Morphology-based classification of functional groups for potamoplankton

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ABSTRACT

Simple morphological traits of phytoplankton have proven to correlate well with the functional properties of taxa. A logical way of distinguishing functional groups of phytoplankton is to cluster species according to their morphological traits, instead of phytosociological traits, which are difficult to measure and incomplete for the vast majority of phytoplankton species. This novel approach inspired the establishment of morphologically based functional groups (MBFG) for lake phytoplankton. This study considers the MBFG as a potentially suitable tool for investigating and comparing the potamoplankton assemblages in riverine ecosystems. This study constructed a special version of MBFG that is aimed at rivers, termed MBFG$_R$. MBFG$_R$ is based on information obtained from more than 800 samples of potamoplankton and zooplankton collected from 101 rivers located in four climate zones. Their habitat templates were also described by the thresholds of the environmental variables that promote group dominance. Despite its similar framework with MBFG, MBFG$_R$ emphasized the presence of flagella, size and shape, exoskeletal structures, life strategy and subdivision of diatoms. Their habitat templates are outlined by additional factors connected to hydrology and river types. Moreover, each discussed template includes consideration of grazing by zooplankton. Theoretically, MBFG$_R$ is better than MBFG because it can sensitively capture the ecological preferences of phytoplankton groups most associated with rivers and provides empirical values to reflect the environmental conditions in riverine ecosystems.

Key words: Functional groups; habitat template; algae; phytoplankton; river; zooplankton predation.

INTRODUCTION

The focus of current research on phytoplankton ecology is on finding an effective substitute for the traditional taxonomic approach to understand phytoplankton patterns in different types of aquatic systems (Salmaso *et al.,* 2012). The phytosociological approach is aimed at linking phytoplankton community and environmental factors (Reynolds, 1980; Reynolds, 1984), and has been highly valued for decades. It stimulated the proposition of functional groups (FG) (Reynolds *et al.*, 2002). Subsequently, morpho-functional groups (MFG) combining morphological traits led to a relative simplification of the *functional scheme* (Salmaso and Padisák, 2007). According to the growing reports by phytoplankton ecologists worldwide, FG and MFG usually provide reliable predictions of environmental conditions in various aquatic ecosystems (Devercelli and O'farrell, 2013), thereby proving the validity of such approaches as effective tools for the assessment of phytoplankton community structure (summarized by Padisák *et al.*, 2009). However, the relationship of morphological traits and phytosociological traits were not evaluated thoroughly until Kruk *et al.* (2010) proposed the morphologically based functional groups (MBFG). The current paucity of information on species-specific

physiological traits limits our ability to arrive at a priori functional classification. Given the expense and practical limitations of systematically investigating physiological traits, arriving at a functional classification of phytoplankton based on simple morphology which could be used for ecological prediction, becomes possible (Kruk *et al.,* 2010). After measuring ten physiological traits, seven (maximum growth, sink velocity, Si half-saturation constant for growth in laboratory, abundance, maximum biomass, mean biomass, and biomass standard deviation in fields) were shown to be closely associated with morphological traits (volume, maximum linear dimension, surface area, and the presence of mucilage, flagella, gas vesicles or aerotopes, heterocysts and siliceous exoskeletal structures). Consequently, MBFG clustered more than 700 species into seven morphologically distinctive groups. These species were from more than 200 lakes situated in climate zones that ranged from subpolar to tropical ones. Differences in simple morphological traits among MBFGs well reflected the significant differences in functional characteristics and habitat templates independently from the geographical location (Kruk *et al.,* 2010; Kruk and Segura, 2012).

Studies applying MBFG (Pacheco *et al.,* 2010; Segura *et al.,* 2013) and those compared MBFG with FG and

MFG (Gallego *et al.,* 2012; Izaguirre *et al.,* 2012; Hu *et al.,* 2013) in a range of lentic water (lakes, ponds, and reservoirs) commended the high simplification and practicality of MBFG. Given that the MBFG was initially proposed for lakes, only a few researches have used it to investigate and compare the phytoplankton assemblages in riverine ecosystems and have concluded low sensitivity (Centis *et al.,* 2010; Stanković *et al.,* 2012; Mihaljević *et al.,* 2015). This consequence can be inferred from the following: i) the predominant presence of only one diatom group; and ii) the poor general-representativeness of some species that are only important for lakes. Such flaws are rooted in the targeted data source (lakes) of MBFG formation rather than the thought process behind the classification. Thus far, existing classification approaches based on ecological functions are all aimed at lakes where phytoplankton communities are relatively abundant and diverse. Rivers are as important as lakes for water management (Wehr and Descy, 1998). MBFG is a potentially suitable tool for riverine study, but its misuse can have serious consequences for interpreting ecological processes (Salmaso *et al.,* 2014). Thus a special version of MBFG for potamoplankton is required and the development of such a version would be meaningful.

Rivers differ from lakes in many aspects, including the water residence time, drainage area, water depth, stream flow, and water clarity, thereby resulting in different ecological structures and functions (Søballe and Kimmel, 1987). Potamoplankton are the primary producers of riverine ecosystems; they directly reflect such differences through biomass proportion, community composition, and morphological traits (Reynolds and Descy, 1996; De Emiliani, 1997). There is an annual cycle of potamoplankton succession (Muylaert *et al.,* 2000) and an analogy of annual cycle in stratified lakes with feedback mechanisms (Gamier *et al.,* 1995). Both studies emphasized the large proportion and long survival of diatoms in rivers compared with lakes. Moreover, other algae such as flagellates (Cloern *et al.,* 1983; De Oliveira and Calheiros, 2000; Gómez *et al.,* 2004), filamentous cyanobacteria (Köhler and Hoeg, 2000), non-diatoms that indicate altered conditions (Mihaljević *et al.,* 2013), are also important. The composition and persistence of potamoplankton assemblages in riverine ecosystems mainly depend on physical (light, water temperature, and turbidity) and hydrological factors (discharge, flow velocity, water level, suspended sediment concentration and water residence time) (Köhler, 1993; Gosselain *et al.,* 1994; Kirk, 1994; Reynolds *et al.,* 1994; Reynolds and Descy, 1996; Train and Rodrigues, 1997; Centis *et al.,* 2010; Salmaso and Zignin, 2010), and resolved silicate concentration (Dortch and Whitledge, 1992; Tavernini *et al.,* 2011). All of these can be distinguished from those in lakes. Therefore, if morphological classification is especially suited for potamoplankton, this approach probably attaches

more importance to the subdivision of diatoms, life forms, traits of flagella, and siliceous structures. Furthermore, habitat templates are supposed to be outlined by additional suitable conditions that indicate river types. Several recent studies showed that the differences among river types (*e.g.,* upper and down streams) are defined in terms of their hydrology and their phytoplankton composition (Várbíró *et al.,* 2007) because of floods (Stanković *et al.,* 2012), climate, topography, and human activity (Abonyi *et al.,* 2014).

