Harmful Algae
July 2019, Volume 87 Pages 101639 (11p.)
https://doi.org/10.1016/j.hal.2019.101639
https://archimer.ifremer.fr/doc/00507/61865/

Demonstrated transfer of cyanobacteria and cyanotoxins along a freshwater-marine continuum in France

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Abstract :

The frequency of cyanobacterial proliferations in fresh waters is increasing worldwide and the presence of associated cyanotoxins represent a threat for ecosystems and human health. While the occurrence of microcystin (MC), the most widespread cyanotoxin, is well documented in freshwaters, only few studies have examined its occurrence in estuarine waters. In this study we evaluated the transfer of cyanobacteria and cyanotoxins along a river continuum from a freshwater reservoir through an interconnecting estuary to the coastal area in Brittany, France. We sampled regularly over 2 years at 5 stations along the river continuum and analysed for phytoplankton and cyanotoxins, together with physico-chemical parameters. Results show that cyanobacteria dominated the phytoplanktonic community with high densities (up to 2 × 106 cells mL−1) at the freshwater sites during the summer and autumn periods of both years, with a cell transfer to estuarine (up to 105 cells mL−1) and marine (2 × 103 cells mL−1) sites. While the temporal variation in cyanobacterial densities was mainly associated with temperature, spatial variation was due to salinity while nutrients were non-limiting for cyanobacterial growth. Cyanobacterial biomass was dominated by several species of Microcystis that survived intermediate salinities. Intracellular MCs were detected in all the freshwater samples with concentrations up to 60 µg L−1, and more intermittently with concentrations up to 1.15 µg L−1, at the most upstream estuarine site. Intracellular MC was only sporadically detected and in low concentration at the most downstream estuarine site and at the marine outlet (respectively <0.14 µg L-1 and <0.03 µg L−1). Different MC variants were detected with dominance of MC-LR, RR and YR and that dominance was conserved along the salinity gradient. Extracellular MC contribution to total MC was higher at the downstream sites in accordance with the lysing of the cells at elevated salinities. No nodularin (NOD) was detected in the particulate samples or in the filtrates.

Highlights

► This study reports on the transfer of both cyanobacteria and cyanotoxins from a freshwater reservoir to the marine outlet in France. ► The very high correlation between the biomass of potentially toxic species and total MC concentrations strongly suggests that the majority of the cyanobacterial present in the estuary were toxic. ► *Microcystis* which dominated the blooms in the freshwater reservoir was the most likely genus responsible for the measured MC concentrations in the estuary, followed by *P. agardhii* both being relatively resistant to the salinity gradient. ► The extracellular contribution to the total MCs increased from upstream to downstream in accordance with cells lysis at elevated salinity. ► Both intracellular and extracellular MC variants did not show specific selection along the salinity gradient but the co-dominance of the highly toxic MC-LR and MC-YR variants is worrisome as it could impact on cyanobacterial consumers.

Keywords : Cyanobacteria, Intracellular and extracellular microcystins, Estuary, Salinity

1. Introduction

Cyanobacterial blooms have been reported worldwide (Merel et al., 2013) and their proliferations have been increasing in recent years as a result of anthropogenic activities including eutrophication and climate warming (O'Neil et al., 2012; Rigosi et al., 2014; Paerl, 2018). This tendency is also recorded in France and in particular in Brittany (AFSSA, 2006; Pitois et al., 2014; Le Moal et al., 2019) where most of the French agricultural lands are located. Freshwater cyanobacteria produce a variety of toxins (i.e. hepatotoxins, neurotoxins, dermatotoxins) which have strong negative impacts on animal and human health (Lance et al., 2010; Metcalf and Codd, 2012; Meriluoto et al., 2017). Reports of toxic cyanobacterial blooms in Brittany have been numerous in freshwater lakes and reservoirs (Vezie et al., 1998; Brient et al., 2009; Pitois et al., 2018). Among the diversity of cyanotoxins, the hepatotoxin microcystin (MC) is largely recognised as the most common and widespread in freshwater ecosystems (Harke et al., 2016). The general structure of that cyclic heptapeptide includes a specific beta amino acid- 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid - Adda (Ortiz et al., 2017; Tillett et al., 2000) as well as two amino acids that can vary leading to the identification of more than 250 MC variants (Puddick et al., 2014). The regulation and synthesis of MC, as well as its ecological role, are complex and not yet fully understood (Neilan et al., 2013; Omidi et al., 2017). Nodularin (NOD) is also a potent cyanobacterial hepatotoxin occurring in brackish waters (Sivonen et al., 1989; Kaebernick and Neilan, 2001). It is a cyclic pentapeptide structurally similar to MC, consisting of Adda, D-glutamicacid (D-Glu), N-methyldehydrobutyrine (MeDhb),D-erythro--methylaspartic acid (D-MeAsp) andL-arginine (L-Arg) (Rinehart et al., 1988).

The transfer of cyanobacteria along the freshwater-marine continuum has been observed worldwide (Preece et al., 2017 for a review), in Africa (Ndlela et al., 2016), USA (Lehman et al., 2005; Peacock et al., 2018), South America (Dörr et al., 2010), Australia (Robson and Hamilton, 2003; Orr et al., 2004), Europe (Verspagen et al., 2006; Tonk et al., 2007; Paldavičiene et al., 2009), and Turkey (Taş et al., 2006). The majority of these studies reported on the cyanobacterial transfer being dominated by *Microcystis aeruginosa* demonstrating a certain salt tolerance of that species. The associated transfer of MC along the river continuum was less often reported (Preece

et al., 2017) and very few studies reported on MC levels high enough to affect recreational activities (Paldavičiene et al., 2009; Albay et al., 2005). In particular, MC transfer to the coastal environment resulting from freshwater discharge from an upstream reservoir has only been reported in Italy (De Pace et al., 2014) and Japan (Umehara et al., 2012). Both MC and NOD have been reported to accumulate in fish and bivalves resulting in a potential risk to humans from consumption of contaminated food (Lopes and Vasconcelos, 2011; Gibble et al., 2016; Karjalainen et al., 2007).

Here we report on the dynamics and transfer of cyanobacteria and cyanotoxins from a freshwater reservoir discharge to the marine environment during a 2 year field study, which to our knowledge is the first study in France reporting on such transfer. This study is part of a larger project aiming at the evaluation of the potential risk of contamination of aquatic organisms (i.e. bivalves) by cyanotoxins during transfer from a freshwater reservoir to an estuary mouth in Brittany, France. We present results on cyanobacterial biomass, species composition, as well as cyanotoxins concentrations of different variants of MC and NOD both in intracellular and extracellular forms. The quantification of those two forms of cyanotoxins are necessary as we anticipate gradual cells lysing along the salinity gradient.

2. Materials and methods

2.1. Study site and sampling strategy

In Brittany, surface water dominated rivers have short residence times (Fraisse et al, 2013) and reservoirs are generally close to marine outlets. The study site is located in the Morbihan (Brittany, France) along a continuum of moderate length (<10 km), from the Pen Mur freshwater reservoir upstream through the estuary and the marine outlet (Fig. 1). This study site was chosen as the Pen Mur reservoir, used for drinking water, is monitored by the Regional Health Agency (ARS) and undergoes recurrent intense cyanobacterial blooms dominated by the genus *Microcystis* (L. Brient, pers. comm.). Freshwater discharge from the reservoir to the estuary is frequently observed but not monitored and therefore not quantified.

From May 2016 to April 2018, we sampled at 5 stations along the freshwater-marine gradient (F1 in the Pen Mur reservoir and F2 in the river downstream of the reservoir

are both in the freshwater section, E1 and E2 are located in the estuarine section and the site M is located at the marine outlet). For each campaign, all 5 stations were sampled on the same day. We consistently sampled the 3 downstream stations within 1 hour of low tide to maximize the freshwater discharge and minimize the tidal contribution. At each station, forthnightly to monthly water sampling was carried out in the water column to: i) describe phytoplankton and cyanobacterial species (identification and enumeration), and (ii) quantify cyanotoxins (MC and NOD) in the cells and in the water. Physico-chemical parameters (temperature, conductivity, dissolved oxygen, phosphorus and nitrogen concentrations) using a YSI 6920 multi-parameter probe (YSI Environmental, Anhydre) and chemical analyses were also determined at a subset of the sampling dates.

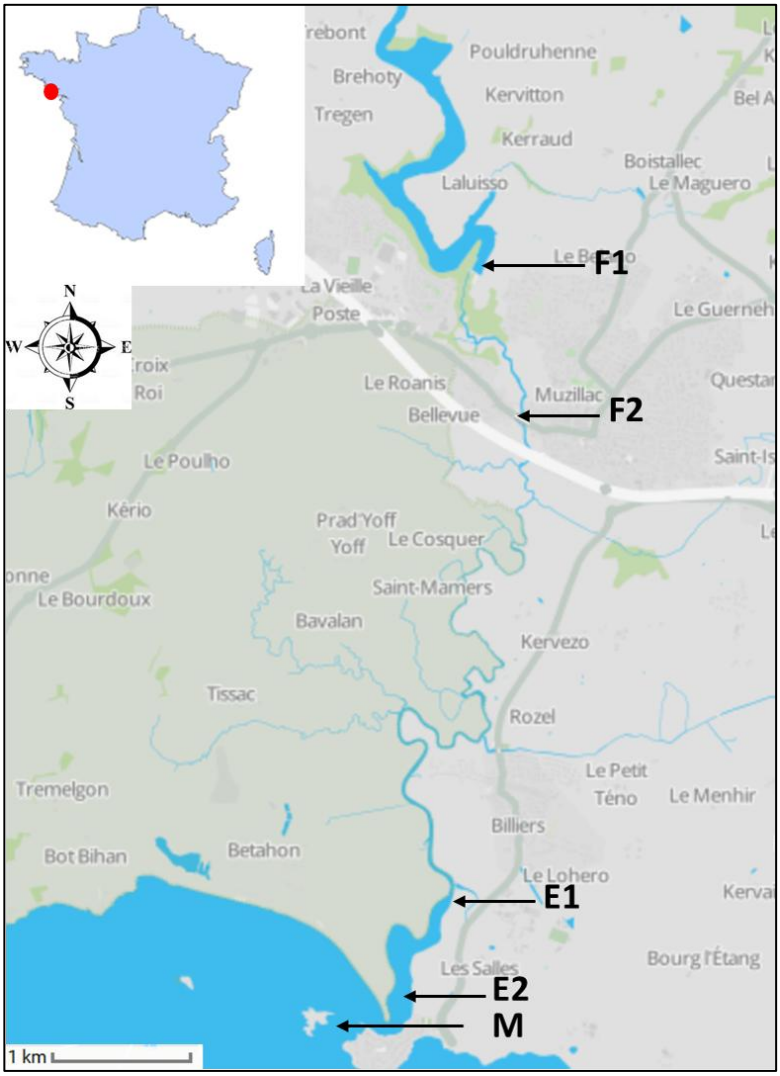


Fig. 1: Map of the study site and sampling stations: F1 and F2 are located in the freshwater section, E1 and E2 in the estuarine section and M at the marine outlet.

2.2. Samples analyses

Samples were filtered upon arrival at the laboratory within a few hours of sampling. Dissolved nutrient concentrations were measured from filtered (GF/F) water using common colorimetric methods (Aminot and Chaussepied, 1983) with a Bran and Luebbe Autoanalyser 3 (Axflow, Norderstedt, Germany). Nitrate was measured after reduction to nitrite on a cadmium-copper column (Henriksen and Selmer-Olsen, 1970). Phosphate was measured following the method of Murphy and Riley (1962). Phytoplankton and cyanobacteria identification and counts were conducted on fresh samples under an optical microscope (100x magnification) using a Nageotte chamber within 24 hours of sampling.

2.3. Chemical analysis of cyanotoxins by LC-MS / MS

Water samples containing cyanobacteria were filtered upon arrival at the laboratory through a 0.45 µm cellulose filter to separate the cell pellet for the intracellular cyanotoxin analysis and the filtrate for dissolved extracellular toxins and frozen at -20°C until chemical analysis. The filtrate was purified on a C_{18} SPE cartridge (Solid Phase Extraction) according to the ISO 20179 standard method (Anon 2005). The fraction containing the toxins was frozen until LC-MS/MS analysis. The cell pellet was ground with 250 mg of glass beads (0.15-0.25 mm) and 1 ml of MeOH so that cells released their toxins. Both fractions (intracellular and extracellular) were filtered by a 0.2 µm filter and analyzed by Ultra Fast Liquid Chromatography (Shimadzu, Marne La Vallee, France) coupled to 5500 QTrap tandem mass spectrometry (ABSciex, Villebon sur Yvette, France). Toxins were separated on a Kinetex XB C18 column (100 x 2.1 mm, 2.6 µm, Phenomenex), with water (A) and acetonitrile (B), both containing 0.1% formic acid at 0.3 mL min^{-1} flow rate. The gradient was raised from 30 to 80% B in 5 min and was held during 1 min before dropping down during 0.5 min to the initial conditions.

Mass spectrometry detection was carried out in multiple reactions monitoring (MRM) mode (positive ions). The electrospray ionization interface (ESI) was operated in positive mode using source setting: curtain gas set at 30 psi, ion spray at 5000 V, a turbogas temperature of 300°C, gas 1 and 2 set at 30 and 40 psi respectively and an entrance potential of 10 V. Each toxin was identified and quantified with two

transitions (Table S1): The toxin concentrations of all 10 lipophilic cyanotoxins were determined using certified standards provided by CNRC (Halifax, NS, Canada). The method was developed and validated internally in the IFREMER Phycotoxins laboratory.

Table S1 : LC-MS/MS transitions for the 9 MC variants and NOD tested with standards

Toxin	Precursor ion (*m/z*)	Transition (*m/z*) - Quantification	Transition *(m/z)* - identification
MC-LR	995.6	213.2	374.5
MC-LW	1025.6	375.2	135.2
MC-LF	986.6	375.2	135.2
MC-LY	1002.6	375.2	135.2
dmMC-LR	981.4	103.0	135.2
MC-RR	520.1	135.2	213.2
dmMC-RR	512.8	135.0	103.0
MC-LA	910.7	375.2	135.2
MC-YR	1045.6	213.2	375.2
NOD	825.5	227.0	163.2

2.4. Statistical analyses

All statistical analyses were carried out using R studio software (R Development Core Team, 2011). A Kruskal-Wallis analysis was used to test the temporal and spatial effects on physico-chemical conditions and dissolved nutrient concentrations. The significance threshold was set at $p < 0.05$. A Spearman correlation was applied between cyanobacterial biomass and overall toxin concentrations. Changes in the physico-chemical and biological conditions were characterized by a principal component analyses (PCA). Analyses were performed on temperature, conductivity, oxygen saturation, cyanobacterial biomass (cell/mL) and Shannon diversity index data.

3. Results

3.1. Dynamics and transfer of cyanobacteria along the freshwater marine continuum

Diatomophyceae and chlorophyceae occurred in winter and spring while cyanobacteria dominated the phytoplankton community in Pen Mur reservoir (F1 site) in summer and early fall during both years (Fig. 2). It is important to note that only few sampling campaigns (6) were performed in winter and spring, while the majority (18) occurred in summer and fall.

