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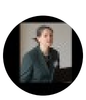

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Development and testing of a phytoplankton index of biotic integrity (P-IBI) for a German lowland river

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A B S T R A C T

We developed a phytoplankton index of biotic integrity (P-IBI) for a German lowland river to assess effects of human disturbances on the biotic condition of riverine phytoplankton community. Six metrics (out of 36 original metrics) were selected from a training data set, based on Cumulative_R^2 and correlation index (CoI) between biotic metrics and environmental variables. The final P-IBI scores were calculated by averaging metrics for a site after transforming them to a discrete 1 (bad), 2 (low), 3 (moderate), 4 (good), 5 (high) scale according to the requirements of the European Water Framework Directive (WFD). We then tested the robustness of P-IBI. The P-IBI and its six metrics were indicative of ecological integrity and water quality as indicated by canonical correspondence analysis and comparisons with other single metrics, although Cumulative_R^2 and CoI values declined in the testing data set. By implementing the developed P-IBI in the study area, we found that the ecological status varied seasonally. The general ecological status of the study region was 'Moderate' regardless of seasonal variations, which was lower than the requirement ('Good' status) of WFD by 2015. The relative lower ecological status was probably caused by point sources, diffuse sources emissions and artificial drainage systems of the study area. Our study was an important trial for the development of IBI in a catchment without reference sites and the constructed P-IBI could be a useful tool to measure the long-term status of streams and the effectiveness of various watershed managements. Besides, further river basin managements are suggested to address point sources, diffuse sources as well as artificial drainage systems in order to gain a better water quality in the study region.

1. Introduction

Multi-metric indices are increasingly used in the assessment of the river ecological status as well as in resource and ecosystem management because (1) they are often more robust than their component metrics (Lacouture et al., 2006), (2) they integrate chemical and physical properties of streams over time that could otherwise be missed by one-time water chemistry sampling (Winter and Duthie, 2000), and (3) furthermore they represent different taxonomic and functional groups within the assemblage, which respond differently to various stressors and can reflect the ecological status in a comprehensive manner (Tang et al., 2006; Blanco et al., 2007; Zalack et al., 2010). The majority of these indices have focused on stream macroinvertebrates, fish, macrophytes and epilithic algae (e.g., Karr, 1981; Prygiel and Coste, 1993; Kerans and Karr, 1994; Hill et al., 2003; Wang et al., 2005; Mattsson and Cooper, 2006; Rothrock et al., 2008; Bae et al., 2010; Hermoso et al., 2010), which have been used as tools for monitoring stream health for a long time in the USA and European countries (Zalack et al., 2010).

Phytoplankton (mainly planktonic algae) constitutes the autochthonous primary producers in aquatic ecosystems and form part of the basis of the food web for other organisms in terms of energy and material input (Hötzel and Croome, 1999). Thus, any changes that affect the biotic integrity of the algal community may impact higher trophic levels as well. In addition, compared to other biotic assemblage indicators of water quality, planktonic algae have shorter regeneration time and life cycle, allowing the community to respond quicker to anthropogenic influences (Domingues and Galvão, 2007; Cabecinha et al., 2009). Moreover, unlike fish and macroinvertebrates, algal communities are usually present before disturbance and generally persist in some form after disturbances. Therefore, applications of algal indicators to rivers are increasing (Borics et al., 2007; Blanco et al., 2007; Plenković-Moraj et al., 2007;

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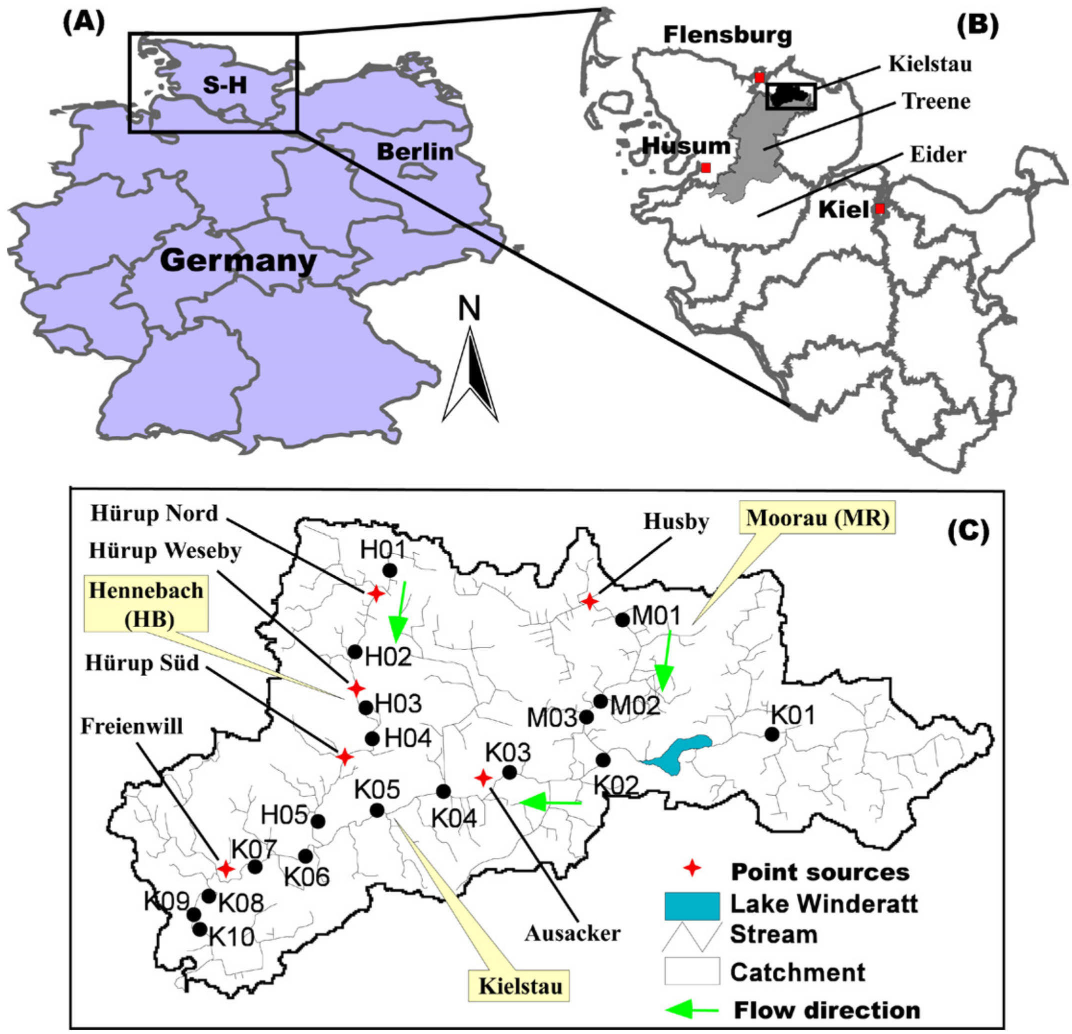

Fig. 1. The location of the Kielstau catchment in Schleswig-Holstein state (B), Northern Germany (A; map source: CDC, 2010) and the sampling sites (C). S-H = Schleswig-Holstein state; M01–M03 = sampling sites collected from Moorau (MR) tributary; H01–H05 = sampling sites from Hennebach (HB) tributary; K01–K10 = sampling sites from main stream Kielstau.

Reavie et al., 2010). However, comparing to the huge investigations in lentic water bodies (e.g., oceans, gulfs, lakes and reservoirs), little attention has been paid to the applications of the phytoplankton in ecological evaluation of rivers so far (Borics et al., 2007). And to our knowledge, a multi-metric based phytoplankton index of biotic integrity (P-IBI) has been rarely considered for river 'health' assessment.

