Phytoplankton dynamics in the Congo River

JEAN-PIERRE DESCY*†, FRANÇOIS DARCHAMBEAU†, THIBAULT LAMBERT†, MAYA P. STOYNEVA-GAERTNER‡, STEVEN BOUILLON§ AND ALBERTO V. BORGES†

*Research Unit in Organismal Biology (URBE), University of Namur, Namur, Belgium
†Unité d’Océanographie Chimique, Université de Liège, Liège, Belgium
‡Department of Botany, University of Sofia St. Kl. Ohridski, Sofia, Bulgaria
§Department of Earth & Environmental Sciences, KU Leuven, Leuven, Belgium

SUMMARY

1. We report a dataset of phytoplankton in the Congo River, acquired along a 1700-km stretch in the mainstem during high water (HW, December 2013) and falling water (FW, June 2014). Samples for phytoplankton analysis were collected in the main river, in tributaries and one lake, and various relevant environmental variables were measured. Phytoplankton biomass and composition were determined by high-performance liquid chromatography analysis of chlorophyll a (Chl a) and marker pigments and by microscopy. Primary production measurements were made using the 13C incubation technique. In addition, data are also reported from a 19-month regular sampling (bi-monthly) at a fixed station in the mainstem of the upper Congo (at the city of Kisangani).

2. Chl a concentrations differed between the two periods studied: in the mainstem, they varied between 0.07 and 1.77 µg L⁻¹ in HW conditions and between 1.13 and 7.68 µg L⁻¹ in FW conditions. The relative contribution to phytoplankton biomass from tributaries (mostly black waters) and from a few permanent lakes was low, and the main confluences resulted in phytoplankton dilution. Based on marker pigment concentration, green algae (both chlorophytes and streptophytes) dominated in the mainstem in HW, whereas diatoms dominated in FW; cryptophytes and cyanobacteria were more abundant but still relatively low in the FW period, both in the tributaries and in the main channel.

3. Daily integrated production measured in the mainstem (n = 15) varied between 64.3 and 434.1 mg C m⁻² day⁻¹ in FW conditions and between 51.5 and 247.6 mg C m⁻² day⁻¹ in HW. Phytoplankton biomass in the Congo River mainstem was likely constrained by hydrological factors (accumulation due to increased retention time during FW, dilution by increased discharge during HW), even though increased nutrient availability in the FW period might have also stimulated phytoplankton production.

4. In contrast to other tropical river systems where connectivity with the floodplain and the presence of natural lakes and man-made reservoirs play a prominent role in the recruitment of phytoplankton to the main river, our results show that phytoplankton growth in the Congo River can take place in the main channel, with hydrological processes allowing maintenance of phytoplankton biomass even during HW.

Keywords: diatoms, green algae, large tropical river, physical control, potamoplankton

Introduction

Most studies on potamoplankton, i.e. the assemblage of suspended algae in flowing water, have been conducted in temperate lowland rivers, starting at the end of the 19th century with the first comprehensive reports on the presence and often substantial amounts of suspended algae in river waters (review by Welch, 1952). These early reports highlighted the role of physical factors in controlling growth and losses of algae during downstream transport, a view which was largely confirmed afterwards (e.g. Reynolds, 1988; Reynolds & Descy,
The origin of this plankton was discussed by several authors, but even though riverine phytoplankton always contains a variable contribution of benthic forms brought into suspension (tychoplankton), inocula of true planktonic taxa from tributaries and retention/accumulation zones (so-called hydrological ‘dead zones’) by Reynolds, Carling & Beven, 1991 and Reynolds, 2006) are needed for the development of cell populations in the main channel. These hydrological dead zones (or retention zones) may be generated by the heterogeneity of flow across a river channel (Reynolds, 1988) or constituted by backwaters with a sufficient hydrological connection with the main channel to provide inocula of cells (Reynolds et al., 1991). In retention zones, phytoplankton photosynthesis may result in net production and growth of phytoplankton, as long as the ratio euphotic depth:river channel depth allows gross primary production to exceed autotrophic respiration losses. Once in the mainstem, cell populations are transported downstream and their survival depends on their capacity to offset advective losses (Reynolds, 1988) and dilution by inputs from the catchment (Descy et al., 1987). Therefore, discharge is a key factor determining transport time and suspended matter load, which influences light penetration in the water column, with mean light exposure depending on channel depth. In other words, it is the physical setting, constrained by hydrology and river morphology, that controls phytoplankton development, and algal populations are submitted to a strong selection by light and temperature: traits such as the ability to achieve net photosynthesis at low light and high specific growth rates are essential for survival and for population increase (Reynolds, 1994). Therefore, in temperate rivers, diatoms, which are well adapted to low temperature and low light, have been generally reported as dominant by biomass in rivers at all times from spring to autumn, with coccal green algae being most species rich and developing whenever conditions allow for greater light availability (Rojo, Alvarez-Cobelas & Arauzo, 1994; Reynolds & Descy, 1996). Besides, evidence for nutrient limitation of phytoplankton growth in rivers has been scarce (Basu & Pick, 1996), even though measures for reducing the nutrient loading to major rivers may have resulted in effective control of phytoplankton biomass (Minaudo et al., 2015).

While it has been stated that ‘tropical rivers are poorly understood compared with their temperate counterparts’ (Davies, Bunn & Hamilton, 2008), the studies conducted in the tropical and subtropical regions suggest that phytoplankton dynamics in tropical rivers obey the same rules as in temperate rivers given above, even though specific features of tropical rivers further constrain development of algal populations within the river channel. The best studied tropical rivers are South American rivers such as the Amazon (Fisher & Parsley, 1979; Wissmar et al., 1981; Forsberg et al., 1993), but also the Uruguay (O’Farrell & Izaguirre, 2014), Paraná (Bonetto, 1983; Garcia de Emiliani, 1990) and Paraguay (Zalocar De Domitrovic, 2002; Zalocar de Domitrovic, Devercelli & Garcia de Emiliani, 2007; Devercelli et al., 2014). The emerging general view is that, in lowland high-order rivers, phytoplankton production in river channels is ‘exceedingly low’ (Lewis, Hamilton & Saunders, 1995) due to extreme light limitation, combined in black water rivers with low nutrient concentrations (Wissmar et al., 1981). In fact, in situ phytoplankton growth seems extremely low in lowland large South American rivers and appears to be actually imported from adjacent floodplain lakes, where high phytoplankton production occurs (Vasquez & Wilbert, 1992; Melack & Forsberg, 2001). Phytoplankton production in floodplain lakes is fuelled by nutrient input from the rising river waters at the onset of the wet season, when settling of suspended matter allows light penetration into the water column. This stresses the major role of the exchange of nutrients and organic matter between the rivers and the floodplain lakes that takes place during the wet season in most tropical catchments (Junk, Bayley & Sparks, 1989; Neiff, 1996).

