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A new type of plankton food web functioning in coastal waters revealed by coupling 1 Monte Carlo Markov Chain Linear Inverse method and Ecological Network Analysis 2 3 4 Marouan Meddeb^{a,b*}, Nathalie Niquil^c, Boutheïna Grami^{a,d}, Kaouther Mejri^{a,b}, Matilda 5 Haraldsson^c, Aurélie Chaalali^{c,e,f}, Olivier Pringault^g, Asma Sakka Hlaili^{a,b} 6 7 ^aUniversité de Carthage, Faculté des Sciences de Bizerte, Laboratoire de phytoplanctonologie 8 9 7021 Zarzouna, Bizerte, Tunisie. 10 ^bUniversité de Tunis El Manar, Faculté des Sciences de Tunis, LR18ES41 Sciences de 11 12 l'Environnement, Biologie et Physiologie des Organismes Aquatiques, Tunis, Tunisie. 13 ^cCNRS, Normandie Université, UNICAEN, UMR BOREA (MNHN, CNRS-7208, Sorbonne 14 15 Universités, Université Caen Normandie, IRD-207, Université des Antilles), CS 14032, Caen, France. 16 17 ^dUniversité de Monastir, Institut Supérieur de Biotechnologie de Monastir, LR-14ES06 18 Bioressources: Biologie Intégrative & Valorisation « BIOLIVAL »., Avenue Taher Hadad, 19 BP 74, 5000, Monastir, Tunisie. 20 ^eIFREMER, Ecology and models applied to fishery resources, Nantes, France. 21 ¹ESE, Ecology and Ecosystem Health, Agro campus Ouest, INRA, 35042 Rennes, France. 22 23 ^gInstitut de Recherche pour le Développement (IRD), MARBEC Marine Biodiversity, Exploitation & Conservation, BP 434 1004 Tunis, Tunisie, UMR IRD (n° 248), Ifremer, 24 Univ. Montpellier, CNRS (n° 9190). 25 26

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30 Abstract

Plankton food webs (PFW) typology is based on different categories of functioning, according 31 to the dominant processes and the role played by heterotrophic bacteria, small vs large 32 33 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 34 35 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 36 37 conventional trophic continuum as previously reported. The "bacterial multivorous food web" present in winter showed the lowest primary production among seasons, but highest bacterial 38 39 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 40 primary production ratio is higher than values reported in global scale (\cong 1); bacterial net 41 production to the potential protozoan prey net production was high (>0.2). In this special food 42 43 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 44 the most organized, specialized, stable and mature, as related to common interpretations of 45 ENA. The spring was characterized by herbivorous food web, with highest activity coinciding 46 with low stability. Although less usual, the herbivorous pathway was also observed during 47 48 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 49 usefulness of food web models derived ratios combined with ecological network analysis 50 indices to conduct evaluation of the structure and functioning of ecosystems and potentially to 51 support management decisions in marine environment. 52

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Keywords: food web modeling, network analysis, trophic structure, coastal waters, seasonal
variations, ecology, bacterial multivorous food web.

57 **1. Introduction**

Marine biological productivity depends on the magnitude of phytoplankton production 58 and its trophic transfer through food webs. The structure and the functioning of the food web 59 can in turn influence the energy flow and the carbon cycling in marine ecosystems (Vargas et 60 61 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 62 and hydrological conditions) can affect the abundance, the size structure and the composition 63 of plankton communities as well as the tropho-dynamics within the plankton system (Sankar 64 and Padmavathi, 2012; Vajravelu et al., 2017; Rasconi et al., 2017). For temperate regions, 65 there exist a conventional scheme describing the food web dynamics over seasons (Legendre 66 and Rassoulzadegan, 1995). The increasing light during spring and the stratification of the 67 water column, which has already been enriched by nutrients during winter mixing, lead to 68 69 phytoplankton blooms dominated by micro-sized algae (i.e. diatoms >10 µm. This high 70 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 71 summer, the persistent water column stability causes a depletion of nutrients in the surface 72 waters, causing a decrease in production and a shift in the primary producers, dominated by 73 74 pico- and nano-sized algae and bacteria. This production is consumed by microbivorous zooplankton (i.e. heterotrophic nanoflagellates and microzooplankton) and transferred to 75 higher consumers through the microbial food web (Calbet and Landry, 2004). In autumn, 76 77 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 78 situation, both the microbial pathways and grazing on larger phytoplankton are important, 79 80 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 81 82 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 83 84 continuum of food web dynamics by Legendre and Rassoulzadegan (1995). They identified the "phyto-microbial food web", where microzooplankton feeding on phytoplankton is more 85 86 important than grazing on bacteria, and the "poly-microbial food web", where microzooplankton consume more or less equally the various types of food. 87