In this paper, we developed and tested a P-IBI using a training data set and a testing data set, respectively, from a German lowland river – the Kielstau catchment. Our specific objectives were to: (1) develop a phytoplankton index of biotic integrity (P-IBI), which can assess effects of human disturbances on the ecological status of the lowland river; (2) test the robustness of the P-IBI by canonical correspondence analysis (CCA) and comparing the performances with other single metrics; (3) deduce the ecological status of the study area by implementing the developed P-IBI.

2. Materials and methods

2.1. Description of the study area

The Kielstau catchment is a lowland watershed with a drainage area of 50 km^2, and located in the Northern part of Germany. It has its origin in the upper part of Lake Winderatt and is a tributary of the Treene River, which is the most important tributary of the Eider River (Fig. 1). Moorau (MR) and Hennebach (HB) are two main tributaries within the Kielstau catchment. Sandy, loamy and peat soils are characteristic for the catchment. Land use is dominated by arable land and pasture (Schmalz et al., 2008b; Schmalz and Fohrer, 2010). The drained fraction of agricultural area in the Kielstau catchment is estimated 38% (Fohrer et al., 2007). The precipitation is 841 mm/a (station Satrup, 1961–1990, DWD, 2010) and the mean annual temperature is 8.2 °C (station Flensburg, 1961–1990, DWD, 2010).

In order to take into account possible inter-seasonal variations, the study was performed seven times at 18 sites (Fig. 1C) along the main stream Kielstau and its tributaries from November 2008 to May 2010. Ten sites (K01–K10) were located along the main stream, three (M01–M03) at the Moorau tributary and five (H01–H05) at the Hennebach tributary. A total of 122 samples were collected.

2.2. Sampling methods and primary procedures

At each site and on every sampling date, three replicate samples of a known volume subsurface (5–40 cm) water were taken with a 10 L bucket and then filtered through a plankton net. The retained

Table 1
Environmental variables and their codes sampled at Kielstau catchment and methods used.

Environmental variables	Codes	Methods
Ammonium–nitrogen (mg/L)	NH_4–N	Nessler's reagent colorimetric method (at 690 nm; DIN38 406-E5-1)
Channel width (m)	Width	Measured with scale in the field
Chloride (mg/L)	Cl^-	Ion chromatography method (DIN38 405-D19)
Conductivity (μS/cm)	COND*	Portable Meter (WTW Multi 340i, Weilheim, Germany)
Dissolved inorganic nitrogen (mg/L)	DIN	Sum of NH_4–N, NO_3–N and NO_2–N
Dissolved oxygen (mg/L)	DO*	Portable Meter (WTW Multi 340i, Weilheim, Germany)
Dissolved silicon (mg/L)	Si	Molybdosilicate method (at 410 nm; DIN38 405-D21)
Mean flow velocity (m/s)	Velocity	FlowSens (FlowSens Single Axis Electromagnetic Flow Meter, SEBA Hydrometrie, Germany)
Mean water depth (m)	Depth*	See text
Nitrate-nitrogen (mg/L)	NO_3–N*	Ion chromatography method (DIN38 405-D19)
Nitrite–nitrogen (mg/L)	NO_2–N	Sulphanilamide and N-(1-naphthyl)-ethylenediamine method (DIN38 405-D10)
Orthophosphate-phosphorus (mg/L)	SRP*	Ammonium molybdate spectrophotometric method (at 880 nm; DIN 1189)
pH value	pH	Portable Meter (WTW Multi 340i, Weilheim, Germany)
Ratio between DIN and TP	N:P*	Calculation by DIN/TP
Sulphate (mg/L)	SO_4^{2-}*	Ion chromatography method (DIN38 405-D19)
Total phosphorus (mg/L)	TP	Ammonium molybdate spectrophotometric method (at 880 nm; DIN 1189); the sample was digested to convert all kinds of P to the orthophosphate form by oxidation reagent, comprised by sodium hydroxide (NaOH), potassium persulfate ($K_2S_2O_8$) and boric acid (H_3BO_3)
Total suspended solid (mg/L)	TSS	Standard Operating Procedure for Total Suspended Solids Analysis (U.S. Environmental Protection Agency, 1997)
Water temperature (°C)	WT	Portable Meter (WTW Multi 340i, Weilheim, Germany)

Variables with * were excluded in the final analyses (see text for details).

organisms were transferred into glass containers and fixed in 5‰ non-acetic Lugol's iodine solution (Sabater et al., 2008). After 48 h, the undisturbed water samples were concentrated to 30 mL for further processes. 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), conductivity (COND) and water temperature (WT) were measured *in situ* by a Portable Meter (WTW Multi 340i, Weilheim, Germany). Water depth, channel width and flow velocity (FlowSens Single Axis Electromagnetic Flow Meter, SEBA Hydrometrie, Germany) were measured at each site as well. We measured several water depths and flow velocities (at least 3 repetitions) along a cross section at each site, and the mean depth and velocity were calculated finally (Table 1).

At each site, water samples were also collected for further laboratory analysis including ammonium–nitrogen (NH_4–N), chloride (Cl^-), dissolved silicon (Si), nitrate–nitrogen (NO_3–N), nitrite–nitrogen (NO_2–N), orthophosphate-phosphorus (SRP), sulphate (SO_4^{2-}), total phosphorus (TP) and total suspended solid (TSS). All these parameters were measured in the lab of the Department of Hydrology and Water Resources Management of Kiel University according to the standard methods (Table 1). Dissolved inorganic nitrogen (DIN) was defined as the sum of NH_4–N, NO_3–N and NO_2–N, and N:P was calculated by DIN:TP. Besides, a known volume of surface water was filtered through WHATMAN GF/C glass-fiber filters for chlorophyll *a* (Chl *a*) determination, which was measured spectrophotometrically following 90% acetone extraction according to APHA (1992).

2.3. Identification under microscope

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 (by 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 its densities were expressed as cell/L. Algal biomass was estimated by Chl *a* and taxa biovolumes (by closest geometric form supposing specific gravity of 1.00 g cm^{-3}) (Hillebrand et al., 1999).

2.4. Development of the phytoplankton index of biotic integrity (P-IBI)

Seventy-one samples (training data set) collected from four dates (November 2008 to August 2009) were used to develop the P-IBI. We first compiled a large pool of attributes (totally 36 metrics), which belonged to community metrics (e.g., Hillebrand et al., 1999), growth form metrics (van Dam et al., 1994; Wang et al., 2005; Porter, 2008) and diversity indices (Shannon and Weaver, 1949; Margalef, 1958; Menhinick, 1964; Ludwig and Reynolds, 1988; Camargo, 2008; Spatharis and Tsirtsis, 2010). Since not all the metrics effectively signaled water quality degeneration, candidate metrics used for the P-IBI were chosen from the pool of original metrics based on the correlations with environmental variables. Nonparametric Spearman rank correlation tests were used to avoid problems associated with non-normal data distribution (Kolmogorov–Smirnov test, $P < 0.05$). Where two or more environmental variables were highly correlated ($r_s > 0.80$), only the greatest correlation with the biotic metrics was included and this criterion reduced the number of environmental variables from 18 to 11. To evaluate the statistical significance of each correlation between biotic metrics and environmental variables, we introduced Cumulative_R^2 and 'correlation index' (CoI) according to Blanco et al. (2007):

$$\text{Cumulative\_}R^2 = \sum_{1}^{y} r_{s,y}^2 \tag{1}$$

where R^2 = sum of $r_{s,y}^2$ with $r_{s,y}$ = Spearman's correlation coefficient, r_s, between a given metric and the environmental variable y.

$$\text{CoI} = \frac{(\text{Cumulative\_}R^2\ S)}{n^2} \tag{2}$$

where CoI = correlation index for a given metric, S = number of $r_{s,y}$ statistically significant at $P < 0.05$, and n = number of environmental variables evaluated.