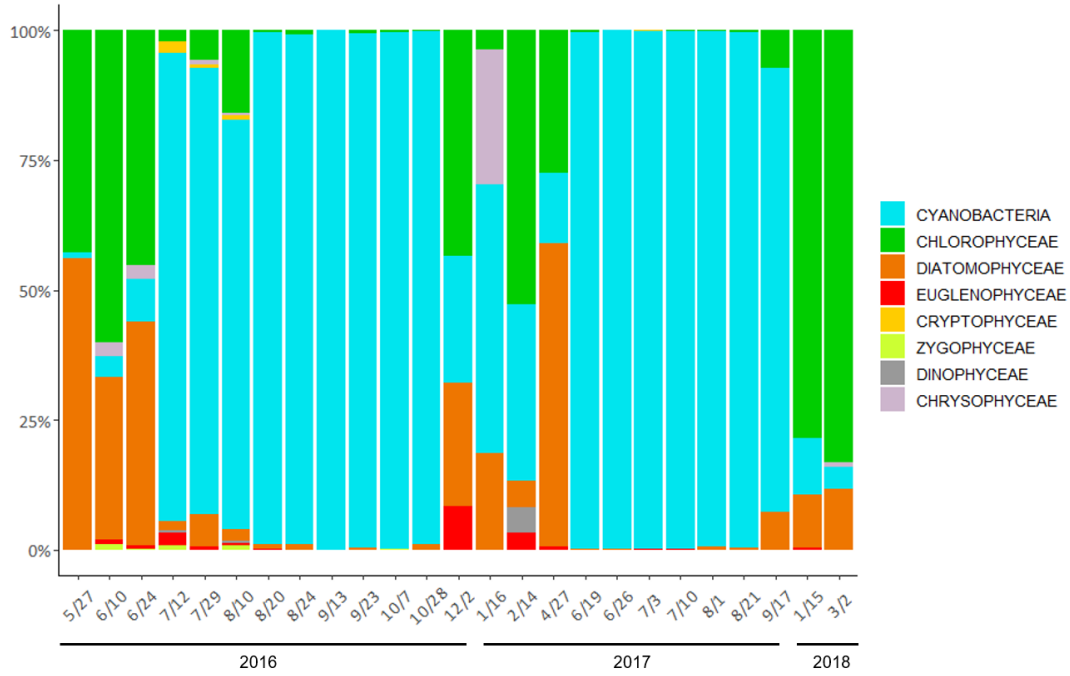


Fig. 2: Dynamics of phytoplankton community structure at the freshwater site F1 over the 2 year field study. The x axis corresponds to sampling dates (and not time).

The proliferation dynamics of cyanobacteria presented in Fig. 3 shows that the biomass reached very high concentrations in the freshwater reservoir (F1 site) (> 10^5 cells mL^{-1}) in summer and fall (from July to October) with a peak of 10^6 cells mL^{-1} in 2016 and of $2x10^6$ cells mL^{-1} in 2017. These intense cyanobacterial blooms were also observed at F2 in the riverine section downstream of the reservoir with a slightly lower biomass.

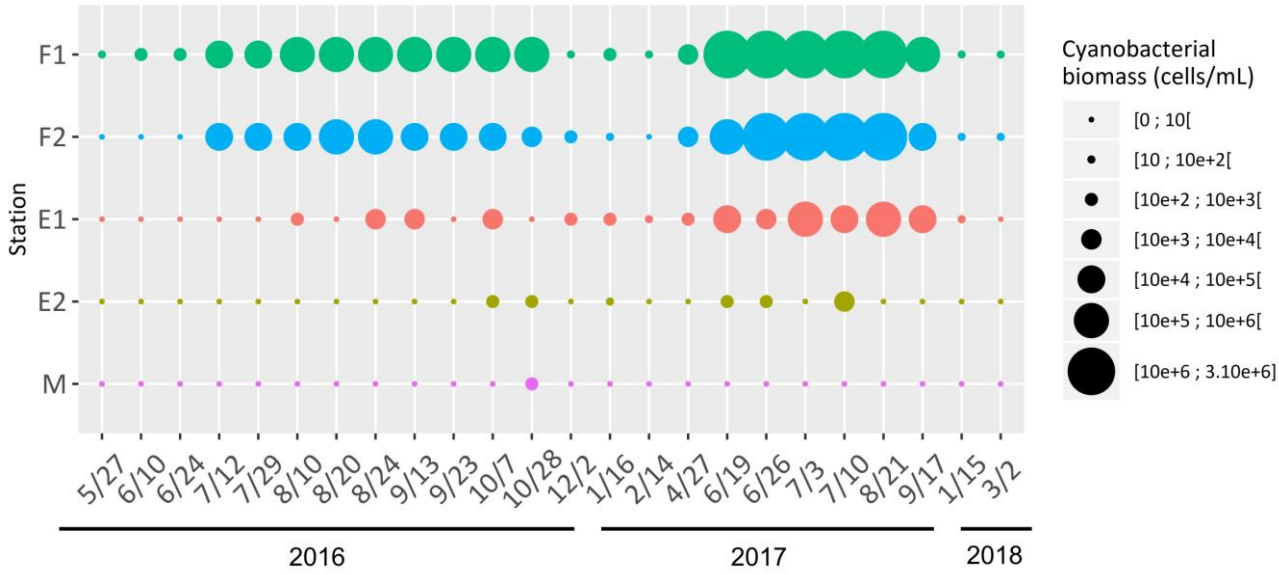


Fig. 3: Dynamics of the cyanobacterial biomass (cells/mL) along the freshwater-marine continuum at the 5 sampling sites. The x axis corresponds to sampling dates (and not time).

Transfer to the estuary was demonstrated with a progressively lower cyanobacterial biomass along the estuary as the distance from the upstream reservoir increased. Downstream cells concentrations were always related to upstream cells concentrations suggesting that they were due to horizontal transfer and not to *in situ* growth. A maximum of $2x10^5$ cells mL^{-1} was recorded at E1 in June 2017 and a maximum of $1.2x10^3$ cells mL^{-1} was observed at E2 on the same day. At the most downstream station M, coinciding with the marine outlet, a maximum of 240 cells/mL was recorded (in October 2016).

3.2. Environmental factors associated with the cyanobacterial biomass

The spatial and temporal variations of cyanobacterial biomass were associated with physico-chemical conditions presented in Fig. 4: a statistically significant seasonal variation in water temperature was observed with maxima in June/August and minima in December/February ($p=10^{-8}$) but relatively little spatial variation along the estuary ($p=0.97$). On the contrary, conductivity exhibited a strong statistically significant spatial variation ($p=3x10^{-11}$) and a relatively low temporal gradient ($p=0.82$). Hence temporal variation in cyanobacterial biomass was essentially correlated with changes in temperature whereas spatial variation was mostly derived from the longitudinal gradient of salinity/conductivity. A threshold of 18°C corresponded to a biomass higher than 10^5 cells mL^{-1} in the reservoir upstream. The

concentration variation in dissolved oxygen gave an indication of photosynthetic activity, values greater than 100% saturation corresponding to photosynthetic production conditions, values around 100% indicating a balance between the water and the atmosphere and values below 100% indicating respiration or consumption by bacteria.

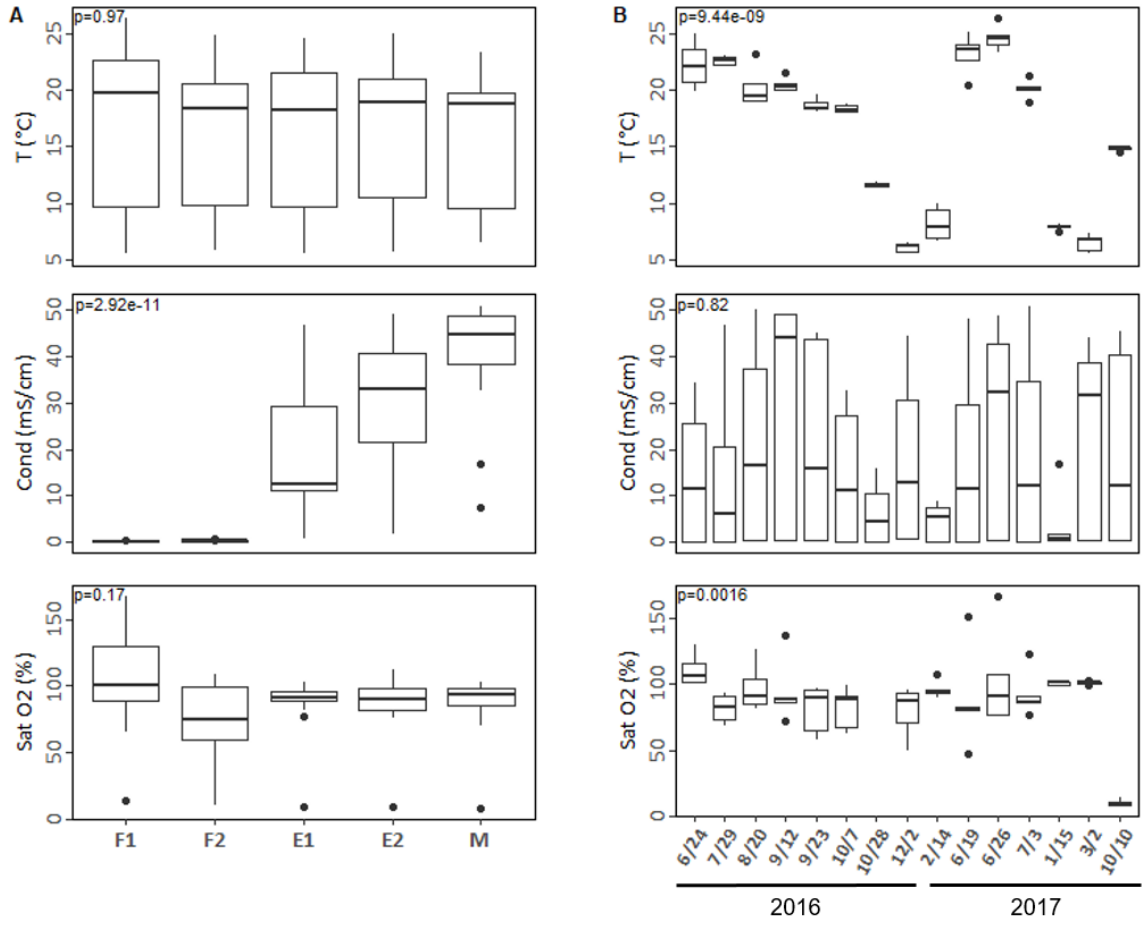


Fig. 4: Spatio-temporal variation of physicochemical conditions measured during the 2 year study along the freshwater to marine continuum.

Dissolved oxygen displayed a statistically significant temporal variation (p=0.0016) while no spatial variation (p=0.17) was observed during the study period. In the reservoir (F1 site) during high cyanobacterial biomass, the dissolved oxygen was oversaturated, while in the shallow F2 site where low water level (< 1m) of near stagnant waters coincided with high biological degradation and bacterial consumption, the dissolved oxygen was strongly undersaturated. Further downstream in the estuary (E1 and E2 sites) the dissolved oxygen was around 80 to 100 % saturation.

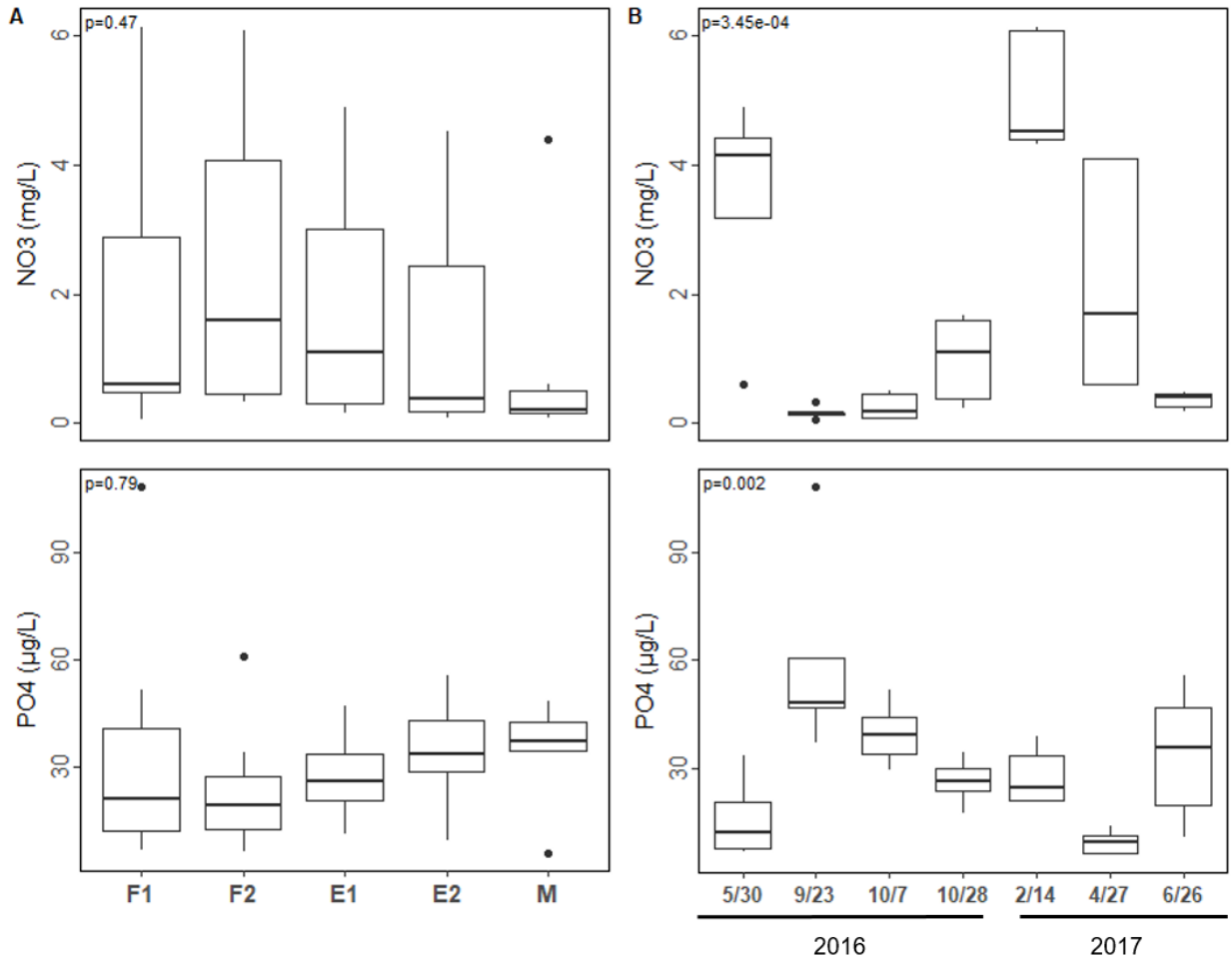


Fig. 5: Spatio-temporal variation of dissolved nutrient concentrations measured during the two year study along the freshwater to marine continuum.

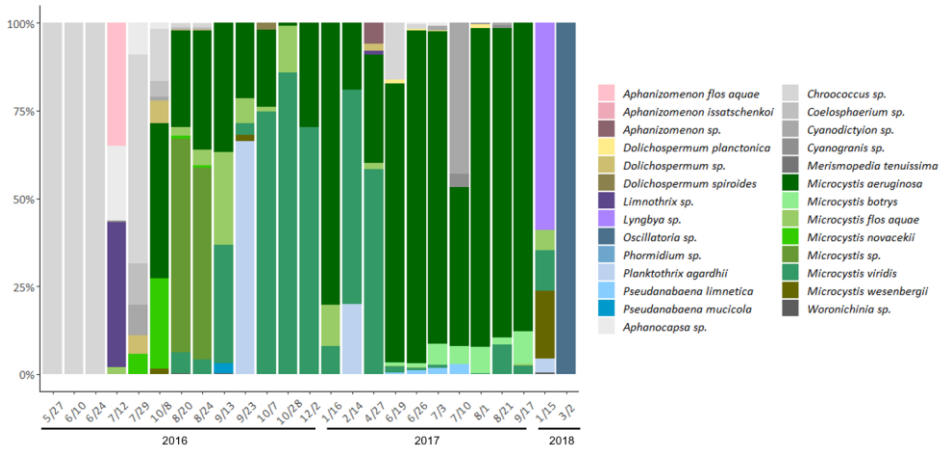
Both dissolved nutrients (NO3 and PO4) displayed a statistically significant temporal variation while the spatial variation only showed tendencies (Fig. 5). Nitrate concentrations presented an upstream to downstream decrease while phosphate concentrations showed the opposing trend with higher values downstream.

