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Plankton food-web functioning in anthropogenically impacted coastal waters (SW Mediterranean Sea): an ecological network analysis.

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Abstract

The study is the first attempt to (i) model spring food webs in three SW Mediterranean ecosystems which are under different anthropogenic pressures and (ii) to project the consequence of this stress on their function. Linear inverse models were built using the Monte Carlo method coupled with Markov Chains to characterize the food-web status of the Lagoon, the Channel (inshore waters under high eutrophication and chemical contamination) and the Bay of Bizerte (offshore waters under less anthropogenic pressure). Ecological network analysis was used for the description of structural and functional properties of each food web and for inter-ecosystem comparisons. Our results showed that more carbon was produced by phytoplankton in the inshore waters ($966\text{--}1234\text{ mg C m}^{-2}\text{ d}^{-1}$) compared to the Bay ($727\text{ mg C m}^{-2}\text{ d}^{-1}$). The total ecosystem carbon inputs into the three food webs was supported by high primary production, which was mainly due to $>10\text{ }\mu\text{m}$ algae. However, the three carbon pathways were characterized by low detritivory and a high herbivory which was mainly assigned to protozooplankton. This latter was efficient in channelling biogenic carbon. In the Lagoon and the Channel, food webs acted almost as a multivorous structure with a tendency towards herbivorous one, whereas in the Bay the herbivorous pathway was more dominant. Ecological indices revealed that the Lagoon and the Channel food webs/systems had high total system throughput and thus were more active than the Bay. The Bay food web, which had a high relative ascendancy value, was more organized and specialized. This inter-ecosystem difference could be due to the varying levels of anthropogenic impact among sites. Indeed, the low value of Finn's cycling index indicated that the three systems are disturbed, but the Lagoon and the Channel, with low average path lengths, appeared to be more stressed, as both sites have undergone higher chemical pollution and nutrient loading. This study shows that ecosystem models combined with ecological indices provide a powerful approach to detect change in environmental status and anthropogenic impacts.

Keywords: Food-web modelling, Ecological Network Analysis, Mediterranean coastal waters, plankton ecology.

1. Introduction

Food webs are by definition simple representations of who eats whom in a considered ecosystem. The common representation of a pelagic food web from the 1920s to the 1970s consisted of a three level compartment: 1. algae as primary producers of organic matter, 2. zooplankton (metazoa) as consumers of primary production (grazers), and 3. Ichthyoplankton (fish larvae) as predators (or secondary consumers). This three-level chain was called “*herbivorous food chain*” (Steele, 1974). With the discovery of the functional role of autotrophic and heterotrophic microorganisms as producers and consumers of organic matter (Pomeroy, 1974), the interactions between the organisms were represented in a more complex way. Azam et al. (1983) introduced the concept of the “*microbial loop*”, which emphasized the importance of heterotrophic microbes in the transfer of organic carbon to larger consumers. Then, Sherr and Sherr (1988) suggested a new food-web structure called the “*microbial food web*” where autotrophic and heterotrophic microorganisms are dominant and sustain the functioning of the ecosystem. Legendre and Rassoulzadegan (1995) suggested complementarily a “*multivorous food web*” where herbivorous and microbial organisms co-act to transfer organic carbon to larger organisms. Legendre and Rivkin (2002) stressed that respiration and downward export of particulate and dissolved organic carbon could be a major way of biogenic carbon export out of the pelagic food web. More recently, food-web modelling approaches have enabled ecologists to describe new food-web structures such as: “*phytomicrobial*” where microzooplankton mostly graze on phytoplankton and “*polymicrobial*” where microzooplankton graze equally on all food resources (Sakka Hlaili et al., 2014).

76 Food-web models summarize data obtained for any environment into a network of
77 compartments connected by trophic links. Using any modelling approach, more integrated
78 and concise information can be obtained on the functioning of pelagic ecosystems and
79 compared with other modelled ecosystems. Ecosystem models provide a powerful approach
80 to analyse and predict the consequence of environmental and anthropogenic pressures on food
81 web structure and functioning. Several methods have been developed to characterize marine
82 food webs. The Linear Inverse Model (LIM), as defined by Vézina and Platt (1988), allows
83 the estimation of unknown trophic flows through the pelagic food web and alleviates the
84 problem of under-sampling using the principle of the conservation of mass, i.e. the quantity of
85 carbon coming into each compartment considered as equal to the amount leaving it.
86 Moreover, this approach is considered as sufficient to describe the structure of aquatic
87 ecosystems (Vézina and Pahlow, 2003; Savenkoff et al., 2004). It has been applied to a wide
88 range of ecosystems: coastal areas (Vézina and Platt, 1988; Jackson and Eldridge, 1992), bays
89 (Richardson et al., 2003; Marquis et al., 2007), lakes (Niquil et al., 2011), oceans (Vézina et
90 al., 1997; Richardson et al., 2004) and gulfs (Vézina et al., 2000; Savenkoff et al., 2004;
91 Chaalali et al., 2015). This deterministic method had limitations (e.g. Eldridge and Jackson,
92 1993; Niquil et al., 1998; Donali et al., 1999; Kones et al., 2006), and was therefore modified
93 into the Monte Carlo Markov Chain Linear Inverse method (LIM-MCMC; Van den Meersche
94 et al., 2009) based on a sampling procedure that attempts a complete coverage of the range of
95 possible solutions. This new approach allows the estimation of likelihood distributions of the
96 flows rather than unique estimates and also takes into account flow variability resulting from
97 uncertainty in sampling data (Grami et al., 2011; Niquil et al., 2012; Rasconi et al., 2014;
98 Saint-Béat et al., 2013; 2015; Chaalali et al., 2015; 2016; Tecchio et al., 2016).
99 Ecological Network Analysis (ENA) is a group of algorithms describing food-web structure
100 and functioning using the output of the calculated flows given by the modelling exercise.

ENA is based on a set of indices (i.e. the ENA indices) that describe (i) the connections between food-web compartments through an analysis of the input and output flows, (ii) the trophic structure, (iii) the topology, (iv) and the recycling in the network (Ulanowicz, 1996). When applied to the estimated flows of the food-web model, ENA allows qualitative and quantitative description of the ecosystem properties (Ulanowicz, 1986; Fath et al., 2007). This tool has been widely used in coastal ecology to characterize the food-web structure, the level of organization, the network dependence on external factors, and to evaluate the ecosystem stress level and its stability (Heymans et al., 2014; Saint-Béat et al., 2015).

