Transitions in diatom assemblages and pigments through dry and wet season conditions in the Red River, Hanoi (Vietnam)

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Background and aims – Biomonitoring is an important tool for assessing river water quality, but is not routinely applied in tropical rivers. Marked hydrological changes can occur between wet and dry season conditions in the tropics. Thus, a prerequisite for ecological assessment is that the influence of 'natural' hydrological change on biota can be distinguished from variability driven by water quality parameters of interest. Here we aimed to (a) assess seasonal changes in water quality, diatoms and algal assemblages from river phytoplankton and artificial substrates through the dry-wet season transition (February–July 2018) in the Red River close to Hanoi and (b) evaluate the potential for microscopic counts and high-performance liquid chromatography (HPLC) analysis of chlorophyll and carotenoid pigments for biomonitoring in large tropical rivers.

Methods – River water (phytoplankton) and biofilms grown on artificial glass substrates were sampled monthly through the dry (February–April) to wet (May–August) season transition and analysed via microscopic and HPLC techniques.

Key results – All phototrophic communities shifted markedly between the dry and wet seasons. Phytoplankton concentrations were low (c. thousands of cells/mL) and declined as the wet season progressed. The dominant phytoplankton taxa were centric diatoms (*Aulacoseira granulata* and *Aulacoseira distans*) and chlorophytes (*Scenedesmus* and *Pediastrum* spp.), with chlorophytes becoming more dominant in the wet season. Biofilm diatoms were dominated by *Melosira varians*, and areal densities declined in the wet season when fast-growing pioneer diatom taxa (e.g. *Achnanthidium minutissimum*, *Planothidium lanceolatum*) and non-degraded Chlorophyll a concentrations increased, suggesting active phytobenthos growth in response to scour damage. Otherwise, a-phorbins were very abundant in river seston and biofilms indicating *in situ* Chlorophyll a degradation which may be typical of tropical river environments. The very large range of total suspended solids (reaching > 120 mg/L) and turbidity appears to be a key driver of photoautotrophs through control of light availability.

Conclusions – Hydrological change and associated turbidity conditions exceed nutrient influences on photoautotrophs at inter-seasonal scales in this part of the Red River. Inter-seasonal differences might be a useful measure for biomonitoring to help track how changes in suspended solids, a major water quality issue in tropical rivers, interact with other variables of interest.

Key words – Chlorophyll and carotenoid pigments, diatom biofilm, water quality monitoring, tropical river, biomonitoring.

INTRODUCTION

Increasing pressure is being placed upon river systems due to the higher demand for water resources, following population growth and greater urbanization (Vörösmarty et al. 2010). Typical of many developing regions, the Red River Delta in northern Vietnam is undergoing rapid changes in industrialization, urbanization, agricultural expansion, irrigation and dam construction. This has led to environmental degradation of the Red River, including pollution by nutrients and toxins, and major changes to sediment loads (Le et al. 2007, 2015, 2018, Dang et al. 2010, Vinh et al. 2014). In such a context, the need for monitoring and assessment of water quality in Vietnam has been recognised, leading to recent developments in legislation (Nguyen 2013). The National Technical Regulation on surface water quality issued in 2015 (QCVN 08-MT:2015/BTNMT) specifies maximum limits for a wide range of water chemistry variables including nutrients and toxic pollutants. There is a Master Plan on the National Environmental and Resource Monitoring Network which aims to establish a uniform network and database for environmental and resource monitoring by 2020. However, whilst biological monitoring has been widely employed as a tool for water quality assessment in some nations, Chlorophyll a (Chl a) and coliforms are the only biotic variables currently included in the Vietnam surface water monitoring guidelines. The potential for the application of diatom-based biomonitoring has been demonstrated in the Red River Delta (Duong et al. 2012, 2014, Hoang et al. 2018), but empirical data linking diatom assemblages with environmental conditions are limited, and other algal monitoring techniques such as chlorophyll and carotenoid pigment analysis have not been explored.

In tropical regions changes between wet and dry monsoonal seasons influence river flows and the transfer of suspended solids and nutrients downstream (Latrubesse et al. 2005). Evaluation of ecological quality in regions of high hydrological variability must distinguish the ecological 'signal' from the 'noise' that results from the scale of variation occurring under 'natural' conditions. Understanding seasonal patterns in large rivers is therefore a necessary prerequisite to water quality assessment to place 'snapshot' measurements into context. Many large rivers develop phytoplankton, which is one potential source for biomonitoring and assessment of river conditions (Basu & Pick 1997, Reynolds 2000). Phytoplankton respond rapidly to hydro-chemical conditions and can be useful indicators of ecological condition in tropical rivers. Alternatively, sampling of biofilm assemblages on benthic (bottom) river habitats is useful as benthos reflect integrated conditions over a more extended period of time than 'snapshot' phytoplankton samples (Kelly & Whitton 1995). Because competition for both nutrients and light occurs between planktonic and benthic habitats, choice of sampling method may depend on the river conditions (Hansson 1988, Henry & Fisher 2003, Jäger & Diehl 2014). Large tropical rivers are often deep with silty substrates and very turbid waters. Here, approaches which sample *in situ* epilithon biofilms may be impractical due to the lack of available substrates (Kelly & Whitton 1995), but the use of artificial 'glass slide' substrates which can be suspended in the photic zone are more feasible (Duong et al. 2012).

Chlorophyll and carotenoid pigment analysis is an alternative to microscopy for rapid screening of the composition of river photoautotrophic protist communities. Analysis of pigments by high-performance liquid chromatography (HPLC) has been successfully used for tracking river phytoplankton blooms (Moorhouse et al. 2018). However benthic algal communities are most commonly enumerated via microscopy, often focusing on diatoms as they have well-established environmental preferences (Kelly & Whitton 1995). More recently, instruments such as the 'Bentho torch' have been used for rapid assessment of the biomass of phototrophic communities on river bottom substrates using fluorescence characteristics of biofilm pigments (Kahlert & McKie 2014). Some studies indicate a mismatch between microscopic and Bentho torch community biomass estimates, caused by difficulties in detecting fluorescence in deeper biofilm layers (Echenique-Subiabre et al. 2016). By sampling entire biofilms from artificial substrates, analysis of chlorophyll and carotenoid pigments should allow rapid screening of overall algal composition at a low taxonomic level using diagnostic biomarker pigments without the need for specialised field instruments (Buchaca & Catalan 2008). Such an approach is attractive in developing countries where cost effective techniques which use commonly available laboratory instruments (in this case HPLC) are required. To date, pigment analysis approaches to examine riverine phytoplankton and biofilms has been overlooked in large tropical river habitats.

The first aim of this paper was to evaluate the changes in phytoplankton, biofilm photoautotrophs from artificial substrates, and water quality parameters in the Red River, Vietnam by tracking shifts across the dry-wet season transition. Secondly, we aimed to evaluate the use of microscopic counts and HPLC analysis of chlorophyll and carotenoid pigments for biomonitoring in tropical river environments.

