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**Distribution of phytoplankton in a German lowland river in relation
to environmental factors**

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Abstract

In comparison to lentic systems, the species composition and community structure of phytoplankton in lotic habitats are still poorly understood. We investigated the spatial and temporal dynamics of the phytoplankton community in a German lowland river, the Kielstau catchment, and the relationships with environmental variables. Among the 125 taxa observed, *Desmodesmus communis*, *Pediastrum duplex* and *Discostella steligera* were dominant species at lentic sites while *Tabellaria flocculosa*, *Euglena* sp., *Planothidium lanceolatum*, *Cocconeis placentula* and *Fragilaria biceps* dominated at lotic sites. Remarkable spatial and temporal variations of the phytoplankton community were revealed by non-metric multidimensional scaling (NMDS). Canonical correspondence analysis (CCA) indicated that physical factors (e.g. hydrological variables) and major nutrients (e.g. total phosphorus, dissolved inorganic nitrogen) were of equal importance controlling the variation in structure of riverine phytoplankton assemblages. Weighted averaging regression (WA) and cross-calibration produced strong models for predicting DIN, WT and TSS, which enabled selection of algal taxa as potentially sensitive indicators: for DIN, *Ulnaria ulna* var. *acus*, *Ulnaria ulna*, *Desmodesmus communis* and *Euglena* sp.; for WT: *Discostella steligera*, *Scenedesmus dimorphus*, *Desmodesmus communis* and *Euglena* sp.; for TSS, *Nitzschia sigmoidea*, *Desmodesmus communis* and *Oscillatoria* sp. The results from this relatively small survey indicate the need for further monitoring to gain a better understanding of riverine phytoplankton and to capitalize on the environmental indicator capacity of the phytoplankton community.

Keywords Canonical correspondence analysis (CCA), Environmental variables, Non-metric multidimensional scaling (NMDS), Weighted averaging regression analysis (WA)

Abbreviations

- COND- electrical conductivity
- DIN- dissolved inorganic nitrogen
- DO- dissolved oxygen
- RMSE- root-mean-squared error of prediction
- TP- total phosphorus
- TSS- total suspended solid
- VSS- volatile suspended solid
- WT- water temperature

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Introduction

Phytoplankton have been studied extensively in lentic fresh waters (lakes and reservoirs) where long residence time and low flow velocity allow sufficient time for growth and reproduction (e.g. Basu and Pick, 1997; Sabater *et al.*, 2008; Torremorell *et al.*, 2009). However, in comparison to lentic systems, the species composition and community structure of phytoplankton in lotic systems (streams and rivers) are still poorly understood (Basu and Pick, 1996; Piirsoo *et al.*, 2008). The spatial and temporal pattern of a community are of crucial importance for understanding ecosystem functioning because they can affect ecosystem processes, functioning and stability, and reflect major shifts in environmental conditions (Suikkanen *et al.*, 2007; Zhou *et al.*, 2009a).

Distribution patterns of phytoplankton are strongly correlated with environmental factors (Lepistö *et al.*, 2004). Possible factors may be physical (climate, water temperature, light intensity), chemical (nutrient concentrations) (Reynolds *et al.*, 1993; Torremorell *et al.*, 2009), hydrological (river morphology, discharge, water residence time, precipitation) (Descy and Gosselain, 1994; Kiss *et al.*, 1994; Skidmore *et al.*, 1998), and biotic (grazing, competition, parasitism) (Moss and Balls, 1989; Ha *et al.*, 1998). Unfortunately, there is no general consensus as to which factors regulate phytoplankton communities in lotic habitats (Basu and Pick, 1995). Besides, contributions of the main environmental factors to phytoplankton variations are also unclear. For example, hydrological factors are thought to be of greater importance to planktonic development in rivers than in lakes (Pace *et al.*, 1992), while other researchers concluded that river phytoplankton is more strongly regulated by nutrient concentrations, like total phosphorus concentration (Soballe and Kimmel, 1987; Moss and Balls, 1989; Basu and Pick, 1996; Van Nieuwenhuysse and Jones, 1996). The response of phytoplankton to environmental factors has become a central topic of current research (Burić *et al.*, 2007), and identification of the main factors controlling phytoplankton in a particular water body is essential for choosing an appropriate management strategy for the maintenance of a desired ecosystem state (Peretyatko *et al.*, 2007).

Lowland rivers, characterized by specific properties, such as low hydraulic gradients, shallow groundwater, and high potential for water retention in peatland and lakes (Schmalz and Fohrer, 2010), are apparently different from the habitats of lakes and mountain streams. Until now, studies

of phytoplankton communities in lowland rivers, to our knowledge, are still scanty. In this paper, we investigated the spatio-temporal variation of the phytoplankton community and environmental variables over a one year period (Nov. 2008 – Aug. 2009) throughout a lowland river ecosystem in northern Germany. The objectives of this study were to: 1) describe the distribution patterns in the species composition and biomass of phytoplankton in the Kielstau catchment; 2) study the relationships between phytoplankton and environmental variables, and establish which factors predominantly structure riverine phytoplankton communities; 3) identify algae species that could potentially be used as indicators of specific water chemistry conditions in this lowland area.

Method

Description of the study area

The Kielstau catchment is located in the Northern part of Germany. It has its origin in the upper part of Lake Winderatt (Fig. 1) and is a tributary of the Treene River, which is the most important tributary of the Eider River. Moorau and Hennebach are two main tributaries within the Kielstau catchment. Sandy, loamy and peat soils are characteristic of the catchment. Land use is dominated by arable land and pasture (Schmalz and Fohrer, 2010). The drained fraction of agricultural area in the Kielstau catchment is estimated to be 38% (Fohrer *et al.*, 2007). The precipitation is 841 mm/a (station Satrup, 1961–1990, DWD, 2009) and the mean annual temperature is 8.2 °C (station Flensburg 1961–1990, DWD, 2009). Many hydrological and morphological studies have been carried out in this catchment, such as Kiesel *et al.* (2009), Schmalz *et al.* (2009), Liu *et al.* (2009), Zhang *et al.* (2009), Kiesel *et al.* (2010), Lam *et al.* (2010), Schmalz and Fohrer (2010).

Samples were collected four times at 20 sites (Fig. 1) along the main stream Kielstau and its tributaries in Nov. 2008, Feb. 2009, May 2009 and Aug. 2009. Ten sites (K01 to K10) were located along the main stream, three (M01 to M03) in the Moorau tributary, five (H01 to H05) in the Hennebach tributary and two lentic sites (L01 and L02) in Lake Winderatt.

Sampling methods and primary procedures

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At each site and on every sampling date, three replicate samples of a known volume of subsurface (5-40 cm) water were taken with a 10 L bucket and then filtered through a plankton net. The organisms retained were transferred into glass containers and fixed in 5‰ non-acetic Lugol's iodine solution (Sabater *et al.*, 2008). After 48 hours, the undisturbed water samples were concentrated to 30 mL for further processing. Considering that nets with very fine meshes (5 or 10 µm) often filter too little water to provide an adequate algal sample, the mesh size chosen in the present study was 20 µm (Paasche and Ostergren, 1980).

Concurrently, the following instream parameters including pH, dissolved oxygen (DO), electrical conductivity (COND) and water temperature (WT) were measured *in situ* by Portable Meter (WTM Multi 340i, Germany). Water depth, channel width and flow velocity (FlowSens Single Axis Electromagnetic Flow Meter, Hydrometrie, Germany) were measured at each site as well.