3.3. Cyanobacterial species composition along the salinity gradient

The composition of cyanobacterial populations was very diverse in the freshwater reservoir with 27 species present during the study. As Fig. 6 shows, during the strongest proliferations (from August to October both years), a dominance of the genus *Microcystis* was observed at the F1 site with up to 7 different species of *Microcystis* i.e. *M. aeruginosa, M. viridis, M. flos aquae, M. wesenbergii, M. novacekii* and *Microcystis sp*. This same diversity and composition was observed at the F2 site

(not shown). There was a shift in the dominant *Microcystis* species between 2016 and 2017 with a mix of *M. viridis, M. flos aquae, M. aeruginosa* and *Microcystis sp* in 2016 and an almost monospecific bloom of *M. aeruginosa* in 2017.

The transfer to the estuary selected for certain species according to their adaptation to the new physicochemical conditions, mainly salinity (recorded as conductivity). Up to half of the cyanobacterial species were present in the estuarine section: 14 species at E1, 7 at E2 and 1 at M. The maximum conductivity recorded during sampling in summer and fall was 12 mS cm^{-1} at E1, 35 mS cm^{-1} at E2 and 50 mS cm^{-1} at M. The species that survived the transfer through the estuary were several species of *Microcystis*, *Aphanizomenon sp., Pseudanabaena limnetica* and *Planktothrix agardhii,* all recorded at the estuarine site E2. *P. agardhii* was the only cyanobacterial species recorded at the marine outlet M. The transfer of *P. agardhii* was observed on 4 October 2016 between the freshwater site F2 (density *P. agardhii* 7400 cells mL^{-1}, *Microcystis sp* 22000 cells mL^{-1}), through the estuarine E1 site (density *P. agardhii* 4480 cells mL^{-1}, *Microcystis sp* 2200 cells mL^{-1}), and the estuarine E2 site (*P. agardhii* density 533 cells mL^{-1}, *Microcystis sp* 267 cells mL^{-1}). Another transfer to the marine outlet was observed at the end of October 2016 between E2 (432 cells mL^{-1} of *P. agardhii*, 480 cells mL^{-1} of total cyanobacteria) and M (240 cells mL^{-1} of *P. agardhii*, 100% cyanobacteria), while *P. agardhii* was not the majority at F2 (160 cells mL^{-1} *P. agardhii*, 4400 cells mL^{-1} *Microcystis sp*.). Among the other filamentous cyanobacteria surviving the estuarine transfer, *Pseudanabaena limnetica* was observed on 26 June 2017 with a decreasing concentration from 165120 cells/mL at F1 to 7680 cells mL^{-1} at E1 down to 192 cells mL^{-1} at E2 while *Aphanizomenon sp* was observed on 27 April 2017 from 220 cells mL^{-1} at F2, down to 132 cells mL^{-1} at E1 and 8 cells mL^{-1} at E2. It is interesting to note that the relative transfer of filamentous cyanobacteria was higher than that of either unicellular (*Aphanocapsa*) or colonial (*Microcystis*). Of the transferred *Microcystis* species, *M. viridis* was observed further downstream than *M. aeruginosa* and *M. flos aquae* in 2016 while the opposite was observed in 2017, in accordance with the relative biomass in the freshwater section.



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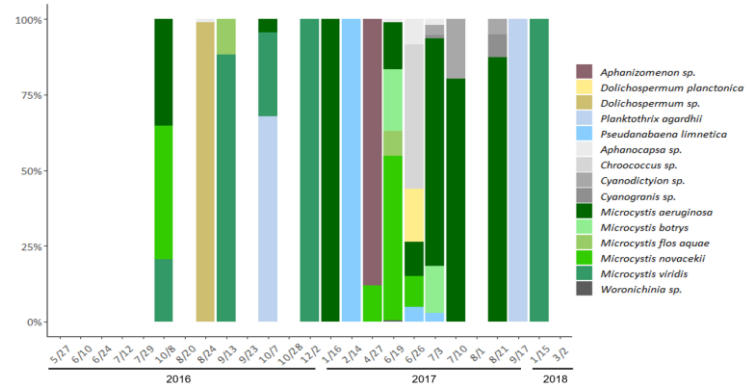

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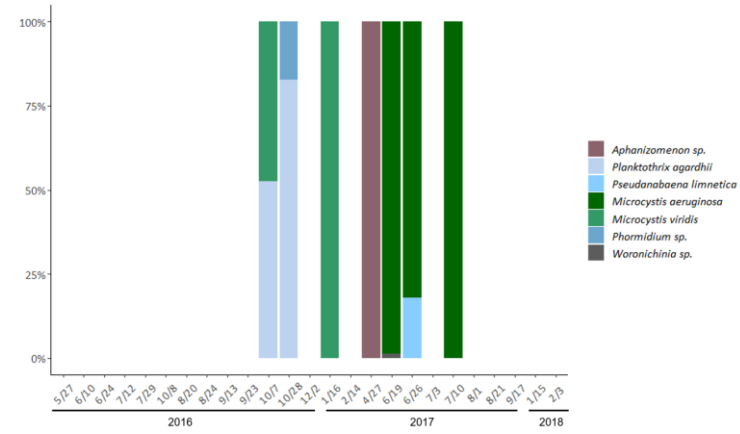

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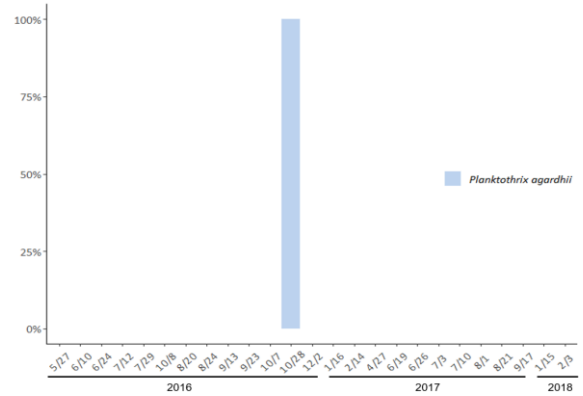

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296 *Fig. 6: Dynamics of cyanobacterial species composition at stations F1, E1, E2 and M*
297 *over the 2 year field study period.*

The cyanobacterial species diversity was also quantified through the cyanobacterial Shannon index which is presented in Fig. S1. A gradual decrease of the Shannon index along the salinity gradient is observed together with higher values in summer and fall.

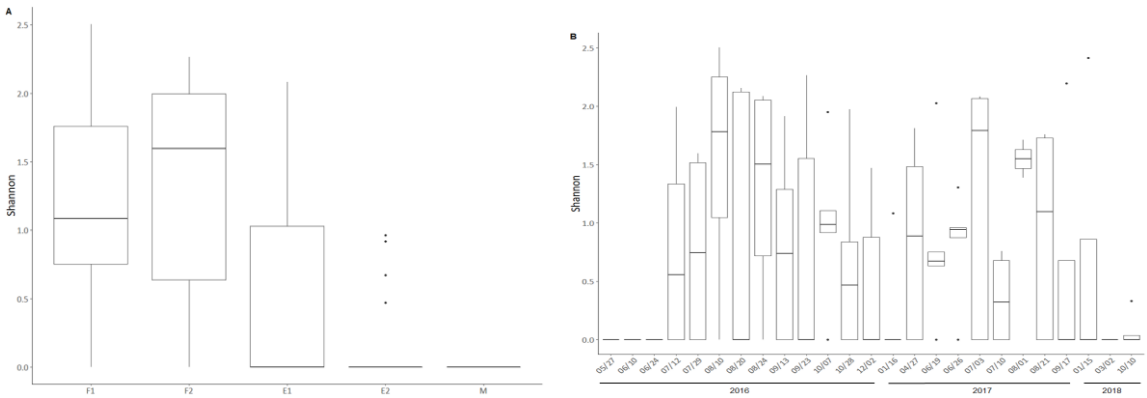


Fig. S1: Spatial and temporal variation of the cyanobacterial Shannon diversity index

A PCA analysis performed on the complete dataset (Fig. S2) confirmed the stronger role of salinity (i.e. conductivity) in structuring the spatial distribution of the observations together with the Shannon diversity index, while the cyanobacterial biomass was most strongly associated with elevated temperature and dissolved oxygen concentration.

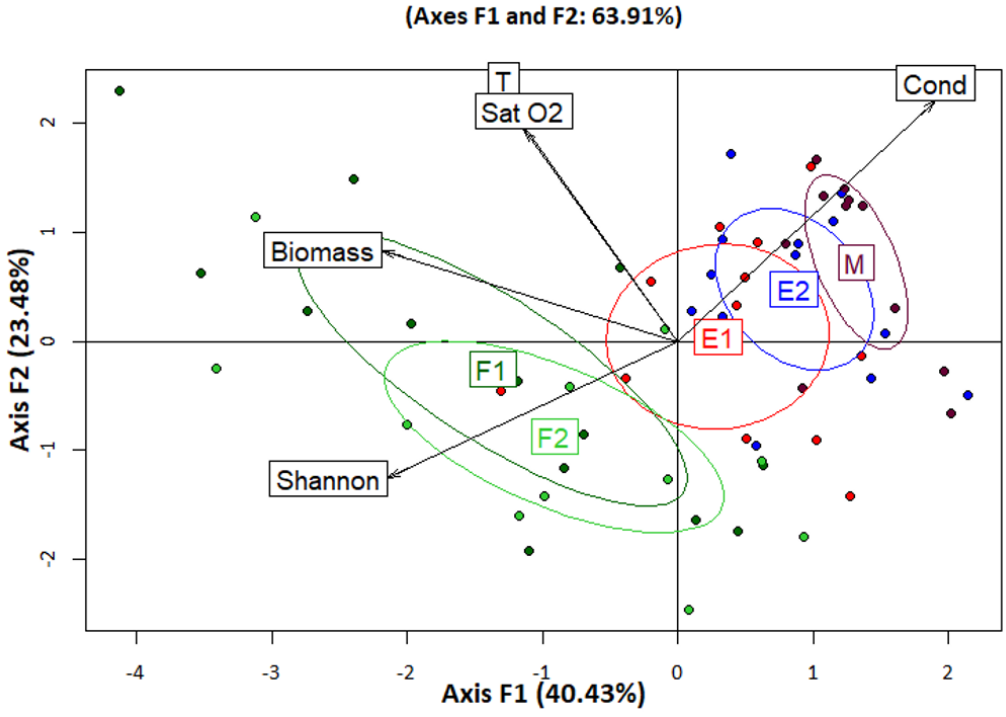


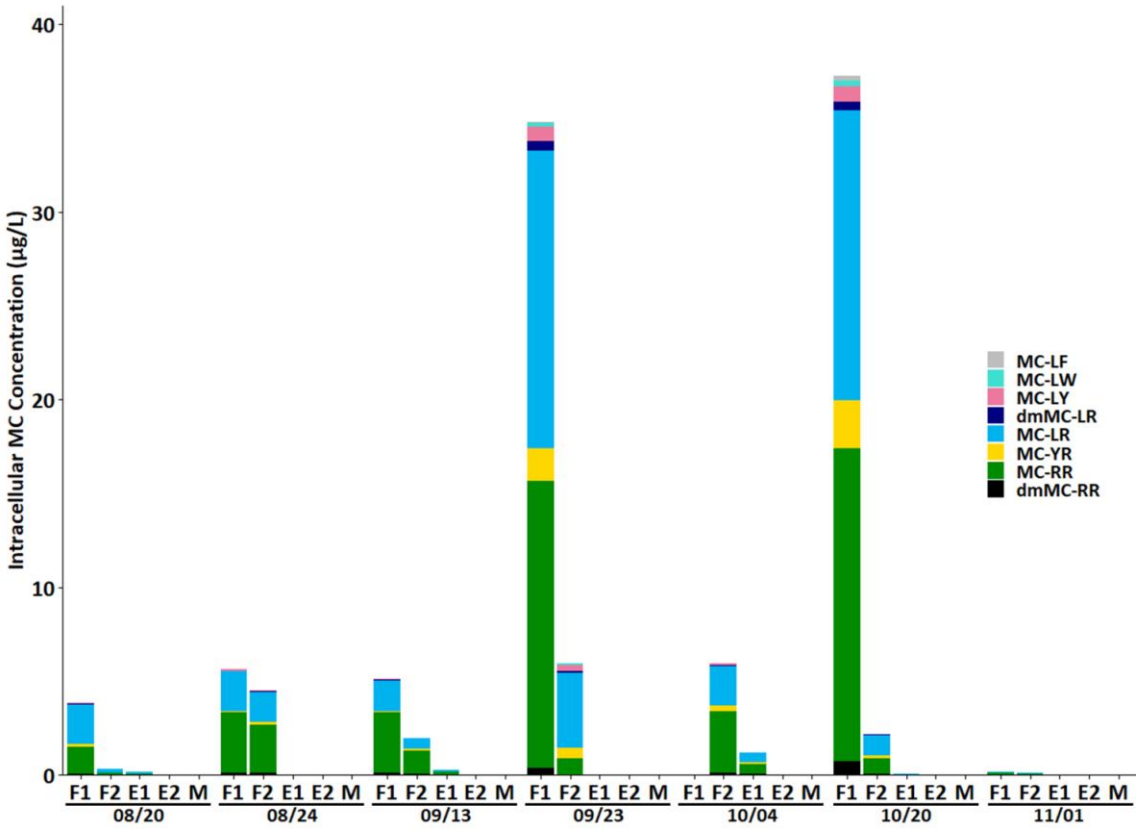
Fig. S2: PCA analysis highlighting the spatial variation in physico-chemical (T, O2, Cond) and biological parameters (cyanobacterial biomass, Shannon index)

3.4 Transfer of cyanotoxins along the freshwater-marine continuum

This study presents the concentrations of non-protein bound lipophilic cyanotoxins, MC and NOD in particulate samples and in the filtrates during the field campaigns of 2016 and 2017. In particular, the intracellular and extracellular forms of MC are separated to consider the potential risk associated with the lysing of the cells at high salinities.

3.4.1. Intracellular toxins

Firstly, the toxins analysis of the particulate samples of the different stations didn't reveal the presence of NOD, which corroborates the observation of cyanobacteria species not potentially producing NOD. The time variation shows the annual reproducibility with measured concentrations and transfer of MC occurring in both 2016 and 2017 with higher toxin concentrations reached in 2017 (Fig. 7).



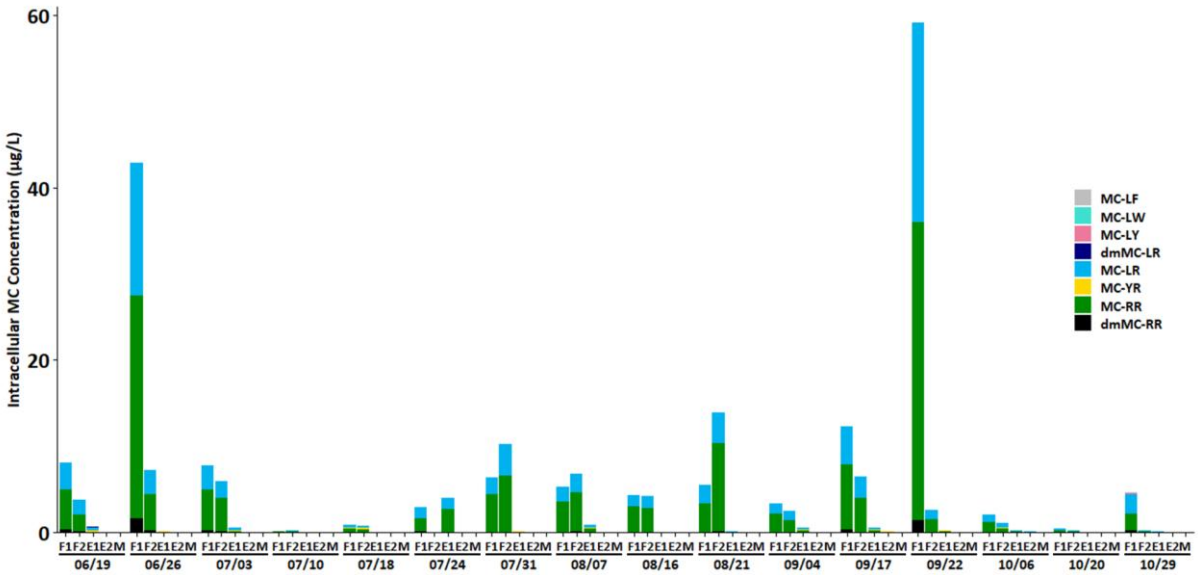


Fig. 7: Intracellular concentrations of MC variants in the cyanobacteria during the field campaigns of A) 2016 and B) 2017. Note that the x axis corresponds to sampling dates (and not time).

