



Phytoplankton and zooplankton in relation to Water Framework Directive status in some regulated lowland watercourses of the Scheldt and Sambre (France and Belgium)

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Abstract Analysis of the composition, abundance and diversity of phytoplankton and zooplankton communities in channelised rivers from the upper Scheldt/Sambre catchment (France/Belgium) is used to assess the extent to which their distribution is related to Water Framework Directive (WFD) water quality criteria. Phytoplankton and zooplankton communities are differently associated with WFD status of the stations: phytoplankton communities differ between good/moderate and good/poor state, while zooplankton communities are different in poor than in moderate and good state stations. Euglenophytes

are characteristic of good status, the rotifer *Brachionus angularis* of moderate status stations, and diatoms and the rotifer *Brachionus calyciflorus* of poor status. Phytoplankton blooms only occur in the poor status stations of the Scheldt, probably due to it having higher SiO₂ concentrations than the other rivers.

Keywords HPLC pigment analysis · CHEMTAX · Eutrophication · WFD · Bioindicators

Introduction

Over the last century, human impact has led to water quality degradation and therefore a decrease of the goods and services provided by aquatic systems (Vitousek et al., 1997; Millenium Ecosystem

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Assesment M.E.A, 2005; Vörösmarty et al., 2010). Recent understanding of the ecological, societal, political, and economic importance of aquatic environments has led to consider their protection as a major concern. The biodiversity of aquatic ecosystems is intrinsically linked to the environmental conditions and water quality of aquatic systems (Preston, 2002; Yüksek et al., 2006; Cardinale, 2011). Therefore, studying the distribution and diversity of organisms living in these environments is useful to understand the functioning and dysfunctioning of these ecosystems.

In this context, the monitoring and restoration of water quality in European Union countries have been conducted since 2000 under the WFD (Directive 2000/60/CE, 2000). For surface waters, the WFD assessment considers several morpho-hydrological, physical, chemical and biological properties as indicators of aquatic system's health. A major problem in many aquatic systems throughout the world is nutrient/organic matter enrichment also called eutrophication (Pinay et al., 2018).

In all types of aquatic systems, phytoplankton reacts directly to nutrient concentrations, making it a useful tool to monitor results of efforts to reduce eutrophication. This is why the WFD includes its biomass, composition as well as the intensity and frequency of blooms (Revilla et al., 2009). Since zooplankton is not included in the WFD, phytoplankton and pelagic fish are the only considered pelagic communities next to several benthic indicators (benthic diatoms and invertebrates, macroalgae and fish).

Monitoring of these pelagic components in rivers focuses on lowland river reaches where residence times are sufficient for significant phytoplankton development (Mischke et al., 2011). Among the four types of water bodies considered within the WFD (lakes, coastal waters, transitional waters and rivers), phytoplankton is least studied in rivers (Mischke et al., 2011; Birk et al., 2012; Poikane et al., 2020). While phytoplankton biomass is generally considered in the WFD assessment through chlorophyll *a* (Chl_a) measurements, several studies (e.g. Friedrich & Pohlmann, 2009; Cereja et al., 2022) also included the microscopic composition of phytoplankton community (e.g. Mischke et al., 2011; Wu et al., 2012; Laplace-Treytore & Feret, 2016; Napiórkowska-Krzebietke & Kobos, 2016; Aubry et al., 2021).

High-pressure liquid chromatography (HPLC) analysis is a long-established method for determining the composition of phytoplankton communities at “group” level (e.g. diatoms, chlorophytes, cryptophytes, cyanophytes) through marker-pigment analysis. Sarmiento & Descy (2008), Lauridsen et al. (2011) and Schlüter et al. (2016) advocated the use of HPLC coupled with CHEMTAX (see details in materials and methods section) (Mackey et al., 1996; Van den Meersche et al., 2008) for WFD phytoplankton monitoring. However, several studies recommend completing HPLC analysis with microscopic verification of phytoplankton taxonomic composition (Sarmiento & Descy, 2008; Friedrich & Pohlmann, 2009). The present study applies these methods on a series of lowland rivers and canals situated in the Scheldt and Sambre catchment (France, Belgium).

This study aims to (1) investigate the extent of which the distribution of phyto- and zooplankton communities is related to the WFD status of the studied stations, using HPLC phytoplankton pigment analyses coupled with zooplankton microscopic analyses; (2) identify whether phytoplankton groups and/or zooplankton taxa can be indicators of WFD status; and (3) in how far relationships between environmental factors and phytoplankton and zooplankton distribution provide ecological information about the ecosystem functioning which complements the WFD assessment.

Materials and methods

Study area and campaigns

The Scheldt catchment covers territories in France, Belgium and the Netherlands. Many of the Scheldt catchment watercourses are actively navigated channels, with relatively low flow velocity (between 0.06 and 1.00 m s⁻¹; Prygiel & Coste, 1993; Le Coz et al., 2017). So, from a hydrological point of view, these systems can be considered as intermediate between lentic and lotic ones. Anthropisation in the Scheldt catchment is strong due to intense farming, industrial activities and the presence of big urban areas such as Lille, Brussels and Antwerp. These pressures have resulted in high nutrient concentrations (nitrogen and phosphorus) and low oxygen concentration in most of the Scheldt catchment waterways (Le Coz et al.,

2017). This is compounded by specific infrastructure works on the waterways themselves (canalisation, locks).

Although canals are monitored under the WFD and the resulting data are publicly available, few scientific papers deal with these systems (e.g. Lampart-Kałuzniacka et al., 2011). This paper used data collected during two projects (Biodiversity and Functionality of Zooplankton, BIOFOZI and Planktonic Functional Biodiversity, BIOFOPLAN). Despite management measures for several waterways in the Scheldt catchment, several stations within the study area remain in moderate, poor or bad ecological status, mainly due to high nitrogen (NO_2 , NO_3 , NH_4), phosphorus (PO_4) and total phosphorus (Ptot) concentrations (www.eau-artois-picardie.fr, last accessed on May 2023). As such, the study area provides a dataset on stations with different WFD states (mainly moderate and poor) to test the performance of HPLC—phytoplankton and zooplankton analysis in WFD context.

Six sampling campaigns were carried out in the upstream part of the Scheldt catchment at stations monitored in the WFD context by the Agence de l'Eau Artois-Picardie (AEAP), the Wallonia Public Service (SPW) and the Vlaamse Milieu Maatschappij (VMM) in France, Wallonia and Flanders, respectively. One station was located on the Sambre basin (Fig. 1). BIOFOZI project campaigns covered 15 stations: Zingem, Berchem, Warcoing, Nivelles, Ferin, Brebieres, Wervicq, Erquinghem-Lys, Aire-sur-la-Lys, Wambrechies, Don, Fresnes-sur-Escaut, Neuville-sur-Escaut, Crevecœur-sur-Escaut and Jeumont. These campaigns took place from 7 to 10 April 2014, 16–23 April 2015, 15–19 June 2015 and 2–7 September 2014. BIOFOPLAN project campaigns took place from 6 to 12 July 2019 and 21–27 August 2018 and covered four stations: Ferin, Fresnes-sur-Escaut, Nivelles and Brebieres. All stations belong to the non-tidal riverine section of the Scheldt continuum such as defined by Le Coz et al. (2017). So, the sampling campaigns, although irregular in time, covered all seasons of significant biological activity in temperate areas: spring, summer and autumn.

There was only one station in good WFD status (Ferin, situated on the Sensée). Moderate and poor status stations are distributed over all watercourses, with a tendency for the moderate stations to be located upstream (Fig. 1). These three WFD states

were sampled in all the campaigns, although some stations were not sampled in July 2019 and August 2018. It should be noted that no station was in bad status.

Environmental factors

pH, dissolved oxygen concentration (O_2), conductivity (Cond) and water temperature (Temp) were measured in situ using a multi-parameter probe (WTW, Multi 3430). Analysis of orthophosphates ($\text{PO}_4\text{-P}$), total phosphorus (Ptot), nitrite ($\text{NO}_2\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), ammonium ($\text{NH}_4\text{-N}$) and dissolved silica (SiO_2) was performed by filtering subsurface water samples on polyvinylidene fluoride (PVDF) filters (porosity 0.2 μm). $\text{PO}_4\text{-P}$ concentration was analysed by ammonium molybdate spectrophotometric method, Ptot concentration by absorption spectrophotometer (Uvi Light XT5, SECOMAM), while $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations by ion chromatography (Dionex Ics-5000+, Dionex Corp., Sunnyvale, CA, USA). SiO_2 was analysed by flow colorimetry on the ALPKEM IV. $\text{DIN-N}/\text{PO}_4\text{-P}$ ratio was calculated as the sum of $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations divided by $\text{PO}_4\text{-P}$ concentration. For suspended particulate matter (SPM) and particulate organic matter (POM), a volume of water collected from the subsurface was filtered through pre-weighed GF-C filters (porosity 1.2 μm) in three replicates, which were kept cool during transport to the laboratory and placed in a freezer at -20°C . Filters were dried in an oven at 60°C for 24 to 48 h and weighed on a precision balance for SPM determination. Filters were subsequently burned at 500°C , cooled and reweighed to determine ash weight, and POM was calculated as $\text{SPM}-\text{ash weight}$. Each factor was sampled once, in triplicate, per campaign.

The physical and chemical WFD status in France and Belgium was obtained from the Ministère de la Transition écologique et solidaire (2019) (www.eaufrance.fr, last accessed on February 2024). These are based on 10% and 90% quartiles of the collected data for each factor. To be able to compare the results of the present study with the WFD status of the sampling stations, 10% (for oxygen concentration) and 90% (for all other factors) quartiles were calculated for all the physical and chemical data of this study. As the samplings occurred during two WFD evaluation,