Table 2
Six selected metrics of the phytoplankton index of biotic integrity (P-IBI) and metric classifications corresponding with scores based on a five-level scaling system (see text for details). Values were obtained from the training data set. Their expected responses (*R*) to deterioration of water quality were also shown: + = indices expected to increase with deterioration, − = indices expected to decrease with deterioration.

Metrics	Response	Five-level scaling system					Reference
		High (5)	Good (4)	Moderate (3)	Low (2)	Bad (1)	
Chl *a*	+	<3.69	3.69–9.04	9.04–23.53	23.53–42.30	>42.30	–
SI_density	+	<1.80	1.8–2.00	2.00–2.28	2.28–2.64	>2.64	–
CyI_density	+	<0.68	0.68–1.97	1.97–5.70	5.70–14.00	>14.00	–
M_density	−	>2.60	2.60–2.21	2.21–1.84	1.84–1.55	<1.55	–
SpR	−	>44	44–38	38–33	33–27	<27	–
Menhinick_density	−	>0.15	0.15–0.09	0.09–0.05	0.05–0.03	<0.03	Spatharis and Tsirtsis (2010)

Note: metrics with '_density' were calculated based on cell density. Chl *a* = Chlorophyll *a*, spectrophotometrically after filtration and extraction with 90% acetone (for details, see text); SI = saprobity index (van Dam et al., 1994); CyI = Cyanobacteria-Index (Mischke and Behrendt, 2007); M = Margalef's diversity index (Margalef, 1958); SpR = species richness; Menhinick = Menhinick diversity index (Menhinick, 1964).

CoI ranged from 0 to 1, while Cumulative_R^2 from 0 to *n*, indicating the theoretical minimum and maximum relationship between a given candidate metric and environmental variables, and the higher values indicating better relationship.

We then used a five-level scaling system to normalize the ranges of selected candidate metrics, which were 1 (bad), 2 (low), 3 (moderate), 4 (good) and 5 (high), based on the requirements of the European Water Framework Directive (WFD) (EC, 2000). The candidate metrics were scored according to 90th, 75th, 50th and 25th percentile of the whole values of training data set, if not available from previous references. For metrics that decreased with impairment, we scored sites as 1, 2, 3, 4 and 5, if the values of the metrics were <25th, 25th–50th, 50th–75th, 75th–90th and >90th percentile of the site values, respectively. For metrics that increased with impairment, we scored sites as 5, 4, 3, 2 and 1, if the values of the metrics were <25th, 25th–50th, 50th–75th, 75th–90th and >90th percentile of the site values, respectively. The final P-IBI scores were the mean of candidate metric values (ranged from 1 to 5) based on above scaling system, which was also classified into 5 scales: 'High' (5.0–4.5), 'Good' (4.5–3.75), 'Moderate' (3.75–2.5), 'Low' (2.5–1.25) and 'Bad' (1.25–1.0).

2.5. Testing the phytoplankton index of biotic integrity (P-IBI)

We tested the P-IBI using a testing data set (51 samples) collected from three dates (November 2009, February 2010 and May 2010). The calculation and scaling system of the candidate metrics for the P-IBI were in the same manner as the previous year. Cumulative_R^2 and CoI were used to evaluate the statistical significance of each correlation between the P-IBI and its metrics with environmental variables. Those results were then used to compare with corresponding values of the training data set. The developed P-IBI was considered acceptable if there was an agreement between Cumulative_R^2 and CoI of the testing and those of the training data sets.

We also applied Q index (QI) (Borics et al., 2007), trophic diatom index (TDI) (Kelly and Whitton, 1995) and trophic index of potamoplankton (TIP) (Mischke and Behrendt, 2007) to both training and testing data sets to determine the relative performance of our P-IBI. These three single metrics were calculated based on both cell density (with a postfix '_density') and taxa biovolumes (with a postfix '_biomass').

A detrended correspondence analysis on the various algal data matrices produced a longest gradient length of 3.26 along the first axis, suggesting that both redundancy analysis (RDA) and canonical correspondence analysis (CCA) were appropriate (Lepš and Šmilauer, 2003). CCA was chosen to test the relationships among species assemblages, environmental variables and P-IBI metrics. CCA is a multivariate ordination technique for direct gradient analysis, and it can be used to evaluate species-environment relationships as well as to derive estimates of the amount of variation in the species data that is explained by measured environmental variables (Reavie et al., 2010). 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 sample and have a total relative abundance >0.5% when all samples were summed; this requirement reduced the number of taxa in the analysis from 96 to 29 (Appendix). During CCA, log $(x+1)$ transformation and downweighting of rare taxa were applied, and forward selection and Monte Carlo permutations (999 iterations) were used to identify a subset of the measured variables that exerted significant and independent effects on phytoplankton assemblages. P-IBI and its metrics were set as passive variables for exploring their relationships with environmental variables and phytoplankton assemblages (Zalack et al., 2010).

Table 3
Spearman rank correlation coefficients ($r_{s,y}$) among metrics (after scoring) or phytoplankton index of biotic integrity (P-IBI) and environmental variables (*y*) using the training data set.

Metrics or P-IBI	Environmental variables										
	Width	Velocity	pH	WT	TSS	NH_4–N	NO_2–N	DIN	TP	Cl^-	Si
Chl *a*	0.199	−0.042	−0.379**	−0.219	−0.275*	−0.183	−0.403***	0.176	−0.177	−0.320**	0.449***
SI_density	0.013	0.345**	−0.374**	−0.396***	−0.003	0.361**	0.257*	0.507***	−0.339**	−0.480***	−0.188
CyI_density	−0.071	−0.366**	−0.043	0.647***	0.226	−0.38**	0.268*	−0.564***	0.289*	0.157	−0.014
M_density	0.284*	0.612***	−0.297*	−0.676***	0.173	0.325**	−0.257*	0.365**	−0.241*	−0.509***	0.163
SpR	0.239*	0.641***	−0.298*	−0.612***	0.325**	0.369**	−0.182	0.346**	−0.141	−0.479***	0.178
Menhinick_density	0.224	0.333**	−0.072	−0.495***	−0.39***	0.124	−0.385***	0.281*	−0.324**	−0.239*	0.079
P-IBI	0.293*	0.515***	−0.479***	−0.573***	0.073	0.212	−0.229	0.351**	−0.284*	−0.624***	0.218

Note: Each metric was scored based on a five-level scaling system (see text for details), with higher scores indicating better water quality. Metrics abbreviations as in Table 2.
$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Table 4
Comparisons of correlation index values (CoI) and Cumulative_R^2 between training and testing data sets for metrics or phytoplankton index of biotic integrity (P-IBI).

Metrics or P-IBI	CoI		Cumulative_R^2	
	Training	Testing	Training	Testing
Chl *a*	0.036	0.065	0.870	1.316
SI_density	0.083	0.026	1.250	0.786
CyI_density	0.062	0.058	1.253	1.176
M_density	0.125	0.031	1.681	0.928
SpR	0.106	0.040	1.608	0.801
Menhinick_density	0.056	0.016	0.974	0.629
P-IBI	0.096	0.035	1.651	1.052
Other single metrics				
QI_density	0.005	0.017	0.283	0.686
QI_biomass	0.008	0.029	0.337	0.887
TIP_density	0.021	0.057	0.639	1.139
TIP_biomass	0.020	0.015	0.592	0.603
TDI_density	0.022	0.009	0.540	0.552
TDI_biomass	0.003	0.028	0.342	0.687

Note: Metrics abbreviations as in Table 2. Metrics with '_density' were calculated based on cell density, while '_biomass' based on taxa biovolumes. QI = Q index (Borics et al., 2007); TIP = trophic index of potamoplankton (Mischke and Behrendt, 2007); TDI = trophic diatom index (Kelly and Whitton, 1995).

