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Impact of a Kraft Pulp and Paper Mill Effluent on Phytoplankton and Macroinvertebrates in River Nzoia, Kenya

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Phytoplankton and macroinvertebrate assemblages were used to assess the impact of a kraft pulp and paper mill effluent in Kenya, on River Nzoia downstream of the discharge point in relation to changes in water quality during May to June and November 2008 (rainy and dry seasons, respectively). Total phosphorus concentration increased from 0.027 mg·L^{-1} upstream to 0.04 mg·L^{-1} downstream. Ammonia nitrogen (NH_3-N) concentration was 0.51 mg·L^{-1} upstream and 0.86 mg·L^{-1} downstream. Nitrate concentration stood at 1.18 mg·L^{-1} upstream compared with the 2.23 mg·L^{-1} downstream. The pH changed from 4.5 to 5.0 upstream to 5.5 to 6.0 downstream, while DO increased from 6.57 to 7.03 mg·L^{-1} downstream. The BOD_5 (biochemical oxygen demand after five days) values remained almost unchanged from 4.63 mg·L^{-1} upstream to 4.67 mg·L^{-1} downstream. Taxon composition of phytoplankton and macroinvertebrates correlated with adverse environmental gradients resulting from the mill's effluent discharge. Overall, there was a shift in composition and abundance of both phytoplankton and macroinvertebrates, with the downstream site recording high numbers of tolerant taxa (i.e., *Microcystis* sp. and *Chironomus* sp.). It was recommended that water quality monitoring with effluents of this nature be done using a combination of chemical analysis and biological indicators such as phytoplankton and macroinvertebrates.

Key words: **macroinvertebrate, biomonitoring, effluent, nutrient, pulp and paper, pollution**

Introduction

Pulp mill effluents have been associated with a number of impact types on water quality and aquatic biota in the receiving water bodies. Several methods have been developed to monitor or assess the impact of paper mill effluents on receiving waters. They include the application of multistable isotope assays (Dubé et al. 2005), chemical analysis, endocrine assessment of fish (McMaster et al. 2005), caging small-bodied fish (Palace et al. 2005), and fathead minnow (*Pimephales promelas*) life cycle tests (Parrott 2009). Most of these methods are quite expensive and very few are in common practice or remain purely experimental. The use of biotic communities such as diatoms (Eloranta 1995, 1999; Eloranta and Kwandrans 1996; Eloranta and Anderson 1998) and fish (Kovacs et al. 2002; Siligato and Böhmer 2002) to survey gradients of aquatic environment variables has several advantages over physical and chemical monitoring (Kwang-Guk et al. 2002). For instance, in running waters where the water quality changes rapidly, biological monitoring has proved to be a very useful tool due to its integrating nature. While most research on pulp and paper mill effluent has focused on the impact on fish and fisheries resources, studies that have investigated the effects on benthic assemblages have reported an increase in abundance, together with some combination of increases, decreases, or no change in taxon richness, depending on the degree of eutrophication (Sprague and McLeese 1968; Marier 1973; Shumway and Palensky 1973; Culp et al. 2000). Other studies have revealed uptake of contaminants by benthic fauna in areas exposed to pulp and paper mill effluents (Etiégni et al. 2007; Meriläinen and Oikari 2008). Results from these studies show that the evaluation of biotic communities offers a comprehensive alternative to the use of physicochemical parameters when assessing the effect of pulp and paper mill effluents on the aquatic environment.

In Kenya, the impact of pulp and paper mill effluent discharge on River Nzoia has been of major concern over the years (Balirwa and Bugenyi 1988; Achoka 1998). Previous studies have indicated a decrease in fish richness (Balirwa and Bugenyi 1988) and deteriorated water quality (Achoka 1998) downstream of the effluent discharge point. However, because of their mobility, fish cannot give a comprehensive account on the impact of pulp mill effluent as opposed to benthic and/or pelagic assemblages which are more sedentary. The purpose of this study was to investigate the impact of pulp and paper mill effluent on the composition and occurrence of phytoplankton and macroinvertebrate assemblages in relation to changes in water quality arising from discharges from a pulp and paper mill effluent. The hypothesis tested in this study was that the mill effluent has no significant effect on physicochemical parameters and the community structure of phytoplankton and macroinvertebrate assemblages. Such information is necessary in assessing, monitoring, and managing the river and other similar water bodies.

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Materials and Methods

Study Area

The study was conducted at the Webuye PanPaper Mills (Fig. 1a) along River Nzoia. The mouth of River Nzoia lies at latitude 0°3'38.8"N and longitude 33°57'47.33"E. River Nzoia originates from the Cherangani Hills at a mean elevation of 2,300 m above sea level and drains into Lake Victoria at an altitude of 1,000 m above sea level. PanPaper (0°39'54.84"S, 34°47'47.44"E), the only kraft pulp and paper mill in Kenya, has an annual production capacity of 120,000 tonnes of paper from kraft cooking of chips from pine (*Pinus patula*), cypress (*Cupressus lusitanica*), and eucalypts (*Eucalyptus saligna*) and stone grinding of pine (*Pinus patula*). The bleaching sequences used are CEHH or CEHP (C = chlorination; E = hot caustic extraction; H = hypochlorite application; P = peroxide application) (Dence and Reeve 1996). The paper products include mainly newsprint, writing, and packaging papers for local and export markets in East and Central Africa and Asia (Orori et al. 2005).

PanPaper is situated at an altitude of 1,200 m above sea level on the western part of Kenya (Fig. 1b). The area experiences two rainy seasons. The long rains fall between March and July while the short rains start from August to October. The mean annual rainfall varies from 1,250 to 1,800 mm. The main farming activities in the catchment upstream of the water intake point into the mill include agriculture, agroforestry, forestry, and livestock rearing. The mill consumes about 40,000 m^3 of water and discharges between 35,000 to 40,000 m^3 daily into the river at a dilution rate varying between 0.3 to 3.2%, depending on the prevailing weather conditions in the area. The mill's effluent takes six weeks to flow through a set of settling tanks (one primary and one secondary), aerated lagoons, and stabilization ponds before being discharged into the river. Over the past 15 years, however, expansion programs within the mill have led to an overloaded wastewater treatment system that was initially designed to treat only 25,000 m^3 of mill effluent per day. As a consequence, partially treated mill wastewater is being discharged into River Nzoia in complete violation of the 2006 Effluent Discharge Standards (Table 1). For example, although PanPaper wastewater pH and total dissolved solids (TDS) are in compliance with regards to Kenya's environmental regulations, the mill's treated effluent biochemical oxygen demand after five days (BOD_5) and chemical oxygen demand (COD) are much higher than the stipulated maximum discharge limits of 30 and 50 $mg \cdot L^{-1}$, respectively. For PanPaper, meeting the 15^0 H for effluent colour has always been elusive, despite numerous attempts at increasing aeration in the aerated lagoons. More work will probably be required in terms of process modification, higher fibre recovery, and more water recycling for the mill to meet the 2006 Effluent Discharge Standards (Table 1).

Study Site, Sampling, and Sample Preparation

An upstream-downstream design was employed in this study. The two sites were separated by a distance of about 1 km. The downstream site was located 50 m below the discharge point after complete mixing of the effluent and river water that occurs after a short stretch from the outfall due to high turbulence. The two sites share similar environmental attributes including both instream and riparian habitat. The substrates in pools were composed of mud, sand, and detritus while the riffles were made of boulders and pebbles. The fast water flow enhanced mixing and oxygenation. Both sites were being used for drinking water for domestic livestock and sand mining. There was no other discernable point source pollution between the sampling sites besides the discharge of the mill's effluent.