While the presence of MC was recorded in all the freshwater samples (at F1 and F2), a high temporal variation in concentration was observed with a maximum concentration of total MC (sum of the MC analogs) of 40 µg L^{-1} in September / October 2016 and 60 µg L^{-1} in September 2017. Among the MC variants observed, some MC analogs (MC-RR, MC-LR, dmMC-RR and MC-YR) were detected in all samples while others (dmMC-LR, MC-LY, MC-LW, MC- LF) were only recorded in some samples. The three dominant variants at F1 were MC-LR, MC-RR and MC-YR. The total MC values at F2 reached a maximum of 14 µg L^{-1} with a dominance of MC-RR, MC-LR, dmMC-RR and MC-YR. A demonstrated transfer of cyanotoxins through the estuary was recorded on many occasions (Table 1) with progressively lower intracellular toxins concentrations reaching 1.15 µg L^{-1} at E1 (comprising of MC-RR, MC-LR, MC-YR, dmMC-RR). In a less frequent manner, a maximum of 0.14 µg L^{-1} was recorded at E2 (comprising of MC-RR, ML-LR) and a maximum of 0.03 µg L^{-1} at site M (MC-RR, MC-LR). In summary, we observed a gradual decrease in occurrence and in concentrations of intracellular toxins from upstream to downstream.

Sites	FO % intra MC (Jun – Nov)		Max intra MC µg/L
	2016	2017	
F1	100	100	60
F2	100	100	14
E1	67	87	1.15
E2	17	38	0.14
M	0	27	0.03

Table 1: Frequency of occurrence (in %) and maximum concentrations of intracellular MC at the 5 stations during the period June to November of both sampling years

The results of this study show relatively strong overall correlation between the amount of toxins in the phytoplankton samples and the total cells density of potentially toxic cyanobacteria (correlation coefficient of 0.85, $p=10^{-11}$). However, when we consider relationships on specific dates, the correlation varies with the dominant cyanobacterial species. For example, in August 2016, a maximum of 5 µg MC L^{-1} was observed at site F1, associated with a cyanobacterial density of 900,000 cells/mL composed mainly of *Microcystis* sp, whereas up to 40 µg MC L^{-1} were measured at the same site in October 2016, associated with a density of 360,000 cells mL^{-1} composed essentially of the species *P. agardhii*. Transfer of *P. agardhii* (4480 cells mL^{-1}) to the estuarine site E1 in early October 2016 coincided with the presence of MC (1.2 µg MC L^{-1}). MC was also measured at E1 in August, September and the end of October, at concentrations close to 1 µg MC L^{-1}. MC in low concentration was measured in the particulate samples at E2 and at M despite the presence of low density cyanobacteria.

3.4.2. Extracellular toxins

The extracellular toxins (measured in the filtered water) were analyzed only from July 2017 to October 2017 (Fig. 8). Similarly to the intracellular fraction, no NOD was detected. The extracellular MC profiles revealed a dominance of MC-LR, MC- RR and MC-YR. Maximum MC concentrations of 0.65 µg L^{-1} were found at F1 in the

upstream freshwater reservoir. We observed higher MC concentrations downstream of F2 during August and September 2017 in the estuarine section and marine site.

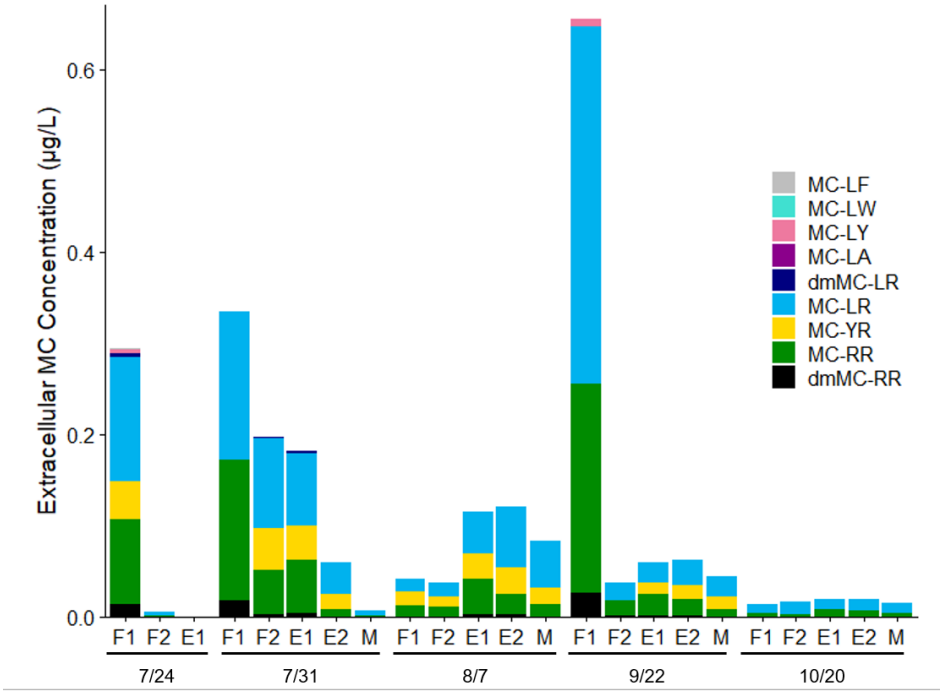


Fig. 8: Extracellular concentrations of MC variants in the filtered water at the 5 sampling sites during summer and fall 2017

When comparing ratios of extracellular MC to the total MC (intracellular + extracellular fractions) along the salinity gradient, we observed a consistent trend with minimal contribution of extracellular toxins in freshwater and a gradual increase with increasing salinities (Fig. 9). At site E1 where the maximum conductivity recorded during sampling during 2017 was 12 mS cm^{-1}, the extracellular contribution was up to 50%, while it increased to 100% at sites E2 and M where conductivities were above 35 and 47 mS cm^{-1} respectively.

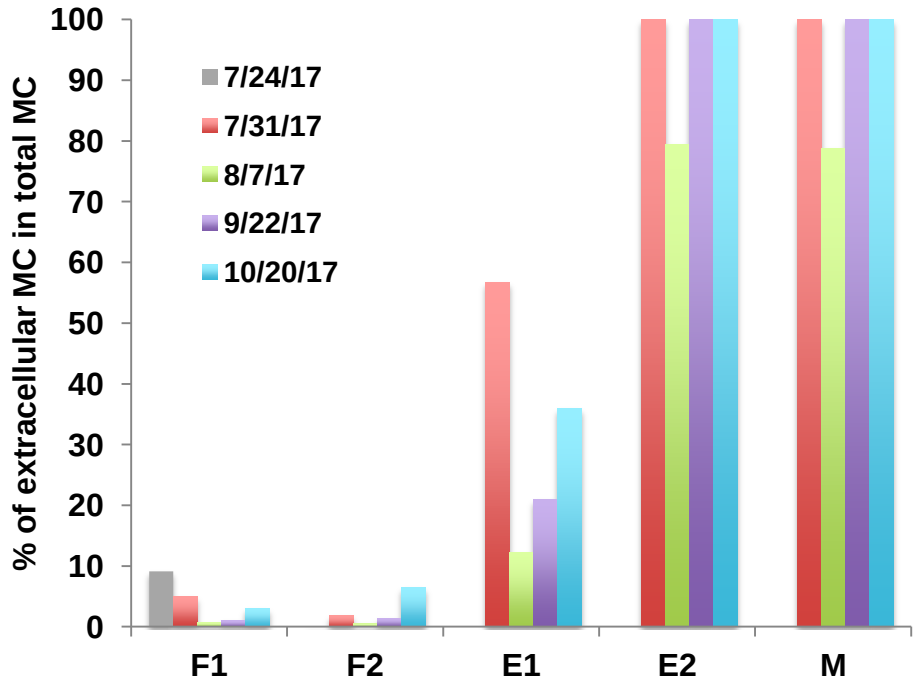


Fig. 9: Contribution of extracellular MC to the total MC concentrations at the 5 sampling sites during summer and fall 2017

4. Discussion

This study demonstrated over 2 successive years the recurrent transfer of cyanobacteria and cyanotoxins along the freshwater – marine continuum in a Brittany estuary, France. This result is in accordance with published studies worldwide. This transfer was first reported in the late 1980's in Europe (Sivonen et al., 1989) and USA (Paerl, 1988) but have gained an interest recently (Preece et al, 2017) and likely to increase in frequency in the future (Paerl et al., 2018). Although the biomass was lower than in the freshwater reservoir, cyanobacterial biomass of up to $2x10^5$ cells mL^{-1} was observed in the estuarine section. These cyanobacteria could therefore contribute significantly to the phytoplankton community structure of the estuary, which has typically a much lower biomass (usually reported in cells L^{-1}) than in freshwater, or serve as food source for invertebrates. While the temporal variation in cyanobacterial densities was mainly associated with temperature, spatial variation was due to salinity while nutrients were unlikely to limit cyanobacterial growth. Nitrate presented an upstream to downstream decrease suggesting a freshwater dominant source. Phosphate values showed the opposing trend with higher values

downstream and in particular at the outlet where we expect sediment resuspension to be the largest, based on the observed high concentrations of suspended particles.

Although the freshwater discharge was not monitored and therefore not quantified, the progressive increase in salinity near the surface was a good indicator of dilution. Possible light limitation could also be important but the short residence time (of the order of one to two days), inferred from observations of the surface velocities during sampling, would not permit *in situ* growth. Hence we suggest that the lower biomass in the estuary results from freshwater discharge dilution with estuarine waters.

The dominant blooming genus in the upstream freshwater reservoir was overwhelmingly *Microcystis*, consistent with the most widespread cyanobacterial occurrence of *Microcystis* in freshwaters worldwide (Harke et al., 2016) and as well as in Brittany (Pitois et al., 2014). While cyanobacterial transfer from freshwaters to estuaries has been reported for around 20 years, the majority of studies worldwide reported that *M. aeruginosa* was the dominant species transferred to coastal waters (Preece et al., 2017 for a review). *M. aeruginosa* is described as one of the freshwater cyanobacteria with the highest salinity tolerances (Verspagen et al., 2006) but variable thresholds have been observed from 4 ppt (Chen et al., 2015), 10 ppt (Lewitus et al., 2008; Tonk et al., 2007) to 35 ppt (Miller et al., 2010 ; Black et al., 2011) possibly showing strong intraspecific variability. In this study, different *Microcystis* species were transferred through the estuary in 2016 and 2017, in accordance with their relative biomass upstream. Therefore we cannot suggest a stronger resistance of either of the *Microcystis* species (*M. aeruginosa*, *M. viridis* and *M. flos aquae*). While the mucilage associated with the colonial form of *Microcystis* is likely to protect the cells from osmotic shock at high salinity, as suggested by Kruk et al.,2017 and Martínez de la Escalera et al., 2017, the relative resistance of the different *Microcystis* species is not known. Filamentous cyanobacteria i.e. *Planktothrix* and *Pseudanabaena* have been shown in this study to also survive the transfer through the estuary and therefore the salinity stress. The salinity tolerance of *P. agardhii* has been recently tested on brackish isolated species and a tolerance value of up to a salinity of 15 has been found (Vergalli et al., 2016). The transfer of *Planktothrix agardhii* through an estuary has been recently reported on one occasion although the bloom was non toxic (Churro et al., 2017). *Pseudanabaena sp.* has

been occasionally reported in the Baltic Sea (Lopes and Vasconscelos, 2011), however, the role of the mucilage/sheath around the filaments, as a potential protective morphological characteristic has not been suggested. On the other hand unicellular species present in relatively high numbers in the freshwater reservoir (i.e. *Aphanocapsa* sp., *Coelosphaerium sp., Cyanodiction sp.* or *Cyanogranis sp.*) were not found in the estuary. A possible explanation besides a sensitivity to salinity is that these species might be difficult to identify in samples with high suspended matter, and therefore not accounted for in the counting.

Intracellular MC was detected in all samples in the freshwater section with concentrations reaching 40 µg L^{-1} in 2016 and 60 µg L^{-1} in 2017. These levels are much higher than the alert 3 warning level of 10-20 µg L^{-1} of the World Health Organization forbidding any recreational activities in waterbodies (Ibelings et al., 2014; Funari et al., 2017). In this study, the measured gradual decrease in cyanobacterial biomass was accompanied by a decrease in intracellular MC concentrations from upstream to downstream indicating a likely dilution effect as expected due to strong tidal influence, even though we minimized that influence by sampling within one hour of low tides. This decrease has also been reported in the San Francisco Estuary (Lehman et al., 2008) and in Monterey Bay (Gibble and Kudela, 2014). A concomitant physiological response to MC production could also be possible at high salinity. Indeed, the effect of salinity on the physiology of *M. aeruginosa* has demonstrated a reduction in the production of MCs under salt stress (Black et al., 2011; Martín-Luna et al., 2015).

A highly significant overall relationship between potentially toxic cyanobacterial biomass and intracellular MC concentrations was found. In 2017, there was an early summer dominance of the small unicellular non-toxic cyanobacteria *Cyanodyction sp.* and *Cyanogranis sp.* which did not occur in 2016 when the summer blooms were dominated by the potentially toxic *M. viridis*. The toxicity of *Microcystis* is also known to vary among species, *M. wesenbergii* being rarely found toxic, while the majority of *M. aeruginosa* and *M. viridis* is reported as toxic (Harke et al., 2016, Otten et al., 2017). The MC concentration in the reservoir and the estuary was most likely due to *Microcystis sp.*, due to its overwhelming dominance and the high percentage of potentially toxic species within that genus in Brittany (Pitois et al., 2014). *P. agardhii*

488 is also known to produce MCs in relatively large quantities (Briand et al., 2009; Lance
489 et al., 2007) and the most toxic bloom reported in Italy's estuarine waters was indeed
490 one of *Planktothrix* (De Pace et al., 2014).
491
492 Few studies report on the different variants of MC during blooms in the natural
493 environment although their identification is important as different variants display
494 different toxicities and health risks (Lehman et al., 2008; Otten et al., 2017).
495 Toxicological studies reported on similar LD_{50} values for MC-LR, MC-LA, MC-YR and
496 MC-YM while the value for MC-RR was 10 times higher (Sivonen and Jones, 1999),
497 while Gupta et al., 2003 reported that MC-LR was twice as toxic as MC-LA. The
498 dominant MCs variants (MC-LR and MC-RR in 2017 and (MC-LR, MC-RR and MC-
499 YR in 2016) were transferred without specific selection along the salinity gradient.
500 The difference between the two years of the dominant variants may be attributed to
501 different dominant *Microcystis* species as it is known that each species and strain
502 produced, in culture conditions or *in situ*, different variants in different proportions and
503 cell quantities (Rios et al., 2014; Briand et al., 2016; Otten et al., 2017). The
504 demonstrated concentrations of the three dominant variants of MC reported in this
505 study might therefore induce potential impacts on cyanobacterial consumers. Our
506 preliminary data (unpublished) show MC accumulation by filter feeding organisms
507 supporting this hypothesis. While we quantified 9 variants of MC it is still possible that
508 other variants were present in the samples.
509
510 In this study, a lysis of the cyanobacterial cells at high salinity was most likely as the
511 ratio of extracellular MC to the total MC concentration increased downstream and in
512 particular accounted for 100 % of the total MC concentrations at the two most
513 downstream sites. In accordance with literature results the dominant form of MC in
514 the freshwater section was intracellular indicating its constitutive nature (Orr and
515 Jones, 1998; Briand et al., 2012), while the dominant form became extracellular as
516 cells lysis increased at elevated salinity (Tonk et al., 2007).
517
518 **5. Conclusion**
519 This study reports on the transfer of both cyanobacteria and cyanotoxins from a
520 freshwater reservoir to the marine outlet in France. Moreover, the transfer through
521 the estuary occurred frequently over the 2 year study period. The higher the

concentrations upstream, the more intense the transfer. The very high correlation between the biomass of potentially toxic species and total MC concentrations suggests that the majority of the cyanobacterial present in the estuary were toxic. *Microcystis* which dominated the blooms in the freshwater reservoir was the most likely genus responsible for the measured MC concentrations in the estuary, followed by *P. agardhii* both being relatively resistant to the salinity gradient. The extracellular contribution to the total MCs increased from upstream to downstream in accordance with cells lysis at elevated salinity. Both intracellular and extracellular MC variants did not show specific selection along the salinity gradient and the dominance of two highly toxic variants (i.e. MC-LR and MC-YR) is worrisome, as it could impact cyanobacterial consumers.