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

primary production (59–150 g C m⁻² y⁻¹, Siokou-Frangou et al., 2010). A large part of fixed 90 carbon is channeled to higher tropic levels through microbial organisms, as small-size 91 autotrophs and heterotrophs are dominant in most seasons (Siokou-Frangou et al., 2002; 92 Zervoudaki et al., 2011; Giannakourou et al., 2014). On the contrary, the coastal waters are 93 more productive environments (200–400 g C m⁻² y⁻¹, Nixon, 1995), mainly due to the high 94 nutrient availability that promotes intense spring diatom-blooms. Here, phytoplankton 95 production is efficiently transferred to higher trophic levels through the herbivorous food web 96 (Caroppo et al., 2000; Auger et al., 2011; Alekseenko et al., 2014). 97

98 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 99 100 materials and flows of energy. The structure of the food web is intimately linked to the size 101 structure of the primary producers, which itself depends on the hydrological conditions that 102 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 103 104 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 105 106 method, evolved from the physical sciences (Vézina and Platt, 1988), has been used to solve 107 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 108 to estimate directly or entirely in field. The LIM approach has been applied to describe food 109 web interactions in various marine ecosystems, as the Gulf of Riga, the Bay of Biscay, the 110 Lagoon of Takapoto, the Northeast Subarctic Pacific and the Baltic Sea (Donali et al., 1999; 111 Vézina and Savenkoff, 1999; Niquil et al., 1998, 2001; Marquis et al., 2007). In the 112 Mediterranean Sea, different modeling analyses have been used to examine the functioning 113 and the structure of food webs and related driving factors. Ecophath with Ecosim (EWE) 114 approach (Coll and Libralato, 2012) is the most used approach in Mediterranean regions, 115 including the Gulf of Gabès (South of Tunisia, Hattab et al., 2013), the Western, Central and 116 117 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 118 119 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 120 Mediterranean waters, such as, the Lagoon of Bizerte (Grami et al., 2008) and coastal waters 121 of the Mediterranean Sea (Olsen et al., 2001). This method was modified into the Monte 122 123 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 124 125 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; 126 Chaalali et al., 2015). Nevertheless, the food web structure and the trophic-interaction 127 functioning have so far been poorly described for the southern Mediterranean regions, despite 128 the high plankton diversity and the pronounced productivity observed there (Sakka Hlaili et 129 al., 2008, Bel Hassen et al., 2008; Hafferssas and Seridji, 2010). Up to date, the food web of 130 Bizerte Lagoon has been modeled during summer (Grami et al., 2008), showing an efficient 131 132 transfer of primary production through the herbivory of microzooplankton. Also, the food 133 webs of the Bay, Channel and Lagoon of Bizerte has been modelled during spring (Meddeb et 134 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 135 136 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 137 138 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 139 in stress levels and stability of the food webs, two attributes of primary importance in the 140 objective of implementing an ecosystem based management of this environment. 141

142 **2. Materials and Methods**

143 **2.1. Study areas**

The Bay of Bizerte is an important fishing region located in the south-western 144 Mediterranean basin (Fig. 1), with depth varying from 16 to 20 m. The Bay is an open 145 embayment facing the Western Mediterranean basin, which is largely influenced by surface 146 Modified Atlantic Water (MAW) (Millot, 1987, 1999). Throughout the year, this water 147 crosses the Straits of Gibraltar and runs along the North African coasts and reaches the 148 northern Tunisian coasts before branching into two veins, one towards the eastern basin and 149 one towards the Tyrrhenian Sea. The MAW caused a mixed layer of 40-50 m with salinity 150 151 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 152 Bay is located in the semi-humid bioclimatic zone, with significant periods of rainfall 153 (especially in winter) causing an important inputs of terrestrial nutrients. Nutrients are 154 commonly abundant in the surface layers throughout the year (0.1–0.7 P μ M; 1–3.5 N μ M; 155 0.5–2.25 Si µM) (Sahraoui et al., 2009). Seasonal dynamics has previously been shown for 156 phytoplankton, with high chlorophyll biomass $(2-4 \ \mu g \ l^{-1})$ during spring and summer, and 157

lower values in the other seasons (<1.5 μ g l⁻¹). This coincided with the predominance of diatoms (10⁵ 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 165 tidal regime in the region is dominated by a semi-diurnal cycle with a low amplitude (<15 166 cm), but the current velocities can reach 1 m s^{-1} in the middle of the Channel (Harzallah, 167 2003; Béjaoui et al., 2008). The Lagoon receives a large amount of freshwater from several 168 rivers and from the big lake of Ichkeul due to strong rains during winter and spring, which 169 170 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 171 172 anthropogenic activities (1–11.5 P µM; 3.5–55 N µM; 2–11Si µM, Bouchouicha et al., 2012), the Channel is considered as a more eutrophied system than the Bay. Pronounced diatom 173 blooms (10⁶ cells l⁻¹) were previously observed in the Channel during summer. Diatoms are 174 also present in spring together with nanoflagellates. These latter predominated during other 175 seasons (Bouchouicha et al., 2012). 176