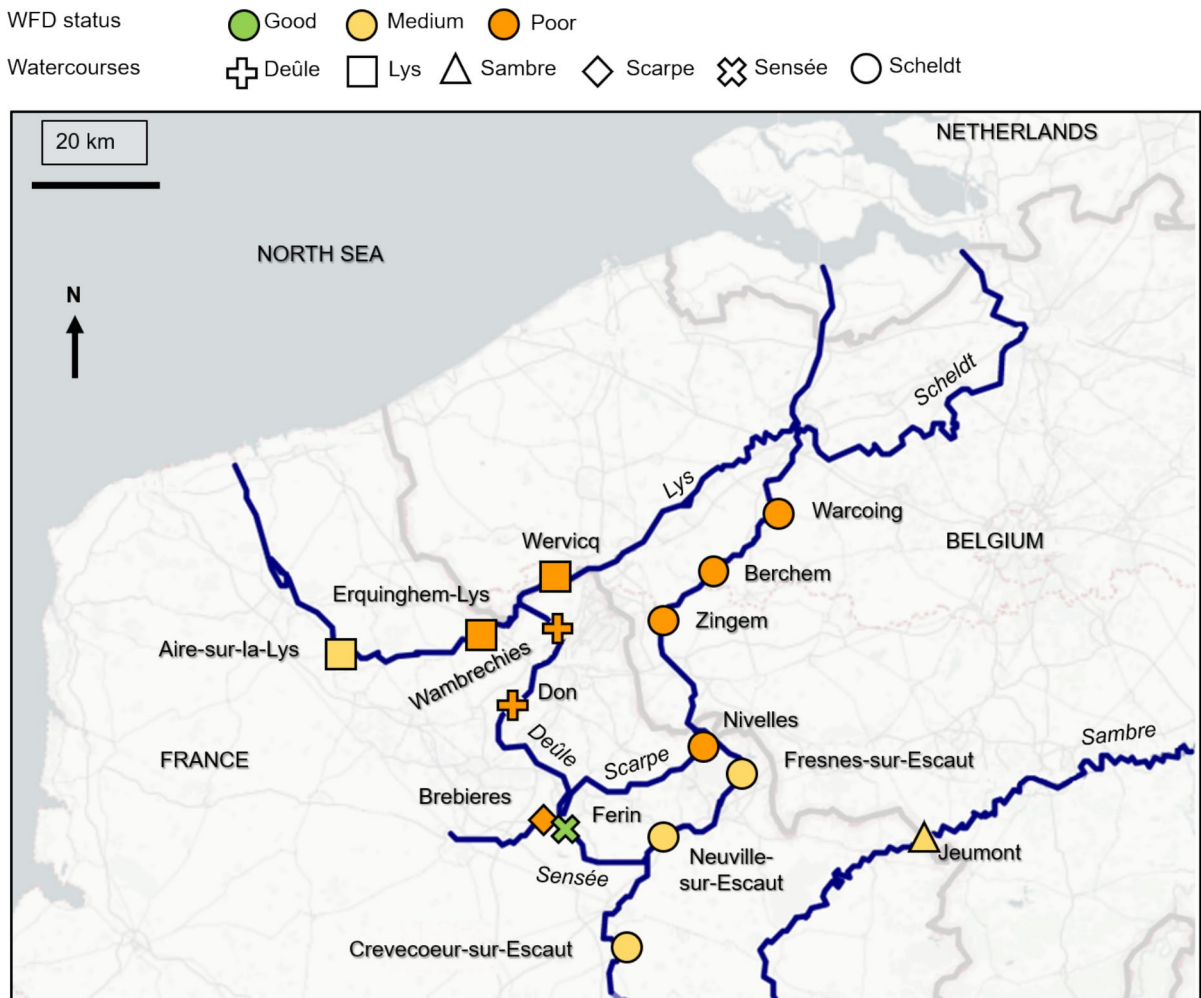


Fig. 1 Map of the upstream part of the Scheldt catchment with a Sambre site. Stations are represented by different shapes depending on the watercourse in which they were sampled. Colours represent the WFD states

these quartiles were calculated for 2014 and 2015 samplings and also for 2018 and 2019 samplings.

10% and 90% quartiles were calculated as:

$$i = a \times N + 0.5$$

where i = rank of the result to be retained; $a = 0.1$ for the 10% quartiles and 0.9 for the 90% quartiles; and N = number of results in ascending order.

Phytoplankton

Subsurface water was sampled from a pontoon with a bucket and a rope at subsurface (≤ 1 m depth). Then subsamples of 200 ml to 1 l (depending on the

samples turbidity) were filtered with $1.2 \mu\text{m}$ GF-C filters for 3 replicates, once a day for the 2014 and 2015 campaigns and twice a day (morning and afternoon) for the 2018 and 2019 ones. The filters and their contents were frozen at -80°C , shredded using pliers on ice and in the dark, in 15-ml conical tubes and stored at -80°C . 5 ml of methanol + 2% ammonium acetate was added to each sample, then sonicated for 2 min, and stored for 15 min at -20°C . This step was performed twice. The obtained pigment extracts were filtered into 2-ml vials using 1-ml syringes and $0.2\text{-}\mu\text{m}$ syringe filters. Pigment concentrations were determined by HPLC analysis at 405 nm, 450 nm, and 665 nm as the majority of phytoplankton pigments

are detectable at these wavelengths (Wright et al., 1991). The HPLC was equipped with a reverse phase C8 5 μm column (MOS-2 HYPERSIL, Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a diode array spectrophotometer (LC1200 series, Agilent Technologies inc., Santa Clara, CA, USA). Barlow's elution programme was used (Barlow et al., 1997). This method uses a gradient of methanol 80%–ammonium acetate 20%. The chromatograms were analysed using the HP ChemStation software (version A, Agilent Technologies Inc.). Pigments were identified by comparison of retention times and absorption spectra with pure pigment standards (supplied by DHI, Denmark). Chl*a* concentration was considered as a proxy of the total phytoplankton biomass. The marker pigments used for each community were:

- Diatoms: fucoxanthin, diadinoxanthin and diatoxanthin (Stauber & Jeffrey, 1988; Roy et al., 2011).
- Chlorophytes: violaxanthin, neoxanthin, zeaxanthin, lutein and chlorophyll b (Gieskes et al., 1988; Stauber & Jeffrey, 1988).
- Cryptophytes: alloxanthin (Pennington et al., 1985).
- Euglenophytes: chlorophyll b, diadinoxanthin and neoxanthin (Jeffrey et al., 1997).

Phytoplankton pigment concentration was calculated as:

$$C = \left(\frac{A \times C_c \times V_{\text{MeOH}} \times 1000}{V_i \times V_f} \right)$$

where C = pigment concentration ($\mu\text{g l}^{-1}$); A = peak area (mv s^{-1}); C_c = calibration coefficient; V_{MeOH} = volume of methanol = 10 ml; V_f = subsample volume (ml); V_i = injected volume of extract (ml).

Zooplankton

50 L of subsurface water was pumped (maximum capacity 750 l min^{-1}) and filtered through a 50 μm mesh. The collected zooplankton was fixed with formalin (4% final concentration). This sampling technique allows the collection of all the mesozooplankton (copepods, cladocerans and rotifers). Zooplankton samples were stained with erythrosine to facilitate the detection of organisms. Several subsamples were

analysed under binocular (90 X magnification), and the different taxa were determined and counted. A minimum of 100 individuals in total were observed per sample. For rotifers and cladocerans, most taxa were determined at genus or species level. Copepods were only determined at the calanoid, cyclopoid and harpacticoid level. Nauplii and copepodite stages were counted separately.

Statistical analyses

All statistical analyses were conducted on the RStudio software (R 4.1.2, R Core Team, 2023). Phytoplankton, SPM and POM data correspond to the mean values based on 3 replicates per station for the 2014 and 2015 sampling campaigns. For the 2018 and 2019 ones, data correspond to the mean values of 6 replicates per station (morning and afternoon).

All other data correspond to one sample per station and campaign. The CHEMTAX method (Mackey et al., 1996; Van den Meersche et al., 2008) was used to calculate the contribution of phytoplankton groups to the total phytoplankton biomass. The analysis optimises the contribution of the different phytoplankton groups to the total phytoplankton biomass on the basis of the marker pigments/Chl*a* ratios (given in a matrix 1) as a starting point. It allows these ratios to vary according to the limits defined in a matrix 2. Matrix 2 values were taken from Carbonnel et al. (2012) who studied silica and phytoplankton dynamics in the Scheldt estuary in Belgium and the Netherlands. This method has been adapted for RStudio by Bach et al. (2020) and uses the function *lsei* in *lim-Solve* package (Soetaert et al., 2009). The script is available on GitHub (<http://github.com>, last accessed on May 2023).

For the zooplankton data, an abundance sorting method (*abund* function from *pastecs* package) (Grosjean & Ibañez, 2018) was used in order to avoid having more species than samples and too many voids (NA) in the data matrix. This method considers both abundant species and rare species (i.e. species that are absent from the majority of stations but abundant at a few stations). A coefficient f between 0 and 1 is used to adjust the weight given to the frequency of zero values. The value of the coefficient f used is 0.2. (Grosjean & Ibañez, 2018).

All analyses were then carried out using the contribution of the phytoplankton groups to total

phytoplankton biomass and the zooplankton taxa selected using the abundance sorting method.

The stacked barplots, scatter plots and combined plots were generated by *geom_bar*, *geom_point* and *geom_line* functions with the package *ggplot2* (Wickham, 2016). Linear correlation was tested by Pearson's method. To determine whether zooplankton richness differed significantly between stations in different WFD states, Kruskal–Wallis tests were performed with non-normal data (*kruskal.test* and *shapiro.test* functions from *stats* package, R Core Team, 2023). If the Kruskal–Wallis test was significant, a pairwise multiple comparison Dunn's test was performed with *dunnTest* function from *FSA* package (Dunn, 1964; Ogle et al., 2023). The *P*-values obtained were adjusted by the Benjamini–Hochberg false discovery method (Benjamini & Hochberg, 1995).

A multiple factor analysis (*MFA function* from *FactoMineR* package, Escofier & Pagès, 1994; Le et al., 2008) was used to analyse the distribution of phytoplankton groups (contribution of the phytoplankton groups to total phytoplankton biomass), zooplankton and environmental factors (scaled to unit variance) in relation to the WFD status of the stations. As the dataset has more objects than parameters, this analysis could be performed, despite the non-normality of the data (David, 2017). The different WFD states were highlighted by 95% confidence ellipses (*stat_ellipse* function from *ggplot2* package, Wickham, 2016). Zooplankton abundance was transformed to $\log_{10} + 1$.

To assess the proportion of variance in contribution of phytoplankton groups to total phytoplankton biomass and zooplankton composition that can be explained by the WFD state, a distance matrix was calculated using the Euclidean distance for phytoplankton and the Hellinger distance for zooplankton with 999 permutations (*vegdist* function from *vegan* package, Oksanen et al., 2022). The dispersion between the groups was tested using the *betadisp* function from *vegan* package (David, 2017; Oksanen et al., 2022). Then a permutational multivariate analysis of variance (permanova) (*adonis2* function from *vegan* package, Oksanen et al., 2022) and an analysis of similarities (ANOSIM) (*anosim* function from *vegan* package, Oksanen et al., 2022) were performed. A pairwise comparison test was then carried out to determine which groups differed (*pairwise*.

adonis function from *pairwiseAdonis* package, Martinez Arbizu, 2017). The *p*-values of the permanova and comparison tests were adjusted by the Benjamini Hochberg method (Benjamini & Hochberg, 1995). The species indicator values method (IndVal) (Dufrene & Legendre, 1997) was implemented (*indval* function from *labdsv* package, Roberts, 2023) to determine whether certain taxa were bioindicators of WFD state.

To corroborate results obtained by HPLC coupled with CHEMTAX, these were compared with an identification of the phytoplankton species by microscopic analysis carried out by AEAP, considering the same months as used for sampling in this study. This analysis was for the same months as the samplings for the study. Unfortunately, no microscopic analysis is available for April 2014 and 2015, although these sampling periods presented the highest *Chla* concentrations and diatom contribution to total phytoplankton biomass. To corroborate the IndVal results, the phosphorus tolerance coefficient (stenoecy coefficient) of Laplace-Tretyure & Feret (2016) was assigned to each species. This coefficient varies between 0 (low tolerance to phosphorus) and 3 (strong tolerance to phosphorus), as is the case in several lowland rivers, such as the Scheldt.

Results

Environmental factors

Only those environmental factors used in this study that are common to those assessed by the WFD are shown (Fig. 2A–H) for all stations. SPM and *Chla* and phaeopigments are considered in the WFD, but present knowledge does not allow reliable moderate, poor and bad thresholds to be set (Ministère de la Transition écologique et solidaire, 2019, www.eaufrance.fr). This is also the case for the poor and bad thresholds of $\text{NO}_3\text{-N}$. For stations Berchem and Zingem, no measurements were made for SPM, $\text{NO}_2\text{-N}$ and *Chla* and phaeopigments. No SPM measurements were taken for Warcoing. Therefore, these stations have no WFD physical and chemical status for these factors (Fig. 2D, F, J).

