Ulanowicz (2000)
Relative Ascendency (A/DC)	Defines the ecosystem degree of development. High A/DC ratios reflect a high degree of organization, highly specialized and less redundant pathways.	$\frac{A}{DC}$	%	Baird et al. (1991, 1998)
Average Path Length (APL)	It is the average number of compartments crossed by a unit of carbon from entering to exiting the ecosystem. Represents a measure of the ecosystem retention capacity.	$APL = \frac{TST - \sum_i \text{Imports}_i}{\sum_i \text{Imports}_i}$	no unit	Kay et al. (1989) and Baird et al. (1991)
Finn Cycling Index (FCI)	Quantifies the fraction of all flows involved in recycling and can be considered as a measure of the retentiveness of the system.	$FCI = \frac{TSTc}{TST}$	%	Finn (1976)
Detritivory/Herbivory (D/H)	Indicates the importance of living trophic interactions compared to detritus chain.	$\frac{D}{H}$	No unit	Ulanowicz (1992)

Table 6

Results of the Cliffs'8 test applied to ecological network indices calculated for the Channel and the Bay over the four seasons. Comparison was made between seasons (spring/summer, spring/autumn, spring/winter, summer/autumn, summer/winter and winter/autumn) for each ecological indices (TST, A, DC, A/DC, APL, FCI and D/H). Asterisk Between brackets represent the magnitude of the effect (***): large and (ns) negligible effects.

		Spring/Summer	Spring/Autumn	Spring/Winter	Summer/Autumn	Summer/Winter	Winter/Autumn
Channel (inshore station)	TST	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)
	A	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)
	DC	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)
	A/DC	0.54 (***)	0.54 (***)	-0.99 (***)	0.54 (***)	-0.99 (***)	0.99 (***)
	APL	-0.92 (***)	0.99 (***)	-1 (***)	0.99 (***)	-1 (***)	0.99 (***)
	FCI	-0.67 (***)	0.99 (***)	-1 (***)	0.99 (***)	-1 (***)	0.99 (***)
	D/H	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)
Bay (marine station)	TST	0.99 (***)	0.99 (***)	0.99 (***)	-0.99 (***)	0.99 (***)	0.99 (***)
	A	0.99 (***)	0.99 (***)	0.99 (***)	-0.99 (***)	0.87 (***)	0.99 (***)
	DC	0.99 (***)	0.99 (***)	0.99 (***)	-0.99 (***)	0.99 (***)	0.99 (***)
	A/DC	0.95 (***)	-0.01 (ns)	-0.94 (***)	-0.96 (***)	-1 (***)	-0.96 (***)
	APL	-0.62 (***)	-0.53 (***)	-1 (***)	0.12 (ns)	-1 (***)	-1 (***)
	FCI	-0.94 (***)	-0.60 (***)	-1 (***)	0.87 (***)	-1 (***)	-1 (***)
	D/H	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)	0.99 (***)