At each site, water samples were also collected for further laboratory analysis including orthophosphate (PO₄-P), ammonium-nitrogen (NH₄-N), total phosphorus (TP), nitrite-nitrogen (NO₂-N), dissolved silicon (Si), nitrate-nitrogen (NO₃-N), chloride (Cl⁻) and sulphate (SO₄²⁻). All these factors were measured according to the standard methods DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). PO₄-P and TP were measured using the ammonium molybdate spectrophotometric method (at 880 nm; DIN 1189). We used Nessler's reagent colorimetric method (DIN 38 406-E5-1) to measure NH₄-N concentrations at 690 nm. NO₂-N was measured by sulphanilamide and N-(1-Naphthyl)-ethylenediamine method (DIN38 405-D10). Si was measured using molybdosilicate (at 410 nm; DIN38 405-D21) method. NO₃-N, Cl⁻ and SO₄²⁻ were measured by an ion chromatography method (DIN38 405-D19). Dissolved inorganic nitrogen (DIN) was defined as the sum of NH₄-N, NO₃-N and NO₂-N, and N:P was calculated by DIN:TP. Total suspended solid (TSS) and volatile suspended solid (VSS) were measured according to Standard Operating Procedure for Total Suspended Solids Analysis (U.S. EPA, 1997).

For chlorophyll *a* (Chl *a*) determinations, a known volume of surface water was filtered through WHATMAN GF/C glass-fiber filters and in the laboratory was determined spectrophotometrically following 90% acetone extraction according to APHA (1992).

Microscope identification

Non-diatom algae were analyzed using a 0.1 ml counting chamber at a magnification of 400× (Zeiss Axioskop microscope). Permanent diatom slides were prepared after oxidizing the organic material (nitric acid and sulfuric acid) and a minimum of 300 valves were counted for each sample using a Zeiss Axioskop microscope at 1000× under oil immersion. Algae were identified to the lowest taxonomic level possible (mainly species level) and abundances were expressed as cell/L. Algal biomass was estimated by Chl *a*.

Data analyses

We calculated the species richness, algal density, relative abundances of dominant species and diatom growth forms (prostrate and mobile taxa) to describe the phytoplankton community. Densities were $\ln(x+1)$ transformed to reduce the effects of extreme values. Besides, % benthic taxa (%) (Porter, 2008), Q index (Borics *et al.*, 2007), Chlorophyte-Index and Pennales-Index (Mischke and Behrendt, 2007) were also calculated based on taxa biovolumes and these indices are widely used for phytoplankton based bio-assessment.

Among-sites separation was evaluated by non-metric multi-dimensional scaling (NMDS) (Kruskal and Wish, 1978), which is an ordination method that is well suited to data that are non-normal or are on arbitrary, discontinuous, or otherwise questionable scales. "Ordination stress" is a measure of departure from monotonicity in the relationship between the dissimilarity (distance) in the original p-dimensional space and distance in the reduced k-dimensional ordination space. Bray-Curtis similarity was used as the distance measure in the analysis.

The relationship between measured environmental variables and phytoplankton assemblages of the catchment was explored using canonical correspondence analysis (CCA). CCA is useful for identifying which environmental variables are important in the determination of community composition as well as spatial variation in the communities (Black *et al.*, 2004). All the biotic data were transformed into relative abundance (0-100%) before analysis. Because of the large number of rare species, individual taxa chosen for analyses had to occur at >1 site and to have a total relative abundance >0.5% when all sites were summed; this requirement reduced the number of taxa in the analysis from 125 to 31. To eliminate the influence of extreme values on ordination scores, species data were logarithmically transformed [$\log(x+1)$] before CCA. Environmental variables with high correlation coefficients ($r > 0.60$) and variance inflation factors ($VIF > 20$) were excluded in the

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3 final CCA analyses (ter Braak and Šmilauer, 1998; Munn *et al.*, 2002). These criteria reduced the
4 number of environmental variables from 19 to 11. Forward selection and Monte Carlo permutations
5 were used to identify a subset of the measured variables that exerted significant and independent
6 effects on algal distributions.
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10 Regression and calibration models were developed to quantify relations between algal
11 abundances and environmental variables strongly expressed in CCA. Taxa optima and tolerances
12 were calculated using weighted averaging regression analysis (WA, Birks *et al.*, 1990). The
13 software calculated species optima and tolerances (respectively, the average and standard deviation
14 of the environmental variables over all sites where a taxon occurs, weighted by the relative
15 abundance of the taxon at each site). The predictive capability of the resulting models was assessed
16 using the jackknife ('leave-one-out') cross-validation procedure, and measured as the coefficient of
17 determination (R^2) between species-inferred and observed environmental variable concentrations
18 and the root-mean-squared error of prediction (RMSE). Because the observed and inferred values
19 used all sites, the R^2 calculated from the regression was termed "apparent" R^2 (R^2_{apparent}). The same
20 model was run using a jackknifing procedure to validate the apparent R^2 values. A model was
21 determined acceptable if there was agreement between apparent and jackknifed R^2 ($R^2_{\text{jackknife}}$)
22 values (Munn *et al.*, 2002). For these data, the procedure was relevant because it also enabled a
23 preliminary identification of taxa that may be suitable as indicators of particular conditions because
24 of their narrow tolerance ranges to environmental variables. Based on Kilroy *et al.* (2006), our
25 criteria were 1) occurrence in at least 30 of the 77 sites, and 2) tolerance to the variable of interest
26 $<0.75 \times$ the mean tolerance for all the species. Untransformed species and environmental data were
27 used for WA.
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46 In our study, two-way analysis of variance (ANOVA) was conducted by STATISTICA 6.0 and
47 $\ln(x+1)$ or square transformation was used if data were not normally distributed; CCA were carried
48 out by CANOCO (Version 4.5); NMDS ordination was performed with PRIMER (Version 5) and
49 WA by C² software.
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56 **Results**

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58 Environmental characteristics

59 River reaches of the study area varied widely in water-quality and habitat characteristics. For
60 example, pH ranged from 6.76 to 9.95 (mean: 7.89), DIN ranged from 0.02 to 43.01 mg/L (mean:

19.99 mg/L), and TP ranged from 0.04 to 1.30 mg/L (mean: 0.41 mg/L). Water temperature averaged 10.56 °C (0.30–21.50 °C), mean TSS was 11.51 mg/L (1.53–58.40 mg/L), mean conductivity was 604 µs/cm (385–803 µs/cm). Stream depth ranged from 4 to 81 cm with an average of 29 cm, and stream width ranged from 0.9 to 4.4 m with a mean value of 2.2 m. The main environmental variable means and two-way ANOVA were summarized in Table I. Eight variables, including pH, WT, TSS, VSS, NO₃-N, DIN, PO₄-P and Si, showed significant differences among the four seasons, while nine variables such as pH, COND, SO₄²⁻, velocity and TSS were considerably different between lentic and lotic sites.

Taxonomic composition and phytoplankton biomass

During our study, a total of 125 algal taxa (mostly to species levels) were identified. Six phytoplankton groups, Bacillariophyta, Chlorophyta, Cryptophyta, Cyanophyta, Euglenophyta and Pyrrophyta, were represented. Diatoms were predominant with 79.61% of the total abundance in lotic sites. In the lentic sites (L01 and L02), Chlorophyta (83.89% of the total abundance) was the most abundant group, followed by Bacillariophyta (13.01%), Cyanophyta (2.05%), Cryptophyta (0.50%), Euglenophyta (0.45%) and Pyrrophyta (0.11%).

The dominant species with relative abundance >1% and main phytoplankton metrics for the four sampling dates are shown in Table II and Table III. *Desmodesmus communis*, *Pediastrum duplex* and *Discostella steligera* were dominant species at lentic sites while *Tabellaria flocculosa*, *Euglena* sp., *Planothidium lanceolatum*, *Cocconeis placentula* and *Fragilaria biceps* prevailed in lotic sites (Table II). Temporal variation of phytoplankton community was also remarkable. At lentic sites, *Pediastrum duplex* was the dominant species in Nov. 2008 and Feb. 2009, however, in May and Aug. 2009, *Desmodesmus communis* was substantially more abundant with more than half of the total abundance. At lotic sites, *Tabellaria flocculosa* was mostly abundant in Nov. 2008, Feb. and Aug. 2009, but in May 2009, phytoplankton was dominant by *Euglena* sp. (Table II). Margalef's diversity index, species richness, total algal density and Pennales-Index were seasonally different ($P < 0.001$) (Table III). The phytoplankton NMDS ordination (Fig. 2) indicated a seasonal trend at both lentic and lotic sites. From Nov. 2008 to Aug. 2009, all the lotic sites moving from bottom to top, and there was a separation of May and Aug. 2009 along Axis 1. Lentic sites, well separated from lotic sites, in four different seasons were also dispersed along Axis 2 (Fig. 2).