In light of the previous studies on the MBFG of lakes, we attempted to construct a similar classification system, namely, $MBFG_R$, whose original data were all river cases, to meet a pressing need to study rivers through the phenotypic variability of potamoplankton. We hypothesized that particular environments will select phytoplankton based on the functional properties that are reflected by the differences in morphological traits among $MBFG_R$. The analysis and habitat template of each $MBFG_R$ environmental preference was established and compared with the MBFG of lakes. Moreover, zooplankton was taken into consideration because grazing exerts critical control over plankton dynamics (Reynolds, 1984; Gosselain *et al.,* 1994; Gamier *et al.,* 1995; Reynolds and Descy, 1996; De Emiliani, 1997).

METHODS

Sampling and measurements

A database of 531 species from 101 rivers located within four climate zones was compiled. This database covered a wide range of environmental characteristics [s, for the two Russian rivers in subpolar zone, information was obtained from unpublished sources (an ecological survey of large rivers of Russia by J. Skakalski, *personal communication*)]. The remaining 99 rivers were distributed in three climate zones of China and sampled for physical, chemical, and biological parameters during 2008-2013. Of the total number of rivers, 67 were sampled only once (12 rivers in spring, 19 in summer, 23 in autumn and 13 in winter), whereas 36 were sampled at least once every season (21 rivers seasonally and 15 bimonthly). The stream order of these rivers ranged from first to sixth. Among these rivers, 68 are located in plains and 33 in mountainous areas. The total drainage area of the 36 large rivers exceeded 50,000 km^2. The investigation was conducted at middle-upstream of 34 rivers and at middle-downstream of 67 rivers (Tab. 1). A complete profile of the rivers studied is reported in Supplementary Tab. 1

The following variables were measured *in situ*: surface water temperature (WT, °C), pH, Chlorophyll-*a* (Chl-*a*, µg L^{-1}), and dissolved oxygen (DO, mg L^{-1}) with a portable YSI model 55 probe (YSI, Yellow Springs, OH, USA). The flow velocity (*v*, m s^{-1}) was determined with a direct reading current meter FP211 (KANGGAOTE, Beijing, China). The light attenuation coefficient (K_d, m^{-1}) was calculated

from *in situ* measurements of irradiance by a spectrum-submersible radiometer (SR9910-PC, Macam, England) (Kirk, 1994). The discharge (Q, m³ s⁻¹) was represented by the mean value for the month from the nearest hydrological station. In the laboratory, the flow injection analyser (Skalar SAN++, Breda, Netherlands) was used to measure total nitrogen (TN, mg L⁻¹) and total phosphorus (TP, mg L⁻¹) concentrations. The soluble reactive silicate (RSi, mg L⁻¹) was measured by plasma emission chromatography ICAP (ICP6300, Thermo Scientific, Waltham MA, USA). The suspended sediment concentration (SSC, g L⁻¹) of upper 100 cm water was measured by an electronic balance (ME104E, Mettler Toledo, Zurich, Switzerland) after vacuum filtration (0.45 μm) and drying.

Quantitative phytoplankton samples were fixed *in situ* with Lugol's solution. Counting was performed with an inverted microscope (OLYMPUS IX-70, Japan) following the Utermöhl method (Lund *et al.,* 1958) at 400× magnification. The counting error was estimated according to Duarte *et al.* (1990). For each sample, at least 400 units (filament, colony, coenobium and single-celled organisms) were enumerated. We estimated the cell numbers per colony or filament, as well as the organism dimensions and maximum linear dimension (MLD, μm). The individual volume (V, μm³) and surface area (S, μm²) were calculated according to the geometric equations of Hillebrand *et al.* (1999). The phytoplankton biomass (mg L⁻¹) was converted from biovolume by using a method described by Hasle (1978) and Javornicky and Komárková (1973). For colonial organisms with mucilage, V and S calculations were made for entire colonies, including mucilage. The presence of aerotopes (Aer), flagella (Fla), mucilage (Muc), unicellularity (Uni), heterocysts (Het), exoskeletal structures (Exs) or siliceous structures (Si) was noted for each relevant organism.

To determine the zooplankton abundance (TZ, org L⁻¹), zooplankton were collected using a 30-cm-diameter plankton net (64-μm mesh) that was horizontally held below the surface in the upper part of the water column. A flow meter was placed next to the mouth of the net so that the total volume of river water filtered for each sample could be determined for use in density measurements. Samples were fixed with buffered formaldehyde (10% final concentration). Before microscopic analysis, the fixed samples were concentrated and re-suspended in filtered river water up to 30 mL in volume. Individuals were enumerated and identified to genus level with an upright microscope (Olympus BX-51, Tokyo, Japan). The core body lengths (μm) of individuals were noted.

Data analysis

A two-step cluster analysis was used to construct the MBFG$_R$ by classifying the species based on the eleven measured morphological traits (V, S, S/V, MLD, Aer, Fla, Muc, Het, Uni, Exs and Si). The Akaike Information Criterion (AIC) can reflect the model parsimony by combining the goodness-of-fit and the number of estimated model parameters (Akaike, 1973). The AIC helped us determine the optimum number of clusters, and each of the cluster solutions was compared. After clustering, outlier selection was performed with noise handling of 25% and no instance was picked out. All analyses were carried out using SPSS for Windows (ver. 20.0). Redundancy analyses (RDA) were used to estimate how much variance of the MBFG$_R$ biomass was explained by the environmental variables. Previously, a detrended correspondence analyses (DCA) was performed. The data showed a linear response, thus RDA was applied. Calculations were performed with the program CANOCO 5.0. The statistical significance of the first axis and of all the axes was tested by a Monte Carlo permutation test. The importance of each variable was assessed by forward selection.

To establish the habitat templates of each MBFG$_R$, a classification and regression tree (CART) was used. Unlike standard regression, this tree is constructed by the recursive

Tab. 1. Average and range (in brackets) of environmental variables of rivers included in this study for the different regions of the northern hemisphere, as follows: Subpolar, Temperate, Subtropical, and Tropical.