Regarding river phytoplankton dynamics, the best studied river in Africa is the Nile, where high phytoplankton biomass and production have been reported (Prowse & Talling, 1958). However, the Nile River is a river–lake system, allowing increased retention times and recruitment from the lakes and relatively high abundance of planktonic species in the main river channel. As such, it behaves very differently from the above-mentioned South American rivers. Sub-Saharan rivers are poorly characterised with regard to phytoplankton dynamics: data have been obtained in small river networks such as rivers in Ivory Coast (Ilitis, 1982; Koné et al., 2009; Ouffoue et al., 2013) and the Tana (Tamoooh et al., 2012) and only in one large river, the Niger (Imevbore & Visser, 1969; Imevbore, 1970).

Regarding the general taxonomic composition of phytoplankton assemblages, selection by turbulence and light availability, rather than by nutrient availability or grazing, is the key process shaping phytoplankton assemblages in most rivers (Reynolds, 1994). According to the review by Rojo et al. (1994), diatoms comprise the majority of species numbers in temperate rivers but are substituted in tropical rivers by desmids and coccol
chlorophytes (when benthic species are not taken into account). In terms of biomass and abundance, diatoms often dominate in tropical rivers, with *Aulacoseira granulata* and other *Aulacoseira* taxa often reported (e.g. Garcia de Emiliani, 1990; Hötzel & Croome, 1996). These diatoms are typical **r** strategists (Reynolds, 1984), able to withstand the variability associated with variations of flow and able to achieve net growth within the time frame imposed by downstream transport and low retention time. Coccal green algae, unicellular and colonial, are also present. They may even be the most important group in terms of abundance and diversity when water transparency and depth allow greater light availability (e.g. in the upper section of the Paraguay River; Zalocar de Domitrovic et al. 2014) than in river sections with high turbidity, where low light adaptation is an absolute requirement for growth and survival (Kilham, Kilham & Hecky, 1986). Other taxonomic groups are also reported, but they become significant only in particular conditions, for instance where substantial interactions take place between the river and the floodplain and backwaters, or with reservoirs.

This study presents data from the Congo River, the second most important river in the world in terms of catchment size and freshwater discharge, covering a 1700-km transect from Kisangani to Kinshasa (Fig. 1). The objectives of this article are to examine phytoplankton longitudinal dynamics in the river mainstem during two contrasting hydrographic conditions (high and falling water) and to identify the factors controlling phytoplankton development. We used marker pigment analyses coupled with identification of the dominant algal species by microscopy and primary production measurements. Phytoplankton primary production is a marginal component of overall carbon flows in tropical rivers that are dominated by inputs from land and from wetlands (macrophytes and flooded forest), as shown in the Amazon (Abril et al., 2014). However, phytoplankton provides organic matter with a high nutritional value compared with more refractory and N and P poor organic matter of terrestrial origin. As such, phytoplankton has been shown to be important in sustaining pelagic food webs in tropical rivers including fish (Aratijo-Lima et al., 1986; Forsberg et al., 1993; Benedito-Cecilio et al., 2000) that can sustain an important economic activity and a source of animal protein for the local populations.

**Methods**

**Study site and sampling**

The Congo River (Fig. 1) is the second largest river in the world in terms of discharge (1457 km³ year⁻¹) and...
drainage basin (3.75 million km²). Flooded areas in the Congo basin largely consist of flooded forest (Bwangoy et al., 2010), and these are mostly permanently flooded unlike the Amazon floodplains that are seasonally flooded. In addition, there are no temporary floodplain lakes but only a handful of relatively large permanent lakes (Lake Mai-Ndombe, 2300 km²; Lake Tumba, 765 km²). Floating macrophytes (mainly hippo grass, Vossia cuspidata) commonly occur along channel edges and within channels and form large meadows in streams, rivers and the mainstem, in all types of waters (white and black). The water height variations in the Congo mainstem are small (3–4 m), because the Congo basin straddles on the equator: the dry season on the northern part of the basin is compensated by the rainy season on the southern part of the basin, and vice versa, leading to a regulation of seasonal water height variations (Runge, 2008).

Two sampling campaigns were carried out, in the high water (HW) period, in December 2013 (75 sites), and in the falling water (FW) period in June 2014 (89 sites). The sampling was carried out in the Congo River mainstem and in tributaries, as well as in the outlet of one of the two main lakes (Lake Tumba). A few additional samples were collected on the lower stretch of the mainstem downstream of the confluence with the Kasai River in May 2015 (FW period). Despite large difference in discharge, the water height in the Congo River mainstem did not differ much between the two main campaigns (Fig. 2 and Table 1). In addition, a temporal survey, with fortnightly sampling of the Congo River, was carried out at Kisangani from November 2012 to May 2014.

Water temperature, conductivity, O₂ and pH were measured in situ with a YSI ProPlus CTD calibrated using standard protocols. As cross-section CTD casts at several sites did not show any variation of pH, O₂, temperature and conductivity with depth, vertical homogeneity of the water column was assumed. Secchi depth was measured with a Secchi disk and water column depth was measured with a portable probe. Water was collected just below the surface in 5-L plastic bottles kept in the dark until further processing within 30 min (at most) of collection. Phytoplankton samples were collected with a 28-μm mesh plankton net and preserved in 20-mL plastic bottles with Lugol’s iodine.