The average value of phytoplankton biomass in the Kielstau catchment was 35.8 µg/L, which

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2 is higher than the corresponding values from Grabia and Brodnia of central Poland (~5 µg/L)
3 (Sumorok *et al.*, 2009). This is comparable to some large European rivers like the Ebro (Spain)
4 (20-45 µg/L in the 1990s) (Sabater *et al.*, 2008) and Rhine (Germany) (21-30 µg/L since 1992)
5 (Friedrich and Pohlmann, 2009), but lower than that for such rivers in Hungary (>740 µg/L) (Kiss
6 *et al.*, 1994), Greece (>740 µg/L) (Montesanto *et al.*, 2000) or Estonia (~740 µg/L) (Piirsoo *et al.*,
7 2008). These differences may be related to the water residence time, which is a useful system-level
8 index that has similar ecological implications for rivers (Soballe and Kimmel, 1987), and is a key
9 parameter controlling the biogeochemical behavior of aquatic ecosystems (Rueda *et al.*, 2006).

20 Relationship between the phytoplankton community and environmental variables

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22 Relations between measured environmental variables and phytoplankton assemblages of the lotic
23 and lentic sites were explored using CCA. The results showed that the variation of phytoplankton
24 was mainly affected by major nutrients (e.g., TP, DIN, NO₂-N), physicochemical parameters (WT,
25 Si, Cl⁻, TSS) and hydrological variables (width and flow velocity) (Monte Carlo test p<0.01) (Fig.
26 3). The eigenvalues of Axis 1 and Axis 2 were 0.130 and 0.122, which accounted for 31.4% and
27 29.5% of the total variance, respectively. The species-environment correlations were 0.770 for Axis
28 1 and 0.861 for Axis 2. Loadings on axis 1 and 2 were substantially larger than those of succeeding
29 axes, and primarily expressed variation in major nutrients and physical variables. Variation
30 expressed on CCA Axis 1 was disproportionately related to lentic sites with high TSS
31 concentrations. For instance, *Scenedesmus dimorphus*, *Desmodesmus communis* and *Cyclotella*
32 *meneghiniana*, typical lentic species, occurred mostly at L01 and L02. CCA Axis 2 probably
33 integrated a seasonal variation of WT, velocity and water depth, which clearly separated wet from
34 dry season sites.

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49 Species weight averaging optima and tolerances and inference models

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52 DIN, WT and TSS weight averaging (WA) species optima were calculated using the full dataset
53 (n=77), and the results were presented for the species with effective numbers of occurrences >30
54 (Table IV). Weight averaging DIN, WT and TSS optima ranged from 2.31~30.77 mg/L, 6.00~15.39
55 °C and 9.14~25.80 mg/L, respectively.

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60 Weighted averaging regression and calibration produced relatively stronger models for
predicting DIN, WT and TSS, by using simple WA regression (no tolerance down-weighting) with

classical de-shrinking. Of the nine variables determined to be important by CCA, DIN demonstrated the best fit between observed and inferred values ($R^2_{\text{apparent}}=0.41$, $\text{RMSE}_{\text{apparent}}=12.71$) and, based upon the jackknifing procedure, was the strongest model ($R^2_{\text{jackknife}}=0.32$, $\text{RMSE}_{\text{jackknife}}=13.57$). WT and TSS were followed (WT: $R^2_{\text{apparent}}=0.34$, $R^2_{\text{jackknife}}=0.24$; TSS: $R^2_{\text{apparent}}=0.32$, $R^2_{\text{jackknife}}=0.20$) (Table V). Jackknife-derived predicted DIN, WT and TSS values matched the measured values well (Fig. 4), and the residuals plotted against predicted values indicated no bias in the models (Fig. 4). Models for other variables performed poorly (not shown).

Four algal species satisfied the indicator selection criteria for DIN: *Ulnaria ulna* var. *acus* (14.04 mg/L), *Ulnaria ulna* (11.92 mg/L), *Desmodesmus communis* (2.31 mg/L) and *Euglena* sp. (10.65 mg/L); meanwhile, there also were four species for WT: *Discostella steligera* (12.85 °C), *Scenedesmus dimorphus* (12.22 °C), *Desmodesmus communis* (12.63 °C) and *Euglena* sp. (12.85 °C); three species for TSS: *Nitzschia sigmaidea* (10.26 mg/L), *Desmodesmus communis* (25.80 mg/L) and *Oscillatoria* sp. (9.14 mg/L) (Table IV).

Discussion

Taxonomic composition

Our study showed the phytoplankton community in the Kielstau catchment is a typical riverine diatom-dominated community and dominated by species of *Achnanthes*, *Cocconeis*, *Cyclotella*, *Fragilaria*, *Navicula* and *Tabellaria*. Most genera observed in the Kielstau catchment were prostrate taxa, whose relative abundance was 47.2% (Table III). Prostrate diatoms, which may indicate high grazing, or early diatom succession (Stevenson, 1996), were predominant at lotic sites (50.5% vs. lentic sites 9.0%) suggesting a high biotic interaction here. It must be pointed out that the use of a plankton net with a mesh size of 20 µm inevitably results in the loss of some species smaller than 20 µm (or in filaments) and may have important consequences for the present results. However, our previous study (unpublished data) indicated that this loss was within the acceptable range from the phytoplankton-based bioassessment point of view.

Historically, it was believed there was no true riverine plankton and the algae found in rivers were believed to come from either upstream lentic waterbodies or the benthos (Hötzel and Croome, 1999). Centis *et al.* (2010) argued that the view that benthic diatom communities are the source of the riverine phytoplankton may be too simplistic, because some species are not necessarily restricted to either habitat. We observed similar algal density and biomass at all the lotic sites, regardless of the influences by the lake. Consistent with Hötzel and Croome (1999), we now have

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confirmation that planktonic algal species do reproduce within rivers and many species develop substantial populations *in situ*. Therefore, we suggest that riverine phytoplankton be considered from a new perspective rather than a historical viewpoint.

Environmental variables influencing the phytoplankton community

DIN was negatively correlated with the second CCA Axis ($r=-0.582$, $p=0.002$) and TP negatively correlated with the first CCA Axis ($r=-0.534$, $p=0.002$), while WT negatively correlated with the third CCA Axis ($r=-0.549$, $p=0.018$). Major nutrients [i.e. nitrogen (N) and phosphorus (P)] concentration of surface waters were a primary factor contributing to variation in phytoplankton assemblages (Unrein *et al.*, 2010). These results were similar to the studies of Suikkanen *et al.* (2007), Burić *et al.* (2007) and Zhou *et al.* (2009b), and they also demonstrated that DIN, TP and WT were the most important factors with respect to changes in the phytoplankton community structure.

TSS was another significant variable affecting the temporal and spatial patterns of phytoplankton, which was positively correlated with the first CCA Axis ($r=0.560$, $p=0.026$). TSS is generally regarded as important environmental parameter because it can reflect the biogeochemical process of aquatic ecosystems (Weyhenmeyer *et al.*, 1997). In general, TSS comprises organic and inorganic particles suspended in the water (such as silt, plankton and industrial wastes), which can affect water transparency and quality, and higher TSS decreases light transmission, thereby influencing the phytoplankton community by reducing light availability.

Results of the CCA indicated the phytoplankton assemblage was also significantly correlated with hydrologic regime parameters like flow velocity and width, which were important factors shaping the structure of phytoplankton assemblages in rivers (Leland *et al.*, 2001; Leland, 2003). However, Ha *et al.* (1998) provided evidence that the phytoplankton periodicity was primarily governed by the hydrologic regime (discharge), and resource supply as well as biotic factors was of equal or greater importance during non-flooding periods. Our study demonstrated that physical factors and major nutrients were of equal importance controlling the structure of riverine phytoplankton assemblages. CCA analysis clearly distinguished samples from lentic and lotic habitats, as well as those collected at different times of the year (Fig. 3). Notwithstanding, the four CCA axes explained only 32.7% (axis1: 12.0%, axis 2: 11.2%, axis 3: 6.1%, axis 4: 3.4%) of the

variance in species data, and besides the ratio (the constrained eigenvalue by the environmental data to the sum of all canonical eigenvalues) was only 0.38, suggesting that other variables may have an important influence on phytoplankton community characteristics.