Temperature reached moderate status ($> 20\text{--}25\text{ }^\circ\text{C}$) at Ferin, Fresnes-sur-Escaut and Nivelles (Fig. 2A). pH values remained mainly below the very good

Stations FER: Ferin ASL: Aire-sur-la-Lys CSE: Crevecoeur-sur-Escaut
 FSE: Fresnes-sur-Escaut JEU: Jeumont NSE: Neuville-sur-Escaut
 BER: Berchem BRE: Brebières DON: Don
 ERQ: Erquinghem-Lys NIV: Nivelles WAM: Wambrechies
 WAR: Warcoing WER: Werwicq ZIN: Zingem

Campaigns years 2014-2015 2018-2019

WFD physico-chemical status Very good Good Medium Poor Bad

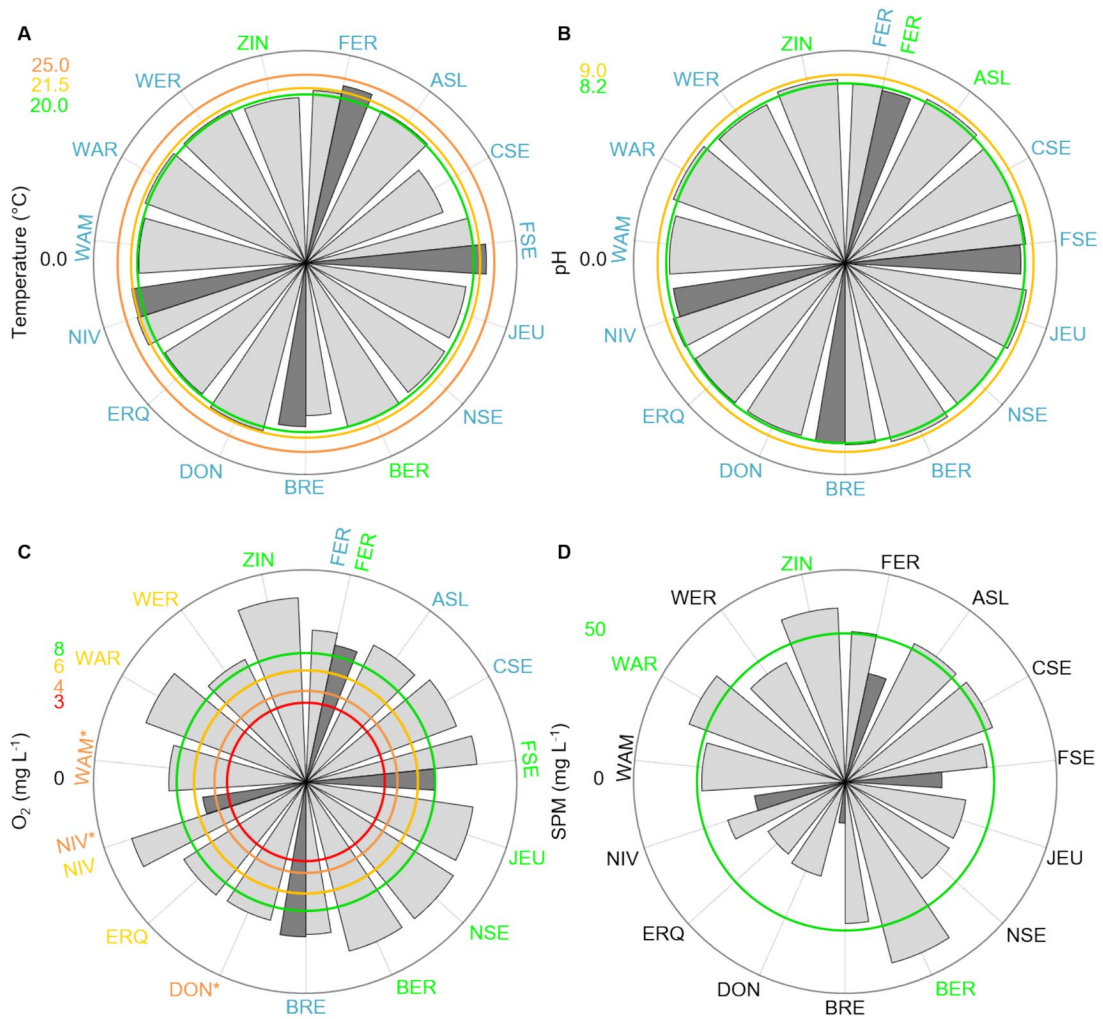


Fig. 2 Comparison of the 90% and 10% quartiles of the environmental factors obtained during the campaigns with the threshold values and WFD physical and chemical status of 3-year datasets (2013–2015) and (2018–2021). Colours of the stations indicate the WFD physical and chemical status of the station for the corresponding factor. Stations in black indicate that the factor was not measured in the WFD assessment. For the Ferin, Fresnes-sur-Escaut, Nivelles and Brebières sta-

tions, the light-shaded pies correspond to BIOFOZI and the dark-shaded ones to BIOFOPLAN campaigns. As the WFD physical and chemical status for some factors was not the same between the WFD (2013–2015) and (2018–2021) assessment, station names may appear in two different colours. "*" indicates the degrading factor for the WFD ecological status of the station

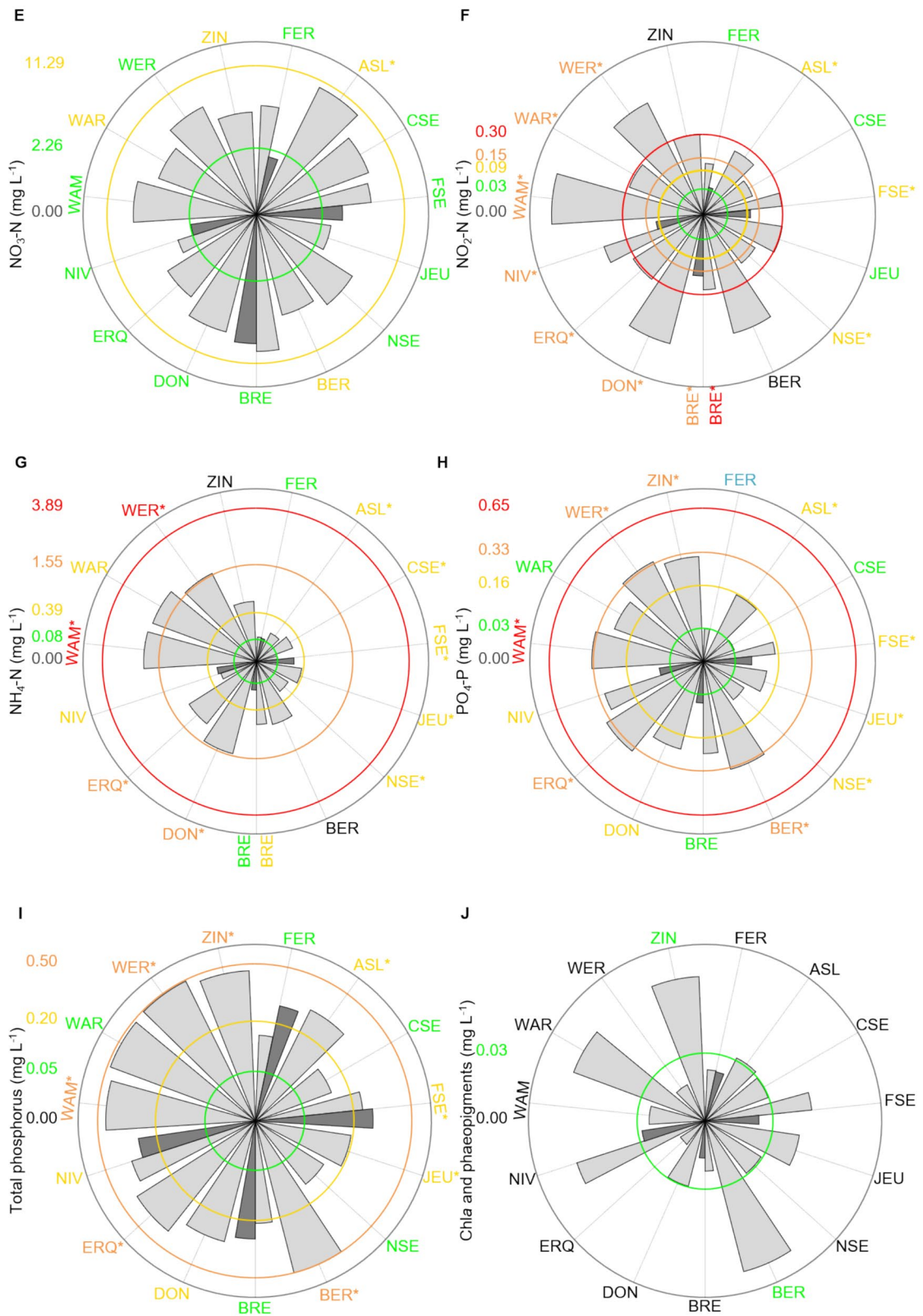


Fig. 2 (continued)

threshold (≤ 8.2), and no value was below 7.7, which corresponds to the minimum pH threshold value for very good status (≥ 6.5) (Fig. 2B). O_2 was a factor downgrading the WFD ecological status of Don, Nivelles in 2018–2019 and Wambrechies (Fig. 2C). For SPM (Fig. 2D), all stations were in very good status ($\leq 50 \text{ mg l}^{-1}$) or in good status ($> 50 \text{ mg l}^{-1}$).

With regard to $NO_3\text{-N}$, no stations in moderate status ($> 11.3 \text{ mg l}^{-1}$) were observed (Fig. 2E). However, Aire-sur-la-Lys (10.25 mg l^{-1}) and Brebieres were close to it for the two periods (9.50 and 8.48 mg l^{-1}). This factor is downgrading for the WFD ecological status of Aire-sur-la-Lys. $NO_2\text{-N}$ downgraded the WFD ecological status of several stations (Fig. 2F), and most of the stations were in poor status ($> 0.15\text{--}0.30 \text{ mg l}^{-1}$). Only one station was in bad status, but many more reached it. $NH_4\text{-N}$ (Fig. 2G) and $PO_4\text{-P}$ (Fig. 2H) are also a downgrading factor for many stations, but only some stations reached poor status ($> 1.54\text{--}3.57 \text{ mg l}^{-1}$ for $NH_4\text{-N}$ and $> 0.3\text{--}0.65 \text{ mg l}^{-1}$ for $PO_4\text{-P}$). Ptot (Fig. 2I) did not exceed the threshold values for poor status ($> 0.5\text{--}1 \text{ mg l}^{-1}$). For Chla and phaeopigments (Fig. 2J), stations were in very good status ($< 0.03\text{--}0.03 \text{ mg l}^{-1}$) or in good status ($> 0.03 \text{ mg l}^{-1}$).

Considering all environmental factors, about half of the measurements in this study (48%) resulted in the same status as the three-year WFD state for the environmental factor tested. 44% indicated one class below or above the WFD status, whereas 8% of the stations indicated more than one class below or above the WFD.