Phytoplankton sampling. Sampling was performed in triplicate at the upstream and downstream sites of the paper mill effluent discharge point on each visit during the months of May, June, and November 2008. Oblique

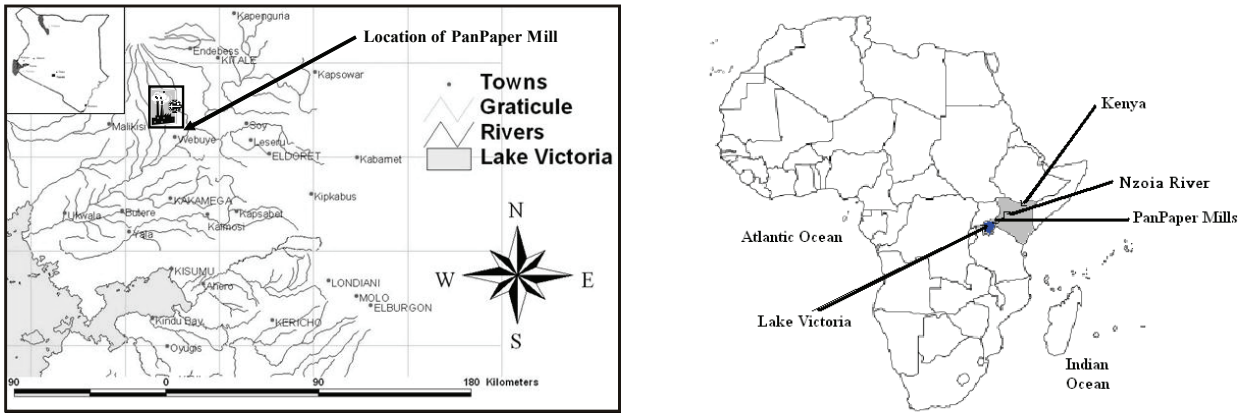

Fig. 1. Map of the study area showing the location of PanPaper Mills at Webuye in Western Kenya. Map of Kenya at the top left corner (left). Map of Africa showing the location of PanPaper Mills (right).

TABLE 1. Raw and treated kraft pulp and paper mill effluent by current treatment system

Parameters [a]	*Primary clarifier overflow*	*Treated after last stabilization lagoon*	*Effluent discharge standards* [b]
pH	8.5–9.3	6.9–7.5	6.5–8.5 (nonmarine)
Alkalinity (mg/L)	330.0–346.4	70.0–88.2	—
Temperature (^{O}C)	39.0–39.6	19.0–21.2	±3
TS (mg/L)	872.4–980.7	440.0–474.5	30.0
TDS (mg/L)	670.0–699.6	670.0–699.6	1,200.0
TSS (mg/L)	212.5–291.5	94.6–133.0	30.0
Colour (^{O}H)	1,280.5–1,867.7	1,600.0–3,263.3	15.0
BOD_5 (mg/L)	182.5–234.7	62.8–117.6	30.0
COD (mg/L)	536.0–591.5	296.7–401.5	50.0
Turbidity (NTU)	130.0–136.1	311.0–351.3	—
Conductivity (μS/cm)	1,339.2–2,109.3	790.0–891.3	—
Dissolved oxygen (mg/L)	0.00	0.40	—
Phosphorus (mg/L)	0.056	0.005	—
Nitrites (mg/L)	0.004	0.008	—
Nitrate (mg/L)	0.02	0.036	—

[a] TS = total solids; TDS = total dissolved solids; TSS = total suspended solids; BOD_5 = biochemical oxygen demand after 5 d; COD = chemical oxygen demand.
[b] Source: Kenya Gazette (2006).

tows were made with a 28-μ plankton net of 50-cm diameter. The net was cast while standing in the water at a safe depth from the bank. Brisk tows were made so that the mouth of the net was not allowed to touch the river bottom. The pay-length of the towing rope was 2.5 m, allowing an effective towing distance of about 2.0 m, thereby giving an efficiency of about 80%. Samples were collected for phytoplankton analysis according to APHA (1998). In the laboratory, the phytoplankton were identified and counted in a Sedgewick Rafter cell (Lund et al. 1958) using an inverted Olympus CK2 Microscope at ×400, while identification of phytoplankton was done at the mechanical stage of a compound microscope at ×800 to the lowest taxonomic unit using several keys and illustrations (Prescott 1962; Vollenweider 1969; Kramer and Lange-Bertalot 1986, 1988, 1991a, 1991b; APHA 1998).

Counting of the phytoplankton was done according to Lund et al. (1958) in the Sedgwick-Rafter cell (50-mm long by 20-mm wide by 1-mm deep with a surface area of 1,000 mm^2) with an Olympus inverted microscope at ×400 magnification (APHA 1998). One (1) mL of the concentrated sample was pipetted into the rafter cell, and phytoplankton were counted in at least ten fields and the number of phytoplankton cells per millilitre was calculated according to a modified procedure by APHA (1998) as shown below:

$$\text{Phytoplankton density mL}^{-1}\ (D) = [(A)(l \times w \times d)] \quad (1)$$

where D = phytoplankton density for subsample of mL^{-1} in number; A = average number of phytoplankton counted in one Sedgwick-Rafter cell; l = length in mm of the Sedgewick Rafter counting cell (50); w = width of the Sedgewick Rafter counting cell (20 mm); d = depth of the Sedgewick Rafter counting cell (1 mm).

The counted number of phytoplankton cells in each 1-mL subsample was converted to the original 50-mL sample by the following relationship:

$$\text{Phytoplankton density in 50 mL } (T) = D \times V_1 \quad (2)$$

where D = phytoplankton density for subsample in numbers per unit volume (mL^{-1}); V_1 = volume of the original sample (50 mL). The 50-mL volume was converted to the total volume filtered during the oblique tows by the following relationship:

$$\text{Final phytoplankton density} = T \times (1000/V_2) \quad (3)$$

where T = phytoplankton density for 50-mL sample; V_2 = original volume filtered during the net tows;

$$\text{Sample volume } (V_2) = \pi r^2 \cdot d \quad (4)$$

where π = pi, with a value of 3.14; r = the radius of the plankton net mouth (25 cm); d = distances moved by the net during towing (200 cm).

The average distance towed was determined from the pay-out tow rope length of 2.5 m resulting in an effective towing distance of 2.0 m (200 cm), and all towings were done while standing in water on the shallow edge of the stream at each sampling site. Identification of phytoplankton was done at the mechanical stage of a compound microscope at ×800 to the lowest taxonomic unit using several keys and illustrations (Prescott 1962; Vollenweider 1969; Kramer and Lange-Bertalot 1986, 1988, 1991a, 1991b; APHA 1998).

Water quality sampling. Water samples were collected in triplicate once in May to June and November 2008 for nutrient analysis following standard procedures (APHA 1998). The samples were packed in fresh ice in a cooler

box then transported to the laboratory for analysis of nitrate nitrogen (NO_3^--N), ammonia nitrogen (NH_3-N), and total phosphorus (TP). Water temperature and dissolved oxygen (DO) were determined in situ at each of the sampling sites using a JENWAY 3405 electrochemical analyzer with an appropriate probe for each of these variables. The standard Winkler Method (APHA 1998) was used to determine BOD_5 in the laboratory.

Determination of nitrite. Nitrite concentration was determined spectrophotometrically after nesslerization using NEDD solution at a wavelength of 543 nm; nitrite-nitrogen (NO2-N) concentrations were obtained from a standard calibration graph and converted into nitrite by multiplication with a factor of 3.28 as follows:

$$1\ mg{\cdot}L^{-1}\ NO_2\text{-}N\ (46.01\ mg\ NO_2/14.01\ mg\ N) = 3.28 \quad (5)$$

Then NO_2 = 3.28 (concentration of NO_2-N from the calibration graph).

Determination of ammonia. Ammonia nitrogen was measured as total Kjeldahl nitrogen. The digest was transferred to a micro Kjeldahl distillate unit to which about 3.5 mL of hypo solution was added. Five (5) mL of baric acid solution containing 3 to 4 drops of indicator was put into a conical flask. The boiling flask was heated to pass the steam into the sample; distillation was continued for about 10 minutes. The conical flask was then removed and the boiling flask cooled so that all wastes were sucked and removed through a tap. The distillate was titrated against hydrochloric acid; turning from blue colour to pink was the end point.

Determination of phosphate. Phosphate concentration was determined by the spectrophotometric method. The absorbance and standard against a reagent blank was read at a wavelength of 882 nm. Phosphate concentration (PO_4^{3-}-P) in $mg{\cdot}L^{-1}$ was given as:

$$F = \text{standard solution } (\mu g\ PO_4^{3-}\text{-}P)/(E_1\ \text{standard} - EB_1) \quad (6)$$

$$\mu g\ PO_4^{3-}\text{-}P = F\ [E_1\ \text{sample} - (E_0 + EB_1)] \quad (7)$$

where E_0 = absorbance of sample without reductant; E_1 = absorbance of sample or standard solution without reductant; EB_1 = absorbance of distilled water + reagent.

Macroinvertebrate sampling. Sampling for macroinvertebrates was carried out where water samples had been collected using a scoop net (0.5-mm mesh size). Triplicate samples were collected by a kick method from runs, riffles, and pools from each site. Sampling was done for a standard three minutes by disturbing a 1-m^2 area for each microhabitat (upstream and downstream at depths of less than 0.5, 0.70, and 1.0 m from the river bank (a total of three microhabitats per site). Specimens were sorted live in white plastic trays, then poured into vials and preserved with 70% ethyl alcohol. Having determined that replications in a site did not exhibit any statistical difference, these replicates were pooled to make one composite sample per site. At the laboratory, samples were sieved using a 300-µm mesh size sieve and sorted further. Specimens were identified down to the genus level according to Merritt and Cummins (1996) and Gerber and Gabriel (2002).