Latitude	No. of rivers	WT (°C)	Ice cover	Q (m³ s⁻¹)	v (m s⁻¹)	SSC (g L⁻¹)	K$_d$ (m⁻¹)	TN (mg L⁻¹)	TP (mg L⁻¹)	Chl-*a* (μg L⁻¹)	RSi (mg L⁻¹)
Subpolar	2	10.2 (3.7-19.5)	Yes	8835 (300-27000)	0.7 (0.2-1.6)	0.06 (<0.001-0.86)	1.3 (36.1-0.4)	2.16 (0.063-20.99)	0.344 (0.015-5.060)	7.5 (<0.3-178)	0.83 (0.10-4.91)
Temperate	25	16.8 (0.5-33.7)	Yes	812 (3.7-10600)	0.4 (<0.05-4.0)	0.78 (<0.001-4.45)	4.5 (37.2-1.4)	2.52 (0.078-23.89)	0.186 (0.038-3.025)	10.4 (0.3-297)	1.52 (0.02-6.89)
Subtropical	56	20.6 (8.0-31.2)	No	1256 (2.8-36060)	1.3 (<0.05-5.7)	0.11 (<0.001-1.93)	2.4 (19.1-0.6)	3.26 (0.054-36.72)	0.443 (0.029-9.411)	20.1 (<0.3-395)	3.63 (0.01-8.90)
Tropical	18	25.0 (12.3-38.5)	No	1652 (12-15070)	1.0 (<0.05-4.3)	0.09 (<0.001-1.65)	1.6 (9.4-0.5)	0.57 (0.028-22.05)	0.096 (0.011-0.493)	4.9 (<0.3-142)	1.49 (0.01-9.01)

*WT, water temperature; Q, discharge; v, flow velocity; SSC, suspended sediment concentration; K$_d$, light attenuation coefficient; TN, total nitrogen; TP, total phosphorus; Chl-*a*, Chlorophyll-*a*; RSi, soluble reactive silicate. The detailed information is shown in the Supplementary Tab. 1.*

binary partitioning of the response variable into regions that are increasingly homogeneous (*i.e.* nodes) until no improvement is possible (Kruk and Segura, 2012). The final nodes are called leaves. At each node, the predictor variable that results in the most homogeneous partition of the response variable (measured by the sum of squared errors, SSE) is selected based on an optimization process (Breiman, 2001). This process continues until reduction of SSE is no longer achieved. The methods are easily interpretable and provide simple yes (>) or no (<) decision trees (Kruk and Segura, 2012). After constructing the full tree, this tree was pruned back by minimizing the cross-validated error to avoid overfitting (De'ath and Fabricius, 2000).

The Gasol model (Gasol, 1994) that was developed for nanoflagellates, allows assessment of the importance of top-down *vs.* bottom-up mechanisms. It helped in identifying the controlling direction when each MBFG$_R$ dominates a river. This model consists of a bivariate space where the (*log*)-transformed phytoplankton and zooplankton abundances are plotted. A *maximum attainable abundance line* (MAA) is computed. MAA depicts the zooplankton abundance that can be attained for a given phytoplankton abundance by assuming that zooplankton feeds only on phytoplankton, which is rarely achieved in natural systems. A *mean realized abundance line* (MRA) is deduced and summarized from observations. MRA represents the average level in nature. The location of data points below the MRA line indicates that the said ecosystem is mainly driven by top-down control, whereas bottom-up regulation is suggested when samples are located above the MAA line.

To evaluate the relative importance of dominant MBFG$_R$ biomass in driving the zooplankton predation, the river-samples were preselected and aggregated according to MBFG$_R$ dominance (>75% cases). Preliminary screening identified that the component river-cases had nearly identical results. To examine the relationships of dominant-group biomass and zooplankton abundance, least-squares linear regression was used. Given the risk of spurious correlation (Jansson *et al.,* 2010), relative comparisons of phytoplankton and zooplankton were prudent to make rather than focusing on the individual r² values. Statistical analyses were performed with SPSS 20.0.

RESULTS

Morphologically based functional groups from rivers

Based on the morphological traits, the two-step cluster analysis separated potamoplankton species into eleven groups, named as MBFG$_R$. As expected, a similar framework and significant differences were observed between MBFG$_R$ and MBFG. Therefore the eleven groups were labelled according to their closest MBFG and marked with a suffix when necessary by considering the comparative study and use. All groups of MBFG$_R$ and their representative taxa

are described in Tabs. 2 and 3, and the basis for classifying species is summarized in the dichotomous key in Tab. 4.

MBFG I was divided into two parts, namely, Groups RIa and RIb based on the absence or presence of flagella. Group RII was re-established for all flagellated organisms with exoskeletal structures and Group RII was subdivided into RIIa and RIIb based on size. Group RIII included all large filaments, whereas the MBFG III was restricted to filaments with aerotopes. Group RV excluded species with hard scales from MBFG V. MBFG VI was subdivided into Groups RVIa, RVIb and RVIc; these groups emphasized size and life form of diatoms. Group RIV and RVII seemed equivalent to MBFG IV and VII, respectively.

Environmental effects on MBFG$_R$ and their dominance

The results of redundancy analysis (RDA) indicated that the biomass of potamoplankton corresponding to the MBFG$_R$ was closely affected by environmental variables. The first two axes accounted for 77.1% of the variance (axis 1, 46.5%; axis 2, 30.6%). The Monte Carlo test indicated that the environmental variables were significantly correlated with the first axis (P=0.018), and the test of significance of all canonical axes was also significant (P=0.006). The first axis was mainly correlated with K$_d$, *v*, and RSi (intra-set correlation coefficients: 0.64, 0.64, and 0.49, respectively), whereas the second axis was mainly defined by WT, SCC, TP, and TN (intra-set correlation coefficient: -0.73, -0.26, -0.48, and -0.42, respectively). Fig. 1 showed the biplots (first two axes) of the
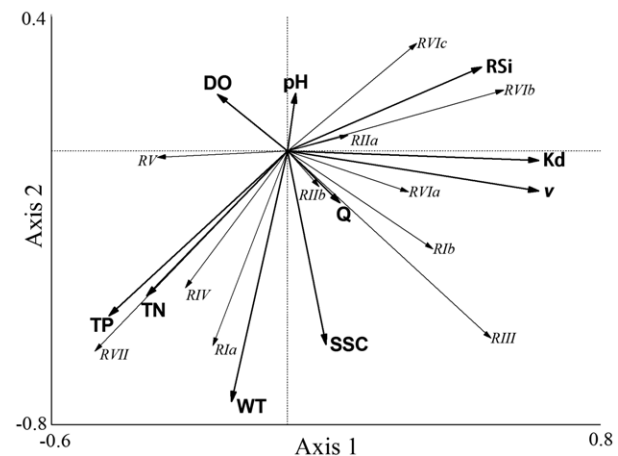

Fig. 1. Biplots of the RDA based on the biomass of the MBFG$_R$ and the environmental variables. WT, surface water temperature; DO, dissolved oxygen; K$_d$, light attenuation coefficient; SSC, suspended sediment concentration; Q, discharge; *v*, flow velocity; TN, total nitrogen; TP, total phosphorus; RSi, soluble reactive silicate.

MBFG$_R$ with respect to the environmental variables. Groups RIa, RIV, and RVII were closely related to TN, TP, and WT. Groups RVIb and RVIc were closely related to RSi. Groups RIb RIII and RVIa were closely related to Q, *v*, and SSC.

From 864 river cases, 245 presented that one MBFG$_R$ reached >75% of the total biomass. Groups RIb and RIIa were dominant only in 6 and 8 river samples, respectively.