MATERIAL AND METHODS

Study area

The Red River has a transboundary watershed of 156 451 km² which is located c. 51% in Vietnam, 47% in China and 0.9% in Laos (fig. 1). Its headwaters lie in the mountainous area of Yunnan Province, China after which it flows 1200 km south-eastward, discharging through four distributaries, into the Gulf of Tonkin in the South Asian Sea at Ba Lat, Lach Gia, Tra Ly and Day. The Red River is formed by three large river branches: the Da, Lo and Thao (sometimes referred to as Hong) rivers. In the upper section of the Delta, the two major tributaries (the Da and the Lo rivers) join the main stream (Thao River) at Viet Tri city and form a large delta area, before discharging into the Tonkin Bay (Luu et al. 2010). The study site (red dot on fig. 1) is situated at the Chuong Duong bridge (Hanoi city), in the main branch of the Red River (21°02′N, 105°51′E) and after the confluence of these three tributaries (the Da, Lo and Thao rivers). The Red River is located within a tropical climatic zone with two clear seasons (dry and rainy season). Total annual rainfall is 1800–2000 mm, with 80% of rain falling during the rainy (wet) season of May to October. Concomitant with the period of highest rainfall, river discharge and suspended solid maxima are usually observed in the middle of the wet season (Le et al. 2007).

Data collection

Daily river discharge (from January to July 2018) were obtained from acoustic doppler current profiler (ADCP) measurements at Hanoi gauging station (21°02′N, 105°51′ E), located in the main channel of the Red River (unpublished data in 2018). Meteorological data (total monthly rainfall, average monthly temperature) at Lang station (Hanoi City 21°01′N, 105°48′E) are derived from a series of monthly publications in the Vietnam Journal of Hydro-Meteorology (2017–2018).

Sampling and laboratory measurements

Water samples were collected monthly from the river surface layer from January to July 2018 at Hanoi gauging station in the main stream Red River (fig. 1). Physicochemical variables (temperature, pH, dissolved oxygen (DO), turbidity and conductivity) were measured *in situ* using a multi-parameter probe (Model WQC-22A, TOA, Japan). Water samples were returned to the laboratory in a cooler (4°C). In the laboratory, a known volume of well-mixed sample was filtered immediately by vacuum filtration through a precombusted (105°C in 2h) glass fiber filter paper (Whatman GF/F, pore size 0.7μm). All filters and filtrates were kept in a freezer (-20°C) after filtration until laboratory analysis. Total suspended solids (TSS) were quantitatively determined (105°C, 2h) on pre-weighed filters (APHA 1995). Nutrients (N, P, Si) were analysed by colorimetric methods with spectrophotometry as described in Le et al. (2007). Chemical oxygen demand (COD) concentration was measured by standard methods and HCO_3^- concentration was determined by titration with 0.01 M HCl within 12 hours of sampling (APHA 1995).

The biofilm monitoring apparatus was deployed between 23 Feb. and 19 Jul. 2018. Glass slides (6 × 30 cm; total of 360 cm² on both sides) were used as artificial substrates for

Figure 1 – Map of the Red River system in Vietnam with the location of the sampling site indicated by the red dot. Map produced by using the 'hydrology' layer of the maps available at http://vrn.org.vn/ten-river-basins-in-vietnam/ and underlying Google API (©Google 2019). This image is not covered by the terms of the Creative Commons licence of this publication. For permission to reuse, please contact the rights holders (Vietnam Rivers Network, http://vrn.org.vn; and Google).

biofilm attachment. A plastic rack containing six vertical glass slides was immersed in the water column, parallel to the current at about 10–15 cm below the water surface and tied to the bank with a rope (see Duong et al. 2012 for details). After a one-month colonisation period, three replicate glass slides were randomly selected for removal from the rack, scraped into a Sterilin tube using a toothbrush, and washed into 100 mL of distilled water. The biofilm samples were then divided into two fractions for diatom identification (10 mL preserved with 37% formaldehyde solution) and pigment analysis (50 mL filtered through a GF/C filter and frozen prior to analysis).

For phytoplankton enumeration, 1L of river water was preserved with 10 mL Lugol's iodine solution and left to settle for 48 h prior to counting. Phytoplankton species were identified according to their morphology and live cells only were identified based on cell contents (Komárek & Anagnostidis 1989, 1999, 2005, Duong 1996, Duong & Vo 1997). The density of phytoplankton cells was calculated using the Utermöhl technique (Edler & Elbrächter 2010) with light microscopy (inverted Zeiss Axiovert microscope, x400 magnification).

A Nageotte counting chamber (Marienfeld, Germany) was used to estimate living diatom density in each biofilm sample by counting the total number of diatoms in 30 fields (1.25 µL each, 0.5 mm depth) using the Axioskop 2 microscope (200x magnification). Data are reported as number of living cells per unit area of artificial substrate (cells/cm²). For identification to species level, diatoms were cleaned with hydrogen peroxide (30%) to remove organic material then rinsed several times and diluted with deionized water. Cleaned frustules were mounted on a microscope glass slide using Naphrax, high resolution mounting medium (Brunel Microscopes Ltd, UK; RI =1.74). Diatom valves were identified under a Zeiss Axioskop 2 plus microscope at x1000 magnification. On each slide, 400 diatom valves were identified following the European Süßwasserflora, with nomenclature updated using online resources (Krammer & Lange-Bertalot 1986–2004, Jüttner et al. 2019).

Chlorophyll (Chl) and carotenoid pigments were extracted overnight in an acetone: methanol: water (80:15:5) mixture, filtered with a PTFE 0.2 µm filter and dried down under nitrogen gas. The dried residues were re-dissolved into an injection solution of a 70:25:5 mixture of acetone, ion-pairing reagent (IPR; 0.75 g of tetrabutylammonium acetate and 7.7 g
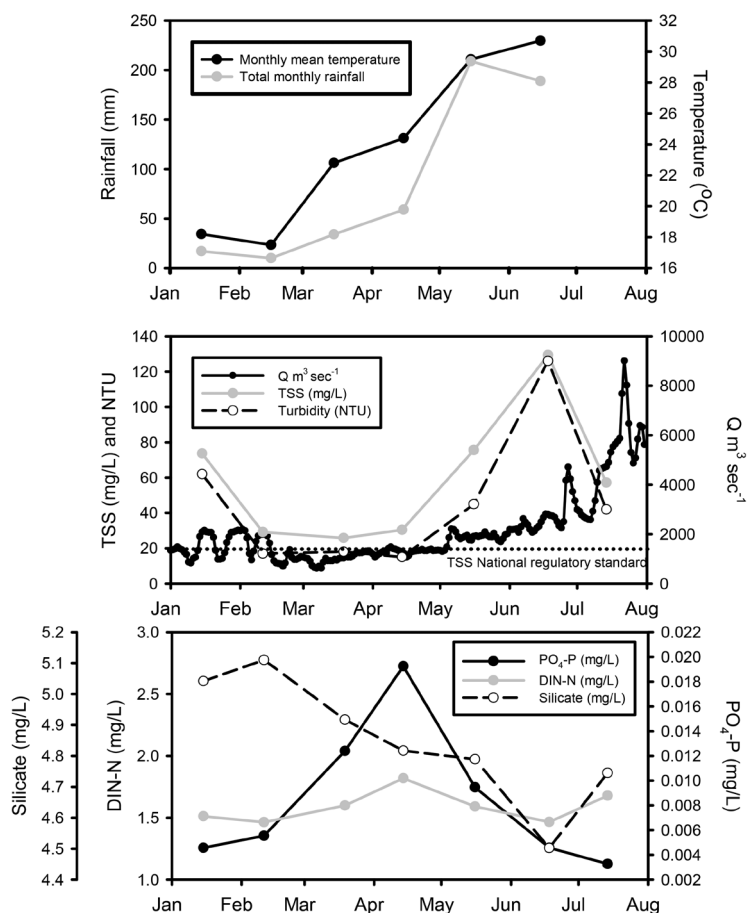

Figure 2 – Total monthly rainfall and mean monthly temperature calculated from daily measurements at Lang Station in Hanoi. Daily river discharge, and monthly turbidity measured at Hanoi hydrological station; total suspended solids (TSS) and turbidity (NTU) silicate (DSi), dissolved inorganic nitrogen and phosphate are also provided.

of ammonium acetate in 100 mL water) and methanol, injected into an Agilent 1200 series HPLC separation module with a ODS Hypersil column (205 × 4.6 mm; 5 µm particle size) and separated using a modification of the conditions of Chen et al. (2001). Eluted pigments passed through a photo-diode array detector scanning at 350–750 nm and peak areas were calibrated to commercial standards (DHI, Denmark). Pigment concentrations are reported as moles of pigments per area of biofilm substrate.