Data Analysis

All the data collected were put in a spreadsheet to facilitate statistical analyses using MINITAB Ver. 13 (Minitab Inc. Corporation 2000) statistical package. Data on physicochemical parameters were tested for significant difference between sites and months using the general linear model (GLM) analysis of variance (ANOVA). Data on phytoplankton and macroinvertebrate abundance were also tested for significant difference between sites, months, replicates, and species using GLM ANOVA. This approach allowed for the use of the Student Newman Keules (SNK) multiple range test if significant differences were detected among the categories in the model. Whenever possible, Least Significant Difference (LSD) tests were used to separate the means. The relative abundance of various macroinvertebrate genera were determined to provide information on the make-up of the assemblage and the relative contribution of the macroinvertebrate populations to the total assemblage according to the following formula:

$$\text{Relative abundance} = \frac{\text{Number of individuals of one taxon}}{\text{Total number of individuals}} \quad (8)$$

Upstream and downstream physicochemical parameters and phytoplankton densities were compared using paired *t*-tests (Zar 2001). The interrelationships between physicochemical parameters, phytoplankton, and macroinvertebrate assemblages were examined using canonical correspondence analysis (CCA) (Braak 1986; Braak and Prentice 1988; Braak and Verdonschot 1995). CCA is a multivariate direct gradient method designed to extract synthetic environmental gradients from ecological datasets. CCA assumes that species have unimodal distributions along environmental gradients (Minchin 1987; Braak and Smilauer 1998). CCA is calculated using a reciprocal averaging form of correspondence analysis. However, at each cycle of the averaging process, a multiple regression of the sample scores is performed on the environmental variables. New site scores are calculated based on this regression, and then the process is repeated until the scores stabilize. The result is that the axes of the final ordination, rather than simply reflecting dimensions of the greatest variability in the species data, are restricted to the linear combinations

of the environmental variables (physicochemical parameters) and the species data (phytoplankton or macroinvertebrate abundance) (Wilson and Mohler 1983; Palmer 1994). In this way these two sets of data are directly related, hence the popularity of this method whose main strength is its robustness to many data types, nonlinear relationships, and some rare species. CCA weakness however remains associated with multiple regression and chi-square distances that can emphasize rare species.

Data on phytoplankton and environmental variables, except pH, were log transformed because of their skewed distributions. Relative abundance values of the macroinvertebrate taxa were arcsine transformed. It is known from statistical theory that percentages or proportions form a binomial rather than a normal distribution, with the deviation from normality being great for small (0 to 30%) or large (70 to 100%) percentages. But if the square root of each proportion is transformed to its arcsine (i.e., the angle whose arcsine is), then the resultant data will have an underlying distribution that is nearly normal. Since count data of macroinvertebrates would follow a binomial distribution, transformation using arcsine and expressing the relative changes of abundance in percentages was appropriate in this study. All statistical tests were carried out at $\alpha = 0.05$.

Results

Physicochemical Water Parameters

The results for treated effluent shown in Table 1 underscore the lack of adequate treatment of the mill's effluent since its expansion programs. There was a marked increase in temperature, BOD_5, nitrites, phosphates, and electrical conductivity downstream as compared with upstream samples. For example, BOD_5 and COD were well above the current Effluent Discharge Standards. Only effluent TDS, temperature, and pH appeared to fall within the acceptable limits. For the river water, there was a significant increase in DO ($mg \cdot L^{-1}$) from 6.57 upstream to 7.03 downstream ($p = 0.024$) and nitrate nitrogen ($mg \cdot L^{-1}$) from 1.18 to 2.23 ($p = 0.008$), BOD_5 ($mg \cdot L^{-1}$) from 4.63 to 4.67 ($p = 0.032$), TP ($mg \cdot L^{-1}$) from 0.03 to 0.04 ($p = 0.045$), and a threefold increase in electrical conductivity ($mS \cdot cm^{-1}$) from 110 upstream to 333 downstream (Table 2). There was an apparent increase in temperature (°C) from 21.7 upstream to 23.0 downstream ($p = 0.383$) and ammonia nitrogen from 0.51 to 0.86 ($mg \cdot L^{-1}$) ($p = 0.108$), but these were not statistically significant (Table 2).

Species Composition and Abundance

Data on phytoplankton genera did not show any significant difference between months, replicates, and sites (upstream and downstream). However, there was a significant difference in abundance among species. A total of 36 different genera, belonging to five classes were recorded: Bacillariophyceae, Cyanophycea, Euglenophycea, Chlorophyceae, and Pyrrophyceae. The Chlorophyceae were the most numerically abundant and they also had the highest number of genera (15), followed by Bacillariophyceae with 10 genera (Fig. 2). The most abundant genera in Chlorophyceae included *Botryococcus, Cyanarcus, Scenedesmus, Botrydium*, and *Monoraphidium*, while the most common and abundant genera in Bacillariophyceae were *Melosira, Synedra, Navicula*, and *Niztchia*. The Cyanophycea had 4 genera consisting of *Microcystis, Synechococcus*, and *Coenococcus* (Fig. 3a). Both Euglenophyceae and Pyrrophyceae had three genera each dominated by *Trachelomonas* and *Netrium*, respectively (Fig. 3b and c).

There was a significant difference in phytoplankton abundance between genera ($F_{0.05(2),34,1} = 3.35$; $p < 0.0005$) but not between sites ($F_{0.05(2),1,174} = 0.29$; $p = 0.858$), although the upstream site exhibited an apparent but not statistically significant high density. The GLM ANOVA showed significant differences in the observed abundance of Bacillariophyceae ($F_{0.05(2),9,1} = 5.43$; $p < 0.0005$) and Chlorophyceae ($F_{0.05(2),14,1} = 2.59$; $p = 0.004$), but not

TABLE 2. Paired *t*-test showing mean physicochemical parameters upstream and downstream during May to June and November 2008 [a]

Physicochemical parameter [b]	*Mean ± SE*		*p-value* [c]
	Upstream	*Downstream*	
Temp (°C)	21.67 ± 0.33	23.00 ± 1.16	0.38
pH	4.75 ± 0.02	5.82 ± 0.03	0.004*
DO (mg/L)	7.03 ± 0.09	6.57 ± 0.07	0.024*
BOD_5 (mg/L)	4.63 ± 0.03	4.67 ± 0.23	0.032
NO_3^--N (mg/L)	1.18 ± 0.01	2.23 ± 0.09	0.008*
NH_3-N (mg/L)	0.51 ± 0.12	0.86 ± 0.021	0.11
TP (mg/L)	0.027 ± 0.001	0.040 ± 0.001	0.045*
Cond. (µS/cm)	109.60 ± 0.60	333.30 ± 21.5	<0.0005*

[a] $n = 9$ for all parameter for all months.
[b] Temp = temperature; DO = dissolved oxygen; BOD_5 = biochemical oxygen demand after 5 d; NO_3^--N = nitrate nitrogen; NH_3-N = ammonia nitrogen; TP = Total phosphorus; Cond. = conductivity.
[c] Asterisk (*) indicates significant differences.

between sites: ($F_{0.05(2),1,49}$ = 0.26; p = 0.613) and ($F_{0.05(2),1,74}$ = 0.25; p = 0.617), respectively (Fig. 2). The same was observed for Cyanophyceae ($F_{0.05(2),3,1}$ = 3.31; p = 0.042) and Euglenophyceae ($F_{0.05(2),2,1}$ = 0.91; p =0.423), but not between sites: ($F_{0.05(2),1,19}$ = 0.22; p = 0.642) and ($F_{0.05(2),1,14}$ = 0.03; p = 0.872), respectively (Fig. 3a,b,c). Further analysis using multiple range tests identified the gradient of abundance between the two sites and facilitated the determination of possible explanations based on physicochemical parameters. It was then possible to identify the sensitive, tolerant, and intolerant groups of phytoplankton in the samples (Table 3).