Benthic algae as indicators: inference model performance

Our results suggest that phytoplankton could be related to environmental variables. Of the 3 parameters we chose to test, the DIN model performed the best, followed by WT, and then TSS. Unfortunately, as typically occurs, the power of these relationships decreases (RMSE increases and R^2 decreases) following jackknifing, a more realistic technique for evaluating our reconstructive model. Compared to publications that specify nitrogen optima in rivers (Christie and Smol, 1993; Leland, 1995; Winter and Duthie, 2000; Leland *et al.*, 2001; Ponader *et al.*, 2007), the DIN WA model present here shows low $R^2_{\text{jackknife}}$ and high $\text{RMSE}_{\text{jackknife}}$. However, there were no bias in the models (residuals plotted against predicted values) (Fig. 4b, d, f) and the differences between apparent and jackknifed correlations ($R^2=0.41$ vs. 0.32 for DIN; 0.34 vs. 0.24 for WT; 0.32 vs. 0.20 for TSS) and RMSEs (12.71 vs. 13.57 for DIN; 7.23 vs. 7.88 for WT; 11.37 vs. 12.60 for TSS) were small (Table V), which indicated the models were reliable.

The lower $R^2_{\text{jackknife}}$ may be caused by the relatively higher dataset number compared to other studies, for example, Leland and Porter (2000): $n=28$, Winter and Duthie (2000): $n=17$. Reavie and Smol (1998) found $R^2_{\text{jackknife}}$ value of 0.23 for SS (suspend solid) when $n=48$, which was comparable to our study for TSS ($R^2_{\text{jackknife}}=0.20$) ($n=77$). There are other factors that might affect the performance of our models, which include the influence of temporal variability in nutrient concentrations (Pan *et al.*, 1996) and the indirect impact of nutrients on diatom species through increasing competition with non-diatom species (Winter and Duthie, 2000; Ponader *et al.*, 2007). Nevertheless, further investigations are needed to explore the reason for these high optimum values for DIN (Table IV).

In general, our DIN, WT and TSS inference models were reliable in terms of their estimation of species optima, although they had relative lower $R^2_{\text{jackknife}}$ when compared to existing models. It is likely that optima and tolerances vary geographically and between habitats (Winter and Duthie, 2000), and that extensive measurements over various eco-regions will be required to develop effective inference models for rivers. The results from this relatively small survey also indicate the

1
2 need for further monitoring in order to gain a better understanding of riverine phytoplankton and
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4 capitalize on the environmental indicator capacity of the phytoplankton community. Poole (2010)
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6 concluded that integrations among ecology, hydrology, geomorphology and hydrogeology (namely
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8 "hydrogeomorphology") would be a basis for future 'Advancing Stream Ecology'. As many
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10 hydrological and morphological studies have been carried out, a combination between already
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12 existed hydrological surveys and hydrobiological data provide the possibility for further 'Advancing
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14 Stream Ecology'. Additionally, our results may supply useful basic data for phytoplankton based
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16 bio-assessment in lowland areas (e.g. Q index, Chlorophyte-Index, Pennales-Index), which are not
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18 well developed as those of benthic diatom, macroinvertebrate and fish.
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Table and Figure legends

Table I: Means (\pm SE) of 19 environmental variables at all sites and different seasons and habitat group in the Kielstau catchment. Summary of two-way analysis of variance (ANOVA) for testing the effects of season and location on the environmental factors are also presented. F-values with significance levels in parentheses (significant differences at $P < 0.05$ are indicated in bold).

Table II: Dominant phytoplankton species collected in four different seasons at lentic and lotic sites in the Kielstau catchment.

Table III: Means (\pm SE) of main phytoplankton metrics at all sites and different seasons and habitat group in the Kielstau catchment. Summary of two-way analysis of variance (ANOVA) for testing the effects of season and location on the environmental factors are also presented. F-values with significance levels in parentheses (significant differences at $P < 0.05$ are indicated in bold).

Table IV: Optimum and tolerance (standard deviation of the optimum) of phytoplankton for DIN (mg/L), WT ($^{\circ}$ C) and TSS (mg/L) in the Kielstau catchment. Except for *Staurastrum* sp., taxa listed the species with number of occurrence > 30 (over all 77 samples). Optima are in **bold** for potential indicator species (see text).

Table V: Comparison of the predictive power of species-based calibration models for DIN, WT and TSS. The data are from lentic and lotic sites sampled in four seasons ($n=77$).

Fig. 1. The location of the Kielstau catchment in Schleswig-Holstein state (B), Germany (A) and the sampling sites (C). A cited from <http://utagerm2313.files.wordpress.com/2009/11/germany-river-map.jpg>; B modified from Schmalz and Fohrer (2010).

Fig. 2. Nonmetric Multidimensional Scaling (NMDS) ordination of phytoplankton community at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study.

Fig. 3. Canonical correspondence ordination of the phytoplankton samples collected at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study and associated significant environmental factors. (a) Biplots of the species and the environmental variables; (b) Biplots of the sampling sites and the environmental variables. Species abbreviations were listed in Table IV.

Fig. 4. Observed DIN (mg/L), WT ($^{\circ}$ C) and TSS (mg/L) values at the 77 sites plotted against predicted values calculated from a WA model. The right three graphs show that there is no bias in the residuals and the solid line shows a LOESS scatter plot smoother ($\text{span}=0.45$).

Table I: Means (\pm SE) of 19 environmental variables at all sites and different seasons and habitat group in the Kielstau catchment. Summary of two-way analysis of variance (ANOVA) for testing the effects of season and location on the environmental factors are also presented. F-values with significance levels in parentheses (significant differences at $P < 0.05$ are indicated in bold).