Mediterranean coastal waters are currently under large anthropogenic pressure, leading to their eutrophication and chemical contamination (Bianchi et al., 2000; Boudouresque et al., 2002; Mzoughi et al., 2005; Sakka Hlaili et al., 2007; Sahraoui et al., 2012). Despite this, a few studies have addressed change in the structure and functioning of the food webs in some Mediterranean northern waters such as those in the Catalan continental slope (Tecchio et al., 2013) and the Gulf of Lions (Auger et al., 2011, Alekseenko et al., 2014). However, ecosystem-level studies in the South of the Mediterranean Sea are very scarce. Indeed, only one study using the LIM approach has modelled the plankton trophic pathways in the semi-enclosed Bizerte Lagoon (Grami et al., 2008), but studies on nearby marine ecosystems are non-existent. So, in the present study, we have applied the approach LIM-MCMC to characterize the trophic food-web status and its associated structural and functional properties in inshore (Lagoon and Channel of Bizerte) and offshore (Bay of Bizerte) coastal waters located in the south-west of the Mediterranean Sea. The work was carried out in these three ecosystems, which differ in environmental conditions and anthropogenic pressures, in order to evaluate if modelling allows us to identify the variability in the structure and the functioning of each planktonic food web. We specifically addressed the following questions: does planktonic food web function differently among these coastal waters during spring? Is their

primary production efficiently channelled to higher trophic levels? What is the environmental status of each site with regard to productivity, trophic structure, carbon transfer, stress, and organisation levels?

The data of standing stocks (bacteria, phytoplankton, protozooplankton, and metazooplankton) and some *in situ* estimated carbon flows (bacterial and phytoplankton production, protozooplankton grazing and metazooplankton grazing) were described in Meddeb et al. (submitted). In the present study, we used these data in addition to a measure of Dissolved Organic Carbon (DOC) and Particulate Organic Carbon (POC) stocks and vertical carbon flowing in each ecosystem. All data were integrated in a common view of ecosystem functioning, and completed, using the inverse analysis LIM-MCMC.

2. Material and Methods

2.1. Study sites

Water sampling was carried out during spring 2012 in three south-western Mediterranean ecosystems, situated in the city of Bizerte (Tunisia, Fig. 1). The sites are the Lagoon (inshore station L: 37°12'46"N, 09°50'48" E), the Channel (inshore station C: 37°15'04" N, 9°52'34" E) and the Bay (offshore station B: 37°16'54" N, 9°53'42" E) of Bizerte. The Lagoon, with a surface area of 121.6 km² and a maximum depth of 12 m, is a semi-enclosed ecosystem that receives a large amount of freshwater, particularly in winter, from several surrounding rivers and mainly from Lake Ichkeul (Fig. 1). The hydrodynamics are mainly driven by wind (Harzallah et al., 2003; Bejaoui et al., 2005). The lagoon is experiencing increasing nutrient concentrations (10.69–17.89 N µM, 0.86–1.33 P µM and 12.30–13.30 Si µM, Sakka Hlaili et al., 2008) from expanding urban, agricultural, and industrial development, leading in turn to high Chl *a* concentrations (4.43–5.48 mg m⁻³, Sakka Hlaili et al., 2008). The anthropogenic activities lead to a contamination of the sediment of the lagoon by several pollutants, including heavy metals (as Zn, Cd, Co, Cu, Pb, Ni, Cd, Mn, and Fe, 20–598 µg g⁻¹, Ben Mna

et al., 2017), organotins (170 ng kg^{-1} , Mzoughi et al., 2005), polycyclic aromatic hydrocarbons (PAHs: $3042\text{--}9948 \text{ ng g}^{-1}$, Zmerli et al., 2017), and pesticides ($1.1\text{--}14.0 \text{ ng g}^{-1}$ of dry sediment, Barhoumi et al., 2013). The Lagoon is one of the most economically important areas in Tunisia, sustaining large populations of planktivorous fish and shellfish (mussels, oysters, and clams). The Lagoon is connected northwards to the Mediterranean Sea through the Bizerte Channel (7 km long, 300 m wide, and 12 m deep). This site supports eutrophied waters ($34.40 \pm 12 \text{ N } \mu\text{M}$, $1.30 \pm 0.3 \text{ P } \mu\text{M}$ and $1.20 \pm 1.05 \text{ Si } \mu\text{M}$, Sahraoui et al., 2012) with high Chl *a* concentrations ($6.20 \pm 4 \text{ mg m}^{-3}$, Sahraoui et al., 2012) and was significantly polluted by heavy metals (especially Zn, Cr, and Pb, $85\text{--}228 \text{ mg g}^{-1}$ of dry sediment) and PAHs ($21\text{--}772 \text{ } \mu\text{g g}^{-1}$ of dry sediment) (Pringault et al., 2016). The Bay of Bizerte, with a depth of 16–20 m, is an open ecosystem directly connected to the Mediterranean Sea. This site supports lower levels of inorganic nutrients ($1.19 \pm 0.9 \text{ N } \mu\text{M}$, $0.53 \pm 0.02 \text{ Si } \mu\text{M}$ and $<1 \text{ P } \mu\text{M}$) and Chl *a* ($0.5\text{--}2.5 \text{ mg Chl } a \text{ m}^{-3}$) (Sahraoui et al., 2012), compared to the two other sites. It is also less contaminated than the Lagoon and the Channel. The PAHs ($78 \text{ } \mu\text{g g}^{-1}$ of dry sediment, Boufahja, 2010) are the main pollutants, which are originated from the oil refinery located on the shore of the Bay (Zrafi-Nouira et al., 2008; Ben Ameer et al., 2013).

2.2. Sampling and water analysis

At each station, water was collected during spring 2012 at four depths using an acid-washed 2.5 L plastic water sampler (Hydro-Bios), which was then pre-filtered through a 200 μm mesh net to remove meso- and macrozooplankton (except water used for DOC determination) before storing it in isothermal containers until processing. Five mL subsamples were filtered on sterilized 0.2 μm polycarbonate filters and then frozen in acid-washed vials at -20°C until analysis for dissolved organic carbon (Knap et al., 1993) using a Shimadzu TOC-5000 carbon analyser. Subsamples for determination of POC were filtered on pre-combusted (at 450°C ,

176 during 2 h) GF/F Whatman filters (21 mm) and analysed on a CHN elemental analyser
177 (Perkin–Elmer 2400), as described in the JGOFS report (Knap et al., 1996). Subsamples were
178 also taken for plankton enumeration and identification.