Group RIa dominated in 11 river samples, Group RIIb in 13, Group RIII in 26, Group RIV in 17, Group RV in 58, Group RVIa in 38, Group RVIb in 45, Group RVIc in 13, and Group RVII in 10. Subsequently, each river case was labelled according to the dominant MBFG$_R$ and was classified according to the environmental variables. The pruned tree had a complexity parameter of 0.01, a relative error of 0.14, and a cross-validated error of 0.87.

Tab. 2. Description of the MBFG$_R$ obtained using more than 800 river samples.

MBFG$_R$	Description	V (μm³)	S (μm²)	S/V (μm⁻¹)	MLD (μm)	Aer	Fla	Muc	Het	Si	Uni	Exs
RIa n=43	Small organisms	20.3 0.6-194	124 2.1-5.6e+3	3.6 1.0-10.4	8.5 0.7-31.7	0.05 0-1	0.00 0	0.03 0-1	0.00 0	0.00 0	0.10 0-1	0.00 0
RIb n=29	Small organisms with flagella	42.4 0.8-283	165 5.8-7.6e+3	5.2 1.4-26.2	20.1 1.2-53.1	0.00 0	1.00 1	0.03 0-1	0.00 0	0.00 0	0.90 0-1	0.00 0
RIIa n=18	Small flagellated organisms with exoskeletal structures	487 1.5-2.63+3	248 4.5-2.1e+3	1.7 0.9-9.7	10.7 2.0-60.5	0.00 0	1.00 1	0.00 0	0.00 0	0.31 0-1	0.83 0-1	1.00 1
RIIb n=35	Medium to large flagellated organisms with exoskeletal structures	3.2e+3 15.7-2.1e+5	906 6.7-2.3e+4	1.4 0.1-5.0	18.5 5.2-186	0.00 0	1.00 1	0.00 0	0.00 0	0.12 0-1	0.75 0-1	1.00 1
RIII n=70	Large filaments	2.9e+3 6.2-1.7e+4	2.9e+3 18.5-8.53+3	1.8 0.4-4.8	125 5.5-877	0.63 0-1	0.00 0	0.24 0-1	0.69 0-1	0.00 0	0.00 0	0.00 0
RIV n=105	Medium sized organisms lacking specialized traits	1.6e+3 12.4-5.1e+4	811 28.6-1.9e+4	1.6 0.1-8.9	35.9 4.7-521	0.00 0	0.00 0	0.00 0	0.00 0	0.00 0	0.44 0-1	0.00 0
RV n=76	Unicellular flagellates of medium to large size	1.2e+3 4.6-1.0e+5	655 7.1-1.9e+4	1.3 0.1-4.7	17.3 6.1-203	0.00 0	1.00 1	0.00 0	0.00 0	0.00 0	1.00 1	0.00 0
RVIa n=87	Small non-flagellated organisms with siliceous exoskeletons	311 7.5-1.5e+4	575 3.4-2.4e+3	1.2 0.4-4.2	21.8 2.8-190	0.00 0	0.00 0	0.00 0	0.00 0	1.00 1	1.00 1	1.00 1
RVIb n=53	Large non-flagellated organisms with siliceous exoskeletons	4.6e+3 11.9-6.4e+4	1.6e+3 16.4-2.1e+4	1.1 0.2-3.2	53.2 10.3-610	0.00 0	0.00 0	0.00 0	0.00 0	1.00 1	1.00 1	1.00 1
RVIc n=47	Colonial non-flagellated organisms with siliceous exoskeletons	1.4e+3 8.1-5.4e+4	1.2e+3 10.5-1.9e+4	1.1 0.1-3.5	40.6 5.4-362	0.00 0	0.00 0	0.00 0	0.00 0	1.00 1	0.00 0	1.00 1
RVII n=41	Large mucilaginous colonies	2.5e+4 4.3-1.3e+4	2.2e+3 15.2-4.6e+4	1.0 0-5.6	21.3 3.5-244	0.15 0-1	0.00 0	1.00 1	0.00 0	0.00 0	0.00 0	0.00 0

V, volume; S, surface area; MLD, maximum linear dimension.

Tab. 3. Representative taxa of MBFG$_R$.

MBFG$_R$	Description	Representative taxa
RIa	Small organisms	*Synechocystis minuscula, Scenedesmus ellipticus, Chroococcus cohaerens, Stichococcus bacillaris*
RIb	Small organisms with flagella	*Chroomonas* spp.
RIIa	Small flagellated organisms with exoskeletal structures	*Kephyrion* spp. *Chrysococcus* spp. *Chrysosphaerella* spp.
RIIb	Medium to large flagellated organisms with exoskeletal structures	*Peridinium* spp. *Peridiniopsis* spp.
RIII	Large filaments	*Osillatoriales, Nostocales, Ulothrix* spp.
RIV	Medium sized organisms lacking specialized traits	*Volvocales,* * *Chlorococcales, Desmidiaceae*
RV	Unicellular flagellates of medium to large sizes	*Cryptomonas* spp. *Euglena* spp. *Gymnodinium* spp.
RVIa	Small non-flagellated organisms with siliceous exoskeletons	*Cyclotella* spp. *Navicula minima, Gomphonema parbulum, Cymbella pusilla*
RVIb	Large non-flagellated organisms with siliceous exoskeletons	*Pinnularia gentilis, Stephanodiscus neoastraea, Cymbella cistula*
RVIc	Colonial non-flagellated organisms with siliceous exoskeletons	*Melosira* spp. *Asterionella formosa, Asterionella* spp.
RVII	Large mucilaginous colonies	*Microcystis* spp. *Botryococcus braunii*

When five or more species from the same genus belong to identical MBFG$_R$, they are not listed individually and just referred to as Genus *spp. Higher taxonomic levels are indicated in a similar manner. *The flagella of this taxon is explained in the Discussion section regarding Group RIV.*

Rivers dominated by all groups were adequately discriminated according to the environmental variables by the tree (Fig. 2). K_d was the first selected variable with a threshold value of 4.5 m^{-1} in the first node (the root node). The next selected variable was WT, but the threshold values were not the same to the left (22°C) and to the right (20°C). The further selected variables included v, SSC and RSi. Meanwhile, TP, TN, pH, DO, and Q were not important based on the analysis. The threshold values of environmental conditions could divide most cases dominated by different groups, though the division was not 100% accurate.

Potential links between MBFG$_R$ and the zooplankton and river types

A total of 36 zooplankton genera were collected, among which 5 copepod, 13 Cladocera, and 18 Rotifera species were included. The individual core body length ranged from 50 to 2850 μm. Based on the probability distribution, zooplankton could be categorized into three groups: small, medium, and large (Fig. 3). The upper 5% of events was designated as large zooplankton (length >1000 μm), the bottom 65% as small (length >330 μm), and the remainder as medium zooplankton (330 < length < 1000 μm).