The vertical light attenuation coefficient (Ke in m⁻¹) was calculated from simultaneous measurements of surface irradiance with a Li-Cor LI-190 Quantum Sensor and underwater PAR measurements with a submersible Li-Cor LI-193SA Spherical Quantum Sensor (Kirk, 1994). The average light to which phytoplankton was exposed was calculated according to Riley (1957) taking into account river depth and vertical light extinction at the site of primary production measurement.

Particulate and dissolved matter analyses

Samples for total suspended matter (TSM) were obtained by filtering a known volume of water on pre-combusted (4 h at 500 °C) and pre-weighed glass fibre filters (47 mm GF/F, 0.7 μm nominal pore size) and air-dried. These were later oven-dried prior to weighing to determine TSM load. Samples for the determination of particulate organic carbon (POC), particulate nitrogen (PN) and particulate phosphorus (PartP) were collected from a known volume of water on pre-combusted 25 mm GF/F filters (0.7 μm nominal pore size). The filtrate from the TSM filtrations was further filtered on 0.2-μm polyethersulfone syringe filters (16532-Q; Sartorius, Goettingen) for dissolved organic carbon (DOC) (40-mL glass vials with polytetrafluoroethylene-coated septa, poisoned with H₃PO₄), coloured dissolved organic matter (CDOM) (20-mL amber glass vials with PTFE-coated septa) and inorganic nutrients (NO₃⁻, NH₄⁺, dissolved Si and soluble reactive phosphorus – SRP) (20-mL PET vials, frozen at −20 °C).

The 25-mm filters for POC and PN were decarbonated with HCl fumes for 4 h, re-dried and packed in Ag cups. POC and PN were determined on an elemental analyser – isotope ratio mass spectrometer (EA-IRMS; ThermoFinnigan DeltaV Advantage, ThermoFischer Scientific, Waltham) using the thermal conductivity detector signal as described by Bouillon et al. (2012). PartP was analysed by spectrophotometric determination of phosphate after digestion with potassium persulfate and boric acid (Valderrama, 1981). DOC was analysed on an Aurora1030 TOC analyser (OI Analytical, College Station) coupled to a Delta V Advantage.
Table 1 Average values and range of the variables relevant to phytoplankton ecology, for the mainstem Congo River and tributaries during high water and low water conditions. POC, particulate organic carbon; DOC, dissolved organic carbon; TSM, total suspended matter; SRP, soluble reactive phosphorus; PartP, particulate phosphorus.

<table>
<thead>
<tr>
<th>Variable</th>
<th>High water (34 sites)</th>
<th>Falling water (41 sites)</th>
<th>Tributaries/lakes (55 sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Mean: 27.4, Min: 26.0, Max: 28.2</td>
<td>Mean: 26.1, Min: 23.4, Max: 27.8</td>
<td>Mean: 26.1, Min: 23.6, Max: 29.1</td>
</tr>
<tr>
<td>DO (% saturation)</td>
<td>60.6, 48.4, 89.2</td>
<td>37.6, 4.2, 111.3</td>
<td>44.5, 0.3, 103.0</td>
</tr>
<tr>
<td>Specific conductivity (μS cm⁻¹)</td>
<td>38.2, 26.6, 48.3</td>
<td>27.5, 7.3, 55.1</td>
<td>31.8, 6.7, 87.6</td>
</tr>
<tr>
<td>pH</td>
<td>6.49, 6.07, 6.92</td>
<td>5.48, 3.91, 6.87</td>
<td>4.89, 3.60, 7.05</td>
</tr>
<tr>
<td>Channel depth (m)</td>
<td>10.2, 3, 38.6</td>
<td>4.9, 1.0, 10.8</td>
<td>4.1, 0.5, 18.9</td>
</tr>
<tr>
<td>Secchi depth (cm)</td>
<td>52</td>
<td>82</td>
<td>73</td>
</tr>
<tr>
<td>Light attenuation coefficient (m⁻¹)</td>
<td>1.54, 1.06, 2.83</td>
<td>1.52, 0.44, 2.79</td>
<td>3.35, 1.48, 5.16</td>
</tr>
<tr>
<td>Euphotic depth (Zeu) (m)</td>
<td>3.13, 1.63, 4.35</td>
<td>3.43, 1.65, 10.36</td>
<td>1.48, 0.89, 3.11</td>
</tr>
<tr>
<td>NH₄⁺ (μmol)</td>
<td>2.27, 0.58, 6.45</td>
<td>2.82, 0.65, 5.68</td>
<td>2.10, 0.38, 20.01</td>
</tr>
<tr>
<td>NO₃⁻ (μmol)</td>
<td>11.31, 0.00, 19.60</td>
<td>4.02, 0.00, 16.29</td>
<td>15.96, 0.00, 89.94</td>
</tr>
<tr>
<td>Si (μmol)</td>
<td>195.7, 169.2, 248.8</td>
<td>162.4, 61.1, 283.0</td>
<td>157.6, 25.2, 295.6</td>
</tr>
<tr>
<td>SRP (μmol)</td>
<td>0.36, 0.11, 1.00</td>
<td>0.31, 0.05, 0.94</td>
<td>0.73, 0.10, 1.73</td>
</tr>
<tr>
<td>TSM (mg L⁻¹)</td>
<td>29.8, 14.0, 99.7</td>
<td>10.0, 0.7, 71.4</td>
<td>8.4, 0.5, 43.0</td>
</tr>
<tr>
<td>POC (mg L⁻¹)</td>
<td>1.81, 0.93, 4.58</td>
<td>1.04, 0.30, 3.56</td>
<td>1.11, 0.23, 3.67</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>8.0, 5.4, 13.9</td>
<td>15.9, 1.8, 47.8</td>
<td>17.9, 1.8, 67.8</td>
</tr>
<tr>
<td>n₃₅₀ (m⁻¹)</td>
<td>29.18, 19.67, 45.39</td>
<td>60.5, 3.6, 180.5</td>
<td>69.2, 6.5, 249.4</td>
</tr>
<tr>
<td>PartP (μmol)</td>
<td>1.46, 0.86, 3.01</td>
<td>0.95, 0.42, 2.92</td>
<td>1.09, 0.08, 2.81</td>
</tr>
<tr>
<td>POC/PN (molar ratio)</td>
<td>12.8, 10.9, 15.5</td>
<td>13.0, 8.8, 16.4</td>
<td>13.5, 8.4, 20.9</td>
</tr>
<tr>
<td>POC/PartP (molar ratio)</td>
<td>100.8, 73.5, 149.4</td>
<td>92, 41, 186</td>
<td>132, 10, 767</td>
</tr>
<tr>
<td>Chlorophyll a (μg L⁻¹)</td>
<td>0.84, 0.10, 1.77</td>
<td>0.44, 0.00, 3.58</td>
<td>0.85, 0.01, 6.39</td>
</tr>
<tr>
<td>Green algae (μg Chl a L⁻¹)</td>
<td>0.75, 0.10, 1.58</td>
<td>0.38, 0.00, 3.14</td>
<td>0.22, 0.00, 1.24</td>
</tr>
<tr>
<td>Cryptophytes (μg Chl a L⁻¹)</td>
<td>0.01, 0.00, 0.06</td>
<td>0.01, 0.00, 0.20</td>
<td>0.10, 0.00, 0.67</td>
</tr>
<tr>
<td>Cyanobacteria (μg Chl a L⁻¹)</td>
<td>0.00, 0.00, 0.00</td>
<td>0.00, 0.00, 0.06</td>
<td>0.01, 0.00, 0.20</td>
</tr>
<tr>
<td>Diatoms (μg Chl a L⁻¹)</td>
<td>0.09, 0.00, 0.22</td>
<td>0.04, 0.00, 0.39</td>
<td>0.71, 0.00, 4.90</td>
</tr>
<tr>
<td>Mean light in the water column (μE m⁻² s⁻¹)</td>
<td>71, 12, 195</td>
<td>5, 108</td>
<td>–, –, –</td>
</tr>
<tr>
<td>Phytoplankton production (mg C·m⁻²·day⁻¹)</td>
<td>109, 51, 248</td>
<td>64, 434</td>
<td>285, 35, 581</td>
</tr>
</tbody>
</table>