179 For the heterotrophic bacteria (bac) count, 20 ml samples were preserved with formaldehyde
180 (final concentration of 4 %) and kept refrigerated at 4°C. Each sample (2 ml) was labelled
181 with 0.2 ml of a solution of orange acridine for 2 minutes (Hobbie et al., 1977; Parsons et al.,
182 1984) and filtered under low pressure on a black filter (0.22 µm, polycarbonate). Counting of
183 bacteria was performed under a CETI Topic-T epifluorescence microscope using a 100x
184 objective and the blue filter. The count was done in 20 random fields and by counting 400
185 cells per slide. To enumerate prokaryotic and eukaryotic picophytoplankton (pic), samples (10
186 ml) were preserved in 0.22 µm prefiltered formaldehyde (2% final concentration) and stored
187 for 1 h in darkness at 4°C. The samples were then filtered on 0.22 µm black polycarbonate
188 filters (Nuclepore), laid over 0.45 µm nitrocellulose backing filters (Millipore). The filters
189 were mounted on slides using low-fluorescence immersion oil and stored immediately at
190 20°C. Abundance was determined under a CETI Topic-T epifluorescence microscope (100 x
191 Fluotar objective), as described in MacIsaac and Stockner (1993), using blue and green
192 excitation and counting at least 200 cells from 30 random squares. Samples for identification
193 and enumeration of phytoplankton [nanophytoplankton: 2-10 µm (nan) and
194 microphytoplankton: 10-200 µm (mic)] and protozooplankton (pro: 5-200 µm, composed of
195 heterotrophic nanoflagellates, dinoflagellates, and ciliates) were fixed with 4% acid Lugol
196 solution (Parsons et al., 1984) and 5% alkaline Lugol solution (Sherr and Sherr, 1993),
197 respectively. Cell abundances were determined under an inverted microscope (100 x
198 objectives) on 50 or 100 ml settled volumes (Lund et al., 1958). At least 200 cells in each
199 sample were counted.

Metazooplankton [two size classes: 200 μm (met1) and 700 μm (met2)] were sampled using two horizontal net tows with 200 μm and 700 μm mesh net. Samples were then preserved in borate-buffered formalin (5% final concentration) for identification and counts of organisms, which were performed under a dissecting stereomicroscope (Leica).

2.3. Areal carbon stocks

The carbon biomasses of plankton were calculated as described in full details in Meddeb et al. (Submitted). Cell volumes (μm^3) of bacteria and picophytoplankton were converted to carbon content (pg C cell^{-1}) using specific conversion factors (Table 1). For phytoplankton and protozooplankton, biovolumes were estimated by applying standard geometric formulae to each taxon, as proposed by Hillebrand et al. (1999). Then, the carbon content was obtained using specific conversion factors or formulae (Table 1). For mesozooplankton, the length and width of organisms were measured and were converted to carbon content using conversion factors or formulae corresponding to each taxonomic group (Table 1). The carbon biomasses of each plankton community (bac, pic, nan, mic, pro, met1, and met2) were estimated by multiplying their cell carbon by their corresponding abundances.

The concentration of dissolved organic carbon (doc) was obtained considering that 1 μM of doc is equal to 12 mg C m^{-3} (Grami et al., 2008). The detrital organic carbon (det) was estimated as total POC, determined from water column, minus the total carbon biomass of all plankton communities.

The final areal carbon stocks (mg C m^{-2}) were calculated by vertically integrating the carbon concentration (mg C m^{-3}) calculated from the sampling depths.

2.4. Areal carbon flows

2.4.1. Production of bacteria and phytoplankton and their grazing by protozooplankton

Dilution experiments were carried out at each site to estimate growth rates (k , d^{-1}) of bacteria and size-fractionated phytoplankton (pic, nan, and mic), as well as their grazing rates (g , d^{-1})

by protozooplankton (Landry and Hassett, 1982; Sakka Hlaili et al., 2007). Heterotrophic nanoflagellates (*Leucocryptos* sp. and *Meringospheara* spp.) and small aloricate ciliates (*Strombidium* spp.) were considered as main grazers of bacteria and pico/nanophytoplankton, while heterotrophic dinoflagellates (species of *Gyrodinium*, *Gymnodinium*, *Ceratium*, *Alexandrium*, and *Protoperidinium*) and tintinnids (*Tintinnopsis corniger*, *Helicostemella kiliensis*, and *Parafavella gigantea*) were assigned as major grazers of microphytoplankton. Details for experimental procedure and calculation are fully provided in Meddeb et al. (submitted). In brief, collected water at each sampling station was filtered on a 200 µm mesh net to remove meso- and macrozooplankton. Very small metazoa (<200 µm, such as copepods nauplii) were extremely rare in the samples and were therefore not considered in the dilution experiment. The 200 µm filtered water was mixed with free-particle sea water to give four dilution factors (100 %, 75 %, 50 % and 25 % of <200 µm prefiltered water). Each dilution mixture was then distributed into three clean 2-l polycarbonate bottles, which were incubated at *in situ* temperature (19–22°C) at the sampling depths for 24 h. 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, and *t* (d) is the incubation time (1 d).

Values of *P* and *G* from the four sampling depths were integrated to determine the areal rates (mg C m⁻² d⁻¹) of production and consumption.

2.4.2. Grazing of phytoplankton by metazooplankton

Grazing impact of mesoplanktonic metazoa was assessed by the gut fluorescence method (Mackas and Bohrer, 1976; Slaughter et al., 2006). Since mesozooplankton are known to have inefficient feeding on < 5 µm prey (Berggreen et al., 1988; Morales et al., 1993), we have assumed that they can only consume the nano- and microphytoplankton in our study. The description of the experiment procedure and calculations have been detailed in Meddeb et al. (submitted). In brief, vertical sampling of mesozooplankton was undertaken from bottom to surface in each site using 200 µm and 700 µm mesh net. This allowed the calculation of phytoplankton grazing by two size fractions of metazoa (200-700 µm and >700 µm). Subsamples (0.5 or 1 l) were filtered onto 47 mm GF/F filters, homogenized in 90% acetone using a motorized tissue homogenizer, 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).

2.4.3. Sinking flux

271 Sediment traps were used to collect organic particles that settle down along the water column.
 272 Two sediment traps (63 cm high, 6 cm internal diameter) were moored at each site. To avoid
 273 re-suspended material bias as possible, the traps were deployed at two or three meters from
 274 the bottom depending on the station's depth (Lagoon: 7-8 m; Channel: 6-7 m; Bay: 14-16 m).
 275 Prior to their deployment, traps were filled with the dense seawater (0.2 μm filtered seawater
 276 + NaCl at final concentration of 5 g l^{-1}) to create a density gradient in order to prevent the
 277 collection of surface particles. After 24 h of mooring, the traps were returned to the laboratory
 278 and stored at 5°C for a night to let the particles settle. For each station, bottom fractions of the
 279 two moored traps were mixed together. Subsamples were taken from the trapped material to
 280 estimate POC as detailed above (section 2.2). The sinking flux ($\text{mg C m}^{-2} \text{ d}^{-1}$) of POC was
 281 estimated as the carbon stock collected in the trap (mg C) divided by the area of the trap
 282 ($6.3 \times 10^{-3} \text{ m}^2$) and the duration of deployment (1 day). Other subsamples from the trapped
 283 material were fixed with acid Lugol solution (final concentration of 4%) for nano- and
 284 microphytoplankton cell identification and abundance estimation. Cell abundances were then
 285 converted to carbon biomass (mg C m^{-3}), as explained above (Table 1), which was multiplied
 286 by the volume of trapped material and divide by 2 to get the algal carbon stock collected in
 287 one trap (mg C). This was then divided by the area of the trap and the duration of deployment
 288 to get sinking flux ($\text{mg C m}^{-2} \text{ d}^{-1}$) for each phytoplankton fraction (i.e. nano- and
 289 microphytoplankton).
 290 Additional subsamples (15–30 ml settled volumes) were preserved in buffered formaldehyde
 291 for faecal pellet enumeration and volume estimation ($\text{mm}^3 \text{ L}^{-1}$) using an inverted microscope
 292 (10 x objectives) following the Utermöhl method (1958). The volume sinking flux ($\text{mm}^3 \text{ m}^{-2}$
 293 d^{-1}) were then calculated for each category of pellet (cylindrical/conical; ovoid/rounded) as
 294 described by Grami et al. (2008). Then the volume vertical flux was converted to carbon

vertical flux ($\text{mg C m}^{-2} \text{ d}^{-1}$) using a factor of $0.057 \text{ mg C mm}^{-3}$ for cylindrical/conical pellets and $0.042 \text{ mg C mm}^{-3}$ for ovoid/ rounded ones (Grami et al., 2008).

