

**Development and testing of a phytoplankton  
index of biotic integrity (P-IBI) to assess the  
ecological conditions of German lowland rivers**

Dissertation

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## Summary

This dissertation developed and tested a phytoplankton index of biotic integrity (P-IBI) to assess the ecological conditions of German lowland rivers, and simulated the daily phytoplankton biomass (as indicated by chlorophyll *a*) dynamics for the purpose of water quality forecasting and watershed management. Prior to that, this study determined whether phytoplankton assemblages generated by the two sampling protocols were related to different environmental variables and therefore might yield different bioassessment, and investigated the relationships between phytoplankton and environmental variables. The study catchment of Kielstau catchment (50 km<sup>2</sup>) is located in the North German lowlands. It is characterized by low hydraulic gradients, shallow groundwater, and flat topography. Sandy, loamy and peat soils are characteristic for this catchment. The water quality is not only influenced by the predominating agricultural land use in the catchment as cropland and pasture, but also by municipal wastewater treatment plants and drainage systems.

At first, phytoplankton assemblages generated by two sampling protocols in the Kielstau catchment were compared to select a better sampling protocol for phytoplankton based bioassessment. Plankton net (PLNET) protocol was a better method compared with sedimentation (SEDIM) protocol because algal data collected by PLNET protocol had higher relationship with environmental variables. Then the spatial and temporal dynamics of phytoplankton community and its relationship with environmental variables were investigated. Phytoplankton community in the Kielstau catchment was a typical riverine diatom-dominated community and prevailed by species of *Achnanthes*, *Cocconeis*, *Cyclotella*, *Fragilaria*, *Navicula* and *Tabellaria*. Remarkable spatial and temporal variations of phytoplankton community were observed and 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.

To assess the ecological conditions of German lowland rivers, a phytoplankton index of biotic integrity (P-IBI) was developed and tested in the Kielstau catchment. It consisted of six metrics (out of 36 original metrics) based on Cumulative\_R<sup>2</sup> and correlation index (CoI) between biotic metrics and environmental variables. The developed P-IBI and its six metrics were indicative for ecological integrity and water quality and it could be a useful tool to measure the long-term status of streams and the effectiveness of various watershed managements. By implementing the developed P-IBI in the study area, the ecological status varied seasonally and the general

ecological status was 'Moderate' regardless of seasonal variations, which was lower than the requirement ('Good' status) of the Water Framework Directive (WFD) by 2015. The relative lower ecological status was probably caused by human disturbances such as point sources, diffuse sources emissions and artificial drainage systems.

Thereafter, based on a 1.5-y measured data set of chlorophyll *a* and environmental variables, two modeling approaches (artificial neural networks, ANN and multiple linear regression, MLR) were developed to simulate the daily chlorophyll *a* dynamics. In general, the developed ANN and MLR models achieved satisfactory accuracy in predicting daily dynamics of chlorophyll *a* concentrations. The predicted and the observed data matched closely by MLR model with coefficient of determination ( $R^2$ ) and Nash-Sutcliffe efficiency (NS) of 0.53 and 0.53 for calibration period and 0.63 and 0.62 for validation period. Likewise, the results of ANN model also illustrated a good agreement between observed and predicted data during calibration and validation periods, which was demonstrated by  $R^2$  and NS values (0.68 and 0.68 for calibration period, 0.55 and 0.66 for validation period). For watershed management, combined with an automatic water quality monitoring device and a telemetry system, the developed models can be used as a real-time forecasting system. The results of prediction can help to assess the future changes of ecological status and develop efficient water management policy.

## Zusammenfassung

In dieser Dissertation wurde ein Phytoplankton-Index der biotischen Integrität (P-IBI) entwickelt und erprobt, um die ökologischen Bedingungen von deutschen Tieflandflüssen zu bewerten, sowie die Dynamik der täglichen Phytoplankton-Biomasse (als Chlorophyll *a* angegeben) für eine Wasserqualitätsprognose und ein Einzugsgebietsmanagement zu simulieren. Vorher hat diese Studie geprüft, ob Phytoplanktongemeinschaften, die durch zwei Probenahmeprotokolle erstellt wurden, mit verschiedenen Umweltvariablen in Beziehung stehen und daher zu unterschiedlichen biologischen Bewertungen führen könnten, und sie untersuchte die Beziehungen zwischen Phytoplankton und Umweltvariablen. Das Untersuchungsgebiet Kielstau-Einzugsgebiet (50 km<sup>2</sup>) liegt in der Norddeutschen Tiefebene. Es wird durch geringe hydraulische Gradienten, oberflächennahes Grundwasser und flache Topographie charakterisiert. Sandige, lehmige und Torfböden sind charakteristisch für dieses Einzugsgebiet. Die Wasserqualität wird nicht nur durch die vorherrschende landwirtschaftliche Nutzung wie Acker- und Grünland in dem Einzugsgebiet, sondern auch durch kommunale Kläranlagen und Dränagesysteme beeinflusst.

Zunächst wurden Phytoplanktongemeinschaften, die von zwei Probenahmeprotokollen im Kielstau-Einzugsgebiet erstellt wurden, verglichen, um ein besseres Probenahmeprotokoll für die Phytoplankton-basierte biologische Bewertung auszuwählen. Verglichen mit dem Sedimentations (SEDIM)-Protokoll war das Planktonnetz (PLNET)-Protokoll eine bessere Methode, da die durch das PLNET-Protokoll gesammelten Algendaten eine engere Beziehung mit den Umgebungsvariablen hatten. Dann wurde die räumliche und zeitliche Dynamik von Phytoplanktongemeinschaften und ihre Beziehung zu Umweltvariablen untersucht. Die Phytoplanktongemeinschaft im Kielstau-Einzugsgebiet war eine typische Diatomeen-dominierte Gemeinschaft der Fließgewässer und vertreten durch die Arten *Achnanthes*, *Cocconeis*, *Cyclotella*, *Fragilaria*, *Navicula* und *Tabellaria*. Bemerkenswerte räumliche und zeitliche Schwankungen der Phytoplanktongemeinschaft wurden festgestellt, und die kanonische Korrespondenzanalyse (CCA) zeigte an, dass physikalische Faktoren (z.B. hydrologische Variablen) und die wichtigsten Nährstoffe (z.B. Gesamt-Phosphor, gelöster anorganischer Stickstoff) von gleicher Bedeutung waren und die Abweichung in der Struktur der Fließgewässer-Phytoplanktongemeinschaften steuert.

Zur Beurteilung der ökologischen Gegebenheiten von deutschen Tieflandflüssen wurde ein Phytoplankton-Index der biotischen Integrität (P-IBI) für des Kielstau-Einzugsgebietes entwickelt und erprobt. Er bestand aus sechs Kenngrößen (von 36 Ursprungskenngrößen) und

basierte auf  $kumulativ\_R^2$  und einem Korrelationsindex (CoI) zwischen biotischen Kenngrößen und Umweltvariablen. Der entwickelte P-IBI und seine sechs Kenngrößen gaben einen Hinweis auf die ökologische Integrität und die Wasserqualität und könnten ein nützliches Werkzeug sein, um den langfristigen Zustand von Fließgewässern und die Wirksamkeit verschiedenen Einzugsgebietsmanagements zu messen. Durch die Anwendung des entwickelten P-IBI im Untersuchungsgebiet fanden wir heraus, dass der ökologische Zustand saisonal variierte. Der generelle ökologische Zustand des Untersuchungsgebietes war "moderat", ungeachtet der saisonalen Schwankungen, und damit schlechter als die Anforderungen ("guter"-Zustand) der WRRL bis 2015. Der relativ schlechte ökologische Zustand wurde vermutlich durch Punktquellen, Emissionen diffuser Quellen und künstliche Entwässerungssysteme (Dränagen) im Untersuchungsgebiet verursacht.

Anschließend, basierend auf gemessenen Chlorophyll *a*-Daten und Umweltvariablen über eine Zeitdauer von 1,5 Jahren, wurden zwei Modellierungsansätze (künstliche neuronale Netze, ANN und multiple lineare Regression, MLR) entwickelt, um die tägliche Dynamik von Chlorophyll *a* zu simulieren. Generell erreichten die entwickelten ANN und MLR-Modelle zufriedenstellende Ergebnisse bei der Vorhersage der täglichen Dynamik der Chlorophyll *a* Konzentrationen. Die vorhergesagten und die gemessenen Werte passten beim MLR-Modell gut zusammen mit Korrelationskoeffizienten ( $R^2$ ) und Nash-Sutcliffe Indizes (NS) von 0,53 und 0,53 für den Kalibrierungszeitraum und 0,63 und 0,62 für den Validierungszeitraum. Auch die Ergebnisse des ANN-Modells zeigten eine gute Übereinstimmung zwischen gemessenen und vorhergesagten Werten für die Kalibrierungs- und Validierungszeiträume, welches durch die  $R^2$  und NS-Werte (0,68 und 0,68 für den Kalibrierungszeitraum, 0,55 und 0,66 für den Validierungszeitraum) aufgezeigt werden konnte. Für ein Einzugsgebietsmanagement, zusammen mit einem Gerät zur automatischen Überwachung der Wasserqualität und einem Telemetrie-System, können die entwickelten Modelle als Echtzeit-Prognose-System verwendet werden. Die Ergebnisse der Vorhersage können helfen, die zukünftigen Veränderungen des ökologischen Zustands zu bewerten und effiziente Wasserwirtschaftspolitik zu entwickeln.

## Table of Contents

<b>Summary .....</b>	<b>I</b>
<b>Zusammenfassung .....</b>	<b>III</b>
<b>Table of Contents .....</b>	<b>V</b>
<b>List of Figures .....</b>	<b>VII</b>
<b>List of Tables .....</b>	<b>X</b>
<b>Chapter I Introduction.....</b>	<b>1</b>
1.1 Background and literature review .....	1
1.2 Study area .....	5
1.3 Objectives and outline .....	7
<b>Chapter II A comparison of phytoplankton assemblages generated by two sampling protocols in a German lowland catchment .....</b>	<b>9</b>
Abstract.....	9
2.1 Introduction.....	9
2.2 Methods .....	11
2.3 Results.....	16
2.4 Discussion.....	24
Acknowledgements.....	26
<b>Chapter III Distribution of phytoplankton in a German lowland river in relation to environmental factors.....</b>	<b>27</b>
Abstract.....	27
3.1 Introduction.....	27
3.2 Materials and methods .....	29
3.3 Results.....	33
3.4 Discussion.....	43
Acknowledgements.....	46
<b>Chapter IV Development and testing of a phytoplankton index of biotic integrity (P-IBI) for a German lowland river .....</b>	<b>47</b>
Abstract.....	47
4.1 Introduction.....	47
4.2 Materials and methods .....	49
4.3 Results.....	54
4.4 Discussion.....	62
Acknowledgements.....	66
<b>Chapter V Modeling daily chlorophyll <i>a</i> dynamics in a German lowland river using artificial neural networks and multiple linear regression approaches .....</b>	<b>67</b>
Abstract.....	67
5.1 Introduction.....	68

## Table of Contents

---

5.2 Methods .....	69
5.3 Results.....	75
5.4 Discussion.....	79
5.5 Conclusions and outlook.....	82
Acknowledgements.....	82
<b>Chapter VI Discussion and conclusion .....</b>	<b>83</b>
6.1 Summary and key achievements.....	83
6.2 Conclusions.....	84
6.3 Outlook .....	86
<b>Bibliography.....</b>	<b>88</b>
<b>Acknowledgements .....</b>	<b>106</b>
<b>Erklärung.....</b>	<b>108</b>



## List of Figures

Figure 1.1: Long-term publication trends of phytoplankton studies searched by (A) Scopus (B) ScienceDirect (C) Springerlink (with an article title of "phytoplankton" or "potamoplankton"). Red bold lines are study trends made by polynomial fit.....	2
Figure 1.2: The proportions of riverine phytoplankton studies compared to other surveys of phytoplankton from (A) JPR (J. Plankton. Res.), (B) EI (Ecol. Indic.), (C) EM (Ecol. Model.) and (D) Total (sum of the three journals).....	3
Figure 1.3: Long-term publication trends of riverine phytoplankton studies in J. Plankton. Res. (JPR), Ecol. Indic. (EI) and Ecol. Model. (EM) and was grouped in 5-y increments. Studies were classified into primary studies, relations with abiotic factors, bioindicators and modeling. The total number of publications was also indicated for each period.....	4
Figure 1.4: Study categories of riverine phytoplankton publications in J. Plankton. Res. (JPR), Ecol. Indic. (EI) and Ecol. Model. (EM). In some cases, a study was classified as having >1 type of categories.....	5
Figure 1.5: Location of the Kielstau catchment (C) in Schleswig-Holstein (B), Germany [A, map source CDC (CDC 2010)] and point sources within the catchment .....	6
Figure 2.1: The location of the Kielstau catchment in Schleswig-Holstein state (B), Germany (A), map source CDC (CDC 2010) and the sampling sites (C). S-H = Schleswig-Holstein state; M01-M03 = sampling sites collected from Moorau tributary; H01-H05 = sampling sites from Hennebach tributary; K01-K10 = sampling sites from main stream Kielstau. ....	12
Figure 2.2: Non-metric multidimensional scaling (NMDS) ordinations of phytoplankton assemblage collected using plankton net (PLNET) and sedimentation (SEDIM) in November 2008 (A), February 2009 (B), May 2009 (C) and August 2009 (D).....	21
Figure 2.3: Relationships between values of total algal densities [Log(TAD)] (A), BeT (B), UnT (C), Ev (D), QI (E) and PeI (F) generated by plankton net (PLNET) and sedimentation (SEDIM). Solid circles are sites with the lowest BC values. Solid lines are regression lines and dashed lines indicate the 95% confidence bands .....	23
Figure 2.4: Regressions of Bray-Curtis (BC) similarity between phytoplankton assemblages generated by plankton net (PLNET) and sedimentation (SEDIM) protocols as a function of total algal density [log(TAD)] (A) and total algal biomass (TAB) (B).....	24

Figure 3.1: The location of the Kielstau catchment in Schleswig-Holstein state (B), Germany (A) and the sampling sites (C). (A) Cited from <http://www.cdc.gov/epiinfo/europe.htm>. (B) Modified from Schmalz and Fohrer (Schmalz and Fohrer 2010)..... 30

Figure 3.2: Nonmetric Multidimensional Scaling (NMDS) ordination of phytoplankton community at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study ..... 37

Figure 3.3: 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) Bioplots of the species and the environmental variables; (b) Bioplots of the sampling sites and the environmental variables. Species abbreviations were listed in Table 3.4 ..... 39

Figure 3.4: 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)..... 40

Figure 4.1: The location of the Kielstau catchment in Schleswig-Holstein state (B), Northern Germany [A; map source: CDC (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 ..... 50

Figure 4.2: 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. .... 59

Figure 4.3: 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. 4.1. 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. .... 60

Figure 4.4: 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 ( $\pm$ SD) scores of the tributaries were used and the thresholds of different ecological status were listed on the right. .... 61

Figure 4.5: 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 ( $\pm$ SD) scores of the tributaries were used and the thresholds of different ecological status were listed on the right. .... 62

Figure 5.1: The location of the Kielstau catchment (C) in Schleswig-Holstein state (B), Northern Germany (A; map source: CDC, 2010) and two photos of the Soltfeld gauging station and automatic sampler for daily chlorophyll *a* samples (D). S-H = Schleswig-Holstein state..... 71

Figure 5.2: Schematic diagram of an artificial neural network (ANN) architecture applied in this study showing input, hidden and output layers. Circles are neurons. Abbreviations (codes) of variables as in Table 5.1..... 74

Figure 5.3: Predicted and observed daily chlorophyll *a* (Chl *a*) concentrations at the Soltfeld gauging station generated by multiple linear regression (MLR) approach..... 77

Figure 5.4: Predicted and observed daily chlorophyll *a* (Chl *a*) concentrations at the Soltfeld gauging station generated by artificial neural network (ANN) approach..... 78

## List of Tables

Table 2.1 Summary of analysed algal metrics .....	15
Table 2.2 Mean ( $\pm$ SE) values of environmental variables for all sites and for sites grouped by Bray-Curtis (BC) similarity between samples collected using plankton net (PLNET) and sedimentation (SEDIM).....	17
Table 2.3 Dominant phytoplankton species collected by plankton net (PLNET) and sedimentation (SEDIM) in Kielstau catchment at 18 lotic and 2 lentic sites. Paired t-test results were also shown.....	19
Table 2.4 Median (range) of 13 algal indices for samples collected using PLNET and SEDIM for all sites and sites with low values of BC similarity between the two protocols. Coefficients of determination and paired t-test are between the two sampling protocols, and <b>bold</b> font indicates statistically significant ( $p < 0.05$ ) .....	20
Table 2.5 Comparisons of CoI values and Cumulative_R <sup>2</sup> between plankton net (PLNET) and sedimentation (SEDIM) protocols .....	22
Table 2.6 Results of canonical correspondence analyses (CCA) relating algal assemblages to environmental variables. Higher percent explained variation indicates stronger algal-environment relationships.....	22
Table 3.1 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).....	34
Table 3.2 Dominant phytoplankton species collected in four different seasons at lentic and lotic sites in the Kielstau catchment .....	35
Table 3.3 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).....	36
Table 3.4 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 <i>Staurastrum</i> sp., taxa listed the species with number of occurrence >30 (over all 77 samples). Optima are in <b>bold</b> for potential indicator species (see text) .....	41
Table 3.5 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) .....	43
Table 4.1 Environmental variables and their codes sampled at Kielstau catchment and methods used .....	51
Table 4.2 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.....	56
Table 4.3 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.....	57
Table 4.4 Comparisons of correlation index values (Col) and Cumulative_R <sup>2</sup> between training and testing data sets for metrics or phytoplankton index of biotic integrity (P-IBI) .....	58
Table 5.1 Variables and their codes and methods used in this study.....	72
Table 5.2 The statistic summary of the environmental variables and chlorophyll <i>a</i> used in this study (n=426) .....	75
Table 5.3 The statistic summary of the chlorophyll <i>a</i> (Chl <i>a</i> ) concentrations in different months .....	76
Table 5.4 Results of multiple linear regression (MLR) based Chl <i>a</i> model using the training data set ..	76

## **Chapter I Introduction**

### **1.1 Background and literature review**

Rivers are among the most threatened habitat types by human-generated pressures (Hering et al. 2006, Beyene et al. 2009) and the major drivers can be summarized as multiple use (such as fisheries, navigation and drinking water extraction), nutrient enrichment and organic pollution, acidification and alterations of hydrology and morphology (Malmqvist and Rundle 2002). Such human pressures can negatively effect the whole ecosystems, including food web simplification, community pauperization, and disappearance of economically important species or nuisance growths, which can ultimately escalate to a public health hazard (Passy 2007). Awareness of these deleterious effects of human pressures on streams has resulted in a long history of monitoring using biological indicators (e.g. Wu 1999, Wang et al. 2005, Zalack et al. 2010, Smucker and Vis 2011, Wu et al. 2012b). At the same time, not surprisingly more and more researchers have focused on developing and testing new metrics, protocols, and programs for faster and more efficient biomonitoring (e.g. Whitton et al. 1991, Whitton and Rott 1996, Barbour et al. 1999, Cao et al. 2007, Philips et al. 2007, Menezes et al. 2010, Zalack et al. 2010, Moya et al. 2011, Wu et al. 2012a).

An accurate assessing system is of great importance for management decision-making and is also essential for choosing an appropriate management strategy for the maintenance of a desired ecosystem state. The assessment of the ecological status of freshwater ecosystems has thus become a key issue for many international laws such as the Water Framework Directive (WFD) (EC 2000, Hermoso et al. 2010). Lowland rivers, characterized by specific properties, such as low flow velocity, low hydraulic gradients, shallow groundwater, flat topography and high potential for water retention in peatland and lakes (Schmalz et al. 2009, Schmalz and Fohrer 2010), are apparently different from the habitats of lakes and mountain streams. Until now, studies of phytoplankton community in lowland rivers, to our knowledge, are still scanty (Wu et al. 2011b).

Phytoplankton (mainly planktonic algae), together with benthic algae and macrophytes, constitute the autochthonous primary producers in aquatic ecosystems and form part of the basis of the food web in terms of energy and material input (Hötzel and Croome 1999). Due to their short life cycle, planktonic algae respond quickly to environmental changes and are thus a valuable indicator of water quality (Domingues and Galvão 2007, Cabecinha et al. 2009). The value of algae as bio-monitors and bio-indicators has already been recognised in the

mid 19th century: the first concept which has been developed was the system of saprobity, which was mainly designed for organic pollution of streams and rivers (Dokulil 2003). Moreover, unlike fish and macroinvertebrates, algal communities are usually present before disturbance and generally persist in some form after disturbances. Therefore, application of algal indicators is increasing (Blanco et al. 2007, Borics et al. 2007, Plenković-Moraj et al. 2007, Reavie et al. 2010). However, most of the investigations were carried out in lentic water bodies (e.g. oceans, gulfs, lakes and reservoirs), and very little attention has been paid to the application of the phytoplankton in ecological evaluation of rivers (Borics et al. 2007). To our knowledge, a multi-metric based phytoplankton index of biotic integrity (P-IBI) has been rarely considered for river 'health' assessment (Wu et al. 2012b).

In this section, by reviewing international scientific literatures, the long-term trends of phytoplankton studies from past to 2010 were described, with emphasis on riverine phytoplankton. Original papers about phytoplankton by means of ScienceDirect (<http://www.sciencedirect.com/>), Springerlink (<http://www.springerlink.com/>) and Scopus (<http://www.scopus.com/home.url>) were searched to inspect the long-term publication trends from 1961 (very few publications before 1960) to 2010. Publications with an article title of "phytoplankton" or "potamoplankton" were searched. The results showed that most of these studies were widely carried out in oceans, coastal areas, gulfs, lakes and reservoirs, and demonstrated an increasing publication trend by the three databases (Fig. 1.1).

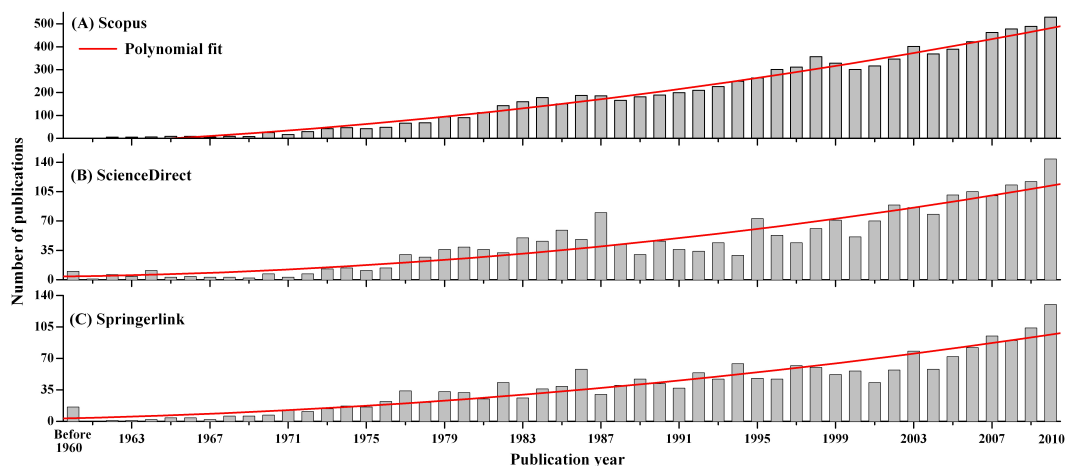


Figure 1.1: Long-term publication trends of phytoplankton studies searched by (A) Scopus (B) ScienceDirect (C) Springerlink (with an article title of "phytoplankton" or "potamoplankton"). Red bold lines are study trends made by polynomial fit

Based on the previous searching results, an additional search was conducted to estimate the proportion of riverine phytoplankton studies. Three journals were taken for example, and they were "J. Plankton. Res. (JPR)", "Ecol. Indic. (EI)" and "Ecol. Model. (EM)", respectively. For the period reviewed, a total of 696 publications (with an article title of "phytoplankton" or "potamoplankton") were examined, and only 14.9% (104 studies) of them focused on riverine phytoplankton (Fig. 1.2). The proportions of riverine phytoplankton studies compared to other surveys of phytoplankton from JPR, EI and EM were 15.9%, 8.8% and 14.9%, respectively (Fig. 1.2).

