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The diversity and abundance of phytoplankton and benthic diatoms in varying environmental conditions in Kok River, Chiang Rai, Thailand as bio-indicators of water quality

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Abstract. *Prasertsin T, Suk-ueng K, Phinyo K, Yana E. 2021. The diversity and abundance of phytoplankton and benthic diatoms in varying environmental conditions in Kok River, Chiang Rai, Thailand as bio-indicators of water quality. Biodiversitas 22: 1853-1862.* The study of living organisms was important information for bio-indicators which was utilized to assess the quality of the environment. In the river ecosystem, the algae with which one organism was interesting accordingly considered the relationship of 2 groups algae, including phytoplankton and benthic diatoms and the physical-chemical parameters of Kok River Chiang Rai, Thailand. Phytoplankton and benthic diatoms were collected from five sampling sites along the Kok River during January (cool dry), March (summer), and June (rainy season) 2018, and assessed as bio-indicators to monitor environmental factors that represent water quality across varying conditions and periods. Fifty-seven species of phytoplankton and thirty-nine species of benthic diatoms were found. The phytoplankton mainly belonged to the phylum Bacillariophyta (diatom group). Physical and chemical factors affecting the dominant phytoplankton and benthic diatom species were subjected to canonical correspondence analysis (CCA). Results showed that planktonic and benthic *Gomphonema lagenula* positively correlated with ammonium nitrogen, recording highest abundance during the rainy season. Abundance of *Achnanthidium straubianum*, a planktonic and benthic diatom, and *Navicula cincta* negatively correlated with river velocity and water conductivity, with lowest numbers during the rainy season. Water trophic status evaluated from the main parameters of the AARL-PC score indicated that during the cool dry and summer seasons all sampling sites were classified as mesotrophic, while during the rainy season they were classified as meso-eutrophic. The combination of phytoplankton and benthic diatom can be used as bio-indicators of water quality in the Kok River and other freshwater ecosystems.

Keywords: AARL-PC score, bioindicator, correlation, environmental factor, standing water

INTRODUCTION

Algae play a significant role in global ecology and ecosystem functioning as important oxygen-producing communities in aquatic environments. Based on habitats, algae can be divided into two groups, i.e. phytoplankton and benthic algae. Phytoplankton is autotrophic organism that lives near the water surface where there is sufficient light to support photosynthesis. Among the more important groups of phytoplankton are the diatoms, cyanobacteria, green algae, euglenoids and dinoflagellates. On the other hand, benthic algae are those attached to all kinds of substrata including rocks, mud, organic and inorganic particles, macrophytes and other living organisms (John et al. 2011).

Two different kinds of communities are usually recognized as microalgae and macroalgae (Lobo et al. 2016). A major group of benthic microalgae comprises the diatoms. Diatoms are abundant in marine and freshwater habitats which in all freshwater habitats including standing and running waters and planktonic and benthic habitats. They dominate both the biomass and biological diversity of the microscopic flora in many aquatic ecosystems. Diatoms inhabit a broad array of habitats but many have specific habitat requirements and have been used as freshwater environment bioassessment indicators to monitor long-term changes in ecological conditions (Blanco and Ector 2009).

Algae is sensitive to changes in their surroundings, as such total algal biomass and certain species are often used as indicators of water quality (Omar 2010). Algae have a long history of use as bio-indicators to determine water quality. The uses of phytoplankton as indicators of water quality were investigated by Peruma et al. (2009); Stepankova et al. (2012); Järvinen et al. (2012); Borics et al. (2014). In Thailand, ecological studies on water quality and its effects on the distribution and diversity of phytoplankton were conducted by Peerapornpisal et al. (2004); Prasertsin et al. (2018) while the applicability of benthic diatom indices to assess river water quality was investigated by Pekthong (2008); Suphan and Peerapornpisal (2010); Yana et al. (2013); Leelahakriengkrai and Peerapornpisal (2014); Nakkaew et al. (2015); Leelahakriengkrai and Kunpradid (2018). While such studies present the use algae as bio-indicators of water quality, each study focused on a particular group of algae, either the phytoplankton or the benthic diatoms. No studies in Thailand have integrated both groups of algae used as bio-indicators.

The Kok River is the main river in Chiang Rai Province, Thailand and one of the tributaries of the Mekong. The characteristics of the river vary from lentic to fast-flowing stretches and this habitat diversity attracts many animals, plants and microorganisms that can be used as bio-indicators. This study aimed to examine the diversity, distribution and abundance of phytoplankton and benthic diatoms, and to investigate their relationships with environmental factors in Kok River. Our data present the first report in Thailand that combines phytoplankton and benthic diatom data as indicators of water quality in the Kok River.

MATERIALS AND METHODS

Study area and period

The Kok River is the main waterway in Chiang Rai Province as a tributary of the Mekong. From its source in Myanmar, the Kok River runs eastward through Chiang Rai and joins the Mekong at Bann Sobkok, Chiang San District. Total length of the river is 290 km, with 114 km running across Chiang Rai Province (Pekthong 2008). Five sampling sites along the Kok River were selected, based on the properties of the water body and environmental impact. Details and locations of each sampling site are shown in Table 1 and Figure 1. Phytoplankton, benthic diatoms and physicochemical water quality properties were determined during January (cool dry), March (summer) and June (rainy season) 2018.

Collection and identification of phytoplankton

Twenty liters of water samples were collected from each sampling site along the Kok River and filtered using a 10 μm mesh size plankton net. The samples were preserved by adding 0.7 mL of Lugol's solution to 100 mL of the sample (Eaton et al. 2005), observed under a light microscope and photographed using an Olympus Normaski Microscope. The phytoplankton specimens were identified based on relevant characteristics, such as color, cell-size, colony or filament, shape of the chloroplast, number and position of the flagella with or without the spine, as well as details of the granular characteristics of the cell wall (John et al. 2011; Prasertsin et al. 2014; Phinyo et al. 2017).