Resulting $DIN\text{-N}/PO_4\text{-P}$ ratios varied between 12.70 and 650.51, with a mean of 46.29. High concentrations of N and P components were generally measured at the same stations: Wambrechies, Warcoing, Wervicq, Don, Berchem, Erquingem-Lys and Nivelles. There was a significant regression between the sum of $NO_2\text{-N}$ and $NH_4\text{-N}$ and $PO_4\text{-P}$ (Fig. S1, in supplementary information). $NO_3\text{-N}$ concentrations being almost always above 2 mg l^{-1} , there was no correlation between $DIN\text{-N}/PO_4\text{-P}$ and $PO_4\text{-P}$ or Ptot (not shown).

Phytoplankton

Chla concentrations varied between 1.85 and $148.65 \mu\text{g l}^{-1}$, and 45% of the concentrations were

above $20 \mu\text{g l}^{-1}$. Highest concentrations were observed during the spring months, lowest during summer, and somewhat higher concentrations in September (Fig. 3).

Considering the whole dataset, diatoms clearly dominated in contribution to phytoplankton biomass (66%), whereas cryptophytes contributed a minimum (4%). Chlorophytes and euglenophytes contributed 17% and 13% to phytoplankton biomass, respectively (Fig. 3). Despite the strong dominance of diatoms, there were differences in the maximum and minimum contribution of the groups to total phytoplankton biomass depending on the stations/campaigns. Specifically, the two April campaigns were characterised by the highest contribution of diatoms (88%). They contributed 100% at eight stations in April 2014 and four stations in April 2015 and one in August 2018. The minimum diatoms contribution (4%) was observed at Jeumont and Neuville-sur-Escaut in June 2015 and September 2014. Diatoms were not observed at Ferin in April 2015. In the other samples, diatoms contributed between 13 and 80%. At Ferin, the only station in good status, the average contribution of diatoms over all campaigns was lower than at the stations in moderate or in poor states (Kruskal–Wallis, P value < 0.01). Chlorophytes were not observed in nine stations in April 2014, six in April 2015 and one in August 2018, but they contributed over 30% to total biomass at three stations in June 2015, two in July 2019, one in August 2018 and September 2014. Cryptophytes were not observed at many stations, but had a maximal contribution at Ferin (100%) in April 2015 followed by Neuville-sur-Escaut (96%) in September 2014. Their contribution was greater than 20% in three stations in September 2014 and one in June 2015. Euglenophytes were not observed in some stations but contributed between 30 and 43% at one station in April 2014, three in June 2015, two in July 2019 and three in September 2014. Their contribution was maximal at Jeumont (60%) in June 2015 and Ferin (45%) in August 2018.

Microscopic analysis showed that diatoms contributed up to 30% of the biovolume in June 2015, 5% in July 2019, 17% in August 2018 and 14% in September 2014. The median diatom stenoecy coefficient was 1.66 for the good status, 1.83 for the moderate status and 1.84 for the poor status stations. The same trend was found for chlorophytes: 1.53, 1.56 and 1.60 for the good, moderate and poor states, respectively. For

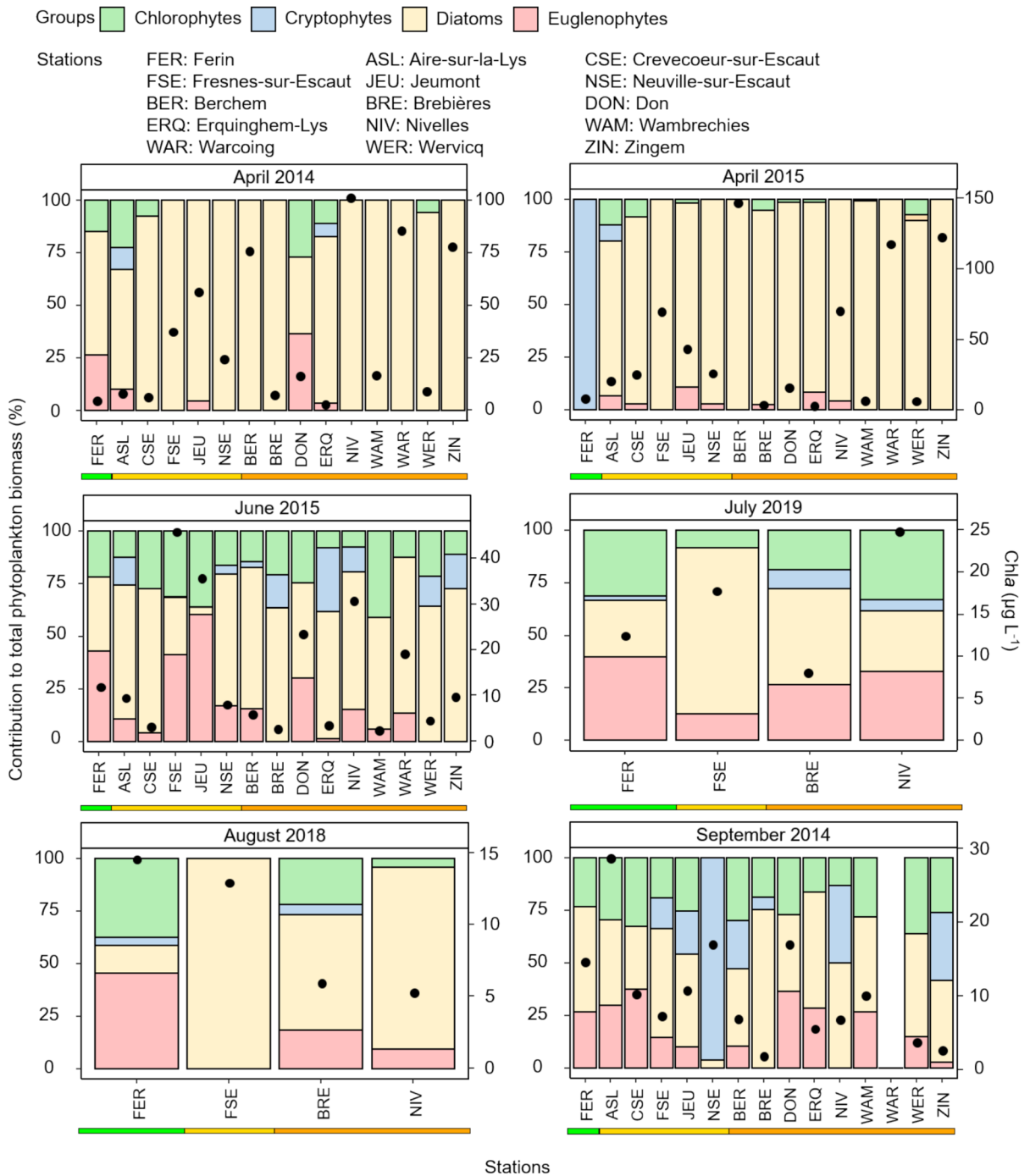


Fig. 3 Chla concentration (black dots, right axis) and percentage contribution of phytoplankton groups to total phytoplankton biomass (HPLC/pigments quantification and CHEMTAX methods) (bars, left axis) for each station and campaign. The

horizontal bars below the stations represent the WFD status of the station (good: FER, moderate: from ASL to NSE, poor: from BER to ZIN). Warcoing September 2014 data are missing

cryptophytes, the median coefficient was higher for the good status (1.52) than for the other states (1.43). Other groups accounted for no more than 20% of biovolume (Fig. S2, in supplementary information).

Zooplankton

Zooplankton abundances varied between 1 486 and 1 124 533 ind. m⁻³ for rotifers and between 2 636 and 316 291 ind. m⁻³ for crustaceans. Cladocerans and copepods (adults and copepodites) had comparable abundances, ranging from 100 to 70 400 and from 80 to 60 214 ind. m⁻³, respectively.

Fifty-eight zooplankton taxa were identified, with rotifers being the most abundant in terms of number (80%) followed by copepod nauplii (15%) and cladocerans (2%). *Brachionus calyciflorus* Pallas, 1766 (19%) was the most abundant rotifer taxon, followed by *Synchaeta* spp. (19%), *Keratella cochlearis* (Gosse, 1851) (17%). The abundance sorting method selected 23 taxa (*Asplanchna* spp. (*Asp*), Bdelloidea (*Bdel*), *Brachionus angularis* Gosse, 1851 (*Bra. ang*), *Brachionus calyciflorus* (*Bra.cal*), *Brachionus leydigii* Cohn, 1862 (*Bra.ley*), *Brachionus urceolaris* Müller, 1773 (*Bra.urc*), *Cephalodella* spp. (*Cep*), *Euchlanis* spp. (*Euc*), *Filinia* spp. (*Fil*), *Keratella cochlearis* (*Ker.coc*), *Keratella quadrata* (Müller, 1786) (*Ker.qua*), *Notholca* spp. (*Not*), *Polyarthra* spp. (*Poly*), *Synchaeta* spp. (*Sync*), *Alona* spp. (*Alo*), *Bosmina* spp. (*Bos*), Calanoid adults and C5 (*Cal*), Calanoid copepodites (*Cal.c*), *Chydorus* spp. (*Chy*), Cyclopoid adults and C5 (*Cyc*), Cyclopoid copepodites (*Cyc.c*), *Daphnia* spp. (*Dap*), Nauplii (*Naup*)). None of these taxa had an abundance < 0.09%.

The composition and abundance of zooplankton exhibited distinct patterns across campaigns/stations. Total rotifers abundance (Fig. 4) was highest in April 2015 and lowest in September 2014. None of the sampling campaigns showed a correlation in total rotifer abundance with station WFD status. *B. calyciflorus* was the most abundant in April 2015 (29%) and June 2015 (22%). *K. cochlearis* was the most abundant in April 2014 (20%), July 2019 (53%), August 2018 (65%) and September 2014 (30%). The percentage of several taxa (*Cephalodella* spp., *Notholca* spp., *B. leydigii*, *B. urceolaris*) never exceeded 1%.

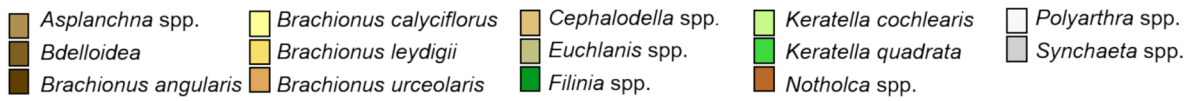
The highest abundance of crustaceans was observed in July 2019 and August 2018, while the lowest abundance was recorded in April 2014. No

trend in the total crustacean abundance was observed in correlation with station WFD status (Fig. 5). Within the crustacean community, copepod nauplii were always the most abundant, followed by cyclopoid copepodites and small bodied cladocerans *Bosmina* spp. and *Chydorus* spp. Other taxa remained below 31% of total crustacean abundance, with a strong variation in their importance among taxa according to station and campaign (Fig. 5). Copepods (calanoid and cyclopoid adults and copepodites) contributed significantly more to total crustacean abundance at Ferin, in good status, than at stations in poor condition (Kruskal–Wallis, $p=0.0352$; Dunn, p -value good vs poor = 0.0435).