Correlation coefficients between the biomass of MBFG$_R$ dominant group and the density of zooplankton showed the significant relationships of potamoplankton and predator taxa and size. Group RIa was related to small, medium, and total zooplankton. Groups RIb and RIV showed relationship with medium and large zooplankton. Group RIIa showed a significant correlation with total zooplankton. Groups RIII and RVIc were positively correlated with copepods. Group RVIa was related to small and medium zooplankton especially rotifers. Group RVII was negatively correlated with the total zooplankton. Groups RIIb, RV, and RVIb showed nearly no correlation with any zooplankton. The synoptic correla-

tion matrix between dominant MBFG$_R$ biomass and the abundance of zooplankton is reported in Tab. 5. A scatter diagram of zooplankton and potamoplankton is reported in Supplementary Fig.1.

Fig. 4 shows the positioning of all river-samples with >95% dominance of a MBFG$_R$ within the framework of the Gasol model (Gasol, 1994). According to this model, Groups RIa, RIII, RVIc, and RVII purely dominated in bottom-up system, whereas only Group RVIa dominated
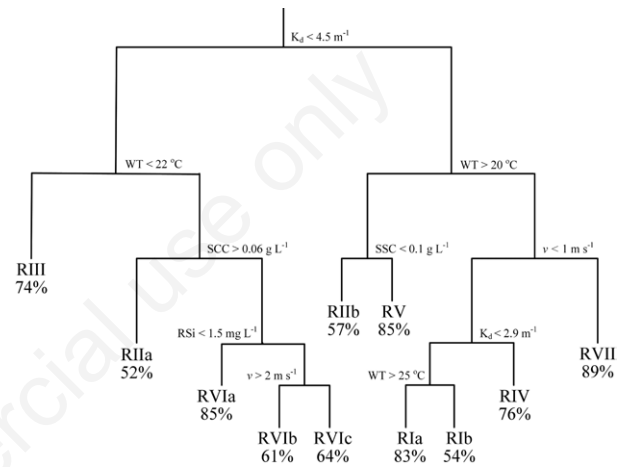

Fig. 2. Classification tree of the environmental variables explaining the dominance (75% over total biomass) of MBFG$_R$. The environmental variable and the corresponding threshold value are shown for each node. At the end of each branch, the MBFG$_R$ with more cases is included as the decisive factor. The percentage representing the proportion of cases where a specific MBFG$_R$ was dominant is shown below each group. K_d, light attenuation coefficient; v, flow velocity; WT, surface water temperature; RS, reactive silicate; SSC, suspended sediment concentration.

Tab. 4. Key for classifying phytoplankton under the MBFG$_R$ approach.

Presence of flagella	Presence of hard exoskeletal structures	V <300 μm^3	Group RIIa	
		V >300 μm^3	Group RIIb	
	Absence of hard exoskeletal structures	MLD <10 μm	Group RIb	
		MLD >10 μm	Group RV	
		Colony	Group RIV	
Absence of flagella	Presence of siliceous structures	Unicell	V <300 μm^3	Group RVIa
			V >300 μm^3	Group RVIb
		Colony	Group RVIc	
	Absence of siliceous structures	Unicell	Group RIV	
		Filaments	Length <20 μm	Group RIa
			Length >20 μm	Group RIII
		Colony	Absence of mucilage	MLD <20 μm	Group RIa
				MLD >20 μm	Group RIV
			Presence of mucilage	MLD <20 μm	Group RIa
				MLD >20 μm	Group RVII

in top-down regulation. Other groups dominated with both kinds of potamoplankton-zooplankton links. The distribution of MBFG$_R$ dominance (>75% cases) in different river types (Tab. 6) showed the significance of topography, stream order, and river sections for Groups RIb, RIII, RV, RVIa, RVIc, and RVII. Group RIb preferred the middle-upper section of higher rivers in mountainous areas. Group RIII preferred the middle-down section of lower rivers. Group RV and RVII preferred the middle-down section of lower rivers in plain. Group RVIa and RVIc preferred middle-downstream in plains. Meanwhile, the other groups (RIa, RIIa, RIIb, RIV, and RVIb) seemed uncorrelated to river type.

DISCUSSION

The concept of using easily determined morphological criteria as an indicators of functional properties may be applied to potamoplankton in rivers just as they are applied to phytoplankton in lakes. The MBFG$_R$ well reflected the different habitat templates. The main ecological processes in lakes are usually evasion of loss processes (mixing and zooplankton), water temperature conditions, and resources acquisition (nutrients and light) (Kruk and Segura, 2012). In addition to these processes, the unique effect from river flow was also represented in the results.

For applying the model, as described by Kruk and Segura (2012), a classification tree was constructed to compare the specific environmental thresholds that determine the dominance of a group in rivers. Most of the group-dominated river cases were successfully separated according to the environmental variables. By eliminating two important factors in RDA analysis (TP and TN), the CART characterized external forces according to five indicators (K_d, WT, v, SCC, and RSi) and developed habitat templates for the eleven groups (Fig. 2). In contrast to the previous MBFG findings (Kruk and Segura, 2012), the MBFG$_R$ model emphasized the importance of flow velocity and suspended sediment. These results showed that the dominance of potamoplankton functional groups depends first on hydrological conditions and second on nutrients. Discharge was not selected which seemed to be counterintuitive. This decision might have been caused by a redundancy of the variables, which were based on long term statistics and not exactly equivalent to that potamoplankton species experienced in nature. In the following Subsections, we describe in detail the morphological characteristics and habitat template of each group, as well as their relationship with zooplankton predation and river types.

Groups RIa and RIb: flagella cause notable differences in small organisms

These r-selected representatives, in which small size and high growth rate were aligned together in MBFG I