	All dates and sites	Nov. 2008		Feb. 2009		May 2009		Aug. 2009		Two-way ANOVA analysis	
		Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	Season (df=3)	Location (df=1)
DO (mg/L)	9.21 \pm 0.4	8.78	8.26 \pm 0.4	8.77	10.49 \pm 0.5	10.53	12.73 \pm 0.8	11.79 \pm 0.2	6.71 \pm 0.5	1.85 (0.151)	0.20 (0.655)
pH	7.89 \pm 0.07	7.77	7.36 \pm 0.05	8.36	8.36 \pm 0.2	8.77 \pm 0.04	7.90 \pm 0.08	9.11 \pm 0.07	7.73 \pm 0.06	3.23 (0.028)	10.23 (0.002)
WT ($^{\circ}$ C)	10.56 \pm 0.6	8.50	9.30 \pm 0.1	0.30	2.93 \pm 0.2	12.95 \pm 0.1	11.97 \pm 0.3	20.35 \pm 0.3	16.96 \pm 0.4	176.31 (0.000)	0.19 (0.662)
COND (μ S/cm)	604 \pm 10	513	602 \pm 20	558	643 \pm 19	404 \pm 1	616 \pm 18	407 \pm 0	610 \pm 21	1.37 (0.258)	16.13 (0.000)
TSS (mg/L)	11.51 \pm 1.2	19.00	6.65 \pm 0.7	5.60	10.23 \pm 1.3	27.1 \pm 0.9	11.42 \pm 2.5	55.23 \pm 3.2	10.99 \pm 1.9	12.73 (0.000)	27.43 (0.000)
VSS (mg/L)	8.61 \pm 1.0	13.43	5.17 \pm 0.4	5.60	7.15 \pm 0.5	19.7 \pm 0.3	9.86 \pm 2.2	46.78 \pm 2.3	6.61 \pm 0.7	17.13 (0.000)	37.9 (0.000)
NH ₄ -N (mg/L)	1.00 \pm 0.17	0.64	0.76 \pm 0.27	0.13	2.15 \pm 0.48	0.01 \pm 0.01	1.30 \pm 0.33	0.01 \pm 0	0.13 \pm 0.03	0.61 (0.609)	2.29 (0.135)
NO ₃ -N (mg/L)	18.92 \pm 1.2	15.8	25.92 \pm 1.4	13.46	25.45 \pm 1.5	0.71 \pm 0.2	19.83 \pm 2.4	0 \pm 0	9.46 \pm 1.1	6.22 (0.001)	16.34 (0.000)
NO ₂ -N (mg/L)	0.07 \pm 0.01	0.07	0.06 \pm 0	0	0.03 \pm 0	0.01 \pm 0	0.12 \pm 0.01	0 \pm 0	0.07 \pm 0.02	0.9 (0.448)	4.85 (0.031)
DIN (mg/L)	19.99 \pm 1.3	16.5	26.74 \pm 1.5	13.59	27.64 \pm 1.6	0.74 \pm 0.2	21.25 \pm 2.6	0.02 \pm 0	9.66 \pm 1.1	6.12 (0.001)	16.81 (0.000)
PO ₄ -P (mg/L)	0.22 \pm 0.02	0.12	0.16 \pm 0.02	0.01	0.24 \pm 0.06	0 \pm 0	0.11 \pm 0.01	0.23 \pm 0	0.4 \pm 0.03	3.75 (0.015)	4.16 (0.045)
TP (mg/L)	0.41 \pm 0.03	0.25	0.26 \pm 0.03	0.10	0.42 \pm 0.07	0.16 \pm 0.01	0.48 \pm 0.07	0.53 \pm 0	0.51 \pm 0.03	1.87 (0.142)	2.57 (0.114)
Si (mg/L)	0.23 \pm 0.01	0.27	0.25 \pm 0.01	0.18	0.24 \pm 0.02	0.03 \pm 0	0.16 \pm 0.02	0.10 \pm 0	0.32 \pm 0.01	6.99 (0.000)	10.97 (0.001)
Cl ⁻ (mg/L)	32.62 \pm 1.0	22.71	25.85 \pm 0.9	15.33	36.51 \pm 3.4	25.32 \pm 0.3	35.68 \pm 1.3	27.97 \pm 0.5	35.49 \pm 1.3	1.00 (0.396)	8.75 (0.004)
SO ₄ ²⁻ (mg/L)	34.71 \pm 1.1	32.41	36.6 \pm 1.9	32.73	34.43 \pm 2	32.46 \pm 1.3	34.11 \pm 2.3	14.25 \pm 0.7	36.43 \pm 2.4	1.42 (0.243)	3.44 (0.068)
N:P	76.82 \pm 11	64.98	133.31 \pm 23	141.59	111.33 \pm 34	4.67 \pm 1.0	59.90 \pm 8.6	0.04 \pm 0.01	18.26 \pm 2.0	2.06 (0.113)	0.53 (0.471)
Width (m)	2.2 \pm 0.11	-	2.46 \pm 0.26	-	2.16 \pm 0.22	-	2.11 \pm 0.21	-	2.07 \pm 0.18	-	-
Depth (m)	0.29 \pm 0.02	-	0.38 \pm 0.05	-	0.3 \pm 0.04	-	0.27 \pm 0.04	-	0.22 \pm 0.03	-	-
Velocity (m/s)	0.17 \pm 0.01	0.00	0.25 \pm 0.03	0.00	0.22 \pm 0.03	0 \pm 0	0.16 \pm 0.02	0 \pm 0	0.09 \pm 0.02	0.59 (0.621)	13.39 (0.000)

Note: - means data absent; values without SE were only one sample.

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Table II: Dominant phytoplankton species collected in four different seasons at lentic and lotic sites in the Kielstau catchment.

	Lentic				
	All dates	Nov. 2008	Feb. 2009	May 2009	Aug. 2009
<i>Desmodesmus communis</i>	49.65	12.84	17.44	52.13	55.45
<i>Pediastrum duplex</i>	26.91	52.22	45.35	24.90	23.81
<i>Discostella steligera</i>	5.04	0.54	0.06	8.14	0.12
<i>Scenedesmus dimorphus</i>	3.34	0.43	2.91	4.44	1.81
<i>Aulacoseira granulata</i>	3.02	3.69	4.22	3.07	2.67
<i>Cyclotella meneghiniana</i>	2.47	2.14	0.18	0.71	6.32
<i>Staurastrum</i> sp.	1.62	2.57	0.58	1.16	2.41
	Lotic				
	All dates	Nov. 2008	Feb. 2009	May 2009	Aug. 2009
<i>Tabellaria flocculosa</i>	12.35	18.08	16.65	4.02	15.46
<i>Euglena</i> sp.	9.64	1.25	1.68	20.23	7.45
<i>Planothidium lanceolatum</i>	8.62	11.32	10.44	4.89	10.00
<i>Cocconeis placentula</i>	6.73	11.68	7.94	0.60	9.48
<i>Fragilaria biceps</i>	6.50	14.51	7.65	6.23	1.57
<i>Cryptomonas erosa</i>	4.15	1.91	4.30	8.57	0.94
<i>Ulnaria ulna</i>	3.99	0.40	0.94	7.12	4.30
<i>Cyclotella meneghiniana</i>	3.77	2.22	1.84	1.52	7.82
<i>Nitzschia sigma</i>	3.72	0.17	3.79	5.27	4.18
<i>Navicula ingapirca</i>	3.34	0.00	0.97	6.67	3.00
<i>Meridion circulare</i>	2.71	0.05	3.40	1.54	5.14
<i>Navicula cryptocephala</i>	2.64	0.22	0.66	4.73	2.85
<i>Oscillatoria</i> sp.	2.36	2.92	9.91	0.70	0.30
<i>Fragilaria crotonensis</i>	1.99	0.00	0.41	2.39	3.46
<i>Gomphonema olivaceum</i>	1.93	1.89	2.61	1.38	2.20
<i>Melosira varians</i>	1.86	0.00	1.40	1.88	3.13
<i>Surirella heidenii</i>	1.85	1.60	1.27	2.05	2.05
<i>Desmodesmus communis</i>	1.17	0.77	1.01	1.82	0.81
<i>Ulnaria ulna</i> var. <i>acus</i>	1.13	0.00	0.24	2.11	1.20
<i>Scenedesmus dimorphus</i>	1.12	0.05	0.25	3.14	0.09
<i>Caloneis amphisbaena</i>	1.11	0.89	1.89	0.96	1.02
<i>Navicula viridula</i>	1.05	1.88	1.18	0.65	0.90

Note: values in tables were relative abundance (%).

Table III: Means (\pm SE) of main phytoplankton metrics at all sites and different seasons and habitat group in the Kielstau catchment. Summary of two-way analysis of variance (ANOVA) for testing the effects of season and location on the environmental factors are also presented. F-values with significance levels in parentheses (significant differences at $P < 0.05$ are indicated in bold).