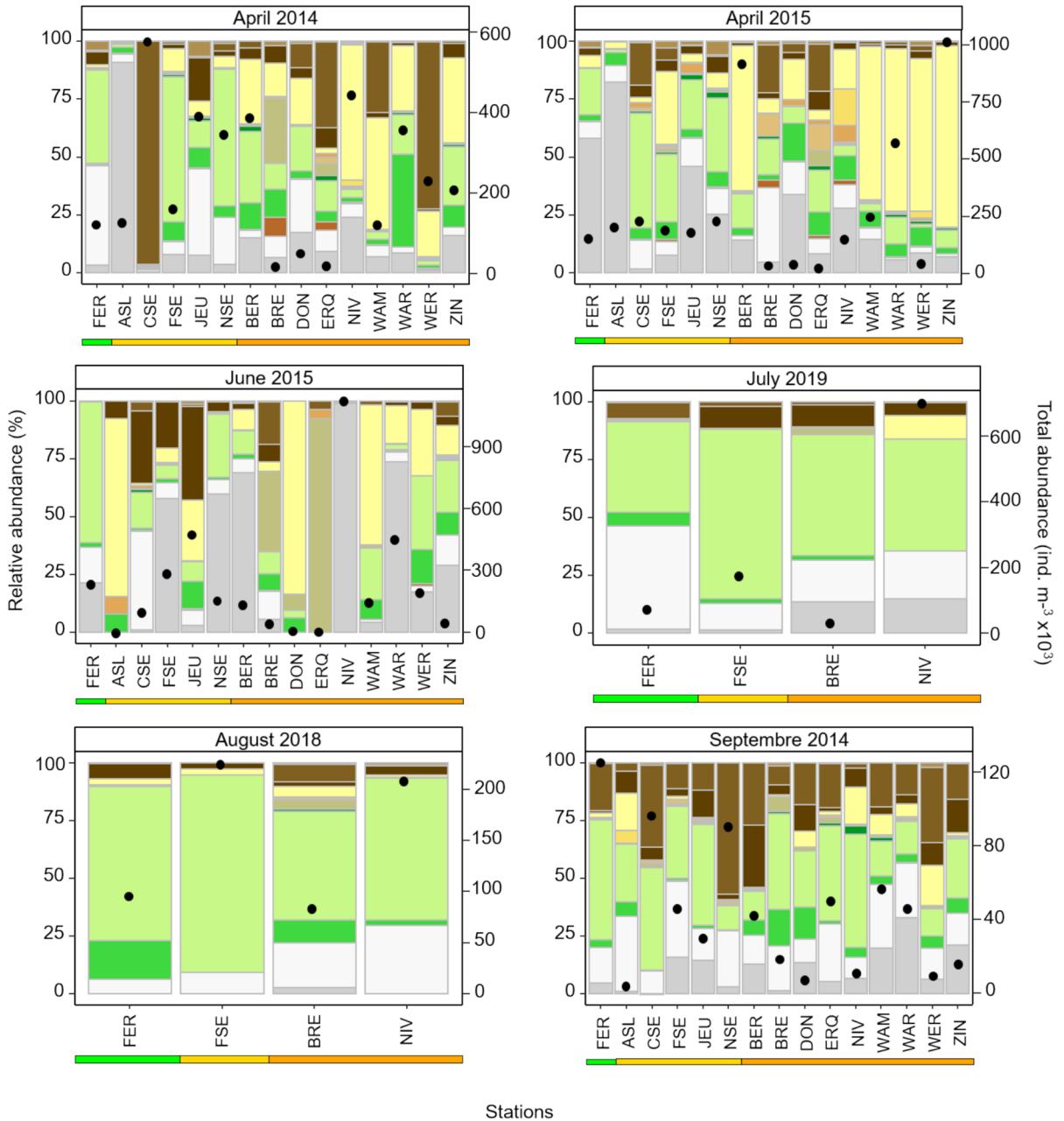
Phytoplankton and zooplankton distribution and WFD status

For phytoplankton, the first principal dimension (Dim 1) of the MFA accounted for 29.24% of the total variance, the second (Dim 2) for 17.87% (Fig. 6A) and the third (Dim 3) for 10.19% (Fig. 6B). In the Dim 1,2 biplot (Fig. 6A), diatoms were opposed to other phytoplankton groups along axis 1 associated with O₂, pH, NO₃-N, DIN-N:PO₄-P and very slightly to POM and Cond.

Chlorophytes, euglenophytes and to a lesser extent cryptophytes were, on the contrary, associated with temperature, SiO₂ and NO₂-N. Except for NO₃-N, NO₂-N, pH, Si and NH₄-N, all environmental factors contributed between 10 and 20% to one of the 1,2 biplot axes. The ordination along Dim 3 (Fig. 6B) showed a notable association of chlorophytes and euglenophytes with temperature and of cryptophytes with SiO₂ and NO₂-N. NO₃-N, O₂, pH and temperature contributed between 10 and 20% to one of the 1,3 biplot axes; the other environmental factors less. The distribution of phytoplankton groups differed significantly according to the WFD status (permanova and ANOSIM, P value = 0.003 and 0.01, respectively) and this difference explained 12.6% of the total variation (permanova, $R^2=0.1256$). The good status differed significantly from the other states (pairwise adonis, p -value good vs moderate = 0.036 and p -value good vs poor = 0.003). Compared to the other phytoplankton groups, diatoms were more associated with the moderate and poor states than with the good status (Fig. 6C and D).



Stations FER: Ferin ASL: Aire-sur-la-Lys CSE: Crevecoeur-sur-Escaut
 FSE: Fresnes-sur-Escaut JEU: Jeumont NSE: Neuville-sur-Escaut
 BER: Berchem BRE: Brebières DON: Don
 ERQ: Erquinghem-Lys NIV: Nivelles WAM: Wambrechies
 WAR: Warcoing WER: Wervicq ZIN: Zingem



◀**Fig. 4** Total rotifer abundance (black dots, right axis) and rotifer taxa relative abundance to the total rotifer abundance (bars, left axis) by station and by campaign. The bars under the stations represent station WFD status (good: FER, moderate: from ASL to NSE, poor: from BER to ZIN)

Concerning zooplankton, Dim 1 and 2 accounted for 22.34% and 11.86% respectively (Fig. 7A) and Dim 3 for 8.39% (Fig. 7B). Dim 1 separated copepod taxa (calanoids, calanoid copepodites, cyclopoid, cyclopoid copepodites) and cladocerans (*Daphnia* spp., *Alona* spp.) associated with temperature and $\text{NO}_2\text{-N}$, from the cladoceran *Chydorus* spp. and rotifers (*B. urceolaris*, *B. leydigii*, *K. quadrata*, *Synchaeta* spp., *Filinia* spp., *Notholca* spp.) mainly associated with O_2 and Chla.

Along Dim 2, the cladoceran *Bosmina* spp., in the upper half of the biplot, was associated to POM, SPM and Cond. The rotifer *Euchlanis* spp. was associated with $\text{DIN-N}:\text{PO}_4\text{-P}$ (N:P) and $\text{NO}_3\text{-N}$. The major part of the zooplankton taxa was situated in the upper right of the biplot, associated with the other environmental factors. Along Dim 3, calanoid and cyclopoid copepods, associated with temperature in the lower left quadrant, were separated from rotifers, distributed in the other three quadrants. *Brachionus* and *Keratella* species were associated with Chla, O_2 and pH in the right lower quadrant.

The poor WFD status differed from the other states in its zooplankton composition (pairwise adonis, P value good vs poor=0.021 and moderate vs poor=0.021) and this difference explained 6.5% of the total variation (permanova, P value=0.007, $R^2=0.0645$). Copepod taxa were more closely associated with good status than cladocerans and rotifers (Fig. 7C and D).

Overall, the IndVal tests highlighted indicators for each status: euglenophytes were indicators of good (P value=0.024), *B. angularis* of moderate (P value=0.048) and diatoms (P value=0.004) as well as *B. calyciflorus* (P value=0.0015) of poor status.

Nutrients–phytoplankton–zooplankton

When plotting the whole Chla dataset concentration as a function of Ptot, a much-used detection method for eutrophication (review by Bennett et al., 2021), a significant linear increase of Chla concentrations with increasing Ptot was detected for Warcoing,

Wervicq, Berchem, Nivelles and to a lesser extent Fresnes-sur-Escaut during the April 2014 and April 2015 campaigns (Fig. 8A). All of these stations were situated on the Scheldt (Fig. 1). The only other station at which Chla exceeded $40 \mu\text{g l}^{-1}$ was Jeumont on the Sambre. Plotting Chla as a function of DIN-N (Fig. 8B) showed that the Scheldt bloom values occurred at DIN-N values between 5 and 7.5 mg l^{-1} , except for Nivelles, which, along with Jeumont on the Sambre, exhibited high Chla values at lower DIN-N concentrations, ($\sim 3 \text{ mg l}^{-1}$) during April.

Total zooplankton abundance followed Chla concentrations during these April blooms (Fig. 8C). This relationship was mainly due to rotifers (Fig. 8D) with *B. calyciflorus* being the main taxa concerned. In contrast, crustaceans (Fig. 8E) and copepod nauplii (Fig. 8F) peaked at lower Chla concentrations, but also mainly in the Scheldt. Plotting crustacean and rotifer abundance as a function of temperature (Fig. 8G) reveals that the former tended to peak at higher temperatures than the latter.

A nutrient not included in the WFD monitoring is Silica. SiO_2 concentrations in the Scheldt were significantly higher than at all the other stations, despite the occurrence of phytoplankton blooms corresponding with dips in Si concentrations (t test, $P < 0.05$, Fig. S3). $\text{SiO}_2/\text{DIN-N}$ ratios were below the generally accepted weight-based Redfield ratio 1.75 in all samples except at Fresnes-sur-Escaut in July 2019 and Nivelles in August 2018 (Fig. S4A, in supplementary information). However, the ratios were higher in the Scheldt than in the Lys and the Scarpe (Kruskal–Wallis, P value=0.02). $\text{Si}/\text{PO}_4\text{-P}$ ratios were always higher than the Redfield value of 12.70 (Fig. S4B, in supplementary information).

Discussion

This paper aimed to determine the extent to which the distribution of phytoplankton and zooplankton communities during the growing season is related to the WFD status of the stations. It tried to identify phytoplankton groups and zooplankton taxa that are specifically related to the station WFD status as well as in order to provide additional ecological information about the ecosystem.