2.5. Model development

Field data were used to construct pelagic food-web models that quantitatively illustrate carbon pathways in the three study sites. Since the unknown flows outnumbered the known flows, 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 three pelagic food webs. The approach is based on four steps: (1) building an *a priori* model including all possible flows between the considered compartments or between the compartments and the outside, (2) setting mass-balance between flows, for each compartment, as equalities, (3) 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 picked from the literature, to reduce the range of possible values for each unknown flow, and (4) calculating the possible solutions for unknown flows.

2.5.1. Compartments

The number of considered compartments and the possible flows/links between them and the outside were considered as the same for the three networks (Channel, Lagoon, and the Bay). Each model contained seven living compartments: heterotrophic bacteria (bac), picophytoplankton $<2 \text{ }\mu\text{m}$ (pic), nanophytoplankton $2\text{-}10 \text{ }\mu\text{m}$ (nan), microphytoplankton $20\text{-}200 \text{ }\mu\text{m}$ (mic), protozooplankton $<200 \text{ }\mu\text{m}$ (including heterotrophic nanoflagellates, dinoflagellates, and ciliates), metazooplankton $200\text{-}700 \text{ }\mu\text{m}$ (met1), and metazooplankton $>700 \text{ }\mu\text{m}$ (met2). The non-living compartments were the dissolved organic carbon (doc) and detrital organic carbon (det).

2.5.2. A priori model

The gross primary production of the three size fractions of phytoplankton (pic, nan, and mic) are the only sources of particulate organic carbon input to the networks (Fig. 2). Carbon output from the network was driven by respiration of all living compartment and sinking of all compartments except protozooplankton, picophytoplankton, and heterotrophic bacteria. Detritus dissolution and doc production by all living compartments contribute to doc input. Detritus and doc can be lost from the system by advection. Bacteria, were the only compartment that uses doc, while protozooplankton the only compartment that graze on them. Production of detritus is assigned to phytoplankton (nan and mic) and protozooplankton through their mortality and metazooplankton *via* their faecal pellets production. The three size fractions of phytoplankton were grazed by protozooplankton, while only nano- and microphytoplankton fractions can be grazed by metazoa. Metazooplankton 200-700 μm (met1) consume protozooplankton and detritus, while metazoa $>700 \mu\text{m}$ (met2) consume protozooplankton, small metazoa (met1) and detritus. The *a priori* model is presented in Fig. 2.

2.5.3. The set of equalities (mass balances)

Setting equations (equalities) is an essential step to impose the mass balances of the network. The mass balance equations for all compartments are given in Table 2. The mass balance equation for each compartment states that the sum of the flows entering any compartment is equal to the sum of the outflows, meaning that we neglected daily variations of biomass compared to daily flow values.

2.5.4. Biological constraints (inequalities)

Imposing ecological limits (maximum and/or minimum) for each unknown flow helps to reduce the range of possible solutions. Two ranges of inequalities were adopted. Firstly, estimated flows derived from field experiments were used as mean values for each model. These mean values did not allow the model to estimate flows with certainty. For this reason,

we proposed to define the minimal and a maximal value for each flow by the calculation of a confidence interval around the field data, i.e. using the minimum and maximum of the average value of each flow (Table 3). The production rates ($\text{mg C m}^{-2} \text{ d}^{-1}$) of bacteria and phytoplankton size fractions (pic, nan, and mic), as well as their grazing rates ($\text{mg C m}^{-2} \text{ d}^{-1}$) by protozoa, derived by dilution technique, were integrated as minimum and maximum flows into the model. Microphytoplankton sedimentation flow was constrained by the minimal and maximal estimated values for each site. Metazooplankton gut content experiments allowed constraining (by a minimum and a maximum value) two flows for each model: grazing of nano- and micro-phytoplankton by metazoan 1 and metazoan 2. Metazoa and detritus sedimentation were respectively constrained by a minimum and a maximum value estimated by field experiment for each site. A total of 26 inequalities derived from field experiments realized for the present study were considered for the Lagoon, the Channel, and the Bay of Bizerte.

A second group of constraints was adopted from previous works to constrain the unknown flows (Vézina and Platt, 1988; Steinberg et al., 2000; Vézina and Pahlow, 2003), including: the minimum and maximum respiration rate of phytoplankton, zooplankton, and bacteria; the minimum and maximum doc production rate by phytoplankton and zooplankton (only the upper limit was fixed for bacteria); the lower and upper limit of zooplankton and bacteria growth efficiency; the lower and upper limit of zooplankton assimilation efficiency, detritus excretion, and detritus dissolution flow upper limit. Forty-four inequalities of this second group were applied in the Lagoon, the Channel, and the Bay model (See details in Table 4).

2.5.5. Solutions

The last step of the inverse analysis was the calculation of unknown flows. The estimates 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 $10 \text{ mg C m}^{-2} \text{ d}^{-1}$ 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 (Weinbauer, 2002; 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).

2.7. Network analysis

To characterize the food web obtained by the LIM-MCMC approach, Ecological Network Analysis (ENA) was applied to the obtained flow values. ENA derived indices are commonly used to describe emergent properties of any ecosystem (Ulanowicz, 1986). The algorithms used here were written for Matlab[®] by Carole Lebreton and Markus Schartau. A number of calculated indices were considered in this study for site comparison and characterization:

- Total system throughput (TST): the sum of all the flows circulating through all compartments and a measure of the total system activity (Kay et al., 1989).

$$TST = \sum_{i=1, j=1}^n T_{ij}$$

where, i: flow out of the system from i, i=1, ... n; j: flow in to the system to j, j=1 ... n; and T_{ij}: flow from compartment i to compartment j.