Table 1. Sampling sites locations and character of water types along the Kok River, Chiang Rai Province, Thailand

Sampling site	Location	Character of water type
Site 1 Huay Mark Liam	Doi Hang Sub-district, (19°57'49.4"N 99°40'40.1"E)	Fast-flowing
Site 2 Had Chiang Rai	Rop Wiang Sub-district, (19°55'03.8"N 99°47'37.2"E)	Fast-flowing
Site 3 Kok River bridge	Ban Rong Suea Ten, Rim Kok Sub-district, Mueang (19°55'18.4"N 99°50'45.6"E)	Almost still
Site 4 Chaloem Phrakiat1	Ban Kwae Wai, Rop Wiang Sub-district, (19°55'29.8"N 99°51'46.5"E)	Almost still
Site 5 Fai Chiang Rai	Ban PA Yang Mon, Rim Kok Sub-district, (19°55'31.4"N 99°53'49.9"E)	Slow-flowing

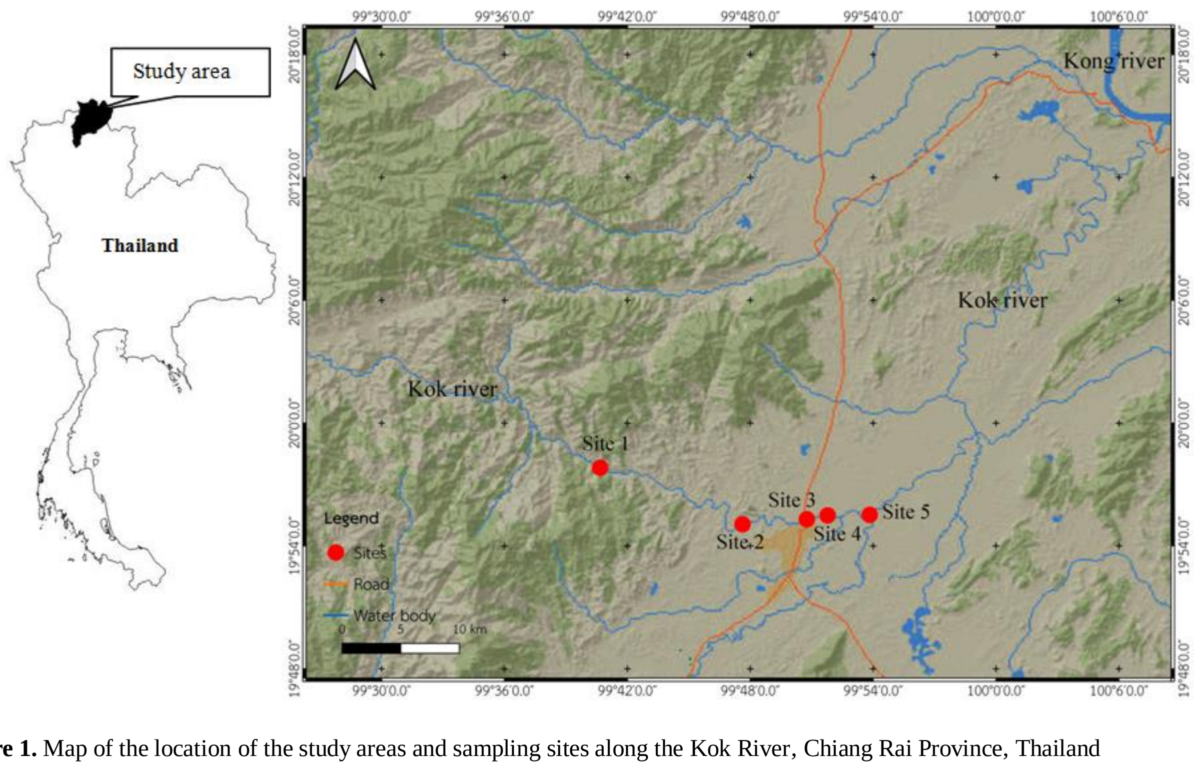

Figure 1. Map of the location of the study areas and sampling sites along the Kok River, Chiang Rai Province, Thailand

Cell enumeration of the phytoplankton species was assessed by the Lackey drop method (Phinyo et al. 2017). This is a simple method that is used to obtain results when studying the density of plankton populations. Dominance (Y) of a species was calculated by the following equation (Yang et al. 2016):

$$Y = \frac{ni}{N} fi$$

Where:
ni = the abundance of species i
fi = the occurrence frequency of species i
N = the total abundance

The occurrence frequency of a species refers to the proportion of the number of stations reporting its occurrence to the total number of sampling stations. A dominant species was defined if Y was greater or equal to 0.02.

Collection and identification of benthic diatoms

Benthic diatom samples were collected by scratching the substrate surface of cobbles and gravel with a toothbrush. The samples were placed in plastic boxes and fixed with Lugol's solution on site. Diatom samples were then taken into the laboratory and cleaned following the concentrated acid digestion method. The collected samples were boiled in hot nitric acid for 15 min, followed by hydrogen peroxide for 15 min before rinsing with distilled water (Yana and Mayama 2015). For light microscopy (LM), cleaned samples were mounted in Naphrax (SPI Supplies, West Chester, USA). All diatom specimens in each slide were counted, identified and photographed using an Olympus Normaski Microscope with a ×100 oil immersion objective. Taxonomy and nomenclature were determined according to Lange-Bertalot (2001); Yana et al. (2013); Nakkaew et al. (2015)

Determination of physical and chemical properties of water

Determination of the relevant physical and chemical properties of the river water was conducted at each sampling site by measuring air temperature, water temperature, velocity, pH, conductivity and dissolved oxygen (DO). Water samples were collected in polyethylene bottles and kept in a cool box at 5-7°C for laboratory analyses of alkalinity, biochemical oxygen demand (BOD), nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus (SRP) (Eaton et al. 2005).

The trophic status of water was evaluated from the main parameters (i.e. conductivity, DO, BOD, ammonium nitrogen, nitrate nitrogen and soluble reactive phosphorus) by the Applied Algal Research Laboratory-Physical and Chemical score: AARL PC-score (Peerapornpisal et al. 2004).

Relationship between phytoplankton content and physical and chemical factors

Comparison between parameters in each season was estimated using ANOVA, followed by Tukey's post hoc test at $p<0.05$. Relationships between phytoplankton, benthic diatoms and physical and chemical factors were explored by canonical correspondence analysis (CCA) using MVSP (Multi-Variate Statistical Package for Windows ver. 3.22. The result was presented as a CCA plot.