(Kruk *et al.,* 2010); they increased with the total nutrients and dominated in dynamic ecosystems (Kruk and Segura, 2012). However, this description might be too general in riverine systems because MBFG$_R$ discovered it was Group RIa without flagella that were strongly associated to nutrients. By contrast, it was flagellated Group RIb that could dominate under a wide variety of trophic conditions involving flushed and transitional systems (Fig. 1), especially the middle-upper stream in mountains (Tab. 6). Furthermore, the following differences were also indicated by the presence or absence of flagella: i) Group RIa had a dominant preference for warm environments (WT >25°C, Fig. 2), which could be a prerequisite to maintaining a high specific growth rate. Meanwhile, Group RIb could dominate at 20-25°C because their flagella-driven allowed for
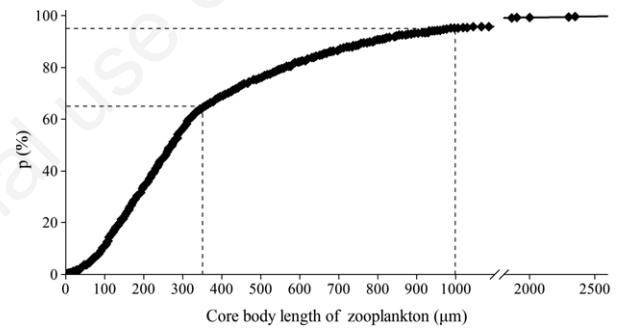
Fig. 3. Probability distribution (P) of zooplankton individual core body length. Dashed lines indicate the division of small, medium, and large predators.
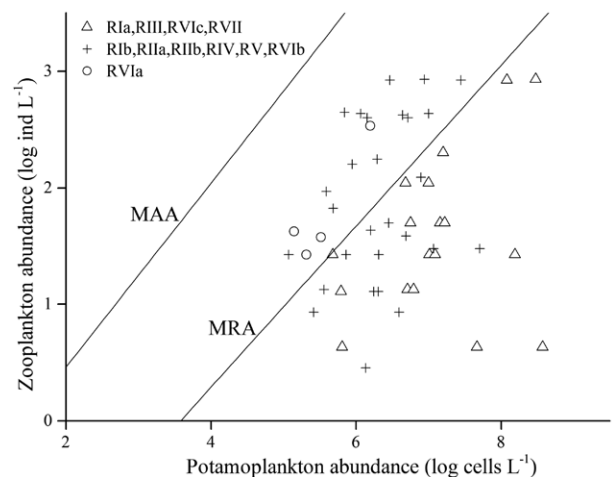
Fig. 4. Position of the river-cases where a specific MBFG$_R$ was dominant (90% over total biomass) within the framework of Gasol's model. MAA, maximum attainable abundance line; MRA, mean realized abundance line.

better resource and nutrient acquisition (Reynolds, 1997). ii) Group RIa were possibly better food for small and medium zooplankton (Tab. 5) and only dominated under bottom-up control (Fig. 4). However, the presence of flagella increased the maximum linear dimension of Group RIb, thereby allowing the species from this group to escape from small invertebrate predators with strict limitations on prey size and shape (Do and On, 1974); the presence of flagella greatly amplified the effect of water viscosity and flow ability on phytoplankton movement, thereby making it difficult for small zooplankton individuals bite or drag (Boukal, 2014). Although predators generally select size-matched prey (Brose *et al.,* 2006), Group RIb species with small sizes were available for large zooplankton (Tab. 5), and the risk is especially high when species from this

group are abundant (Van Donk *et al.,* 2011). Our data-set was composed of a low number of rivers dominated by Group RIb (only 6 samples), which precluded further analysis of their dominance.

Groups RIIa and RIIb: coexistence of flagella and exoskeletal structures

According to the description by Kruk and Segura (2012), Group RIIa was closer to MBFG II: small flagellates which have exoskeletal structures and favor cold oligotrophic conditions. Most species in RIIb belonged to MBFG V which had the typical flagella and large size (*e.g.*, *Ceratium hirundinella*). But Group RIIb appeared closer to Group RIIa in the RDA biplots, thereby indicat-

Tab. 5. Correlation coefficients of dominant MBFG$_R$ biomass (dominance >75%) and zooplankton abundance (P<0.001).

MBFG$_R$	No. of samples	Total zooplankton	Rotifera	Cladocera	Copepoda	Small zooplankton	Medium zooplankton	Large zooplankton
RIa	11	0.60				0.62	0.54	
RIb	6						0.66	0.60
RIIa	8	0.84						
RIIb	13							
RIII	26				0.78			
RIV	17						0.74	0.51
RV	58							
RVIa	38		0.61			0.59	0.51	
RVIb	45							
RVIc	13				0.67			
RVII	10	-0.82						

The categories of individual core body length of zooplankton are defined as follows: small <330 μm; 330< medium <1000 μm; large >1000 μm. Least-squares linear regression lines are shown in the Supplementary Fig. 1.

Tab. 6. Distribution of MBFG$_R$ dominance (>75% cases) in different river types based on topography, scale (indicated by the drainage area of whole river), stream order, and section.

MBFG$_R$	Topography		Drainage area (km²)		Stream order		River section	
	Mountainous	Plain	>5e+4	<5e+4	1-3	4-6	Middle-upper	Middle-down
Sampled rivers	33	68	36	65	61	40	29	72
RIa	3/11	8/11	4/11	7/11	4/11	7/11	5/11	6/11
RIb	6/6*	0/6	2/6	4/6	1/6	5/6*	5/6*	1/6
RIIa	4/8	4/8	2/8	6/8	2/8	6/8	5/8	3/8
RIIb	5/13	8/13	4/13	9/13	7/13	6/13	3/13	10/13
RIII	10/26	16/26	8/26	18/26	24/26*	2/26	3/26	23/26*
RIV	8/17	9/17	9/17	8/17	10/17	7/17	8/17	9/17
RV	4/58	54/58*	20/58	38/58	48/58*	10/58	3/58	55/58*
RVIa	3/38	35/38*	10/38	28/38	22/38	16/38	4/38	34/38*
RVIb	24/45	21/45	25/45	20/45	30/45	15/45	22/45	23/45
RVIc	2/13	11/13*	5/13	8/13	8/13	5/13	1/13	12/13*
RVII	1/10	9/10*	4/10	6/10	8/10*	2/10	2/10	8/10

*The underlined numbers indicate the total number of studied rivers; *significant MBFG$_R$ dominance in the river types (P<0.05).*

ing that they shared favorable environments; this trend was probably caused by the coexistence of flagella and exoskeletal structures as important morphological traits. First, Groups RIIa and RIIb both could withstand some flushes and impacts under the protection of hard exoskeletal structures. Second, they both benefited from the effective nutrient foraging and permitted mixotrophy (Salmaso and Padisák, 2007), which increased their adaptability to lower trophic levels (Kruk and Segura, 2012). However, Group RIIb showed no relationship with any zooplankton (Tab. 5), which might be an indicator of substantial grazing tolerance (in accordance with the results of Kruk and Segura, 2012). Besides physical defence, some species in Group RIIb could release chemicals upon mechanical damage (*e.g.*, *Alexandrium minutum*). Chemical cues associated with the risk of herbivory allowed for resistance to further zooplankton grazing and potentially affected fertilization success or embryonic fitness (Van Donk *et al.,* 2011). The key distinction of Groups RIIa and RIIb was sinking loss. For RIIa, the so-called exoskeletal structures of some species were in the form of scales and/or spines that just covered part of the cell body, thereby extremely reducing the side effects of heavy structures. The small size of species in this group and the presence of flagella facilitated buoyancy, especially in rivers. Meanwhile, the large size and teardrop shape of species in Group RIIb worsened the sinking problem (Padisák *et al.,* 2003). Therefore, the dominance of Group RIIb was not achieved in turbulent environments with $K_d > 4.5$ m^{-1}.