Acknowledgments

The authors thank Alexandra Lepoutre, Maxime Georges des Aulnois and the PME Minyvel Environnement for help with field work and Marion Lengronne for nutrient analyses. Biochemical analyses and microscopy were supported by the Experimental Ecology (ECOLEX) and the Analytical (PLAY) platforms from the UMR Ecobio. This project was financed by the ANSES (Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'environnment et du travail) under the Bieautox project EST-2015-191.

References

AFSSA, 2006. Rapport sur l'évaluation des risques liés à la présence de cyanobactéries et de leurs toxines dans les eaux destinées à l'alimentation, à la baignade et autres activités récréatives. 300 p.

Albay, M., Matthiensen, A., Codd, G.A., 2005. Occurrence of toxic blue green algae in the Kucukcekmece Lagoon (Istanbul, Turkey). Environ. Toxicol. 20 : 227-284.

Aminot, A., Chaussepied, M., 1983. Manuel des analyses chimiques en milieu marin. [Chemical analyses in marine waters guide]. Centre National pour l'Exploration des Océans CNEXO Editeur, Brest, France; 395 p.

Anon, 2005. Anonyme ISO 20179:2005(E). Water quality – Determination of microcystins – Method using solid phase extraction (SPE) and high performance

liquid chromatography (HPLC) with ultraviolet (UV) detection. International Standard ISO 20179, first edition 2005-10-01, 17p.

Black, K., Yilmaz, M., Phlips, E.J., 2011. Growth and toxin production by *Microcystis aeruginosa* PCC 7806 (Kutzing) Lemmerman at elevated salt concentrations. J. Environ. Prot. 2, 669–674.

Briand, E., Escoffier, N., Straub, C., Sabart, M., Quiblier, C., 2009. Spatiotemporal changes in the genetic diversity of a bloom-forming *Microcystis aeruginosa* (cyanobacteria) population. ISME J 3: 419–429.

Briand, E., Bormans, M., Quiblier, C., Salençon, M.J., Humbert, J.F., 2012. Evidence of the cost of the production of microcystins by *Microcystis aeruginosa* under differing light and nitrate environmental conditions. PLoS One 7 (1), e29981.

Briand, E., Bormans, M., Gugger, M., Dorrestein, P.C., Gerwick , W.H., 2016. Changes in secondary metabolic profiles of *Microcystis aeruginosa* strains in response to intraspecific interactions. Environmental microbiology 18 (2), 384-400.

Brient, L., Lengronne, M., Bormans, M., Fastner, J., 2009. First occurrence of cylindrospermopsin in freshwater in France. Environmental Toxicology 24 (4), 415-420.

Chen, L., Maoa, F., Chira Kirumba, G., Jiang, C., Manefield, M., He, Y., 2015. Changes in metabolites, antioxidant system and gene expression in *Microcystis aeruginosa* under sodium chloride stress. Ecotoxicology and Environmental Safety 122 :126–135.

Churro, C., Azevedo, J., Vasconcelos, V., Silva, A., 2017. Detection of a *Planktothrix agardhii* Bloom in Portuguese Marine Coastal Waters. Toxins 9, 391 ; doi:10.3390/toxins9120391

De Pace, R., Vita, V., Bucci, M.S., Gallo, P., Bruno, M., 2014. Microcystin contamination in sea mussel farms from the Italian southern Adriatic coast following cyanobacterial blooms in the artificial reservoir. J. Ecosyst. 2014,1–11. doi.org/10.1155/2014/374027.

Dörr, F.A., Pinto, E., Soares, R.M., Azevedo, S.M.F.O., 2010. Microcystins in South American aquatic ecosystems: occurrence, toxicity and toxicological assays. Toxicon 56, 1247–1256.

Fraisse, S., Bormans, M., Lagadeuc, Y., 2013. Morphofunctional traits reflect differences in phytoplankton community between rivers of contrasted flow regime. Aquatic Ecology 47: 315–327. doi:10.1007/s10452-013-9446-z

Funari, E., Manganelli, M., Buratti, F.M., Testai, E., 2017. Cyanobacteria blooms in water: Italian guidelines to assess and manage the risk associated to bathing and recreational activities. Science of the total environment 598: 867-880.

607 Gibble, C.M., Kudela, R.M., 2014. Detection of persistent microcystin toxins at the
608 land-sea interface in Monterey Bay, California. Harmful Algae 39, 146–153.

609 Gibble, C.M., Peacock, M.B., Kudela, R.M., 2016. Evidence of freshwater algal toxins
610 in marine shellfish: Implications for human and aquatic health. Harmful Algae, 59:59-
611 66.

612 Gupta, N., Pant, S.C., Vijayaraghavan, R., Rao, P.V.L., 2003 Comparative toxicity
613 evaluation of cyanobacterial cyclic peptide toxin Microcystin variants (LR, RR, YR) in
614 mice Toxicology 188(2-3):285-96

615 Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A.,
616 Paerl, H. W., 2016. A review of the global ecology, genomics, and biogeography of
617 the toxic cyanobacterium *Microcystis spp*. Harmful Algae 54, 4–20.
618
619 Henriksen A, Selmer-Olsen AR. 1970. Automatic methods for determining nitrate and
620 nitrite in water and soil extracts. Analyst. 95:514–518.
621
622 Ibelings, B.W., Backer, L.C., Kardinaal, W.E.A., et Chorus, I., 2014. Current
623 approaches to cyanotoxin risk assessment and risk management around the globe:
624 Harmful Algae, 40, 63-74.

625 Kaebernick, M. and Neilan, B.A., 2001. Ecological and molecular investigations of
626 cyanotoxin production. FEMS Microbiol Ecol. 35(1):1-9

627 Karjalainen, M., Engstrom-Ost, J., Korpinen, S., Peltonen, H., Paakkonen, J.P.,
628 Ronkkonen, S., Suikkanen, S., Viitasalo, M., 2007. Ecosystem Consequences of
629 Cyanobacteria in the Northern Baltic Sea. Ambio, 36 : 2-3.
630
631 Kruk, C., Segura, A.M., Nogueira, L., Alcántara, I., Calliaria, D., Martínez de la
632 Escalerad, G., Carballoa, C., Cabreraa, C., Sarthoua, F., Scavone, P., Piccini, C.,
633 2017. A multilevel trait-based approach to the ecological performance of *Microcystis*
634 *aeruginosa* complex from headwaters to the ocean. Harmful Algae 70 : 23–36.
635
636 Lance, L., Paty, C., Bormans, M., Brient, L., Gérard, C., 2007. Interactions between
637 cyanobacteria and gastropods: II. Impact of toxic *Planktothrix agardhii* on the life-
638 history traits of *Lymnaea stagnalis*. Aquatic Toxicology 81 (4), 389-396
639
640 Lance, E., Brient, L., Carpentier, A., Acou, A., Marion, L., Bormans, M., Gérard, C.,
641 2010. Impact of toxic cyanobacteria on gastropods and microcystin accumulation in a
642 eutrophic lake (Grand-Lieu, France) with special reference to Physa (= Physella)
643 acuta. Science of the Total Environment 408, 3560–3568.
644
645 Lehman, P.W., Boyer, G., Hall, C., Waller, S., Gehrts, K., 2005. Distribution and
646 toxicity of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay
647 Estuary, California. Hydrobiologia 541, 87–99.
648
649 Lehman, P.W., Boyer, G., Satchwell, M., Waller, S., 2008. The influence of
650 environmental conditions on the variation of *Microcystis* cell density and microcystin
651 concentration in San Francisco Estuary. Hydrobiologia 600, 187– 204.

Le Moal, M., Gascuel-Odoux, C., Menesquen, A., Souchon, Y., Etrillard, C., Levain, A., Moatar, F., Pannard, A., Souchu, P., Lefebvre, A., Pinay, G., 2019. Eutrophication: A new wine in an old bottle? Science of the total environment. DOI:10.1016/j.scitotenv.2018.09.139

Lewitus, A.J., Brock, L.M., Burke, M.K., DeMattio, K.A., Wilde, S.B., 2008. Lagoonal stormwater detention ponds as promoters of harmful algal blooms and eutrophication along the South Carolina Coast. Harmful Algae 8, 60–65.

Lopes, V.R. and Vasconscelos, V., 2011. Planktonic and benthic cyanobacteria of European brackish waters: A perspective on estuaries and brackish seas European Journal of Phycology 46(3), 292-304. DOI: 10.1080/09670262.2011.602429

Martín-Luna, B., Sevilla, E., Bes, M.T., Fillat, M.F., Peleato, M.L., 2015. Variation in the synthesis of microcystin in response to osmotic stress in *Microcystis aeruginosa* PCC7806. Limnetica 34 (1), 205–214.

Martínez de la Escalera, G., Kruk, C., Segurad, A.M., Nogueira, L., Alcántara, I., Piccini, C., 2017. Dynamics of toxic genotypes of *Microcystis aeruginosa* complex (MAC) through a wide freshwater to marine environmental gradient. Harmful Algae 62 :73–83

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurés, E., Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ. Int. 59, 303–327.

Meriluoto, J., Blaha, L., Bojadzija, G., Bormans, M., Brient, L., Codd, G.A., Drobac, D., Faassen, E.J., Fastner, J., Hiskia, A., Ibelings, B.W., Kaloudis, T., Kokocinski, M., Kurmayer, R., Pantelić, D., Quesada, A., Salmaso, N., Tokodi, N., Triantis, T.M., Visser, P.M., Svirčev, Z., 2017. Toxic cyanobacteria and cyanotoxins in European waters - recent progress achieved through the CYANOCOST Action and challenges for further research. Advances in Oceanography and Limnology, 8(1) DOI: 10.4081/aiol.2017.6429

Metcalf, J.S., Codd, G.A., 2012. Cyanotoxins: In B. A. Whitton (ed.) The Ecology of Cyanobacteria II. Their Diversity in Space and Time, Second Edition, Springer, Dordrecht, 651-675pp.

Miller, M.A., Kudela, R.M., Mekebri, A., Crane, D., Oates, S.C., Tinker, M.T., Staedler, W. A., Miller, M., Toy-Choutka, W.A., Dominik, S., Hardin, C., Langlois, D., Murray, G., Ward, M.K., Jessup, D.A., 2010. Evidence for a novel marine harmful algal bloom: cyanotoxins (microcystin) transfer from land to sea otters. PLoS One 5 (9), 1–11.

Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 27:31–36.

Ndlela, L.L., Oberholster, P.J., Van Wyk, J.H., Cheng, P.H., 2016. An overview of cyanobacterial bloom occurences and research in Africa over the last decade. Harmful Algae 60, 11–26.

Omidi, A., Esterhuizen-Londt, M., Pflugmacher, S., 2017. Still challenging: the ecological function of the cyanobacterial toxin microcystin – What we know so far. Toxin Reviews, 37, Issue 2.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change Harmful Algae 14 : 313–334

Orr, P.T., Jones, G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnol Oceanogr 43: 1604–1614.

Orr, P.T., Jones, G.J., Douglas, G.B., 2004. Response of cultured *Microcystis aeruginosa* from the Swan River, Australia, to elevated salt concentration and consequences for bloom and toxin management in estuaries. Mar. Freshw. Res. 55 (3), 277– 283.

Ortiz et al., 2017: A high throughput targeted and non-targeted method for the analysis of microcystins and anatoxin-A using on-line solid phase extraction coupled to liquid chromatography–quadrupole time-of-flight high resolution mass spectrometry. Analytical and Bioanalytical Chemistry. 409 (21): 4959–4969.

Otten, T.G., Paerl, H.W., Dreher, T.W., Kimmerer, W.J., and Parker, A.E., 2017. The molecular ecology of *Microcystis sp.* blooms in the San Francisco Estuary. Environmental Microbiology, 19(9), 3619–3637.

Paerl, H., 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. Limn. Ocean. 33: 823-847.

Paerl, H., 2018. Mitigating Toxic Planktonic Cyanobacterial Blooms in Aquatic Ecosystems Facing Increasing Anthropogenic and Climatic Pressures. Toxins, 10, 76; doi:10.3390/toxins10020076

Paerl, H.W., Otten, T.G., Kudela, R., 2018. Mitigating the Expansion of Harmful Algal Blooms Across the Freshwater-to-Marine Continuum. Environ. Sci. Technol. 52 : 5519−5529

Paldavičiene A., Mazur-Marzec, H., Razinokovas, A., 2009. Toxic cyanobacteria blooms in the Lithuanian part of the Curonian Lagoon. Oceanologia 51 (2), 203– 216.

Peacock, M.B., Gibble, C.M., Senn, D.B., Cloern, J.E., Kudela, R.M., 2018. Blurred lines: Multiple freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California. Harmful Algae 73, 138-147.

Pitois, F., Thoraval, I., Baurès, E., Thomas, O., 2014. Geographical Patterns in Cyanobacteria Distribution: Climate Influence at Regional Scale. Toxins, 6, 509-522.

Pitois, F., Fastner, J., Pagotto, C., Dechesne, M., 2018. Multi-Toxin Occurrences in Ten French Water Resource Reservoirs. Toxins, 10, 283.

Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in Estuarine and Marine waters: Environmental implications and human health risk. Harmful Algae 61, 31–45.

Puddick, J., Prinsep, M.R., Wood, S.A., Kaufononga, S.A.F., Cary, S.C., Hamilton, D.P., 2014. High Levels of Structural Diversity Observed in Microcystins from *Microcystis* CAWBG11 and Characterization of Six New Microcystin Congeners. Mar. Drugs, 12, 5372-5395; doi:10.3390/md12115372

R Development Core Team. 2011. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing, Vienna, Austria http://www.r-project.org/

Rigosi, A., Carey, C.C., Ibelings, B.W., Brookes, J.D., 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa Limnol. Oceanogr., 59(1), 99–114.

Rinehart, K.L., Harada, K., Namikoshi, M., Chen, C., Harvis, C.A., Munro, M.H .G., Blunt, J.W., Mulligan, P.E., Beasley, V.R., Dahlem, A.M., Carmichael, W.W.,1988. Nodularin, microcystin, and the configuration of Adda. *J. Am. Chem. Soc.* 110 25 : 8557-8558

Ríos, V., Moreno, I., Prieto, A. I., Soria-Díaz, M. E, Frías J.E. and Cameán, A.M., 2014. Comparison of *Microcystis aeruginosa* (PCC7820 and PCC7806) growth and intracellular microcystins content determined by liquid chromatography–mass spectrometry, enzyme-linked immunosorbent assay, anti-Adda and phosphatase bioassay. Journal of Water and Health.12.1, 69-80.

Robson, B.J., Hamilton, D.P., 2003. Summer flow event induces a cyanobacterial bloom in a seasonal western Australia estuary. Mar. Freshw. Res. 54, 139–151.

Sivonen, K., Jones, G., 1999. Cyanobacterial Toxins. In: Chorus, I. and Bartram, J., Eds., Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management, E & FN Spon, London, 41-111.