177 2.2. Sampling and hydrological structure of the water column

Data for the study were obtained from several surveys conducted during four seasons 178 (spring, summer, and autumn 2012, and winter 2013) at a marine station located in the Bay 179 (station B, 37°16'54" N,9°53'42" E) and an inshore station in the Channel (station C, 180 37°15'04" N, 9°52'34" E) (Fig.1). During each season, the sampling was carried out at four 181 depths of each station (0.5, 2.5, 5/6 and 8/16 m) using an acid-washed 2.5-L plastic water 182 sampler (Hydro-Bios). Sampled water was filtered through a 200 µm mesh screen to remove 183 meso- and macrozooplankton (except water used for DOC determination) and stored in 184 polyethylene containers until analyses. Metazooplankton, for two size classes (200-700 µm 185 and $> 700 \,\mu\text{m}$), were sampled using two vertical net tows with 200 μm and 700 μm screen 186 mesh. Vertical profiles of salinity and temperature were measured in situ with a 187 microprocessor conductivity meter (LF 196) (Fig. 2). 188

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).

192 2.3. Linear Inverse Method-Monte Carlo Markov Chain analysis (LIM-MCMC)

193 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 194 method (Van Den Meersche et al., 2009), derived from the inverse analysis method of Vézina 195 and Platt (1988), was adopted to reconstruct trophic carbon flows through the eight planktonic 196 197 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 198 outside; (ii) setting mass-balance between flows, for each compartment, as equalities; (iii) 199 200 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 201 202 the range of possible values for each flow; and (iv) calculating a large sample of possible 203 solutions for unknown flows.

204 2.3.1. A priori model

The *a priori* model describes all possible carbon flows among the trophic 205 206 compartments. For all seasons, the model (Fig. 3) includes nine compartments: heterotrophic bacteria (bac), picophytoplankton (pic, $<2 \mu m$), nanophytoplankton (nan, 2-10 μm), 207 208 microphytoplankton (mic, 10-200 µm), protozooplankton (pro = heterotrophic 209 nanoflagellates, dinoflagellates and ciliates, <200 µm), metazooplankton 200-700 µm (met1), metazooplankton >700 µm (met2), dissolved organic carbon (doc) and detritus (det). Forty-210 three flows were considered in the *a priori* model of each season (Fig. 3, Table 3). 211

The gross primary production (gpp) of the three phytoplankton size fractions (pic, nan, 212 and mic) is the only source of carbon input to the network. Carbon loss is driven by 213 respiration of all living compartments and sinking of all compartments except 214 protozooplankton, picophytoplankton and bacteria. We assumed that the very small size of pic 215 and bac did not allow them to generate sinking flux. Detritus dissolution, phytoplankton 216 217 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 218 219 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 220 221 (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 222 223 several coastal areas, including our study sites (Meddeb et al., 2018). Metazooplankton, known as inefficient grazers on particles $< 5 \,\mu m$ (Fortier et al., 1994), consumed nano- and 224 225 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).

228 **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 239 240 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 241 242 Savenkoff, 1999; Steinberg et al., 2000; Vézina et al., 2000; Vézina and Pahlow, 2003). They 243 constrain the respiration of bacteria, phytoplankton, protozooplankton and metazooplankton; the doc production by phytoplankton, bacteria and zooplankton; the growth efficiency of 244 bacteria and zooplankton; the assimilation efficiency of zooplankton; the detritus production 245 by zooplankton and the dissolution of detritus (Table 4). 246

247 **2.3.4. Solutions**

The last step of the inverse analysis was the calculation of unknown flows. The 248 estimations of each unknown flow were obtained by the LIM-MCMC method, based on the 249 mirror technique defined by Van Den Meersche et al. (2009). A jump value of 10 mgC m⁻² d⁻¹ 250 and 300000 iterations were adopted to run the models in order to optimize the coverage of all 251 the possible solutions (i.e. correct polytope exploration). A Matlab[©] translation by Alain 252 Vézina and Lauriane Campo of the R-CRAN project package LIM-Solve was used (Van den 253 Meersche et al., 2009). More details on the method are available in Van Den Meersche et al. 254 (2009) and Niquil et al. (2012). 255

256 **2.4. Data**

Data used in the eight models were collected during seasonal samplings atthe 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 260 more details on the preservation of samples and analyses. Briefly, Samples for doc 261 measurement were filtered on sterilized 0.2 µm polycarbonate filters before being analyzed 262 using a Shimadzu TOC-5000 carbon analyser (Knap et al., 1993). The carbon concentration 263 of doc was obtained considering that 1 µM of doc is equal to 12 mg C m⁻³ (Grami et al., 2008; 264 Meddeb et al., 2018). POC was analysed on a CHN elemental analyser (Perkin–Elmer 2400) 265 after filtration of samples through pre-combusted (at 450°C, during 2 h) GF/F Whatman 266 filters (21 mm) (Knap et al., 1996). 267

