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Factors driving the seasonal distribution of planktonic and epiphytic ciliates in a eutrophicated Mediterranean Lagoon

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ABSTRACT

We studied the distribution of planktonic and epiphytic ciliates coupled with environmental factors and microalgae abundance at five stations in Ghar El Melh Lagoon (Tunisia). Planktonic ciliates were monitored for a year and epiphytic ciliates were sampled during summer 2011 in concordance with the proliferation of the seagrass *Ruppia cirrhosa*. Ciliate assemblage was largely dominated by Spirotrichea followed respectively by Tintinnida and Strombidiida. No significant difference was found in the distribution of ciliate species among the stations. Redundancy analysis indicates that abiotic factors (temperature and nutrients) have a significant effect on the dynamics of certain ciliates. For epiphytic ciliates, 4 species were identified: *Tintinnopsis campanula*, *Aspidisca* sp., *Strombidium acutum* and *Amphorides amphora*. Based on PERMANOVA analyses, ciliates exhibit significant correlations among months and stations. According to ACP, epiphyte distribution follows roughly those of *R. cirrhosa* and pH. Significant correlations were found between harmful dinoflagellates and both planktonic and epiphytic ciliates.

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1. Introduction

Coastal lagoons are considered to be distinct systems rather than adjoining ones (Knoppers, 1994). As interfaces between land and sea, they exhibit high primary and secondary productions that promote the development of extensive fisheries and aquaculture (Kjerfve, 1994; Chessa et al., 2005; Pérez-Ruzafa et al., 2007). As semi-enclosed systems, coastal lagoons are strongly influenced by freshwater input (Valiela et al., 1997; De Wit et al., 2005) and are usually impacted by agricultural, industrial and tourism activities (Cloern, 2001; Verlaque, 2001; Lloret et al., 2008). These unique features allow lagoon waters to acquire significantly different characteristics compared to the nearby seawater, which leads to greater diversity in the biological communities in these ecosystems. Despite their economic and environmental importance, lagoons remain insufficiently studied (Alvarez-Borrego, 1994; Danovaro and Pusceddu, 2007) and little is known about the factors driving ciliate dynamics in their ecosystems. Ciliates are one of the major functional groups in the aquatic food web (Landry and Calbet, 2004; Pomeroy et al., 2007; Fenchel, 2008; Sherr and Sherr, 2008) with numerous works reporting ciliate feeding on bacterioplankton (Simek et al., 1998; Karayanni et al., 2008) and phytoplankton (Gismervik et al., 1996; Loder et al., 2011),

making them a likely link in the transfer of energy from microbial components to higher trophic levels (Azam et al., 1983; Sherr et al., 1986). Furthermore, ciliates are valuable bioindicators of water quality considering their specific eco-physiological properties as rapid sensors of variation in environmental changes (Aleya et al., 1992; Foissner and Berger, 1996; Jiang, 2006).

It is noteworthy that in the Mediterranean, the majority of studies on the ciliate community structure have dealt with coastal and/or oceanic plankton communities (Admiraal and Venekamp, 1986; Cariou et al., 1999; Dolan, 2000; Dolan et al., 2002; Kršenić and Grbec, 2006) while lagoons have been neglected. Yet, the nutrient dynamics at the pelagic/benthic interface in the lagoons are likely to be complex with regenerated nutrients released from the sediment when disturbed. In Tunisia, most studies of ciliates have been reported from coastal and open sea ecosystems (Hannachi et al., 2009, 2011; Kchaou et al., 2009; Drira et al., 2009; Rekić et al., 2012; Ben Brahim et al., 2013), or solar salterns (Eloumi et al., 2006, 2009) whereas, as far as we know, no studies have been conducted in lagoons despite their abundance in Tunisia and their many interesting characteristics. This is particularly true for the shallow Ghar El Melh Lagoon (GML) which: (i) has limited connection with the sea and is now under stress from both natural and anthropogenic pressures such as pollution, rising sea level and silting, and also has episodes of harmful dinoflagellate blooms (Romdhane et al., 1998; Turki et al., 2007; Dhib et al., 2013), (ii) is a well-known breeding site for birds (grebes, herons, storks, flamingos, gulls...) (Kraiem et al., 2009), (iii) is

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permanently connected to Sabkhet El Ouafi; sabkha is an Arabic term for a coastal and inland saline mud flat built up by the deposition of silt, clay and sand in shallow, sometimes extensive, depressions (Kchaou et al., 2009), and (iv) has a benthic vegetation dominated by a *Ruppia cirrhosa* meadow (Shili et al., 2002) which spreads spectacularly in summer and whose leaves overwhelm the surface water in different parts of the lagoon; we hypothesized that these leaves may harbour ciliates whose structure and abundance should be explored.

Altogether, these conditions prompted us to explore over the period of a year the species composition abundance, and biomass of planktonic ciliates in GML waters in relation to physical and chemical factors as well as phytoplankton abundance. In addition, in order to obtain a good overview of the lagoon's entire ciliate community, epiphytic ciliates of *R. cirrhosa* were investigated in summer due to the meadow's massive proliferation.

We hypothesized that ciliates should exhibit interspecific differences in relation to both environmental variability and potential phytoplankton prey.