Sivonen, K., Kononen, K., Carmichael, W., Dahlem, A.M., Rinehart, K.L., Kiviranta, J., Niemela, S.I., 1989. Occurrence of the hepatotoxic cyanobacterium Nodularia spumigena in the Baltic Sea and structure of toxin. Applied and Environmental Microbiology 55(8):1990-5

Taş, S., Okus, E., Aslan-Yilmaz, A., 2006. The blooms of a cyanobacterium, *Microcystis cf. aeruginosa* in a severely polluted estuary, the Golden Horn, Turkey. Estuar. Coast. Shelf Sci. 68, 593–599.

Tatters , A.O., Howard , M.D .A., Nagoda , C., Busse, L., Gellene , A.G., Caron, D.A., 2017 Multiple stressors at the land-sea interface: cyanotoxins at the land-sea

interface in the Southern California Bight. Toxins, 9, 95.

Tillett, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T., Neilan, B.A., 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide polyketide synthetase system. Chemistry & Biology, 7:753-764

Tonk, L., Bosch, K., Visser, P.M., Huisman, J., 2007. Salt tolerance of the harmful cyanobacterium *Microcystis aeruginosa*. Aquat. Microb. Ecol. 46, 117–123.

Umehara, A., Tsutsumi, H., Takahashi, T., 2012. Blooming of *Microcystis aeruginosa* in the reservoir of the reclaimed land and discharge of microcystins to Isahaya Bay (Japan). Environ. Sci. Pollut. Res. 19, 3257–3267.

Vergalli, J., Bertrand, C., Borla, A., Franquet, E., Fayolle, S., 2016. Salt tolerance of *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek (cyanoprokaryota) between freshwater and brackish strains in batch cultures. Algological Studies 150 : 3–20.

Verspagen, J.M.H., Passarge, J., Jöhnk, K.D., Visser, P.M., Peperzak, L., Boers, P., Laanbroek, H.J., Huisman, J., 2006. Water management strategies against toxic *Microcystis* blooms in the Dutch delta. Ecol. Appl. 16, 313–327.

Vezie, C., Brient, L., Sivonen , K., Bertru, G., Lefeuvre, J.C., Salkinoja-Salonen, M., 1998. Variation of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). Microb Ecol 35:126–35.

Figure

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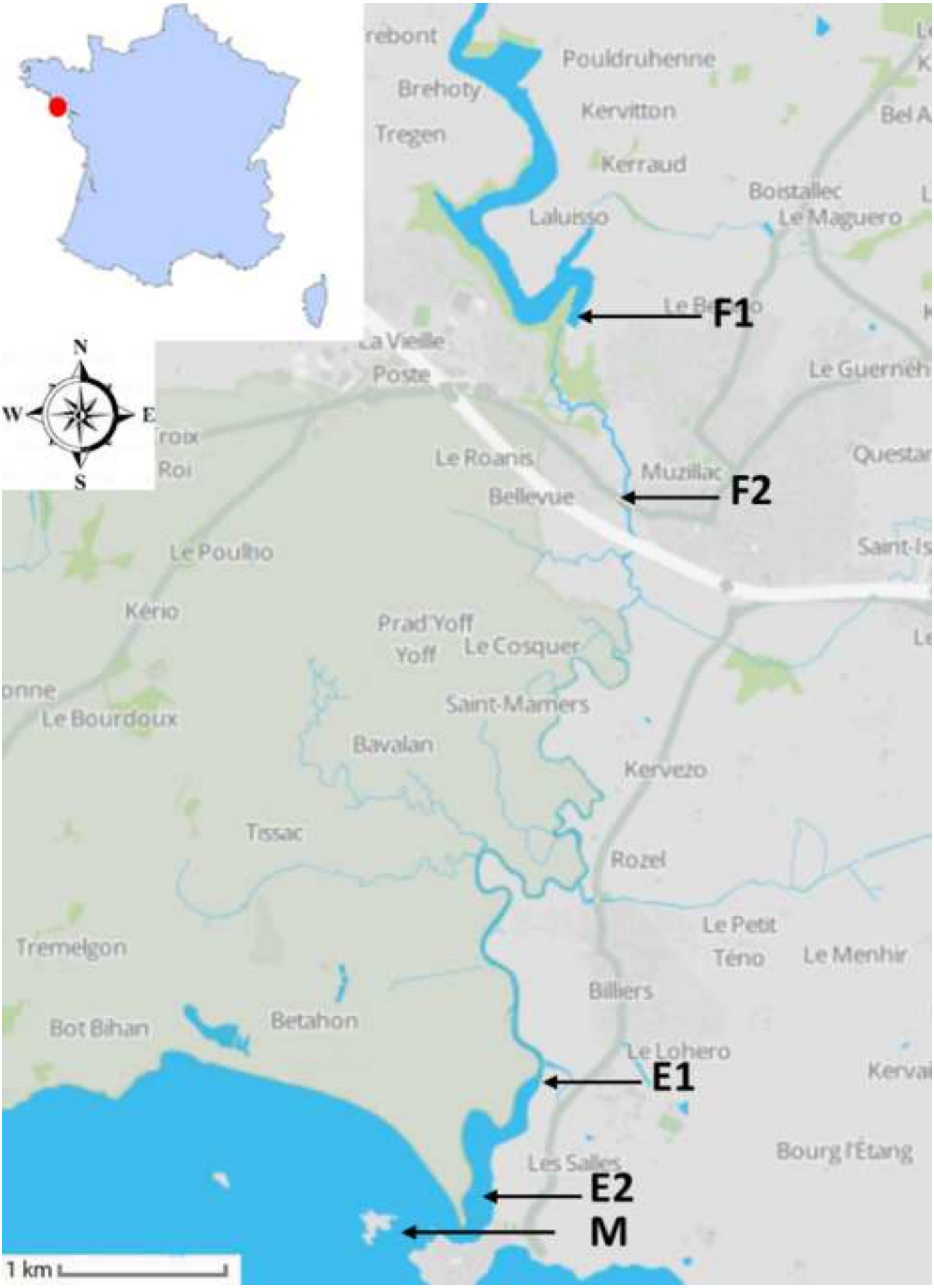

Figure 2

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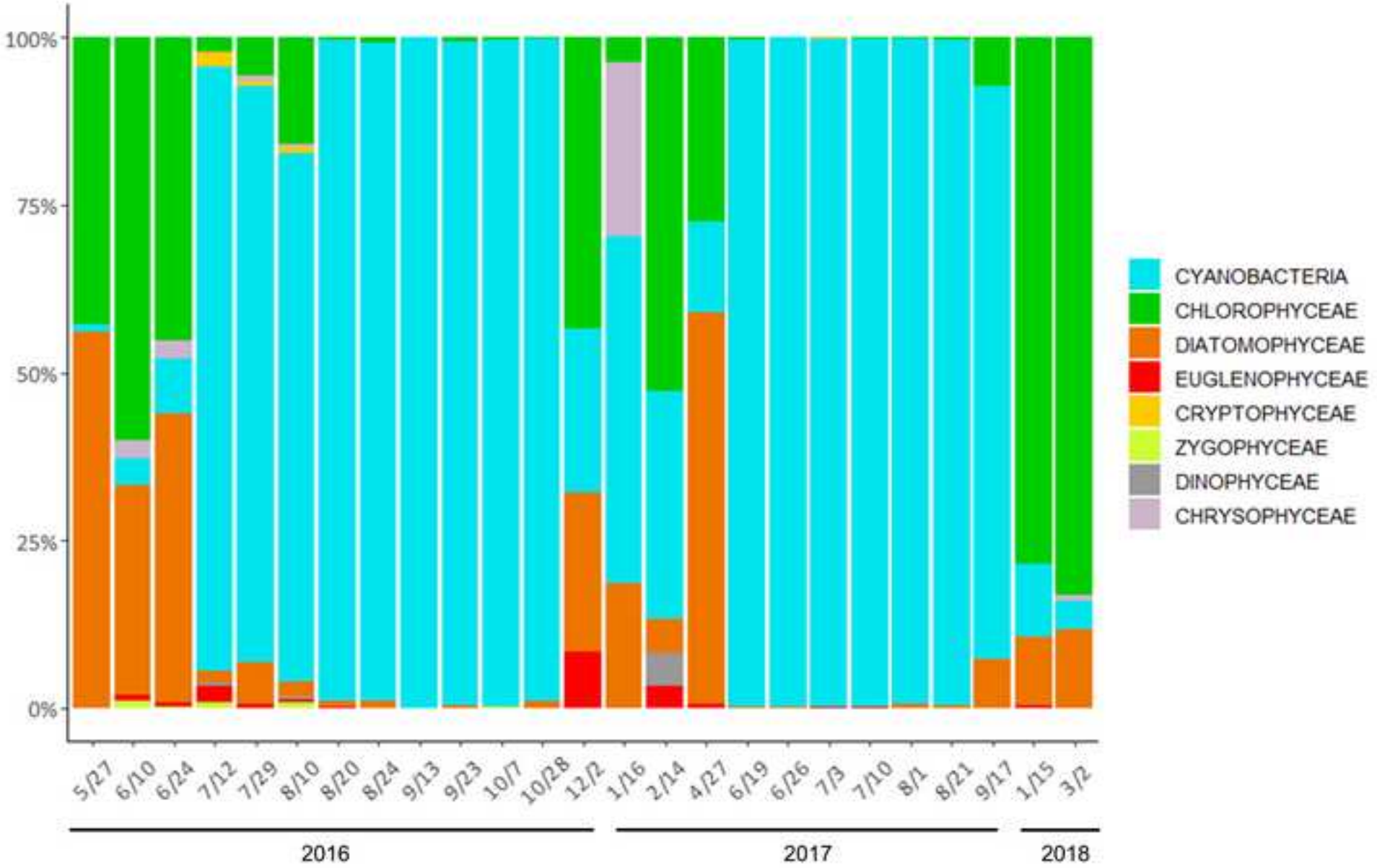

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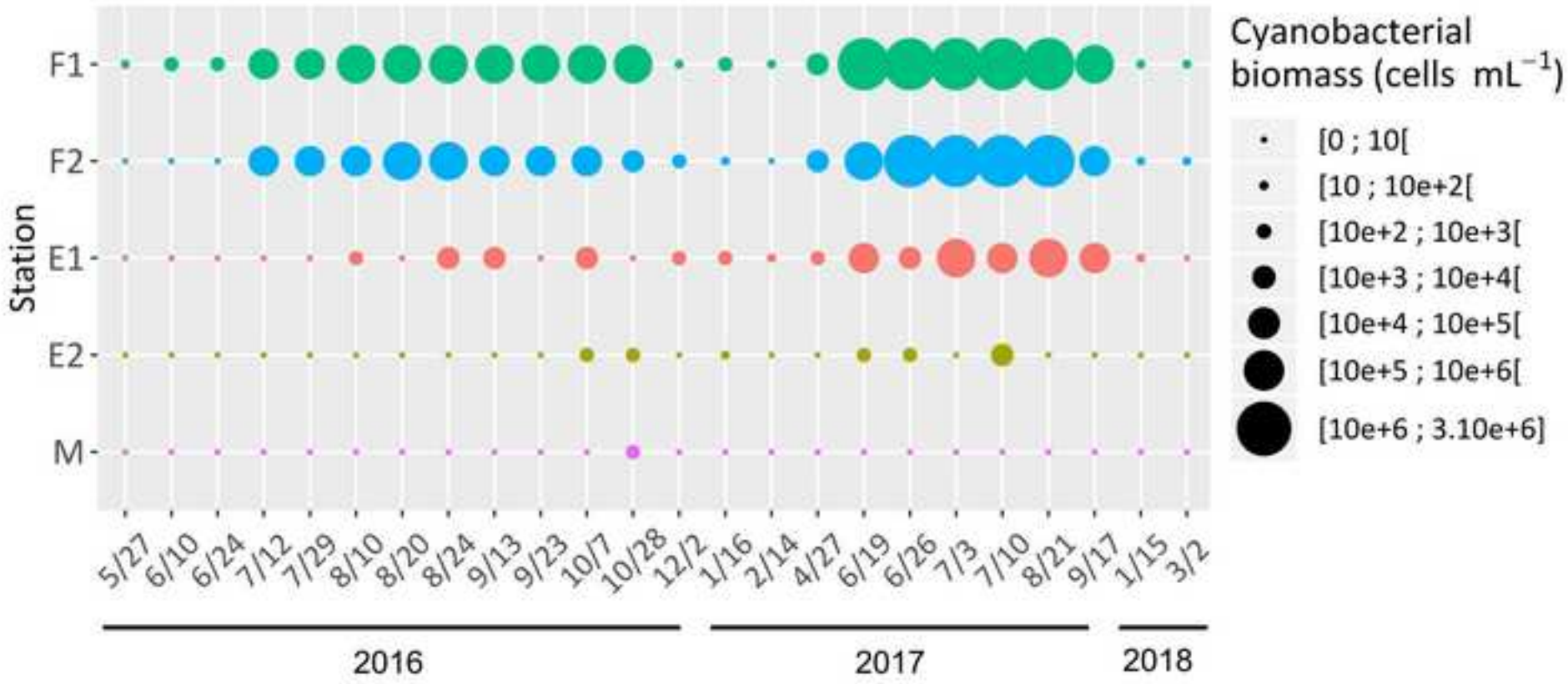

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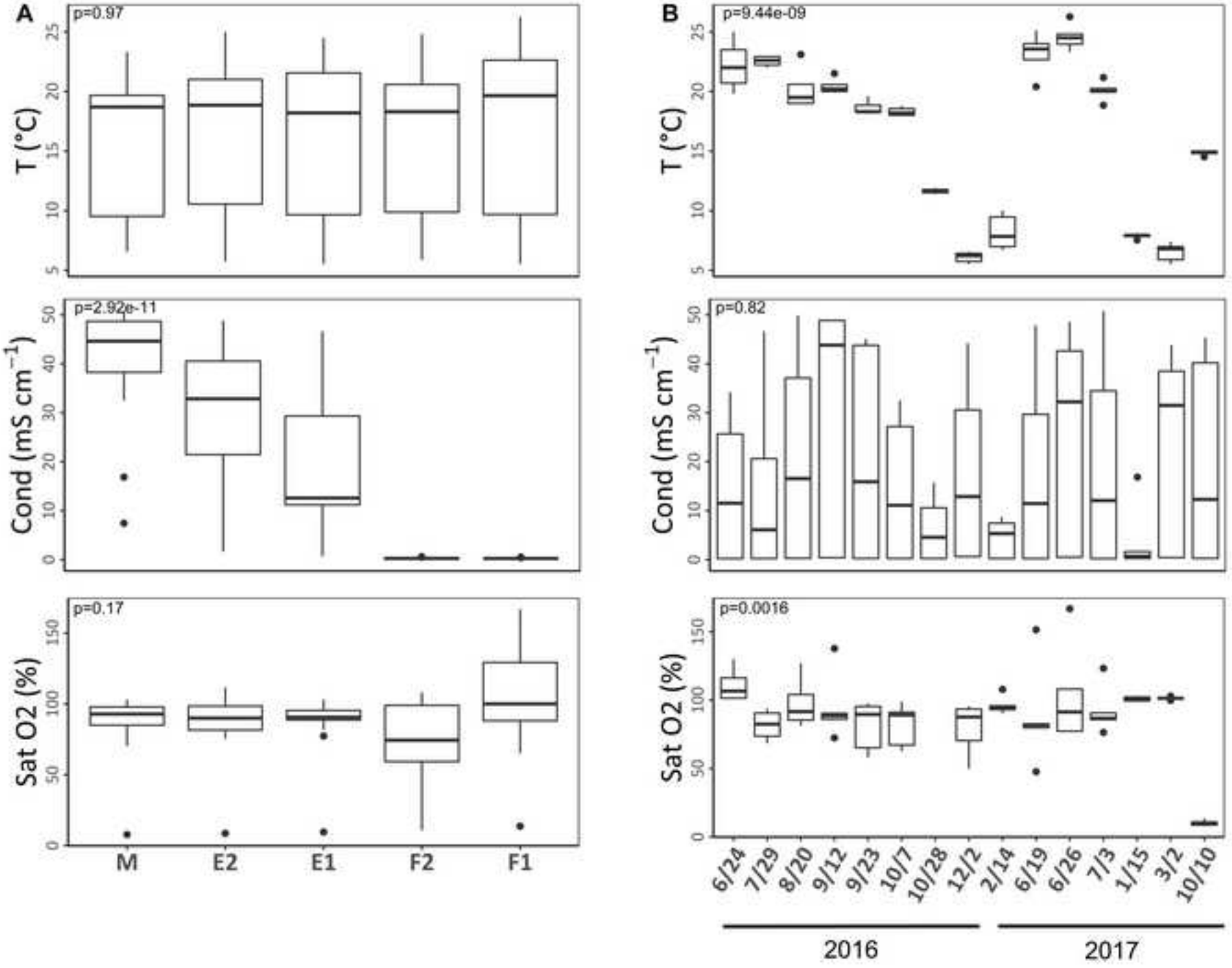