Numerical analyses

Differences between wet and dry season water chemistry variables were evaluated using t-tests in SPSS version 24.0. General patterns of water chemistry, diatom relative abundances (> 1%) and pigment concentrations were explored using multivariate analyses conducted in CANOCO version 5.04 (ter Braak & Šmilauer 2012). Detrended Correspondence Analysis (DCA) gradient lengths (< 2 SD) indicated that Principal Components Analysis (PCA) should be used for analysis of water chemistry and pigments whereas the unimodal method of DCA was used for diatom assemblage analysis. Pigments were log (x+1)-transformed before analysis.

RESULTS

Meteorological conditions, hydrological regime and environmental parameters

The transition from 'dry' (January–April) to 'wet' (May–July) seasons was marked by large increases in rainfall (from 10 mm in February to 209 mm in May) and air temperature (from 18°C in February to 31°C in June) (fig. 2). The increase in rainfall was concurrent with a gradual increase in discharge (Q) starting in May from a dry season (January–April) mean of 1305 m³/s to a wet season (May–July) mean of 3164 m³/s.

Water temperatures tracked air temperatures, ranging from 18.4°C to 30.9°C with significant differences ($p < 0.05$) between dry and wet seasons (table 1). pH was circumneutral, (between 7.0 to 7.8, not significantly different between seasons), dissolved oxygen (DO) was below saturation, varying between 4.2 and 6.2 mg/L, with an average of 5.2 mg/L over the study period and conductivity ranged from 17.7 to 26.6 µS/m. Turbidity and TSS varied similarly, ranging from 15 to 126 NTU and from 26 to 129 mg/L respectively, and both were consistently higher in the rainy season (fig. 2, table 1). Highest values of turbidity and TSS were reached in June, following the start of the rainy season. Through the monitoring period, concentrations of dissolved inorganic nitrogen (DIN) and silicate consistently exceeded 1.4 mg/L and 4.5 mg/L respectively, whereas phosphate concentrations ranged between 0.003 and 0.019 mg/L with the majority of P being present in particulate and/or soluble unreactive forms (0.075–0.130 mg/L total phosphorus; TP) (fig. 2, table 1). Dissolved silicate (DSi) concentrations were higher in the dry than the wet season, whereas maximum phosphate and DIN concentrations were present in April. When nutrient fluxes were

Table 1 – Mean (± standard deviation) physio-chemical characteristics and water quality of the Red River at Hanoi site during the period from January to July 2018.
*Indicates a significant difference ($p < -.05$) in variables between wet and dry seasons.

Time period	pH	*T (°C)	DO (mg/L)	*Turbidity (NTU)	Conductivity (µS/m)	*TSS (mg/L)	N-NO$_2$ (mg/L)	N-NO$_3$ (mg/L)	N-NH$_4$ (mg/L)	DSi (mg/L)	P-PO$_4$ (mg/L)	Ptotal (mg/L)	HCO$_3^-$ (mg/L)	COD (mg/L)
Whole period	7.48±0.31	25.6±4.8	5.2±0.8	46.4±39.2	19.8±3.1	60±37	0.02±0.01	1.45±0.1	0.12±0.05	4.85±0.2	0.01±0.01	0.08±0.03	112.9±4.1	13.8±5.6
Dry season	7.71±0.07	22.8±4.4	5.6±0.7	28±22.7	18.7±0.9	40±23	0.02±0.01	1.44±0.1	0.13±0.06	4.97±0.13	0.01±0.01	0.08±0.04	113.3±4.1	12.3±4.7
Rainy season	7.19±0.25	29.4±1.6	4.7±0.7	71±47.7	21.3±4.6	87±37	0.02±0.01	1.45±0.11	0.10±0.01	4.68±0.15	0.01±0.005	0.08±0.005	112.5±5.1	15.8±7.1

calculated using the discharge measurements (mg/sec; not shown) there was an increase in silicate and DIN delivery in June-July, whereas the maximum phosphate flux was in April. Water chemistry patterns are summarized by PCA (fig. 3) which shows that the wet season water samples are characterized by higher discharge, temperature, turbidity, TP concentrations and suspended solids. Silicate concentrations are strongly negatively correlated with axis 1, indicating higher silicate concentrations in the dry season samples (although absolute changes in concentrations were quite small). Dry season samples also tended towards higher pH and ammonium-N. COD and bicarbonate ion concentrations appeared to be negatively correlated with one another and represented a secondary gradient along axis 2; samples from July had higher COD.

Characteristics of phytoplankton and biofilm assemblages

The most common taxonomically-diagnostic pigments from river phytoplankton were from Chlorophyta (pheophytin b, Chl b, lutein) and Bacillariophyta (fucoxanthin, diadinoxanthin) (fig. 4A). Ubiquitous pigments representing all photoautotrophic taxa in the plankton were dominated by degradation products of Chl a, with an epimer of Chl a being