The fact that only one station is in good state and no stations in bad condition were sampled, could be

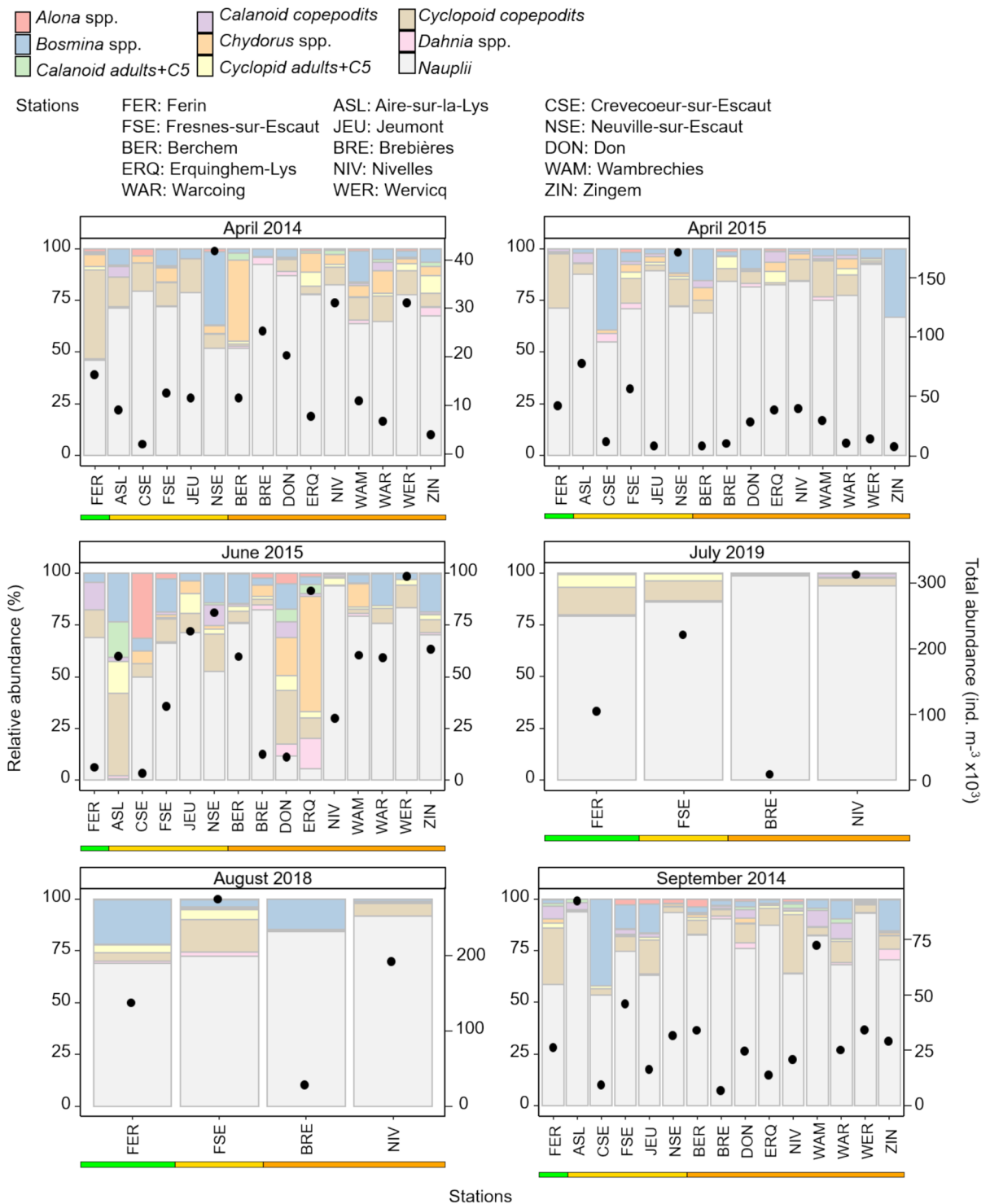


Fig. 5 Total crustacean abundance (black dots, right axis) and crustacean taxa relative abundance to the total crustacean abundance (bars, left axis). The bars under the stations repre-

sent WFD status (good: FER, moderate: from ASL to NSE, poor: from BER to ZIN)

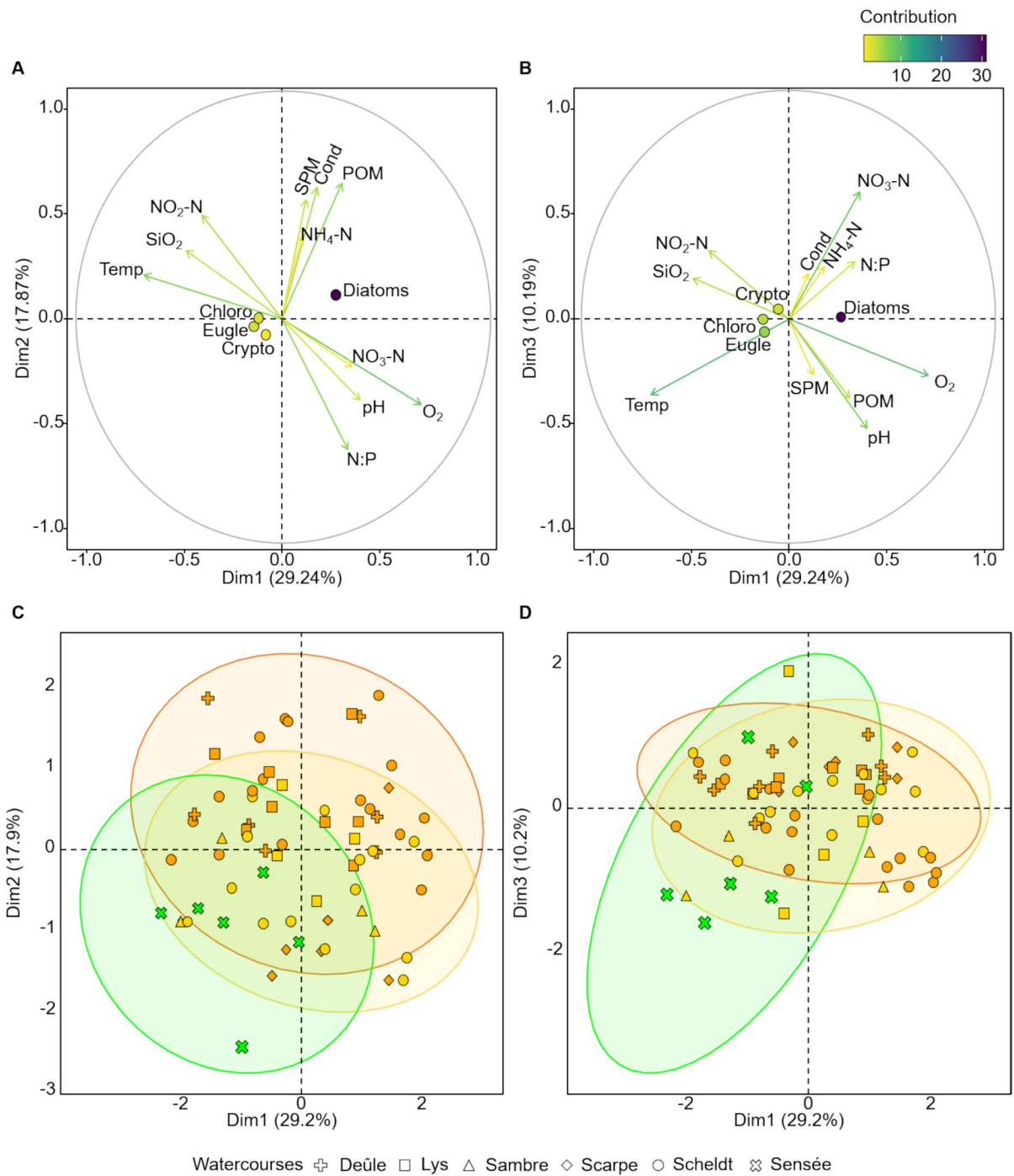


Fig. 6 MFA biplots of phytoplankton and environmental factors data (**A** and **B**: phytoplankton and environmental factors, **C** and **D**: samples). Chloro=chlorophytes, Eugle=euglenophytes, Crypto=cryptophytes, N:P=DIN-N/PO₄-P. 95% confidence ellipses highlight WFD states of stations (green = good,

yellow = moderate, orange = poor). The maximum contribution of the phytoplankton and environmental factors to the dimensions of the MFA is represented by a colour gradient for arrows and points in A and B (legend on top right of graph)

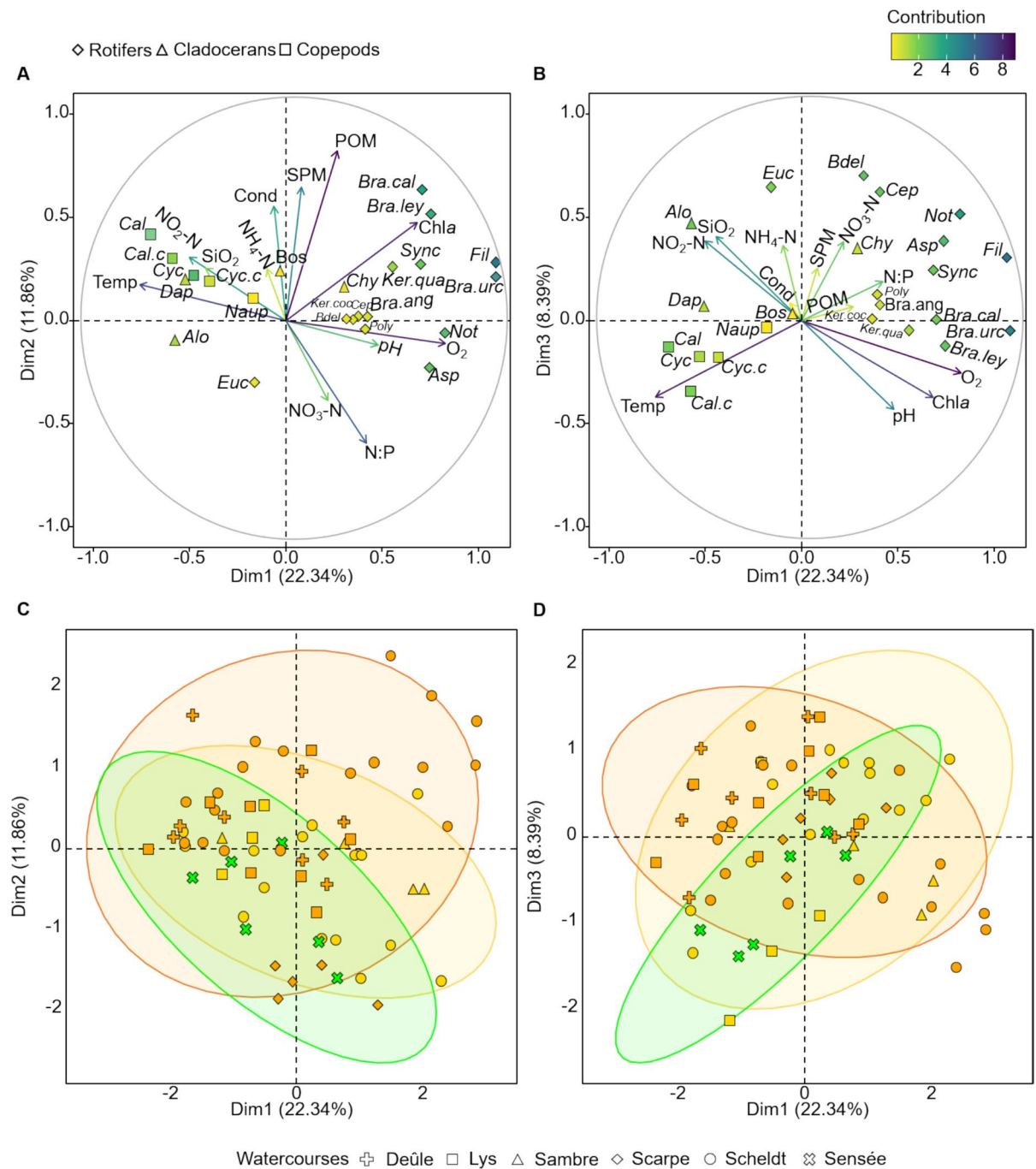


Fig. 7 MFA biplots of zooplankton and environmental factors data (**A** and **B**: zooplankton and environmental factors data, **C** and **D**: samples). N:P=DIN–N:PO₄–P. 95% confidence ellipses highlighted WFD states of stations (green = good, yellow = moderate, orange = poor).

The maximum contribution of the zooplankton and environmental factors to the dimensions of the MFA is represented by a colour gradient for arrows and points in **A** and **B** (legend on top right of graph)

seen as a drawback of the study. However, this distribution of WFD states is representative of the study area, where the majority of rivers are in moderate or poor ecological status. In fact, 31% of all stations in rivers studied are in moderate status and 40% in poor status (www.eau-artois-picardie.fr).