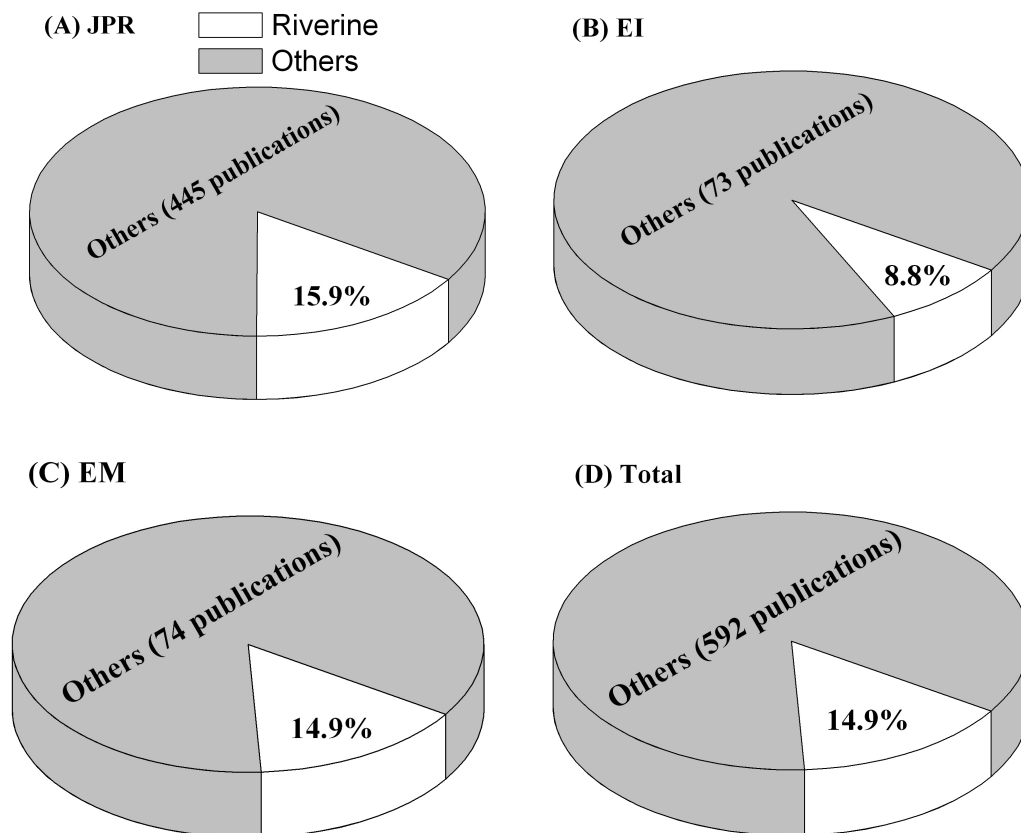


Figure 1.2: The proportions of riverine phytoplankton studies compared to other surveys of phytoplankton from (A) JPR (J. Plankton. Res.), (B) EI (Ecol. Indic.), (C) EM (Ecol. Model.) and (D) Total (sum of the three journals)



Furthermore, we classified these (104) studies into four major categories: (I) primary studies (including taxonomic composition, temporal and spatial distribution, biovolume, sampling methods, etc.), (II) relations with abiotic factors, (III) bio-indicator and (IV) modeling. We examined studies in 5-y increments (Fig. 1.3). Overall, the number of riverine phytoplankton publications increased from 5 (first 5-y, 1981-1985) to 34 (the last 5-y, 2006-2010), except for a small number of 1991-1995. Most studies of them so far, however, were primary studies, whose percentage was 70.2% (73 out of 104) (Fig. 1.4). There were only 15 publications (14.4%) studying "relations with abiotic factors", but an increasing trend was found from 1986-1990 (1 publication) to 2006-2010 (6 publications). As well as modeling studies, the utilizations of riverine phytoplankton as bio-indicator were still poorly investigated and available studies were those mainly published after the year of 2000 (Fig. 1.3, Fig. 1.4).

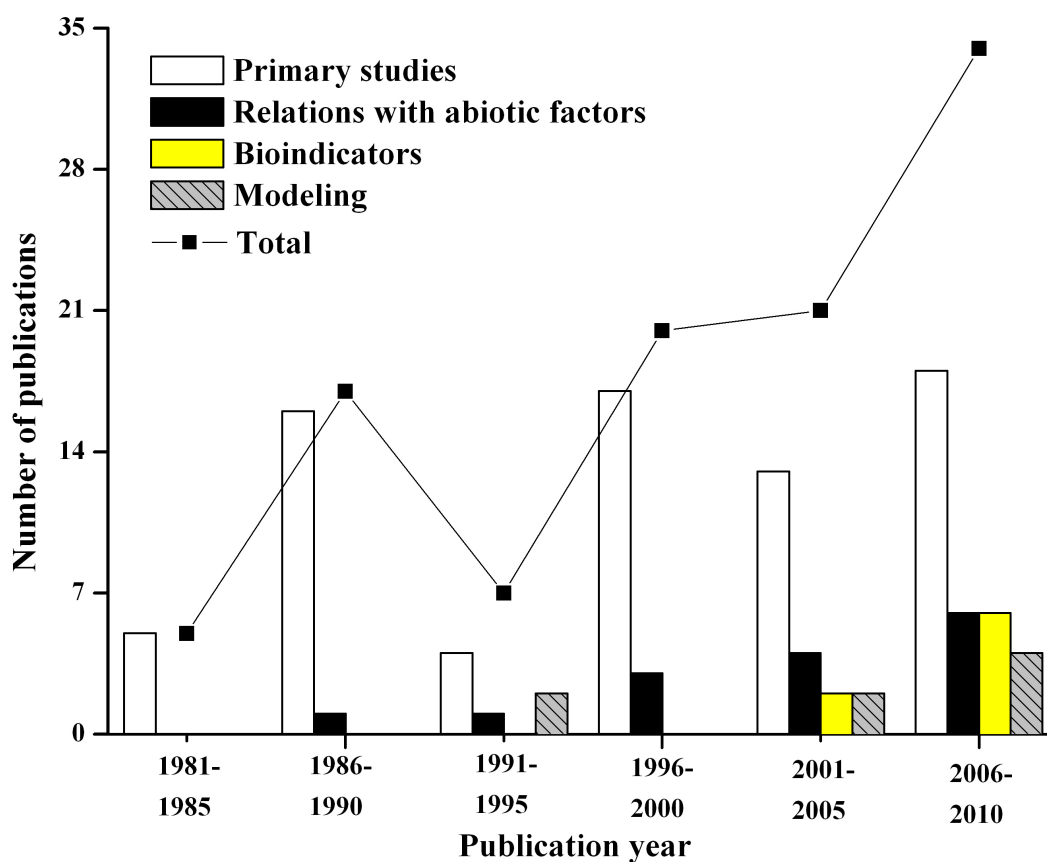


Figure 1.3: Long-term publication trends of riverine phytoplankton studies in J. Plankton. Res. (JPR), Ecol. Indic. (EI) and Ecol. Model. (EM) and was grouped in 5-y increments. Studies were classified into primary studies, relations with abiotic factors, bioindicators and modeling. The total number of publications was also indicated for each period

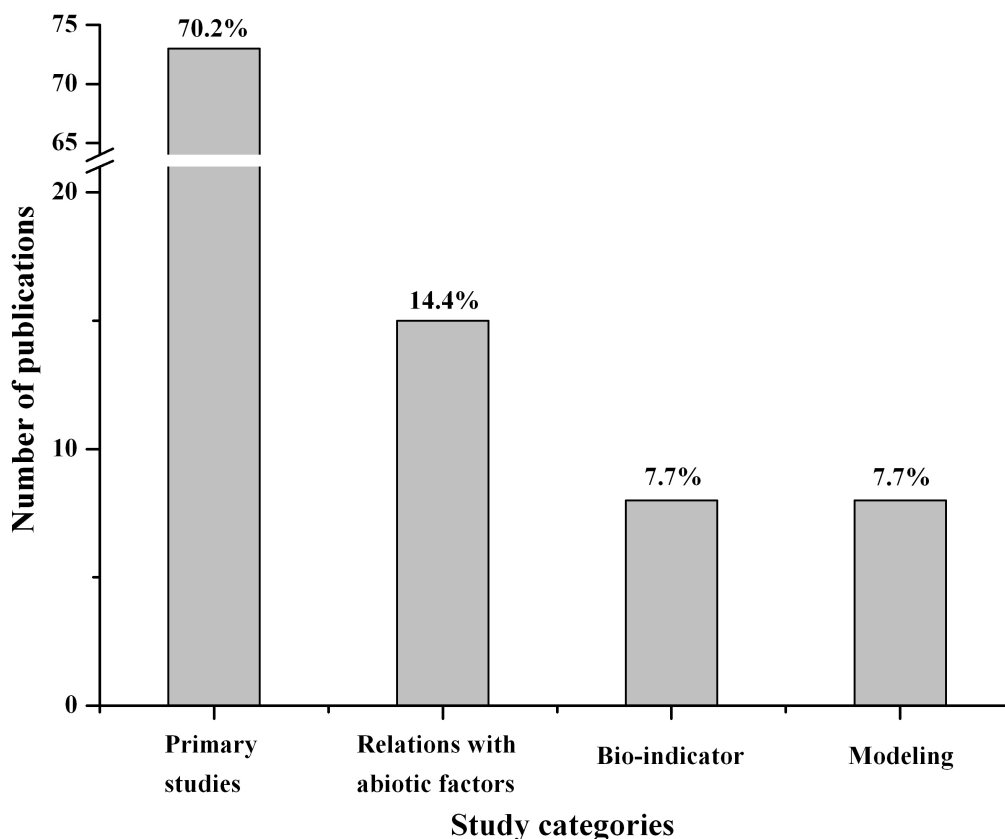


Figure 1.4: Study categories of riverine phytoplankton publications in J. Plankton. Res. (JPR), Ecol. Indic. (EI) and Ecol. Model. (EM). In some cases, a study was classified as having >1 type of categories

## 1.2 Study area

The study area Kielstau catchment, an UNESCO demosite for ecohydrology since 2010, is located in the Northern part of Germany (Schleswig-Holstein) (Fig. 1.5). With a drainage area of 50 km<sup>2</sup> and a length of 17 km, it is a part of the Treene catchment, which is the most important tributary of the Eider river (Fig. 1.5B). It has its origin in the upper part of Lake Winderatt (Fig. 1.5C). 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). 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 (~55% and 26%, respectively, of the catchment area) (Fohrer et al. 2007, Schmalz et al. 2008b).

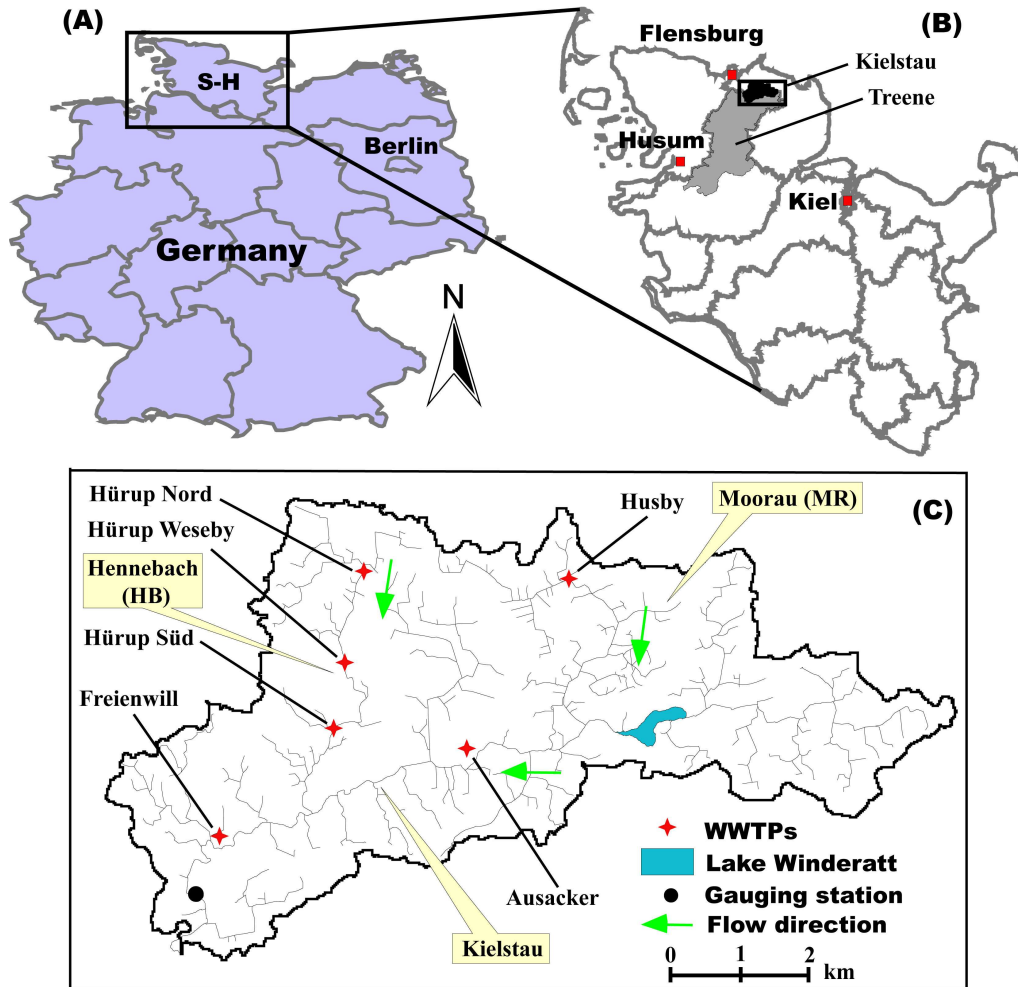


Figure 1.5: Location of the Kielstau catchment (C) in Schleswig-Holstein (B), Germany [A, map source CDC (CDC 2010)] and point sources within the catchment

There are three main human disturbances in the Kielstau catchment: point sources, artificial drainage systems and diffuse sources by agricultural practices. The combination of the three pressures have influenced instream water quality considerably (Wu et al. 2012b). Six wastewater treatment plants are located within the Kielstau watershed: Ausacker, Freienwill, Husby, Hürup Nord, Hürup Weseby and Hürup Süd (Fig. 1.5C). Ausacker and Freienwill are located on the main stream with 1880 and 350 population equivalents (PE), respectively. Husby is situated at the beginning of the Moorau tributary with 3000 PE. Hürup Nord, Hürup Weseby and Hürup Süd are located along the longitudinal Hennebach tributary (461, 447 and 240 PE, respectively). Besides, the drained fraction of agricultural area in the Kielstau catchment is estimated 38% (Fohrer et al. 2007) and various small tributaries and water from drainage pipes and ditches flow into the river Kielstau. In addition, agricultural practices, which

are the dominant contributor of diffuse sources in the Kielstau catchment (Lam et al. 2010), have strong influences on nutrient loads and water quality, leading to the high dissolved inorganic nitrogen and total phosphorus concentrations.

Previously, a lot of studies, ranging from hydrology to hydrobiology, have been carried out in this catchment by the Department of Hydrology and Water Resources Management of Kiel University (e.g. Hörmann et al. 2007, Schmalz et al. 2008a, Schmalz et al. 2008b, Kiesel et al. 2009, Liu et al. 2009, Schmalz et al. 2009, Zhang et al. 2009, Kiesel et al. 2010, Lam et al. 2010, Schmalz and Fohrer 2010, Lam 2011, Lam et al. 2011, Liu et al. 2011). Moreover, the Soltfeld gauging station, installed at the outlet of the Kielstau catchment (Fig. 1.5C), is a part of the official gauging network of the Federal State Schleswig-Holstein. Important hydrological data such as water discharge can be downloaded from this station. Close to this gauging station, there is an automatic sampler, with which daily water samples can be collected. All of these previous works and facilities provide a solid baseline for this research. The Kielstau catchment is therefore a suitable study area for the aims of this investigation.

### **1.3 Objectives and outline**

This research aims to develop and test a phytoplankton index of biotic integrity (P-IBI) that can assess effects of human disturbances on the ecological status of the lowland rivers, and to simulate the daily phytoplankton biomass (as indicated by chlorophyll *a*) dynamics for the purpose of water quality forecasting and watershed management. Prior to that, this study also aims to determine whether phytoplankton assemblages generated by the two sampling protocols are related to different environmental variables and therefore may yield different bioassessment. Also it investigates the relationships between phytoplankton and environmental variables and compares which factors predominantly structure riverine phytoplankton communities.

The dissertation is cumulatively organized as stand-alone manuscripts that are published or awaiting publication in international peer-reviewed journals. These papers are reproduced here unmodified except for cross-references.

Chapter I reviews the background of riverine phytoplankton and its use as bio-indicators in rivers, and introduces the study area of the Kielstau catchment, the statement of problems, and the summary of objectives as well as the main contents mentioned from chapters II - VI.

Chapter II compares phytoplankton assemblages generated by two sampling protocols in the

Kielstau catchment. The aim of this chapter is to determine how phytoplankton sampling protocols may influence bioassessment. Bray-Curtis (BC) and Jaccard's similarities are used to examine phytoplankton assemblages generated by the two protocols. In addition, 'Correlation Index' (CoI), Cumulative\_ $R^2$  and canonical correspondence analysis (CCA) are employed to analyze algal assemblages generated by two protocols to environmental variables, with the goal of selecting a better sampling method for the consequent studies.

Chapter III mainly investigates the spatial and temporal dynamics of phytoplankton community in the Kielstau catchment, and the relationships with environmental variables (conducted by canonical correspondence analysis, CCA). To quantify relations between algal abundances and environmental variables that strongly expressed in CCA, regression and calibration models were developed using weighted averaging regression analysis (WA). Furthermore, CCA compares the contributions of physical factors (e.g. hydrological variables) and major nutrients (e.g. total phosphorus, dissolved inorganic nitrogen) to the structure of riverine phytoplankton communities.

Chapter IV presents the development and testing of a phytoplankton index of biotic integrity (P-IBI) for a German lowland river of the Kielstau catchment to assess effects of human disturbances. The objectives of this chapter are to develop an integrated index (P-IBI) that can effectively assess the human disturbances on the ecological status of the lowland rivers; test the robustness of the P-IBI by canonical correspondence analysis (CCA) and comparing the performances with other single metrics; and deduce the ecological status of the study area by implementing the developed P-IBI.

Chapter V concentrates on the simulation of daily chlorophyll *a* dynamics at the outlet of the Kielstau catchment for the purpose of water quality forecasting and watershed management. Based on a 1.5-y measured data set of chlorophyll *a* and environmental variables, this chapter aims to develop and compare two modeling approaches (artificial neural networks, ANN and multiple linear regression, MLR) to simulate the daily chlorophyll *a* dynamics. In this chapter, the performances of the two developed models are compared and evaluated by  $R^2$  and Nash-Sutcliffe efficiency (NS).

Chapter VI composed by summary and conclusion summarizes the key findings and important results from this study. Likewise, an outlook for possible future works is also presented.

## **Chapter II A comparison of phytoplankton assemblages generated by two sampling protocols in a German lowland catchment**

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### **Abstract**

The Research in the phytoplankton community has become an important part of the overall water quality monitoring. However, to date studies in small rivers and streams were still scarce and sampling methods were also diverse and not as well-developed as in lakes or large rivers. We investigated whether two sampling protocols collected different phytoplankton assemblages within a lowland catchment and, consequently, influenced the outcome of bioassessment. Data collected from 77 sites by plankton net collection (PLNET) and sedimentation (SEDIM) protocols were analyzed. Median Bray-Curtis (BC) similarity between phytoplankton assemblages generated by the two protocols was 48.5% (range: 7.5-82.0%), and sites with the lowest BC similarities tended to have lower chlorophyll *a* (Chl *a*), water temperature (WT), total-suspended-solid (TSS) and volatile-suspended-solid (VSS), but higher channel width and water depth, than other sites with higher BC similarities. Reduced total algal density and biomass, but higher species richness, were observed by the PLNET protocol. However, overall phytoplankton assemblages generated by the two protocols were similar, as indicated by dominant species (paired t-test,  $p > 0.05$ ) and non-metric multidimensional scaling (NMDS) ordination. Nevertheless, from the phytoplankton-based bioassessment point of view, PLNET protocol was a better method compared with SEDIM protocol because algal data collected by PLNET protocol had higher relationship with environmental variables as indicated by 'Correlation Index' (CoI), Cumulative\_R<sup>2</sup> and canonical correspondence analysis (CCA).

Keywords: Algal metrics; Canonical correspondence analysis (CCA); Correlation Index (CoI); Plankton net (PLNET); Sedimentation (SEDIM)

### **2.1 Introduction**

Phytoplankton, together with benthic algae and macrophytes, constitute the autochthonous primary producers in aquatic ecosystem and form part of the basis of the food web in terms of energy and material input (Hötzel and Croome 1999). Due to their short life cycle, planktonic

algae are sensitive to respond quickly to a broad range of stressors, and thus an ideal indicator for water quality (Domingues and Galvão 2007, Cabecinha et al. 2009). The investigation of the phytoplankton community has become an important part of the overall water quality monitoring (Majaneva et al. 2009). From the management decision-making point of view, Jeong et al. (Jeong et al. 2008) even thought that if an accurate autoregressive model for phytoplankton dynamics was reliable, then forecasting would be possible with only phytoplankton data instead of monitoring a wide range of limnological changes, which usually has exorbitant costs.

A complete list of species and reliable quantitative data on species densities are of primary importance for bioassessment development. Only with precise species composition we can get a great deal of community information from different kinds of indices available, like community diversity, growth forms, etc (Wang et al. 2005). However, the precision obtained in the field may vary greatly due to the differences in sampling methods. Two sampling protocols of phytoplankton were widely used in lakes or large rivers: plankton net collection (PLNET) and sedimentation method (SEDIM) (Hötzel and Croome 1999, Huang 2000). PLNET protocol is labor saving, fast, easy to handle and can capture rare species, but allows real nannoplankton to pass through its meshes (Kraatz 1940, Tangen 1978). It is thus a preferred method for clean water with low phytoplankton density. In contrast, SEDIM is usually used in water bodies with high phytoplankton density (e.g. Ha et al. 1998, Köhler et al. 2002, Zhou et al. 2006, Sabater et al. 2008, Friedrich and Pohlmann 2009, Xu et al. 2009). Nevertheless, in small rivers and streams with relative higher current velocity, the studies on phytoplankton were very scarce (Piiroso et al. 2008, Sumorok et al. 2009, Centis et al. 2010) and the sampling methods in these systems were also diverse and not as well-developed as in lakes or large rivers. For example, Trifonova and Pavlova (Trifonova and Pavlova 2004), Trifonova et al. (Trifonova et al. 2007) and Wu et al. (Wu et al. 2007) used SEDIM to explore the riverine phytoplankton community in the river of the Lake Ladoga basin, Russia and the Xiangxi River, China, respectively, while PLNET was employed by Paasche and Ostergren (Paasche and Ostergren 1980) in the inner Oslofjord, Norway, and Plenković-Moraj et al. (Plenković-Moraj et al. 2007) in the Drava River, Croatia. Although the above mentioned methods were both applied in the stream systems, to our knowledge, the influence of two sampling protocols on the outcome of bioassessment in streams has not been investigated systematically yet. Thus, the main purpose of our study is to determine how phytoplankton sampling protocols may influence bioassessment.

In this study, we compared phytoplankton assemblages generated from PLNET and SEDIM in a German lowland catchment - the Kielstau catchment. The main aim was to determine whether phytoplankton assemblages generated by the two sampling protocols were related to different

environmental variables and therefore might yield different bioassessment. Our specific objectives were to: 1) compare phytoplankton assemblages collected using PLNET and SEDIM protocols, 2) quantify and compare the relationships between phytoplankton assemblages and environmental variables shown by the two sampling protocols, and 3) compare the relationship of algal metrics, commonly used in bioassessment, between PLNET and SEDIM protocols.