especially prevalent throughout the monitoring period (fig. 4B). Pigment concentrations were highest in April (dry season), and there were much higher concentrations of degradation products pheophorbide a and pheophytin b during that month that in the wet season samples. Microscopic counts of river phytoplankton also recorded the dominance of Bacillariophyta (*Aulacoseira granulata* (Ehrenb.) Simonsen, *Aulacoseira distans* (Ehrenb.) Simonsen, *Cyclotella ocellata* Pant., *Cyclotella fottii* Hust., *Melosira varians* C.Agardh, *Fragilaria ulna* (Nitzsch) Lange-Bert.) and chlorophytes (*Scenedesmus acuminatus* (Lagerh.) Chodat, *Pediastrum duplex* Meyen, *Pediastrum simplex* Meyen, *Crucigenia* spp.) were common. Cryptophytes (*Cryptomonas* spp.), and cyanobacteria (*Oscillatoria* spp.) comprised a small proportion, and dinoflagellates (*Peridinium* spp.) and euglenophytes (*Phacus* spp., *Euglena* spp.) constituted a negligible proportion of the community. Seasonal patterns of microscopic enumerations also indicated that cell densities were highest in April with a maximum of 5850 cells/mL and lower thereafter during the wet season (minimum of 3214 cells/mL) (fig. 4C). Proportionally, the pigments and microscope counts suggest an increased contribution of Bacillariophyta to the overall assemblage during the dry season and a rise in predominance of Chlorophyta during the wet season.
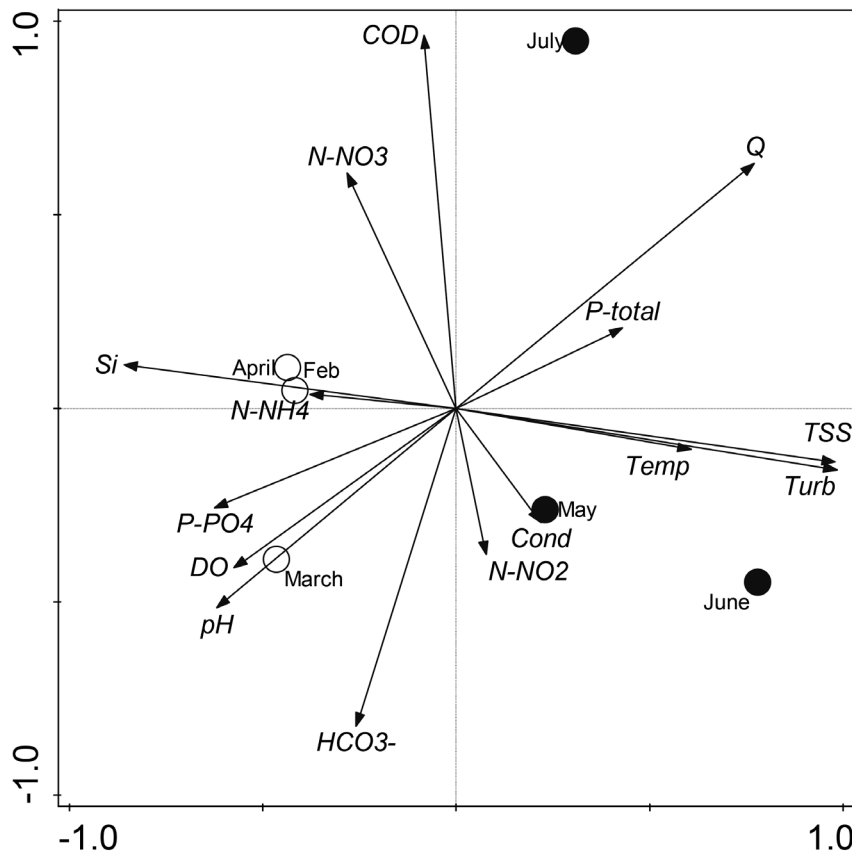

Figure 3 – PCA of water chemistry and physicochemical variables with samples from dry season (February–April; open circles) and wet season (May–July; closed circles). Eigenvalues are λ_1 0.7948 and λ_2 0.1613.
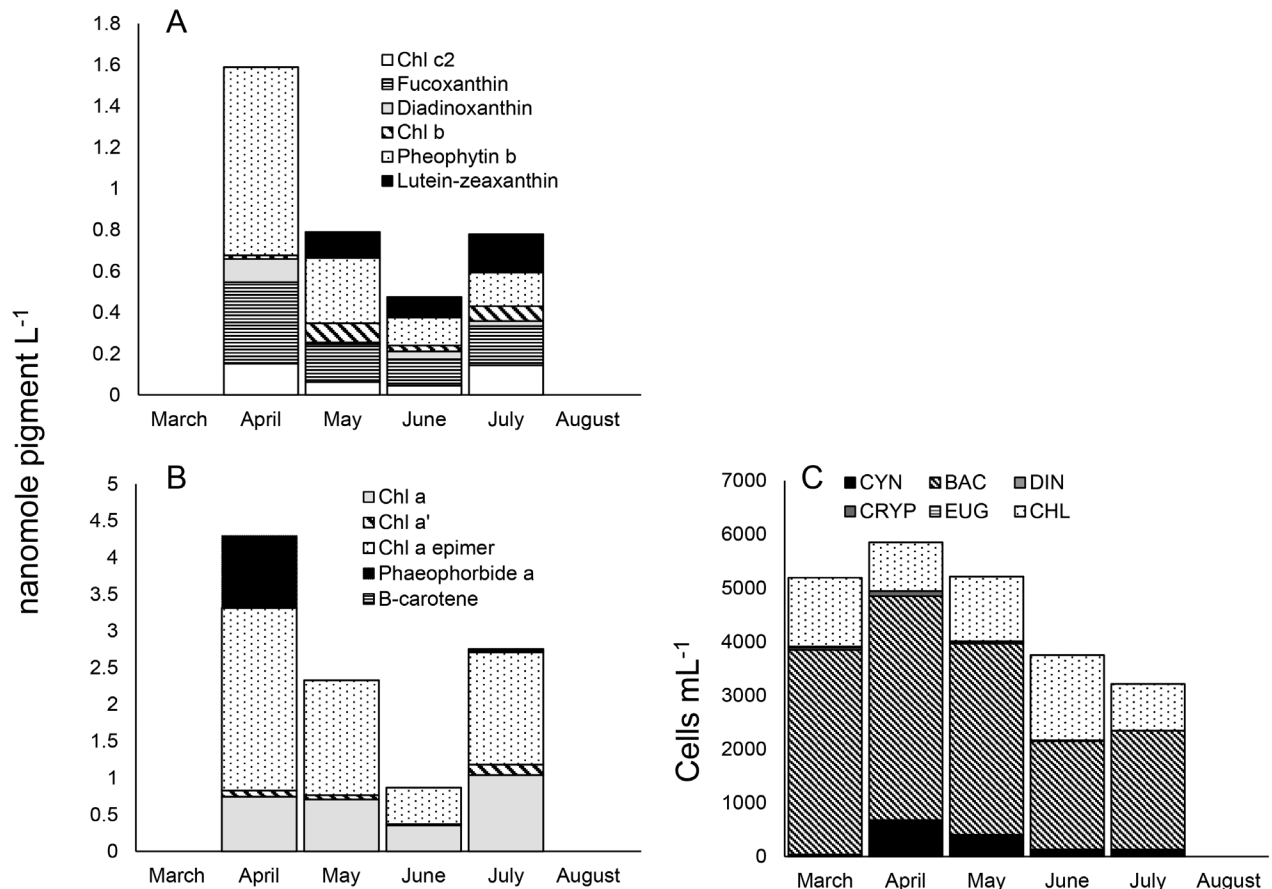
Figure 4 – Phytoplankton enumerations separated into: A, taxonomically diagnostic pigment indicators; B, ubiquitous pigments (Chl a and b-carotene) and a-phorbin degradation products; C, microscopic phytoplankton enumerations grouped by family including cyanobacteria (CYN), Bacillariophyta (BAC), Dinoflagellates (DIN), Cryptophyta (CRYP), Euglenophyta (EUG), Chlorophyta (CHL) monitored across the dry (March–April) and wet (May–July) seasons.