	All dates and sites	Nov. 2008		Feb. 2009		May 2009		Aug. 2009		Two-way ANOVA analysis	
		Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	Season (df=3)	Location (df=1)
Margalef's index	1.92 \pm 0.06	2.45	2.35 \pm 0.07	1.32	2.36 \pm 0.13	1.32 \pm 0.10	1.62 \pm 0.05	0.85 \pm 0.03	1.58 \pm 0.06	11.49 (0.000)	10.85 (0.002)
Richness	33.00 \pm 1.03	46.00	39.67 \pm 1.34	23.00	39.06 \pm 2.45	28.00 \pm 2.00	28.83 \pm 0.88	17.50 \pm 0.50	26.89 \pm 1.35	8.26 (0.000)	2.85 (0.096)
Chl <i>a</i> (μ g/L)*	2.57 \pm 0.14	4.07	1.73 \pm 0.13	3.57	2.06 \pm 0.16	4.09 \pm 0.03	3.82 \pm 0.27	4.86 \pm 0.02	2.06 \pm 0.2	2.20 (0.095)	21.99 (0.000)
Total density (cell/L)*	11.58 \pm 0.12	12.74	11.39 \pm 0.12	11.52	11.09 \pm 0.2	14.22 \pm 0.04	11.87 \pm 0.14	13.5 \pm 0.05	11.37 \pm 0.33	3.26 (0.027)	15.00 (0.000)
Shannon-Wiener index**	6.33 \pm 0.25	3.42	7.32 \pm 0.23	3.39	7.11 \pm 0.67	2.32 \pm 0.22	5.94 \pm 0.42	2.06 \pm 0.01	6.24 \pm 0.44	0.63 (0.600)	20.25 (0.000)
Evenness**	0.52 \pm 0.02	0.23	0.54 \pm 0.02	0.35	0.53 \pm 0.04	0.21 \pm 0.01	0.53 \pm 0.04	0.26 \pm 0.01	0.58 \pm 0.04	0.24 (0.870)	19.80 (0.000)
% prostrate taxa (%)	47.23 \pm 2.09	23.21	45.82 \pm 2.45	5.68	50.42 \pm 2.93	11.68 \pm 3.52	51.4 \pm 4.71	0.93 \pm 0.30	54.2 \pm 3.6	0.23 (0.874)	36.78 (0.000)
% mobile taxa (%)	11.08 \pm 0.87	5.91	8.72 \pm 1.03	4.55	12.45 \pm 1.41	3.39 \pm 0.33	14.9 \pm 2.06	0.41 \pm 0.41	11.03 \pm 2.2	0.29 (0.832)	6.35 (0.014)
% benthic taxa (%)	46.23 \pm 21.09	2.58	50.02 \pm 11.15	0.66	48.11 \pm 17.39	5.92 \pm 3.21	48.94 \pm 22.84	0.56 \pm 0.20	52.57 \pm 17.60	0.18 (0.911)	42.11 (0.000)
Q index	3.69 \pm 0.80	2.65	4.06 \pm 0.28	2.26	3.48 \pm 0.90	2.99 \pm 0.01	3.74 \pm 0.98	2.77 \pm 0.11	3.77 \pm 0.72	1.79 (0.158)	10.46 (0.002)
Chlorophyte-Index (%)	13.45 \pm 19.73	71.08	10.51 \pm 12.93	53.39	7.05 \pm 5.10	74.91 \pm 5.47	10.89 \pm 11.60	76.50 \pm 4.77	5.39 \pm 8.05	1.33 (0.270)	217.08 (0.000)
Pennales-Index (%)	84.64 \pm 21.70	34.53	92.63 \pm 4.73	4.96	91.45 \pm 4.72	32.03 \pm 3.29	92.73 \pm 6.00	2.26 \pm 2.43	84.70 \pm 13.14	6.12 (0.001)	395.15 (0.000)

Note: * Ln(x+1) transformation; ** Square transformation; values without SE were only one sample.

Table IV: Optimum and tolerance (standard deviation of the optimum) of phytoplankton for DIN (mg/L), WT (°C) and TSS (mg/L) in the Kielstau catchment. **Except for *Staurastrum* sp.**, taxa listed the species with number of occurrence >30 (over all 77 samples). Optima are in **bold** for potential indicator species (see text).

Taxon	Code	Number of occurrences	DIN	WT		TSS		
			Optimum	Tolerance	Optimum	Tolerance	Optimum	Tolerance
BACILLARIOPHYTA								
<i>Achnantheidium minutissimum</i> (Kütz.)								
<i>Czarnecki</i>	Acmi	42	16.22	10.92	11.47	5.80	17.23	9.63
<i>Aulacoseira granulata</i> (Ehr.) Ralfs	Augr	36	9.15	12.43	11.00	3.89	20.56	9.84
<i>Caloneis amphisbaena</i> (Bory) Cleve	Caam	53	21.50	11.62	10.76	5.26	13.46	8.37
<i>Cocconeis placentula</i> Ehrenberg	Copl	63	15.65	9.42	12.14	6.29	12.76	8.64
<i>Cyclotella meneghiniana</i>	Cyme	71	12.11	9.38	15.39	5.69	19.00	11.56
<i>Discostella steligera</i> (Cleve & Grunow)								
<i>Håkansson</i>	Dist	36	3.75	8.26	12.85	3.22	24.33	8.68
<i>Fragilaria biceps</i> (Kütz.) Lange-Bertalot	Frbi	68	24.02	9.04	9.80	4.23	9.67	7.89
<i>Fragilaria crotonensis</i> Kitton	Frcr	42	14.94	12.70	14.88	4.21	17.06	11.72
<i>Gomphonema olivaceum</i> (Lyngb.) Kütz.	Gool	72	19.46	10.40	11.80	5.80	12.75	8.25
<i>Melosira varians</i> Ag.	Meva	44	15.94	11.80	13.84	5.23	17.03	11.74
<i>Meridion circulare</i> (Grev.) Ag.	Meci	42	14.02	14.80	13.15	6.17	12.82	7.53
<i>Navicula cryptocephala</i> Kütz.	Nacr	47	16.02	9.74	13.68	4.35	11.63	9.52
<i>Navicula ingapirca</i> Lange-Bertalot & U.								
<i>Rumrich</i>	Nain	48	21.28	13.54	13.19	3.74	13.60	8.16
<i>Navicula rhynchocephala</i> Kütz.	Narh	42	18.17	13.42	12.75	5.02	16.03	9.74
<i>Navicula viridula</i> (Kütz.) Ehr.	Navi	55	20.24	10.18	10.79	4.99	12.40	6.92
<i>Nitzschia dissipata</i> (Kütz.) Grun.	Nidi	53	19.97	10.34	11.39	4.99	10.86	6.78
<i>Nitzschia sigma</i> (Kütz.) W. Sm.	Nisi	58	18.61	11.55	12.61	5.00	14.05	10.73
<i>Nitzschia sigmoidea</i> (Nitz.) W. Sm.	Nisd	42	24.65	8.47	9.29	3.61	10.26	5.43
<i>Planothidium lanceolatum</i> (Breb.) Round et								
<i>Bukhtiyarova</i>	Plla	74	19.52	11.12	11.51	5.13	12.60	8.02
<i>Pleurosigma delicatulum</i> W. Sm.	Plde	49	16.88	12.69	12.90	5.06	17.53	9.25
<i>Surirella heidenii</i> Hust.	Suhe	62	21.75	12.55	12.25	4.56	14.23	8.55

<i>Tabellaria flocculosa</i> (Roth) Kütz.	Tafl	66	16.84	8.66	12.10	6.76	14.28	9.54
<i>Ulnaria ulna</i> (Nitzsch) Comperé var. <i>acus</i> (Kütz.) Lange-Bertalot	Ulac	29	14.04	7.22	13.78	3.82	17.11	14.06
<i>Ulnaria ulna</i> (Nitzsch) Comperé	Ulul	59	11.92	6.27	13.60	3.84	17.05	13.46
CHLOROPHYTA								
<i>Desmodesmus communis</i> (Hegew.) Hegew.	Deco	50	2.31	6.87	12.63	2.42	25.80	6.26
<i>Pediastrum duplex</i> Meyen	Pedu	41	5.35	8.84	11.40	3.91	23.55	7.65
<i>Scenedesmus dimorphus</i> (Turp.) Kütz.	Scdi	31	12.99	16.85	12.22	2.32	19.50	10.28
<i>Staurastrum</i> sp.	Stsp.	9	4.82	8.78	11.77	2.91	24.28	6.37
CRYPTOPHYTA								
<i>Cryptomonas erosa</i> Ehr.	Crer	48	30.77	12.46	9.67	4.24	13.03	6.89
CYANOPHYTA								
<i>Oscillatoria</i> sp.	Ossp.	66	25.55	7.95	6.00	4.48	9.14	5.61
EUGLENOPHYTA								
<i>Euglena</i> sp.	Eusp.	43	10.65	7.73	12.85	2.87	23.85	13.38

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Table V: Comparison of the predictive power of species-based calibration models for DIN, WT and TSS. The data are from lentic and lotic sites sampled in four seasons (n=77).