In our study, nonparametric Spearman rank correlation tests and Kolmogorov–Smirnov tests were conducted by SPSS 11.5. CCA were carried out by CANOCO (Version 4.5).

3. Results

3.1. Metric selection and training the phytoplankton index of biotic integrity (P-IBI)

The final selected 11 environmental variables (Table 1; Appendix) in the training data set reflected the water quality and habitat gradients and showed a wide range of values. For example, pH ranged from 6.76 to 9.95 (mean: 7.83), NH_4–N ranged from 0.02 to 8.48 mg/L (mean: 1.07 mg/L), NO_2–N ranged from 0 to 0.30 mg/L (mean: 0.07 mg/L), DIN ranged from 0.02 to 14.50 mg/L (mean: 5.68 mg/L), and TP ranged from 0.04 to 1.30 mg/L (mean: 0.42 mg/L). WT averaged 10.39 °C (1.10–21.50 °C), mean TSS was 9.82 mg/L (1.53–37.33 mg/L), mean Si was 0.24 mg/L (0–0.38 mg/L), Cl^- averaged 33.34 mg/L (17.81–71.93 mg/L). Flow velocity ranged from 0 to 0.60 m/s with an average of 0.18 m/s, and stream width ranged from 0.9 to 4.4 m with a mean value of 2.2 m.

Preliminary evaluation of the 36 original metrics indicated weak relationships with environmental variables in the study area. The P-IBI was built utilizing six metrics based on higher CoI values and Cumulative_R^2, and they were as follows: Chl *a*, saprobity index (SI_density), Cyanobacteria-Index (CyI_density), Margalef's diversity index (M_density) and species richness (SpR). We normalized the scoring criteria of each metric based on a five-level scaling system (Table 2), so the final P-IBI scores could be calculated. These metrics represented different aspects of phytoplankton assemblages and were significantly correlated with numerous environmental variables. The final P-IBI were strongly correlated with channel width ($r_s = 0.293$, $P < 0.05$), mean velocity ($r_s = 0.515$, $P < 0.001$), pH ($r_s = -0.479$, $P < 0.001$), WT ($r_s = -0.573$, $P < 0.001$), DIN ($r_s = 0.351$, $P < 0.01$), TP ($r_s = -0.284$, $P < 0.05$) and Cl^- ($r_s = -0.624$, $P < 0.001$). The six metrics were also highly correlated with the similar environmental variables as the P-IBI (Table 3).

3.2. Testing the P-IBI and comparison with other single metrics

Cumulative_R^2 and CoI values in the testing data set decreased compared with values in the training data set (except for Chl *a*; Table 4). However, with respect to the other single metrics evaluated in our study (QI_density, QI_biomass, TDI_density, TDI_biomass, TIP_density and TIP_biomass), except for TIP_density of the testing samples, P-IBI provided higher correlation with environmental variables in terms of Cumulative_R^2 and CoI at both training and testing data sets (Table 4).

Among the 11 environmental variables used in the final CCA (Appendix), seven parameters (Width, Si, NO_2–N, pH, DIN, WT and Cl^-) explained 42.3% of the variation of species data with first four axes being significant ($P < 0.05$, 999 Monte Carlo permutations). There was no problem with multicollinearity, since variable inflation factors were all <5. The first CCA axis was associated with width, DIN and Cl^- and represented 18.0% variance of species data. CCA axis 2 explained 13.2% of the variation indicated by pH, WT and Si (Fig. 2). All the six metrics and P-IBI, except for CyI_density and

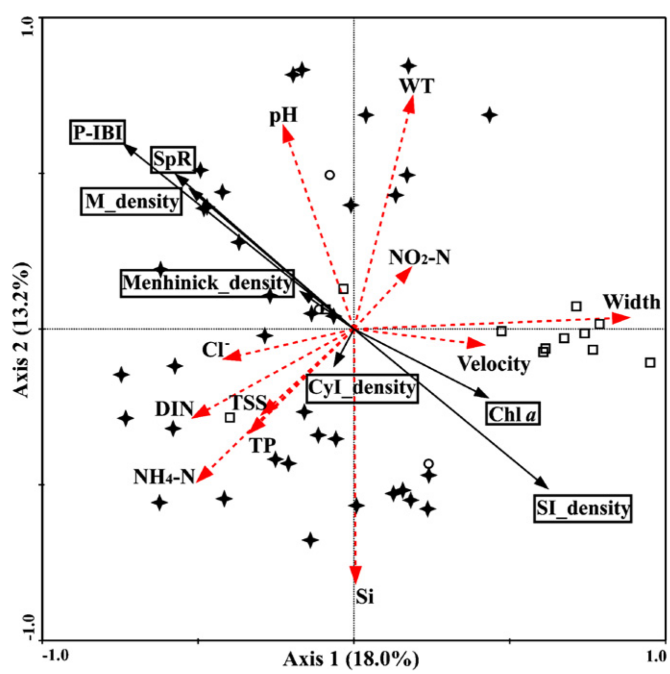

Fig. 2. Canonical correspondence analysis of the 51 samples in the testing data set showed relationships among species assemblages, environmental variables and phytoplankton index of biotic integrity (P-IBI) metrics. Letters in boxes are metrics and P-IBI as passive variables in the analysis. Solid arrows are metrics and P-IBI, while dashed arrows are environmental variables. Circles are sites scored as good by the final P-IBI scores, stars = moderate, squares = low. Metrics with '_density' were calculated based on cell density. M = Margalef's diversity index; SpR = species richness; SI = saprobity index; CyI = Cyanobacteria-Index; Menhinick = Menhinick diversity index.