IRMS, with a precision better than ±5%. Absorbance was recorded on a UV/Vis 650S spectrophotometer (Perkin-Elmer, Waltham, MA) using a 1-cm quartz cuvette. Absorbance spectra were used to report CDOM as the absorption coefficient at 350 nm (a₃₅₀). Details on data acquisition and corrections can be found in Lambert et al. (2015). NO₃⁻ and NH₄⁺ concentrations were estimated by spectrophotometry, using the dichloroisocyanurate-salicylate-nitroprussiate colorimetric method for NH₄⁺ (Westwood, 1981), the sulfanilamide colorimetric after vanadium reduction method for NO₃⁻ (American Public Health Association, 1998). SRP was determined by spectrophotometry using the ammonium molybdate-potassium antimonyl tartrate method (American Public Health Association, 1998). Si was measured with inductively coupled plasma mass spectrometry (Agilent 7700x, Santa Clara). The detection limits were 0.30, 0.15, 0.03 and 8.0 μmol L⁻¹ for NH₄⁺, NO₃⁻, SRP and Si, respectively.

Phytoplankton analyses

Pigments were analysed by high-performance liquid chromatography (HPLC) after acetone extraction (90%) of the material filtered on Macherey-Nagel 47 mm diameter GF-5 filters. Filters were frozen (−20 °C), and within a few days, the extractions were carried out in 5-mL acetone (90%) and a 1-mL extracts subsamples were kept in a portable deep-freezer until transport to Belgium. Chl a and marker pigments were determined according to Descy et al. (2005), using a HPLC system (Waters, Milton) equipped with a Waters 996 PDA detector and a Water 470 fluorescence detector. The contribution of phytoplankton classes was calculated using CHEMTAX (Mackey et al., 1996), with input ratio matrices defined from microscopic examination of net samples (see below), allowing to verify the presence of diatoms, green algae (chlorophytes and streptophytes), cyanobacteria, cryptophytes, chrysophytes, dinoflag-
ellates and euglenophytes. Owing to the low concentrations of pigment in the HW samples, only a few markers were used for assessing the biomass of the main phytoplankton classes: fucoxanthin (diatoms and chrysophytes), lutein and chlorophyll b (green algae), alloxanthin (cryptophytes) and zeaxanthin (cyanobacteria and green algae). For the FW samples, more marker pigments were used, including neoxanthin and violaxanthin (green algae), myxoxanthophyll (cyanobacteria type 2; Wright & Jeffrey, 2006), diadinoxanthin and diatoxanthin (diatoms), and chlorophyll c1–c2 (diatoms and cryptophytes).

Phytoplankton samples were examined at the microscope (Leitz Laborlux S, Leitz, Oberkochen and Motic BA 400, Motic, Wetzlar) for identification to species level whenever possible. We are aware that these samples may be biased towards the largest phytoplankton taxa, which were retained by the net. Therefore, the microscope data were used here for assessing taxa frequency of occurrence, and for all quantitative phytoplankton assessments, we used biomass estimates at the class level derived from processing the marker pigments concentrations with CHEMTAX.