	R^2_{apparent}	$\text{RMSE}_{\text{apparent}}$	$R^2_{\text{jackknife}}$	$\text{RMSE}_{\text{jackknife}}$
DIN (mg/L)	0.41	12.71	0.32	13.57
WT (°C)	0.34	7.23	0.24	7.88
TSS (mg/L)	0.32	11.37	0.20	12.60

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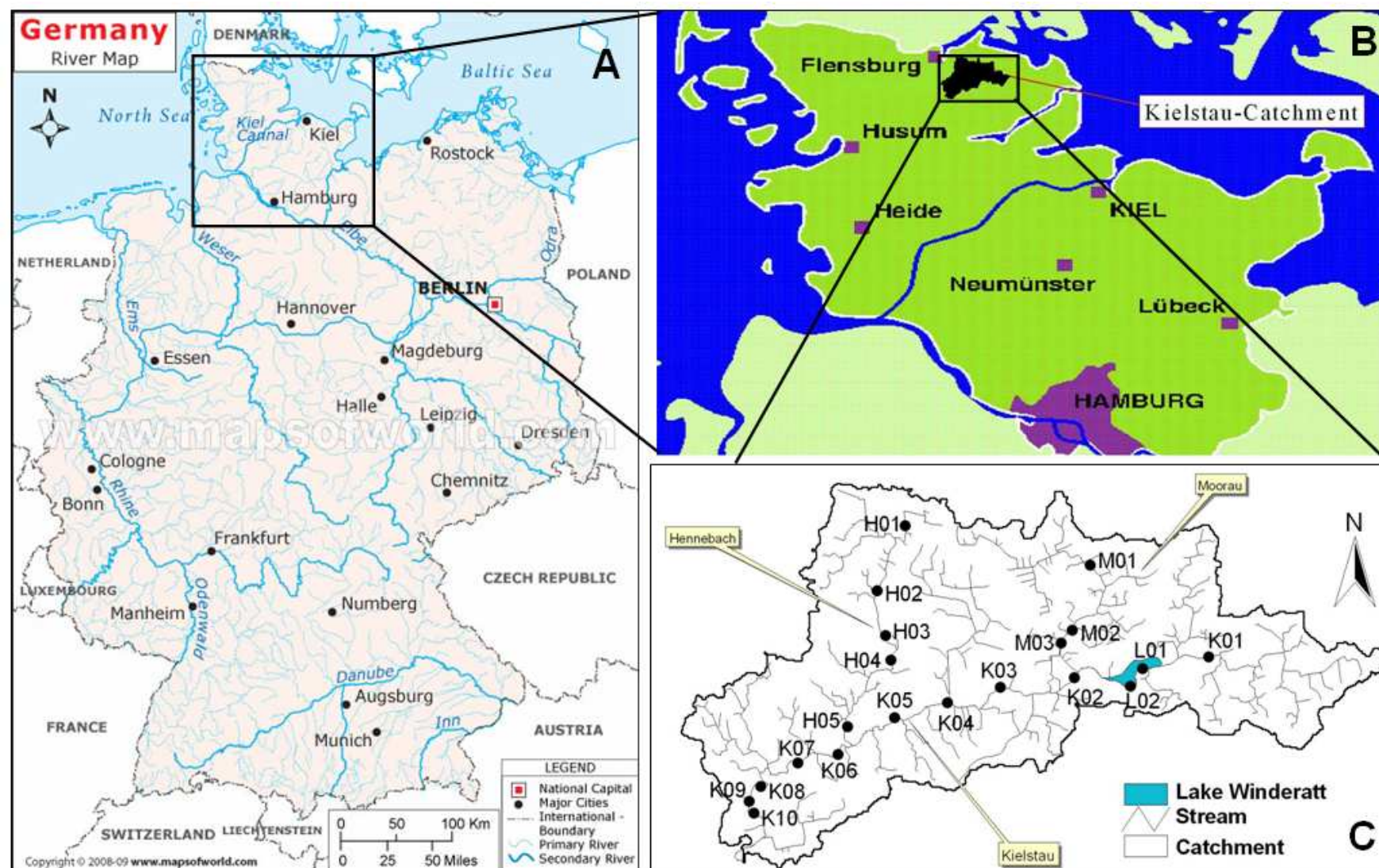


Fig. 1. The location of the Kielstau catchment in Schleswig-Holstein state (B), Germany (A) and the sampling sites (C). A cited from <http://utagerm2313.files.wordpress.com/2009/11/germany-river-map.jpg>; B from Schmalz and Fohrer (2010).

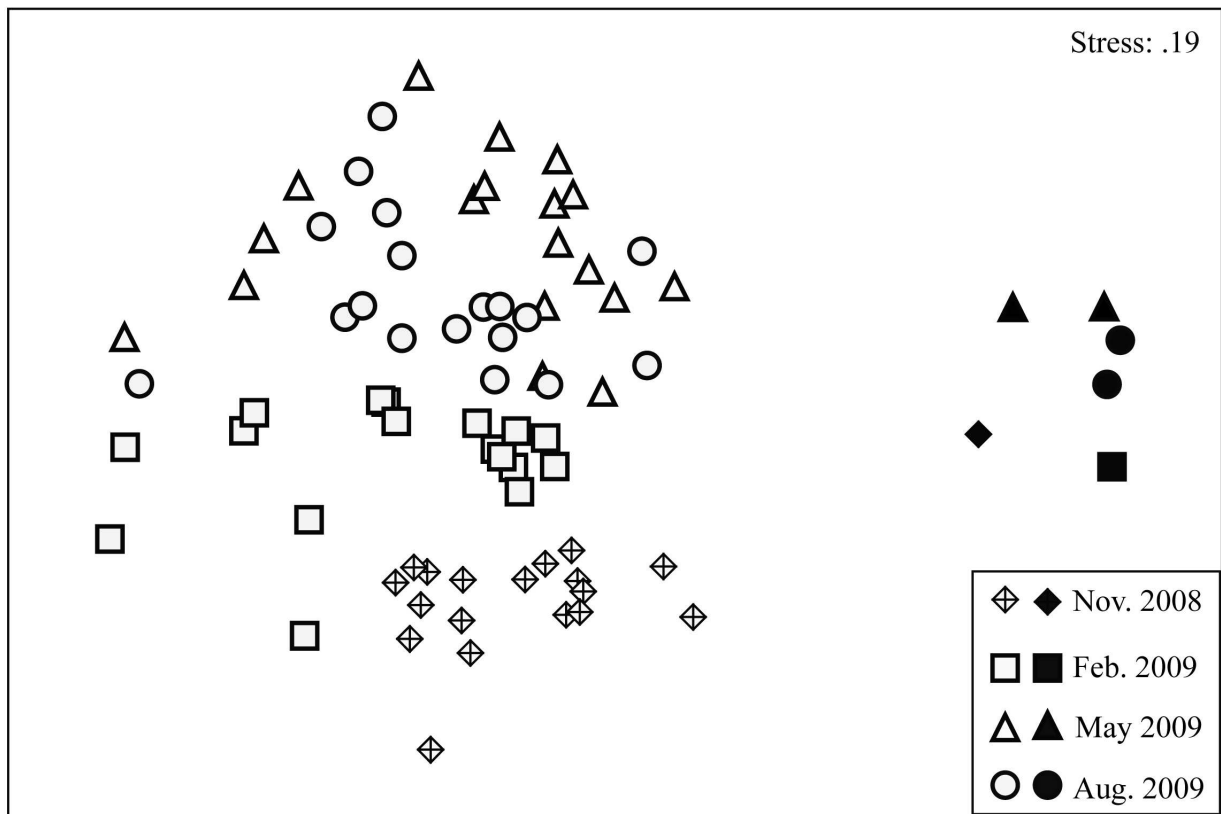


Fig. 2. Nonmetric Multidimensional Scaling (NMDS) ordination of phytoplankton community at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study.