RESULTS AND DISCUSSION

Distribution of phytoplankton and benthic diatoms in the Kok River

Four Phyla containing 57 species of phytoplankton were found in the Kok River. The most diverse Phylum was Chlorophyta (31 species), followed by Bacillariophyta (18 species), Cyanobacteria (7 species) and Euglenozoa (1 species) (Table 2). All phytoplankton species found in the Kok River were acknowledged as common species that are typically found in standing water throughout Thailand (Pollution Control Department 2010). Each species showed different distribution at the sampling sites. All species in the Phyla Chlorophyta, Cyanobacteria and Euglenozoa had the highest distribution in almost still and slowly flowing water (Sites 1, 2 and 3), while Phylum Bacillariophyta in planktonic form was found at all five sampling sites. Each species was recorded at a different percentage of relative abundance during each season. Relative abundance can be used to consider the dominant species (Yang et al. 2016). Dominant phytoplankton in the Kok River was from the Phylum Bacillariophyta (diatom group). These were found at all sampling sites and included *Achnanthidium straubianum* (Lange-Bertalot) Lange-Bertalot, *Cymbella tugidula* Grunow, *Gomphonema lagenula* Kützing and *Navicula cincta* (Ehrenberg) Ralfs.

Thirty-nine species of benthic diatoms were found in the Kok River (Table 3 and Figure 2). Most were common species found in lotic ecosystems throughout Thailand (Pekthong 2008; Yana et al. 2013; Nakkaew et al. 2015; Leelahakriengkrai and Kunpradid 2018). The majority of benthic diatoms were pennate (97%), while the remaining 3% were centric diatoms. This finding concurred with Leelahakriengkrai and Peerapornpisal (2010) who noted that pennate diatoms dominated freshwater bodies, whereas centric diatoms were more abundant in marine ecosystems. *Achnanthidium straubianum* (Lange-Bertalot), *Cocconeis placentula* Ehrenberg, *Gomphonema lagenula* Kützing, *Planothidium frequentissimum* (Østrup) Lange-Bertalot, *Nitzschia inconspicua* Grunow, *Nitzschia palea* (Kützing) W.Smith and *Nitzschia supralitorea* Lange-Bertalot were the seven dominant species of benthic diatoms recorded in the Kok River. *Achnanthidium straubianum* (Lange-Bertalot) Lange-Bertalot and *Gomphonema lagenula* Kützing were found in planktonic and benthic diatoms, agreeing with (Guiry and Guiry (2020), who reported that these two species occurred in all freshwater biospheres including standing and flowing waters and planktonic and benthic habitats. *Cocconeis placentula* Ehrenberg, *Cymbella tugidula* Grunow and *Nitzschia palea* (Kützing) W.Smith were the dominant species in the Kok River. This result concurred with Leelahakriengkrai and Peerapornpisal (2011) who indicated that these three species were the dominant benthic diatoms in all the main rivers of Thailand. *Cocconeis placentula* Ehrenberg, *Cymbella tugidula* Grunow, *Nitzschia palea* (Kützing) W.Smith, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot, *Navicula cryptotenella* Lange-Bertalot in Krammer & Lange-Bertalot, *Achnanthidium exiguum* (Grunow) Czarnecki, *Seminavis strigose* (Hustedt) Danieledis & Economou-Amilli in Danielidis & D.G.Mann, and *Navicula germainii* J.H.Wallace were most abundant species found in the Ping River Chiang Mai Province, Thailand (Leelahakriengkrai and Kunpradid 2018).

Water quality based on physical and chemical properties

Different values of physical and chemical parameters at each sampling site along the Kok River were recorded during the cool dry, summer and rainy seasons (Table 4). Air and water temperatures ranged between 22.00±0.00 and 31.00±0.00 °C and 20.67±0.29 and 27.00±0.00 °C, respectively. The highest value occurred during the summer season at site 4, while the lowest was recorded during the cool dry season at site 1. Water turbidity gave diverse readings, ranging between 16.73±0.47 and 537.00±35.59 NTU. Highest value occurred during the rainy season at site 2, while the lowest was recorded during the summer season at site 4. Turbidity was reported at less than 10 NTU in some clean and clear headwater streams. Differences in turbidity were recorded throughout the sampling period, with maximum turbidity value at every sampling site during the rainy season. Alkalinity in the Kok River ranged between 56.67±4.16 and 142.67±4.62 mg L^{-1}. The value of alkalinity in the cool dry season indicated low impact of effluents, while during the summer and rainy seasons the impact of effluents was higher. Natural water alkalinity ranges between 50 and 100 mg L^{-1} and is frequently less than 100 mg L^{-1} in clean resources. The pH in the Kok River ranged between 6.42±0.07 and 7.33±0.15. On average, all sampling sites were neutral throughout the summer and rainy seasons and slightly acid during the cool dry season. Water at all sampling sites water was livable for living organisms and suitable for human consumption as it did not exceed the quality standard (6-9) of surface water (Simachaya 2000; Evans et al. 2012). Overall conductivity was between 104.43±2.68 µS cm^{-1} and 133.13±0.95 µS cm^{-1}. All sampling sites were normal for general water resources, meaning that the water was livable for living organisms and suitable for human consumption as it did not exceed the quality standard (<300 µS cm^{-1}) of surface water. Conductivity is a quality parameter that is used to assess water status. The range for oligo-mesotrophic status is 50-100 µS cm^{-1}, with 100-250 µS cm^{-1} for mesotrophic (Shekha et al. 2017). Value of the dissolved oxygen (DO) was between 2.73±1.08 mg L^{-1} and 8.60±0.35 mg L^{-1}. The highest and lowest values were found during the cool dry season at sites 1 and 3 and at site 5 during the rainy season, respectively. All sampling sites met the standard of surface water quality for general water resources (2 mg L^{-1}) (Simachaya 2000). The BOD was between 1.87±1.50 and 11.53±1.29 mg L^{-1}. The highest value occurred during the rainy season at site 2, while the lowest was recorded in the cool dry season at site 1. Results showed that the standard of surface water was not exceeded (Simachaya 2000). Amounts of nutrients, such as nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus, were between 0.5±0.29 and 6.4±0.75 mg L^{-1}, 0.23±0.03 and 0.87±0.32 mg L^{-1}, and 0.35±0.07 and 4.21±1.32 mg L^{-1}, respectively. The highest value occurred during the rainy season, while the lowest was recorded in the cool dry season. The levels of nitrate nitrogen and ammonium nitrogen found at all sites did not exceed the values of Thailand's prescribed surface water quality standards (Simachaya 2000).