## **2.2 Methods**

### **2.2.1 Study area and sites**

The Kielstau catchment is located in northern Germany, with a length of 17 km and a drainage area of 50 km<sup>2</sup>. 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. 2.1). Moorau and Hennebach are two main tributaries within the Kielstau catchment. In addition, various smaller tributaries and water from drainage pipes and open ditches flow into the Kielstau. Sandy, loamy and peat soils are characteristic for 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 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 four times in November 2008, February 2009, May 2009 and August 2009, respectively. At each date, 20 sites (Fig. 2.1C) along the main stream Kielstau and its tributaries were sampled. Ten sites (K01 to K10) were located along the main stream, three (M01 to M03) at the Moorau tributary, five (H01 to H05) at the Hennebach tributary and two (L01 and L02) at Lake Winderatt. Due to some absent samples, we totally collected 77 samples in the four sampling dates.

### **2.2.2 Sampling methods and primary procedures**

For PLNET collection, 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 the water was filtered through a plankton net. The retained organisms in the plankton net were transferred into glass containers and concentrated to 30 mL. 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).



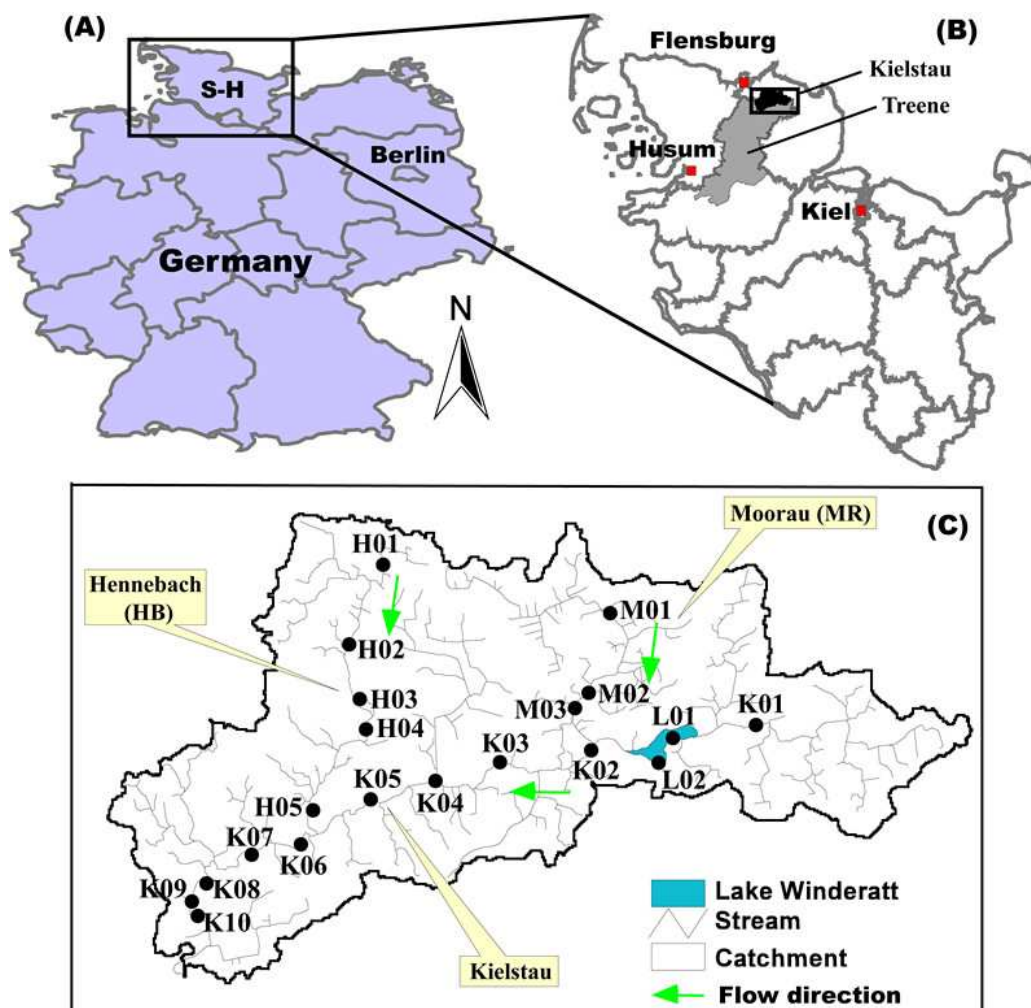


Figure 2.1: The location of the Kielstau catchment in Schleswig-Holstein state (B), Germany (A), map source CDC (CDC 2010) and the sampling sites (C). S-H = Schleswig-Holstein state; M01-M03 = sampling sites collected from Moorau tributary; H01-H05 = sampling sites from Hennebach tributary; K01-K10 = sampling sites from main stream Kielstau.

Concurrently, the SEDIM protocol was also used at each site. A sample of 1 L of subsurface (5-40 cm) river water was taken and fixed in 5% non-acetic Lugol's iodine solution (Sabater et al. 2008). Forty eight hours later, the supernatant liquid was removed and the retained organisms were transferred into glass containers. After sedimentation again, samples were concentrated to 30 mL for further processing.

Besides, 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, Weilheim, 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.

### **2.2.3 Chemical variables and chlorophyll a measurement**

The factors including orthophosphate-phosphorus ( $\text{PO}_4\text{-P}$ ), ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ), total phosphorus (TP), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ), dissolved silicon (Si), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), chloride ( $\text{Cl}^-$ ) and sulphate ( $\text{SO}_4^{2-}$ ) were measured according to the standard methods DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung).  $\text{PO}_4\text{-P}$  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  $\text{NH}_4\text{-N}$  concentrations at 690 nm.  $\text{NO}_2\text{-N}$  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.  $\text{NO}_3\text{-N}$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were measured by ion chromatography method (DIN38 405-D19). Dissolved inorganic nitrogen (DIN) was defined as the sum of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$ , 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). 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 (APHA 1992).

### **2.2.4 Algae identification under microscope**

Non-diatom algae were analyzed using a 0.1 ml counting chamber at a magnification of 400 $\times$  (Zeiss Axioskop microscope). Permanent diatom slides were prepared after oxidizing the organic material (by nitric acid and sulfuric acid) according to the methods of Abrantes (Abrantes 1988) and Abrantes *et al.* (Abrantes *et al.* 2005) with some modifications. A minimum of 300 valves were counted for each sample using a Zeiss Axioskop microscope at 1000 $\times$  under oil immersion. Algae were identified to the lowest taxonomic level possible (mainly species level) according to Simonsen (Simonsen 1987), Round *et al.* (Round *et al.* 1990) and Lange-Bertalot (Lange-Bertalot 2000a, b, 2005, 2007). and their densities were expressed as cell/L. Total algal biomass (TAB) for each sample was estimated based on multiplication of density data with volume (closest geometric form) supposing specific gravity of 1.00 g  $\text{cm}^{-3}$  (Hillebrand *et al.* 1999, Padisák and Adrian 1999).

### 2.2.5 Data calculation and analyses

Phytoplankton taxon counts were expressed as the proportion of total individuals at each site (relative abundances). Bray-Curtis (BC; relative abundance data) and Jaccard's (presence/absence data) similarities were calculated between PLNET and SEDIM protocols at each site. Sites were then ranked based on their BC similarity and divided into four groups of equal number (low-BC group: n=20, all other groups: n=19). Paired t-test was used to compare dominant phytoplankton species collected by PLNET and SEDIM protocols at lotic and lentic sites, respectively. Stream sites were ordinated on the basis of phytoplankton composition (BC similarity as the distance measure) by non-metric multidimensional scaling (NMDS) ordination.

To test the influence generated by PLNET and SEDIM protocols on the outcome of bioassessment, totally 13 algal metrics, which were commonly used for phytoplankton based bioassessment, were calculated at each site. These metrics included assemblage diversity measures [e.g. species richness (SpR), Simpson's diversity index (SiD), Shannon-Wiener diversity index [H'] and evenness (Ev)], morphological metrics [%benthic taxa (BeT), %mobile taxa (MoT) and %unattached taxa (UnT)], saprobity index (SaI), Q index (QI), Chlorophyte-Index (ChI), Pennales-Index (PeI), total algal density (TAD) and total algal biomass (TAB). All the algal metrics were briefly described in Table 2.1, and all of them (except for TAD) were calculated based on taxa biovolumes. To evaluate the statistical significance of each correlation between biotic metrics and environmental variables, we used 'Correlation Index' (CoI) and Cumulative\_R<sup>2</sup> according to Blanco et al. (Blanco et al. 2007):

$$\text{Cumulative\_R}^2 = \sum(r_s^2) \quad (1)$$

where R<sup>2</sup>= sum of r<sub>s</sub><sup>2</sup> with r<sub>s</sub>= Spearman's correlation coefficient between a given metric and the environmental variable.

$$\text{CoI} = (\text{Cumulative\_R}^2 \cdot S) / n^2 \quad (2)$$

where CoI= Correlation Index for a given metric, S= Number of r<sub>s</sub> statistically significant at p<0.05, and n= number of environmental variables evaluated.

CoI ranges from 0 to 1, while Cumulative\_R<sup>2</sup> from 0 to n, indicating the theoretical minimum and maximum relationship between a given candidate metric and environmental variables, with high values indicating better relationship. Paired t-test was also used to compare the differences of CoI and Cumulative\_R<sup>2</sup> collected by PLNET and SEDIM, respectively.

Table 2.1 Summary of analysed algal metrics

Abbreviation	Metric	Description	Reference
TAD	Total algal density	Measures of algal numbers per liter	-
TAB	Total algal biomass	Measures of total algal biomass per liter, and is estimated based on multiplication of density data with volume (closest geometric form) supposing specific gravity of 1.00 g cm <sup>-3</sup>	Hillebrand et al. (Hillebrand et al. 1999)
BeT	%benthic taxa (%)	Measures of microhabitat traits (pelagic or benthic life-styles) of taxa	Algal Analysis System (ADAS) using an attribute file of published values (van Dam et al. 1994, Wang et al. 2005, Porter et al. 2008)
MoT	%mobile taxa (%)	Measures of movement capable of taxa in water or on submerged surface	
UnT	%unattached taxa (%)	Measures of algal growth forms	
Sal	Saprobity index	Measures of saprobic status of the water	
SpR	Species richness	Numbers of specific taxa in each sample	-
SiD	Simpson's diversity index		
H'	Shannon-Wiener diversity index	Measures ecological diversity in the community	
Ev	Evenness		
QI	Q index	A new evaluation technique of potamoplankton for the assessment of the ecological status of rivers; QI>4.75 = Excellent, 4.75-4.50 = Good, 4.50-4.00 = Moderate, 4.00-3.50 = Poor, <3.50 = Bad	Borics et al. (Borics et al. 2007)
ChI	Chlorophyte-Index	The German method to assess rivers by phytoplankton with a list of indicator taxa	Mischke and Behrendt (Mischke and Behrendt 2007)
PeI	Pennales-Index	for routine monitoring in Germany	

Note: all the algal metrics (except for TAD) were calculated based on algal biomass.

Moreover, canonical correspondence analysis (CCA) was used to relate algal assemblages generated by PLNET and SEDIM protocols to environmental variables. CCA was chosen because detrended correspondence analyses on the algal data matrices produced longest gradient length of 4.27 and 3.74 for PLNET and SEDIM, respectively (Lepš and Šmilauer 2003). 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 algal 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 to 31 and 20, respectively for PLNET and SEDIM. Environmental variables with high correlation coefficients ( $r > 0.60$ ) and variance inflation factors ( $VIF > 20$ ) were excluded in the final CCA analyses (Wu et al. 2011b). This criterion reduced the number of environmental variables to 11. 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.

NMDS ordination was performed with PRIMER (Version 5). Correlation analyses were conducted by STATISTICA 6.0 (version 6.0, StatSoft, Tulsa, Oklahoma), and the data not normally distributed were  $\log(x+1)$  transformed prior to analysis. CCA were carried out by CANOCO (Version 4.5).

## **2.3 Results**

### **2.3.1 Environmental characteristics**

River reaches of the study area varied widely in water-quality and habitat characteristics (Table 2.2). For 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  $\mu\text{s}/\text{cm}$  (385-803  $\mu\text{s}/\text{cm}$ ). 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.

Table 2.2 Mean ( $\pm 1$  SE) values of environmental variables for all sites and for sites grouped by Bray-Curtis (BC) similarity between samples collected using plankton net (PLNET) and sedimentation (SEDIM)

Environmental variables	All sites	BC>60.3	BC=48.5-60.3	BC=36.8-48.5	BC<36.8
Chl <i>a</i> ( $\mu\text{g/L}$ )	35.76 $\pm$ 9.64	92.18 $\pm$ 35.91	16.80 $\pm$ 4.02	19.48 $\pm$ 5.72	15.42 $\pm$ 3.38
DO (mg/L)	9.21 $\pm$ 0.38	9.98 $\pm$ 0.97	8.92 $\pm$ 0.46	8.52 $\pm$ 0.78	9.38 $\pm$ 0.76
Channel width (m)	2.2 $\pm$ 0.11	2.06 $\pm$ 0.21	2.41 $\pm$ 0.22	1.92 $\pm$ 0.19	2.42 $\pm$ 0.25
Water depth (m)	0.29 $\pm$ 0.02	0.29 $\pm$ 0.05	0.33 $\pm$ 0.04	0.22 $\pm$ 0.02	0.34 $\pm$ 0.05
Flow velocity (m/s)	0.17 $\pm$ 0.01	0.10 $\pm$ 0.02	0.22 $\pm$ 0.03	0.18 $\pm$ 0.03	0.17 $\pm$ 0.03
pH	7.89 $\pm$ 0.07	8.02 $\pm$ 0.14	7.81 $\pm$ 0.16	7.77 $\pm$ 0.08	7.97 $\pm$ 0.17
WT ( $^{\circ}\text{C}$ )	10.56 $\pm$ 0.61	13.15 $\pm$ 1.03	9.82 $\pm$ 1.15	10.9 $\pm$ 1.33	8.37 $\pm$ 1.19
COND ( $\mu\text{s/cm}$ )	604.49 $\pm$ 10.59	589.16 $\pm$ 28.13	608.37 $\pm$ 19.9	611.4 $\pm$ 19.63	608.68 $\pm$ 16.95
TSS (mg/L)	11.51 $\pm$ 1.2	20.97 $\pm$ 3.61	9.29 $\pm$ 1.52	9.34 $\pm$ 1.14	6.55 $\pm$ 0.98
VSS (mg/L)	8.61 $\pm$ 0.96	16.01 $\pm$ 3.21	6.07 $\pm$ 0.61	6.91 $\pm$ 0.7	5.55 $\pm$ 0.6
NH <sub>4</sub> -N (mg/L)	1 $\pm$ 0.17	0.64 $\pm$ 0.21	1.24 $\pm$ 0.36	1.43 $\pm$ 0.47	0.66 $\pm$ 0.15
NO <sub>3</sub> -N (mg/L)	18.92 $\pm$ 1.17	17.09 $\pm$ 3.18	21.19 $\pm$ 2.29	18.63 $\pm$ 1.93	18.8 $\pm$ 1.87
NO <sub>2</sub> -N (mg/L)	0.07 $\pm$ 0.01	0.06 $\pm$ 0.01	0.08 $\pm$ 0.02	0.09 $\pm$ 0.02	0.05 $\pm$ 0.01
DIN (mg/L)	19.99 $\pm$ 1.25	17.79 $\pm$ 3.25	22.5 $\pm$ 2.52	20.15 $\pm$ 2.2	19.51 $\pm$ 1.94
PO <sub>4</sub> -P (mg/L)	0.22 $\pm$ 0.02	0.23 $\pm$ 0.04	0.17 $\pm$ 0.03	0.29 $\pm$ 0.05	0.18 $\pm$ 0.03
TP (mg/L)	0.41 $\pm$ 0.03	0.48 $\pm$ 0.06	0.37 $\pm$ 0.05	0.49 $\pm$ 0.06	0.3 $\pm$ 0.03
Si (mg/L)	0.23 $\pm$ 0.01	0.22 $\pm$ 0.03	0.23 $\pm$ 0.02	0.24 $\pm$ 0.02	0.23 $\pm$ 0.02
Cl <sup>-</sup> (mg/L)	32.62 $\pm$ 1.04	32.39 $\pm$ 1.6	31.95 $\pm$ 1.3	34.73 $\pm$ 2.28	31.28 $\pm$ 2.83
SO <sub>4</sub> <sup>2-</sup> (mg/L)	34.71 $\pm$ 1.05	34.63 $\pm$ 2.73	36.2 $\pm$ 1.91	35.57 $\pm$ 2.04	32.39 $\pm$ 1.66
N:P	76.82 $\pm$ 10.76	77.06 $\pm$ 32.93	91.04 $\pm$ 24.32	52.96 $\pm$ 7.79	87.48 $\pm$ 13.63

### 2.3.2 Phytoplankton assemblage

A total of 127 taxa (mostly to species levels) were identified from the four sampling dates and two protocols. These taxa belonged to six phytoplankton groups - Bacillariophyta, Chlorophyta, Cryptophyta, Cyanophyta, Euglenophyta and Pyrrophyta. Diatoms were predominant with 59.52% of the total abundance in lotic sites. In the lentic site (L01 and L02), Chlorophyta (88.35% of the total abundance) was the most abundant group and Cryptophyta (4.99%), Bacillariophyta (3.96%), Cyanophyta (2.43%), Euglenophyta (0.26%) and Pyrrophyta (0.004%)

followed. 125 taxa were identified in the PLNET counts and 95 taxa were observed in the SEDIM counts. However, the dominant species with relative abundance >1% were similar at both lotic and lentic sites between counts generated by the two sampling protocols (paired t-test,  $p > 0.05$ ; Table 2.3).

In general, BC similarities ranged from 7.5 to 82.0% (lower quartile = 36.8%, median = 48.5%, upper quartile = 60.3%), while Jaccard's similarities ranged from 10.5 to 64.9% (lower quartile = 32.4%, median = 42.1%, upper quartile = 50.0%). Jaccard's similarities were less than BC similarities in 55 sites, indicating that rare species influenced similarities between PLNET and SEDIM protocols in these sites. The PLNET protocol yielded higher SpR than the SEDIM sampling protocol in 72 of 77 sites. 32 taxa identified in the PLNET counts were not found in the SEDIM counts, whereas all taxa observed in the SEDIM counts were found in the PLNET counts. Besides, reduced total algal density (TAD) and biomass (TAB) were observed by PLNET protocol (Table 2.4).

Most sites clustered more closely in the NMDS ordination of PLNET-generated data than in that of SEDIM-generated data (Fig. 2.2A-D). However, sites of PLNET and SEDIM were not separated along NMDS axis 1 or 2 in the four sampling dates (Fig. 2.2A-D), indicating that the two sampling protocols yielded similar phytoplankton assemblages.

### **2.3.3 Sites with low values of BC similarity**

Phytoplankton assemblages from the subset of sites with the lowest values of BC similarity were examined further. Sites were regarded as having a low BC similarity if the values was in the lowest quartile of similarities ( $BC < 36.8$ ,  $n=20$ ). For this group, median SpR was much lower for counts generated by SEDIM than those by PLNET protocol (Table 2.4). Individual values of SpR were lower by SEDIM protocol than those by PLNET protocol at all the 20 low BC sites. Sites in the low BC group tended to have lower Chl *a*, WT, TSS and VSS, but higher channel width and water depth, than other sites (Table 2.2).

Table 2.3 Dominant phytoplankton species collected by plankton net (PLNET) and sedimentation (SEDIM) in Kielstau catchment at 18 lotic and 2 lentic sites. Paired t-test results were also shown

Species	Overall	Lotic sites		Lentic sites	
		PLNET	SEDIM	PLNET	SEDIM
<b>BACILLARIOPHYTA</b>					
<i>Cocconeis placentula</i> Ehrenberg	1.51	6.73	3.96	0.07	0.02
<i>Cyclotella meneghiniana</i>	3.02	3.77	4.43	2.47	2.33
<i>Fragilaria biceps</i> (Kütz.) Lange-Bertalot	1.28	6.50	3.24	0.02	0.00
<i>Fragilaria crotonensis</i> Kitton	0.39	1.99	0.91	0.11	0.03
<i>Gomphonema olivaceum</i> (Lyngb.) Kütz.	0.93	1.93	2.86	0.05	0.01
<i>Meridion circulare</i> (Grev.) Ag.	0.51	2.71	1.29	0.00	0.00
<i>Navicula cryptocephala</i> Kütz.	0.96	2.64	2.83	0.04	0.00
<i>Navicula ingapirca</i> Lange-Bertalot & U. Rumrich	1.29	3.34	3.80	0.14	0.01
<i>Navicula viridula</i> (Kütz.) Ehr.	0.40	1.05	1.17	0.05	0.00
<i>Nitzschia sigma</i> (Kütz.) W. Sm.	0.85	3.72	2.22	0.18	0.01
<i>Planothidium lanceolatum</i> (Breb.) Round et Bukhtiyarova	2.86	8.62	8.21	0.35	0.04
<i>Tabellaria flocculosa</i> (Roth) Kütz.	2.12	12.35	4.97	0.36	0.07
<i>Ulnaria ulna</i> (Nitzsch) Comperé	0.95	3.99	2.54	0.03	0.01
<b>CHLOROPHYTA</b>					
<i>Coelastrum</i> sp.	0.87	0.21	0.19	0.72	1.25
<i>Desmodesmus communis</i> (Hegew.) Hegew.	48.20	1.17	6.15	49.65	71.28
<i>Pediastrum duplex</i> Meyen	7.06	0.93	0.25	26.91	9.85
<i>Scenedesmus dimorphus</i> (Turp.) Kütz.	3.67	1.12	1.88	3.34	4.72
<b>CRYPTOPHYTA</b>					
<i>Cryptomonas erosa</i> Ehr.	6.30	4.15	9.65	0.50	5.18
<b>CYANOPHYTA</b>					
<i>Oscillatoria</i> sp.	1.66	2.36	4.25	0.60	0.46
<i>Stigonema</i> sp.	1.26	0.00	0.00	0.27	1.99
<b>EUGLENOPHYTA</b>					
<i>Euglena</i> sp.	6.70	9.64	20.79	0.45	0.25
Paired t-test		t=-0.401 p=0.693		t=-0.391 p=0.700	

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



Table 2.4 Median (range) of 13 algal indices for samples collected using PLNET and SEDIM for all sites and sites with low values of BC similarity between the two protocols. Coefficients of determination and paired t-test are between the two sampling protocols, and **bold** font indicates statistically significant ( $p < 0.05$ )

Algal metrics	All sites				Low BC sites		
	PLNET	SEDIM	r	paired t-test	PLNET	SEDIM	r
Log (TAD)*	5.03 (3.66-6.19)	5.84 (4.75-7.47)	<b>0.58</b>	<b>0.000</b>	4.91 (3.73-5.53)	5.71 (5.00-6.57)	0.44
TAB (mg/L)	0.78 (0.02-4.24)	5.01 (0.08-54.4)	<b>0.60</b>	<b>0.000</b>	0.50 (0.02-2.26)	2.73 (0.21-8.80)	0.35
BeT (%)*	46.03 (0.42-86.77)	38.09 (0-90.28)	<b>0.63</b>	<b>0.001</b>	41.89 (0.66-86.77)	24.35 (0-73.03)	<b>0.59</b>
MoT (%)	25.35 (1.01-92.61)	43.80 (0.61-100)	<b>0.63</b>	<b>0.000</b>	26.92 (1.01-90.03)	61.93 (19.98-100)	<b>0.67</b>
UnT (%)*	25.08 (2.35-98.73)	22.95 (0-98.21)	<b>0.82</b>	0.183	30.30 (3.85-89.77)	27.25 (0-81.25)	<b>0.66</b>
Sal	1.04 (0.29-2.86)	1.11 (0-2.55)	<b>0.43</b>	0.208	1.15 (0.45-2.86)	1.32 (0.53-2.55)	<b>0.61</b>
SiD	7.61 (1.58-14.03)	6.35 (1.25-15.05)	<b>0.66</b>	<b>0.000</b>	6.19 (1.58-11.87)	4.18 (1.59-8.78)	0.31
SpR	33 (17-54)	20 (3-41)	<b>0.46</b>	<b>0.000</b>	35.05 (18-54)	15 (3-23)	<b>0.57</b>
H'	2.47 (1.01-3.10)	2.09 (0.48-3.09)	<b>0.79</b>	<b>0.000</b>	2.34 (1.01-3.03)	1.76 (0.71-2.41)	<b>0.68</b>
Ev*	0.71 (0.28-0.91)	0.71 (0.19-0.97)	<b>0.69</b>	0.788	0.67 (0.28-0.82)	0.66 (0.38-0.92)	<b>0.66</b>
QI*	3.67 (1.40-4.73)	3.14 (1.01-4.96)	<b>0.69</b>	<b>0.000</b>	3.29 (1.76-4.42)	2.67 (1.03-3.96)	0.43
ChI (%)	13.28 (0-79.87)	12.32 (0-94.52)	<b>0.67</b>	0.635	17.12 (0-71.08)	10.99 (0-63.87)	0.33
PeI (%)*	84.56 (0.54-99.47)	78.09 (0.84-100)	<b>0.76</b>	<b>0.001</b>	78.82 (4.96-97.05)	69.31 (2.55-100)	<b>0.63</b>

Metrics with \* were presented at Fig. 2.3, metric abbreviations were listed in Table 2.1.