Group RIII: large filaments

MBFG III was composed of large filaments with aerotopes, which confer greater tolerance to limited light and nutrients (Kruk *et al.,* 2010; Kruk and Segura, 2012). Group RIII in MBFG$_R$ included absorbed species of similar morphology, even those without aerotopes, and were located closely related to environmental variables concerning hydrology (Q, *v*, and SCC) in the RDA biplots (Fig. 1). Their probable key to success in rivers was the flexibility of filamentous life forms instead of the buoyancy from aerotopes. Filamentous algae, whether possessing aerotopes (*e.g.*, *Planktothrix agardhii*) or otherwise (*e.g.*, *Anabaenopsis circularis*), were important and common in potamoplankton communities; these algae often dominated in the middle-down stream of lower rivers (Tab. 6). Similar to MBFG III, Group RIII could dominate in low-light environments, but only at WT >22°C (Fig. 2). The high optimum temperature seemed to be another preferred condition, which was not seriously considered as a threshold of dominance for this group (Kruk and Segura, 2012). Their large size, occasionally coiled shape, and potential toxicity (Kruk *et al.,* 2010) increased resistance to grazing. Group RIII only had a close relationship with copepods according to the results (Tab. 5). Besides the matched sizes, copepods showed a high de-

gree of feeding selection by maximizing the ingestion of the most nutritious food from a mixture of particles by using algae secondary metabolites as detection cues to prevent toxin ingestion (Koski *et al.,* 2002). Consequently, selective feeding usually allows copepods to uninhibitedly feed on alternative food in the presence of toxic species, thereby resulting in grazers coexisting with blooms (Ger *et al.,* 2014).

Group RIV: medium sized organisms lacking specialized traits

The composition and characteristics of this group were basically similar in MBFG and MBFG$_R$. As high quality food, this group contained the most varied species and shapes which covered a spectrum from typically r-selected and K-selected types (Kruk *et al.,* 2010). In accordance with the habitat template of MBFG IV, Group RIV was liable to high light. However, no evidence was observed in the river cases supporting low nutrient and temperature conditions (Figs. 1 and 2). Both Group RIV and MBFG IV included coenobium, which comprised many flagellated cells (*e.g., Pandorina morum*), that cannot reach high unidirectional speed because of the unsynchronized flagellar movement of individual cells in the colony is not synchronized (Padisák *et al.,* 2003). In this way, the functions (*e.g.*, motility and flotation) of morphological variability (*e.g.*, shapes, spines, and protuberances) that were observed among cells in the colony were most likely handicapped by cybotactic life forms. This phenomenon seemed to be an ecological cost of grazing resistance. Small zooplankton did not show an obvious correlation with the biomass of Group RIV (Tab. 5) probably because of algal colony formation and size, which were influenced by chemicals released by predators (Van Donk *et al.,* 2011).

Group RV: unicellular flagellates of medium to large sizes

In MBFG$_R$, Group RV was an aggregation of Cryptophyceae, Euglenophyceae, Volvocales and Chlorococcales, and excluded Dinophyceae (with exoskeletal structures). This group matched the characteristics described for MBFG V, as follows: good motility, preference for low temperature (WT <20°C, Fig. 2), and substantial tolerance to grazing (Tab. 5). However, two points are worthy of discussion. First, MBFG V mixed species of Groups RV and RIIb by ignoring the absence and presence of exoskeletal structures, whereas this morphological difference might lead to observable discrepancies in terms of tolerance to suspended sediment in rivers. Potamoplankton showed sensitivity to inorganic (non-algal) turbidity, which is a function of SSC (May *et al.,* 2003). Group RV was possibly vulnerable to high SSC because it lacked hard armour (*e.g.*, dinoflagellates) (Lau *et al.,*

2007) to protect against the direct physical impact of suspended sediment along with the current; thus, this group dominated in the middle-down stream of plain lower rivers (Tab. 6) with only SSC <0.1 g L^{-1} (Fig. 2). Second, MBFG V achieved dominance at high K_d (> 3.9 m^{-1}) whereas Group RV required K_d <4.5 m^{-1}. These results might be a reflection of the hydrological differences between rivers and lakes. In river systems, the light attenuation coefficient can be influenced by turbulence, basin morphology and tripton (Phlips *et al.,* 2000; Christian and Sheng, 2003). Group RV largely preferred the slight effects from slow flow with little amounts of sediment, as indicated by the low K_d.

Groups RVIa, RVIb, and RVIc: non-flagellated organisms with siliceous exoskeletons

The obligate presence of a siliceous wall was probably the main constraining trait of species from these groups (Kruk *et al.,* 2010). MBFG VI collected all diatoms, thereby indicating their special common characteristics, as follows: immobility, silicate requirement, preference for low temperature, and resistance to viral infection (Kruk and Segura, 2012). $MBFG_R$ exerted a high amount of stress on the individual volume and life forms, which subdivided them into three groups.

Group RVIa mainly composed of small unicellular diatoms. The small size weakened the effect of the high exoskeleton density on sinking loss, and reduced the silicate requirements for reproduction (RSi < 1.5 mg L^{-1}; Figs. 1 and 2). Correspondingly, Group RVIa was easily grazed by rotifers and other small or medium sized zooplankton (Tab. 5). However, this group was the only group that could dominate the middle-down stream of plain rivers (Tab. 6) under top-down control (Fig. 4) largely due to their high growth rate (*e.g.,* *Cyclotella meneghiniana*).

Group RVIb mainly represented large unicellular diatoms. According to their morphology, this group had hiah aptitude for high flow velocity (v >2 m s^{-1}) in turbulent environments. Their relatively high maximum linear dimension might give substantial tolerance to grazing, but their large size increased sinking. In addition, grazing-induced silicification (*e.g.,* *Fragilariopsis kerguelensis*) might increase the mechanical resistance of the diatom (not limited to species of this group) frustule (Hamm *et al.,* 2003). To date, the chemical compound that induces this defence mechanism remains unknown.

Group RVIc mainly represented colonial diatoms. The formation of a colony was greatly induced by heavy walls (Padisák *et al.,* 2003). Some species have been shown to be readily ingested by zooplankton taxa (*e.g.,* copepods; Tab. 5), but survived gut passage when arranged as chains following ingestion by predators (Van Donk *et al.,* 2011). The advantages of this life form included defences to grazing and the reduction of sinking loss (Padisák *et al.,*

2003). However, the formation of colonies negatively affected photosynthesis and the uptake of nutrients because it reduced the available surface-per-volume (Verschoor *et al.,* 2009). Consequently, colonial diatoms preferred the middle-down stream of plain rivers (Tab. 6) and seldom dominated turbulent current (v >2 m s^{-1}, Fig. 2). The fragmentation of diatom colonies would occur with the beginning of population decline, during which these colonies usually split and sink into the hypolimnion to allow new development (Padisák *et al.,* 2003).