Table 2. Distribution and abundance (%) of phytoplankton in the Kok River at each sampling site during the three seasons

Taxonomic categories	Site distribution	Abundance (%)		
		Cool dry	Summer	Rainy
Phylum Cyanobacteria/Class Cyanophyceae /Order Synechococcales				
Family Merismopediaceae				
Aphanocapsa sp.	1, 2, 3	0.9	0.0	0.3
Family Coelosphaeriaceae				
Coelomoron pusillum (Van Goor) Komárek	1, 2, 3, 4, 5	0.2	0.8	2.1
Family Merismopediaceae				
Merismopedia punctata Meyen	3, 5	0.0	0.2	0.1
Family Leptolyngbyaceae				
Planktolyngbya contorta (Lemmermann) Anagnostidis & Komárek	1, 3, 5	0.0	0.0	0.1
Family Pseudanabaenaceae				
Pseudanabaena sp.	4	0.0	0.0	2.4
Order Nostocales/Family Aphanizomenonaceae				
Cylindrospermopsis raciborskii (Woloszyńska) Seenayya & Subba Raju	1, 3, 4	0.0	0.0	0.5
Phylum Chlorophyta/Class Chlorophyceae/Order Chlamydomonadales				
Family Chlamydomonadaceae				
Chlamydomonas gloeopara Rodhe & Skuja	3, 5	0.0	0.1	0.0
Family Goniaceae				
Gonium pectosale O.F.Müller	2, 3, 4, 5	1.1	0.0	0.0
Family Volvocaceae				
Eudorina elegans Ehrenberg	1, 3	0.3	0.3	0.5
Pandorina morum (O.F.Müller) Bory	1, 2, 3, 5	0.0	1.6	2.0

Order Sphaeropleales/Family Hydrodictyaceae				
Pediastrum duplex var. *subgranulosum* Raciborski	3, 4, 5	0.3	3.1	3.4
Pediastrum simplex var. *simplex* Meyen	3, 4, 5	1.2	1.2	0.0
Pediastrum tetras (Ehrenberg) Ralfs	3, 4, 5	2.2	0.1	0.5
Class Trebouxiophyceae/Order Chlorellales/Family Chlorellaceae				
Actinastrum hantzchii Lagerheim	3, 4, 5	0.0	1.7	0.9
Chlorella sp.	3, 4, 5	3.3	0.0	4.5
Dictyosphaerium granulatum Hindák	3, 4, 5	0.2	0.4	3.4
Dictyosphaerium tetrachotomum Printz	3, 4, 5	2.0	5.0	0.7
Micractinium quadrisetum (Lemmermann) G.M.Smith	3, 4, 5	0.5	1.1	2.0
Family Oocystaceae				
Crucigeniella crucifera (Wolle) Komárek	3, 4, 5	0.0	0.3	1.8
Nephrocytium limneticum (G.M.Smith) G.M.Smith	3, 5	0.0	0.0	0.6
Order Sphaeropleales/Family Neochloridaceae				
Golenkinia sp.	1, 2, 3, 4	0.6	0.0	1.6
Family Selenastraceae				
Ankistrodesmus bibraianus (Reinsch) Korshikov	3	0.0	0.4	0.0
Ankistrodesmus spiralis (W.B.Turner) Lemmermann	1, 3, 4, 5	0.0	0.9	0.0
Family Selenastraceae				
Kirchneriella lunaris (Kirchner) Möbius	1, 2, 3, 4, 5	0.9	2.2	0.6
Family Scenedesmaceae				
Coelastrum astroideum De Notaris	3, 4, 5	2.2	2.8	3.1
*Coelastrum reticulatum (*Dangeard) Senn	1, 2, 3, 4, 5	1.8	3.2	2.7
Coelastrum cf. *verrucosum* (Reinsch) Reinsch	3, 4, 5	0.0	0.7	0.1
Comasiella arcuata var. *platydisca* (G.M.Smith) E.Hegewald & M.Wolf	3, 4, 5	0.6	0.9	1.5
Dimorphococcus lunatus A.Braum	1, 5	1.0	0.2	0.4
Desmodesmus opoliensis (P.G.Richter) E.Hegewald	3, 4, 5	0.7	3.3	4.8
Tetradesmus acuminatus (Lagerheim) M.J.Wynne	3, 4, 5	1.8	2.0	2.0
Class Zygnematophyceae/Order Desmidiales/Family Closteriaceae				
Closterium parvulum Nägeli	1, 2, 3, 4, 5	1.2	1.1	0.9
Family Desmidiaceae				
Cosmarium contractum var. *contractum* Kirchner	2, 3	0.6	0.0	0.8
Cosmarium askeasyi Schmidle	5	0.0	0.1	0.2
Euastrum turneri W.West	5	0.0	0.1	0.0
Staurastrum cf. *longbrachiatum* (Borge) Gutwinski	2, 3, 4, 5	0.7	0.0	2.0
Phylum Euglenozoa/Class Euglenophyceae/Order Euglenida/Family Phacidae				
Phacus longicauda (Ehrenberg) Dujardin	4, 5	0.2	0.1	0.0
Phylum Bacillariophyta/Class Bacillariophyceae/Order Bacillariales/Family Bacillariaceae				
Nitzschia palea (Kützing) W.Smith	2, 4, 5	0.0	1.1	1.1
Nitzschia inconspicua Grunow	3, 4, 5	1.2	0.9	0.7
Order Cocconeidales Family Achnanthidiaceae				
*Achnanthidium straubianum (*Lange-Bertalot) Lange-Bertalot	1, 2, 3, 4, 5	7.4	7.6	6.1
Family Cocconeidaceae				
Cocconeis pediculus Ehrenberg	3, 4	0.5	0.9	0.7
Order Cymbellales/Family Cymbellaceae				
Cymbella tugidula Grunow	1, 2, 3, 4, 5	6.9	6.6	6.5
Cymbella tumida (Brébisson) Van Heurck	1, 2, 3, 4, 5	5.2	3.4	3.3
Family Gomphonemataceae				
Gomphonema lagenula Kützing	1, 2, 3, 4, 5	6.8	10.4	12.5
Gomphonema pumilum (Grunow) E.Reichardt & Lange-Bertalot	1, 2, 3, 4, 5	1.2	2.6	1.1
Order Fragilariales/Family Fragilariaceae				
Synedra ulna (Nitzsch) Ehrenberg	1, 2, 3, 4, 5	5.5	2.2	3.7
Order Mastogloiales/Family Achnanthaceae				
Achnanthes inflata (Kützing) Grunow	2, 3, 4	0.9	0.0	0.0
Order Naviculales/Family Naviculaceae				
Gyrosigma scalproides (Rabenhorst) Cleve	1, 2, 3, 4, 5	3.0	3.4	3.3
Gyrosigma spenceri (Bailey ex Quekett) Griffith & Henfrey	1, 2, 3, 4, 5	1.2	2.2	1.1
Navicula cincta (Ehrenberg) Ralfs	1, 2, 3, 4, 5	12.7	10.1	5.3
Navicula germainii J.H.Wallace	1, 2, 3, 4, 5	5.3	3.8	2.8
Order Surirellales/Family Surirellaceae				
Surirella sp.	1, 2, 3, 4, 5	4.4	2.9	2.9
Order Thalassiophysales/Family Catenulaceae				
Amphora sp.	2, 5	0.9	0.7	0.0
Class Coscinodiscophyceae/Order Melosirales/Family Melosiraceae				
Melosira varians C.Agardh	1, 2, 3, 4, 5	3.0	3.4	4.2
Class Mediophyceae/Order Stephanodiscales/Family Stephanodiscaceae				
Cyclotella meneghiniana Kützing	1, 2, 3, 4, 5	3.7	3.9	3.9