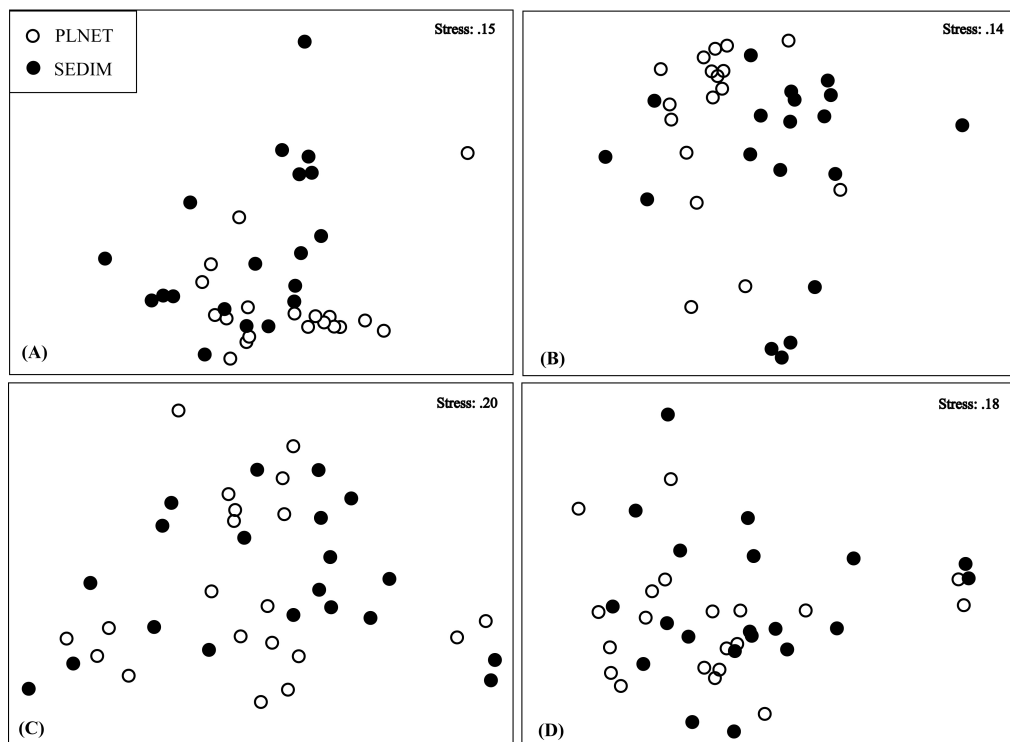


Figure 2.2: Non-metric multidimensional scaling (NMDS) ordinations of phytoplankton assemblage collected using plankton net (PLNET) and sedimentation (SEDIM) in November 2008 (A), February 2009 (B), May 2009 (C) and August 2009 (D)

### 2.3.4 Influences on algal metrics and bioassessment

All the 13 algal metrics were significantly correlated between PLNET and SEDIM protocols within all sites ( $p < 0.05$ ; Table 2.4, Fig. 2.3A-F). Divergence in SpR, TAD, TAB, QI and ChI tended to be greater at sites with the lowest BC values (Table 2.4, Fig. 2.3A, E). BC similarity values were only positively related to total algal density (TAD) (log transformed) and total algal biomass (TAB) ( $r = 0.30$  and  $0.33$ , respectively), but these relationships were statistically significant in all cases ( $p < 0.05$ , Fig. 2.4A-B).

However, CoI and Cumulative\_ $R^2$  between algal data collected by PLNET and environmental variables were significantly higher than those of SEDIM, respectively (paired t-test,  $p < 0.05$ ; Table 2.5). Furthermore, results of CCA relating algal assemblages to environmental variables demonstrated that higher percent of algal variation was explained by PLNET (38.2%) than by SEDIM (31.4%) (Table 2.6).

Table 2.5 Comparisons of CoI values and Cumulative\_R<sup>2</sup> between plankton net (PLNET) and sedimentation (SEDIM) protocols

Metrics	CoI		Cumulative_R <sup>2</sup>	
	PLNET	SEDIM	PLNET	SEDIM
Log (TAD)	0.029	0.008	0.882	0.477
TAB (mg/L)	0.027	0.014	0.805	0.562
BeT (%)	0.009	0.010	0.374	0.392
MoT (%)	0.011	0.012	0.451	0.474
UaT (%)	0.047	0.042	1.137	0.850
SaI	0.057	0.007	1.152	0.395
SiD	0.010	0.011	0.388	0.439
SpR	0.098	0.010	1.478	0.387
H'	0.048	0.010	0.822	0.416
Ev	0.000	0.014	0.248	0.411
QI	0.021	0.005	0.632	0.299
ChI (%)	0.023	0.003	0.546	0.335
PeI (%)	0.034	0.025	0.685	0.600
Mean	0.032	0.013	0.738	0.464
Paired t-test	t=2.704 p=0.018		t=3.071 p=0.009	

Metrics abbreviations were listed in Table 2.1.

Table 2.6 Results of canonical correspondence analyses (CCA) relating algal assemblages to environmental variables. Higher percent explained variation indicates stronger algal-environment relationships

Axes	PLNET				SEDIM			
	1	2	3	4	1	2	3	4
Eigenvalues	0.130	0.122	0.066	0.037	0.126	0.111	0.068	0.033
Species-environment correlations	0.770	0.861	0.820	0.698	0.764	0.751	0.656	0.678
Cumulative percentage variance								
of species data	12.0	23.2	29.3	32.7	10.4	19.6	25.1	27.8
of species-environment relation	31.4	60.9	76.8	85.7	33.2	62.3	80.1	88.7
Sum of all eigenvalues		1.085				1.210		
Sum of all canonical eigenvalues		0.414				0.380		
% explained variation in species data		38.2				31.4		

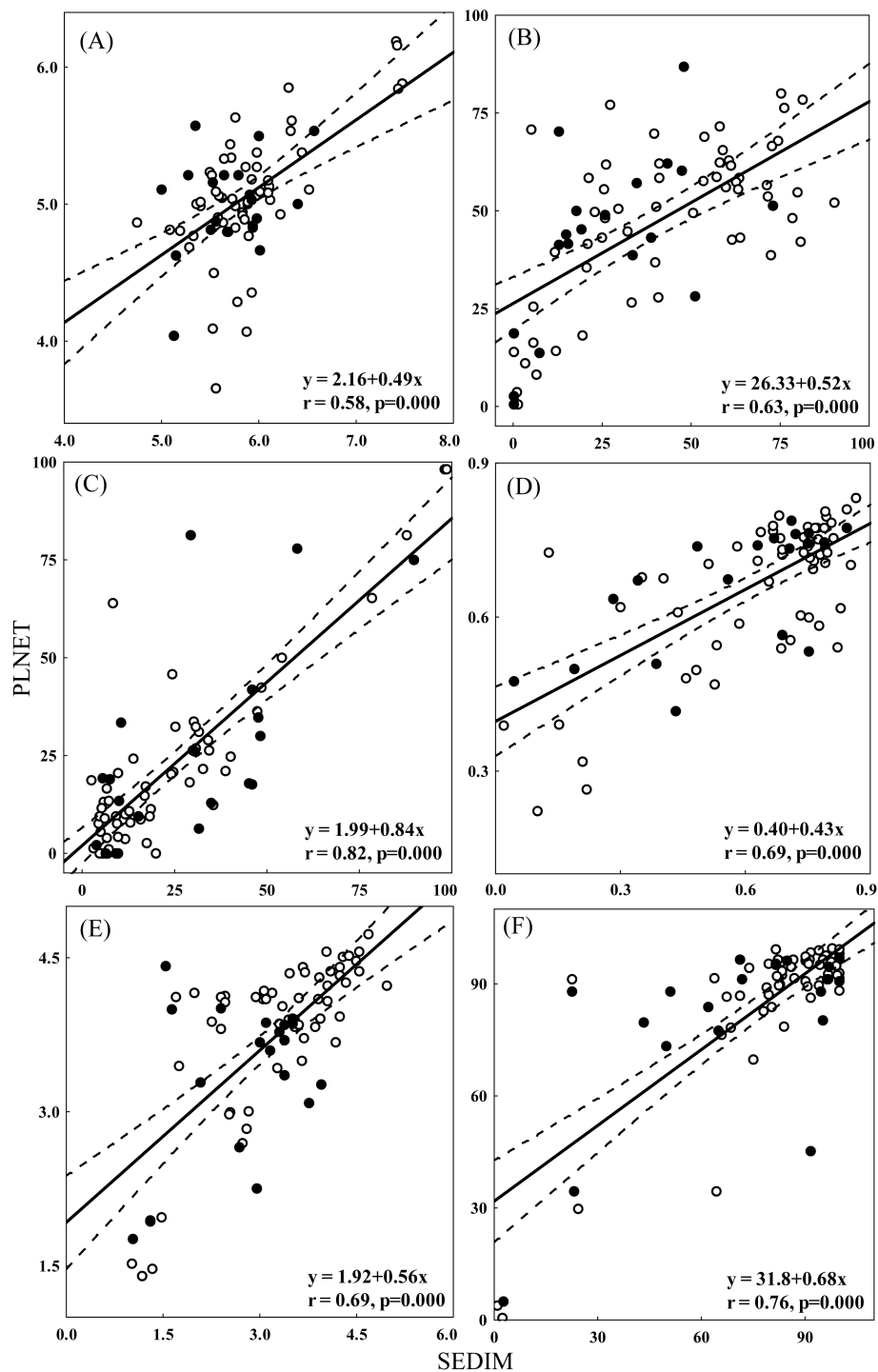


Figure 2.3: Relationships between values of total algal densities [Log(TAD)] (A), BeT (B), UnT (C), Ev (D), QI (E) and PeI (F) generated by plankton net (PLNET) and sedimentation (SEDIM). Solid circles are sites with the lowest BC values. Solid lines are regression lines and dashed lines indicate the 95% confidence bands

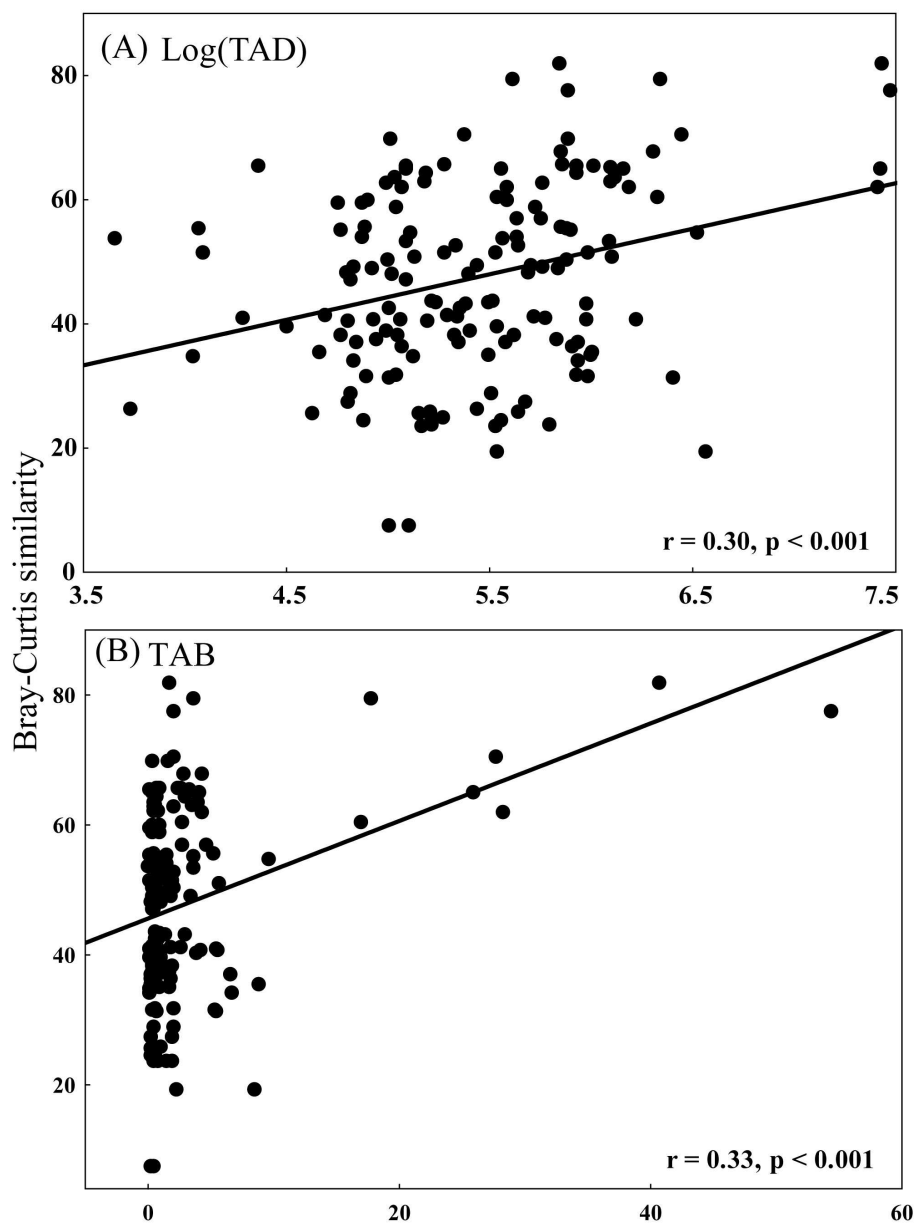


Figure 2.4: Regressions of Bray-Curtis (BC) similarity between phytoplankton assemblages generated by plankton net (PLNET) and sedimentation (SEDIM) protocols as a function of total algal density [ $\log(\text{TAD})$ ] (A) and total algal biomass (TAB) (B)

## 2.4 Discussion

In our study, the median BC similarity between PLNET and SEDIM protocols was 48.5%, and it's lower than those of previous studies. For example, mean BC similarity between diatom assemblages from different habitats within the same stream sampled multiple times was 68%

(Stevenson and Hashim 1987). Weilhoefer and Pan (Weilhoefer and Pan 2007), who compared two periphyton sampling protocols in 3 states of the USA, found a median BC value of 70%. The potential reason was that the phytoplankton density in our study area was much lower than that in lakes, reservoirs and riverine periphyton. As BC similarity values were significantly positively correlated with TAD and TAB (Fig. 2.4), in the low-algae density water bodies, SEDIM sampling protocol is usually not suitable to collect enough species (particularly rare species) to reflect the actual algal characteristics, because it can only collect phytoplankton from 1-L water (compared to 40-60 L of PLNET). Consequently, the similarities between the two sampling protocols were reduced and most of all samples were beyond the 95% limits (Fig. 2.3). This was also why PLNET sampling protocol yielded higher SpR than the SEDIM sampling protocol. Because SpR is highly correlated to the volume of sub sample which has been investigated (Padisák et al. 1999), with increasing volume of sample examined, the number of phytoplankton will increase.

As we used 20- $\mu\text{m}$  net for PLNET, theoretically species smaller than 20  $\mu\text{m}$  can surpass the plankton net and PLNET protocol would induce reduced TAD and TAB than SEDIM protocol. In the present study, we did find this pattern (Table 2.4), demonstrating the loss of small taxa as well as TAD and TAB by using PLNET protocol. However, the dominant species generated by the two sampling protocols were similar, as indicated by paired t-test (Table 2.3) and NMDS ordination (Fig. 2.2). Although the 13 algal metrics were significantly correlated between the two protocols within all sites (Table 2.4, Fig. 2.3), paired t-test showed that most metrics were significantly different between the two sampling protocols ( $p < 0.05$ ; Table 2.4), indicating that the two sampling protocols yielded different information on phytoplankton based bioassessment. Furthermore, the PLNET protocol seemed to be a better method than the SEDIM protocol for bioassessment, as suggested by the results of CoI, Cumulative\_ $R^2$  and CCA (Table 2.5, Table 2.6). Many investigators (e.g. Tangen 1978, Hötzel and Croome 1999) suggested that the PLNET protocol could only provide qualitative data and should be combined with quantitative methods. Nevertheless, on the other hand, Kraatz (Kraatz 1940) argued that sometimes the PLNET protocol was better for quantitative purposes than the SEDIM protocol. Indeed the SEDIM protocol was less accurate than the PLNET collection because based on fewer actual counted specimens and involving more multiplication in the calculation (Kraatz 1940). From the phytoplankton based bioassessment point of view, the present study was consistent with Kraatz (Kraatz 1940) and found that the PLNET protocol provided more accurate phytoplankton information on algal metrics, although the two sampling protocols generated similar dominant algal species. It must be pointed out that, in rivers, phytoplankton is less used for bio-indicator than other communities, such as periphyton and benthic invertebrates, because planktonic algae

are believed to come from either upstream lentic waterbodies or the benthos (Hötzel and Croome 1999). However, the present study found high correlations between algal communities and environmental variables and highlighted the importance of riverine phytoplankton based bioassessment.

One of the basic premises of bioassessment is that the species assemblage has been characterized accurately within the study unit. Both sampling protocols provided similar information about phytoplankton assemblages, but our study did not inform us as to whether the reaches had been sampled adequately. To our knowledge, no published studies have determined the best way to sample a stream reach to represent its phytoplankton assemblage adequately. Several studies have demonstrated that the observed composition and richness of macroinvertebrate and fish assemblages changed as more subsamples were collected within a reach (e.g. Cao et al. 1997, Cao et al. 2002), but no well-developed method existed to determine how many subsamples were enough for representative. Samples representativeness tended to increase with the number of samples collected, but the stabilization point varied among habitat types (Weilhoefer and Pan 2007). Thus, phytoplankton-based stream bioassessment would greatly benefit from further studies on the number of samples needed to characterize phytoplankton adequately in stream reaches with habitat heterogeneities. For instance, multiple samples from different habitats within a reach could be collected and analyzed respectively to determine how habitat heterogeneity impacts descriptors of phytoplankton assemblages. Those data could be used to detect the relationship between the number of samples and species richness to provide insight into the number of samples necessary to characterize the riverine phytoplankton assemblage.

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## **Chapter III 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 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 phytoplankton community were revealed by non-metric multidimensional scaling. Canonical correspondence analysis indicated that physical factors (e.g. hydrological variables) and major nutrients [e.g. total phosphorus, dissolved inorganic nitrogen (DIN)] were of equal importance controlling the variation in structure of riverine phytoplankton assemblages. Weighted averaging regression and cross-calibration produced strong models for predicting DIN, water temperature (WT) and total suspended solid (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 sigmaidea*, *Desmodesmus communis* and *Oscillatoria* sp. The results from this relatively small survey indicate the need of further monitoring to gain a better understanding of riverine phytoplankton and capitalize on the environmental indicator capacity of phytoplankton community.

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

### **3.1 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 ones (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 (WT), 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 community in lotic habitats (Basu and Pick 1995). Besides, contributions of main environmental factors to phytoplankton variations are also conflictive. For example, hydrological factors are thought to be of greater importance to planktonic development in rivers than in lakes (Pace et al. 1992), whereas other researchers concluded that river phytoplankton is more strongly regulated by nutrient concentrations, such as 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 in the surrounding environment has become a central topic of present researches (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 community in lowland rivers, to our knowledge, are still scanty. In this paper, we investigated the spatio-temporal variation of phytoplankton community and environmental variables over 1-year period (November 2008 – August 2009) throughout a lowland river ecosystem in northern Germany. The objectives of this study were to: (i) describe the distribution patterns in the species composition and biomass of phytoplankton in the Kielstau catchment; (ii) study the relationships between phytoplankton and environmental variables, and compare which factors predominantly structure riverine phytoplankton communities; (iii) identify algae species that could potentially be used as indicators of specific water chemistry

conditions in this lowland area.

## 3.2 Materials and methods

### 3.2.1 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. 3.1) 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 for 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 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). Many hydrological and morphological studies have been carried out in this catchment (Kiesel et al. 2009, Liu et al. 2009, Schmalz 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. 3.1) 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) at the Moorau tributary, five (H01 to H05) at the Hennebach tributary and two lentic sites (L01 and L02) at Lake Winderatt.

### 3.2.2 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 organisms 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 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), 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.

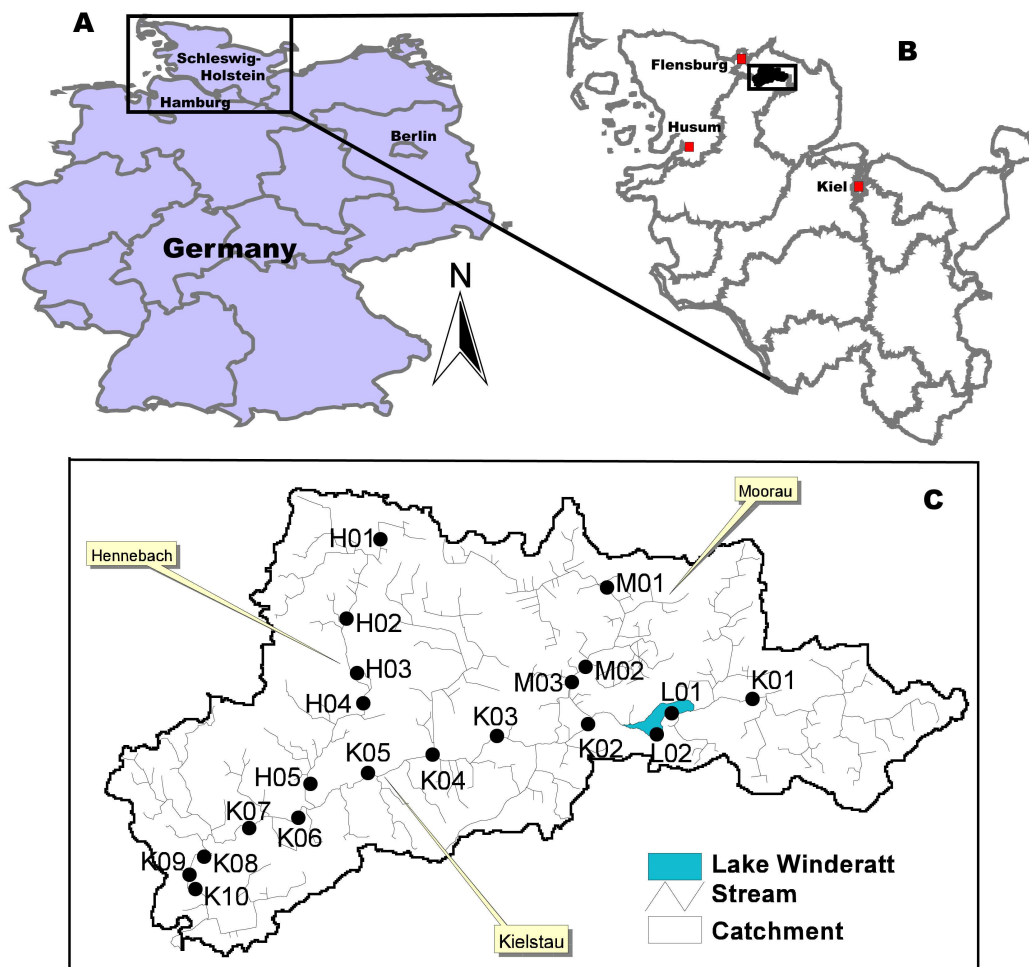


Figure 3.1: The location of the Kielstau catchment in Schleswig-Holstein state (B), Germany (A) and the sampling sites (C). (A) Cited from <http://www.cdc.gov/epiinfo/europe.htm>. (B) Modified from Schmalz and Fohrer (Schmalz and Fohrer 2010)

At each site, water samples were also collected for further laboratory analysis including orthophosphate ( $\text{PO}_4\text{-P}$ ), ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ), total phosphorus (TP), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ), dissolved silicon (Si), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), chloride (Cl) and sulphate ( $\text{SO}_4^{2-}$ ). All these factors were measured according to the standard methods DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung).  $\text{PO}_4\text{-P}$  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  $\text{NH}_4\text{-N}$  concentrations at 690 nm.  $\text{NO}_2\text{-N}$  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.  $\text{NO}_3\text{-N}$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were measured by ion chromatography method (DIN38 405-D19). Dissolved inorganic nitrogen (DIN) was defined as the sum of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$ , 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 (APHA 1992).