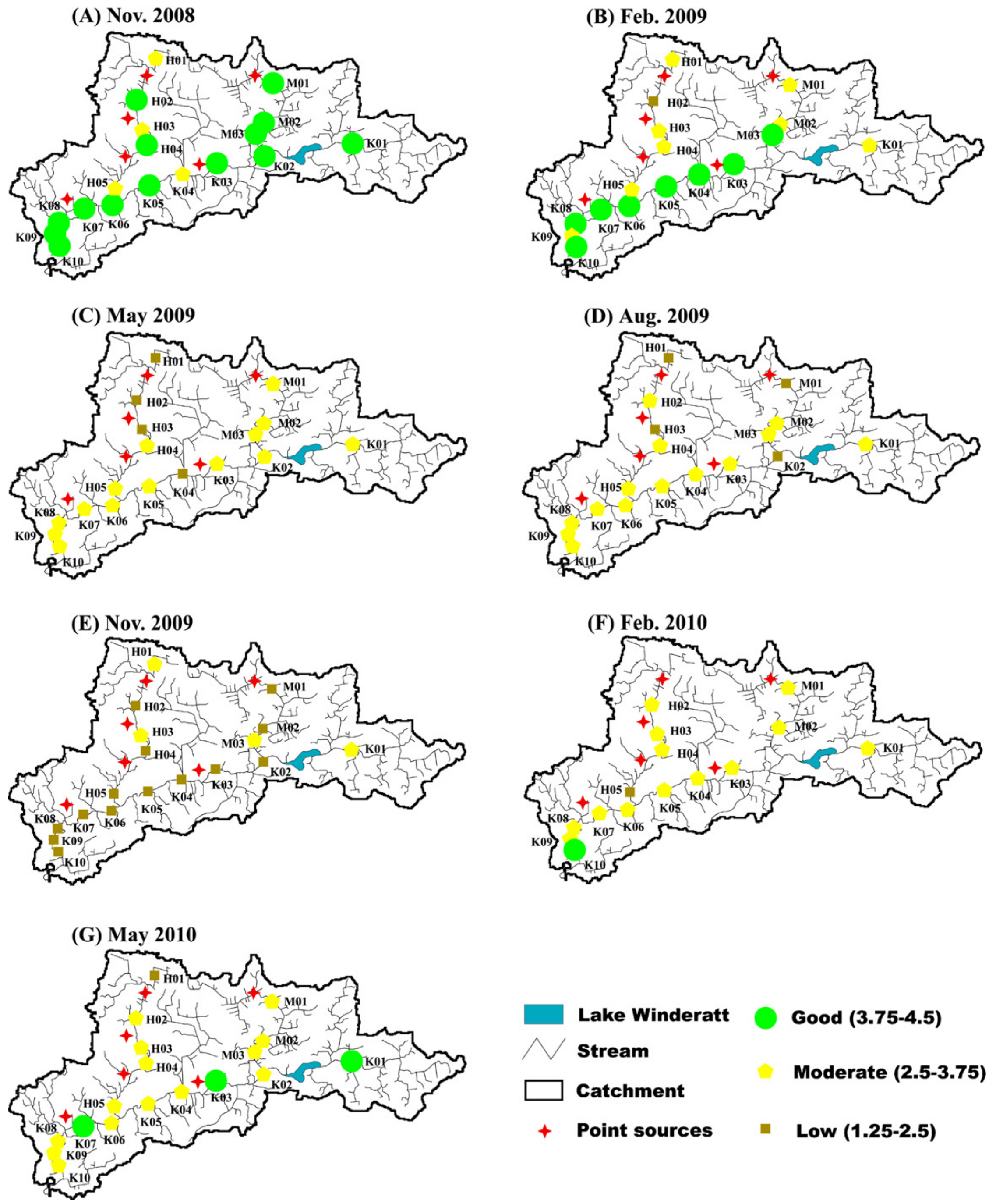

Fig. 3. The spatial distribution of the final phytoplankton index of biotic integrity (P-IBI) scores of different samples in the training and testing data sets from (A) November 2008 to (G) May 2010. Stars are point sources as shown in Fig. 1. The thresholds of different ecological status are indicated by different shapes and larger symbols indicate better ecological status. The legends of "high" and "bad" are absent because no sites fell into these categories.

Menhinick_density, were strongly correlated with the first two CCA axes. Chl *a* and SI_density increased with more positive CCA axis 1 indicative of lower water quality where samples with 'low' status were located (Fig. 2). M_density, SpR and P-IBI vectors, correlated negatively with axis 1 and positively with axis 2, were in left of the ordination which was mostly comprised of 'good' and 'moderate' samples (Fig. 2).

Generally, the final P-IBI and its six metrics were indicative of the ecological status, as indicated by CCA results (Fig. 2) and comparisons with other single metrics (Table 4), and could be used for bioassessment of the study region, although Cumulative_R^2 and CoI values declined in the testing data set.

3.3. Assessing results of the study area

The final P-IBI scores showed a wide range of values from 1.50 to 4.50 (5.0 max) with an average value of 3.17 ('Moderate' status) (Appendix), and varied in different sampling seasons (Fig. 3). Overall, most (63.9%) of the samples were in 'Moderate' status, 14.8% in 'Low' condition, 21.3% in 'Good' condition and no 'Bad' or 'High' samples. Except for the 'Good' status of samples collected from November 2008 and February 2009 (Fig. 3A and B), most samples from four times (May 2009, August 2009, February 2010 and May 2010) were in 'Moderate' status (Fig. 3C, D, F and G). Most samples collected in November 2009 were only in 'Low' status (Fig. 3E).

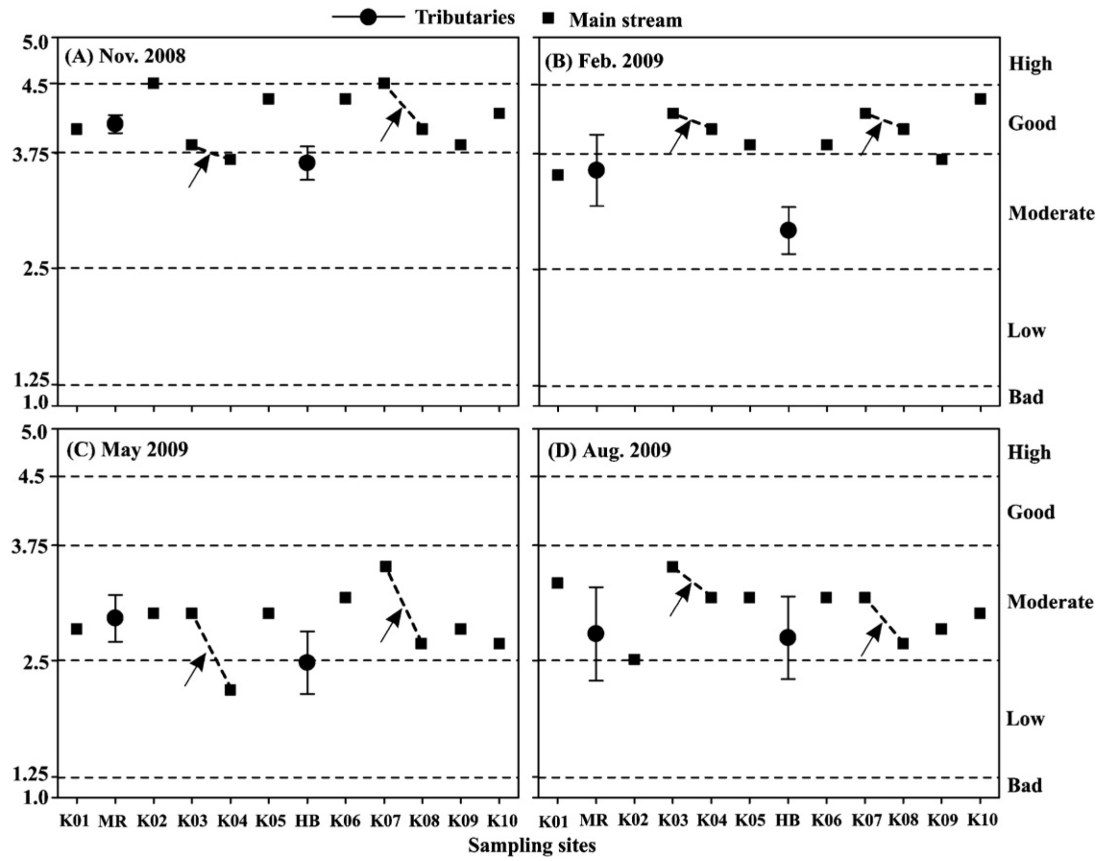

Fig. 4. The final phytoplankton index of biotic integrity (P-IBI) scores of different samples in the training data set from (A) November 2008 to (D) August 2009. Black arrows indicated point sources of Ausacker (between K03 and K04) and Freienwill (between K07 and K08). Mean (±SD) scores of the tributaries were used and the thresholds of different ecological status were listed on the right.