Table 3. Distribution and abundance (%) of benthic diatoms in the Kok River at each sampling site during the three seasons

Taxonomic categories	Site distribution	Abundance (%)		
		Cool dry	Summer	Rainy
Phyla Bacillariophyta				
Order Bacillariales, Family Bacillariaceae				
Nitzschia amphibia Grunow	1, 3,4,5	1.1	0.3	0.9
Nitzschia inconspicua Grunow	1, 2, 3, 4, 5	6.2	6.1	3.8
Nitzschia intermedia Hantzsch	1, 2, 3, 4, 5	0.6	0.6	0.2
Nitzschia palea (Kützing) W.Smith	1, 2, 3, 4, 5	2.5	7.4	9.7
Nitzschia supralitorea Lange-Bertalot	1, 2, 3, 4, 5	7.2	4.0	6.0
Tryblionella jelineckii (Grunow) Mann	3	0.2	0.2	0.2
Order Cocconeidales, Family Achnanthidiaceae				
Achnanthidium pseudoconspicuum var. *yomensis* Yana & Mayama	1, 2, 3, 4, 5	1.4	2.1	0.9
Achnanthidium straubianum (Lange-Bertalot)	1, 2, 3, 4, 5	43.7	14.9	14.0
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	1, 2, 3, 4, 5	5.4	7.0	4.7
Planothidium rostratum (Østrup) Lange-Bertalot	1, 2, 3, 4, 5	0.8	0.9	1.1
Order Cocconeidales, Family Cocconeidaceae				
Cocconeis placentula Ehrenberg	1, 2, 3, 4, 5	4.8	12.1	1.1
Order Cymbellales, Family Cymbellaceae				
Cymbella tugidula Grunow	1, 2, 3, 4, 5	0.8	2.8	2.4
Cymbella tumida (Brébisson) Van Heurck	1, 3, 4, 5	0.1	0.1	0.4
Order Cymbellales, Family Gomphonemataceae				
Gomphonema lagenula Kützing	1, 2, 3, 4, 5	3.7	5.8	13.3
Gomphonema pumilum var. *rigidum* E.Reichardt & Lange-Bertalot	1, 2, 3, 4, 5	0.7	4.0	1.0
Gomphonema subclavatum (Grunow) Grunow	3, 4, 5	0.0	0.2	0.1
Order Fragilariales, Family Fragilariaceae				
Fragilaria capucina Desmazières	1, 2, 3, 4, 5	0.1	2.7	0.1
Order Licmophorales, Family Ulnariaceae				
Ulnaria lanceolata (Kützing) Compère	1, 3, 4, 5	0.1	0.2	0.8
Order Naviculales, Family Diadesmidaceae				
Diadesmis confervacea Kützing	1, 2, 3, 4, 5	2.4	1.4	0.7
Luticola mutica (Kützing) D.G.Mann	1, 2, 3, 4, 5	1.3	1.6	7.2
Order Naviculales, Family Naviculales incertae sedis				
Mayamaea agrestis (Hustedt) Lange-Bertalot	1, 2, 3, 4, 5	0.5	0.5	8.1
Order Naviculales, Family Naviculaceae				
Navicula cincta (Ehrenberg) Ralfs	1, 2, 3, 4, 5	1.0	2.4	1.0
Navicula cryptotenella Lange-Bertalot	1, 3, 4, 5	0.0	0.5	0.1
Navicula germainii J.H.Wallace	1, 2, 3, 4, 5	1.4	3.5	1.6
Navicula simulata Manguin	1, 2, 3, 4, 5	2.0	1.9	1.7
Navicula sp.1	1, 2, 3, 4, 5	1.1	0.1	0.4
Navicula sp.2	1, 2, 3, 4, 5	2.3	0.7	1.0
Navicula sp.3	1, 2, 3, 4, 5	0.1	0.8	0.3
Naviculadicta nanogomphonema Lange-Bertalot & U.Rumrich	1, 2, 3, 4, 5	1.0	0.1	3.2
Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli	1, 2, 3, 4, 5	1.1	6	1.1
Fallacia sp.1	3	0.3	0.1	0.1
Fallacia sp.2	3, 4, 5	0.3	0.0	0.3
Sellaphora lanceolata D.G.Mann & S.Droop	1, 2, 3, 4, 5	0.1	0.0	1.4
Sellaphora pupula (Kützing) Mereschkovsky	1, 2, 3, 4, 5	0.2	1.6	0.5
Sellaphora sp.1	1, 2, 3, 4, 5	1.7	0.5	5.7
Surirella fonticola Hustedt	2, 3,4	0.0	0.5	0.1
Amphora montana Krasske	1, 2, 3, 4, 5	1.8	0.3	3.6
Amphora pediculus (Kützing) Grunow	1, 2, 3, 4, 5	2.2	5.9	1.3
Cyclotella meneghiniana Kützing	2, 3, 4, 5	0.3	0.3	0.2