Periphytic diatom densities on the artificial substrates after a one-month incubation are presented in fig. 5. The density of living cells in the diatom biofilms was lower in the wet than the dry season. The highest values were recorded in March and April (mean densities of $617\,532 \pm 91\,837$ cells/cm^2), and the lowest densities were observed in May–July (mean densities of $299\,835 \pm 123\,087$ cells/cm^2). A total of 104 species and subspecies were identified in the biofilm diatom assemblages, and were characterized by an association of Centrophycideae, Araphideae, Naviculaceae, and Nitzschiaceae (table 2). Relative abundances of the main periphytic diatom species and DCA indicates that wet and dry season diatom assemblages were distinct from one another, with shifts in the dominant taxa (fig. 6, table 2). The colonial river diatom *Melosira varians* was abundant in all months. *Planothidium lanceolatum* (Bréb. ex Kütz.) Lange-Bert. increased in the wet season at the expense of *Planothidium rostratum* (Østrup) Lange-Bert., which was more abundant during the dry season. *Navicula recens* (Lange-Bert.) Lange-Bert. increased markedly in July when the community was dominated by benthic taxa (*P. lanceolatum*, *Bacillaria pax-*
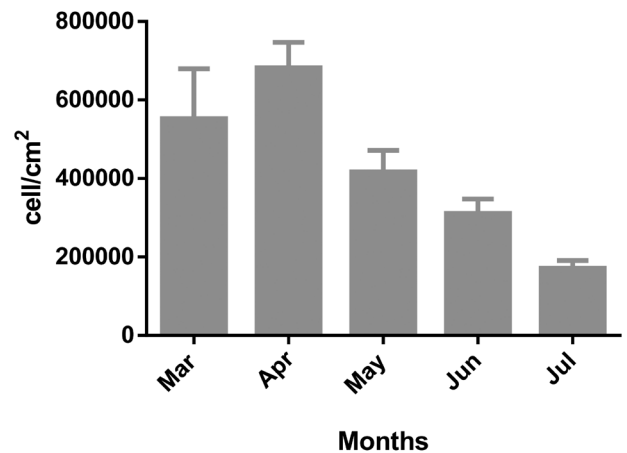

Figure 5 – Diatom densities (cells/cm^2) of artificial glass substrate biofilms sampled after a one-month incubation through the sampling period (average $\pm$ standard deviations of three replicates) monitored across the dry (March–April) to wet (May–July) seasons.

Table 2 – **Diatom taxa in biofilm samples collected from Hanoi station (Red River) during the study period from 23 Feb. to 19 Jul. 2018.**
Percentage average data are presented for each species with minimum and maximum occurrences in parentheses. (> 1% abundance in the diatom assemblage) (average values and minimum –maximum values).

Taxon name	Code	March	April	May	June	July
Melosira varians C.Agardh	MVAR	26.6 (18.8–28.5)	26.1 (14.3–30.9)	5.8 (2.9–9.6)	36.4 (34.2–38.7)	4.3 (0.3–7.7)
Planothidium lanceolatum (Bréb. ex. Kütz.) Lange-Bert.	ALAN	2.3 (1.2–3.1)	0.84 (0.44–1.05)	18.8 (11.7–27)	33.1 (27–27)	23.9 (21– 29.1)
Navicula recens (Lange-Bert.) Lange–Bert.	NRCS	3.3 (2.5–4.3)	2.2 (0.63–2.8)	4.02 (3.7–5.02)	4.6 (3.6–5.9)	23.1 (20.4–25)
Planothidium rostratum (Østrup) Lange-Bert.	ALAR	7.4 (4.8–11.3)	13.4 (5.1–26.4)	20.6 (13.3–26.9)	3.2 (2.9–3.4)	0.8 (0–1.3)
Fragilaria ulna var. *acus* Kütz. (Lange-Bert.)	FUAC	11.2 (7.3–13.1)	17.6 (2.4–36)	2.8 (0.4–6.1)	3.9 (2.3–5.9)	1.3 (0.4–1.7)
Bacillaria paxillifer (O.F.Müll.) T.Marson	BPAX	15.6 (8.4–24.3)	8.2 (7.0–7.9)	4.2 (0.5–8.1)	4.2 (3.2–6)	13.2 (8.8–16.6)
Achnanthidium minutissimum (Kütz.) Czarn.	ADMI	1.6 (0.9–2.1)	2 (0.4–3.4)	12.1 (3.7–18.6)	1.8 (0.8–2.7)	3.1 (1.3–3.9)
Cocconeis placentula Ehrenb.	CPLA	4.4 (2.2–8.2)	1.9 (0–3.4)	9.7 (9.07–10.9)	1.1 (0.8–1.3)	2 (1.5–2.8)
Fragilaria nanana Lange-Bert.	FNAN	2 (0.64–3.3)	6.6 (4.2–7.9)	0.64 (0.14–0.62)	0.4 (0.13–0.8)	0.3 (0.18–0.5)
Fragilaria crotonensis Kitton	FCRO	5.7 (5.3–6.1)	0.7 (0.4–1.1)	0.2 (0–0.31)	0.4 (0.13–0.8)	0.3 (0–0.9)
Eolimna minima (Grunow) Lange-Bert., nom. illeg.	EOMI	0.5 (0–0.9)	0.7 (0–1.4)	1.3 (0.3–2.3)	2.6 (0.82–3.8)	5.1 (0.57–11.3)
Amphora pediculus (Kütz.) Grunow	APED	0	0.3 (0–0.89)	3.9 (0–5.02)	1.3 (0.4–1.7)	0
Nitzschia palea (Kütz.) W.Sm.	NPAL	0.5 (0–1.4)	0.7 (0.2–1.04)	0.36 (0.14–0.8)	0.1 (0–0.17)	3.5 (2.6–4.9)
Nitzschia filiformis (W.Sm.) Van Heurck	NFIL	1.5 (07–2.1)	0	0.13 (0–0.38)	0	0
Amphora veneta Kütz.	AVEN	0.8 (0–2.4)	0.3 (0–0.88)	0.3 (0–0.74)	0.4 (0.17–0.8)	2.5 (2.2–2.8)
Gomphonema parvulum Kütz. (Kütz.)	GPAR	1.5 (1.2–1.7)	2.33 (1.2–3.5)	1.8 (1.5–2.2)	0.5 (0.4 – 0.58)	2.2 (1.4–3.7)
Aulacoseira granulata (Ehrenb.) Simonsen	AUGR	2.2 (1.4–2.5)	0.21 (0–0.4)	0	0.4 (0 – 0.87)	0.7 (0.4–0.75)
Cyclotella ocellata Pant.	COCE	0.2 (0–0.32)	1.9 (1.7–2.4)	0.1 (0–0.15)	0	0.1 (0– 0.3)
Amphora sp. 1	AMPS1	0.5 (0–1.2)	1.7 (0.4–2.6)	1.1 (0–2.5)	0.9 (0.2 – 1.3)	0.4 (0–0.6)
Cymbella tumida (Bréb.) Van Heurck	CTUM	1.6 (0.8–2.8)	0.5 (0.2–0.88)	0.3 (0.14–0.5)	0.1 (0–0.2)	0.4 (0.31–0.56)
Gyrosigma scalproides (Rabenh.) Cleve	GSCA	0.1 (0–0.15)	0.07 (0–0.2)	0.4 (0–0.8)	0.5 (0.38 – 0.6)	1.5 (0.9–2.25)
Aulacoseira distans (Ehrenb.) Simonsen	AUDI	0.6 (0–1.9)	0	0	0.1 (0 – 0.34)	1.4 (0.9 – 1.89)
Gyrosigma acuminatum (Kütz.) Rabenh.	GYAC	0	0	0.1 (0–0.15)	0.3 (0.17 – 0.38)	1.2 (0.15–2.08)

illifer (O.F.Müll.) T.Marson, *Eolimna minima* (Grunow) Lange-Bert., nom. illeg.) with a minor contribution from *Melosira varians* (fig. 6).