Heterotrophic bacteria and picophytoplankton (pic= prokaryotes and eukaryotes) were 268 enumerated under a CETI Topic-T epifluorescence microscope on samples filtered through 269 0.22 µm black polycarbonate filters (Nuclepore) laid over 0.45 µm nitrocellulose backing 270 filters (Millipore) (Parsons et al., 1984; Mac Isaac and Stockner, 1993). The mean cell volume 271 of bac and pic was converted into cell carbon using specific conversion factors, 0.35 pg C um 272 ³ for bacteria (Bjørnsen, 1986), 0.22 pg C µm⁻³ for prokaryotic pic (Søndergaard et al., 1991) 273 and 0.22 pg C cell⁻¹ for eukaryotic pic (Mullin et al., 1966). Phytoplankton (nan and mic) and 274 protozooplankton were counted and identified under an inverted microscope (Lund et al., 275 276 1958) on 100 ml settled samples fixed with 3% acid Lugol solution and 5% alkaline Lugol 277 solution, respectively. The mean cell volume of each taxon of phytoplankton and protozooplankton was determined by applying standard geometric shape, as proposed by 278 Hillebrand et al. (1999). Then, the mean cell volumes were converted to cell carbon using 279 specific formula or factors (see Table 1, Meddeb et al., 2018). 280

281 Metazooplankton samples were preserved in borate-buffered formalin (5% final concentration) for identification and counts of organisms under a dissecting stereomicroscope 282 283 (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 284 285 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 286 287 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 288 carbon stocks (mg C m⁻²) for each living and non-living compartments were calculated by 289 vertically integrating the carbon biomass calculated for each sampling depth. More details of 290 291 calculations are given in Meddeb et al. (2018).

The gross production rate of size-fractionated phytoplankton (pic, nan and mic) and 292 bacteria as well as consumption rate of these living components by protozooplankton were 293 seasonally assessed at each sampling depth of each site by in situ dilution experiments 294 (Landry and Hassett, 1982). This method is relatively simple to set up, it minimizes the 295 296 manipulation and disruption of the micrograzer population and is an appropriate technique for 297 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. 298 (2018) and Meddeb et al. (submitted). 299

300 Dilution experiments could be described as mixing free-particle sea water (filtred on 0.2 µm) with pre-filtred water on 200 µm mesh net (to remove meso- and macrozooplankton 301 302 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 303 sampling depths fortnight. The estimated rates k and g were used to calculate gross production 304 rates for phytoplankton and bacteria (P, mg C $m^{-3} d^{-1}$) and their consumption rates (G, mg C 305 m⁻³ d⁻¹) as suggested by Moigis (2000) and in more recent works (Sakka Hlaili et al., 2008, 306 307 Grattepanche et al., 2011):

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

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

308 where $C_0 \text{ (mg C m}^{-3})$ is the initial biomass of phytoplankton or bacteria, k is the growth rates 309 of bacteria and size-fractionated phytoplankton (d⁻¹), g is the grazing rates by 310 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 μ m and >700 μ m). 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

- 321 pigment were measured before and after acidification with 10% hydrochloric acid using the
- 322 spectrophotometric method of Lorenzen (1967).
- 323 Gut pigment content (GP, mg pigment m^{-3}) was calculated using the following equation:

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

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.

327 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 μ m 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).

333 Sediment traps were used to measure sinking rates of particles and faecal pellets, during334 each season and at each site, as described in Meddeb et al. (2018).

335 2.5. Ecological Network Analysis

Ecological network analysis (Ulanowicz, 1997) was used to describe and to characterize 336 the structural properties and function of plankton food webs resulted from the LIM-MCMC 337 analysis. The MATLAB[©] algorithms, written by Carole Lebreton and Markus Schartau 338 (GKSS Research Center, Geesthacht Germany), were used to calculate the indices that 339 characterize food webs in term of activity, organization, specialization, recycling, maturity 340 and stability (Table 5). The ENA indices used in this work as trophic functioning indicators 341 342 were the total system throughput (TST), the ascendency (A), the development capacity (DC), the relative ascendency (A/DC), the average path length (APL) the Finn cycling index (FCI) 343 and the detritivory/herbivory ratio (D/H). The definition and the formulae calculation of these 344 indices are presented in Table 5. 345

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 toRomano (2006). No p values can be calculated based on delta.