- Ascendency (A) and Development Capacity (DC):

Ascendency represents the organized part of the ecosystem. It is the product of the throughput and the Average Mutual Information (AMI: degree of specialization of flows in a network) (Ulanowicz, 2004), and merges the quantification of the system activity and the degree of specialization (Ulanowicz and Wulff, 1991; Ulanowicz, 2000).

$$A = \sum_{ij} (T_{ij}) \times \log \left(\frac{T_{ij} \times TST}{T_j \times T_i} \right)$$

389 where, T_i : Total outflow from compartment i; T_j : Total inflow for compartment j; and T_{ij} :
 390 Flow from compartment i to compartment j.

391 The development capacity (DC) is the sum of A and Overhead (O), which represents the
 392 maximum possible value of Ascendency that an ecosystem may reach.

$$DC = -TST \times \sum_{ij} \frac{T_{ij}}{TST} \times \log \left(\frac{T_{ij}}{TST} \right)$$

393 where, i: flow out of the system from i; j: flow in to the system to j; and T_{ij} : flow from
 394 compartment i to compartment j.

395 Ascendency is a more informative metric when it is expressed in relation to development
 396 capacity (relative Ascendency, A/DC). It defines the degree of ecosystem development. High
 397 A/DC ratios are thought to reflect a high degree of organization, highly specialized and less
 398 redundant pathways (Baird et al., 1991; Baird et al., 1998).

$$AC = \frac{A}{DC}$$

399 - Average path length (APL): is the average number of compartments crossed by a unit of
 400 carbon from entering to exiting the ecosystem. It represents a measure of the ecosystem
 401 retention capacity (Kay et al., 1989).

$$APL = \frac{\sum d_{i \leftrightarrow j}}{\frac{N(N-1)}{2}}$$

402 where, i: flow out of the system from i; j: flow in to the system to j; and N is the number of
 403 vertices in graph.

404 - Finn Cycling Index (FCI): is the ratio of carbon flowing in loops to the sum of all carbon
 405 flows. It quantifies the fraction of all flows involved in recycling (Finn, 1976) and can be
 406 considered as a measure of the retentiveness of the system.

$$FCI = \frac{TST_c}{TST}$$

where, TSTc is the total flow that is recycled.

2.8. Cliff's δ test for comparing network indices between sites

As suggested by Tecchio et al. (2016), we use the Cliff δ method for statistically testing the ENA index differences between the models. This method is necessary when we have large sample sizes (here 300000 values for each flow), which precludes us from using other tests like ANOVA or *t*-tests. For each ENA index, three pairwise comparisons were performed. Then, the following values were used to define small, medium, and large effects (i.e. $|\delta|$ <0.147 "negligible"; $|\delta|$ 0.147-0.33 "small"; $|\delta|$ 0.33-0.474 "medium"; >0.474 "large" according to Tecchio et al. (2016).

3. Results

3.1. Input, output and throughput

Calculated flows are given in Table 5 and Figure 3. At each site, the food web was only powered by the phytoplankton gross primary production. The phytoplankton production reached 1234 mg C m⁻² d⁻¹ in the Lagoon and 966 mg C m⁻² d⁻¹ in the Channel, but only 727 mg C m⁻² d⁻¹ in the Bay. For the three networks, microphytoplankton (mic) exhibited the highest contribution to total carbon input (70–83%). Picophytoplankton contribution fluctuated between 8–16%, while nanophytoplankton production varied between 8% and 13% of total carbon input.

Carbon exited the food webs (outputs) either through respiration (res) or dissolved organic carbon (doc) loss or downward sedimentation flows of detritus (det), phytoplankton (nan and mic), and zooplankton (met1 and met2). Carbon loss by respiration reached 14–16% of total carbon circulating for the three sites. It represents the main factor of carbon output at the site level, i.e. in the Lagoon (39%), the Channel (46%), and the Bay (45%). The highest respiration flows within a compartment were those from the protozooplankton and metazooplankton (19–20% and 13–19% of total carbon outputs, respectively, Fig. 4A).

Carbon sinking contributed 31% of total carbon loss in the Lagoon, 29% in the Channel, and 25% in the Bay. Microphytoplankton and metazooplankton $>700\ \mu\text{m}$ (met2) were the main living compartment contributing to sinking flows (Fig. 4B).

Throughput was defined as the sum of carbon flows incoming or leaving a compartment. Microphytoplankton followed by protozooplankton and metazooplankton showed the highest values of carbon throughput ($609\text{--}868\ \text{mg C m}^{-2}\ \text{d}^{-1}$) among the living compartments of the three networks (Fig. 5). Detritus also presented relatively high throughput of carbon ($230\text{--}531\ \text{mg C m}^{-2}\ \text{d}^{-1}$).

3.2. Production of dissolved organic carbon and detritus

The doc production at each site amounted to 334, 274, and 208 $\text{mg C m}^{-2}\ \text{d}^{-1}$ in the Lagoon, the Channel, and the Bay, respectively (Fig. 6A). The metazooplankton (met1 and met2) were the main compartment that contributed (36–40%) to doc in the Lagoon ($130\ \text{mg C m}^{-2}\ \text{d}^{-1}$) and the Bay ($76\ \text{mg C m}^{-2}\ \text{d}^{-1}$) (Table 5, Fig. 6A). In the Lagoon, the phytoplankton exudation ($115\ \text{mg C m}^{-2}\ \text{d}^{-1}$, i.e. 34% of doc production) was higher than the protozooplankton excretion ($89\ \text{mg C m}^{-2}\ \text{d}^{-1}$, i.e. 26% of doc production), whereas both flows were similar in the Bay (65.9 and $66.4\ \text{mg C m}^{-2}\ \text{d}^{-1}$). For the Channel, the three algal compartments (mic, nan, and picophytoplankton) contributed almost equally (32, 34, and 35%) to doc (84 , 94 , and $96\ \text{mg C m}^{-2}\ \text{d}^{-1}$). It is important to highlight here that the microphytoplankton contributed 66–81% to total algal exudation.

Detritus production by all living compartments was high in the Lagoon ($531\ \text{mg C m}^{-2}\ \text{d}^{-1}$) compared to the Channel ($229\ \text{mg C m}^{-2}\ \text{d}^{-1}$) and the Bay ($285\ \text{mg C m}^{-2}\ \text{d}^{-1}$) (Fig. 6B). Half of the detritus production derived from microphytoplankton in the Lagoon and the Bay. In these sites, protozooplankton (16 and 22%) and metazooplankton (i.e.; met1 and met2, 23 and 25% respectively) presented almost similar contributions to detritus production. In the Channel, most of the detritus production resulted from protozooplankton (41%) and

metazooplankton (40%) activities whereas phytoplankton had a lower contribution (19%) (Fig. 6B).

3.3. Zooplankton diets

The protozooplankton diet was mainly based on herbivory, as microalgae constituted the main prey for these protozoa in the three sites (60–77% of the protozooplankton diet, Fig. 7A). Nanophytoplankton formed only 3–13% of total carbon consumed by protozooplankton in all sites, while picophytoplankton contributed more to the consumption of protozooplankton (11–23%). Protozooplankton showed very weak bacterivory, since bacteria formed only 10% of their diet in the Channel and not more than 5% in the Lagoon and the Bay.