Pigment composition of the river biofilms was dominated by compounds from siliceous algae (Chl c2 and c3, fucoxanthin) and chlorophytes (Chl b, lutein), with only minor contributions from other algal biomarkers (the cryptophyte pigment alloxanthin) and an absence of pigments from cyanobacteria (fig. 7). Although Chl c3 tracked the decline in diatom abundance between the wet and dry season as monitored by the microscopic cell counts, Chl c2 increased during the June monitoring. The chlorophyte pigment Chl b was highly variable among replicates in the dry season (March–April), peaked in May and then declined as the wet season progressed. In contrast to the other chlorophyll pigments, and to the diatom microscopic counts, Chl a (used as an indicator of total algal biomass) increased in the wet season relative to the dry season, and there was a high degree of variability among the three replicates. The ratios of Chl a: a-phorbins indicates the presence and dominance of a diverse range of chlorophyll a degradation production including an epimer, the allomer Chl a', pheophorbide a and several pheophytins. Chl a was a minor component of the a-phorbins throughout much of the year, but increased in relative abundance in July. The grazing indicator pheophorbide a was the most abundant a-phorbin between May and August. The PCA suggests some community separation of the wet and dry season pigment assemblages, with wet season biofilms containing greater concentrations of Chl a', Chl a, and Chl a epimer, as well as the carotenoids lutein and alloxanthin (fig. 7C). The dry season samples are more strongly associated with pheophytins and pheophorbides and Chl b and c.

DISCUSSION

Similar to other large rivers (Soballe & Kimmel 1987, Gamier et al. 1995, O'Farrell & Izaguirre 2014, Simić et al. 2015), diatoms (mostly centric forms) were numerically dominant in the phytoplankton of the lowland course of the Red River, accounting for 54–73% of the total phytoplankton assemblage. Phytoplankton enumerations on tropical river systems are rare, limiting our ability to generalize across other similar river systems. However, the magnitudes of phytoplankton densities in other river sites range between hundreds (10^2) and hundreds of thousands (10^5) of cells/mL in US rivers (Soballe & Kimmel 1987), compared with maximum diatom abundances of tens of thousands of (10^4) cells/mL in the UK (Moorhouse et al. 2018), 10^3–10^4 cells/mL in northwestern Spain (Vasconcelos & Cerqueira 2001), 10^2–10^3 cells/mL in subtropical Paraguay (De Domitrovic 2002) and 10^2–10^5 cells/mL in tropical/ subtropical Argentina (O'Farrell 1994). Therefore, our phytoplankton densities in the range of thousands (10^3) of cells/mL (fig. 4) are within the lower end of these ranges, and lower than densities in the Day River, downstream of this site where average densities of c. 10^4 cells/mL (Hoang et al. 2018) were recorded. Nutrient concentrations of the main branch Red River at Hanoi were quite low, in comparison to urban rivers (Nhue, To Lich) in the Red River Delta (Duc et al. 2007). At the same time, TSS concentrations (and hence turbidity) averaging 60 ± 37 mg/L consistently exceeded permitted values (20 mg/L) of the Vietnam National Technical Regulation on surface water quality (QCVN 08:2015/BTNMT column A1). Because bioavailable nutrient concentrations remained detectable throughout the monitoring period, we attribute the low phy-
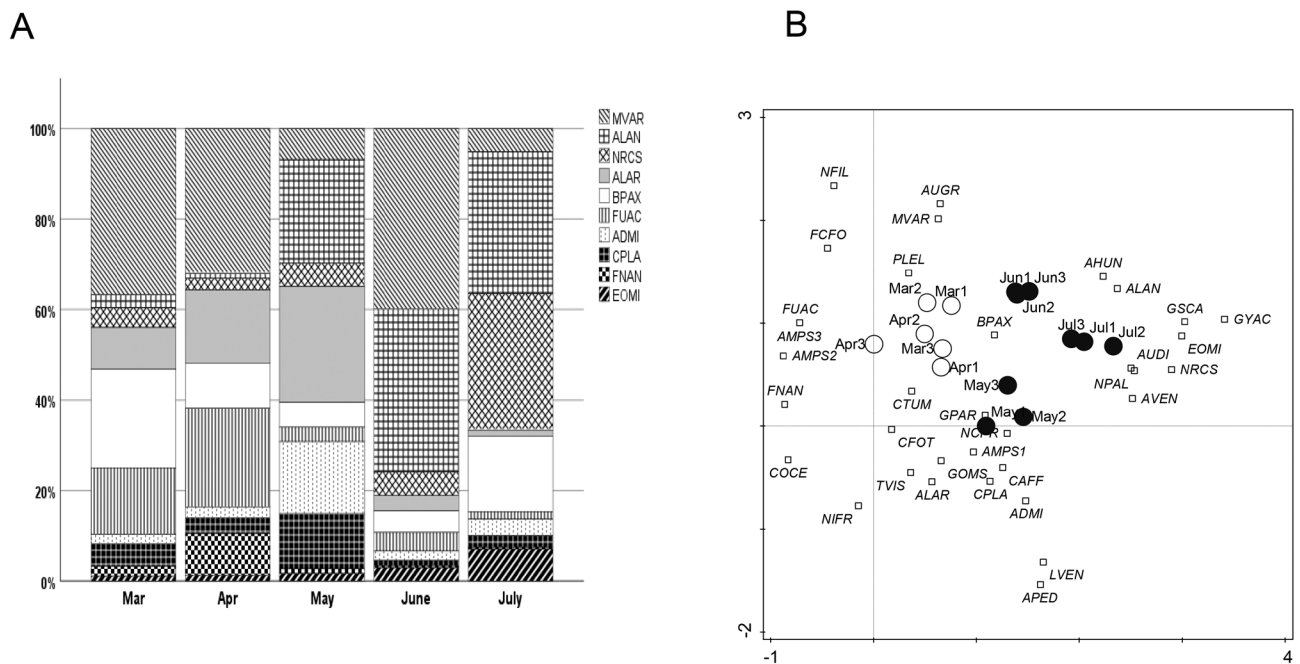
A

B

Figure 6 – Artificial substrate biofilm samples: A, relative abundance of the main diatom species (> 5% abundance); B, DCA of diatom assemblage (species > 1% relative abundance) with the samples from dry season (March–April; open circles) and wet season (May–July; closed circles). Eigenvalues are λ_1 0.3222 and λ_2 0.1121. Abbreviations for taxa are given in table 2.

toplankton densities in our samples mainly to limited light availability due to high TSS concentrations.

Phytoplankton changes between dry and wet seasons

Phytoplankton densities evaluated by both pigment and microcopic techniques showed an approximately two-fold reduction in cell densities/ pigment concentrations between dry and wet periods. This decline was associated with an increase in river discharge, suggesting that dilution of phytoplankton biomass was probably partly responsible for the patterns observed. Diatoms constituted a mean of 72% of the total community in the dry season and 63% of the total community in wet season (fig. 4). Correspondingly, chlorophytes became relatively more important in the phytoplankton assemblage during the wet season (31% in May–July) compared to the dry season (20% in March–April). Seasonal variation in river phytoplankton is driven by changes in the water environment, such as changes in nutrients, disturbance patterns, zooplankton grazing pressure, and hydrodynamics (Lucas et al. 2009, Bussi et al. 2016). Our results were consistent with studies from across bioclimatic zones, which showed distinct river algal succession: centric diatoms to green algae in the Berounka River (Czech Republic; Desortová & Punčochář

2011); centric diatoms to cyanobacteria in the Uruguay River (O'Farrell & Izaguirre 2014). TSS concentrations can substantially alter the temporal and spatial pattern of phytoplankton in temperate rivers (Wu et al. 2010) through light limitation and by the physical flocculation of algal cells which enhances sinking beyond the photic zone (Reynolds 2006). In the Red River, where turbidity and discharge are very high during the wet season, it seems likely that a combination of dilution, light limitation and algal cell flocculation has led to the reduction in phytoplankton cell density after May 2018 (average of 4059 ± 1035 cells/mL) (fig. 4). The implications for biomonitoring are that although turbidity changes usually correlate with hydrology, fluxes of suspended solids are exacerbated by human influences (Taylor & Owens 2009). Therefore, seasonal shifts in phytoplankton might be developed as indicators of suspended solids, an important contaminant in tropical regions.