354 **3. Results**

355 3.1. Hydrodynamic status

Water temperatures, which were similar among stations and over depths, showed a 356 seasonal cycle, ranging between a minimum in winter (12-13.7 °C) and a maximum in 357 summer (25.7–27.1 °C). In the Bay, the salinity showed little variation within and between 358 depths and seasons (36.9–37.4 psu). The water column of the Channel was vertically 359 homogenous regarding the salinity during summer, autumn and winter (36.5-37.5 psu). In 360 contrast, there was a haline stratification of the inshore water during spring due to low saline 361 surface water coming from the Bizerte Lagoon. During this season, there was an exceptional 362 decrease of salinity in the Lagoon (14-24 psu) caused by important river runoff associated 363 with a large amount of rain during the winter 2012 (213 mm, INM, 2013). Further, high levels 364 of nutrients were recorded in the Channel (Fig. 4). In both stations, pronounced 365 concentrations of NO_2^- , NO_3^+ , and NH_4^+ were detected in spring, summer and autumn (10– 366 24 μ M) compared to the winter (3–4 μ M) (Fig. 4A). PO₄³⁻ (0.14–0.99 μ M) and Si(OH)₄(0.84– 367 368 2.86 μ M) were available throughout the year in both stations (Fig. 4 B, C).

369 **3.2. Primary and bacterial production**

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

The primary production was relatively high during spring (966–727 mg C m⁻² d⁻¹), moderate 372 in summer and autumn (394–571 mg C m⁻² d⁻¹) and low in winter (160–256 mg C m⁻² d⁻¹) 373 (Fig. 5). During most seasons and in both stations, microphytoplankton (mic) contributed 374 significantly to total production (Bay: 48-84%; Channel: 60-82%), except in the inshore 375 station during autumn, when the proportion of picophytoplankton production was high 60%. 376 377 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 378 lower (0.29–3.03%) in autumn-winter in all stations. 379

In both sites, bacterial production was low from spring to autumn (48–117 mg C m⁻² d⁻¹) and significantly increased in winter (135–274 mg C m⁻² 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.

384 **3.3. Carbon outflows**

Respiration constituted the major carbon outflow from the eight model systems, with 385 higher proportion during winter (66-84% of gross primary production) compared to the other 386 seasons (45-53%) (Fig. 6). Carbon sinking contributed from 26-31% and 14-29% of gross 387 primary production, respectively, in the inshore and marine stations (Fig. 6A, B). Added to 388 these two carbon loss pathways, the remaining carbon could be available for trophic transfer 389 *via* the predation on metazooplankton. This trophic transfer was high during spring, summer 390 and autumn (21-31% of gross primary production), but did not exceed 2% in winter at both 391 392 stations (Fig. 6A, B).

393 **3.4. Carbon flows to grazers**

In the eight plankton food webs, the diet of protozooplankton was mainly composed of 394 autotrophs during spring (90-97%), summer (86-88%) and autumn (86-93%), but equally 395 shared between bacteria and phytoplankton in winter (i.e. mic and pic) (Fig.7A). 396 397 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 398 399 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 400 401 protozoan diet was low in spring and summer (3-22 %) and even lower during autumn and 402 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).

410 **3.5. Structural and functional proprieties of food webs**

The total system throughput (TST) varied proportionally to the total input in the 411 plankton food web system. This index, related to the total activity of the system, was high in 412 spring (2934–3818 mg C m⁻² d⁻¹), moderate in summer and autumn (1733–1743 and 1710– 413 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 414 spring exhibited the highest development capacity (DC)(Channel: 11.106 mg C m⁻² d⁻¹; Bay: 415 8489 mg C m⁻² d⁻¹, Fig. 8, Table 6) compared to the other seasons (2634–6951 mg C m⁻² d⁻¹) 416 (Fig. 8). The ascendency showed similar pattern as DC, with higher values in spring (4838– 417 $6054 \text{ mg C m}^{-2} \text{ d}^{-1}$) in comparison to the rest of the year (1566–3963 mg C m⁻² d⁻¹). Fig. 8. 418

Table 6). The A/DC ratio, which quantifies the degree of organization (information) of the 419 system, reached higher values in winter (59.4-59.6%, Fig. 8) and decreased from autumn 420 (57-58%) to spring (54.5-56%) and summer (53.4-53.9%) (Fig. 8). The highest values of 421 422 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– 423 3.03) and during summer in the Channel (3.20). Similarly, the Finn cycling index was highest 424 in winter (41–46%). During autumn (for both sites) and summer (for the channel), this index 425 reached higher values (16–17%) than in spring (for both sites) and summer (for the Bay) (13– 426 427 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, 428 Fig. 8, Table 6) to lower values (0.05–0.12, Fig. 8, Table 6) were observed respectively 429 during the spring, autumn and winter in all study sites. 430