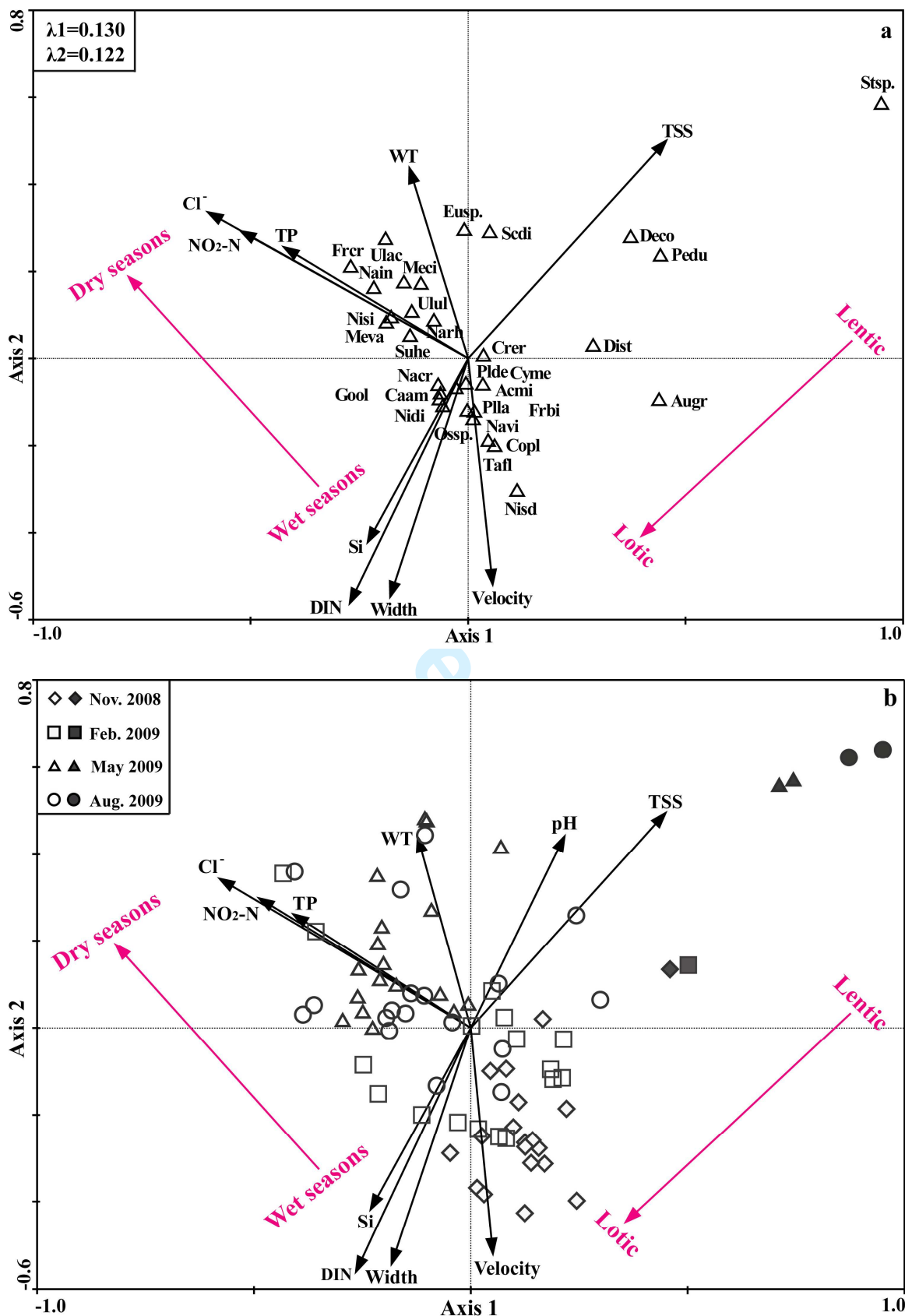


Fig. 3. Canonical correspondence ordination of the phytoplankton samples collected at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study and associated significant environmental factors. (a) Biplots of the species and the environmental variables; (b) Biplots of the sampling sites and the environmental variables. Species abbreviations were listed in Table IV.

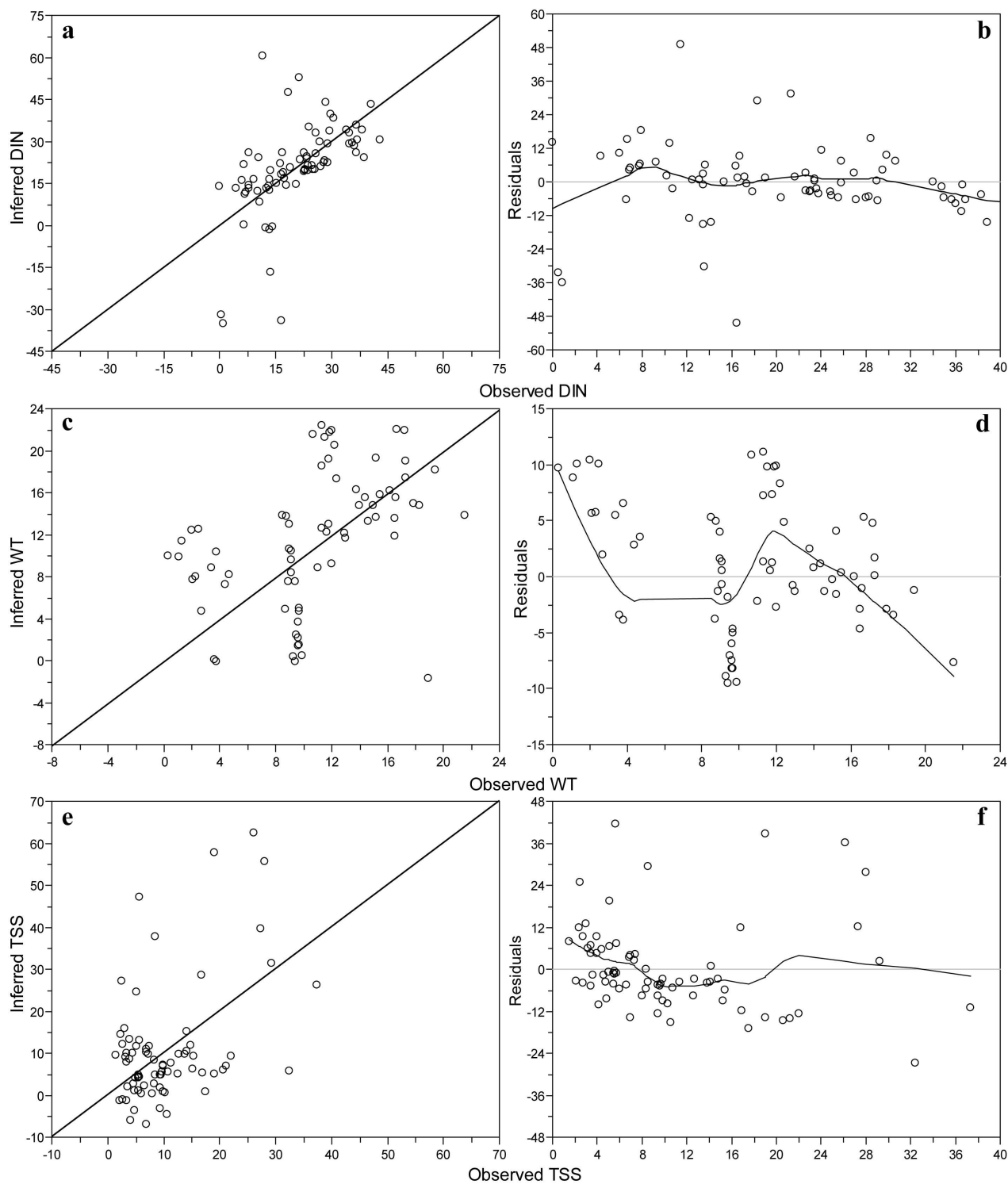


Fig. 4. Observed DIN (mg/L), WT (°C) and TSS (mg/L) values at the 77 sites plotted against predicted values calculated from WA models. The right three graphs show that there is no bias in the residuals and the solid line shows a LOESS scatter plot smoother (span=0.45).