The diatom assemblages in Red River phytoplankton appeared to be well suited to turbid conditions. The most abundant diatom taxa were *Aulacoseira granulata*, *Aulacoseira distans*, *Cyclotella ocellata*, *Cyclotella fottii* and *Melosira varians*. *Aulacoseira granulata* is well known for being tolerant of both turbulent (Nardelli et al. 2014) as well as turbid
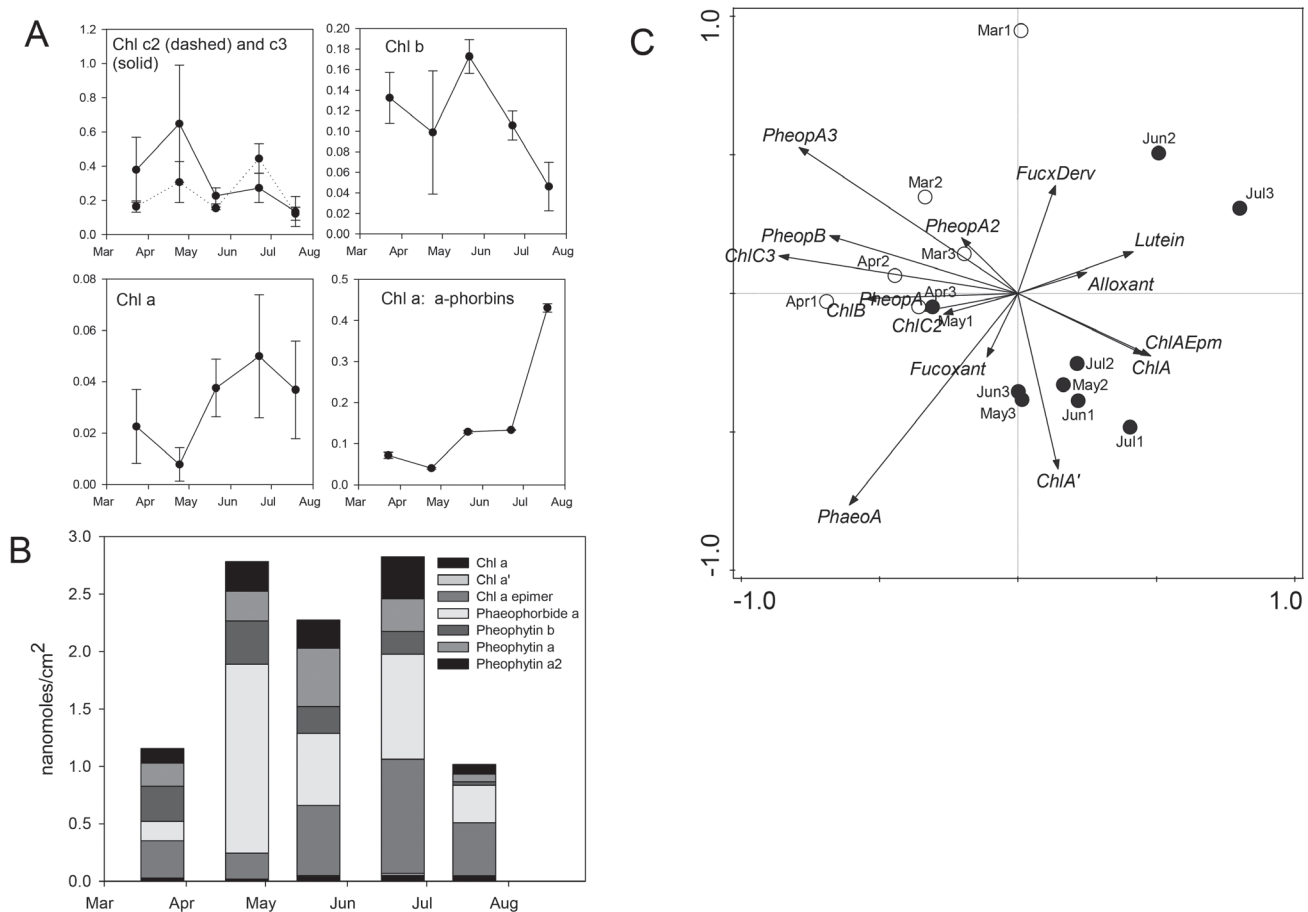

Figure 7 – Pigment measurements of artificial glass substrate biofilms: A, changes in mean pigment concentrations (± standard deviations); B, changes in concentrations of a-phorbins; C, PCA of algal pigments with replicates 1, 3 and 5 from dry season (March–April; open circles) and wet season (May–July; closed circles). Eigenvalues are λ_1 0.3129 and λ_2 0.2275.

conditions (Davey 1987) and certain subspecies of *A. granulata* have high silicate demands (Gómez et al. 1995). *Aulacoseira* species are very common in river-floodplain systems (Yang et al. 2008). However, increasing concentrations of TSS in the Red River were associated with a decline in these diatoms at the expense of chlorophytes. The decline in bioavailable silicate observed after February 2018 (fig. 2) may have altered the nutrient stoichiometric balance in favour of chlorophytes during the wet season (fig. 4). The seasonal shift from diatoms to chlorophytes has also been identified in temperate river systems such as the Thames (Moorhouse et al. 2018). Here, diatoms and some large chlorophytes dominate spring algal production, with a community shift to pico-chlorophytes and cyanobacteria in summer when temperatures increase (Moorhouse et al. 2018). It is probable that the increasing temperatures after February, coincident with higher TSS and shifts in the nutrient balance can all account for the community shift to chlorophytes after diatoms in the Red River (fig. 2). Further research into seasonal shifts across nutrient gradients should be able to disentangle the extent to which hydrology, turbidity and nutrient stoichiometry influences seasonal community succession of phytoplankton.

Biofilm changes between dry and wet seasons

Total diatom densities within artificial substrate biofilms reflect their developmental potential over time within a specific physico-chemical environment and hydrological conditions (Kelly et al. 1998). Although biofilm development on artificial substrates may differ from that on natural surfaces (Lamberti & Resh 1995, Barbiero 2000), uniform substrates can provide more reproducible results for biomonitoring, and may be the only feasible option in deep and silty rivers (Eulin & Le Cohu 1998). In the present study, we observed higher cell densities of living diatoms in March–April than in May–July. Increased rainfall combined with an increase in discharge (fig. 2) during May–July (with a mean of 3164 m^3/sec) may explain the lower total densities observed in the wet season (fig. 5). High current velocity is recognized to cause shearing and shading, along with other detrimental physical effects on periphytic biofilm immigration and colonization, which can alter the structure of the colonized periphytic community (Ghosh & Gaur 1998, Plenković-Moraj et al. 2008). The majority of diatom biofilm taxa identified are cosmopolitan in distribution and their ecological preferences are consistent with those described in the literature (Van Dam et al. 1994). Most species (*Aulacoseira granulata*, *Melosira varians*, *Eolimna minima*, *Nitzschia palea* (Kütz.) W.Sm.) have additionally been recorded in eutrophic sites in the Red River basin and are considered to be bioindicators for moderate pollution in the region (Duong et al. 2012). The presence of *A. granulata*, which is usually considered to be planktonic, may indicate that river seston is trapped within the biofilm matrix; a potentially useful way of capturing information on planktonic taxa. In the present study, there are no very large differences in nutrient (N, P and Si) concentrations or delivery between dry and wet seasons in the Red River at Hanoi (fig. 2). This is consistent with studies which show that water discharge in eutrophic rivers may cause greater differences in benthic diatom communities, than sea-

sonal chemical compositional changes in waters (Kwandrans et al. 1998).