431 **4. Discussion**

432 **4.1. Hydrodynamics and nutritional status over seasons**

433 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 434 the year. Even in winter, the freshwater discharges from the rivers surrounding the Lagoon of 435 Bizerte only partly reaches the Bay. These conditions can lead to little variation of 436 hydrological structure throughout the year. Consequently, nutrients were available throughout 437 the year inside the Bay. In contrast, the Channel was influenced both by the marine water 438 from the Bay (mostly in summer) and by lagoonal water (mostly in winter). Hence, the spring 439 situation within the Channel was characterized by a strong halocline resulting from low saline 440 surface water coming from the Lagoon of Bizerte, which was caused by an important river 441 runoff during late winter (Fig.2A). In summer, the direction of the current was reversed, with 442 an inflow of marine water from the Bay resulting in well mixed water (Fig. 2A). In autumn 443 vertical mixing became more important, resulting in a well-mixed water column rich in 444 nutrients (Fig. 3). This hydrological situation was maintained in winter with lower nutrient 445 446 levels (particularly for nitrogenous nutrients).

447 **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 453 Bay (R7 = 0.07) functioned as herbivorous pathways. During the summer food web 454 functioned as herbivorous pathway in the Channel (R7 = 0.1), and as multivorous in the Bay 455 (R7 = 0.27). The situation in autumn was different between the sites (Fig. 9A, B). In the Bay, 456 the food web did not change much in structure from that in summer and continued to function 457 as a multivorous pathway (R7 = 0.21), while, it functioned approximately as a microbial 458 pathway in the Channel (R7 = 0.7). More explanations in the evolution of food webs in both 459 460 sites during spring, summer and autumn were reported in the supplement annex.

461 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 462 lowest rates registered throughout the year (160–256 mg C m⁻² d⁻¹, Fig. 9). These levels were 463 very low compared to the one found in coastal areas. Instead, the bacterial production 464 465 increased (especially in the Bay) and almost equalled the primary production (bacterial to primary production ratio, PB:PP = 0.85-1.07). This differed from that observed for the other 466 467 seasons (PB:PP ratio 0.06–0.21), 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 468 (0.23, Marquis et al., 2007, 2011: spring), and the lagoon of Bizerte (0.23–0.26, Grami et al., 469 2008: summer). Bacteria supplied 50% of the C demand of protozooplankton, which were 470 mainly composed of aloricate ciliates and HNF in both sites during winter (50-70%, Meddeb 471 et al., submitted). Consequently, the microbivorous activity of protozooplankton increased 472 relatively to their herbivory (Fig. 9 B), while metazooplankton consumed more 473 microphytoplankton than during the other seasons. For these reasons, we were cautious to use 474 only the R7 ratio to identify the dominant food web during winter, because bacteria seemed to 475 476 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 477 478 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 479 480 (i.e. sum of net production of bacteria, phytoplankton, detritus and doc). R5 values showed 481 that, in all sites, the bacteria contributed largely to the production of potential 482 microzooplankton food during winter (0.26–0.29) than the other seasons (0.03–0.1). The R7 ratio calculated for winter (0.20 and 0.28 for the Channel and the Bay, respectively) suggested 483 484 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 485

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 491 researchers and managers, new criteria that they could use to identify quantitatively the 492 trophic pathways that dominate the multivorous food web. The first criterion was the R7 ratio 493 494 (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 495 496 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 497 498 discriminates between the multivorous (low R5 < 0.1) and bacterial multivorous food web 499 (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". 500

Despite the impact of many factors on the seasonal evolution of planktonic food webs 501 functioning, it is possible to identify an overall pattern of evolution. The accumulation of 502 503 nitrates in the water column during winter allows the development of a spring bloom of 504 phytoplankton when light and temperature conditions become more favorable. Thereafter, the mesozooplankton species develop rapidly and the 'herbivorous' food web is set up. This 505 carbon pathway cannot persist in time (Legendre and Rassoulzadegan, 1995). The grazing 506 activity of heterotrophic organisms enriches the pool of carbon and dissolved nitrogen (DOC 507 and DON) and ammonium (excretion). DON promotes remineralization of ammonium by 508 509 bacteria and their associated predators (protozoa) and a microbial food web settles while the 510 classical herbivorous food web persists, leading to the formation of a 'multivorous food web' 511 (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 512 more suitable for non-limiting conditions of nitrates as was previously observed in other 513 systems (Reynolds 2000, Alves de Souza et al., 2008). Smaller producers dominate when 514 515 nitrates decrease, which was observed in our inshore station during autumn. Other conditions 516 may favor proliferation of small producers leading to their dominance in relation the total 517 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, 518

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).