**Primary production**

The primary production rate was determined from photosynthesis-irradiance incubations using H$^{13}$CO$_3^-$ as a tracer for incorporation of dissolved inorganic carbon (DIC) into biomass. A solution was prepared with 500 mL of surface water spiked with NaH$^{13}$CO$_3$. Subsamples of the spiked solution were preserved in triplicate in 12 mL Exetainer vials (Labco, Lampeter) and poisoned with a saturated solution of HgCl$_2$, for the determination of the $\delta^{13}$C-DIC value. Eight 50-mL polycarbonate flasks were filled with the spiked solution and were placed into a floating incubator providing a range from 0 to 90% of natural light energy and incubated $\textit{in situ}$ around mid-day for 24 h just below the surface. Incident light was monitored by a LI-190SB quantum sensor (Li-Cor, Lincoln) during daytime (5-min interval acquisition) for the entire duration of the cruises. The incubation was stopped by adding neutral formaldehyde and water was filtered on pre-combusted Macherey-Nägel GF5 filters (25 mm diameter). Filters were rinsed with HCl 0.1 N and preserved dry. For the analysis of $\delta^{13}$C-DIC, a 2-mL helium (He) headspace was created, and H$_3$PO$_4$ was added to convert all DIC species to CO$_2$. After overnight equilibration, part of the headspace was injected into the He stream on the above-mentioned EA-IRMS. Given the high $^{13}$C-enrichments, the obtained $\delta^{13}$C data were not corrected for isotopic equilibration between gaseous and dissolved CO$_2$. The $\delta^{13}$C-POC was determined on the above-mentioned EA-IRMS using an internally calibrated acetanilide and sucrose (IAEA-C6) to calibrate the $\delta^{13}$C-POC (for the natural abundance data).

The specific photosynthetic rate in an individual bottle, $P_i$ (in $\mu$g C L$^{-1}$ h$^{-1}$) was calculated following Dau-chez, Legendre & Fortier (1995) based on the initial and final $\delta^{13}$C-POC values and $\delta^{13}$C-DIC of the spiked incubated solution, and considering that isotopic discrimination is negligible (Legendre & Gosselin, 1996), at most 2.5% according to Hama et al. (1983). For each experiment, the maximum specific photosynthetic rate $P_m$ (in $\mu$g C L$^{-1}$ h$^{-1}$) and the irradiance at the onset of light saturation $I_k$ ($\mu$E m$^{-2}$ s$^{-1}$) were determined by fitting $P_i$ to the irradiance gradient provided by the incubator $I_i$ ($\mu$E m$^{-2}$ s$^{-1}$), using the following equation:

$$P_i = 2P_m \left[ \frac{I_i/2I_k}{1 + (I_i/2I_k)^2} \right]$$

which is the Vollenweider’s equation with $a = 1$ and $n = 1$, allowing for photoinhibition (Vollenweider, 1965), with $P_i$ the photosynthetic rate in bottle $i$ during the incubation, and with $I_i$ the corresponding mean light during the incubation. Fitting was performed using the Gauss–Newton algorithm for nonlinear least squares regression with TATISTICA software (StaSoft, Tulsa).

Daily depth-integrated primary production (mg C m$^{-2}$ day$^{-1}$) was calculated according to Kirk (1994) using the following equation:

$$P(z, t) = 2P_m \left[ \frac{I(z, t)/2I_k}{1 + (I(z, t)/2I_k)^2} \right]$$

where $P(z, t)$ is the photosynthesis at depth $z$ and time $t$, and $I(z, t)$ is the underwater light determined from Ke and surface irradiance recorded every 5 min, and assuming a vertically homogenous Chl $a$ profile. Assuming that short-term incubations provide an estimate which is close to gross primary production (GPP), we calculated water column daily respiration (R, mg C m$^{-2}$ day$^{-1}$) as in Reynolds (2006), considering a respiration rate of 0.16 mg C mg Chl $a$ h$^{-1}$ at 18 °C (based on Lopez-Sandoval et al., 2014), a $Q_{10}$ of 2 for adjusting for river temperature, a constant respiration rate over 24 h, and the whole river depth at the study sites. Subtracting R from GPP provided net primary production estimates, which in turn provided estimates of phytoplankton growth rate. Community growth rate was calculated using a C:Chl $a$ ratio of 40, according to
Harris (1986). This is an estimate of the maximal growth rate, not taking into account mortality, sedimentation and grazing losses, nor possible nutrient limitation.

Statistical and multivariate analyses

A principal component analysis (PCA) was performed on scaled variable using the `prcomp` function in the R software (R Development Core Team, 2008). Physical and chemical data from the mainstem were used as the variables for the PCA, with the objective of determining their correlation to chlorophyll $a$ and to explore the biogeochemical and ecological gradients during the two main sampling series. The following variables (see Table 1 for acronyms) were included: Rkm (River km, or distance downstream of Kisangani), Secchi depth, total suspended solids, POC, PN, PartP, $a_{350}$, SRP, nitrate, ammonia, dissolved oxygen, conductivity and pH. A similar analysis was run for all sites, including the tributaries. Given the different units of the variables used in the PCA, data were scaled to zero-mean and unit-variance as recommended (Borcard, Gillet & Legendre, 2011).

Results

The data are summarised in Table 1, presenting the mean and the range of the environmental data, of phytoplankton biomass and production, in the sampled tributaries and lakes, and in 34 sites along the mainstem, under both hydrological conditions (HW; FW).

Physical and chemical data

Below we focus on the variables which could influence phytoplankton development in the Congo River mainstem. The Congo River main channel is relatively shallow and its depth varies little between FW and HW (Fig. 2 and Table 1): in most sites, the depth did not exceed 10 m and was often lower, except in the lower part (between the mouth of the Kasai and the entrance of the Malebo pool), where the river channel is narrower and deeper (22–37 m). TSM concentrations (Fig. 3) decreased longitudinally and were higher in FW conditions, resulting in lower Secchi depth (Fig. 3) and depth of the euphotic zone (Zeu), compared to HW conditions (mean Zeu 3.1 m in HW versus 1.2 m in FW). Generally, TSM was lower in the tributaries than in the mainstem, with the exception of the Kasai River (confluence 30-km upstream of Kinshasa), and no distinct seasonal variation in the TSM of tributaries was apparent. SRP and DIN concentrations were higher in the mainstem at FW than at HW (Fig. 4). This pattern was also observed in the tributaries. The POC:PN and POC:PartP ratios (Table 1) did not vary much among sites in both hydrological conditions: the molar POC:PN averaged 12.8 (FW) and 12.9 (HW), and the mean molar POC:PartP was lower in FW (78) than in HW (101). Extremely low pH values (<4) and $O_2$ concentrations close to zero were measured in some of the small and slow-moving tributaries draining the Cuvette Centrale (Table 1), in accordance with extremely high DOC content, and $CO_2$ and $CH_4$ concentrations (Borges et al., 2015a,b; Lambert et al., 2016).