The ecological status of the two tributaries (MR and HB) (except for 'Good' status in November 2008 and February 2009) located always in 'Moderate' or 'Low' status demonstrating the worse water quality (Figs. 3–5). In addition, relative low influences of tributaries were found on the P-IBI scores of the main stream (Figs. 4 and 5), which probably was due to the much smaller discharges of the two tributaries (MR: $\sim$0.08 m^3/s, calculated from M03; HB: $\sim$0.06 m^3/s, calculated from H05) than that of the main stream (0.43 m^3/s, Lam et al., 2010). Furthermore, the P-IBI showed high sensitivity to water quality impairment caused by point source emissions and decreased dramatically after inputs of wastewater treatment plants (K04 and K08) during the seven sampling dates by comparing with upstream corresponding sites (K03 and K07, respectively) (Figs. 4 and 5).

4. Discussion

4.1. Development and testing of the P-IBI

Historically, it was believed that there was no true riverine plankton and the algae found in rivers were believed to come from either upstream lentic water bodies or the benthos (Hötzel and Croome, 1999). However, Centis et al. (2010) argued that benthic diatom communities as the source of the riverine phytoplankton may be too simplistic, because some species are not necessarily restricted to either habitat. The previous study (Wu et al., 2011) has confirmed that planktonic algal species did reproduce within rivers and many species developed substantial populations *in situ*. Consequently the riverine phytoplankton based water quality assessment should not be ignored and probably is a perspective in the European Water Framework Directive (WFD).

The assessment of the ecological status of freshwater ecosystems is a key issue for WFD (Hermoso et al., 2010). A multi-metric approach – the index of biotic integrity (IBI), originally developed by Karr (1981), has become the most common indicator of stream condition in use today. And many assessment methods based on IBI have been developed to date in several countries and regions for different impairments (e.g., Hill et al., 2003; Wang et al., 2005; Tang et al., 2006; Zhu and Chang, 2008; Bae et al., 2010; Zalack et al., 2010). Reference sites were a critical element of those IBIs to assess the quality or health of the aquatic ecosystem (Karr, 1981; Zhu and Chang, 2008). However, due to the specific properties of lowland rivers, such as low hydraulic gradients, high potential for water retention (Schmalz et al., 2008b; Schmalz and Fohrer, 2010) and relatively larger population within the watershed, reference sites were normally impossible to find, which was apparently different from the mountain streams. In such situation, we used correlations with environmental variables to select candidate metrics for the phytoplankton index of biotic integrity (P-IBI). Similar selection methods have been employed in lacustrine wetlands (Rothrock et al., 2008), lake assessment (Kane et al., 2009) and acid mine drainage (AMD) impacted streams (Zalack et al., 2010). Our study was an important trial for the development of IBI in a catchment without reference sites, but the P-IBI was very responsive and sensitive to water quality impairment as indicated by the testing results (Fig. 2, Table 4).

Multimetric indices have been criticized because they reduce data into a single number, Gerritsen (1995), however, argued that data simplification is the goal of a multimetric index, and its this feature that allows to be used by resource managers who may not be expert in stream ecology. The current study supported the conclusions of Gerritsen (1995) and many other authors (Lydy

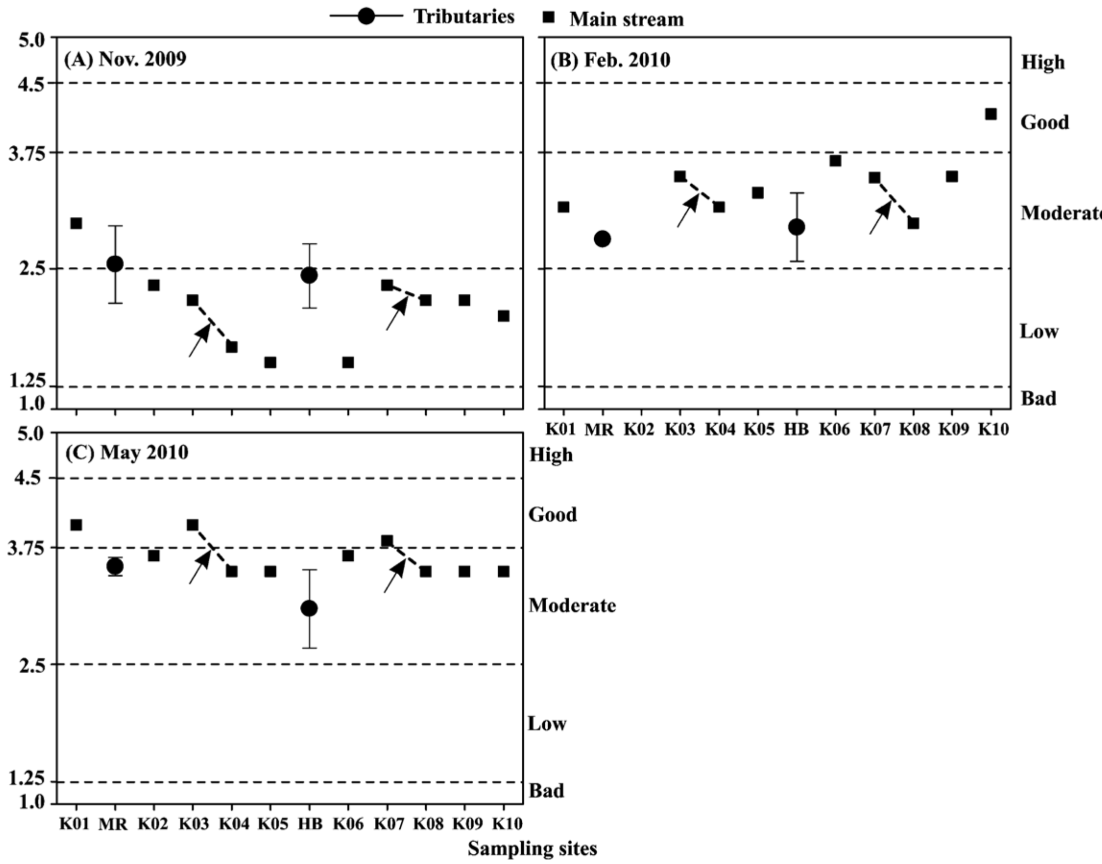

Fig. 5. The final phytoplankton index of biotic integrity (P-IBI) scores of different samples in the testing data set from (A) November 2009 to (C) May 2010. Black arrows indicated point sources of Ausacker (between K03 and K04) and Freienwill (between K07 and K08). Mean (±SD) scores of the tributaries were used and the thresholds of different ecological status were listed on the right.

et al., 2000; Triest et al., 2001; Blanco et al., 2007), showing that the use of a combination of metrics and analytical tools in the analysis of biological data would ensure the reliable assessment of water quality. The advantages of using a multimetric system over a univariate assessment include: (1) different responses to multi-stressors occurring within the region of interest since metrics represent various taxonomic and functional groups within the assemblages (biotic integrity) (Zalack et al., 2010), (2) the transferability of multi-metrics among habitats both within and among regions (Barbour et al., 1999), and (3) compensation for erroneous responses of a few metrics and incorporation of metrics related to multiple ecological attributes that are valued by decision makers (Wang et al., 2005). Overall, our developed P-IBI effectively signaled water quality impairments of the study area, and had higher correlations with environmental variables compared to single metrics such as Q index (QI), trophic index of potamoplankton (TIP), and trophic diatom index (TDI) in both training and testing data sets (Table 4). Besides, the P-IBI showed high sensitivity to water quality impairment caused by point source emissions and its scores decreased obviously after Ausacker (at site K04) and Freienwill (at site K08) (Fig. 1C, Figs. 3–5).