524 **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 530 531 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 532 Fig 11. The highest ecosystem activity, indicated by TST, was observed in spring (2933–3818 533 mg C $m^{-2} d^{-1}$), when algal blooms occurred and primary production dominating by 534 microphytoplankton (Table 3) resulting to the herbivorous food web. In the opposite, the 535 winter "bacterial multivorous web" showed the lowest activity. The ascendency (A) and the 536 development capacity (DC) are strongly related to the TST (Table 5, Ulanowicz, 2004) and 537 showed the same pattern of variation. The relative ascendency (A/DC) is an essential 538 indicator of the degree of ecosystem organization and specialization (Ulanowicz, 1997; 539 Patrício et al., 2004). The A/DC decreased from spring to summer and then rose in 540 autumn/winter, which perfectly followed the seasonal change in the activity of the observed 541 542 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 543 544 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 545 546 emergent properties of ecosystems (i.e. stability, maturity, resilience...) should also vary 547 among seasons. For example, Legendre and Rassoulzadegan (1995) stated that an herbivorous 548 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 549 550 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 551

have increased from spring (FCI: 13-14%; APL: 2.95-3.20) to summer/autumn (FCI: 16-552 17%; APL: 3.27-3.33) and then showed the highest value in winter (FCI: 41-46%; APL: 553 4.48–5.09). Moreover, during the same succession, the A/DC values have slightly increased, 554 and the maximum value reached is slightly inferior to 60%, hence. With this situation, the 555 maximum possible specialization was not reached, letting a reserve of redundancy, important 556 for the robustness of the food web as underlined by Ulanowicz et al. (2009). This indicates a 557 concomitant increase of ecosystem stability and maturity, when the trophic typology has 558 evolved from the herbivorous web to multivorous and microbial webs and reached the 559 "bacterial multivorous web". This new trophic pathway based on a high heterotrophic 560 561 bacterial production had the strongest carbon recycling, which lead to a higher retention of 562 carbon inside the ecosystem and then appears to be the most stable and mature.

563 The D/H ratio is often considered as another index that could bring an important 564 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 565 566 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: 567 568 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 569 570 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 571 detritivory unknown flows before investigating the link between plankton food web typology 572 573 and D/H index.

An important emergent property of each food web, highly related to the internal 574 organization described with the ENA indices, is the distribution of carbon as transfer from the 575 planktonic system, to non-planktonic pelagic consumers or down to the benthic consumers. 576 577 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; 578 579 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 580 581 channel C to higher pelagic and benthic consumers, whereas in microbial food webs, most 582 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). 583 Interestingly, a specific situation has been found in winter during which the "bacterial 584 multivorous web" was acting that differs from others seasons (Fig. 6). In spring, summer and 585

autumn, the respiration was around 50% of gpp and a substantial fraction of primary 586 production could reach higher pelagic consumers (21–31% of gross primary production, gpp). 587 Meanwhile, another significant part of the production sank down to the benthic food web (20-588 589 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 590 assumptions made above for the herbivorous and multivorous food webs found in the Channel 591 (during spring/summer) and the Bay (during spring/summer/autumn), but was unexpected for 592 the Channel in autumn. During this season, the microbial pathway was observed and 593 594 picophytoplankton dominated the primary production, which typically results in carbon leaving the plankton food web mainly through respiration (Legendre and Rassoulzadegan, 595 596 1995; Rivkin et al., 1996; Legendre and Le Fèvre, 1995). In our case, it seems that the 597 capacity of the food web to channel carbon to higher trophic levels does not depend so much 598 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 599 600 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 601 602 recycling (FCI) and the low retention of carbon associated with low path length (APL) in the 603 system (Fig. 11).

When the "bacterial multivorous food web" was observed, during winter, most 604 produced carbon was lost by respiration (66–84% of gpp), leaving a little amount available for 605 higher pelagic consumers (2–2.32% of gpp, Fig. 6). Obviously, this was a consequence of the 606 functioning of the "bacterial multivorous web", which was characterized by a strong recycling 607 activity and long internal food chains, as supported by the highest values of FCI and APL 608 609 (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 610 611 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 612 613 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 614 615 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 616 617 activities in both study sites. This is of first importance for environment managers.

618 **4.4. Ecological indicators, ecosystems health monitoring and management outlook**

The present results highlight the importance of selecting the right indicators and that 619 620 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 621 ecosystems (Baird et al., 2009; Tecchio et al., 2016; Pezy et al., 2017; de la Vega et al., 622 2018a, b). In line with these former studies, our results showed significant implications of 623 these approaches for the understanding of the ecosystem dynamics and for adaptive 624 management. Some types of food webs can be considered as indicators of trophic status (Fath 625 et al., 2019, Safi et al., 2019) and ecosystem services (Schückel et al., 2018). Thus, the present 626 627 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 628 criteria (R7 and R5) to consider, in complement to ENA, and that they could use to identify 629 quantitatively the newly defined food web type, "bacterial multivorous food web" which we 630 631 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). 632