### 3.2.3 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 (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*.

### 3.2.4 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 et al. 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 final CCA analyses (ter Braak and Šmilauer 1998, Munn et al. 2002). These criteria reduced the number of environmental variables from 19 to 11. Forward selection and Monte Carlo permutations were used to identify a subset of the measured variables that exerted significant and independent effects on algal distributions.

Regression and calibration models were developed to quantify relations between algal abundances and environmental variables strongly expressed in CCA. Taxa optima and tolerances were calculated using weighted averaging regression analysis (WA) (Birks et al. 1990). The software calculated species optima and tolerances (respectively, the average and standard deviation of the environmental variables over all sites where a taxon occurs, weighted by the relative abundance of the taxon at each site). The predictive capability of the resulting models was assessed using the jackknife ('leave-one-out') cross-validation procedure, and measured as the coefficient of determination ( $R^2$ ) between species-inferred and observed environmental variable concentrations and the root-mean-squared error of prediction (RMSE). Because the observed and inferred values used all sites, the  $R^2$  calculated from the regression was termed "apparent"  $R^2$  ( $R^2_{\text{apparent}}$ ). The same model was run using a jackknifing procedure to validate the apparent  $R^2$  values. A model was determined acceptable if there was agreement between apparent and jackknifed  $R^2$  ( $R^2_{\text{jackknife}}$ ) values (Munn et al. 2002). For these data, the procedure was relevant because it also enabled a preliminary identification of taxa that may be suitable as indicators of particular conditions because of their narrow tolerance ranges to environmental variables. Based on Kilroy et al. (Kilroy et al. 2006), our criteria were 1) occurrence in at least 30 of the 77 sites, and 2) tolerance to the variable of interest  $< 0.75$  \* the mean tolerance for all the species. Untransformed species and environmental data were used for WA.

In our study, two-way analysis of variance (ANOVA) was conducted by STATISTICA 6.0 and  $\ln(x+1)$  or square transformation was used if data were not normally distributed; CCA were

carried out by CANOCO (Version 4.5); NMDS ordination was performed with PRIMER (Version 5) and WA by C<sup>2</sup> software.

### 3.3 Results

#### 3.3.1 Environmental characteristics

River reaches of the study area varied widely in water-quality and habitat characteristics. For 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 3.1. Eight variables, including pH, WT, TSS, VSS, NO<sub>3</sub>-N, DIN, PO<sub>4</sub>-P and Si, showed significant differences among the four seasons, while nine variables such as pH, COND, SO<sub>4</sub><sup>2-</sup>, velocity and TSS were considerably different between lentic and lotic sites.

#### 3.3.2 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 in the four sampling dates were shown in Table 3.2 and Table 3.3. *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 3.2). Temporal variation of phytoplankton community was also remarkable. At lentic sites, *Pediastrum duplex* was dominant species in November 2008 and February 2009, however, in May and August 2009, *Desmodesmus communis* was substantially more abundant with more than a half of the total abundance.

Table 3.1 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	November 2008		February 2009		May 2009		August 2009		Two-way ANOVA analysis	
	and sites	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 ( <b>0.028</b> )	10.23 ( <b>0.002</b> )
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 ( <b>0.000</b> )	0.19 (0.662)
COND ( $\mu$ 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 ( <b>0.000</b> )
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 ( <b>0.000</b> )	27.43 ( <b>0.000</b> )
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 ( <b>0.000</b> )	37.9 ( <b>0.000</b> )
NH <sub>4</sub> -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 <sub>3</sub> -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 ( <b>0.001</b> )	16.34 ( <b>0.000</b> )
NO <sub>2</sub> -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 ( <b>0.031</b> )
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 ( <b>0.001</b> )	16.81 ( <b>0.000</b> )
PO <sub>4</sub> -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 ( <b>0.015</b> )	4.16 ( <b>0.045</b> )
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 ( <b>0.000</b> )	10.97 ( <b>0.001</b> )
Cl <sup>-</sup> (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 ( <b>0.004</b> )
SO <sub>4</sub> <sup>2-</sup> (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 ( <b>0.000</b> )

Note: - means data absent; values without SE were only one sample. Summary of two-way 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 3.2 Dominant phytoplankton species collected in four different seasons at lentic and lotic sites in the Kielstau catchment

	All dates	November 2008	February 2009	May 2009	August 2009
<b>Lentic</b>					
<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
<b>Lotic</b>					
<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 amphibaena</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 3.3 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	November 2008		February 2009		May 2009		August 2009		Two-way ANOVA analysis	
	and sites	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 ( <b>0.000</b> )	10.85 ( <b>0.002</b> )
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 ( <b>0.000</b> )	2.85 (0.096)
Chl <i>a</i> ( $\mu$ 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 ( <b>0.000</b> )
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 ( <b>0.027</b> )	15.00 ( <b>0.000</b> )
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 ( <b>0.000</b> )
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 ( <b>0.000</b> )
% 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 ( <b>0.000</b> )
% 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 ( <b>0.014</b> )
% 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 ( <b>0.000</b> )
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 ( <b>0.002</b> )
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 ( <b>0.000</b> )
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 ( <b>0.001</b> )	395.15 ( <b>0.000</b> )

Summary of two-way ANOVA for testing the effects of season and location on main phytoplankton metrics are also presented. F-values with significance levels in parentheses (significant differences at  $P < 0.05$  are indicated in bold)

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

At lotic sites, *Tabellaria flocculosa* was mostly abundant in November 2008, February and August 2009, but in May 2009, phytoplankton was dominant by *Euglena* sp. (Table 3.2). Margalef's diversity index, species richness, total algal density and Pennales-Index were seasonally different ( $P < 0.001$ ) (Table 3.3). The phytoplankton NMDS ordination (Fig. 3.2) indicated a seasonal trend at both lentic and lotic sites. From November 2008 to August 2009, all the lotic sites moving from bottom to top, and there was a separation of May and August 2009 along Axis 1. Lentic sites, well separated from lotic sites, in four different seasons were also dispersed along Axis 2 (Fig. 3.2).

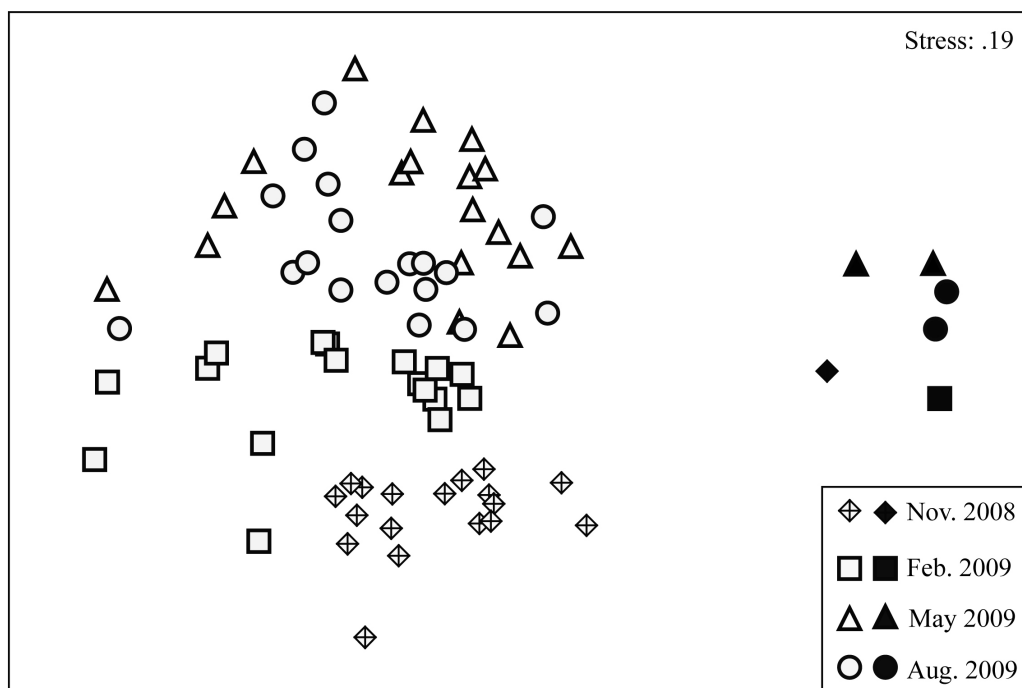


Figure 3.2: Nonmetric Multidimensional Scaling (NMDS) ordination of phytoplankton community at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study

The average value of phytoplankton biomass in the Kielstau catchment was  $35.8 \mu\text{g/L}$ , which is higher than the corresponding values from Grabia and Brodnia of central Poland ( $\sim 5 \mu\text{g/L}$ ) (Sumorok et al. 2009). It is comparable to some large European rivers like Ebro (Spain) ( $20\text{-}45 \mu\text{g/L}$  in the 1990s) (Sabater et al. 2008) and Rhine (Germany) ( $21\text{-}30 \mu\text{g/L}$  since 1992) (Friedrich and Pohlmann 2009), but lower than that for such rivers in Hungary ( $>740 \mu\text{g/L}$ ) (Kiss et al. 1994), Greece ( $>740 \mu\text{g/L}$ ) (Montesanto et al. 2000) or Estonia ( $\sim 740 \mu\text{g/L}$ ) (Pirsoo et al. 2008). These differences may be related to the water residence time, which is a useful

system-level index that has similar ecological implications for rivers (Soballe and Kimmel 1987), and is a key parameter controlling the biogeochemical behavior of aquatic ecosystems (Rueda et al. 2006).

### 3.3.3 Relationship between phytoplankton community and environmental variables

Relations between measured environmental variables and phytoplankton assemblages of the lotic and lentic sites were explored using CCA. Its results showed that the variation of phytoplankton was mainly affected by major nutrients (e.g., TP, DIN, NO<sub>2</sub>-N), physicochemical parameters (WT, Si, Cl, TSS) and hydrological variables (width and flow velocity) (Monte Carlo test  $p < 0.01$ ) (Fig. 3.3). The eigenvalues of Axes 1 and 2 were 0.130 and 0.122, which accounted for 31.4% and 29.5% of the total variance, respectively. The species-environment correlations were 0.770 for Axis 1 and 0.861 for Axis 2. Loadings on Axes 1 and 2 were substantially larger than that of succeeding axes, and primarily expressed variation in major nutrients and physical variables. Variation expressed on CCA Axis 1 was disproportionately related to lentic sites with high TSS concentrations. For instance, *Scenedesmus dimorphus*, *Desmodesmus communis* and *Cyclotella meneghiniana*, typical lentic species, occurred mostly at L01 and L02. CCA Axis 2 was likely integrating a seasonal variation of WT, velocity and channel width, which well separated wet from dry season sites.

### 3.3.4 Species WA-optima and tolerances and inference models

DIN, WT and TSS weight averaging (WA) species optima were calculated using the full dataset ( $n=77$ ), and its results were presented for the species with effective numbers of occurrences  $>30$  (Table 3.4). Weight averaging DIN, WT and TSS optima ranged from 2.31~30.77 mg/L, 6.00~15.39 °C and 9.14~25.80 mg/L, respectively.

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 deshrinking. 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 3.5). Jackknife-derived predicted DIN, WT and TSS values matched the measured values well (Fig. 3.4), and the residuals plotted

against predicted values indicated no bias in the models (Fig. 3.4). Models for other variables performed poorly (not shown).

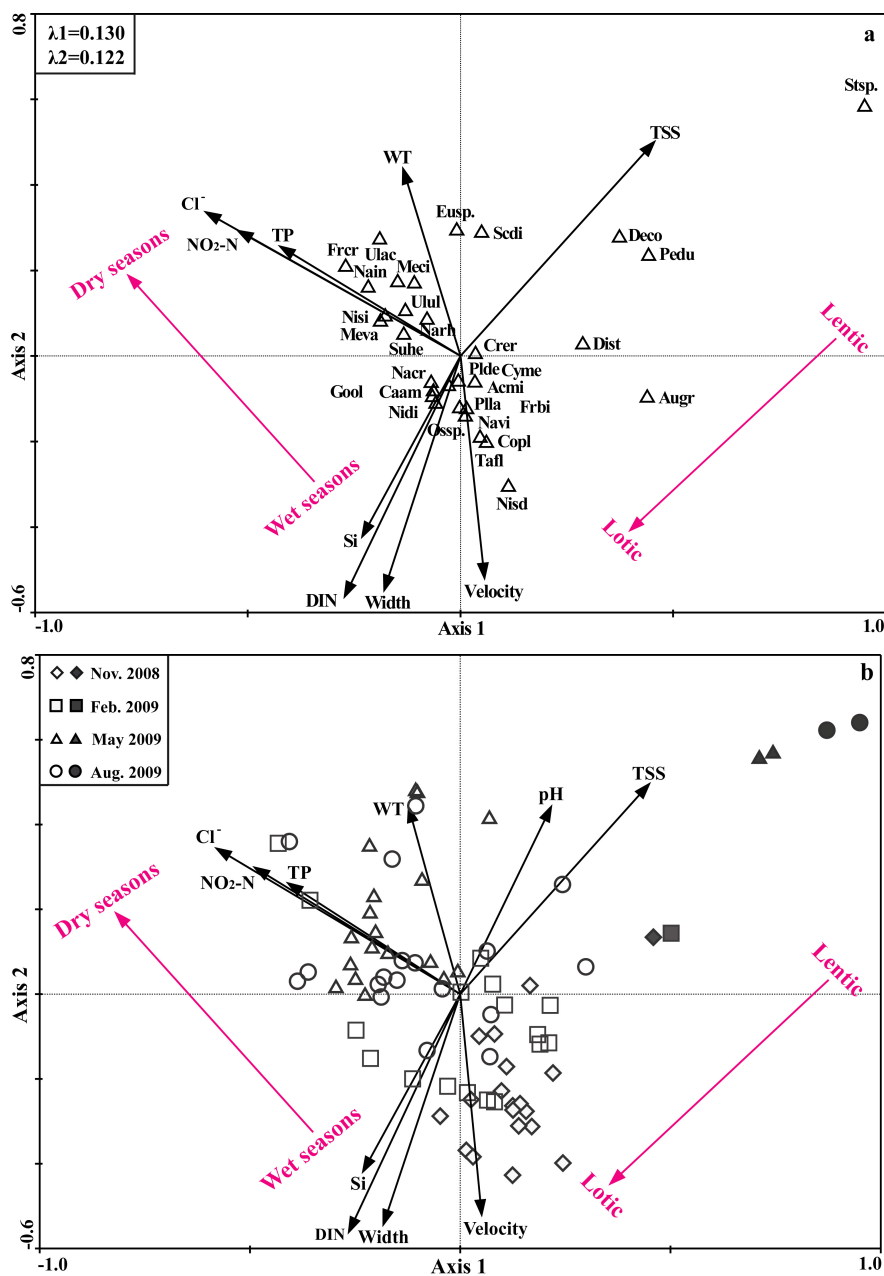


Figure 3.3: Canonical correspondence ordination of the phytoplankton samples collected at lentic (solid symbols) and lotic sites (open symbols) in the Kielstau catchment throughout the study and associated significant environmental factors. (a) Biplots of the species and the environmental variables; (b) Biplots of the sampling sites and the environmental variables. Species abbreviations were listed in Table 3.4

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 3.4).

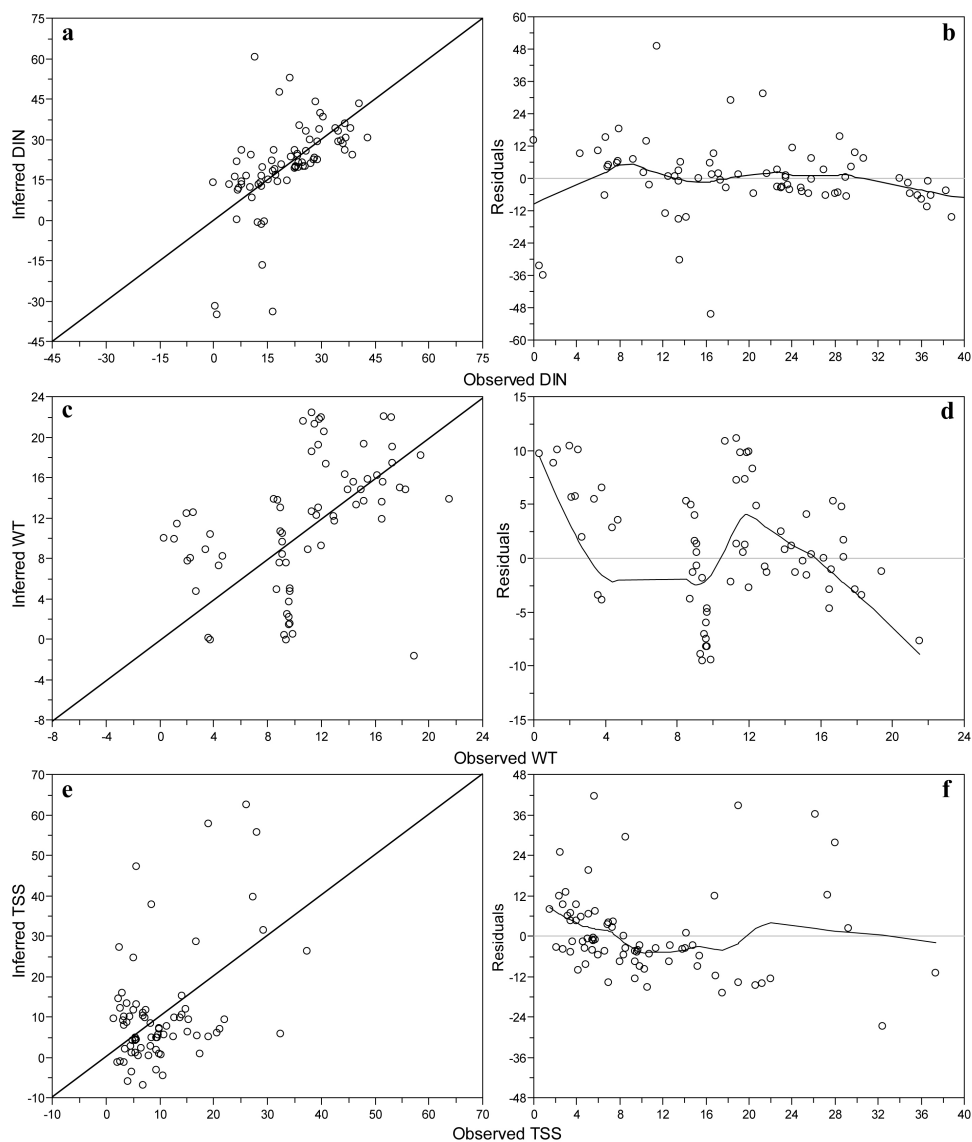


Figure 3.4: Observed DIN (mg/L), WT (°C) and TSS (mg/L) values at the 77 sites plotted against predicted values calculated from WA models. The right three graphs show that there is no bias in the residuals and the solid line shows a LOESS scatter plot smoother (span=0.45)

Table 3.4 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
<b>BACILLARIOPHYTA</b>								
<i>Achnanthydium minutissimum</i> (Kütz.)								
Czarnecki	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)								
Häkansson	Dist	36	3.75	8.26	<b>12.85</b>	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	Frer	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.								
Rumrich	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

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

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<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	<b>10.26</b>	5.43
<i>Planothidium lanceolatum</i> (Breb.) Round et Bukhtiyarova	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	<b>14.04</b>	7.22	13.78	3.82	17.11	14.06
<i>Ulnaria ulna</i> (Nitzsch) Comperé	Ulul	59	<b>11.92</b>	6.27	13.60	3.84	17.05	13.46
CHLOROPHYTA								
<i>Desmodesmus communis</i> (Hegew.) Hegew.	Deco	50	<b>2.31</b>	6.87	<b>12.63</b>	2.42	<b>25.80</b>	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	<b>12.22</b>	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	<b>9.14</b>	5.61
EUGLENOPHYTA								
<i>Euglena</i> sp.	Eusp.	43	<b>10.65</b>	7.73	<b>12.85</b>	2.87	23.85	13.38

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Table 3.5 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)

	$R^2_{\text{apparent}}$	RMSE <sub>apparent</sub>	$R^2_{\text{jackknife}}$	RMSE <sub>jackknife</sub>
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

### 3.4 Discussion

#### 3.4.1 Taxonomic composition

Our study showed phytoplankton community in the Kielstau catchment was a typical riverine diatom-dominated community and prevailed by species of *Achnanthes*, *Cocconeis*, *Cyclotella*, *Fragilaria*, *Navicula* and *Tabellaria*. Most genera observed in the Kielstau catchment were prostrate taxa, whose relative abundance was 47.23% (Table 3.3). Prostrate diatoms, which may indicate high grazing, or early diatom succession (Stevenson 1996), were predominant at lotic sites (50.46% vs. lentic sites 9.02%) suggesting a high biotic interaction here. It must be pointed out that 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 (Wu et al. 2011a) 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. (Centis et al. 2010) argued 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. At the meantime, we observed similar algal density and biomass at all the lotic sites, regardless of the influences by the lake. Consistent with Hötzel and Croome (Hötzel and Croome 1999), we now have confirmation that planktonic algal species do reproduce within rivers and many species develop substantial populations *in situ*. Therefore, we strongly appeal to look at the riverine phytoplankton from a new perspective rather than historical viewpoint.



### 3.4.2 Environmental variables influencing 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 nutrient [i.e. nitrogen (N) and phosphorus (P)] concentration of surface waters was a primary factor contributing to variation in phytoplankton assemblages (Unrein et al. 2010). These results were similar with the studies of Suikkanen et al. (Suikkanen et al. 2007), Burić et al. (Burić et al. 2007) and Zhou et al. (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 ecosystem (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 decrease the passage of light through water, 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 was an important factor shaping structure of phytoplankton assemblages in rivers (Leland et al. 2001, Leland 2003). However, Ha et al. (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 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. 3.3). Notwithstanding, the 4 CCA axes explained only 32.7% (axis 1: 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.

### 3.4.3 Phytoplankton 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. 3.4b, 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 3.5), which indicated the models were reliable.

The lower  $R^2_{\text{jackknife}}$  may be caused by the relative higher dataset number compared to other studies, for example, Leland and Porter (Leland and Porter 2000):  $n=28$ , Winter and Duthie (Winter and Duthie 2000):  $n=17$ . Reavie and Smol (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 performances 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 of these high optimum values for DIN (Table 3.4).

In general, our DIN, WT and TSS inference models were reliable in terms of their estimation of species optima, although 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 need of further monitoring in order to gain a better understanding of riverine phytoplankton and capitalize on the environmental indicator capacity of phytoplankton community. Poole (Poole 2010) convinced that integrations among ecology, hydrology, geomorphology and hydrogeology (namely "hydrogeomorphology") would be a basis for future 'Advancing Stream Ecology'. As many hydrological and morphological studies have been carried out, a combination between already existed hydrological surveys and hydrobiological data provide possibility of

further 'Advancing Stream Ecology'. Besides, our results may supply useful basic data for phytoplankton based bio-assessment in lowland areas (e.g. Q index, Chlorophyte-Index, Pennales-Index), which are not well developed as those of benthic diatom, macroinvertebrate and fish.

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

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### **Abstract**

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<sup>2</sup> 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<sup>2</sup> 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.

**Key Words:** Bioassessment; the European Water Framework Directive (WFD); Ecological status; Watershed management

### **4.1 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 (Blanco et al. 2007, Borics et al. 2007, Plenković-Moraj et al. 2007, 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.