As an evaluation it is worth mentioning the weaknesses of the P-IBI and further applications to other environments. First, high correlations among metrics of the P-IBI both in training and testing data sets were found, which were caused probably by the dominance of a single stressor of water pollution (nitrogen, Schmalz et al., 2008a; Lam et al., 2010) in the study region. Similar high correlations were found by Wang et al. (2005). Second, the sensitivity of the P-IBI and its metrics to discriminate water quality can be further enhanced by expanding to a larger catchment, since we only used seasonal data instead of a large study area. Third, many of the metrics would be useful in other regions, but need further testing and assessment of their applicability. Besides, the compositive metrics for a concrete IBI approach may be different among impairment types and study regions, which depends highly on anthropogenic stressors. For example, saprobity index (SI) was mainly designed for organic pollution of rivers (Dokulil, 2003), while TDI and TIP for eutrophication (Kelly and Whitton, 1995; Mischke and Behrendt, 2007); Chl *a* metric was widely used to assess nutrient enrichment of streams (Hill et al., 2000). Anyway, the developed index should supply a quick assessment of the overall condition of a stream, and the individual metrics should provide insight into the causes of impairment (Hill et al., 2003). Fourth, the implication of the plankton net with a mesh size of 20 μm inevitably results in the loss of some species smaller than 20 μm (or in filament) and may have important consequences for the present results. However, our previous study (unpublished data) indicated that this loss was within the acceptable range and in addition plankton net protocol was a better method compared with sedimentation protocol from the phytoplankton-based bioassessment point of view. Lastly, it is suggested to develop a more comprehensive IBI including all kinds of possible metrics for assessing water quality impairment (e.g., fish, macroinvertebrate, periphyton and zooplankton).

4.2. Assessing results of the study area

The European Water Framework Directive (WFD) establishes a framework for the protection of all waters including inland surface waters, transitional waters, coastal waters and groundwater and in particular assumes the development of a five-level water quality classification scheme (High, Good, Moderate, Poor and Bad) with the environmental objective to achieve "good" ecological water status for all European waters by 2015 (EC, 2000). Our results (Figs. 3–5), however, indicated that the ecological status of most samples were 'Moderate' in the study area. Possible reasons of the relative lower ecological status were severe human interferences including point source emissions, diffuse sources (mainly agricultural practices such as fertilizer, pesticides utilization) and artificial drainage systems.

Both six wastewater treatment plants built within the Kielstau watershed (main stream: Ausacker and Freienwill; Moorau:Husby; Hennebach:Hürup Nord; Hürup Weseby and Hürup Süd) and point source emissions from the six villages (Fig. 1C) resulted in a high mean NH_4–N of 1.18 mg/L, and caused a dramatical decline of water quality as indicated by the final P-IBI scores in the main stream and two tributaries (Figs. 3–5). Besides, agricultural practices, which were the dominant contributor of diffuse sources in the study area (Lam et al., 2010), have strong influences on nutrient loads and water quality, leading to the high dissolved inorganic nitrogen (DIN) and total phosphorus (TP) concentrations (averaged 6.6 mg/L and 0.35 mg/L respectively). Furthermore, the drainage fraction of agricultural area in the Kielstau catchment was estimated at 38% (Fohrer et al., 2007), which have been proved to have great impacts on nutrient concentrations by faster transportation (Evans et al., 1995; Riley et al., 2009). The combination of these diffuse sources, point sources and artificial drainage systems influenced instream water quality considerably, and thus induced lower ecological status.

Another important finding of this study was that we found the ecological status of rivers varied seasonally. Several factors may play important and potential confounding roles governing such phenomenon. One possible reason was the seasonal fertilizer applications for different crops in the Kielstau catchment (Lam et al., 2011). Besides, seasonal rainfall can also cause negative or positive impacts on stream nutrients by either flushing the arable lands or diluting stream water. Moreover, habitat shifts caused by seasonal hydraulic changes may also influence the phytoplankton community remarkably. For example, hydrological variables (channel width and flow velocity, indicated by CCA) were supposed to have the same importance as major nutrients controlling the structure of riverine phytoplankton assemblages (Wu et al., 2011). This may be due to the important recruitment functions of inshore retention zone (Schiemer et al., 2001), which was primarily determined by hydrological variables. Furthermore, seasonal differences of environmental variables like water temperature (WT) and major nutrients (Wu et al., 2011) were another possible reason. Nevertheless, contributions of different factors to the seasonal variations were still unclear. Regardless of the reasons, one of the implications for bioassessment is that normal annual or 1-time sampling data should be improved to address seasonal variations.

Our results indicated that the general ecological status of the Kielstau catchment was 'Moderate' regardless of seasonal variations, which was lower than the requirement ('Good' status) of WFD by 2015. The present study demonstrated the need of further river basin management about point sources, diffuse sources as well as artificial drainage systems in order to gain a better water quality in the Kielstau catchment. Besides, due to the well performance, the constructed phytoplankton index of biotic integrity (P-IBI) could be a useful tool to measure the long-term status of streams and the effectiveness of various watershed managements.

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Appendix A.

Supplementary data (Appendix) associated with this article can be found online: www.hydrology.uni-kiel.de/download/files/wu_appendix.xls.

References

APHA, 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, New York.
Bae, D., Kumar, H., Han, J., Kim, J., Kim, K., Kwon, Y., An, K., 2010. Integrative ecological health assessment of an acid mine stream and in situ pilot tests for wastewater treatments. Ecological Engineering 36, 653–663.
Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols For Use In Wadeable Streams And Rivers. EPA 841-B-99-002. Office of Water, US Environmental Protection Agency, Washington DC.
Blanco, S., Bécares, E., Cauchie, H., Hoffmann, L., Ector, L., 2007. Comparison of biotic indices for water quality diagnosis in the Duero Basin (Spain). Archiv für Hydrobiologie (Supplement) 161, 267–286.
Borics, G., Várbíró, G., Grigorszky, I., Krasznai, E., Szabó, S., Kiss, K., 2007. A new evaluation technique of potamo-plankton for the assessment of the ecological status of rivers. Archiv für Hydrobiologie (Supplement) 161, 465–486.
Cabecinha, E., Cortes, R., Cabral, J., Ferreira, T., Lourenço, M., Pardal, M., 2009. Multiscale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs. Ecological Indicators 9, 240–255.
Camargo, J., 2008. Revisiting the relation between species diversity and information theory. Acta Biotheoretica 56, 275–283.
CDC. 2010. Centers for Disease Control and Prevention. http://www.cdc.gov/epiinfo/europe.htm (downloaded 10 October 2010).
Centis, B., Tolotti, M., Salmaso, N., 2010. Structure of the diatom community of the River Adige (North-Eastern Italy) along a hydrological gradient. Hydrobiologia 639, 37–42.
Dokulil, M., 2003. Algae as ecological bio-indicators. In: Markert, B.A., Breure, A.M., Zechmeister, H.G. (Eds.), Trace Metals and other Contaminants in the Environment. Elsevier, pp. 285–327, Chapter 9.
Domingues, R., Galvão, H., 2007. Phytoplankton and environmental variability in a dam regulated temperate estuary. Hydrobiologia 586, 117–134.
DWD. 2010. Mean values of the precipitation and temperature for the period 1961–1990. www.dwd.de (last accessed 18 June 2010).
EC, 2000. European Commission Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of European Communications, Brussels, L327.
Evans, R., Skaggs, R., Gilliam, J., 1995. Controlled versus conventional drainage effects on water quality. Journal of Irrigation and Drainage Engineering – ASCE 121, 271–276.
Fohrer, N., Schmalz, B., Tavares, F., Golon, J., 2007. Ansätze zur integration von landwirtschaftlichen drainagen in die modellierung des landschaftswasserhaushalts von mesoskaligen tieflandeinzugsgebieten. Hydrologie and Wasserbewirtschaftung 51, 164–169.
Gerritsen, J., 1995. Additive biological indices for resource management. Journal of the North American Benthological Society 14, 451–457.
Hermoso, V., Clavero, M., Blanco-Garrido, F., Prenda, J., 2010. Assessing the ecological status in species-poor systems: a fish-based index for Mediterranean Rivers (Guadiana River, SW Spain). Ecological Indicators 10, 1152–1161.
Hill, B.H., Herlihy, A.T., Kaufmann, P.R., Stevenson, R.J., McCormick, F.H., Johnson, C.B., 2000. Use of periphyton assemblage data as an index of biotic integrity. Journal of the North American Benthological Society 19, 50–67.
Hill, B.H., Herlihy, A.T., Kaufmann, P.R., DeCelles, S.J., Borgh, M.A.V., 2003. Assessment of streams of the eastern United States using a periphyton index of biotic integrity. Ecological Indicators 2, 325–338.
Hillebrand, H., Dürselen, C., Kirschtel, D., Pollingher, U.T.Z., 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35, 403–424.
Hötzel, G., Croome, R., 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.
Kane, D., Gordon, S., Munawar, M., Charlton, M., Culver, D., 2009. The planktonic index of biotic integrity (P-IBI): an approach for assessing lake ecosystem health. Ecological Indicators 9, 1234–1247.
Karr, J., 1981. Assessment of biotic integrity using fish communities. Fisheries 6, 21–27.
Kelly, M., Whitton, B., 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. Journal of Applied Phycology 7, 433–444.
Kerans, B., Karr, J., 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications 4, 768–785.
Lacouture, R., Johnson, J., Buchanan, C., Marshall, H., 2006. Phytoplankton index of biotic integrity for Chesapeake Bay and its tidal tributaries. Estuaries and Coasts 29, 598–616.