In the Lagoon and the Bay, the metazooplankton 200–700 μm (met1) were mainly detritivorous, since detritus represented 52–58% of their diet (Fig. 7B). At both sites, protozooplankton contributed 32–39% of the met1 consumption while phytoplankton guaranteed only 5%. In the Channel, the smaller metazooplankton was carnivorous, with a diet that relied mainly on protozooplankton (54%) (Fig. 7B). Detritus and phytoplankton formed 31 and 10% of met1 diet, respectively, in this site.

At the three stations, the metazooplankton >700 μm (met2) was mainly carnivorous, since 48–62% of their diet were composed of protozoa and metazoa 200–700 μm (Fig. 7C). The remaining source of carbon came principally from detritus in the Lagoon (42%) and the Bay (38%), where herbivory did not represent more than 10%. In the Channel, phytoplankton and detritus contributed almost equally (21 and 26%, respectively) to the carbon demand of met2 (Fig. 7C).

3.4. Ecosystem indicators and Ecological Network Analysis indices.

Flow estimates obtained from the LIM-MCMC were used to calculate ecological networks indices (Fig. 8). This showed that the total system throughput (TST) was higher in the Lagoon (4798 $\text{mg C m}^{-2} \text{ d}^{-1}$) compared to the Channel (3818 $\text{mg C m}^{-2} \text{ d}^{-1}$) and the Bay (2934 mg C

$\text{m}^{-2} \text{d}^{-1}$), due to higher primary production by phytoplankton. Also the development capacity (DC) in the Lagoon ($14195 \text{ mg C m}^{-2} \text{d}^{-1}$) significantly exceeded those of the Channel ($11106 \text{ mg C m}^{-2} \text{d}^{-1}$) and the Bay ($8489 \text{ mg C m}^{-2} \text{d}^{-1}$) (Table 6, Fig. 8). Ascendency showed a similar pattern as DC, with higher values for the Lagoon ($7686 \text{ mg C m}^{-2} \text{d}^{-1}$) and the Channel ($6054 \text{ mg C m}^{-2} \text{d}^{-1}$) compared to the Bay ($4839 \text{ mg C m}^{-2} \text{d}^{-1}$). Conversely, a high relative ascendency was founded in the Bay ($A/DC = 57\%$, Fig. 8) in comparison to the Lagoon and the Channel ($A/DC = 55\%$).

The average path length (APL) differed significantly between the Lagoon (2.95) and the Channel (2.88) (Table 6). While the Bay had the highest APL value (3.03), suggesting that a unit of carbon crossed more compartments during its path through the trophic system (from input e.g. gross primary production, to output: e.g. respiration) compared to the Lagoon and the Channel. The Finn cycling index (FCI) showed slightly higher and significant cycling activity in the Channel (14%) compared to the Bay (13%) or the Lagoon (12%) (Table 6, Fig. 8).

4. Discussion

4.1. Plankton food webs of the study sites

Mediterranean coastal waters are currently under several anthropogenic pressures, including eutrophication and pollution, but few studies have addressed the impact of these stressors on their functioning and food web structure. Here, we have presented new results concerning the food web and its associated properties in three coastal waters habitats in the SW Mediterranean Sea. Although these sites are closely located, they are different in eutrophic status, contamination levels, hydrology, and geomorphology (Bejaoui et al., 2008; Boufahja et al., 2012; Louati et al., 2013; Barhoumi et al., 2014a, b). These factors may impact plankton diversity and dynamics as well as productivity (Smayda and Reynold, 2001; Sakka Hlaili et

al., 2006; Leboulanger et al., 2011; Lafabrie et al., 2013). Therefore, variability in the structure and functioning of the plankton food web among sites may be suspected. Indeed, the LIM-MCMC approach revealed that the three ecosystems differed in their productivity which could be reflected in their planktonic system functions.

The photosynthetic carbon input was sufficient to support the functioning of the three food-web models. As expected, the inshore waters with substantial nutrients loading (Sakka Hlaili et al., 2008; Ben Garali et al., 2010; Sahraoui et al., 2012) exhibited high production rate ($1234 - 966 \text{ mg C m}^{-2} \text{ d}^{-1}$). Similar production rates were previously found for the Lagoon of Bizerte ($1872 \text{ mg C m}^{-2} \text{ d}^{-1}$, Grami et al., 2008) and the Bay of Biscay (French Atlantic Gulf; $1145 - 1666 \text{ mg C m}^{-2} \text{ d}^{-1}$, Marquis et al., 2007). These values are however low compared to other values recorded in temperate ecosystems, like the shelf of the Gulf of Cadiz (Spanish South Atlantic Gulf; $4500 \text{ mg C m}^{-2} \text{ d}^{-1}$; Huertas et al., 2005). The production rate in the Bay ($727 \text{ mg C m}^{-2} \text{ d}^{-1}$) was similar to those of Arcachon Bay (French Atlantic Coast; $433 \text{ mg C m}^{-2} \text{ d}^{-1}$, Tortajada et al., 2012) and the Bay of Biscay during late spring (French Atlantic Gulf; $631 \text{ mg C m}^{-2} \text{ d}^{-1}$, Marquis et al., 2007). For all study sites, phytoplankton production ($727 - 1234 \text{ mg C m}^{-2} \text{ d}^{-1}$) was higher than the bacterial production ($34.5 - 116.7 \text{ mg C m}^{-2} \text{ d}^{-1}$). The bacterial to primary production ratio exhibited very low values in the Lagoon (0.03) and the Bay (0.06), which was close to those found in other coastal systems such as the Bay of Biscay (0.03–0.04, Marquis et al., 2007), the northern Spanish coast (0.04; Teira et al., 2003), and the Arcachon Bay (0.02, Tortajada et al., 2012). In the Channel, this ratio amounted to 0.12 but it was still lower than the value reported at a global scale (0.3; Cole et al., 1988).

In these three locations, the primary production was mainly sustained by microphytoplankton (70–80%). Diatoms and autotrophic dinoflagellates were the main microphytoplankton taxa at all stations and dominated the algal biomasses (Sakka Hlaili et al., 2007; Meddeb et al.,

submitted). Since microphytoplankton known to be selected by herbivorous zooplankton, the dominance of the herbivorous pathway from the typology of Legendre and Rassoulzadegan (1995, 1996; redefined in Sakka Hlaili et al. 2014) is suspected at the three sites. Nevertheless, a previous study has reported the dominance of the microbial food web in the Bizerte Lagoon during summer, although diatoms were prevailing during this season (Grami et al., 2008). Similarly, the microbial food web was shown to act in other coastal regions, where diatoms were abundant (Umani and Beran, 2003; Berglund et al., 2005).