The SNK multiple range test and LSD indicated that for Bacillariophyceae, *Melosira* was significantly more abundant at the two sites than all the other genera (p = 0.004), possibly indicating that it is tolerant to the pulp and paper mill effluents. *Synedra* and *Eunotia* were significantly more abundant than *Bacillaria, Eunotia*, and *Pinnularia,* especially at the downstream site, also indicating high tolerance to kraft paper mill effluent (Fig. 2a). *Synedra* and *Eunotia* abundance increased by almost a similar proportion of 100% from upstream to downstream sites (Table 3). Other genera such as *Rhoicosphenia*, *Gomphocymbella,* and *Cocconeis,* which were absent upstream, suddenly appeared downstream in relatively high abundance, indicating their sensitivity in determining the impact of pollutants from the pulp and paper effluent (Fig. 2a). *Sychococcus* increased twofold downstream. For the Chlorophyceae the abundance of *Botryococcus* downstream was significantly higher than all the other genera at the two sites, thereby showing tolerance to the effluents (Fig. 2b). However, *Botrydium*

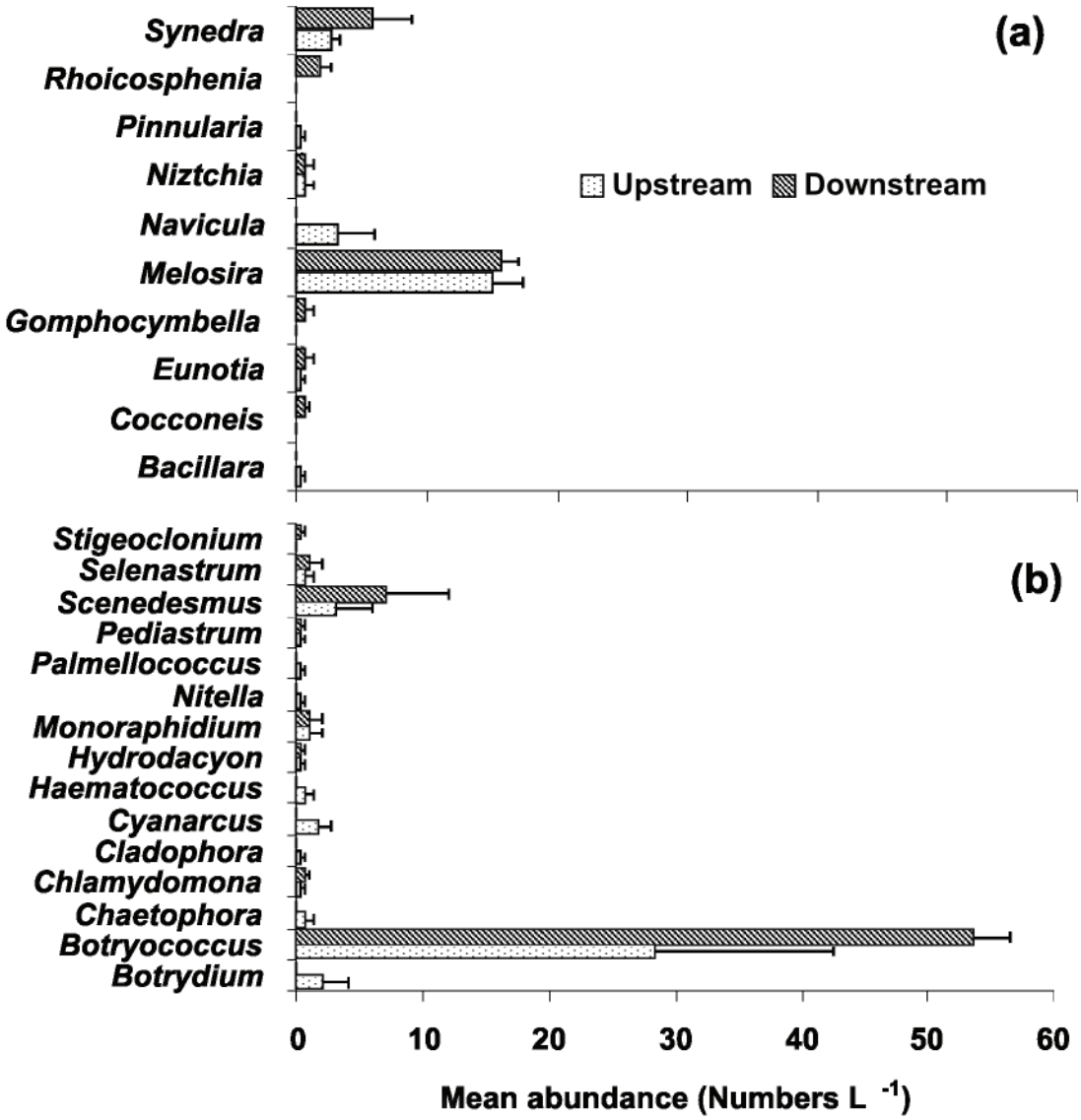

Fig. 2. Variation in phytoplankton genera of Bacillariophycea (a) and Chlorophyceae (b) for two sampling stations above the intake and below the discharge into the river. (Horizontal bars are standard errors).

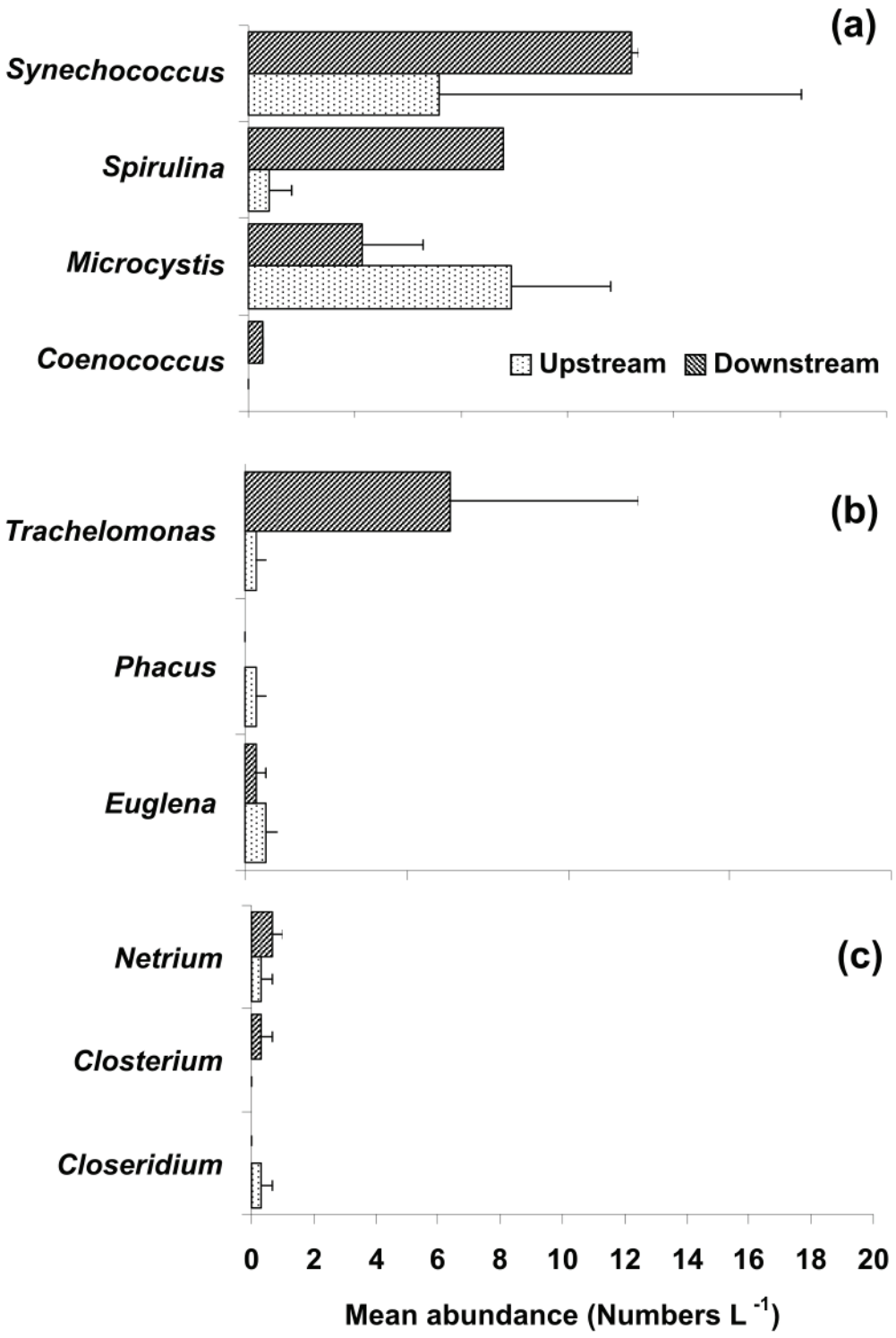

Fig. 3. Variation in phytoplankton genera of Cyanophyceae (a), Euglenophyceae (b), and Pyrrophyceae (c) for two sampling stations above the intake and below the discharge into River Nzoia and environmental gradients (horizontal bars are standard errors).