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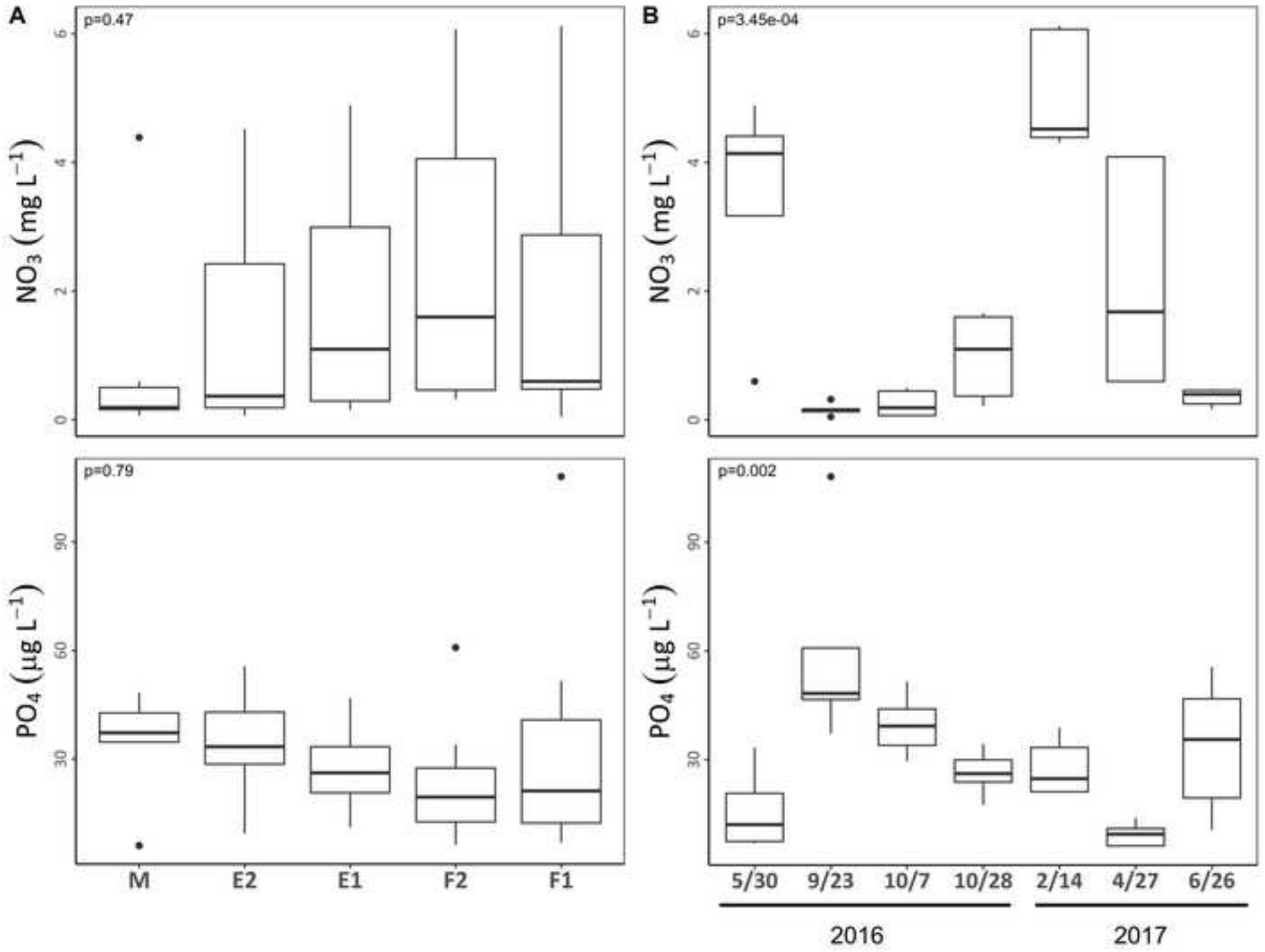

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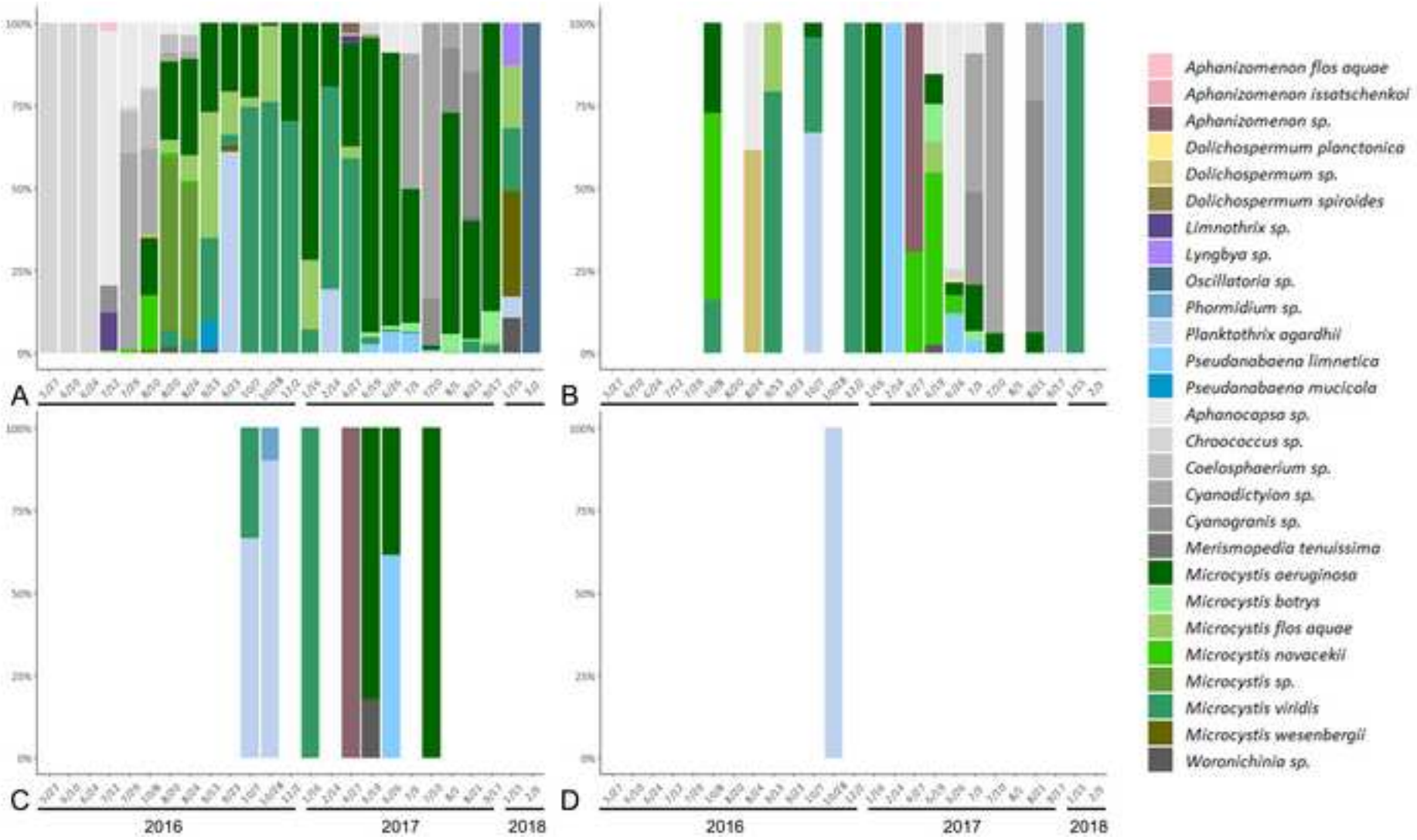

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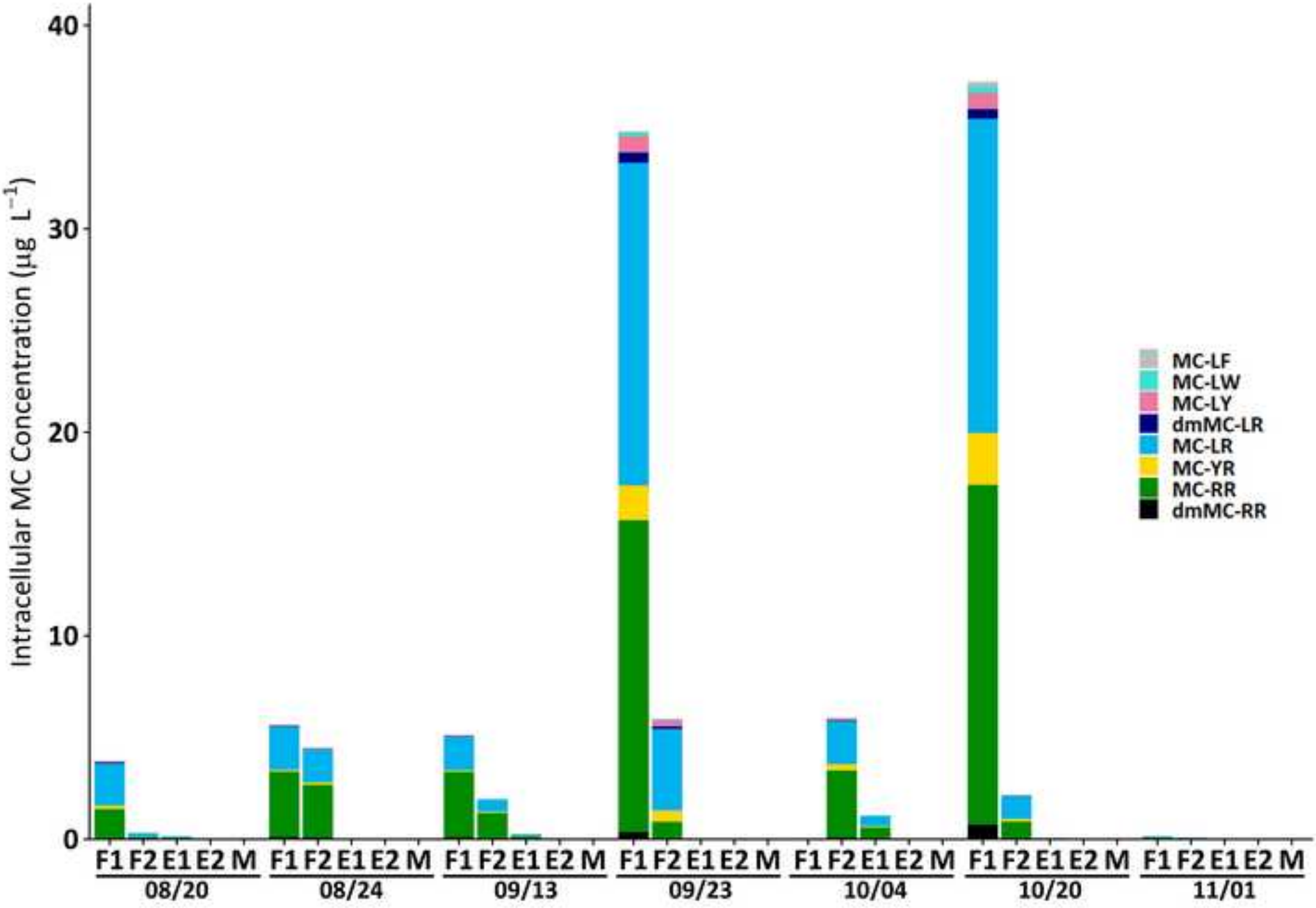

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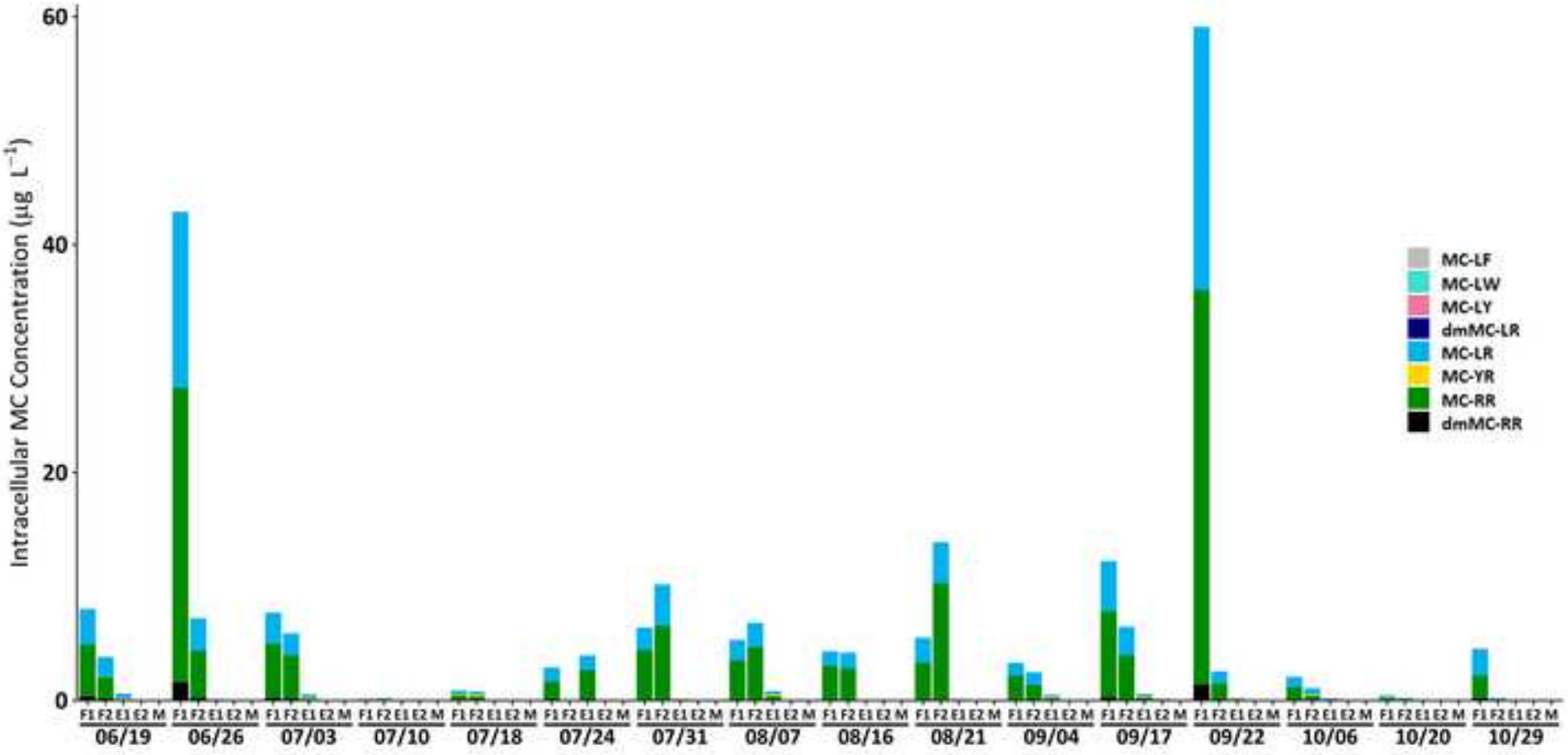