At each site, carbon left the pelagic ecosystem firstly by respiration, then by sinking vertically and finally through doc losses, each loss represented by 39–46%, 25–31% and 24–30% of total outputs, respectively. Respiration was mainly driven by metazooplankton and protozooplankton activities (19–20% and 13–19% of total carbon outputs, respectively). Phytoplankton respiration had a low contribution (5–6%) to total carbon losses. This was comparable to a previous study in the Bizerte Lagoon (~5%, Grami et al., 2008), but much less so than in the Bay of Biscay (57–82%; Marquis et al., 2007) and at Lake Pavin (26%, Grami et al., 2011). Bacterial respiration was very weak, contributing only to 1–3% of carbon output, which may be related to low bacterial production rates. The two size classes of metazooplankton (met1 and met2) exhibited the highest vertical sinking flux that represented 14 – 19% of total carbon output. These trophic components produced important fraction (23–40%) of total detritus.

In the Channel, the sinking of phytoplankton (particularly of microphytoplankton) represented 12% of total carbon losses. However, it did not exceed 7% in the Lagoon and the Bay despite 50% of total detritus production made up by microphytoplankton at these sites. This change may be caused by the high detritivory activity of metazooplankton at these two sites (Fig. 6). In addition, detritus sinking accounted for only 3.5–5.5% of total carbon output, which highlighted the important occurrence of detritivorous metazooplankton.

556 When considering the carbon throughput (i.e. activity of each compartment) in the three sites,
557 the most active compartments were microphytoplankton, followed by protozooplankton and
558 metazooplankton, suggesting their important role, as producers and grazers. In contrast, the
559 bacteria exhibited the lowest Throughput value (Fig. 5) which was consistent of their low
560 production and respiration. The bacterial uptake of doc was also low (Table 5) corresponding
561 to 8–30% of doc, which resulted from plankton exudation/excretion and from detritus
562 dissolution. In parallel to the low activity of bacteria, a low fraction of bacterial production
563 was grazed by protozooplankton at the three sites (29–49% of their total grazing). These
564 values were in the same range of those reported in previous study for Bizerte Lagoon during
565 summer (25–50%, Grami et al., 2008), Arcachon Bay (50%, Tortajada et al., 2012) and the
566 Lake Pavin (48%, Grami et al., 2011). During our study, small protozooplankton, including
567 aloricate ciliates (*Strombidium* spp.) and heterotrophic nanoflagellates (*Leucocryptos* spp.),
568 represented less than 5% of total protozooplankton carbon biomass (Meddeb et al.
569 (submitted). Since protozooplankton have a weak bacterivory, they would satisfy their carbon
570 demand by consuming large fraction (>80%) of picphytoplankton production. During the
571 sampling season (i.e. spring), the protozooplankton were mostly composed by >50 µm
572 heterotrophic dinoflagellates (i.e. *Protoperidinium* spp.) and tintinnid ciliates (i.e. *Paravavella*
573 *gigantea* and *Tintinnopsis corniger*) (70–95% of total biomass, Meddeb et al., submitted).
574 This means that their herbivory was suspected to be high. At the same time, these large
575 protozooplankton could be potential prey for the metazooplankton and hence can contribute to
576 channel carbon in each site. To elucidate the real role of each grazer's group in the studied
577 pelagic ecosystems, the composition of each diet of the proto- and metazooplankton were
578 analysed. This may also permit a characterization of the dominant pathways of biogenic
579 carbon.

580 As suspected in our hypothesis, during spring the diet of protozooplankton relied mainly on
581 phytoplankton (90–98%), with large contribution of microphytoplankton (60–77%). The
582 remaining carbon demand of protozooplankton was supported by bacteria (5–10%). A similar
583 result was previously reported in the Bizerte Lagoon during the summer season (with 62% of
584 autotrophs and 17% of bacteria; Grami et al., 2008), in the spring season at Arcachon Bay
585 (with 84% of autotrophs and 16% of bacteria; Tortajada et al., 2012), and again in spring at
586 the Bay of Biscay (with 88% of autotrophs and 12% of bacteria; Marquis et al., 2007). In
587 contrast, in other regions, such as Lake Biwa, the main carbon input to protozooplankton was
588 detritus (45%) and bacteria (52%) (Niquil et al., 2006). In the Bay of Biscay during spring,
589 the major food source was picophytoplankton, which contributed 64–83% of total carbon
590 input; while in the late spring the protozooplankton diet was composed of bacteria and
591 phytoplankton in roughly equal shares (Marquis et al., 2007).

592 Small metazooplankton (200-700 μm) seemed to be mainly detritivorous in the Lagoon and
593 the Bay of Bizerte during spring (detritus formed 52–58% of their diets), whereas in the
594 Channel, protozooplankton constituted important fraction of their ingested carbon (54%).
595 Larger mesozooplankton ($>700 \mu\text{m}$) were mostly carnivorous, as protozooplankton and small
596 metazooplankton (200-700 μm) formed half of their diets at all sites, but detritus represented a
597 non-negligible carbon source for the met2 (21–42%). In all sites, diets for both size classes of
598 metazooplankton relied less on phytoplankton (10, 21, and 5% for Lagoon, Channel and Bay,
599 respectively). When considering all zooplankton, the detritivory:herbivory (D:H) ratio varied
600 from 0.25 to 0.77 for the three sites during spring. These values were very similar to those
601 already reported for the Bizerte Lagoon in spring (0.23–0.74, Grami et al., 2008). Then,
602 detritus do not have a principal role in channelling carbon through the three planktonic food
603 webs. The planktonic food webs rely mostly on the herbivory of zooplankton. To stress the
604 importance of protozooplankton compared to metazooplankton as autotrophic carbon

consumer, two specific ratios were adapted from Sakka Hlaili et al. (2014): (1) the ratio of the consumption rate of phytoplankton by protozooplankton to the total consumption rate by protozooplankton (R1 ratio) and (2) the ratio of consumption rate of phytoplankton by protozooplankton to the consumption rate of phytoplankton by proto- and metazooplankton (R8 ratio). In our case, the R1 ratio varied from 0.83 to 0.94, indicating that protozooplankton grazed on more phytoplankton than other prey. In parallel, primary producers were consumed more-so by protozooplankton than metazooplankton, since our R8 ratio varied from 0.82 to 0.99. According to both ratios, the protozooplankton mainly assured the herbivory in the three pelagic ecosystems. Moreover, the protozooplankton removed 40 –54% of nano- and microphytoplankton production per day compared to the 3–14% grazed by metazooplankton. These mesozooplankton seemed to have a small role in herbivory, but instead they can impose important predatory pressure on protozooplankton and then participate in channelling biogenic carbon.

To identify the dominant trophic pathway in the three planktonic ecosystems, a specific ratio was proposed by Sakka Hlaili et al. (2014): 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 ($R7 \geq 0.6$). In the Lagoon and the Channel, the ratio values (0.13 and 0.11, respectively) are very close to the lower limit of R7 (0.1) that discriminates the multivorous structure of the herbivorous one. Therefore, food webs in both sites during spring can be considered to be approximately multivorous but tend towards the herbivorous pathway. In the Bay of Bizerte, the herbivorous pathway was more dominant, at 0.08.