Phytoplankton abundance and composition

Chlorophyll $a$ concentrations differed between the two periods studied: in the mainstem, they varied between 0.07 and 1.77 mg L$^{-1}$ in HW and between 1.13 and 7.68 mg L$^{-1}$ in FW conditions (Table 1). The range in the tributaries was even larger: 0–3.58 mg L$^{-1}$ in the HW period, with maxima in the Oubangui River (3.58 mg L$^{-1}$), in the outlet of Lake Tumba (1.85 mg L$^{-1}$) and in the Lefini River (1.71 mg L$^{-1}$) downstream of the Imboulou dam. In the FW period, Chl $a$ in the tributaries varied between 0.02 and 6.39 mg L$^{-1}$, with maxima recorded in the Oubangui River (6.39 mg L$^{-1}$) and lake
Tumba (2.28 μg L⁻¹), but with higher concentrations than in HW in many tributaries, suggesting that phytoplankton development occurred throughout the Congo catchment. Substantial amounts of Chl a degradation products (chlorophyllide a, phaeophytins a and phaeophorbides) were detected in most samples (on average 23% of total phorbins-a at FW), indicating the transport of dead and senescent cells among living, healthy cells and possibly of plant detritus. The longitudinal variation of Chl a and the contribution of the main phytoplankton groups are shown in Fig. 5. In the mainstem, Chl a exhibited a hump-shaped pattern, with a maximum between 200 and 600 km from Kinshasa in the HW period, and a maximum around 700 km from Kinshasa in the FW period. Chl a in most tributaries (mostly black waters) was generally lower than in the mainstem, with few exceptions (e.g. Oubangui River and outlet of Lake Tumba in the HW period).

The difference in phytoplankton composition in the two periods is striking: both pigment data and microscopic examination of samples showed that green algae dominated in the mainstem in HW, whereas diatoms dominated in FW (Fig. 6). Green algae dominance in HW was also observed in the tributaries, where they represented on average 88% of Chl a (Table 1); a sample taken in a *Vassia* sp. patch in HW contained only green algae, with 98% of contribution to total Chl a. Cryptophytes and cyanobacteria were more abundant in the FW period, both in the tributaries and in the main channel. Overall, taxonomic diversity was greater in the FW period. The most frequent phytoplankton taxa in the Congo mainstem were *Aphanocapsa* spp., *Aphanothece* spp., *Limnoococcus limneticus*, *Planktolyngbya* spp. (cyanobacteria/cyanoprokaryotes), *Actinastrum rhaphidioides*, *Coelastrum* spp., *Coenochloris* spp., *Desmodesmus magnus*, *D. perforatus*, *Staurosirella* spp. and *Thalassiosira rudolfii* (diatoms).

The PCA (Fig. 7) performed on the physical and chemical variables and Chl a from the main channel (70.9% of cumulated variance associated with the two first principal components) showed a clear discrimination between the sampling series (sites from the FW campaign in the upper part of the diagram), resulting from higher inorganic nutrient concentrations (except NH₄⁺), dissolved oxygen, pH, particulate phosphorus and Chl a in the FW conditions, and from higher a₃₅₀ in...
HW conditions. Within each sampling series, the sites are distributed along the first axis of the PCA, which represents a longitudinal gradient, well-marked for the HW series, depending mainly on TSM, POC, PN and Secchi depth. The PCA (Figure S1) performed on all sites, including the tributaries, essentially discriminated the tributaries from the mainstem, based on higher DOM concentration (measured by $a_{350}$ and DOC) and lower TSM, pH, POC and PN.

The temporal survey at Kisangani provided additional data, allowing to observe a strong variation of Chl $a$ (Fig. 8) over time. Phytoplankton biomass was low (0.49–1.43 µg Chl $a$ L$^{-1}$) during the HW period and increased up to 5.72 µg Chl $a$ L$^{-1}$ when discharge and TSM decreased.

**Phytoplankton photosynthesis and production**

The average light to which phytoplankton was exposed in the mainstem varied between 5 and 108 µE m$^{-2}$ s$^{-1}$ in FW and between 12 and 195 µE m$^{-2}$ s$^{-1}$ in HW (Table 1). Phytoplankton photosynthesis parameters were similar in the two sampling series: the mean maximal photosynthetic rate was 10 µg C L$^{-1}$ h$^{-1}$ in HW and 11.5 µg C L$^{-1}$ h$^{-1}$ in FW, with mean I$_{k}$ (light at the onset of saturation of photosynthesis) of 311 µE m$^{-2}$ s$^{-1}$. Daily integrated GPP measured in the mainstem varied between 64.3 and 434.1 mg C m$^{-2}$ day$^{-1}$ in FW conditions and between 51.5 and 247.6 mg C m$^{-2}$ day$^{-1}$ in HW. Phytoplankton production was close to that of the mainstem in the main tributaries, i.e. in the rivers Tshopo (FW: 261.6 mg C m$^{-2}$ day$^{-1}$), Lomami (FW: 245.6 mg C m$^{-2}$ day$^{-1}$), Oubangui (HW: 417.6 mg C m$^{-2}$ day$^{-1}$; FW: 236 mg C m$^{-2}$ day$^{-1}$) and Kasai (HW: 34.7 mg C m$^{-2}$ day$^{-1}$; FW: 168 mg C m$^{-2}$ day$^{-1}$). In both sampling series, primary production increased regularly in the Congo River (Fig. 9) between 1200 and 800 km (from Kinshasa) and dropped around the confluence with the river Oubangui, at c. 500 km. This was related to a decrease of Chl $a$ (Fig. 5) likely resulting from dilution and TSM input by this tributary, despite that it carried significant phytoplankton biomass during FW. During HW, the decrease of Chl $a$ occurred further downstream after the confluence of the Kasai River that brought high TSM to the mainstem.