From an efficiency of monitoring viewpoint, it would be interesting to compare these results with those of an all year-round phytoplankton and zooplankton monitoring, to evaluate in how far sampling only during the growing season really suffices to characterise pelagic communities as a function of WFD status.

Environmental factors

Eutrophication remains a significant challenge in water quality management. While the WFD does not set specific objectives for nutrient concentration, it does require EU member states to determine criteria for each type of water in order to guarantee/support good ecological status (Poikane et al., 2019). The WFD guidelines refer to the Drinking Water Directive (Directive 98/83/CE, Légifrance, 1998) and the Nitrates Directive (Directive 91/676/ CEE, Légifrance, 1991). The Drinking Water Directive set a threshold value for $\text{NO}_3\text{-N}$ for the good status at values $\leq 11.3 \text{ mg l}^{-1}$ as well as the Nitrates Directive for ground water. The Nitrates Directive defines surface freshwater where $\text{NO}_3\text{-N}$ value exceeds 4.07 mg l^{-1} as suffering or likely to suffer from eutrophication (Articles R. 211–75, R. 211–76 and R. 211–77 of the Environment Code, Légifrance, 2015).

The current WFD guidelines on NO_3 have been the subject of criticism from several authors who have argued that they are unsuitable for water quality assessment (Pinay et al., 2018; Poikane et al., 2021). Several studies in USA have demonstrated that the first signs of eutrophication, manifested as changes in the composition of benthic or planktonic algal communities and macroinvertebrates, are observed for total nitrogen concentrations between 0.7 mg l^{-1} at 1.8 mg l^{-1} (Dodds et al., 1997; Stevenson et al., 2006; Ponader et al., 2008; Smith & Tran, 2010; Enache et al., 2012; Charles et al., 2019). Poikane et al. (2021) estimate threshold values corresponding to good status for total nitrogen using macrophytes and phytobenthos for lowland rivers with low alkalinity in

the Central-Baltic region of Europe between 0.9 and 3.5 mg l^{-1} .

Indeed, although these thresholds are for total nitrogen, they are below the norm for good status ($< 2.26 \text{ mg l}^{-1} \text{ NO}_3\text{-N}$) and moderate status ($> 2.26\text{--}11.3 \text{ mg l}^{-1} \text{ NO}_3\text{-N}$) in France and Belgium. $\text{NO}_3\text{-N}$ at all the stations exceeded the good status threshold, but not the moderate one, even not at the stations with poor WFD ecological status (Fig. 2). These include Erquinghem-Lys, Wervicq, Wambrechies, Nivelles, Don and Brebieres, all of which have a poor ecological status due to nutrient concentrations (Fig. 2F, G, H, I). So, WFD guidelines on NO_3 do not seem to reflect the real ecological state of the stations.

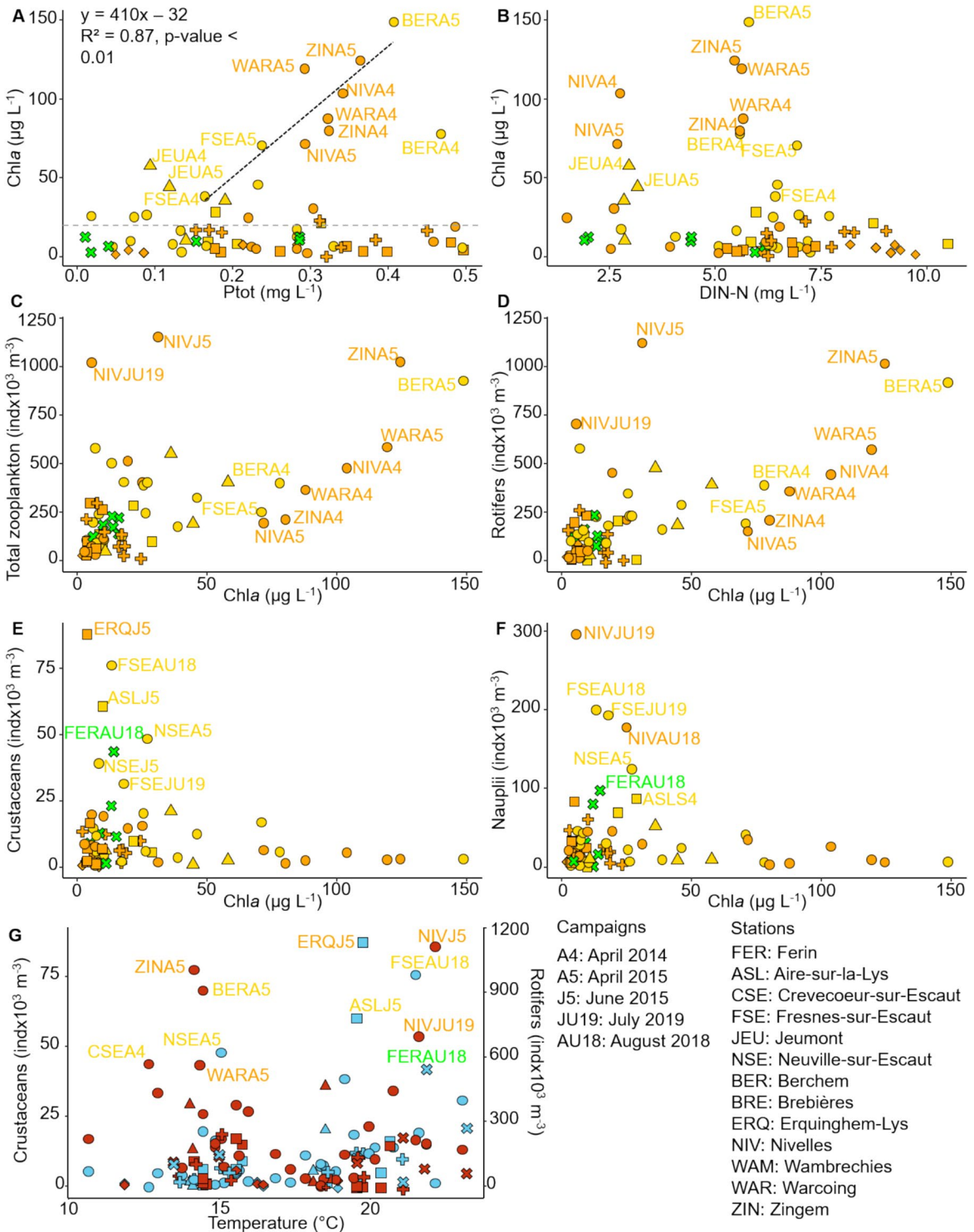
It should be noted that the present study investigated the abundance and distribution of phytoplankton and zooplankton at a number of occasions during seasons with environmental conditions allowing/favouring their development. These conditions differed in 52% of the evaluations from the three-year-round conditions as represented by the WFD status for each station studied. This, however, does not prevent considering the relationship between the WFD status of a study site and the planktonic communities which develop there during the growing season. Further comments concerning the environmental factors are given in the nutrient–phytoplankton–zooplankton section.

Phytoplankton

The stations studied yield a considerable range of phytoplankton biomass ($2\text{--}149 \mu\text{g l}^{-1} \text{ Chla}$). The maximum Chla concentrations reported from temperate rivers in UK and Ireland vary in the $150\text{--}590 \mu\text{g l}^{-1}$ range (Young et al., 1999; Neal et al., 2006; Hutchins et al., 2010; Bowes et al., 2012; Smith, 2016; Moorhouse et al., 2018). Also Mischke et al. (2011) report Chla concentrations ranging from 20 to $300 \mu\text{g l}^{-1}$, with a P_{tot} gradient between 0.05 and 0.4 mg l^{-1} for small, lowland rivers in Germany. So, the maxima Chla concentrations observed in this study can be considered as not excessive for freshwater lotic systems.

Diatoms and chlorophytes are the two communities with the highest contribution to total phytoplankton biomass, as is the case in several lowland rivers, such as the Meuse and the Moselle (Luxemburg/Germany; Gosselain et al., 1998) and the Seine (France; Billen

Watercourses \oplus Deûle \square Lys \triangle Sambre \diamond Scarpe \otimes Sensée \circ Scheldt
 Zooplankton \bullet Rotifers \bullet Crustaceans
 WFD \bullet Good \bullet Medium \bullet Poor



◀**Fig. 8** Chla concentration as a function of Ptot **A** and DIN-N **B**. Abundance of: **C** total zooplankton, **D** rotifers, **E** crustaceans (adult and copepodite copepods and cladocerans) and **F** copepod nauplii as a function of Chla. **G** Abundance of crustaceans and rotifers as a function of temperature. The value of BER A4 is not included in the regression in **A**. The grey dotted line in **A** represents Chla concentration $< 20 \mu\text{g l}^{-1}$

et al., 2001). Phytoplankton blooms are observed during both April 2014 and 2015 in the downstream Scheldt stations which are in poor WFD status. No bloom is observed at the more upstream stations Crevecoeur-sur-Escaut and Neuville-sur-Escaut, in moderate status. A moderate bloom is observed at Fresnes-sur-Escaut, also in moderate status. So, it seems that high nutrient concentrations, which are responsible for the classification in moderate status are a necessary factor to allow phytoplankton blooms. However, this is only the case for the Scheldt river. Many other stations on other rivers, also classified poor because of nutrient concentrations (e.g. Brebieres, Berchem, Don, Wambrechies, Warcoing, Wervicq and Zingem, Fig. 8A), do not show phytoplankton development above $20 \mu\text{g l}^{-1}$. So, other factors should evidently be considered such as chemical pollution, hydrology or grazing by zooplankton (see also below in the nutrient–phytoplankton–zooplankton section).

The MFA analysis showed that phytoplankton groups in the study area are differentially associated with environmental variables. Diatoms are mostly associated with O_2 , pH, $\text{NO}_3\text{-N}$ and $\text{DIN-N/PO}_4\text{-P}$, characteristic of moderate and poor status stations. In contrast, chlorophytes, cryptophytes and euglenophytes are more associated with temperature, SiO_2 and $\text{NO}_2\text{-N}$ and good status (Fig. 6). Diatoms, which constituted the majority of the phytoplankton biomass at 82% of the stations, are indicators of poor status. Euglenophytes, which thrive best in summer and autumn, contributing up to 60% to total phytoplankton biomass, are indicator of the good WFD status. The IndVal values for diatoms and euglenophytes are greater than 0.25 (0.43 and 0.49, respectively). This indicates their presence in at least 50% of the stations of the poor (for diatoms) and good (for euglenophytes) WFD status and/or their relative abundances reach at least 50%.

In several literature reports, diatoms are on the contrary found to be associated with good water quality (e.g. Palmer, 1969; Mischke et al., 2011).

In a study conducted by Charles et al. (2019), the majority of the common diatom species in 95 river and stream sites representing five different ecoregions in New Jersey (USA) exhibited tolerance to P and N pollution. While this large tolerance range makes diatoms useful as bioindicators, unfortunately, the WFD routine evaluation in many European countries, including France, considers only benthic diatoms, while phytoplankton indicator systems are being tested (Laplace-Treytore & Feret, 2016). Recently, Meyer et al. (2024) proposed a WFD compatible phytoplankton-based multimetric index, but specifically for large rivers.