TABLE 3. Upstream to downstream algal genera comparisons: loss and appearance of genera as well as significant increase or decrease in the abundance of sensitive and tolerant indicator genera

Effects	*Genera*	*Percent change*	*Implication*	*Reference*
Loss	*Bacillaria*	100	sensitive	this study
	Botrydium	100	sensitive	this study
	Chaetophora	100	sensitive	this study
	Cladophora	100	sensitive	this study
	Closeridium	100	sensitive	this study
	Cyanarcus	100	sensitive	this study
	Haematococcus	100	sensitive	this study
	Navicula	100	pollution indicator	Soininen 2002
	Nitella	100	sensitive	this study
	Palmellococcus	100	sensitive	this study
	Phacus	100	sensitive	this study
	Pinullaria	100	sensitive	Arimoro et al. 2008
Appearance	*Closterium*	100	tolerant to nutrients	Arimoro et al. 2008
	Coenococcus	100	tolerant to nutrients	Arimoro et al. 2008
	Gomphonema	100	pollution indicator	Lung'ayiah et al. 2000
	Rhoicosphenia	100	pollution indicator	Unni and Pawar 2000
	Stigeoclonium	100	tolerant to nutrients	this study
	Cocconeis	100	tolerant to nutrients	Lung'ayiah et al. 2000
Increase	*Botryococcus*	89	tolerant to nutrients	Soininen 2002
	Chlamydomonas	100	tolerant to nutrients	this study
	Synechococcus	100	tolerant to nutrients	Akbay et al. 1999
	Melosira	0.9	tolerant to nutrients	Lung'ayiah et al. 2000
	Eunotia	100	tolerant to nutrients	Soininen 2002
	Netrium	100	tolerant to nutrients	this study
	Scenedesmus	133	tolerant to nutrients	Unni and Pawar 2000
	Spirulina	1,100	tolerant to nutrients	Okoth et al. 2009
	Synedra	112	tolerant to nutrients	Akbay et al. 1999
	Trachelomonas	1,800	tolerant to nutrients	this study
Decrease	*Euglena*	50	sensitive	Arimoro et al. 2008
	Microcystis	57	tolerant to nutrients	Akbay et al. 1999

density was significantly lower than that of *Botrycoccus* ($p < 0.0005$) but not for any other genera. *Botrydium* could be considered as a sensitive species that disappeared with deteriorating water quality downstream (Table 3). The abundance of *Botryococcus*, *Scenedesmus*, and *Chlamydomonas* had significantly increased (by 37, 154, and 100%, respectively) downstream compared with their abundance upstream (Fig. 2b; Table 3). These genera also are indicators of the deteriorating water quality. The Pyrrophyceae genus *Closeridium* was completely absent downstream (Fig. 3c; Table 2). For Euglenophycea, the genus *Phacus* also appeared only in the upstream station but not downstream (Fig. 3b). These genera are also considered to be sensitive (Table 3). The Cyanophytcea genera *Coenococcus* appeared downstream (Fig. 3a). *Coenococcus* is generally considered an indicator of pollution and/or eutrophication (Table 3). *Synechococcus*, *Spirulina*, and *Microcystis,* which are also considered indicators of pollution and/or eutrophication, were also observed upstream (Fig. 3a; Table 3).

For macroinvertebrate samples, having determined that replications in a site did not exhibit any statistical difference, replicates were pooled to make one composite sample per site for each of the sampling dates (months). Further analysis indicated no significant differences among the months, hence the data were further pooled. The relative abundance of macroinvertebrate taxa was thus compared only between upstream and downstream sites. Macroinvertebrate samples at the upstream site were dominated by Ephemeroptera taxa which together with Plecoptera and Trichoptera (EPT) formed more than 45% of the total number of individuals collected from the site (Table 4). At the downstream site the abundance of EPT was reduced while that of Diptera increased, constituting more than 58% of the total number of individuals sampled. There was a reduction in the number of taxa from 18 at the upstream site to 15 at the downstream site where most of the Ephemeroptera taxa were replaced by the Diptera (Table 5).

Canonical Correspondence Analysis (CCA)

Based on the *t*-test performed on the river physicochemical parameters, it was observed that the variation in populations of certain phytoplankton genera coupled with changes in river water quality were illustrated using the multivariate approach, CCA.

TABLE 4. The distribution and relative abundance (arcsine numbers) of macroinvertebrate taxa upstream and downstream in the River Nzoia during May-June and November 2008[a]

Order	*Family*	*Genus*	*Upstream*	*Downstream*
Ephemeroptera	Baetidae	*Baetis*	23.1	4.7
	Baetidae	*Centroptilum*	3.1	–
	Caenidae	*Caenis*	1.0	–
	Ecdyonurida e	*Ecdyunurus*	3.1	9.2
	Heptageniidae	*Heptagenia*	6.1	–
	Ecdyonuridae	*Rithrogena*	4.6	–
Plecoptera	Perlidae	*Dinocras*	1.5	4.2
Trichoptera	Leptoceridae	*Athropsades*	–	3.1
	Philopotamidae	*Philopotamus*	3.1	2.1
Amphipoda		*Nyctiphanes*	–	5.7
Branchyura		*Caecinus*	–	0.5
Coleoptera	Dytiscidae	*Platambus*	9.2	
	Elmidae	*Elmis*	4.6	7.3
	Gyrinidae	*Gyrinus*	13.9	2.1
	Hydreanidae	*Hydronchus*	–	1.6
Diptera	Chironomidae	*Chironomus*	–	36.5
	Simuliidae	*Simulium*	–	1.0
	Tabanidae	*Tabanus*	–	19.8
	Tipulidae	*Pedicia*	–	1.6
		Elliptera	3.1	
Hemiptera	Belostomatidae	*Belostoma*	6.2	4.2
	Gerridae	*Gerris*	7.7	–
	Veliidae	*Velia*	1.5	–
Lamellibranchiata	Sphaeriidae	*Sphaerium*	1.0	–
Odonata	Agriidae	*Agrion*	3.1	–
	Corduliidae	*Epiconelulis*	0.5	–

[a] $n = 9$ for all months.

TABLE 5. Upstream to downstream benthic macroinvetebrate genera comparisons; loss and appearance of genera as well as significant increase or decrease in the abundance of a genera are indicated

Effect	*Genera*	*Percent change*	*Pollution tolerance*	*Reference*
Loss	*Centroptilum*	100	Sensitive	Barbour et al. 1999
	Heptagenia	100	Sensitive	Raburu et al. 2009
	Rithrogena	100	Sensitive	Raburu et al. 2009
	Platambus	100	Sensitive	Barbour et al. 1999
	Elliptera	100	Sensitive	Barbour et al. 1999
	Gerris	100	Moderately tolerant	Barbour et al. 1999
	Agrion	100	Moderately tolerant	Masese et al. 2009a
Appearance	*Hydronchus*	100	Pollution tolerant	Barbour et al. 1999
	Chironomus	100	Pollution tolerant	Barbour et al. 1999
	Simulium	100	Moderately tolerant	Masese et al. 2009a
	Tabanus	100	Pollution tolerant	Dickens and Graham 2002
	Arthropsades	100	Pollution tolerant	Barbour et al. 1999
	Nyctiphanes	100	Pollution tolerant	Barbour et al. 1999
	Pedicia	100	Pollution tolerant	Dickens and Graham 2002
Increase	*Ecdyonurus*	197	Sensitive	Barbour et al. 1999
	Dinocras	180	Sensitive	Barbour et al. 1999
	Elmis	59	Moderately tolerant	Buss et al. 2002
Decrease	*Baetis*	80	Moderately tolerant	Thorne and Williams 1997
	Philopotamus	32	Moderately tolerant	Thorne and William s 1997
	Gyrinus	85	Moderately tolerant	Barbour et al. 1999

Bacillariophyceae. The CCA triplot showed that Bacillara and Navicula were associated with high loading of nitrates on the first CCA axis (Fig. 4), whereas Gomphocymbella and Synedra were affected by high loading of phosphorus. Negative loading of BOD_5 and ammonia was associated with Nitzchia at both upstream and downstream sampling sites. Pinnularia was however not associated with any factor loading, but showed negative abundance on the first axis upstream.