Net primary production estimated for the Congo River sites ($n$ = 30) varied between −442.6 and 148.4 mg C m$^{-2}$ day$^{-1}$ in FW conditions and between −64.7 and 204.0 mg C m$^{-2}$ day$^{-1}$ in HW. Community
growth rates were comprised between $-0.13$ and $0.43$ day$^{-1}$ in FW and $-0.07$ and $0.63$ day$^{-1}$ in HW.

**Discussion**

The results of this study provide evidence of significant phytoplankton production in the main channel in the second largest river of the world, the Congo River. Phytoplankton biomass developed mainly in the period of low water and FW, when the lower discharge (resulting in higher residence time and lower dilution by tributaries) and the lower TSM load allowed sufficient light penetration for substantial primary production to occur. Chl $a$ reached a maximal concentration (up to c. $8 \mu g L^{-1}$) after a gradual increase along a c. 1000-km transect in the FW period, resulting from net phytoplankton increase, most probably allowed by lower dilution by tributaries than in the HW period. In the Congo River, phytoplankton biomass may be controlled by dilution rather than by residence time. Indeed, given the relatively high production rates of the phytoplankton, the river is long enough to allow sufficient residence time for biomass increase, irrespective of the discharge: based on a simple calculation, the water residence time from Kisangani to Kinshasa was c. 25 days in HW, and it did not increase much (c. 30 days) in FW. By contrast, the discharge of all tributaries, estimated from the few data available, was about twice as high in HW than in FW, and the main tributaries contributed a significant proportion of the main river flow. For instance, it could be estimated that the Kasai River, the Oubangui River and the Ruki River contribute, respectively, to 21, 8 and 12% of the discharge recorded in Kinshasa, whereas the river network located upstream of Kisangani contributes to 20% of the total river flow. The temporal survey at Kisangani provided further evidence that phytoplankton development was controlled by hydrological factors and turbidity.

In the most downstream section of the Congo River, phytoplankton biomass declined, as a result from combined effects of dilution, TSM inputs by major tributaries (Oubangui and Kasai rivers) and greater channel depth. The region after the confluence of the Kasai also corresponds to a narrowing and deepening of the Congo upstream of Pool Malebo (Runge, 2008; O’Loughlin et al., 2013). Schematically, the longitudinal profile of phytoplankton biomass in the Congo River is, therefore, similar to that described in the early publications on phytoplankton in large temperate rivers (e.g. Welch, 1952): an absence or small amount of true plankton in an upstream section, followed by a downstream increase up to a maximum in a middle section, and decline in the lower section. This similarity clearly stems from the same controlling factor, i.e. hydrology, influencing residence time, dilution during downstream transport and suspended matter loading, which determines the availability of light.

Our $^{13}$C uptake measurements and estimates of daily primary production provide evidence for significant phytoplankton production in the mainstem of the Congo River, particularly in shallow sections of the river between 400 and 1400 km from Kinshasa, where organic C losses from respiration were reduced, allowing positive net production to take place. The substantial
Phytoplankton production occurring in the main channel may be a major difference between the mainstem of the Congo River and that of most other large tropical rivers. The general pattern in the rivers of South America (e.g. Amazon, Orinoco, Paraná) is that phytoplankton growth is not possible in their mainstem, due to a very low euphotic depth:river depth ratio (e.g. Fisher & Parsley, 1979), resulting from high TSM loading in a deep river channel. Instead, in the South American floodplains, the bulk of planktonic primary production takes place in lakes that receive nutrients from the river during the ‘flood pulse’ (Junk et al., 1989) in the rising water period, and most phytoplankton present in the rivers is imported from the lakes. In a similar way, in catchments without extensive seasonally inundated floodplains, natural lakes and man-made reservoirs in the catchment act as reactors where phytoplankton is produced, resulting from improved light penetration from sedimentation of suspended matter, and their overflow provides planktonic algae and cyanobacteria to the downstream river section. As described by Talling & Prowse (2010), these inocula can be substantial and are transported far downstream, but the maintenance of populations in the river depends on ability to grow in a turbulent, usually turbid, environment with short retention time. However, transport from floodplain lakes and reservoirs is not the only process allowing phytoplankton presence in the main channel of tropical rivers. Studies conducted in tropical rivers have shown that a substantial part of the phytoplankton production can take place in slow-flowing areas of the main channel, as in the Orinoco River (Lewis, 1988) or in the Daly River, tropical Australia (Townsend, Przybylska & Miloshis, 2012). Moreover, the success of Aulacoseira species, which are abundant in the turbulent and turbid waters of the Paraná river, while being absent from the floodplain lakes (O’Farrell, Izaguirre & Vinocur, 1996; Devercelli et al., 2014), suggests that they originate from the river environment rather than being imported from lakes.

In contrast to the Nile and to its South American counterparts, the Congo River has only a few lakes in its catchment and lacks an extensive seasonal floodplain (Borges et al., 2015a). The floodplain consists of permanently flooded forest, with little variation in water depth between the extremes of the hydrograph. A factor explaining the possibility of net phytoplankton growth in the river main channel is the relatively low TSM load over most of the studied transect, compared for instance with the Amazon. Indeed, the average TSM in the Amazon mainstem at Obidos of 230 mg L\(^{-1}\) (range 93–385 mg L\(^{-1}\), Richey et al., 1986) is distinctly higher than the average TSM in the Congo mainstem at Kinshasa of 26 mg L\(^{-1}\) (range 15–40 mg L\(^{-1}\), Coynel et al., 2005) and at Kisangani of 53 mg L\(^{-1}\) (range 13–111 mg L\(^{-1}\), A.V. Borges, own unpubl. data). Another factor is the low mean depth in most of the Congo River mainstem that allowed a euphotic depth:channel depth ratio favourable for sufficient net photosynthesis to sustain algal growth.