## 4.2 Materials and methods

### 4.2.1 Description of the study area

The Kielstau catchment is a lowland watershed with a drainage area of 50 km<sup>2</sup>, 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. 4.1). 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. 4.1C) along the main stream Kielstau and its tributaries from November 2008 to May 2010. Ten sites (K01 to K10) were located along the main stream, three (M01 to M03) at the Moorau tributary and five (H01 to H05) at the Hennebach tributary. A total of 122 samples were collected.

### 4.2.2. 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 organisms 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 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 4.1).

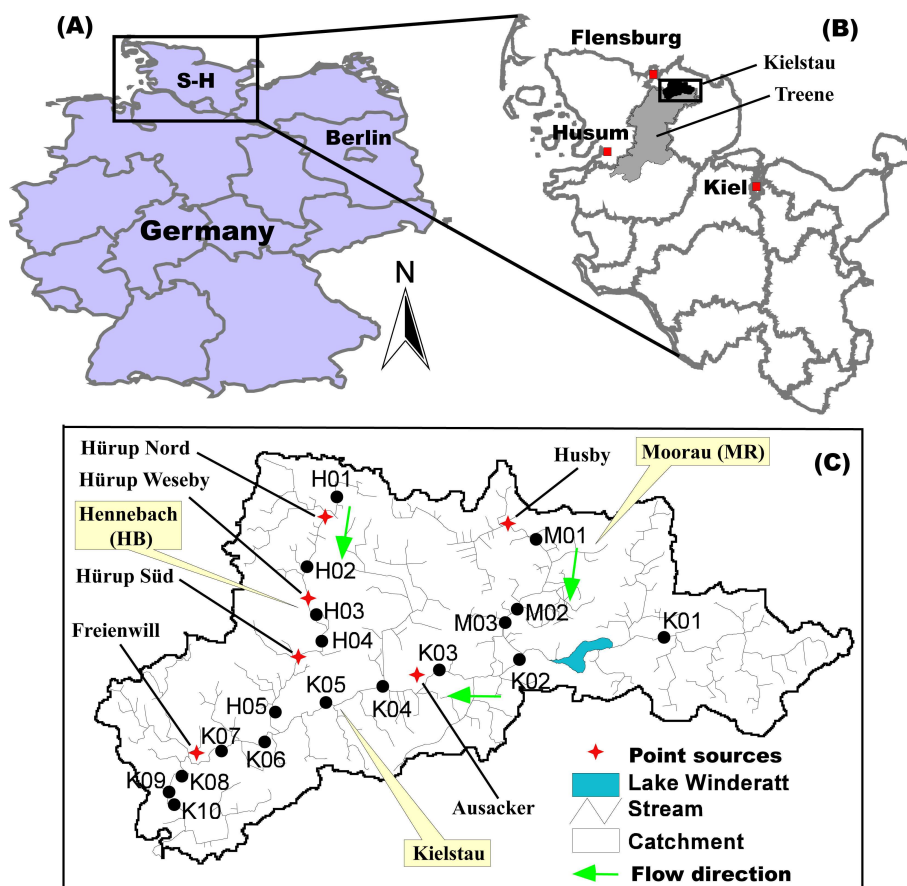


Figure 4.1: The location of the Kielstau catchment in Schleswig-Holstein state (B), Northern Germany [A; map source: CDC (CDC 2010)] and the sampling sites (C). S-H = Schleswig-Holstein state; M01-M03 = sampling sites collected from Moorau (MR) tributary; H01-H05 = sampling sites from Hennebach (HB) tributary; K01-K10 = sampling sites from main stream Kielstau

At each site, water samples were also collected for further laboratory analysis including ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ), chloride ( $\text{Cl}^-$ ), dissolved silicon ( $\text{Si}$ ), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ), orthophosphate-phosphorus (SRP), sulphate ( $\text{SO}_4^{2-}$ ), 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 4.1). Dissolved inorganic nitrogen (DIN) was defined as the sum of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$ , and N:P was calculated by  $\text{DIN}:\text{TP}$ . 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 (APHA 1992).

Table 4.1 Environmental variables and their codes sampled at Kielstau catchment and methods used

Environmental variables	Codes	Methods
Ammonium-nitrogen (mg/L)	NH <sub>4</sub> -N	Nessler's reagent colorimetric method (at 690 nm; DIN 38 406-E5-1)
Channel width (m)	Width	Measured with scale in the field
Chloride (mg/L)	Cl <sup>-</sup>	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 <sub>4</sub> -N, NO <sub>3</sub> -N and NO <sub>2</sub> -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 <sub>3</sub> -N*	Ion chromatography method (DIN38 405-D19)
Nitrite-nitrogen (mg/L)	NO <sub>2</sub> -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 <sub>4</sub> <sup>2-</sup> *	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 <sub>2</sub> S <sub>2</sub> O <sub>8</sub> ) and boric acid (H <sub>3</sub> BO <sub>3</sub> )
Total suspended solid (mg/L)	TSS	Standard Operating Procedure for Total Suspended Solids Analysis (U.S. EPA 1997)
Water temperature (°C)	WT	Portable Meter (WTW Multi 340i, Weilheim, Germany)

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



### 4.2.3. 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<sup>-3</sup>) (Hillebrand et al. 1999).

### 4.2.4. 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 firstly 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 et al. 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. (Blanco et al. 2007):

$$\text{Cumulative } R^2 = \sum_1^y r_{s,y}^2 \quad (1)$$

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  $y$ .

$$\text{CoI} = (\text{Cumulative } R^2 \bullet S) / n^2 \quad (2)$$

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.

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 90<sup>th</sup>, 75<sup>th</sup>, 50<sup>th</sup> and 25<sup>th</sup> 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  $< 25^{\text{th}}$ ,  $25^{\text{th}} - 50^{\text{th}}$ ,  $50^{\text{th}} - 75^{\text{th}}$ ,  $75^{\text{th}} - 90^{\text{th}}$  and  $> 90^{\text{th}}$  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  $< 25^{\text{th}}$ ,  $25^{\text{th}} - 50^{\text{th}}$ ,  $50^{\text{th}} - 75^{\text{th}}$ ,  $75^{\text{th}} - 90^{\text{th}}$  and  $> 90^{\text{th}}$  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).

#### **4.2.5. 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. 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).

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).

## **4.3 Results**

### **4.3.1 Metric selection and training the phytoplankton index of biotic integrity (P-IBI)**

The final selected 11 environmental variables (Table 4.1) 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<sub>4</sub>-N ranged from 0.02 to 8.48 mg/L (mean: 1.07 mg/L), NO<sub>2</sub>-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<sup>-</sup> 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<sup>2</sup>, 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 4.2), 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<sup>-</sup> ( $r_s = -0.624$ ,  $P < 0.001$ ). The six metrics were also highly correlated with the similar environmental variables as the P-IBI (Table 4.3).

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

Cumulative\_R<sup>2</sup> and CoI values in the testing data set decreased compared with values in the training data set (except for Chl *a*; Table 4.4). 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<sup>2</sup> and CoI at both training and testing data sets (Table 4.4).

Among the 11 environmental variables used in the final CCA, seven parameters (Width, Si, NO<sub>2</sub>-N, pH, DIN, WT and Cl<sup>-</sup>) 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<sup>-</sup> and represented 18.0% variance of species data. CCA axis 2 explained 13.2% of the variation indicated by pH, WT and Si (Fig. 4.2). All the six metrics and P-IBI, except for CyI\_density and 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. 4.2). 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. 4.2).

Table 4.2 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 <i>a</i>	+	<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).

Table 4.3 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 <sub>4</sub> -N	NO <sub>2</sub> -N	DIN	TP	Cl <sup>-</sup>	Si
Chl <i>a</i>	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 4.2. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001.

Table 4.4 Comparisons of correlation index values (CoI) and Cumulative\_R<sup>2</sup> between training and testing data sets for metrics or phytoplankton index of biotic integrity (P-IBI)

Metrics or P-IBI	CoI		Cumulative_R <sup>2</sup>	
	Training	Testing	Training	Testing
Chl <i>a</i>	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 4.2. 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).

Generally, the final P-IBI and its six metrics were indicative of the ecological status, as indicated by CCA results (Fig. 4.2) and comparisons with other single metrics (Table 4.4), and could be used for bioassessment of the study region, although Cumulative\_R<sup>2</sup> and CoI values declined in the testing data set.

### 4.3.3 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), and varied in different sampling seasons (Fig. 4.3). 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. 4.3A and Fig. 4.3B), most samples from four times (May 2009, August 2009, February 2010 and May 2010) were in ‘Moderate’ status (Fig. 4.3C, Fig. 4.3D, Fig. 4.3F and Fig. 4.3G). Most samples collected in November 2009 were only in ‘Low’ status (Fig. 4.3E).

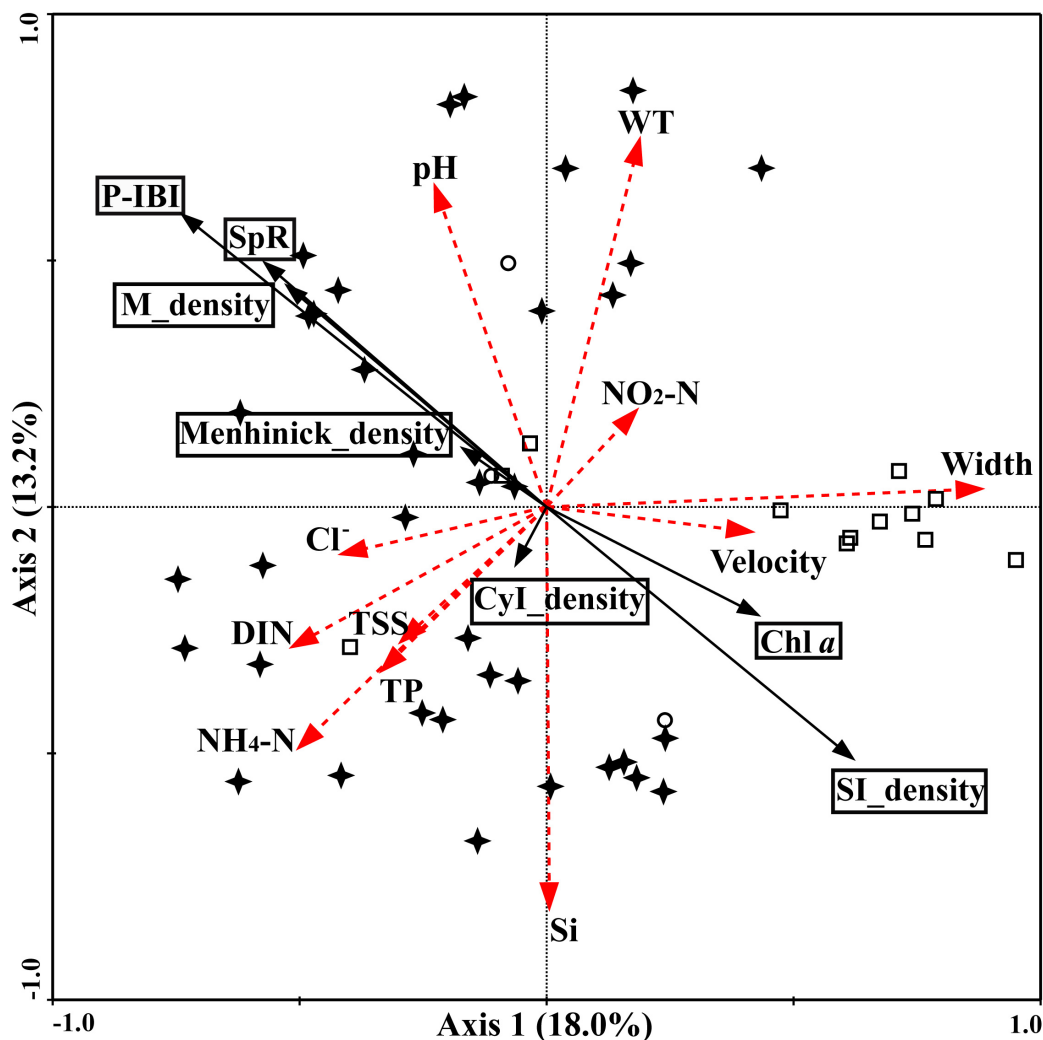


Figure 4.2: Canonical correspondence analysis of the 51 samples in the testing data set showed relationships among species assemblages, environmental variables and phytoplankton index of biotic integrity (P-IBI) metrics. Letters in boxes are metrics and P-IBI as passive variables in the analysis. Solid 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.



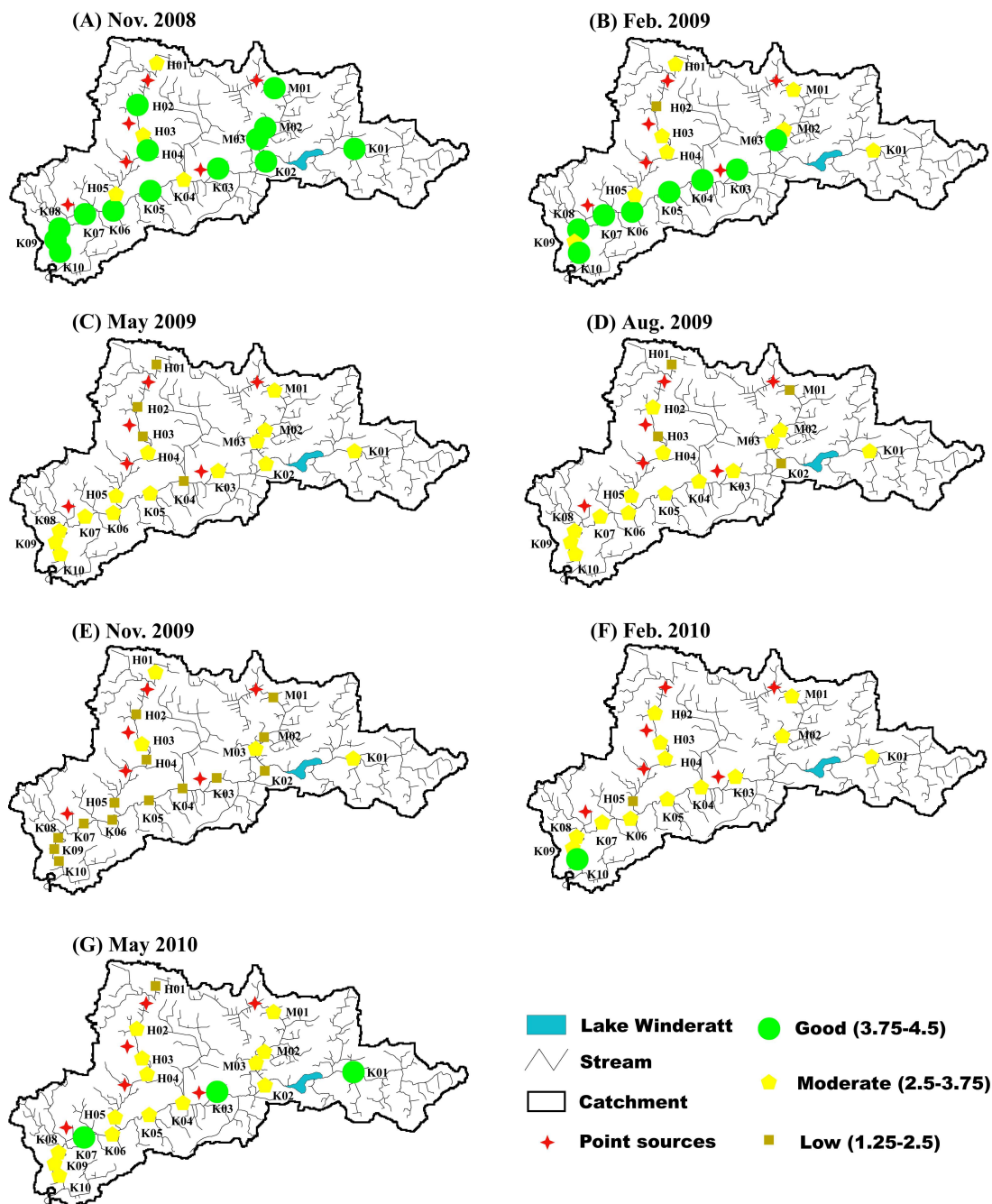


Figure 4.3: The spatial distribution of the final phytoplankton index of biotic integrity (P-IBI) scores of different samples in the training and testing data sets from (A) November 2008 to (G) May 2010. Stars are point sources as shown in Fig. 4.1. The thresholds of different ecological status are indicated 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.

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 (Fig. 4.3, Fig. 4.4 and Fig. 4.5). In addition, relative low influences of tributaries were found on the P-IBI scores of the main stream (Fig. 4.4 and Fig. 4.5), which probably was due to the much smaller discharges of the two tributaries (MR:  $\sim 0.08 \text{ m}^3/\text{s}$ , calculated from M03; HB:  $\sim 0.06 \text{ m}^3/\text{s}$ , calculated from H05) than that of the main stream ( $0.43 \text{ m}^3/\text{s}$ ) (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) (Fig. 4.4 and Fig. 4.5).

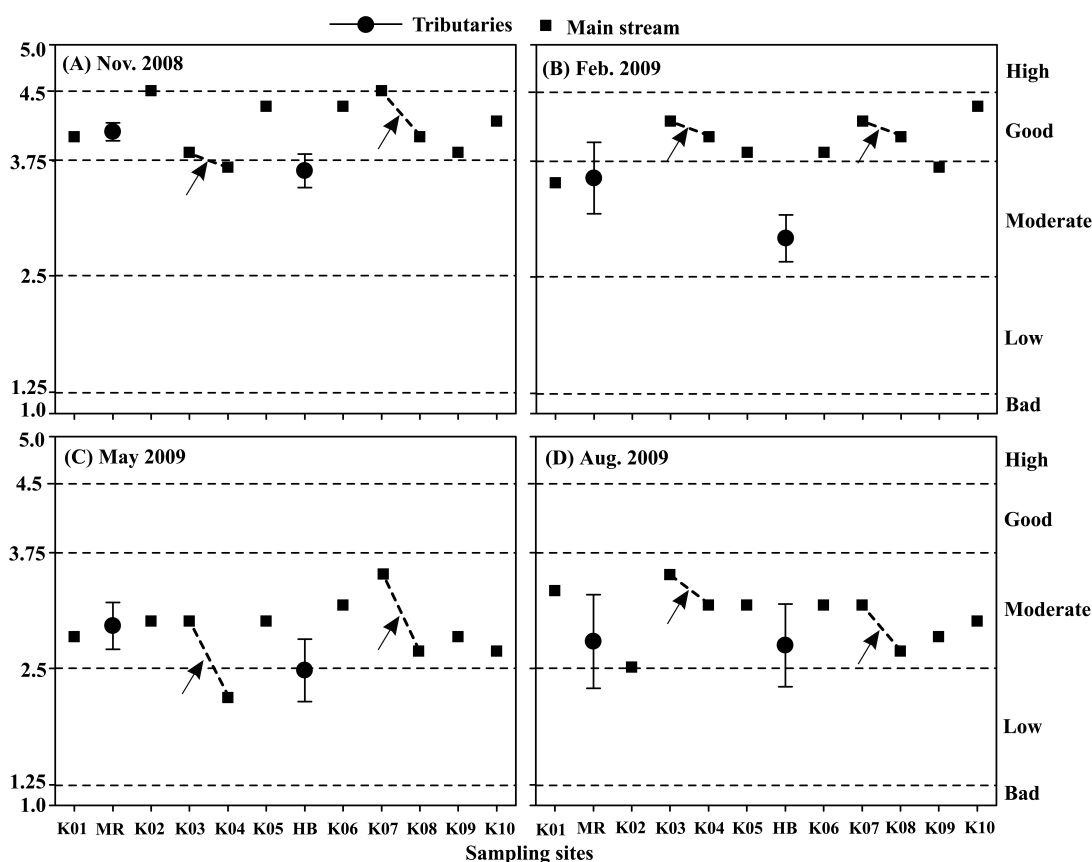


Figure 4.4: The final phytoplankton index of biotic integrity (P-IBI) scores of different samples in the training data set from (A) November 2008 to (D) August 2009. Black arrows indicated point sources of Ausacker (between K03 and K04) and Freienwill (between K07 and K08). Mean ( $\pm$ SD) scores of the tributaries were used and the thresholds of different ecological status were listed on the right.

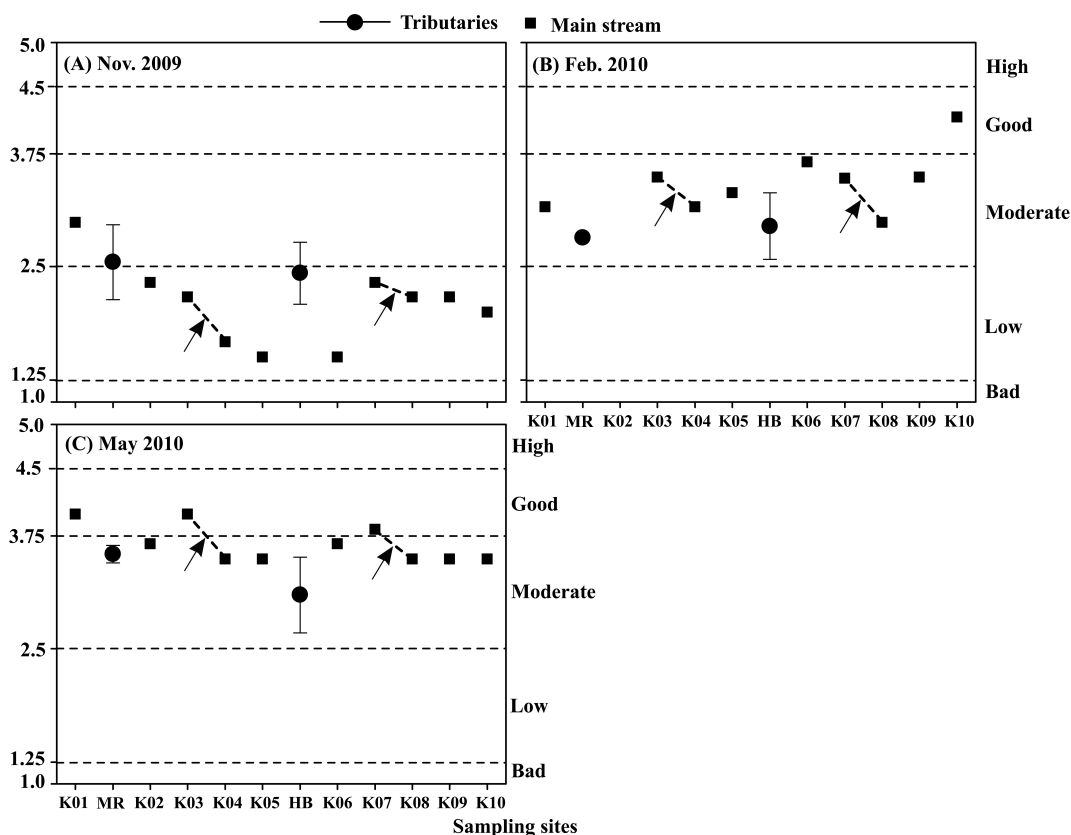


Figure 4.5: The final phytoplankton index of biotic integrity (P-IBI) scores of different samples in the testing data set from (A) November 2009 to (C) May 2010. Black arrows indicated point sources of Ausacker (between K03 and K04) and Freienwill (between K07 and K08). Mean ( $\pm$ SD) scores of the tributaries were used and the thresholds of different ecological status were listed on the right.

## 4.4 Discussion

### 4.4.1 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. (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. 2011b) 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 (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. 4.2, Table 4.4).

Multimetric indices have been criticized because they reduce data into a single number, Gerritsen (Gerritsen 1995), however, argued that data simplification is the goal of a multimetric index, and it's 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 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 an 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 4.4). 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. 4.1C, Fig. 4.3, Fig. 4.4 and Fig. 4.5).

As an evaluation it is worth mentioning the weaknesses of the P-IBI and further applications to other environments. Firstly, 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. (Wang et al. 2005). Secondly, 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. Thirdly, 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). Fourthly, 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 study (Wu et al. 2011a) 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).

#### **4.4.2 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 (Fig. 4.3, Fig. 4.4 and Fig. 4.5), 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. 4.1C) resulted in a high mean  $\text{NH}_4\text{-N}$  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 (Fig. 4.3, Fig. 4.4 and Fig. 4.5). 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. 2011b). 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. 2011b) 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.