Group RVII: large mucilaginous colonies

Group RVII in $MBFG_R$ matched the composition and characteristics of MBFG VII. The presence of mucilage provided buoyant properties, grazing avoidance, and sensitivity to low resource supply (Kruk and Segura, 2012). This group tended to dominate rivers with low K_d (<4.5 m^{-1}) and slow flow (v <1 m s^{-1}) in warm seasons (WT > 20°C) like the middle-down stream of plain lower rivers (Tab. 6). Closely linked to TP and TN (Fig. 1), the massive occurrence of Group RVII might indicate an out-of-balance state in the ecosystem, which was mainly due to eutrophication. Uniquely, the biomass of this group was negatively related to the total zooplankton density (Tab. 5) under bottom-up control (Fig. 4) in rivers, which was obviously an evidence of toxic defences in phytoplankton species. Previously, Van Donk *et al.* (2011) demonstrated that several strains (*e.g.,* *Microcystis*) increase their toxin production in response to direct and indirect exposure to herbivorous zooplankton. Some species of copepods and Cladocera can maximize adaptation via selective feeding or detoxification mechanisms (Ger *et al.,* 2014). However, zooplankton taxa with detoxification mechanisms are challenged by the limited ability to handle individual food particles (*e.g.,* *Daphnia*) (Ger *et al.,* 2014) or the metabolic cost of maturing at a slower rate (*e.g.,* *Boeckella*) (Twombly *et al.,* 1998). Apparently, neither existing predators nor their future evolved forms would actually use phytoplankton of this group for food (Ger *et al.,* 2014).

Limitations of the $MBFG_R$ study

The veracity and completeness of data are crucial to the study of potamoplankton. The establishment of $MBFG_R$ was purely based on field sampling, thereby leaving much to consider: i) the physical and chemical conditions at the point of sampling will not necessarily reflect the conditions created by river variability that have affected the phytoplankton; ii) riverine phytoplankton is actually a mixture of various planktonic elements that arrive from different water types of the watershed, such as shallows (Stoyneva, 1994), inflows (Istvànovic and Honti, 2011), and reservoirs. Therefore, the potamoplankton composition frequently reflects the effects of the key

processes in the watershed and not just those that act in the main channel of the river; this is especially true for highly modified watersheds; iii) more frequent sampling in some rivers might cause pseudo-replication, especially in cases with small differences in the hydrological characteristics of sampling periods.

These limitations are impossible to eliminate thoroughly when field sampling is conducted across wide areas. Thus, highly manipulative laboratory experiments would be valuable as an additional complement.

CONCLUSIONS

The characteristics and habitat templates of all groups of MBFG$_R$ in terms of biomass distribution among rivers was described in this study and presented in Tab. 7. The conditions for significant dominance of most groups (RIa, RIIb, RIII, RIV, RV, RVIa, RVIb, RVIc and RVII) were well discriminated by the environmental variables and constituted homogeneous groups. Compared with previous studies on lakes, the separation of Group RIb from Group RIa in MBFG I revealed their differences in terms of optimum temperature, predator composition and favourable river types. The separation of Group RIIb from MBFG V showed the importance of armour, as well as adaptability to flushing, turbulence, and suspended sediment. The different sizes and life forms (unicell or colony) divided the diatoms belonging to MBFG VI into several groups, as follows: Group RVIa (small size), Group RVIb (large size), and Group RVIc (colony). This subdivision revealed that only Group RVIa had low-silicate requirements and were grazed by most zooplankton. Group RVIb experienced high sinking loss. Group RVIc preferred the downstream regions of plain rivers, which were charac-

terized by slow flow. However, the discriminatory power of the conditions was low for the dominance of Groups RIb and RIIa. Rivers in various geographical conditions and extreme environments should be included to improve the results presented here.

The MBFG$_R$ might fit potamoplankton classification better because of the following reasons: i) it did not mix species with and without flagella in any group; ii) it refined the classification of diatoms; iii) it included the various exoskeletons; iv) it disregarded the aerotopes of filaments.

These alterations are possibly reasonable: i) The rotation of flagella can move algae (Wetzel, 2001) to allow them to actively regulate their position (Fraisse *et al.,* 2013). Motility may help phytoplankton find more favourable light and nutrient conditions in heterogeneous environments. ii) Diatoms are the primary components of potamoplankton communities. When more groups of this class are present, the classification approach for rivers can attain higher sensitivity. Individual volume and life forms (unicellular or colony) are major explanatory variables that should be given priority. iii) The exoskeletal structures of phytoplankton can greatly affect survival in rivers. Although the weight of these structures leads to increased sinking loss, their hardness effectively prevents phytoplankton from damage in turbulent flow (Lau *et al.,* 2007). iv) The crucial advantage of large filaments in rivers is probably more related to flexibility rather than buoyancy. The importance of aerotopes, which reduce sinking loss, may be more evident in still water columns of lakes rather than river ecosystems.

In conclusion, a large data set from riverine ecosystems was used to verify that morphological traits instead of phytosociological traits would better represent the functional

Tab. 7. Summary of the MBFG$_R$ habitat templates.

MBFG$_R$	Compare to MBFG	K$_d$ (m^{-1})	WT (°C)	SSC (g L^{-1})	v (m s^{-1})	RSi (mg L^{-1})	Main predators	Suitable river types for dominance
RIa	MBFG I without flagella	2.9-4.5	>25		>1		Small and medium zooplankton	
RIb	MBFG I with flagella	2.9-4.5	20-25		>1		Medium and large zooplankton	Upperstream of higher rivers in mountains
RIIa	MBFG II	>4.5	<22	<0.06			All zooplankton	
RIIb	MBFG V with exoskeleton	<4.5	<20	>0.1				
RIII	MBFG III but ignoring aerotopes	>4.5	>22				Copepods	Downstream of lower rivers
RIV	MBFG IV	<2.9	>20		>1		Medium and large zooplankton	
RV	MBFG V without exoskeleton	<4.5	<20	<0.1				Downstream of lower rivers in plains
RVIa	MBFG VI of small size	>4.5	<22	>0.06		<1.5	Small and medium rotifers	Downstream of plain rivers
RVIb	MBFG VI of large size	>4.5	<22	>0.06	>2	>1.5		
RVIc	MBFG VI of colony	>4.5	<22	>0.06	<2	>1.5	Copepods	Downstream of plain rivers
RVII	MBFG VII	<4.5	>20					Downstream of lower rivers in plains

K$_d$, light attenuation coefficient; WT, water temperature; SSC, suspended sediment concentration; v, flow velocity; RSi, soluble reactive silicate.

properties among phytoplankton. Morphological traits are closely related to the water environment. MBFG$_R$ overcomes the low sensitivity of MBFG for better application in rivers. Undoubtedly, the morphological approach is an efficient tool to explore the effects of hydrological, physicochemical, and food web changes on potamoplankton independently from the geographical location and specific composition, although such approaches are not consummate (Mihaljević *et al.,* 2013). The use of a morphological approach can lead to a better understanding of the ecological processes concerning riverine phytoplankton.

ACKNOWLEDGMENTS

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