In common with other lotic systems, we found that hydrologic factors appeared to be highly important in influencing the distribution, succession and structure of diatom biofilm communities (Soininen et al. 2004, Bergey & Resh 2006, Zębek 2013). Changes in assemblages of the main diatom taxa at the Hanoi study site were clearly associated with shifts in dry-wet season water flows, as separated by axis 1 on the DCA (fig. 6). During the low flow periods, the dominant diatom community composition remained quite stable with chain-forming centric diatom *Melosira varians* as well as large *Fragilaria* species and halophilous taxa such as *Bacillaria paxillifer* (O.F.Müll.) T.Marsson. In contrast, during high flows, the diatom community showed a marked change in composition towards a greater contribution of the small monoraphid *Achnanthidium* spp., *Cocconeis placentula* Ehrenb. and *Navicula recens* (Lange-Bert.) Lange-Bert. Like *Achnanthidium* spp. and *Cocconeis placentula*, some *Navicula* species have also been previously reported as pioneering species on artificial substrata and ability to withstand higher current velocity (Kelly et al. 1998, Plenković-Moraj et al. 2008). High abundances of *Achnanthidium* species in the wet period can be explained by their ability to respond well to disturbance and to reproduce at relatively high growth rates, which enables them to colonise and populate the surface before their competitors (Plenković-Moraj et al. 2008). Additionally, *Navicula* and *Achnanthidium* may dominate in areas where the water flow is fast (Soininen 2005, Everest & Aslan 2016).

Assessment of pigments for monitoring of artificial substrates

Pigment assemblages on biofilms allow a more holistic quantification of the entire algal community including diatoms and other algal groups. As observed in the phytoplankton assemblages, the dominant biofilm pigments were representative of siliceous algae (Chl c2, Chl c3, fucoxanthin) and chlorophytes (Chl b, lutein) (fig. 7A). The dominant siliceous algal pigment, Chl c3, suggests a decline in the abundance of diatoms in the wet compared to the dry season, and increases in Chl b suggests a succession towards chlorophytes in May (similar to pigment successional sequences in temperate rivers; Moorhouse et al. 2018). As biofilms are unaffected by direct dilution effects that can influence phytoplankton densities, other factors must be considered to explain this pattern. The assemblage change may, in part, be driven by the decline in dissolved silicate availability relative to phosphate and inorganic nitrogen (fig. 2) which may provide a competitive advantage for non-siliceous algae. However, the main shifts in algae are between dry and wet season assemblages, which seem to be overwhelmingly driven by turbidity and light intensity (fig. 2). It was surprising that Chl a, which is broadly used as an estimate of total periphyton biomass, increased during the wet season, peaking in May–July when the main taxonomically specific biomarker pigments declined. A possible explanation for this pattern is that the increase in turbidity in the wet season, which was exceptionally high in June, reduced light availability and led to adjustment of cel-

lular pigment production. Changes in algal pigment content can occur over very short time scales as a photoacclimation response, with cellular pigment content usually increasing as light intensity declines (Falkowski 1980). The turbid and light-limited conditions therefore seem a plausible explanation for the increase in Chl a concentrations through cellular adjustments. Such photoacclimation may mean that using Chl a concentrations as a direct indicator of biofilm biomass could be problematic in very turbid rivers. Adaptation to low light conditions may result in an increase in the Chl c / Chl a ratio in diatoms and Chl b / Chl a ratio in chlorophytes (Falkowski 1980), but such a pattern was not observed in our samples. An alternative explanation for these shifts in Chl composition may be the changes in biofilm composition between the wet and dry seasons, because different algal taxa are known to have different pigment contents per cell. The compositional shift led to an increase in chlorophytes and a greater proportion of smaller monoraphid diatoms, well known colonisers of substrates during conditions of high scour. The rapid growth rates of these small colonizing taxa may further explain the production of Chl a, which is produced when algae are in the growth phase (Klein 1988).

One obvious feature of these biofilms is that the concentrations of intact Chl a is very low relative to other a-phorbins, suggesting that degradation within the biofilm was exerting a heavy influence on the Chl a concentrations. The most dominant a-phorbin, pheophorbide a, is considered to be produced when Chl a is consumed by grazers (Cartaxana et al. 2003) whereas pheophytins are usually produced during algal senescence (Buchaca & Catalan 2008), although significant debate about their relative specificity to these processes exists (Ford & Honeywill 2002). The allomers and epimers of Chl a are considered to be oxidation products, and their production is exacerbated by microbial decomposition in the presence of oxygen (Szymczak-Żyla et al. 2008). The very high proportions of degradation-related compounds are consistent with a high proportion of senescent algal materials on the biofilm, which may be influenced by invertebrate grazers and microbial attack. Another likely cause is the constant supply of particulate detritus from the river, which contains degraded algal materials. Increasing scour during the wet season leads to disturbance of the algal biofilms and may partly explain the high proportions of senescence indicators. These conditions lead to fast-growing smaller diatoms probably providing conditions for the production and retention of more intact Chl a relative to the dry season. Taken together, it is clear that pigment assemblages provide information that is complementary to microscopic counts, and that can lead to further understanding of river biofilm communities. The pigment assemblage also helps to explain possible discrepancies between assessments of algae using *in situ* fluorometry (Bentho torch) which uses pigment composition to calculate algal biomass and other methods (Echenique-Subiabre et al. 2016). This work suggests that decomposition of pigments in senescent biofilm biomass, and shifts in pigment composition associated with periods of new growth and community change might be important considerations.

CONCLUSIONS

We have used a combination of approaches to assess the application of phytoplankton and benthic biofilms as biomonitoring tools in a tropical river close to Hanoi, Vietnam. Microscopic and pigment methods provided complementary information that broadens the understanding of photoautotrophic seasonal responses and potential drivers. Our monitoring period encompasses a transition between wet and dry season conditions to demonstrate that shifts in phytoplankton and biofilm communities appear to be driven more strongly by hydrological conditions and associated turbidity changes rather than nutrient concentrations (Royer et al. 2008). The implications of these seasonal shifts for biomonitoring are that changes in the magnitude and composition of phytoplankton and benthic algae might be useful to incorporate into monitoring programmes to assess the combined effects of hydrological and turbidity changes with other ecological drivers such as nutrients. Shifts from diatom dominance towards an increase in chlorophytes in the dry season in both habitats, compare similarly with seasonal changes in other rivers from diverse geographic regions. Future and ongoing impacts of damming, land use change and climate change will alter river hydrological regimes, transport of particulates and nutrient loads and so biomonitoring could be vital in tracking changes in such locations.

ACKNOWLEDGEMENTS

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