4.2. Structural and functional proprieties of identified food webs

The ENA indices have proved to be very useful to characterize ecosystem functioning, to describe the activity and the retention capacity as well as the level of organization and

maturity of the food webs, and to detect any change in their environments (Proulx et al., 2005; Heymans et al., 2007). These indicators have been frequently used, especially to assess the impact of natural and anthropogenic pressures on coastal marine ecosystems (Belgrano et al., 2005; Niquil et al., 2014; Piroddi et al., 2015; Chaalali et al., 2016). They also provide useful information on the degree of stress and stability of food webs (Grami et al., 2008; Heymans et al., 2014; Saint-Béat et al., 2015). Besides, ENA indices were potentially very valuable in inter-ecosystem comparisons.

Considering the total system throughput (TST), the highest values were observed for the Lagoon ($4798 \text{ mg C m}^{-2} \text{ d}^{-1}$) and the Channel ($3818 \text{ mg C m}^{-2} \text{ d}^{-1}$) (large and medium difference) compared to the Bay ($2934 \text{ mg C m}^{-2} \text{ d}^{-1}$) (Table 6; Fig. 8). This means that the Lagoon and the Channel were most active as they had high productivity, which can be related to their high degree of eutrophication. These semi-enclosed ecosystems are closer to sources of nutrient discharges compared to the Bay that has larger exchange with the Mediterranean Sea (Fig. 1). Previously, Grami et al. (2008) have also shown high TST within the Lagoon of Bizerte, which was directly related to the proximity of the study station to sources of nutrient discharge (urban and shellfish farming input). Our results are in line with the estimates obtained for other eutrophic ecosystems. For example, some South African ecosystems are very active with high TST values (Niquil et al., 2012) reflecting high production, which rely on the strong activity of the benthic compartments (macrophytes as main primary producers in salt marshes) (Scharler et al., 2005).

The relative ascendancy (A/DC) represents the total activity that has been converted into organized complexity. It equalled 57% in the Bay and 55% in the Lagoon and the Channel of Bizerte (Fig. 8), suggesting that the Bay is more organized than the two other ecosystems. Some ecosystems, such as Kromme Estuary (South Africa), exhibited low A/DC (35%, Scharler et al., 2005) because of severe limitations in freshwater inflow that caused a decline

of the internal organization and maturity (Baird and Heymans, 1996). On the Italian coast, higher relative ascendancy values appeared to be related to the impact of human and industrial activities focused in the southern part of the Apulian peninsula (Vassallo et al., 2006).

According to Monaco and Ulanowicz (1997), recycling is considered to be an important indicator of an ecosystem's ability to maintain its structure and integrity through positive feedbacks, and has been used as an indicator of stress (Ulanowicz, 1986) and ecosystems maturity (Odum, 1969; Christensen, 1995; Vasconcellos et al., 1997). Cycling reduces the impact of a perturbation on an ecosystem by acting as a buffer and it seems to increase the resistance of the system (Saint-Béat et al., 2015). Therefore, the Finn's cycling index (FCI, Finn, 1976) has been shown to be correlated with ecosystem maturity, resilience, and stability and high FCI is a feature of mature ecosystem (Christensen et al., 2005; Duan et al., 2009, Niquil et al., 2012). In our study, the FCI was generally quite low (12–14%), suggesting that the study sites are disturbed ecosystems and that they are not mature. This may be due to anthropogenic stresses, such as eutrophication and chemical contamination. Patrício et al. (2004) has shown that FCI decreased from 20.24% to 19.46% between moderate and strongly eutrophic waters. Similarly, Duan et al. (2009) have reported a drastic decrease of the FCI for the Pearl River Estuary between 1981 (9.21%) and 1998 (2.72%) due to a continuing pollution since 1980. Piroddi et al. (2015) showed that the Amvrakikos Gulf (Western Greece) was an immature and perturbed ecosystem (FCI = 16%), typical of “closed” ecosystems (e.g., like estuaries, lagoons, and bays) where bottom-up prevail over top-down processes, and where possibly high levels of community stress are induced by anthropogenic and environmental forces. In a previous study, conducted in the Bizerte Lagoon in 2004 (Grami et al., 2008), the FCI was found to be higher (21%) than our estimate (12%). During last decade, the Lagoon has undergone increasing anthropogenic pressure, as several new industrial factories are located on its shores and the number of shellfish farms has tripled. This

has obviously contributed to an increase of eutrophic and polluted status of the Lagoon, which was confirmed by a decrease of the FCI from 2004 to 2012. Unfortunately, no previous data existed for the Channel and the Bay, so our study is a first report modelling the perturbed status of these waters.

To compare the degree of perturbation among the study sites, the average path length (APL) was used. This descriptor is expected to be longer in systems with high degree of flow diversity and cycling (Christensen, 1995; Thomas and Christian, 2001). Increasing the number of links increases the robustness of interactions of an ecosystem (Lafferty et al., 2008), and its overall organization (Lafferty et al., 2006). The APL reached higher value in the Bay (3.03 components per average food cycle) than the two other sites (2.88 –2.95). So, compared to the Bay, the Lagoon and the Channel were more stressed. As mentioned, both sites have undergone higher chemical pollution and nutrient loading than the Bay (Bejaoui et al., 2010; Sahraoui et al., 2012; Barhoumi et al., 2014a, b; Pringault et al., 2016)

5. Conclusion

Modelling can be used to investigate, analyse, and compare the functioning of ecosystems subjected to various anthropogenic stresses at different periods. This integrated approach provides many indications on ecosystem health and stability. Several modelling technique exists and are already used for management objectives (e.g: Ecopath, Osmose, Atlantis). More specifically, the LIM-MCMC approach has important complementary features, since it takes into account microbial processes and which can provide confidence intervals for statistical comparisons. Our study has highlighted the status and the functioning of three interconnected sites during the spring of 2012 (Lagoon, Channel, and Bay of Bizerte), subjected to various anthropogenic pressures. The main results showed that (i) carbon input came from autotrophic primary production, by microphytoplankton, (ii) herbivorous and almost multivorous food-webs were the dominant carbon pathways in the study sites during spring, and (iii) the

herbivorous activity was mainly driven by the protozooplankton, which played a major role in the transfer of biogenic carbon to higher trophic levels.

The different indices used in our study showed that the Lagoon and the Channel seemed to be more stressed, more active, and less organized compared to the Bay of Bizerte. This directly reflects the difference in the degrees of anthropogenic pressure (as eutrophication and chemical pollution) among these ecosystems. Finally, this study supports that inverse modelling combined with ecological network analysis may offer an effective tool for management and assessment of ecosystems health, and to diagnose the occurrence of anthropogenic pressures.

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