633 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 634 635 recommendations for environmental managers and industry in terms of monitoring the effects 636 on marine ecosystems by the limitation of nutrient and pollutant loads in coastal zone and ensuring restoration and increasing ecosystem resilience. Effectively, some ecological 637 indicators, such as TST, A/DC, APL and FCI are already used to examine the impact of 638 anthropization on marine ecosystems. Previous studies have considered TST as an indicator of 639 eutrophication (Niquil et al., 2012; Grami et al., 2008; Scharler and Baird, 2005). Our results 640 reported also very high TST values in spring, related to the high productivity of the 641 ecosystems during this season, and especially to that of the autotrophic organisms (i.e. 642 phytoplankton). The increase in TST could be interpreted as eutrophication and affiliated to 643 the presence of a higher biodiversity of sources of carbon in the ecosystem and also to the 644 proximity of the study stations to sources of nutrient discharges (urban, agricultural and 645 646 shellfish farming input). Effectively, eutrophication events has been always reported for the Bay and the Channel sites (Sakka Hlaili et al., 2007). The relative ascendency (A/DC), 647 648 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 649 theory. The observed pattern mean that the least disturbed systems (autumn, winter) appeared 650 to be less vulnerable to disturbance, more tightly organized, resistant and more efficient in 651 652 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 653 indices (e.g. A). This confirms that the relative information indices appear more suitable for 654 monitoring the effects of anthropogenic impact (as pollution, eutrophication...) on an 655 ecosystem level than the absolute indices (Ulanowicz et al., 2009) and are preferred by many 656 authors (Baird et al., 1991; Baird and Ulanowicz, 1993; Christensen 1995; Monaco and 657 Ulanowicz, 1997; Rybarczyk and Elkaïm 2003; Rybarczyk et al., 2003; Patricio et al., 2004) 658 because it excludes throughputs effects and allows comparison between ecosystems 659 660 (Heymans et al., 2002).

661 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 662 663 (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. 664 665 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 666 667 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 668 669 good candidate index for comparing stress states of many ecosystems. In the Bay and the 670 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 671 values of the winter (0.41-0.46) and those lower but still high compared to literature values, 672 in other seasons (0.13–0.17), revealed that both systems could be considered as stressed over 673 all the year. Furthermore, for both sites and over all seasons the APL responded to the 674 environmental perturbation to the same gradient as FCI, thus suggesting that either index 675 would be sufficient for evaluating the ecological status of studied ecosystems. 676

The differences observed in the functioning of the studied sites (the Channel and the 677 Bay of Bizerte) indicate that those ecosystems, strongly impacted by human activities, must 678 be monitored based on several representative sites, chosen regarding their main habitat type 679 680 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 681 682 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 683 data, in particular for higher trophic levels such as fish, birds, mammals ... and for lower 684 trophic levels such as meiofauna or benthic diatoms. For this purpose, we advise managers to 685 686 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 687 other trophic strategies are important to be considered and studied for trophic transfer in 688 planktonic environment (e.g. mixotrophy, parasitism, saprophitism). Integrating all these 689 690 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 691 holistic ecosystem management strategy. Finally, we propose the use of well-studied areas as 692 reference sites, selected according to available data (research projects) and existing 693 monitoring programs (sampling takes place regularly, e.g. at least annual basis) for 694 695 comparison.

696 **5. Conclusion**

697 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 698 699 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 700 701 hydrological conditions that were reflected in the food web structures, which showed seasonal dynamics that diverged from the conventional scheme proposed by Legendre and 702 703 Rassoulzadegan (1995). The herbivorous food web dominated during spring, and showed the 704 highest gross primary production that was largely dominated by microphytoplankton. This 705 coincided with high availability of nutrients and light and stratification of the water column, 706 caused by an increased freshwater inflow in the inshore waters. More surprisingly, the 707 herbivorous food web was also identified during summer, as nutrient and light conditions remained favorable for microphytoplankton production. The multivorous pathway was 708 709 dominant in the marine site during summer and autumn, when microalgal production decreased and picophytoplankton became more productive. In contrast, the autumn food web 710 in inshore site functioned as microbial pathway, as primary production was mainly dominated 711 by picophytoplankton. The microphytoplankton exhibited the lowest production in autumn, 712 probably due to the decrease of light. The winter situation corresponded to a new type of 713 pathway, the "bacterial multivorous food web" which we suggest can be added to the existing 714 typologies of plankton food webs. In this newly defined food web, bacterial production 715 equalled that of primary production, hence bacteria and microphytoplankton were the main 716 actors that together fuelled the system. 717

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 720 the most active with pronounced total system throughput (TST). In opposite, they showed 721 lower stability in comparison to the systems encountered during summer (multivorous 722 723 pathway) and autumn (microbial and multivorous pathways), with relatively high cycling activity and path length. The strongest carbon recycling coincided with the "bacterial 724 multivorous food webs" observed in winter. This pathway was the most stable and mature, 725 with pronounced capacity to retain carbon inside the system, leading to low export. The 726 winter ecosystem also seemed to be most organized and specialized, as indicated by the high 727 728 relative ascendency (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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