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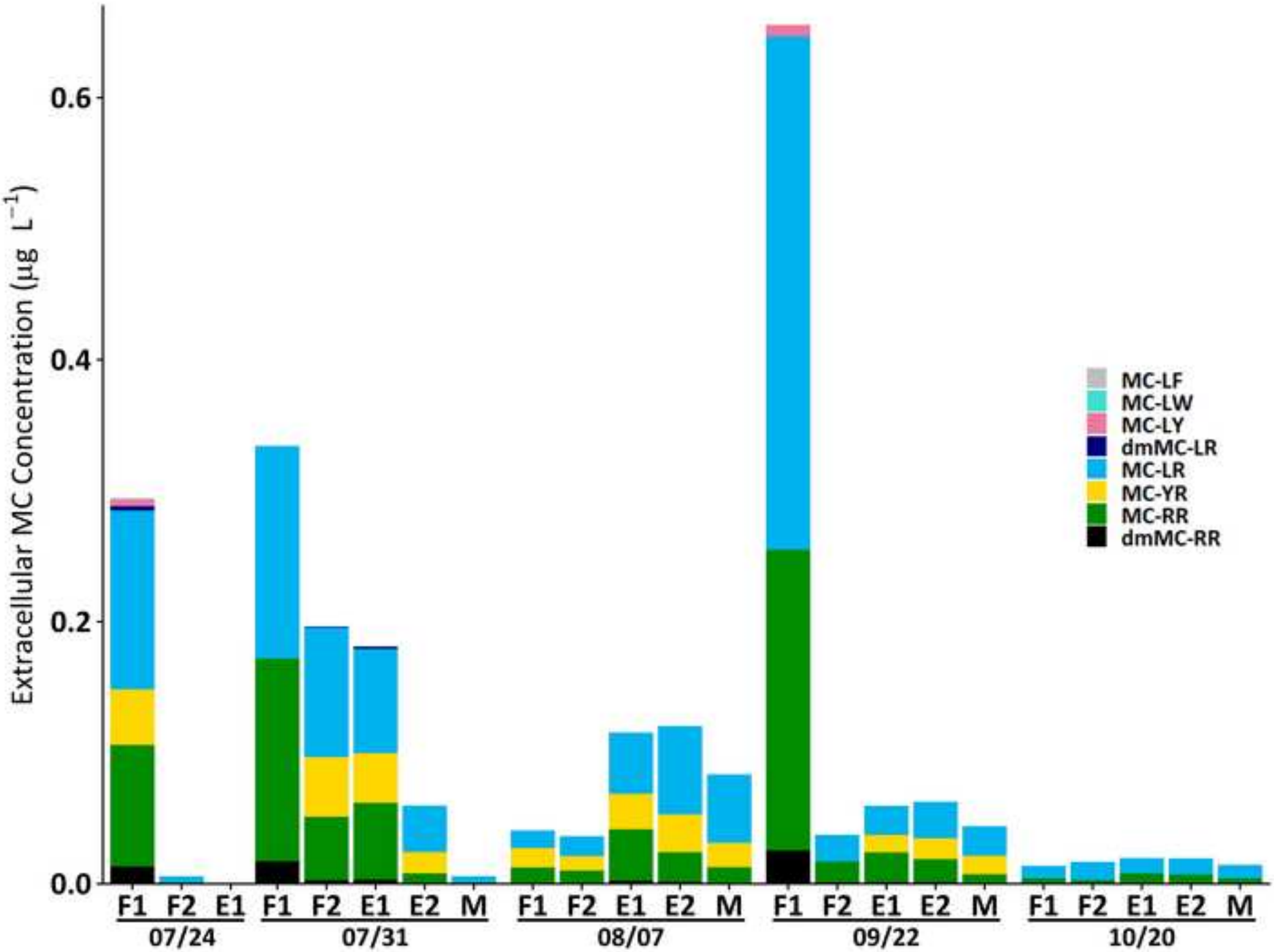

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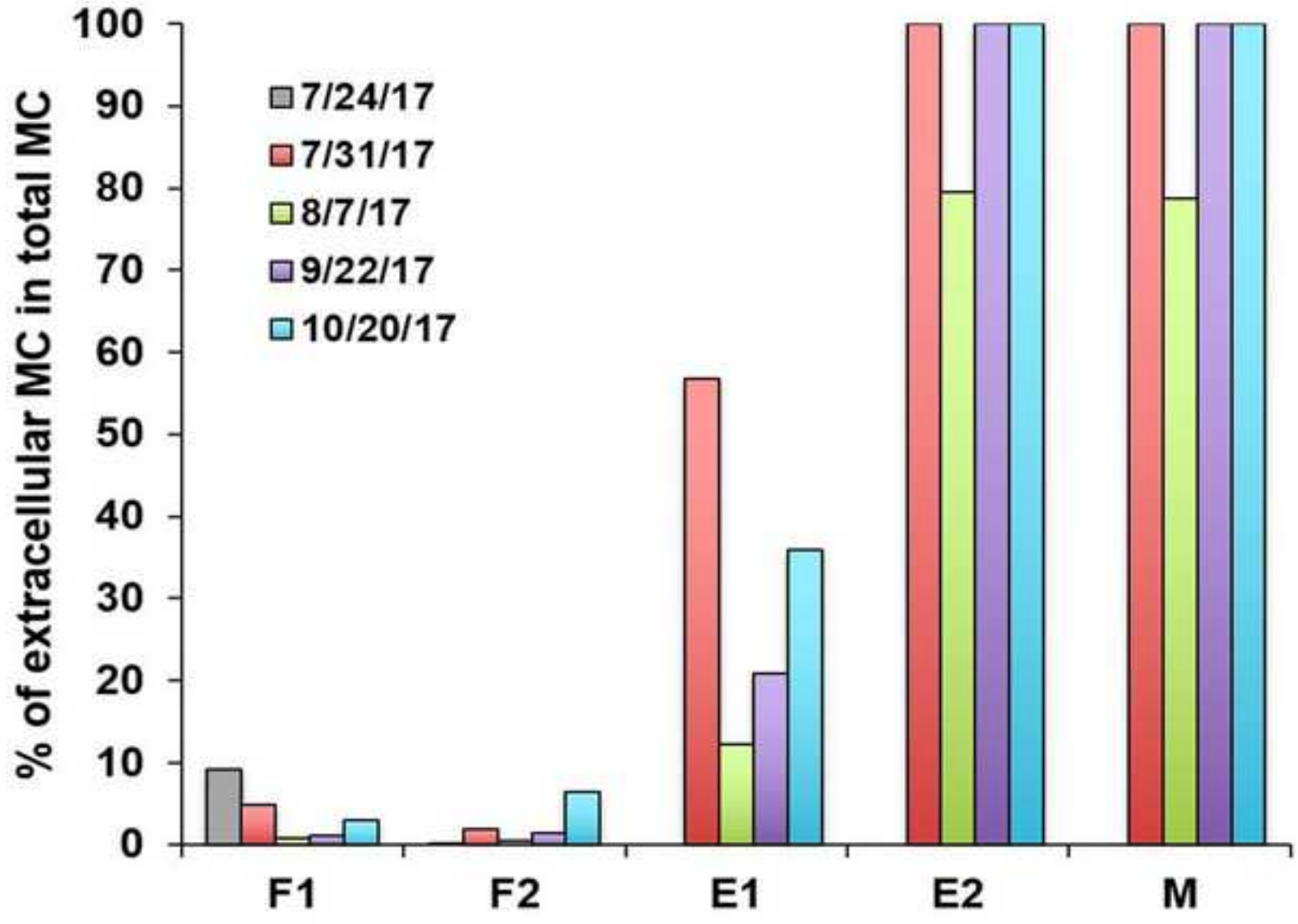

Table

Sites	FO % intra MC (Jun – Nov)		Max intra MC µg/L
	2016	2017	
F1	100	100	60
F2	100	100	14
E1	67	87	1.15
E2	17	38	0.14
M	0	27	0.03

Table 1: Frequency of occurrence (in %) and maximum concentrations of intracellular MC at the 5 stations during the period June to November of both sampling years

Table S1 : LC-MS/MS transitions for the 9 MC variants and NOD tested with standards

Toxin	Precursor ion (*m/z*)	Transition (*m/z*) - Quantification	Transition *(m/z)* - identification
MC-LR	995.6	213.2	374.5
MC-LW	1025.6	375.2	135.2
MC-LF	986.6	375.2	135.2
MC-LY	1002.6	375.2	135.2
dmMC-LR	981.4	103.0	135.2
MC-RR	520.1	135.2	213.2
dmMC-RR	512.8	135.0	103.0
MC-LA	910.7	375.2	135.2
MC-YR	1045.6	213.2	375.2
NOD	825.5	227.0	163.2

Supplementary Material

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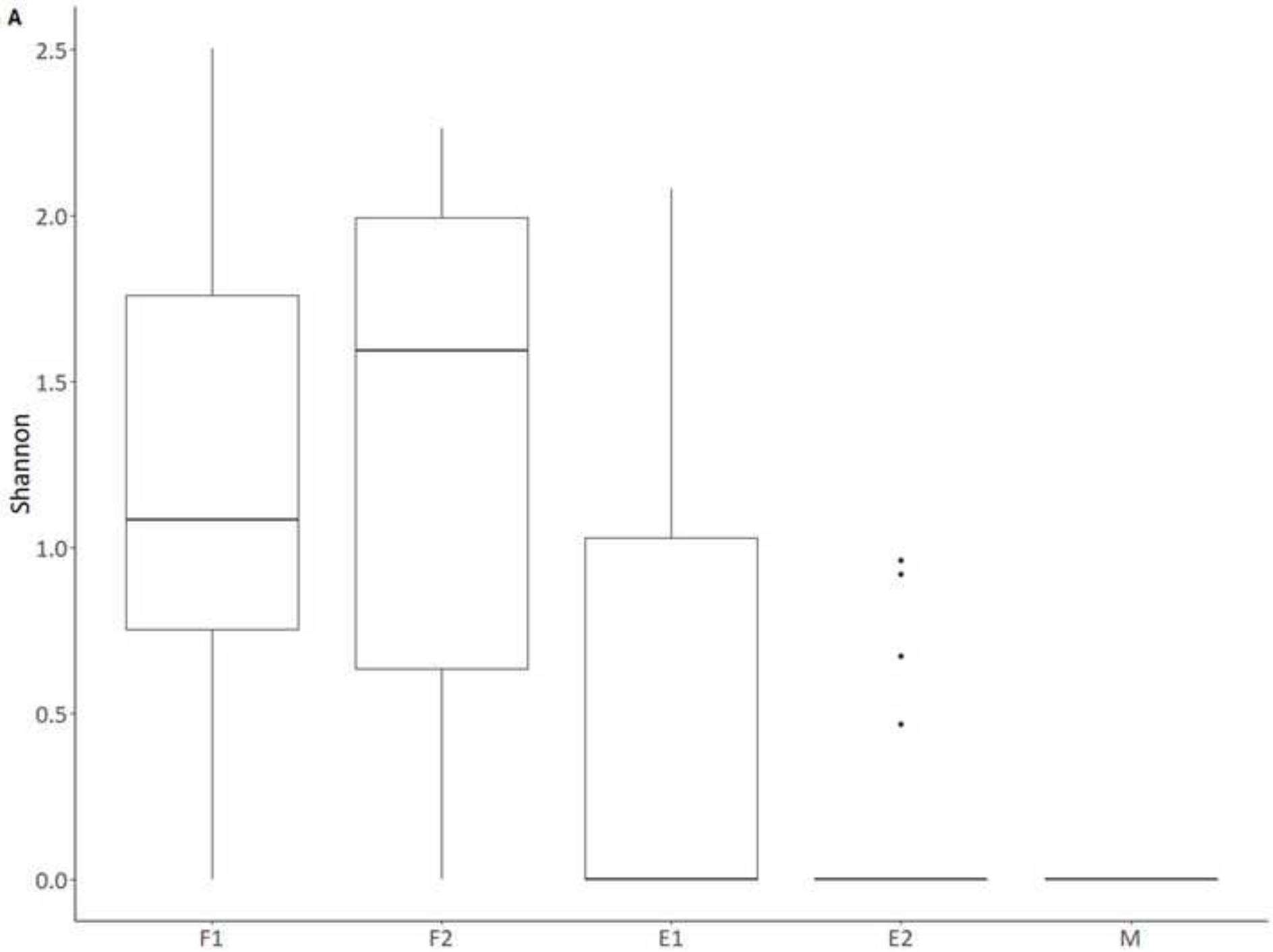

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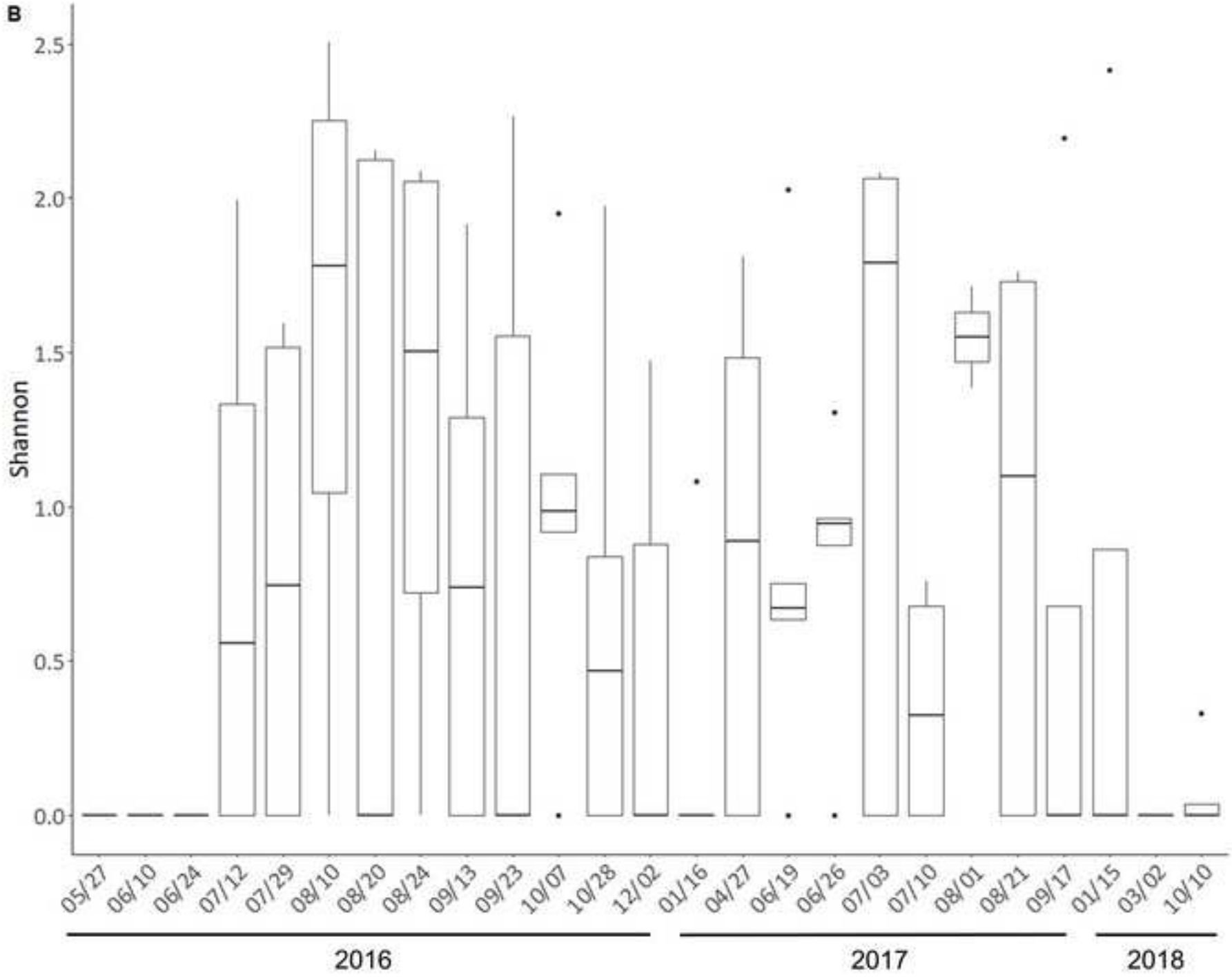

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