### **Acknowledgements**

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## **Chapter V Modeling daily chlorophyll *a* dynamics in a German lowland river using artificial neural networks and multiple linear regression approaches**

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### **Abstract**

Phytoplankton biomass is an important indicator for water quality, and predicting its dynamics is thus regarded as one of the important issues in the domain of river ecology and management. However, the vast majority of models in river systems focused mostly on flow prediction and water quality with very few applications to biotic parameters such as chlorophyll *a*. Based on a 1.5-y measured data set of chlorophyll *a* and environmental variables, we developed two modeling approaches [artificial neural networks (ANN) and multiple linear regression (MLR)] to simulate the daily chlorophyll *a* dynamics in a German lowland river. In general, the developed ANN and MLR models achieved satisfactory accuracy in predicting daily dynamics of chlorophyll *a* concentrations. Although some peaks and valleys were not predicted, the predicted and the observed data matched closely by MLR model with coefficient of determination ( $R^2$ ) and Nash-Sutcliffe efficiency (NS) of 0.53 and 0.53 for calibration period and 0.63 and 0.62 for validation period. Likewise, the results of ANN model also illustrated a good agreement between observed and predicted data during calibration and validation periods, which was demonstrated by  $R^2$  and NS values (0.68 and 0.68 for calibration period, 0.55 and 0.66 for validation period). We concluded that it was possible to predict the daily chlorophyll *a* dynamics in the German lowland river based on relevant environmental factors using either ANN or MLR models. ANN model is well suited for solving non-linear and complex problems, while MLR model can explicitly explore the coefficients between independent and dependent variables. Further studies are still needed to improve the accuracy of developed models. For watershed management, combined with an automatic water quality monitoring device and a telemetry system, the developed models can be used as a real-time forecasting system. The results of prediction can help to assess the future changes of ecological status and develop efficient water management policy.

**Key Words:** Artificial neural networks; Daily chlorophyll *a*; Multiple linear regression; Models; Watershed management



## 5.1 Introduction

As one of the autochthonous primary producers, phytoplankton serves as an important functional role within aquatic ecosystems, providing a significant resource for grazers and forming part of the basis of the food web in terms of energy and material input (Hötzel and Croome 1999). Due to their short life cycle, planktonic algae respond rapidly to environmental changes, and thus they are an ideal indicator for water quality (Domingues and Galvão 2007, Cabecinha et al. 2009, Wu et al. 2012b). The investigation of the phytoplankton community has thus become an important part of the overall water quality monitoring so far (Majaneva et al. 2009) and predicting freshwater phytoplankton dynamics is regarded as one of the important issues in the domain of river ecology and management (Jeong et al. 2008). The successful prediction by multivariate processes either for short or long terms of monitoring could drive the underlying mechanisms between phytoplankton and their environments. From the management decision-making point of view, Jeong et al. (2008) thought that if an accurate model for phytoplankton dynamics was reliable, forecasting would be possible with only phytoplankton data instead of monitoring a wide range of limnological changes, which usually has exorbitant costs. There has been an implicit desire among limnologists to be able to construct reasonable models that simulate the dynamics of phytoplankton populations successfully (Elliott et al. 2010) and many successful efforts have been undertaken in lake and reservoir systems (Jeong et al. 2001, Huang et al. 2012).

In practice, however, microscopic examination and counting of phytoplankton species in collected samples is time-consuming and requires extensive taxonomic experience by the investigators (Krienitz et al. 1996, Kasprzak et al. 2008). Chlorophyll *a* concentration, a proxy of phytoplankton biomass, is therefore monitored and simulated widely in reservoirs and lakes. Chlorophyll is the major light-absorbing pigment in green plants that absorbs sunlight to synthesize carbohydrates from CO<sub>2</sub> and water (photosynthesis). Chlorophyll *a* is used to indicate the amount of phytoplankton present in water bodies and commonly employed to assess nutrient enrichment of streams (Hill et al. 2000). As concluded by Kalff (Kalff 2002), the empirical relationships between phosphorus and chlorophyll *a* are well known and used to manage lakes, rivers and catchments all over the world.

Modeling in ecology has greatly increased in the past decade, and major advances have been made in terrestrial landscape ecology, including forestry and plant ecology (Waite et al. 2010). While advanced mechanistic models that include spatial and temporal processes have been developed for fluvial hydrology, the application of models in stream ecology at present are

primarily descriptive models and they are not as well developed as in many other research areas (Turak et al. 2011). Modeling the relations among chlorophyll *a* and various parameters will not only help researchers to understand and rank the importance of different linkages but has the potential of the prediction of the condition for unsampled rivers. The latter aspect may be especially important for resource managers with the task of reporting on the overall condition of all rivers and streams in their responsibility. Most previous work on predictive models for chlorophyll *a* concentration were carried out in lentic waters such as reservoirs and lakes such as Kowe et al. (Kowe et al. 1998), Elliott et al. (Elliott et al. 2007), Jeong et al. (Jeong et al. 2008), Lu et al. (Lu et al. 2008), where long residence time and low flow velocity allow sufficient time for growth and reproduction (Torremorell et al. 2009, Wu et al. 2011b). However, in comparison, the vast majority of models in river systems focused mostly on flow prediction and water quality (e.g. Kiesel et al. 2010, Lam et al. 2010, Lam et al. 2011) with very few applications to biotic parameters such as chlorophyll *a* (Maier et al. 2010).

In this study, we monitored the daily dynamics of chlorophyll *a* concentrations and physicochemical parameters in a German lowland river – Kielstau catchment over a 1.5-y period (July 27, 2009 – December 31, 2010). The objective of this study was to develop and compare two models: artificial neural networks (ANN) and multiple linear regression (MLR), to simulate the daily chlorophyll *a* dynamics for the purpose of water quality forecasting and watershed management.

## 5.2 Methods

### 5.2.1. Description of the study area

The Kielstau catchment, an UNESCO demosite for Ecohydrological since 2010, is a lowland watershed with a drainage area of 50 km<sup>2</sup>, and located in the Northern part of Germany (Schleswig-Holstein). 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. 5.1). 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). Moorau (MR) and Hennebach (HB) are two main tributaries within the Kielstau catchment. There are six wastewater treatment plants within the Kielstau watershed: Ausacker, Freienwill, Husby, Hürup Nord, Hürup Weseby and Hürup Süd (Fig. 5.1C). Ausacker and Freienwill are located on the main stream with 1880 and 350 population equivalents (PE), respectively. Husby is situated at the beginning of the Moorau tributary with 3000 PE. Hürup Nord, Hürup Weseby

and Hürup Süd are located along the longitudinal Hennebach tributary (461, 447 and 240 PE, respectively). In addition, the drained fraction of agricultural area in the Kielstau catchment is estimated 38% (Fohrer et al. 2007) and various small tributaries and water from drainage pipes and ditches flow into the river Kielstau. Besides, agricultural practices, which are the dominant contributor of diffuse sources in the Kielstau catchment (Lam et al. 2010), have strong influences on nutrient loads and water quality, leading to the high dissolved inorganic nitrogen and total phosphorus concentrations. The combination of these point sources, artificial drainage systems and diffuse sources have influenced instream water quality considerably (Wu et al. 2012b). Sandy, loamy and peat soils are characteristic for the catchment. Land use is dominated by arable land and pasture (~55% and ~26%, respectively, of the catchment area) (Fohrer et al. 2007, Schmalz et al. 2008b).

### 5.2.2. Data aggregation and measuring methods

The Soltfeld gauging station, installed at the outlet of the Kielstau catchment (Fig. 5.1C), is a part of the official gauging network of the Federal State Schleswig-Holstein. Close to the gauging station is the automatic device for the collection and analysis of mixed daily chlorophyll *a* (Chl *a*) samples, which were taken during the period from July 27, 2009 to December 31, 2010. With the automatic device (Fig. 5.1D), mixed daily water samples were collected and taken back once a week (normally on Monday) to the lab of the Department of Hydrology and Water Resources Management of Kiel University. In the lab, the water samples were divided into several parts, one of which was for Chl *a* measurement while others were used for water quality analyses, which included ammonium-nitrogen (NH<sub>4</sub>-N), chloride (Cl<sup>-</sup>), nitrate-nitrogen (NO<sub>3</sub>-N), nitrite-nitrogen (NO<sub>2</sub>-N), orthophosphate-phosphorus (SRP), sulphate (SO<sub>4</sub><sup>2-</sup>) and total phosphorus (TP) (Table 5.1). Dissolved inorganic nitrogen (DIN) was defined as the sum of NH<sub>4</sub>-N, NO<sub>3</sub>-N and NO<sub>2</sub>-N. Additionally, the average daily discharge data (ADD) (LLUR, 2011), as well as daily precipitation (DP) and air temperature (AT) (DWD, 2011), were downloaded from this station or nearby gauging stations (if it is not available at Soltfeld Soltfeld gauging station) (Table 5.1). For Chl *a* determination, a known volume of sampled water (normally 1 L) was filtered through WHATMAN GF/C glass-fiber filter, and was measured spectrophotometrically following 90% acetone extraction according to APHA (APHA 1992). Sampling was not possible on 96 dates because of the lower temperature or other technical factors, and a total of 426 samples were collected.

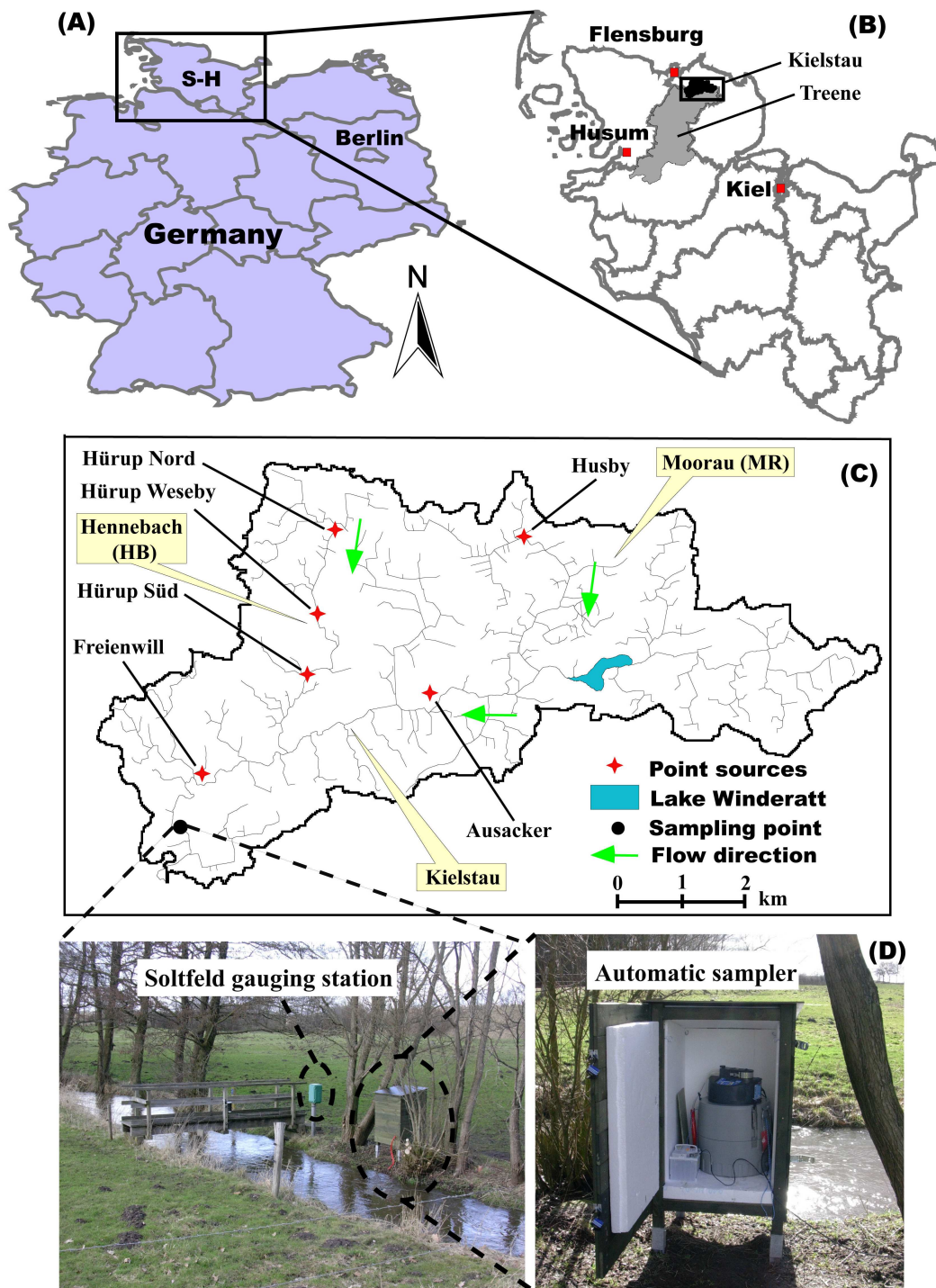


Figure 5.1: The location of the Kielstau catchment (C) in Schleswig-Holstein state (B), Northern Germany (A; map source: CDC, 2010) and two photos of the Soltfeld gauging station and automatic sampler for daily chlorophyll *a* samples (D). S-H = Schleswig-Holstein state

Table 5.1 Variables and their codes and methods used in this study

Variables	Codes	Methods
Air temperature (°C)	AT	Downloaded from a nearby gauging station (Meierwik) (DWD, 2011)
Ammonium-nitrogen (mg/L)	NH <sub>4</sub> -N	Nessler's reagent colorimetric method (at 690 nm; DIN 38 406-E5-1)
Average daily discharge (m <sup>3</sup> /s)	ADD	Downloaded from the Soltfeld gauging station (LLUR, 2011)
Chloride (mg/L)	Cl <sup>-</sup>	Ion chromatography method (DIN38 405-D19)
Chlorophyll <i>a</i> concentration (µg/L)	Chl <i>a</i>	Spectrophotometrically following 90% acetone extraction according to APHA (1992)
Daily precipitation (mm/d)	DP	Downloaded from nearby gauging stations (Satrup and Schuby) (DWD, 2011)
Dissolved inorganic nitrogen (mg/L)	DIN	Sum of NH <sub>4</sub> -N, NO <sub>3</sub> -N and NO <sub>2</sub> -N
Nitrate-nitrogen (mg/L)	NO <sub>3</sub> -N	Ion chromatography method (DIN38 405-D19)
Nitrite-nitrogen (mg/L)	NO <sub>2</sub> -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)
Sulphate (mg/L)	SO <sub>4</sub> <sup>2-</sup>	Ion chromatography method (DIN38 405-D19)
Total phosphorus (mg/L)	TP	Ammonium molybdate spectrophotometric method (at 880 nm; DIN 1189)

### 5.2.3. Multiple linear regression (MLR) modeling approach

The MLR modeling approach, conducted by SPSS 11.5, was used to determine the linkage between Chl *a* and environmental variables. All the 426 days were firstly classified into either training or test sets. Each tenth day was designated as test set, i.e. the tenth day, the twentieth day, the thirtieth day, and so on. We thus had a training set of 384 dates while a test set of 42 dates. The dependent variable was Chl *a*, while independent variables were 11 environmental parameters (Table 5.1) and autoregressive chlorophyll *a* concentrations (Auto-Chl *a*). Different linear methods (Amiri and Nakane 2009) (enter, stepwise, remove, backward and forward) were applied to a training set in order to achieve a best fit model for daily Chl *a* dynamics. The appropriate model was selected based on regression statistics (higher  $R^2$  and lower *p* value). Finally, the goodness-of-fit of the selected regression model was evaluated by the coefficient of determination ( $R^2$ ) and Nash-Sutcliffe efficiency (NS) (Nash and Sutcliffe 1970).

Several statistics, including sum of squared errors (SSE), the root mean square error (RMSE),  $R^2$  and NS, have been commonly used to evaluate the accuracy of model predictions (Nash and Sutcliffe 1970, Maier et al. 2010). In this study,  $R^2$  and NS were selected.  $R^2$  is the square of the Pearson's correlation coefficient, which is an indicator of strength of relationship between measured and simulated values. NS has been widely used to evaluate the performance of hydrologic models. The values of NS and  $R^2$  range from 0 to 1, respectively, with high values indicating better performance of the model. In general, according to Santhi et al. (Santhi et al. 2001) and Moriasi et al. (Moriasi et al. 2007), model simulation can be judged as satisfactory if a value greater than 0.5 for these variables.

### 5.2.4. Artificial neural networks (ANN) modeling approach

For ANN modeling, a three-layer back-propagation neural network, performed by MATLAB 7.5.0 (R2007b), was applied to simulate the daily Chl *a* dynamics. ANN models simulate the cognitive process of the human brain and are well suited for solving non-linear and complex problems (Neyamadpour et al. 2009). Fig. 5.2 shows a schematic diagram of the ANN architecture, which consists of an input, a non-linear mapping processing component (hidden layer) and an output. ANN has become a popular and useful tool for modeling environmental systems because of the ability to find non-linear patterns in data (Amiri and Nakane 2009). The processing elements in the network, called neurons, were arranged in a layered structure. In this study, the input layer constituted twelve neurons corresponding to independent variables in

MLR modeling approach, and the output layer included a single neuron relating to the dependent variable (Chl *a*). The layer between the input and output layers is referred as the hidden layer. The network parameters, such as the node number of the hidden layer and the number of hidden layer, were determined empirically by testing various combinations and selecting the optimal one (Geman et al. 1992, Hou et al. 2004). Some network properties were as follows: the number of nodes in hidden layer was 6; maximum iteration number was 1000; training algorithm was Levenberg-1 Marquardt backpropagation algorithm (mentioned as “trainlm” in the ANN code). The detailed introduction and calculation of ANN could be found at Hou et al. (Hou et al. 2004), Amiri and Nakane (Amiri and Nakane 2009) and Maier et al. (Maier et al. 2010). In the present study, the data were also classified as either training or test set, which was done in the same manner as the MLR modeling approach. Likewise,  $R^2$  and NS were employed to evaluate the performance of the ANN model.

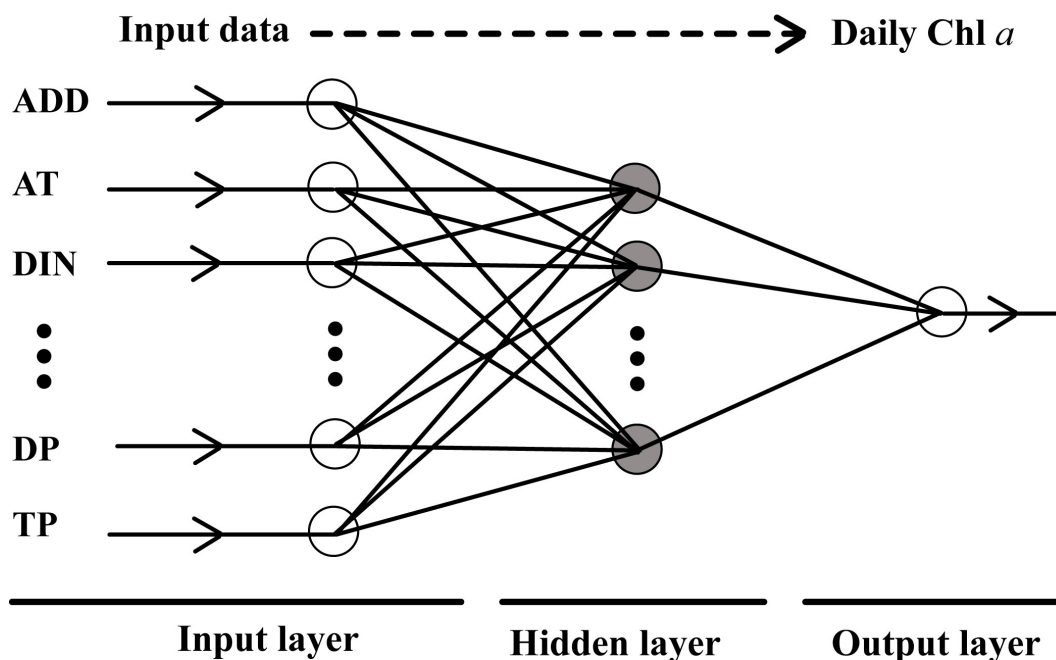


Figure 5.2: Schematic diagram of an artificial neural network (ANN) architecture applied in this study showing input, hidden and output layers. Circles are neurons. Abbreviations (codes) of variables as in Table 5.1

## 5.3 Results

### 5.3.1 Dynamics of chlorophyll *a* (Chl *a*) and environmental variables

The basic statistics of the environmental variables and Chl *a* used in this study from July 27, 2009 to December 31, 2010 were presented in Table 5.2. Similar with the environmental variables, the daily dynamics of Chl *a* showed high temporal variability with a mean value of 4.04 µg/L and ranged from 0.02 to 22.05 µg/L. The monthly average Chl *a* concentrations varied significantly from January to December (Table 5.3) (one-way ANOVA test:  $F=11.86$ ,  $p=0.000$ ). In September, October, November, April and May, higher Chl *a* concentrations were observed while lower values were found in February, June, July, August and December. The monthly changes of environmental variables were also remarkable ( $p<0.05$ ) (not shown).

Table 5.2 The statistic summary of the environmental variables and chlorophyll *a* used in this study (n=426)

Variables	Mean	Median	Std. Deviation	Minimum	Maximum
AT (°C)	10.21	10.50	6.31	-7.70	25.40
NH <sub>4</sub> -N (mg/L)	0.23	0.12	0.33	0.00	1.92
ADD (m <sup>3</sup> /s)	0.48	0.25	0.51	0.04	2.90
Cl <sup>-</sup> (mg/L)	27.14	26.62	6.25	13.66	78.46
Chl <i>a</i> (µg/L)	4.04	2.82	3.95	0.02	22.05
DP (mm/d)	2.85	0.20	5.02	0.00	29.40
DIN (mg/L)	4.61	4.38	2.02	0.76	11.65
NO <sub>3</sub> -N (mg/L)	4.33	4.08	1.90	0.77	11.25
NO <sub>2</sub> -N (mg/L)	0.05	0.00	0.08	0.00	0.45
SRP (mg/L)	0.13	0.11	0.07	0.03	0.46
SO <sub>4</sub> <sup>2-</sup> (mg/L)	35.68	35.04	9.20	17.29	85.36
TP (mg/L)	0.22	0.20	0.11	0.06	1.32

Note: Variable abbreviations (codes) and order as in Table 5.1.

### 5.3.2 Statistic MLR model and performance

Based on regression statistics ( $R^2$  and  $p$  value), the “backward” MLR method was used to represent the linkage between Chl *a* and independent variables. The result of MLR based Chl *a* model was summarized in Table 5.4. In the Chl *a* regression model ( $R^2 = 0.53$ ,  $p < 0.001$ ), 53%



of total variations in Chl *a* could be explained by the change of the four selected variables (Auto-Chl *a*, ADD, Cl<sup>-</sup> and TP) (Table 5.4). Fig. 5.3 showed the predicted and observed daily Chl *a* concentrations at the Soltfeld gauging station generated by MLR modeling approach. On the whole, the predicted results and the observed data matched closely, although some peaks and valleys were not predicted. The linear regression between predicted and observed data found that the R<sup>2</sup> and NS values were 0.53 and 0.53 for calibration period, while 0.63 and 0.62 for validation period.

Table 5.3 The statistic summary of the chlorophyll *a* (Chl *a*) concentrations in different months

Month	Count (n)	Mean (µg/L)	Median (µg/L)	Std. Deviation	Minimum (µg/L)	Maximum (µg/L)
Jan	21	2.98	2.13	4.36	0.23	20.83
Feb	7	0.90	0.50	0.86	0.23	2.39
Mar	29	3.49	1.65	3.48	0.49	13.20
Apr	29	5.97	5.61	2.04	3.14	11.43
May	28	6.31	5.40	3.00	0.81	12.28
Jun	30	1.82	1.70	0.94	0.54	5.00
Jul	32	1.98	1.67	0.94	0.72	4.09
Aug	61	2.02	0.98	2.27	0.02	9.03
Sep	58	5.05	2.60	5.60	0.71	22.05
Oct	59	5.51	4.30	3.50	0.75	16.33
Nov	47	6.63	5.09	4.83	0.47	17.74
Dec	25	1.21	0.89	0.82	0.15	3.21

Table 5.4 Results of multiple linear regression (MLR) based Chl *a* model using the training data set

Variable		Unstandardized Coefficients	Statistics	
Dependent	Independent		R <sup>2</sup>	p value
Chl <i>a</i>	Constant	3.242	0.53	0.000
	Auto-Chl <i>a</i>	0.674		
	ADD	0.056		
	Cl <sup>-</sup>	-0.055		
	TP	-2.723		

Note: Regression method is “Backward”.