Chlorophyceae. DO and ammonia had positive factor loading on the first axis but negative loading on the second axis (Fig. 5). On the first axis, the factor loading at the upstream site was correlated with high abundance of Monoraphidium, Hydrodactyon, Cladophora, Botrydium, and Haematococcus. Negative factor loading for nitrates and phosphorus on the first axis at the upstream site was associated with high densities of Palmellococcus and Nitella. Chaetophora was closely associated with the upstream site without any significant factor loading on the first axis, but with high negative factor loading for DO, BOD_5 and ammonia.

Cyanophyceae. There was high positive factor loading for nitrates on the first axis at the upstream station for Synechococcus and at the downstream site for Microcystis (Fig. 6). High factor loading of phosphorus and temperature in the downstream promoted high Coenococcus density.

Euglenophyceae and Pyrrophyceae. The Euglenophyceae and Pyrrophyceae were represented by only three genera each. There was a positive factor loading of phosphorus, nitrates, and temperature on the first axis downstream (Fig. 7). *Closterium* was closely associated with nitrates while *Netrium* was associated with temperature factor loading downstream. High negative factor loading of ammonia, DO, and BOD_5 upstream were associated with high densities of *Phacus* and *Closeridium* on both axes (Fig. 7).

Macroinvertebrates. The downstream site was associated with increased pH, BOD_5, and conductivity while the upstream site recorded higher DO values (Fig. 8). The CCA grouped taxa into three clusters: those associated with the downstream site and higher pH, BOD_5, and conductivity values (*Chironomus* sp., *Tabanus* sp., *Limnius* sp., *Gyrinus* sp., and *Pedicia* sp.), and those associated with the upstream site and higher DO values (e.g., *Heptagenia*, *Agrio* sp., *Gerris* sp., and *Rhithrogena* sp.). The third group displayed no particular preference for either of the two sites (*Ecdyonurus* sp., *Belostoma* sp., and *Philopotamus* sp.). As a general observation, Fig. 2 and 3 show that there was a reduction of taxon richness as one moved from upstream to the downstream sites.

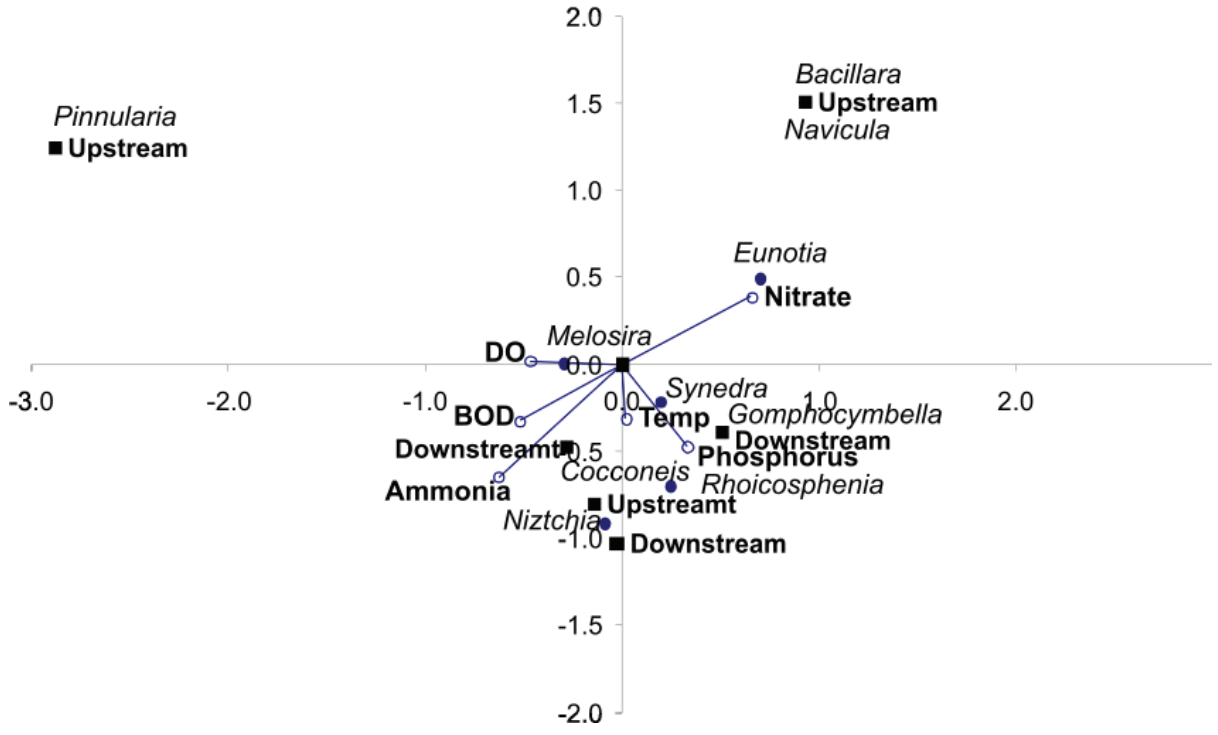

Fig. 4. Cannonical correspondence analysis (CCA) ordination plot for the distribution of Bacillariophyceae genera above the intake and below the discharge into River Nzoia and environmental gradients.

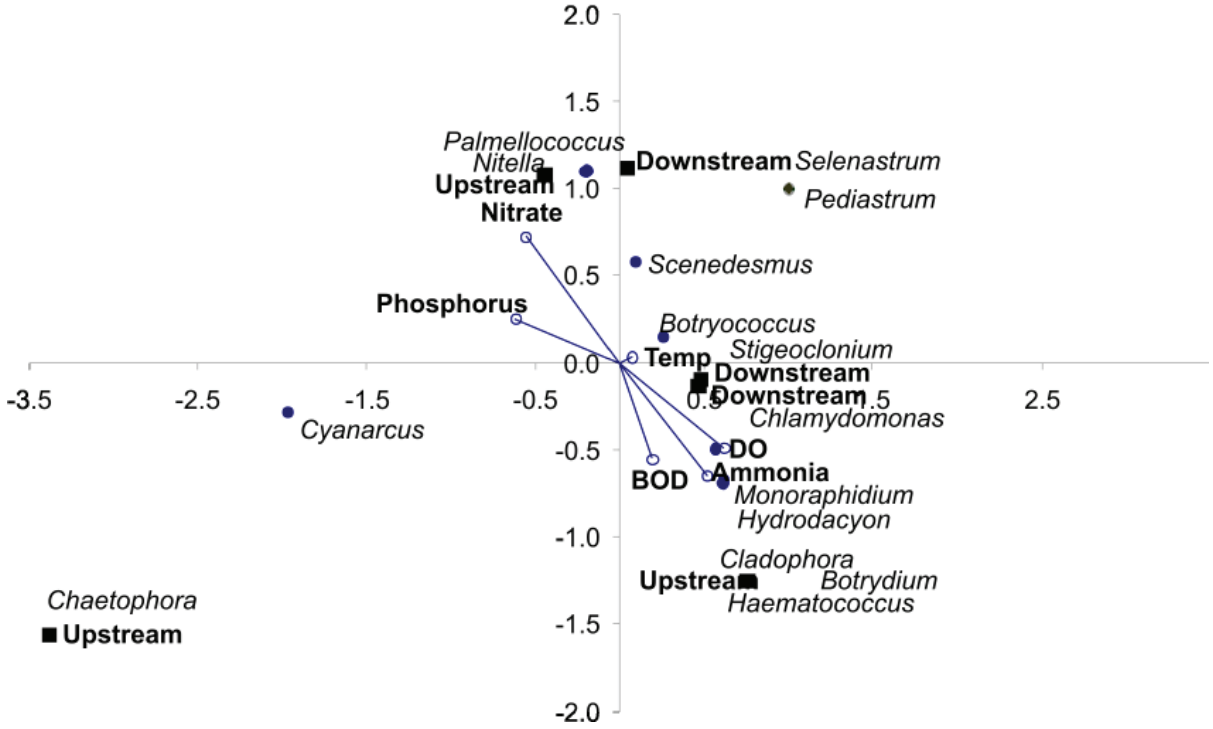

Fig. 5. Cannonical correspondence analysis (CCA) ordination plot for the distribution of Chlorophyceae genera above the intake and below the discharge into River Nzoia and environmental gradients.