Note: Data are expressed as mean ± standard deviation (SD) of four replicates. Different letters (a, b and c) represent statistical comparisons between groups in each row using ANOVA and Tukey's post hoc test ($p<0.05$)

The trophic status of the Kok River water was classified as mesotrophic to meso-eutrophic. Results showed that all sampling sites during the cool dry and summer seasons were classified as mesotrophic, while during the rainy season all sites were classified as meso-eutrophic. This result concurred with Pekthong (2008) who indicated that water qualities in Kok River were mesotrophic status in most months, with the exception of the rainy season when water quality changed to meso-eutrophic status. The physical and chemical parameters among the sampling sites showed that conductivity, BOD, nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus had highest values in the rainy season and lowest values in the cool dry season.

Table 4. Physical and chemical parameters of water quality (average) in Kok River, Chiang Rai Province, Thailand

Parameters	**Cool dry**					**Summer**					**Rainy**				
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Water temp. (°C)	22.00±0.00^{b}	22.70±0.58^{b}	22.67±0.29^{b}	20.67±0.29^{a}	22.17±0.29^{b}	27.00±0.00^{c}	26.00±0.00^{c}	26.17±0.29^{c}	26.00±0.00^{c}	26.33±0.58^{c}	26.03±0.25^{c}	25.97±0.06^{c}	25.17±0.15^{bc}	24.63±0.06^{bc}	26.00±0.58^{c}
Air temp. (°C)	22.00±0.00^{a}	25.33±0.58^{b}	23.87±1.76^{ab}	22.33±0.29^{a}	25.00±0.00^{b}	31.00±0.00^{c}	30.00±0.00^{c}	26.33±0.58^{b}	26.33±0.58^{b}	25.67±1.15^{b}	27.00±0.00^{bc}	30.00±0.00^{c}	28.00±0.00^{bc}	25.00±0.00^{b}	28.53±1.27^{bc}
Velocity (m s^{-1})	0.25±1.10^{ab}	0.30±1.50^{ab}	0.23±1.01^{ab}	0.36±0.57^{ab}	0.14±0.20^{a}	0.16±0.65^{a}	0.25±1.74^{ab}	0.15±0.29^{a}	0.29±1.90^{ab}	0.12±0.06^{a}	0.26±0.50^{ab}	0.28±2.48^{ab}	0.20±0.38^{a}	0.46±3.21^{b}	0.18±0.20^{a}
Turbidity (NTU)	31.10±2.93^{b}	26.77±1.91^{ab}	26.90±4.45^{ab}	24.67±3.99^{ab}	20.73±3.55^{a}	23.73±2.11^{ab}	32.23±3.93^{b}	18.27±1.05^{a}	16.73±0.47^{a}	17.73±0.91^{a}	196.67±3.06^{d}	537.00±35.59^{e}	176.00±33.18^{d}	122.67±5.86^{c}	103.00±4.58^{c}
pH	7.10±0.71^{a}	6.42±1.50^{a}	6.42±0.07^{a}	6.55±0.12a	6.43±0.10^{a}	7.33±0.15^{a}	7.27±0.15^{a}	7.23±0.15^{a}	7.23±0.06^{a}	7.17±0.31^{a}	7.23±0.21^{a}	6.97±0.15^{a}	7.10±0.10^{a}	7.20±0.10^{a}	7.03±0.15^{a}
Alkalinity (mg L^{-1})	56.67±4.16^{a}	67.30±1.15^{b}	68.67±3.06^{b}	80.00±5.29^{c}	72.00±3.46^{bc}	125.33±2.31^{c}	130.67±15.14^{cd}	133.33±18.04^{cd}	134.67±6.11^{cd}	142.67±4.62^{d}	140.00±2.00^{d}	121.33±1.15^{c}	132.00±5.29^{cd}	123.33±3.06^{c}	133.33±5.77^{cd}
Conductivity)µS cm^{-1})*	107.70±0.30^{a}	104.77±6.88^{a}	104.57±1.78^{a}	104.43±2.68^{a}	109.27±4.48^{a}	123.10±0.95^{ab}	129.37±1.22^{ab}	126.40±0.62^{ab}	131.93±10.46^{b}	132.10±0.87^{b}	133.13±0.95^{b}	117.60±0.53^{ab}	112.03±1.70^{a}	117.47±14.01^{ab}	120.63±6.47^{ab}
DO (mg L^{-1})*	8.60±0.00^{c}	8.30±0.27^{c}	8.60±0.35^{c}	7.27±0.31^{bc}	7.40±0.53^{bc}	6.87±0.31^{b}	6.67±0.23^{b}	6.27±0.42^{ab}	5.47±0.23^{a}	6.20±0.53^{ab}	3.27±0.06^{a}	3.20±0.00^{a}	3.10±0.20^{a}	3.03±0.21^{a}	2.73±1.08^{a}
BOD_5 (mg L^{-1})*	1.87±1.50^{a}	6.90±0.64^{b}	5.27±0.81^{ab}	4.53±1.14^{ab}	3.2±0.72^{ab}	5.20±1.20^{ab}	10.93±0.83^{c}	6.53±3.61^{b}	5.87±0.23^{ab}	6.80±2.43^{b}	6.93±1.21^{b}	11.53±1.29^{c}	10.73±1.03^{c}	11.40±1.97^{c}	10.13±4.31^{bc}
Nitrate nitrogen)mg L^{-1})*	0.5±0.29^{a}	0.8±0.59^{a}	0.8±0.35^{a}	0.6±0.20^{a}	1.0±0.06^{a}	0.5±0.15^{a}	0.7±0.35^{a}	1.4±1.01^{a}	1.3±0.32^{a}	0.8±0.20^{a}	3.3±0.67^{a}	4.8±0.65^{ab}	4.1±0.74^{ab}	6.1±1.56^{b}	6.4±0.75^{b}
Ammonium nitrogen)mg L^{-1})*	0.46±0.25^{ab}	0.84±0.24^{b}	0.30±0.03^{a}	0.23±0.03a	0.33±0.15^{a}	0.39±0.06^{a}	0.37±0.02^{a}	0.48±0.33^{a}	0.68±0.58^{ab}	0.35±0.03^{a}	0.35±0.09^{a}	0.87±0.32^{b}	0.55±0.20^{ab}	0.63±0.16^{ab}	0.29±0.08^{a}
Soluble reactive phosphorus (mg L^{-1})*	0.53±0.07^{a}	0.54±0.14^{a}	0.61±0.28^{a}	0.35±0.07^{a}	0.56±0.09^{a}	0.57±0.28^{a}	0.79±0.15^{a}	0.75±0.23^{a}	0.67±0.12^{a}	0.71±0.15^{a}	0.79±0.27^{a}	3.52±0.54^{b}	3.86±0.08^{b}	4.21±1.32^{b}	4.03±0.79^{b}
Trophic status	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Mesotrophic	Meso - eutrophic	Meso - eutrophic	Meso - eutrophic	Meso - eutrophic	Meso - eutrophic