Two different parts can be distinguished in the Kisangani–Kinshasa transect: the upper sector of about 200 km in length where TSM is higher during the HW period and the whole river downstream of this upper sector, where TSM is actually lower in HW conditions than in FW. This could be explained by deposition of sediments in the ‘Cuvette Centrale Congolaise’ region during HWs, whereas sediments and nutrients were transported back to the rivers during FWs. Several results of our study point towards this process: higher TSM in most of the river transect and increased inorganic macro-nutrients (NO\(_3\) and SRP) both in the main river and the tributaries during FW. This nutrient increase might have favoured phytoplankton growth, although there was no clear evidence for a change in phytoplankton nutrient status from elemental ratios. Indeed, POC:PN and POC:PartP ratios cannot be relied on as indicators of the phytoplankton nutrient status, as POC:Chl \(a\) ratios were quite high, showing that phytoplankton carbon was a small fraction of the POC (2–8%, using a Chl \(a\):C ratio of 40; Harris, 1986).

Phytoplankton composition was quite different between HW and at FW. In FW conditions in the mainstem, diatoms contributed on average to 60% of Chl \(a\) and were the dominant group at all sites, in agreement to what was reported in most studies on tropical river phytoplankton. Accordingly, Hughes et al. (2011) found that the largest fraction of biogenic silica (BSi) in the Congo River was composed of diatoms, with significant uptake of dissolved silicon, affecting its concentration in the river, as well as silicon isotopic fractionation. This confirms that significant diatom production does occur in the Congo River mainstem at falling and probably low waters.

The most abundant taxa were those adapted to low light conditions, such as diverse taxa of Aulacoseira, described as the most competitive at high Si:light ratio (Kilham et al., 1986). The second most important phytoplankton taxa in the FW period were the green algae, which accounted for 10% of Chl \(a\), while the other groups (cyanobacteria and cryptophytes) accounted for 8% of Chl \(a\) on average. In contrast, during HW, green algae contributed to 88% of Chl \(a\) in the Congo River mainstem conditions, and 87% in the tributaries.
whereas diatoms barely reached 10% of total phytoplankton biomass. This is quite different from most reports on phytoplankton composition in large tropical rivers, particularly of South America (e.g. Garcia de Emiliani, 1990; Lewis et al., 1995; Davies et al., 2008; Devercelli et al., 2014; O’Farrell & Izaguirre, 2014) but also from Africa (Prowse & Talling, 1958; Talling & Rzóska, 1967): one would have expected dominance of diatoms during both discharge conditions, owing to light limitation resulting from the TSM concentration in the mainstem and large tributaries, and from high DOC in the blackwater tributaries (Mayora, Devercelli & Frau, 2016). Indeed, considering a mean daily surface irradiance of 750 µE m⁻² s⁻¹, calculated from continuous recordings during the cruises, the mean light exposure of phytoplankton would have been on average 35 µE m⁻² s⁻¹ in FW versus 71 µE m⁻² s⁻¹ in HW (Table 1). Under such conditions, diatoms, which are highly efficient at photosynthesis at low light (Kilham et al., 1986; Kirk, 1994), would have been able to grow, so that the most likely explanation for their lack of development at HW is dilution by water inputs and lack of time to build populations in the fast-flowing mainstem. In contrast, green algae typically need high light for growth: Dauta (1982) measured optimal light for photosynthesis (I_opt) at different temperatures for some coccal green taxa and found I_opt values between 180 and 281 µE m⁻² s⁻¹ in the 25–30 °C range. The question thus arises how did green algae manage to maintain significant populations during HW. The most likely mechanism by which they can access these light levels and achieve high growth rates may be that they grew in the well-developed littoral/wetland aquatic vegetation of the main river, thereby providing inocula to the mainstem. This hypothesis, suggested by the presence and high abundance of green algae in a sample taken at HW in the fringing aquatic meadow, should be further substantiated by specific observations.

The general descriptions of the ecology and distribution of key coccal and filamentous conjugate genera found in the Congo River show their belonging to the metaphyton, that is, algae loosely attached to the submerged macrophytes, from which they may be dislodged by the waves and current and then occur as tychoplankters in open waters (e.g. Behre, 1956; John et al. 2011). In these shallow environments, green algae may have benefited from adequate light conditions for growth, and water exchanges with the main channel may have supplied cells to the mainstem, according to the process described by Reynolds et al. (1991) involving the hydrological ‘dead zones’. Similarly, the observations of Stoyneva (1994) suggest the role of macrophytes growing in the shallow river margin for sustaining the planktonic green algae in the Danube River. Once in the mainstem, green algae may have been able to maintain populations, provided that they were able to sustain a high photosynthetic rate. More research would be needed to assess whether plankton retention in the fringing aquatic vegetation actually plays a role in the maintenance of phytoplankton production in the mainstem of the Congo River during the different hydrological periods.

The fact that phytoplankton production occurs in the Congo River mainstem is of some biogeochemical significance, such as the effect of diatom silicon uptake on the Si cycle (Hughes et al., 2011), but this production remains marginal in terms of the overall river network carbon budget. For instance, the emission of CO₂ to the atmosphere from the Congo River fuelled by processing of organic matter from land and wetland (13 800–18 250 mg C m⁻² day⁻¹, Borges et al., 2015b) is two orders of magnitude higher than primary production (<500 mg C m⁻² day⁻¹). The major contribution of phytoplankton primary production and biomass may lie in its role in sustaining the food web. Indeed, as for the Amazon River (Forsberg et al., 1993) where a key role of autochthonous C production by phytoplankton in fish diet was evidenced, there are indications that phytoplankton is an important component of the diet of some fish species in the Congo River. This is the case, based on gut content analysis, in two cyprinid species (Pwema et al., 2015) and in a catfish (Tembeni Makiai et al., 2013) in the Malebo pool. This stresses the importance of understanding food-web processes, and in particular, the role of allochthonous versus autochthonous C, in large tropical rivers, where the fisheries contribute to the local economies and provide food for the human populations.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Results of a principal component analysis carried out on the physical and chemical data in the Congo River mainstem and its tributaries, from the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW). The dots are the sampling sites; their shading depends on the distances from Kinshasa. See Table 1 for acronyms.

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