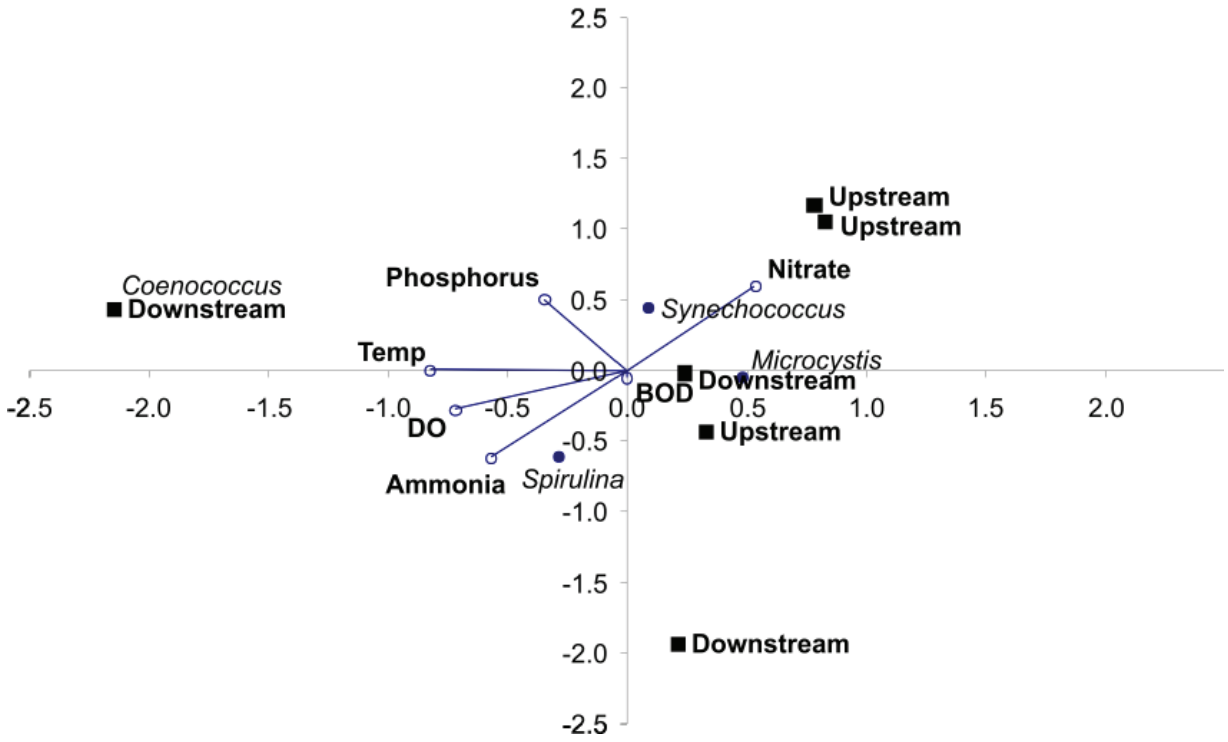

Fig. 6. Cannonical correspondence analysis (CCA) ordination plot for the distribution of Cyanophycea genera above the intake and below the discharge into River Nzoia and environmental gradients.

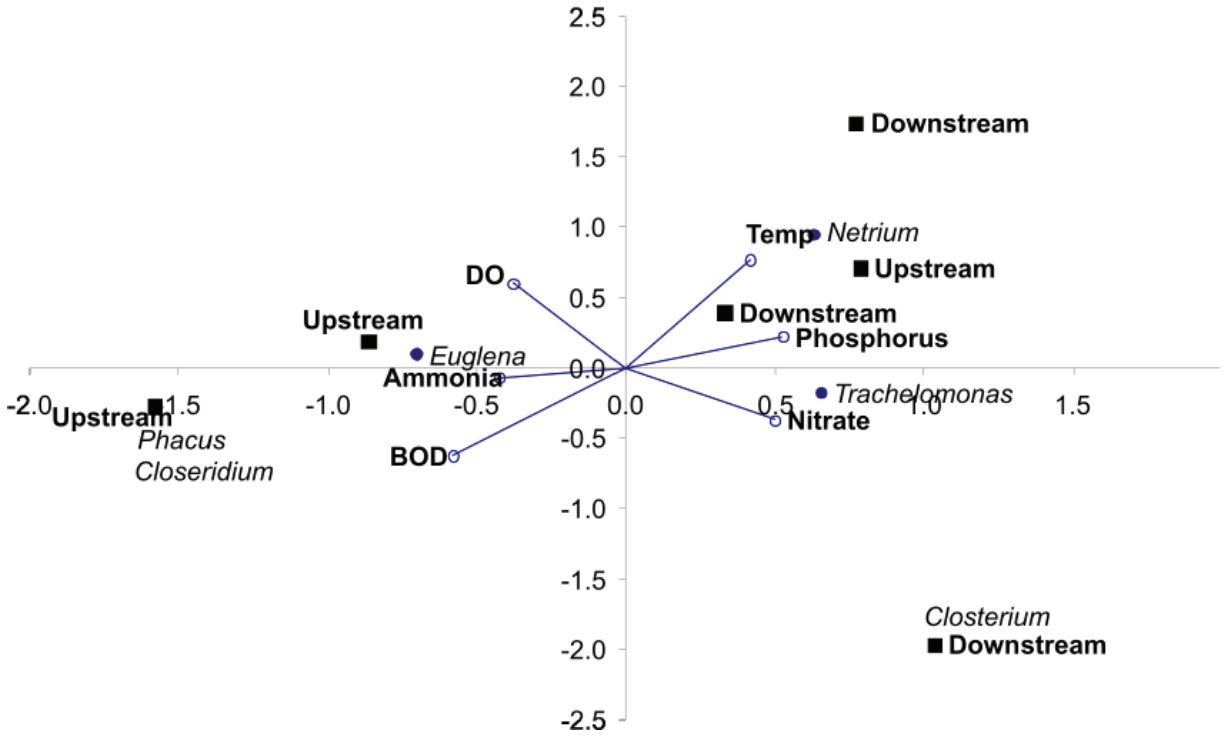

Fig. 7. Cannonical correspondence analysis (CCA) ordination plot for the distribution of Euglenopohyta and Pyrrophyceae genera above the intake and below the discharge into River Nzoia and environmental gradients.

Discussion

Kramer and Lange-Bertalot (1986) have grouped diatoms into three categories based on their sensitivity to pollution: highly pollution tolerant, moderately pollution tolerant, and pollution-sensitive species. In this study, among the Bacillariophyceae, *Navicula* (which is known to be sensitive and hence a pollution indicator [Soininen 2002]) disappeared from the downstream site (Table 3). *Niztchia* (Bacillariophyta) *Hydrodacyon*, *Monoraphidium*, and *Pediastrum* (Chlorophyta) (which are known to be cosmopolitan and insensitive to environmental change) (Lung'ayiah et al. 2000) displayed no particular site preference, with some slight increase downstream. However, nutrient tolerant groups such as *Closterium, Coenococcus, Stigeoclonium* (Arimoro et al. 2008), and *Cocconeis* (Lung'ayiah et al. 2000) were found only in the downstream site (Table 2). Similarly, two species that are known to be indicators of pollution, *Gomphonema* (Lung'ayiah et al. 2000) and *Rhoicosphenia* (Unni and Pawar 2000), showed increased abundance downstream. The increase in density in the downstream site of most of these pollution tolerant taxa was probably associated with high loadings of both nitrates and phosphorus.

Even though there was a decline in *Microcystis* and *Synechococcus* abundance downstream, these species are known to be tolerant to high levels of nutrients (Akbay et al. 1999). This shows that the level of nutrient (nitrogen and phosphorus) in the river from the upper catchment was already high. The high nitrogen and phosphorus was likely the result of intensive application of N:P fertilizers for wheat and maize farming within the upper catchment area (Osano et al. 2003; Raburu et al. 2009). High abundance of *Rhoicosphenia* and *Gomphocymbella,* believed to be good indicators of pollution (Lung'ayiah et al. 2000; Unni and Pawar 2000), were also found downstream probably as a result of increased phosphorus and high temperature (Table 3). High phosphorus in the river at the downstream site may probably result from the incomplete consumption of the diammonium phosphate (DAP) applied by PanPaper to assist the biodegradation of organic matters in its effluent.