While the stenoecy coefficient is developed for lake phytoplankton in France, several of the species listed in Laplace-Treytore & Feret (2016) also occur in the water courses treated in the present study. The stenoecy range of diatoms found in this study area is consistent with the IndVal results, which indicate that diatoms are associated with high nutrients (in *casu* phosphorus) concentrations, characteristic of moderate and poor status. Indeed, all of the diatom species present in the poor status for all the months sampled have a stenoecy coefficient ≥ 1.78 . Furthermore, diatoms species particularly found in poor (and moderate) states are *Cyclostephanos dubius* (Hustedt) Round 1988 and *Melosira varians* C. Agardh 1827, which have a high stenoecy coefficient of 2.18 and 1.85, respectively. *Ulnaria ulna* (Nitzsch) Compère 2001, the species most present in the good status stations, has a lower stenoecy coefficient (1.00). No stenoecy coefficient is available for the genus *Fragilaria*, also found specifically in poor water. However, in a review by Chandel et al. (2023), this genus is described as tolerant to industrial and organic pollution and present in eutrophic waters (Palmer, 1969; Malik et al., 2021; Uthirasamy et al., 2021).

On the other hand, in the good status station Ferin, unlike the results obtained by HPLC, no euglenophyte species were identified in the microscopic analyses conducted in July 2019 and September 2014 and no stenoecy coefficient is available (Laplace-Treytore & Feret, 2016). Several studies have demonstrated that HPLC analysis can identify a larger number of phytoplankton groups than microscopy, particularly for small, non-dominant species that are more difficult to identify by microscopy (Rodríguez et al., 2003; Brewin et al., 2010; Schlüter et al., 2016; Lee et al.,

2020). This could explain why HPLC analysis reveals more euglenophytes than microscopic analysis.

The differences in results between both methods illustrate the complementarity of HPLC and microscopic analysis. On the one hand, HPLC analyses are limited to the identification of groups, whereas analysis at genera/species level allows the acquisition of information through the sensitivity gradients within the algal groups. On the other hand, HPLC analysis is less work intensive, allowing for more intense monitoring and the detection of small taxa not observed by microscopy. Our results are in line with the recommendation of carrying out HPLC analyses with determining the genera/species present in samples without necessarily carrying out a detailed count (Schlüter et al., 2006; Sarmiento & Descy, 2008; Lauridsen et al., 2011; Seoane et al., 2011).

Zooplankton

Rotifers, with *B. calyciflorus* as the major taxon, were most abundant within the zooplankton community. The dominance of rotifers can be explained by their shorter generation times than crustaceans, which renders them more suited to ecosystems with short retention times such as rivers (Kobayashi et al., 1998; Gosse, 2000; Lionard et al., 2005; Lair, 2006). Many of the dominant taxa found in this study (*B. calyciflorus*, indicator of poor state), *B. angularis* (indicator of moderate state) and *K. cochlearis* are also reported from other, partially canalised lowland rivers such as the Meuse and Moselle (Luxemburg/Germany) (Gosselain et al., 1998) and the Nakdong river (Korea; Kim & Joo, 2000).

The 1,3 biplot (Fig. 6D) shows copepods (calanoids and cyclopoids) and some cladocerans (*Bosmina* spp., *Daphnia* spp., *Alona* spp.) to be more associated with good status, in contrast to rotifers (Brachionidae and Synchaetidae) which are more associated with moderate and poor conditions, but the percentage explanation of axis 3 is small (8.39%). At a higher taxonomic level however, some specific associations occur. *B. angularis* is an indicator of moderate status and *B. calyciflorus* of poor status. They have an IndVal value close to 1 (0.71 for *B. angularis* and 0.78 for *B. calyciflorus*), indicating that these species are almost exclusively present in the moderate and poor states and almost in all the stations in these states.

Brachionidae are described in the literature in Europe, Brazil and China as indicators of eutrophic and Chla-rich conditions (Sládeček, 1983; Bērziņš & Pejler, 1989; Van Tongeren et al., 1992; Attayde & Bozelli, 1998; Čeirāns, 2007) and particularly well adapted to life in the current (up to 20 cms⁻¹; Lair, 2006). *B. angularis* appears as an indicator taxon of hypertrophic state in reservoirs and lakes (Bērziņš & Pejler, 1989; Radwan & Popiolek, 1989; Lougheed & Chow-Fraser, 2002; García-Chicote et al., 2019) but also as an indicator of organic pollution (Sládeček, 1983). *B. calyciflorus* appears to be a reliable indicator of eutrophic conditions in lakes and rivers (Sousa et al., 2008; Gao et al., 2021).

Copepod and cladoceran abundance ranged the same orders of magnitude, with cyclopoid copepods being far more abundant than calanoids. This corresponds to a general trend in river-estuarine continua, where calanoid copepods mainly develop in the tidal more saline reaches (Soetaert & Van Rijswijk, 1993; Modéran et al., 2010). Their association to rather good water quality can be in part explained by the fact that drainage basin-derived pollution is diluted downstream (Gutierrez et al., 2022). Several studies in Europe show that zooplankton has a high water quality indicator value that is not represented in the pelagic biota studied in the WFD (phytoplankton and fish) (Caroni & Irvine, 2010; Jeppesen et al., 2011; García-Chicote et al., 2019; Min et al., 2021; Muñoz-Colmenares et al., 2021). In the present study, phytoplankton and zooplankton communities during the growing season are equally distinctive of the WFD states of the stations, although in a different way. Phytoplankton communities differ between good and moderate states as well as between good and poor states, while zooplankton communities differ between poor and moderate and poor and good states. Nevertheless, the eigenvalue and the total explained variance by WFD states of the zooplankton MFA are lower than those of the phytoplankton (42.5 vs 57.3% and 6.5 vs 12.6%, respectively).

Nutrients–phytoplankton–zooplankton

As explained above, the Chla concentrations observed in this study can be considered as moderate. Several studies on longitudinal evolution of phytoplankton biomass report an increase along the head water-downstream gradient (Basu & Pick, 1997).

Low upstream planktonic production/biomass in river-estuarine continua are consistent with the River Continuum Concept (RCC, Vannote et al., 1980). However, Le Coz et al. (2017), studying the spring phytoplankton biomass and zooplankton distribution along the Scheldt continuum from source to mouth, show that Chl a concentration can be as high in the upper basin (corresponding to the stations of this paper) and then in the adjacent estuarine tidal reach, the most productive section of the continuum.

While phosphorus has long time been considered to be a limiting factor for phytoplankton in fresh water (Vollenweider, 1976; Hecky & Kilham, 1988; Mischke et al., 2011), there is increasing evidence that both nitrogen and phosphorus should be considered to study phytoplankton development in rivers (Dodds et al., 2002; Dodds, 2007; Dodds & Smith, 2016; Poikane et al., 2019). In the studied area, 92% of the samples have DIN–N/PO $_4$ –P ratios > 7, suggesting a P limitation in regulating the rate of primary production, rather than nitrogen (N). However, a clear (linear) relationship between Chl a concentration and P $_{tot}$ representing a considerable incorporation of P in phytoplankton biomass, was only observed in April at the Scheldt poor status stations (Fig. 8A).

The observed phytoplankton bloom gave rise to the development of important rotifers communities, while this association was not observed for crustaceans. One potential explanation for this is that crustaceans are known to develop later in the year, at higher temperatures (Sommer et al., 1986). This is the case in the Scheldt stations, where the majority of crustacean peaks occurred at temperatures > 17 °C, corresponding to lower Chl a concentrations (Fig. 8G). The extent to which the observed lower phytoplankton concentrations are a consequence of crustacean grazing will be addressed in a subsequent publication.

It is unclear why phytoplankton blooms develop in the Scheldt moderate quality stations and not in other stations also in moderate status. The high diatom biomass observed at most of the stations suggests that dissolved silica may play an important role in phytoplankton dynamics. While all silicate concentrations are consistently above the limiting value for uptake of 0.6 mg l $^{-1}$ (Maris et al., 2022), they are higher in the Scheldt, where phytoplankton blooms have been observed. The SiO $_2$ /N ratio is also less removed from the optimal ratio in the Scheldt than in the other rivers. So, it is possible that Si concentrations regulate

primary production and are responsible for avoidance of phytoplankton blooms in the majority of the study area stations. Cryptophytes and euglenophytes are more associated with temperature, NO $_2$ –N and good status stations and rarely give rise to Chl a concentrations > 20 μ g l $^{-1}$. It would be interesting to include current velocities or residence times of the different rivers in the analysis. Unfortunately, these data are not available. Nevertheless, if lower current velocities in the Scheldt would be responsible for its phytoplankton development, one would expect non-siliceous species, which generally have a shorter development time than diatoms, to develop better than diatoms in the other rivers with shorter residence times. However, the ratio diatoms/other groups contribution to total phytoplankton biomass is not different between the Scheldt and the other rivers (Kruskal–Wallis, $P > 0.05$).

Apart from general trends found in several lowland rivers, such as a dominance of diatoms and of rotifer taxa, it was difficult to find comparable systems to the upper Scheldt basin studied here. Lowland river studies often consider big, high-order rivers and small, low-order rivers are often studied at high-altitude/strongly vegetated sites. As said in the introduction, the study zone of this paper, with a regulated hydrology and high agricultural and anthropogenic impact, falls in a little documented category in open literature, at least as far as plankton is concerned. The high variation in ecological situations along upper basin-mouth continua has given rise to several conceptual views (e.g. Vannote et al., 1980; Thorp et al., 2006), which all illustrate the particularity, but also the importance of the upstream sections to the downstream system functioning. So, we plead for more publications on these upstream, lowland sections, using WFD data and preferably including zooplankton. For a summary of the continuum concepts and an application to the Scheldt, the reader is referred to Le Coz et al. (2017).

Conclusion

The study showed that in channelised water courses of the lower Scheldt basin, the distribution of phytoplankton and zooplankton communities differ significantly according to WFD status. Phytoplankton communities differ between good and moderate states

as well as good and poor states, while zooplankton communities differ between poor and moderate states and poor and good states. Phytoplankton groups and zooplankton taxa are indicators of WFD status: euglenophytes are characteristics of good status, the rotifer *B. angularis* of moderate status stations, diatoms and the rotifer *B. calyciflorus* of poor status.

Dissolved silica should be considered to study phytoplankton development in WFD assessment, indeed in this study, dissolved silica plays an important role in phytoplankton dynamics and maybe regulate primary production. While phytoplankton blooms observed in the river Scheldt gave rise to the development of important rotifers communities, crustaceans peaks occurred to lower *Chla* concentrations. So, it will be interesting to investigate on the potential regulation of phytoplankton blooms by crustacean grazing.

Similar studies on a larger scale, including more variety of WFD status stations and year-round sampling, would be useful to validate the complementarity of phytoplankton and zooplankton data to the WFD ecological evaluation of aquatic systems.

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Author contributions The conceptualisation and methodology were realised by Elisa Bou, Céleste Mouth and Anaëlle Bernard. Database construction and analysis was done by Elisa Bou. Michèle Tackx took care of funding acquisition, project administration and supervision. Frédéric Azémar, Maïwen Le Coz, Sophie Chambord, Julien Ovaert and Claudine Sossou contributed to the field work, sample and data analysis in various stages of the BIOFOZI and BIOFOPAN projects and the realisation of this manuscript. Christophe Lesniak and Jean Prygiel provided essential elements and interpretation about the WFD evaluation system and the microscopic analysis data. Elisa Bou was responsible for visualisation of results, draft writing and finalisation of the manuscript. All authors commented on previous versions of the manuscript and have read and approved the final version.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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