Note: * = used for assessment of AARL - PC Score

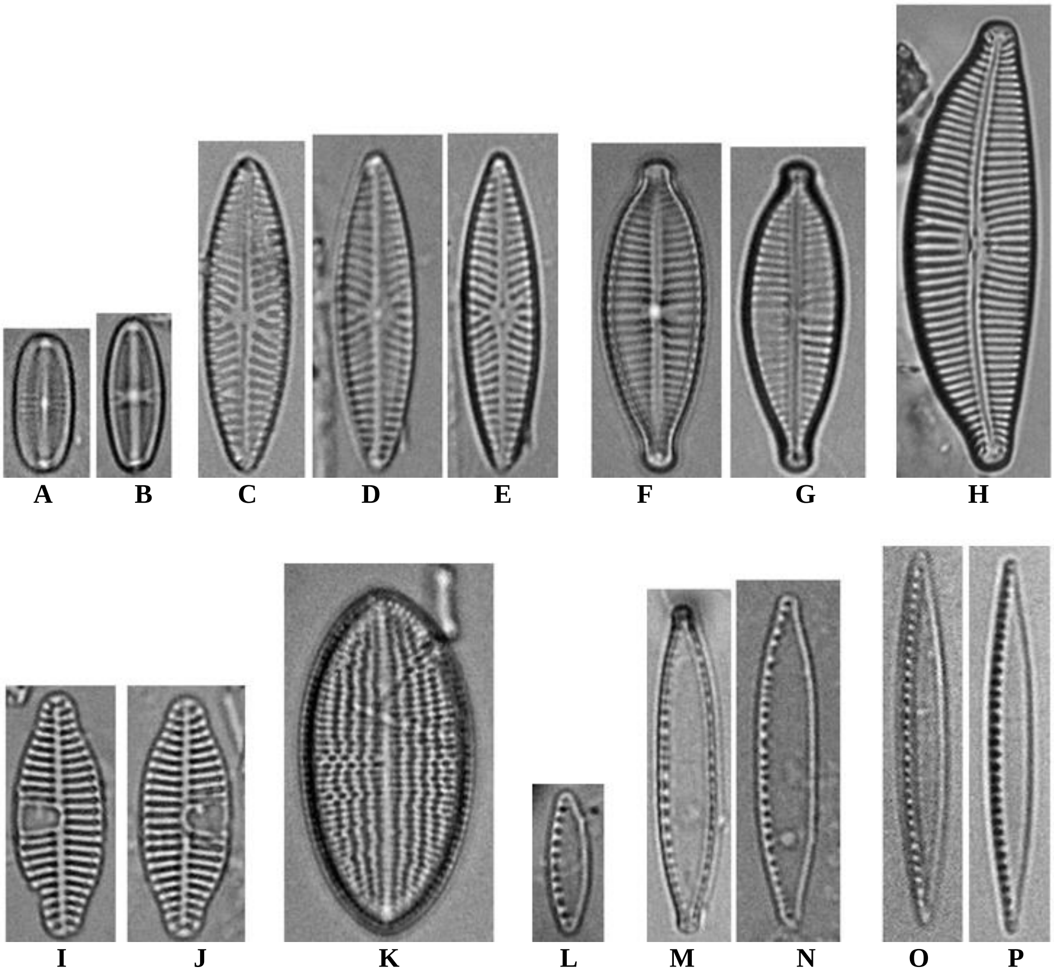

Figure 2. Light micrographs of dominant phytoplankton and benthic diatoms in the Kok River (scale bar = 10 µm). A-B. *Achnanthidium straubianum* (Lange-Bertalot)*♥, C-E. *Navicula cincta* (Ehrenberg) Ralfs*, F-G. *Gomphonema lagenula* Kützing*♥, H. *Cymbella tugidula* Grunow*, I-J. *Planothidium frequentissimum* (Østrup) Lange-Bertalot♥, K. *Cocconeis placentula* Ehrenberg♥, L. *Nitzschia inconspicua* Grunow♥, M-N. *Nitzschia palea* (Kützing) W.Smith♥, O-P. *Nitzschia supralitorea* Lange-Bertalot♥. Note: The symbol represent to living characteristic: * Phytoplankton, ♥ Benthic diatoms and *♥ Phytoplankton and benthic diatoms

The Kok River is one of the tributaries of the Mekong, and runs for 114 km across Chiang Rai Province through the general community, restaurants, fish ponds, and agricultural activities. These conditions impact various water quality parameters including nutrient loading (Xu et al. 2015; Withers et al. 2014). Frequent rainfall occurring during June resulted in discharge of nitrogen and phosphorus from the soil and discharge of agricultural fertilizers and wastewater from the community into the water body (Sharpley et al. 2013). Flushing of inorganic nitrogen from N-enriched upper soils horizons has been suggested as the primary mechanism for increasing N concentrations during rainfall events (Howden et al. 2011). This finding was similar to results reported by Liu et al. (2014), indicating that total nitrogen and nitrate nitrogen under heavy rainfall conditions were higher than measured under conditions of moderate rainfall. Pekthong (2008) found that during the rainy season months, the Kok River water was highly turbid, with high nitrate nitrogen and ammonium nitrogen concentrations.