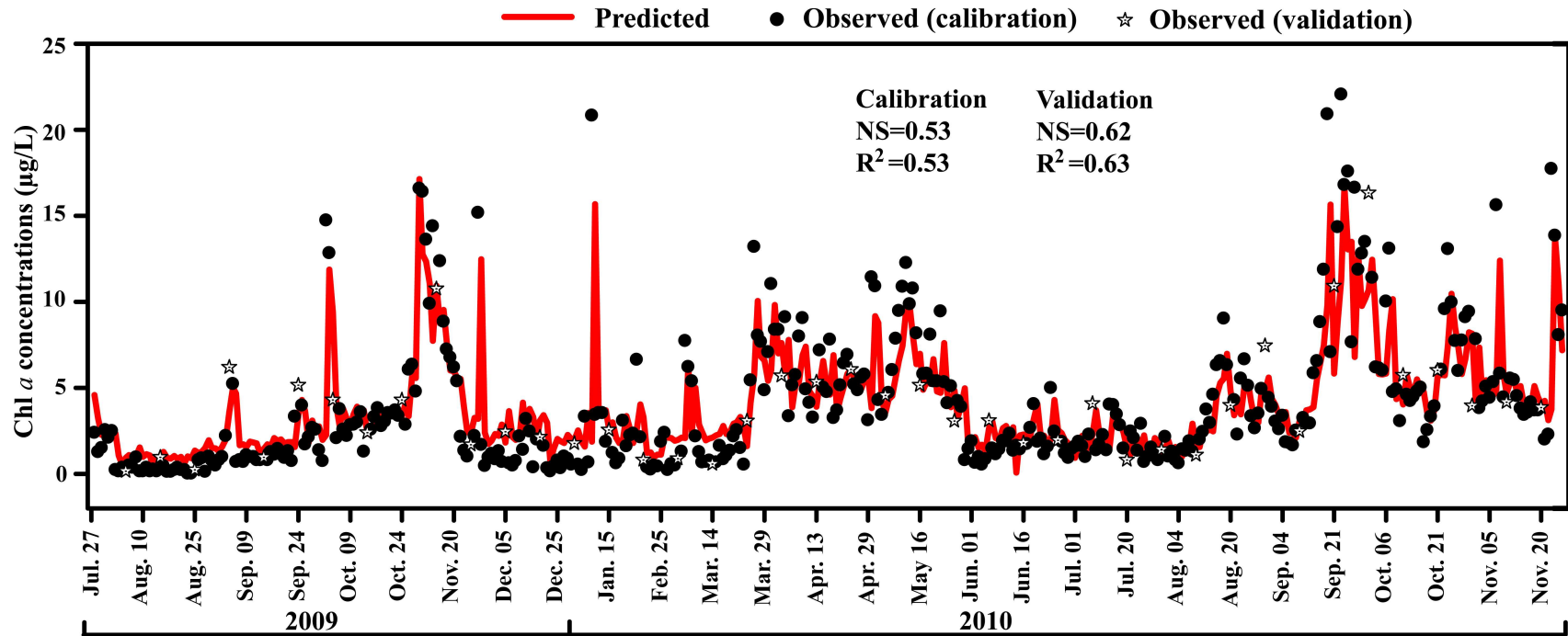


Figure 5.3: Predicted and observed daily chlorophyll *a* (Chl *a*) concentrations at the Soltfeld gauging station generated by multiple linear regression (MLR) approach

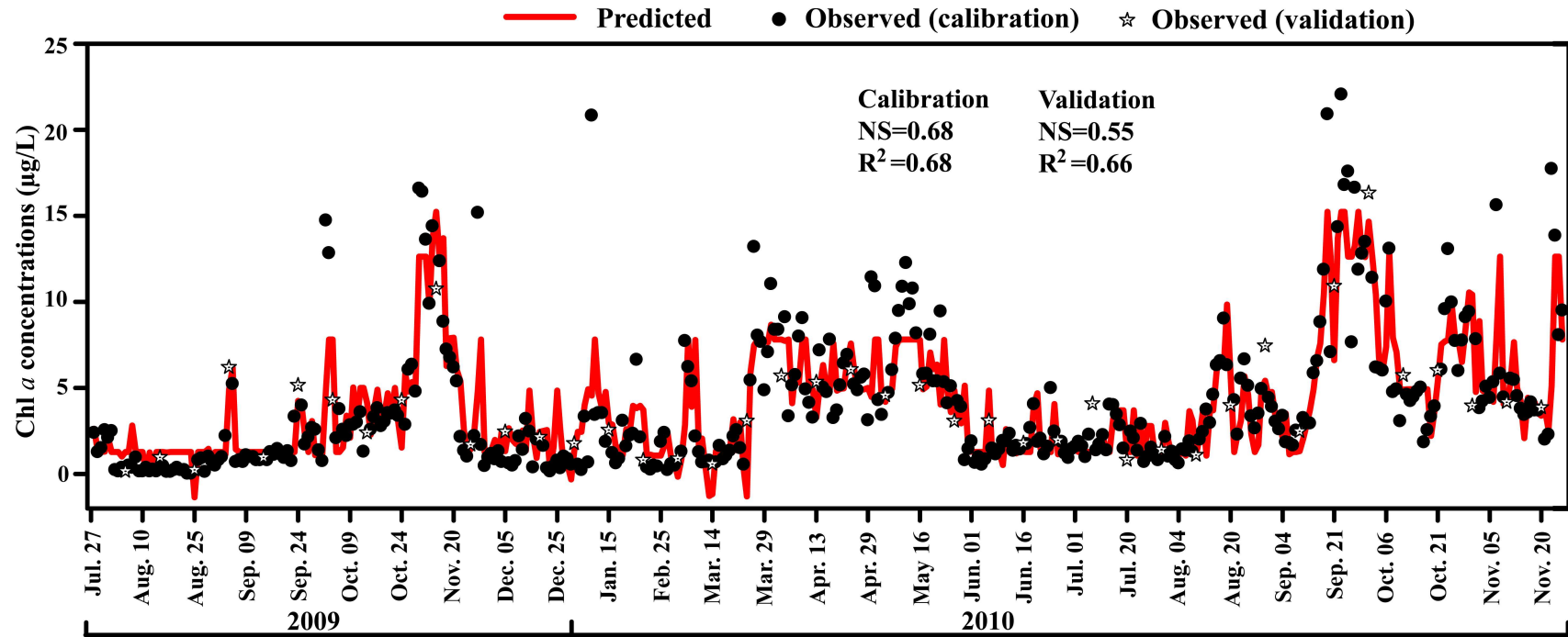


Figure 5.4: Predicted and observed daily chlorophyll *a* (Chl *a*) concentrations at the Soltfeld gauging station generated by artificial neural network (ANN) approach

### 5.3.3 ANN modeling approach and predictability

The results of ANN model also illustrated a good agreement between observed and predicted daily Chl *a* for both calibration and validation at the Soltfeld gauging station (Fig. 5.4). This was demonstrated by the correlation coefficient ( $R^2 = 0.68$ ) and Nash-Sutcliffe efficiency (NS = 0.68) values during the calibration period. For validation, the NS and  $R^2$  values were calculated to be 0.55 and 0.66 respectively, though relatively lower, indicating acceptable agreement between observed and predicted data according to Santhi et al. (2001) and Moriasi et al. (2007).

## 5.4 Discussion

### 5.4.1 Performance of the developed models

By performing the artificial neural networks (ANN) and multiple linear regression (MLR) based models to simulate daily dynamics of Chl *a*, the results of this study were satisfactory in terms of  $R^2$  and NS (Fig. 5.3, Fig. 5.4), which were within acceptable ranges (Santhi et al. 2001, Moriasi et al. 2007). However, compared to most previous studies (e.g. Jeong et al. 2008, Amiri and Nakane 2009, Ye and Cai 2009), the present results exhibited relatively lower  $R^2$  and NS at both calibration and validation periods. For example, Jeong et al. (2008) used temporal autoregressive recurrent neural networks (TARNN) to model freshwater phytoplankton dynamics, and they found that the TARNN models outperformed the statistical methods in accuracy of prediction ( $R^2 > 0.9$  for both seasonal and non-seasonal testing data sets). Amiri and Nakane (2009) employed ANN and MLR to simulate total nitrogen concentrations in Chugoku district of Japan and got accurate results between observed and predicted values ( $R^2 = 0.94$  and  $0.85$ , respectively, for ANN and MLR). Ye and Cai (2009) forecasted the chlorophyll *a* concentrations during a spring phytoplankton bloom in a bay of the Three-Gorges Reservoir by means of a recurrent artificial network and yielded  $R^2$  values of  $0.89$  and  $0.66$ , respectively, for validation periods of 0-day-ahead and 7-days-ahead models.

One possible reason of the relative lower  $R^2$  and NS is a lower Chl *a* concentrations than those of reservoir, lake and coastal waters, where most of the previous works were carried out. (Fielding 1999) insisted that many zero values would make statistical models insufficient in predicting or explaining the systems. Although there was no zero Chl *a* value in this study, the periods with low values were mostly over-estimated by both MLR and ANN models (Fig. 5.3, Fig. 5.4). Besides, the extremely high values were also not well captured. It is easier to predict

data with low variation close to the long-term mean than highly variable observations (Obach et al. 2001). During the study period, abrupt changes were commonly found in adjacent days (Fig. 5.3, Fig. 5.4), which, regardless of the reason, addressed a great challenge to be predicted. The inevitable errors induced during the process of Chl *a* (e.g. sampling, filtration and measurement) may create such abrupt changes that we can no longer ignore. Nevertheless, further investigations are needed to explore the reason for such phenomenon.

In addition, the complex nature of phytoplankton data sets could partially contribute to the lower  $R^2$  and NS. In such situation, process-based models may be a good choice to simulate Chl *a* accurately. However, for process-based models, we should take physical and biological processes that may influence phytoplankton dynamics into account. This requires data on all variables, which are often more difficult to obtain, such as phytoplankton growth (by solar energy flow and nutrient uptake) and loss (by zooplankton grazing, settling into sediment, respiration, mortality and excretion) (Huang et al. 2012). In rivers, phytoplankton succession is mainly determined by changes in hydrological, abiotic parameters (e.g. discharge, water residence time, climate, nutrients) and biotic factors (e.g. grazing, competition, parasitism) both in space and time (Pirsoo et al. 2008). Unfortunately, there is no general consensus as to which factors regulate phytoplankton community in lotic habitats (Basu and Pick 1995). Besides, contributions of different factors to phytoplankton variations are still unclear (Wu et al. 2011b). Previous to the development of process-based models, above outlined problems should be clarified. Moreover, MLR and ANN are data-driven models, and their performance relies heavily on the amount and quality of the data (Jeong et al. 2001). The restricted number of input variables may have limited the quality of the results. It is necessary to optimize the sampling rate and increase the number of variables, e.g. river morphology, predation pressure, since an increasing number of predictors may improve the results.

In general, however, our MLR and ANN models were reliable in terms of their estimation of daily Chl *a* concentrations, although had relative lower  $R^2$  and NS when compared to previous studies. Monitoring in ecology based on daily observation, to our knowledge, is rarely found, so information from the data would be valuable in the development of a systematic and robust model for Chl *a* prediction.

MLR models found that ADD,  $Cl^-$  and TP were important factors for daily Chl *a* dynamics. These results were not surprising since TP, as a major nutrient in rivers, was a primary factor contributing to variation in phytoplankton assemblages (Unrein et al. 2010), which were comparable with the studies of Suikkanen et al. (Suikkanen et al. 2007), Burić et al. (Burić et al.

2007) and Zhou et al. (Zhou et al. 2009b). As crucial physical and hydrological factors, impacts of CI and river discharge (ADD) on phytoplankton community have been widely proved (e.g. Brink et al. 1993, Kristiansen 1998, Tadashi et al. 2000).

#### **5.4.2 Comparison between ANN and MLR based models**

ANN is a powerful method that can be used to solve many complex problems in various fields (Hou et al. 2004) and proved to be very successful to predict noisy and non-linear ecological phenomena such as algal blooms (Paruelo and Tomasel 1997, Recknagel 1997, Recknagel et al. 1997, Teles et al. 2006, Ye and Cai 2009). MLR based models have repeatedly been applied to many fields for predictions e.g. cyanobacteria bloom dynamics (Jeong et al. 2003), tropospheric ozone concentrations (Sousa et al. 2007), phytoplankton dynamics (Jeong et al. 2008) and total nitrogen concentrations (Jeong et al. 2003, Amiri and Nakane 2009). Of the two models we developed, the ANN based model performed generally better than MLR model (Fig. 5.3, Fig. 5.4). Similar results were observed by Paruelo and Tomasel (Paruelo and Tomasel 1997), Jeong et al. (Jeong et al. 2003), Jeong et al. (Jeong et al. 2008) and Amiri and Nakane (Amiri and Nakane 2009). However, according to Santhi et al. (Santhi et al. 2001), we could conclude that it is possible to predict the daily Chl *a* dynamics in the study area based on relevant environmental factors using either ANN or MLR models.

It must be pointed out that the major shortcoming of ANN is the difficulty of interpreting relationship between input and output variables because it is a “black-box” model. Even an ANN able to make perfect predictions could not tell the relationship between the input and output variables. In contrast, the MLR model explicitly determined the coefficients of selected independent variables (see Table 5.4). However, the data-driven models ANN and MLR are able to make best use of existing data, which is an advantage over process-driven models (Maier et al. 2010). The best results may be obtained with specific combinations of linear and non-linear techniques, and this is probably an aim of future improvement. For example, by quadratic and logarithmic transformations, non-linear terms could be possibly incorporated in the regression models. Besides, the parameters of many models (e.g. data division, selection of input variables, architecture and structure, methods for calibration and validation) were empirically determined and different setting may induce distinct results. There was no suitable way to resolve above problem so far (Maier et al. 2010).

## 5.5 Conclusions and outlook

With the development of economy and increase of population, freshwater bodies are widely polluted and over-exploited. Rivers are therefore among the most threatened habitat types by human-generated pressures (Hering et al. 2006, Beyene et al. 2009). Awareness of these deleterious effects of human pressures on rivers has resulted in a long history of monitoring using biotic parameters, which integrate chemical and physical properties over time that could otherwise be missed by one-time water chemistry sampling (Winter and Duthie 2000). The assessment of properties and process of running waters has thus become a major issue in aquatic environmental management (Schleiter et al. 1999). However, as we outlined above, because the knowledge of species-habitat interactions remains insufficient, an integrative and in consequence, prognostic assessment of ecosystem properties is not presently available (Schleiter et al. 2006). The prediction of aquatic biotic parameters (such as chlorophyll *a*) with models is promising to jump over this step and has advantages if the relationships are unknown, very complex or non-linear. The development of models to predict biotic parameters is thus very useful because it can provide early warnings to the population and resource managers.

In recent years, the technology of database systems has been highly developed and is evolving in various ways. For instance, a consecutive automatic monitoring device could decrease sampling efforts and facilitate a denser sampling interval, which, combined with modeling and remote sensing, has created the possibility of on-line prediction systems (Iglesias et al. 2007, Pulido-Calvo et al. 2007). For phytoplankton prediction, Jeong et al. (Jeong et al. 2008) suggested that an automatic water quality monitoring device with a telemetry system could be adopted for remote sensing. Combined with an appropriate model, the data obtained would be used as a real-time forecasting system, which will enable monitoring efficiency to be maximized (Jeong et al. 2008). The results of prediction will then help to assess the future changes of ecological status and develop efficient water management policy.

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## Chapter VI Discussion and conclusion

### 6.1 Summary and key achievements

This dissertation was conducted in the lowland Kielstau catchment in North Germany, and aimed to develop a phytoplankton index of biotic integrity (P-IBI) that can assess effects of human disturbances on the ecological status of the lowland rivers, as well as to simulate the daily chlorophyll *a* dynamics for the purpose of water quality forecasting and watershed management. Prior to that, this study determined whether phytoplankton assemblages generated by the two sampling protocols were related to different environmental variables and therefore might yield different bioassessment. Also it investigated the relationships between phytoplankton and environmental variables, as well as compared which factors predominantly structure riverine phytoplankton communities. The main achievements are summarized as follows:

#### 6.1.1 A comparison of phytoplankton assemblages by two sampling protocols

Data collected from 77 sites by plankton net collection (PLNET) and sedimentation (SEDIM) protocols were analyzed. Median Bray-Curtis (BC) similarity between phytoplankton assemblages generated by the two protocols was 48.5% (range: 7.5-82.0%), and sites with the lowest BC similarities tended to have lower chlorophyll *a* (Chl *a*), water temperature (WT), total-suspended-solid (TSS) and volatile-suspended-solid (VSS), but higher channel width and water depth, than other sites with higher BC similarities. Reduced total algal density and biomass, but higher species richness, were observed by the PLNET protocol. However, overall phytoplankton assemblages generated by the two protocols were similar, as indicated by dominant species (paired t-test,  $p > 0.05$ ) and non-metric multidimensional scaling (NMDS) ordination. Nevertheless, from the phytoplankton-based bioassessment point of view, PLNET protocol was a better method compared with SEDIM protocol as indicated by ‘Correlation Index’ (CoI), Cumulative\_ $R^2$  and canonical correspondence analysis (CCA).

#### 6.1.2 Distribution of phytoplankton in relation to environmental factors

The spatial and temporal dynamics of phytoplankton community in a German lowland river and the relationships with environmental variables were investigated. Phytoplankton community in the Kielstau catchment was a typical riverine diatom-dominated community and prevailed by species of *Achnanthes*, *Cocconeis*, *Cyclotella*, *Fragilaria*, *Navicula* and *Tabellaria*. Among the 125 taxa observed, *Desmodesmus communis*, *Pediastrum duplex* and *Discostella steligera* were



the 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 phytoplankton community were observed by non-metric multidimensional scaling. 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 (WA) regression and cross-calibration produced strong models for predicting DIN, water temperature (WT) and total suspended solid (TSS), which enabled selection of algal taxa as potentially sensitive indicators.

### **6.1.3 Development of a phytoplankton index of biotic integrity (P-IBI)**

A phytoplankton index of biotic integrity (P-IBI) for a German lowland river was developed and tested, and it consisted of six metrics (out of 36 original metrics) based on Cumulative\_R<sup>2</sup> and correlation index (CoI) between biotic metrics and environmental variables. 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.

### **6.1.4 Modeling daily chlorophyll *a* dynamics in a German lowland river**

Based on a 1.5-y measured data set of chlorophyll *a* and environmental variables, two modeling approaches (artificial neural networks, ANN and multiple linear regression, MLR) were developed to simulate the daily chlorophyll *a* dynamics in the Kielstau catchment. In general, the developed ANN and MLR models achieved satisfactory accuracy in predicting daily dynamics of chlorophyll *a* concentrations. Although some peaks and valleys were not predicted, the predicted and the observed data matched closely by MLR model with coefficient of determination (R<sup>2</sup>) and Nash-Sutcliffe efficiency (NS) of 0.53 and 0.53 for calibration period and 0.63 and 0.62 for validation period. Likewise, the results of ANN model also illustrated a good agreement between observed and predicted data during calibration and validation periods, which was demonstrated by R<sup>2</sup> and NS values (0.68 and 0.68 for calibration period, 0.55 and 0.66 for validation period).

## **6.2 Conclusions**

From the phytoplankton-based bioassessment point of view, this study was consistent with

Kraatz (Kraatz 1940) and found that PLNET protocol was a better method compared with SEDIM protocol because algal data collected by PLNET protocol had higher relationship with environmental variables as indicated by 'Correlation Index' (CoI), Cumulative\_R<sup>2</sup> and canonical correspondence analysis (CCA).

Historically, it was believed there was no true riverine plankton and the algae found in rivers come from either upstream lentic waterbodies or the benthos (Hötzel and Croome 1999). However, this study (Wu et al. 2011b) 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 developed P-IBI and its six metrics were indicative for ecological integrity and water quality as indicated by canonical correspondence analysis and comparisons with other single metrics, although Cumulative\_R<sup>2</sup> and CoI values declined in the testing data set. This 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. Furthermore, the relative low ecological status in the study area ('Moderate' status) was probably caused by point sources, diffuse sources emissions and artificial drainage systems of the study area. In addition, an important finding was that the ecological status of rivers varied seasonally, which demonstrated that normal annual or 1-time sampling data should be improved to address seasonal variations.

It was possible to predict the daily chlorophyll *a* dynamics in the German lowland rivers based on relevant environmental factors using either artificial neural networks (ANN) or multiple linear regression (MLR) models. ANN model is well suited for solving non-linear and complex problems, while MLR model can explicitly explore the coefficients between independent and dependent variables. Further studies are still needed to improve the accuracy of developed models. For watershed management, combined with an automatic water quality monitoring device and a telemetry system, the developed models can be used as a real-time forecasting system. The results of prediction can help to assess the future changes of ecological status and develop efficient water management policy.

### 6.3 Outlook

This dissertation presented the results of a comparison between two sampling protocols, relations between phytoplankton community and environmental variables, development of a phytoplankton index of biotic integrity (P-IBI), as well as modeling daily chlorophyll *a* dynamics. Nevertheless, the works done by this study are only at the beginning stage of phytoplankton based assessment in German lowland rivers. To fully understand the behavior of phytoplankton community and their usage as bio-indicators, the following issues should be considered for further research and development:

- This study demonstrated that the PLNET protocol was a better method compared with the SEDIM protocol, but did not inform us whether the reaches had been sampled adequately. Further studies on the number of samples needed to characterize phytoplankton adequately in stream reaches with habitat heterogeneities will greatly benefit the phytoplankton based bioassessment in lowland rivers.
- Further monitoring is also needed to gain a better understanding of long-term variations of riverine phytoplankton and capitalize on the environmental indicator capacity of phytoplankton community. For example, changes in hydrology and suspended sediments, riparian vegetation composition, macroinvertebrate assemblages, as well as nutrient concentrations, will affect riverine phytoplankton dynamics. Moreover, specific landuse changes, such as conversion from forest land to agricultural uses or from agricultural land to suburban uses, can have multiple effects on instream organic matter dynamics, with potentially cascading effects on the aquatic food web (Tank et al. 2010). Understanding organism dynamics and resilience of river ecosystems in a changing landscape will continue to pose a challenge to biologists.
- 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). Besides, the sensitivity of the P-IBI and its metrics to discriminate water quality can be further enhanced by expanding to a larger catchment, since this study only used seasonal data instead of a large study area. Moreover, many of the metrics would be useful in other regions, but need further testing and assessment of their applicability.
- Although the developed ANN and MLR models achieved satisfactory accuracy in predicting daily dynamics of chlorophyll *a* concentrations, process-based models may be a better choice to simulate chlorophyll *a* accurately. This requires data on all variables

that influence phytoplankton growth (by solar energy flow and nutrient uptake) and loss (by zooplankton grazing, settling into sediment, respiration, mortality and excretion).

- An automatic water quality monitoring device with a telemetry system could be adopted for remote sensing. Combined with an appropriate model, the data obtained would be used as a real-time forecasting system, which will help to assess the future changes of ecological status and develop efficient water management policy.

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Naicheng Wu



## **Erklärung**

Hiermit erkläre ich, dass ich die vorliegende Dissertation, abgesehen von der Beratung durch meine akademischen Lehrer, selbstständig verfasst habe und keine weiteren Quellen und Hilfsmittel als die hier angegebenen verwendet habe. Diese Arbeit hat weder ganz, noch in Teilen, bereits an anderer Stelle einer Prüfungskommission zur Erlangung des Doktorgrades vorgelegen, und ist nicht veröffentlicht worden oder wurde zur Veröffentlichung eingereicht. Ich erkläre, dass die vorliegende Arbeit gemäß der Grundsätze zur Sicherung guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft erstellt wurde.

Kiel, Januar 2012

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