2. Materials and methods

2.1. Study area

Ghar El Melh Lagoon is a Ramsar site (Bureau, 2007), located in the southern Mediterranean Sea on the north-eastern coast of Tunisia (37°06–37°10N and 10°08'–10°15E) and influenced by the regional water circulation (Ben Ismail et al. 2012, 2013) (Fig. 1). GML has an area of about 3000 ha including 2 small sub-lagoons, namely Sabkhet El Ouafi and Sabkhet Sidi Ali El Mekki. The main lagoon is permanently connected with Sabkhet El Ouafi but isolated from Sabkhet Sidi Ali El Mekki by embankments. It is connected to the Mediterranean Sea via a permanent channel called El Boughaz that passes through the coastal sand bars. The lagoon exhibits different levels of salinity with the highest recorded in stagnant areas within the lagoon. Freshwater inflows are seasonal, limited in summer and high in winter, sometimes with the occurrence of exceptional floods creating a connexion between the lagoon and the Mejerda River. Benthic vegetation currently consists of *R. cirrhosa* and *Cladophora* (Shili et al., 2002). Birds including grebes, herons, storks, flamingos and gulls are frequent in the shallow south-eastern part of GML. The small fishery in GML comprises eel, mullet, sea-bass and sole (Kraïem et al., 2009).

2.2. Sampling

Five stations (S1–S5) were chosen to cope with the different environmental conditions found within GML (Fig. 1). S1 is located in the north-eastern part of the lagoon, a shallow area influenced by a supply of freshwater from 'El Ayoune', with a mat of limnetic plants (reeds) around the edges. S2 faces the El Boughaz Channel which is a permanent 70-m wide connection with the Gulf of Tunis. The deepest area of GML, it is influenced by strong hydrodynamics despite continuous silting of the channel. S3, located in the south-east of the lagoon in the middle of Sabkhet El Ouafi, is a semi-closed zone and one of the most stagnant areas. S4 in the south-west of GML is affected by heavy industrial discharge (form 43 industrial units) and is drained by tributaries mainly in winter. S5, located in the northern part of the lagoon, is affected by agricultural and urban discharge. Leachate from rural zones, rainwater and the wastewater from the city of Ghar El Melh arrive directly into this part of the lagoon.

Samples for planktonic ciliate and microalgae identification and enumeration were collected using a polyvinylchloride (PVC) tube twice a month, from January 2011 to January 2012 at the five sampling sites ($N = 110$). Additionally, three replicates of epiphytic

ciliate and microalgae of *R. cirrhosa* were collected ($N = 45$) at each station during the summer (early June, mid-July and late August). To detach the micro-communities, 100 g of leaf samples were placed in plastic bottles; the epiphytes were separated by vigorous shaking and washing, then mixed with 200 ml of seawater and filtered through a 0.2 μm pore-size membrane (Millipore). The obtained solution was then passed through 250 μm mesh sieves to remove large particles and was then fixed with formaldehyde (5%). Concomitantly with epiphyte sampling, shoot density of *R. cirrhosa* was measured *in situ* using a quadrat (40 cm \times 40 cm) that was randomly placed over the shoots before carefully collecting them. At each station, three replicate quadrats were sampled. Meadow density (shoot m^{-2}) was calculated as the number of shoots bearing leaves in each quadrat (Ott, 1980).

Environmental variables were measured in the field concomitantly with ciliate and phytoplankton sampling. A WTW multiparameter was used for water temperature ($^{\circ}\text{C}$) and salinity. Transparency was measured using a Secchi disk. Water samples were collected in 1000-ml polypropylene bottles at 30–50 cm depth. In the laboratory, nutrients (ammonium, nitrite, nitrate, phosphate, silicate) were analysed with a BRAN and LUEBBE type 3 autoanalyser and concentrations were determined colourimetrically using a UV-visible (JENWAY 6705) spectrophotometer (APHA, 1992).

2.3. Ciliate and microalgae enumeration

Sub-samples (25 ml) of planktonic communities were counted under an inverted microscope after fixation with lugol solution (final concentration 1% v/v) and settling for 48 h using the Utermöhl method (1958).

Sub-samples (1 ml) of epiphytic communities (Turki, 2005) were also enumerated according to the Utermöhl method by means of an inverted microscope.

Ciliate identification was performed using the keys of Corliss (1961), Petz (1999) and Strüder-Kypke and Montagnes (2002). Tintinnids were identified using lorica morphology and species description according to Kofoid and Campbell (1929, 1939) and Balch (1959). Naked ciliates were identified based on the methods of Lynn and Small (1997), Petz (1999), Alder (1999) and Strüder-Kypke and Montagnes (2002). Cell numbers were expressed as cells l^{-1} . Mean biovolume of each ciliate taxon was estimated from length and width measurements of more than 20 individuals for the abundant taxa, and converted to carbon biomass with the conversion factor proposed by Putt and Stoecker (1989): 1 $\mu\text{m}^3 = 0.19 \text{ pgC}$. The level of community structure was assessed with Shannon and Weaver's (1949) H' diversity index.

$$H' = - \sum ni/N * \log_2 ni/N$$

ni/N : is the frequency of species i in the sample. N : number of species in the community.

2.4. Statistical analysis

For the planktonic community, we used one-way variance analysis (ANOVA) to assess the variability of parameters among stations and months. Potential relationships between variables were tested by Spearman's correlation coefficient. Redundancy analysis (RDA) was performed to define the structuring effects of sampling sites and seasons, along with environmental conditions on ciliate species abundances. To prevent any disproportionate influence of rare species in the subsequent analysis, the only species considered were those having an abundance >2% of total ciliate abundance. Ciliate abundances were normalised prior to analysis. Statistical analysis was produced by R 2.15.0 (R Core Team development,

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