High levels of ammonia (0.86 $mg \cdot L^{-1}$), nitrates (2.23 $mg \cdot L^{-1}$), and phosphorus (0.04 $mg \cdot L^{-1}$) favoured the survival and multiplication of *Scenedesmus* and *Pediastrum* downstream as compared with upstream. However, lower loadings of the same factors on a secondary axis favoured *Palmellococcus* and *Nitella* upstream, which are pollution sensitive (Lung'ayiah et al. 2000). High DO levels downstream can be attributed to a high reaeration rate of the river water at the point of effluent discharge because of the high turbulence maintained at this point by design to ensure good mixing and dilution of the mill effluent. However, the impact of the mill nutrients (e.g., deteriorating physicochemical parameters) was still reflected in the biotic communities of phytoplankton and macroinvertebrates. *Stigeoclonium* and *Monoraphidium* appeared to be insensitive to

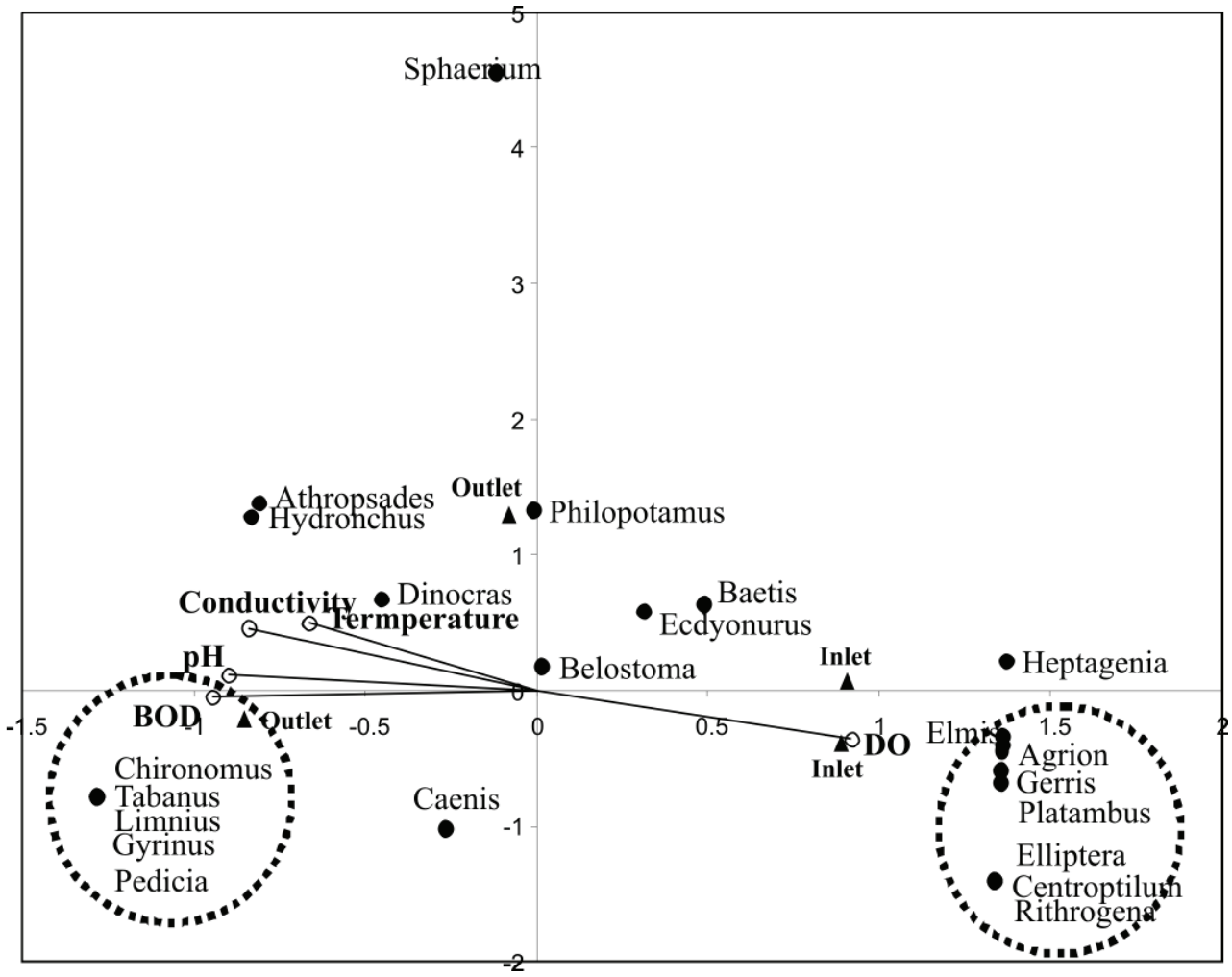

Fig. 8. Cannonical correspondence analysis (CCA) ordination plot for the macroinvertebrate taxa and environmental gradients at the two sites showing clear separation of sites in the primary axis. The dashed circles encompass taxa associated with environmental conditions peculiar to the two sites.

changes in the river water quality, while *Botryococcus*, *Scenedesmus*, and *Chlamydomonas* seemed to thrive with an increase of 37, 154, and 100%, respectively, in the face of deteriorating water qualities (Table 5). The cumulative percentage eigenvalues of the CCA explained up to 60% density variation for Bacillariophyta, 70% for Chlorophyta, 95% for Cyanophyta, 77% for Euglenophyta, 85% for Pyrrophyta, and 90% density variation for the macroinvertebrates (*Y* variables) in relation to the water quality parameters (*X* variables). The results from this study show that multivariate analysis can be used to link the effects of environmental gradients to phytoplankton and macroinvertebrate assemblages with good analytical results (Miesch 2005). Even though there is need for additional water quality parameters to be determined, only a few of these parameters can be effectively used to explain changes in community structures, and this study has provided a good example of that based on the high cumulative eigenvalues of at least 60%.

High densities of *Microcystis* were correlated with high levels of nitrates, while the densities of *Coenococcus* were correlated with high phosphorus load (Fig. 6). This means that the growth of some genera of Bacillariophyceae, Chlorophyceae, and Cyanophyceae was favoured by nitrate, while others were helped by phosphates and to some extent elevated temperatures. Low levels of ammonia and BOD_5 upstream seemed to favour *Closteridium* and *Phacus*, which were completely absent downstream (Fig.7). Increases in temperature and DO resulted in the appearance of *Closterium*, and an increase in both *Netrium* (100%) and *Trachelomonas* (160%) numbers at the downstream site.

The composition of macroinvertebrates at the downstream site was different from that at the upstream site, indicating a probable direct effect of the mill's discharges on the composition of macroinvertebrate assemblages. The taxa recorded at the upstream site mainly belonged to the pollution sensitive Ephemeroptera (Raburu et al. 2009). As a consequence of the effect of

the effluent discharges on water quality, the sensitive taxa were replaced by tolerant *Chironomus* sp., *Pedicia* sp., *Tabanus* sp. (Diptera), which have been found to be tolerant to high organic loads from industrial discharges (Raburu and Tonderskii 2004).

It can be stated that the impact of effluents from PanPaper Mills on water quality was likely responsible for the reduction in taxon richness at the downstream site. This is based on the fact that the macroinvertebrate taxa recorded at the two sites were low for a sixth-order river, indicating an overall degraded water quality in the river. A relatively unperturbed upstream tributary of the same river had been found to have more taxa than the number recorded in this study (Masese et al. 2009b). River Nzoia water quality has also been found to be significantly impacted by agriculture and land use practices in the upper catchment of the river system (Osano et al. 2003; Masese et al. 2009a). These pollutant loads might have compounded or exacerbated the effects of the mill's wastewater discharges, resulting in the overall low taxon numbers at the two sites. This can be confirmed by the fact that the Ephemeroptera taxa recorded at the upstream site (e.g. *Baetis* sp., *Philopotamus* sp., and *Caenis* sp.), are among the most tolerant groups to eutrophication (Thorne and Williams 1997), as is the case also for most Cyanophytes (APHA 1998). However, the marked shift in composition from pollution sensitive taxa at the upstream site to pollution tolerant taxa at the downstream site is indicative of the additional effects of the pulp mill effluents on macroinvertebrate and phytoplankton assemblages. Findings in this study are in agreement with results from studies elsewhere on the effects of pulp mill effluent on benthic assemblages in mesocosms in which the composition of benthic invertebrates was significantly altered (Culp et al. 2003). The fact that in this study the abundance of tolerant taxa such as *Chironomus* and *Microcystis* was increased while intolerant groups were either eliminated or their abundance was considerably depressed downstream of the mill outfall, may be a clear indication of the impact of PanPaper Mills' effluent on River Nzoia.

Conclusion

From this study it can be concluded that: (1) PanPaper Mills produces effluents that change both physicochemical parameters of the receiving water and contribute to nutrient loading, especially phosphorus and nitrate; (2) the mill effluent affects the taxon richness and abundance of both phytoplankton and macroinvertebrates; and (3) the deteriorating water quality and eutrophication eliminates some taxa of both phytoplankton and macroinvertebrates, whereas others such as *Microcystis* sp. and *Chironomus* sp. appear to thrive due to their tolerance to changing water quality. It is therefore recommended that water quality monitoring that involves effluents of this nature be done using a combination of chemical analysis and biological indicators such as phytoplankton and macroinvertebrates.

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