Correlation between phytoplankton, benthic diatoms and water quality

Many variables influence the growth of freshwater algae. Environmental factors affect stream conditions and freshwater algal seasonality. Algae possess different physical and chemical requirements, whereby each species has a different set of favorable conditions that promote its growth and reproduction (Prasertsin and Peerapornpisal 2018). Algal communities are sensitive to changes in their environment (Li et al. 2018). As a result, algae total biomass and certain designated species are often used as indicators of water quality (Järvinen et al. 2012). In a lotic ecosystem, almost all algae live as benthic forms (Bere and Tundisi 2010).

The relationship between the dominant phytoplankton, benthic diatoms and the physical and chemical characteristics of the water body are shown by the results of the CCA plot (Figure 3).

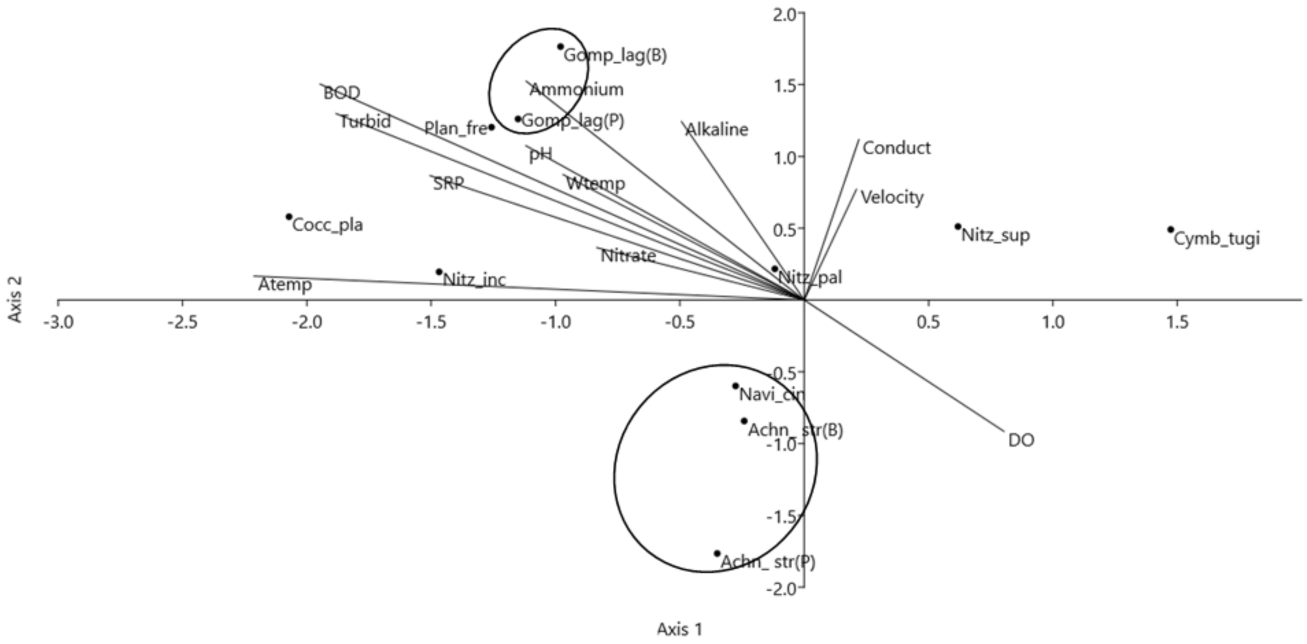

Figure 3. Canonical Correspondence Analysis (CCA) of the physical and chemical parameters and the phytoplankton and benthic diatoms. The results revealed a correlation between the physicochemical parameters and the dominant phytoplankton and benthic diatom in the Kok River, Chiang Rai province, Thailand (Eigenvalues percentage of axis 1 = 43.2, axis = 27.62

Gomphonema lagenula (Gomp_lag) in both planktonic and benthic forms showed a positive correlation with ammonium nitrogen. The highest percentage of abundance of this species was found during the rainy season, with the highest ammonium nitrogen. This result concurred with Leelahakriengkrai and Peerapornpisal (2010) who reported that the species occurred in moderate water quality. Moreover, *Achnanthidium straubianum* (Achn_str) in both planktonic and benthic forms and *Navicula cincta* (Navi_cin) showed a negative correlation between velocity and conductivity. The abundance of these species was the lowest during the rainy season with the highest water velocity and conductivity, similar to Ivanov (2018) who reported that these species occurred in moderate water quality.

Nitzschia palea (Nitz_pal) also showed a positive correlation with alkalinity, while *Planothidium frequentissimum* (Plan_fre) had a positive correlation with pH. *Nitzschia inconspicua* (Nitz_inc) had a positive correlation with air temperature (Atemp) but a negative correlation with DO (Fig. 3). Temperature was the most important factor controlling which phytoplankton taxa were present in freshwater lakes (Lv et al. 2014). Dominant planktonic and benthic diatoms, such as *Nitzschia palea*, *Gomphonema lagenula and Cocconeis placentula*, were good indicator species for moderate water quality. This result was similar to Leelahakriengkrai and Peerapornpisal (2010) findings who reported that these species occurred in moderate water quality.

In conclusion, phytoplankton and benthic diatoms can be found in a wide variety of water qualities that possess different physical and chemical requirements. Water temperature, turbidity, pH, alkalinity, velocity, conductivity, DO, BOD, ammonium nitrogen and soluble reactive phosphorus were the main factors that influenced the composition of phytoplankton and benthic diatoms in Kok River. The combination of phytoplankton and benthic diatom can be used as bio-indicators of water quality in the Kok River and other freshwater ecosystems.

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