Lam, Q., Schmalz, B., Fohrer, N., 2010. Modelling point and diffuse source pollution of nitrate in a rural lowland catchment using the SWAT model. Agricultural Water Management 97, 317–325.
Lam, Q., Schmalz, B., Fohrer, N., 2011. The impact of agricultural best management practices on water quality in a North German lowland catchment. Environmental Monitoring and Assessment, doi:10.1007/s10661-011-1926-9.
Lepš, J., Šmilauer, P., 2003. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, New York.
Ludwig, A.J., Reynolds, J.F., 1988. Statistical Ecology: A Primer on Methods and Computing. John Wiley and Sons, New York.
Lydy, M., Crawford, C., Frey, J., 2000. A comparison of selected diversity, similarity, and biotic indices for detecting changes in benthic-invertebrate community structure and stream quality. Archives of Environmental Contamination and Toxicology 39, 469–479.
Margalef, R., 1958. Information theory in ecology. General Systems 3, 36–71.
Mattsson, B., Cooper, R., 2006. Louisiana waterthrushes (*Seiurus motacilla*) and habitat assessments as cost-effective indicators of instream biotic integrity. Freshwater Biology 51, 1941–1958.
Menhinick, E., 1964. A comparison of some species-individuals diversity indices applied to samples of field insects. Ecology 45, 859–861.
Mischke, U., Behrendt, H., 2007. Handbuch zum Bewertungsverfahren von Fließgewässern mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie in Deutschland. WeißenseeVerlag, Berlin, ISBN 978-3-89998-105-6, 88 (in German).
Paasche, E., Ostergren, I., 1980. The annual cycle of plankton diatom growth and silica production in the inner Oslofjord. Limnology and Oceanography 25, 481–494.
Plenković-Moraj, A., Gligora, M., Kralj, K., Mustafić, P., 2007. Diatoms in monitoring of Drava River, Croatia. Archiv für Hydrobiologie (Supplement) 161, 511–525.
Porter, S.D., 2008. Algal attributes: an autecological classification of algal taxa collected by the National Water-Quality Assessment Program. U.S. Geological Survey Data Series 329, http://pubs.usgs.gov/ds/ds329/ (accessed October 2010).
Prygiel, J., Coste, M., 1993. The assessement of water quality in the Artois-Picardie water basin (France) by use of diatom indices. Hydrobiologia 269/270, 343–349.
Reavie, E., Jicha, T., Angradi, T., Bolgrien, D., Hill, B., 2010. Algal assemblages for large river monitoring: comparison among biovolume, absolute and relative abundance metrics. Ecological Indicators 10, 167–177.
Riley, K., Helmers, M., Lawlor, P., Singh, R., 2009. Water balance investigation of drainage water management in non-weighing lysimeters. Applied Engineering in Agriculture 25, 507–514.
Rothrock, P., Simon, T., Stewart, P., 2008. Development, calibration, and validation of a littoral zone plant index of biotic integrity (PIBI) for lacustrine wetlands. Ecological Indicators 8, 79–88.
Sabater, S., Artigas, J., Duran, C., Pardos, M., Romani, A., Tornes, E., Ylla, I., 2008. Longitudinal development of chlorophyll and phytoplankton assemblages in a regulated large river (the Ebro River). Science of Total Environment 404, 196–206.
Schiemer, F., Keckeis, H., Reckendorfer, W., Winkler, G., 2001. The "inshore retention concept" and its significance for large rivers. Archiv für Hydrobiologie (Supplement) 135, 509–516.
Schmalz, B., Fohrer, N., 2010. Ecohydrological research in the German lowland catchment Kielstau. IAHS Publications 336, 115–120.
Schmalz, B., Bieger, K., Fohrer, N., 2008a. A method to assess instream water quality – the role of nitrogen entries in a North German rural lowland catchment. Advances in Geosciences 18, 37–41.
Schmalz, B., Tavares, F., Fohrer, N., 2008b. Modelling hydrological processes in mesoscale lowland river basins with SWAT – capabilities and challenges. Hydrological Sciences Journal 53, 989–1000.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, Illinois.
Spatharis, S., Tsirtsis, G., 2010. Ecological quality scales based on phytoplankton for the implementation of Water Framework Directive in the Eastern Mediterranean. Ecological Indicators 10, 840–847.
Tang, T., Cai, Q., Liu, J., 2006. Using epilithic diatom communities to assess ecological condition of Xiangxi River system. Environmental Monitoring and Assessment 112, 347–361.
Triest, L., Kaur, P., Heylen, S., De Pauw, N., 2001. Comparative monitoring of diatoms, macroinvertebrates and macrophytes in the Woluwe River (Brussels, Belgium). Aquatic Ecology 35, 183–194.
U.S. Environmental Protection Agency (U.S. EPA), 1997. Lake Michigan Mass Balance Methods Compendium (volume 3): LMMB 065 (ESS Method 340.2). Wisconsin State Lab of Hygiene, Madison, http://www.epa.gov/greatlakes/lmmb/methods/methd340.pdf.
van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology 28, 117–133.
Wang, Y., Stevenson, R., Metzmeier, L., 2005. Development and evaluation of a diatom-based index of biotic integrity for the Interior Plateau Ecoregion, USA. Journal of the North American Benthological Society 24, 990–1008.
Winter, J., Duthie, H., 2000. Epilithic diatoms as indicators of stream total N and total P concentration. Journal of the North American Benthological Society 19, 32–49.
Wu, N., Schmalz, B., Fohrer, N., 2011. Distribution of phytoplankton in a German lowland river in relation to environmental factors. Journal of Plankton Research 33, 807–820.
Zalack, J., Smucker, N., Vis, M., 2010. Development of a diatom index of biotic integrity for acid mine drainage impacted streams. Ecological Indicators 10, 287–295.
Zhu, D., Chang, J., 2008. Annual variations of biotic integrity in the upper Yangtze River using an adapted index of biotic integrity (IBI). Ecological Indicators 8, 564–572.