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Spatial variability of chromophoric dissolved organic matter in a large floodplain river: control factors and relations with phytoplankton during a low water period

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ABSTRACT

Chromophoric dissolved organic matter (CDOM) affects ecological processes in freshwater environments. Few studies assessed its spatial variability and relations with phytoplankton in floodplain rivers. Therefore, these topics were examined in a hydrological connectivity gradient in the Middle Paraná system. Absorption coefficients at 250 and 365 nm were measured to estimate CDOM concentration and molecular weight (MW), to find their explanatory limnological variables (Redundancy Analysis, RDA), and to assess their contribution to the explanation of phytoplankton structure (Canonical Correspondence Analysis, CCA). Conductivity and turbidity explained CDOM significantly. Increased conductivity from the main channel to floodplain lakes was associated with increased CDOM concentration. The lake indirectly connected to the river showed the highest turbidity associated with high-MW-CDOM, while the isolated lake showed the highest conductivity associated with low-MW-CDOM. Phytoplankton structure was significantly explained by conductivity, turbidity, and high-MW-CDOM (CCA). Environments directly connected to the river (with the lowest conductivity) were associated with diatoms. Phytoflagellates were associated with turbidity and high-MW-CDOM in the lake indirectly connected to the river, whereas Cyanobacteria were associated with conductivity and low-MW-CDOM in the isolated lake. The combined effect of physical factors and CDOM explained the highest fraction of species variation (partial CCA). As a conclusion: (1) CDOM increases as hydrological connectivity with the river decreases; (2) lake sediment resuspension is linked to an increase of high-MW-CDOM; (3) lake isolation from the river corresponds to an increase in low-MW-CDOM; and (4) CDOM explains phytoplankton structure significantly and should be considered as an important variable in studies of microalgal assembly. Copyright © 2015 John Wiley & Sons, Ltd.

KEY WORDS hydrological connectivity; CDOM optical estimators; limnological variables; phytoplankton structure

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INTRODUCTION

In floodplain fluvial systems, limnological characteristics are strongly influenced by the hydrological connectivity among aquatic environments (Junk *et al*., 1989; Neiff, 1990). During high water phases, the higher connectivity among floodplain water bodies and the main channel enhances the exchange of material among them. During low water phases, such exchanges decrease and limnological conditions become mostly determined by local processes (Forsberg *et al*., 1988; Hamilton and Lewis, 1990; Thomaz *et al*., 2007). The topographic position of the water bodies also influences their degree of hydrological connectivity and therefore their limnological conditions (Drago, 1989). Several authors observed, in a hydrological connectivity gradient from the main channel to the isolated floodplain water bodies, a decrease in depth, nitrate (NO_3^-), pH, and dissolved oxygen (DO); and an increase in conductivity, transparency, and phytoplankton biomass (Izaguirre *et al*., 2001; Unrein, 2002; Zalocar de Domitrovic *et al*., 2007; Cardoso *et al*., 2012; Mayora *et al*., 2013).

Chromophoric dissolved organic matter (CDOM) is also affected by hydrological connectivity in floodplain fluvial systems (Depetris and Kempe, 1993; Mladenov *et al*., 2005; Peduzzi *et al*., 2008; Cawley *et al*., 2012; Sieczko and Peduzzi, 2014). Most of these studies focus on CDOM temporal variability. During periods of higher hydrological connectivity, CDOM concentration decreases or increases depending on the importance of CDOM dilution and input from flooded environments. In comparison with other limnological variables, CDOM spatial variability has been less studied in floodplain rivers. The analysis of the spatial patterns of CDOM could contribute to the understanding of the functioning of

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these systems. CDOM affects multiple limnological characteristics, such as light climate (Williamson *et al*., 1996; Costa *et al*., 2013) and bioavailability of inorganic nutrients (Shaw, 1994), toxic compounds (Sakkas *et al*., 2002), and carbon sources for microorganisms (Granéli *et al*., 1999; Teixeira *et al*., 2011a). Hence, spatial variations of CDOM influence ecological and biogeochemical processes.

In freshwater systems, CDOM has different sources related to its molecular weight (MW). Bacteria, phytoplankton, and aquatic macrophytes are important sources of autochthonous CDOM, which is characterized by low MW. Contrarily, allochthonous CDOM is transported from the catchment and is mainly originated from the degradation of terrestrial vegetation that produces compounds of higher MW (Steinberg, 2003). The importance of different sources of CDOM influences both its concentration and the predominance of low or high MW fractions. In this respect, the predominance of high-MW-CDOM in water bodies with low hydrological connectivity has been associated with the contribution of terrestrial sources (Mladenov *et al*., 2005; Cawley *et al*., 2012).

CDOM is degraded over time through microbiological metabolism and photobleaching. Water residence time (Curtis, 1998; Mazzuoli *et al*., 2005), water column depth (Pęczuła, 2014), and transparency (Osburn *et al*., 2009) can affect the intensity of the degradation process, and consequently the concentration and MW of CDOM. In addition, pH, DO, and ionic strength of water can modify the conformation of CDOM molecules (De Haan *et al*., 1987; Karanfil *et al*., 1994; Reche *et al*., 1999).

The changes that CDOM causes on light climate and nutrient availability affect phytoplankton development. The influence of CDOM on phytoplankton growth is not conclusive: both stimulating and inhibiting effects have been documented (Jones, 1992; Arvola and Tulonen, 1998; Carpenter *et al*., 1998; Nürnberg and Shaw, 1999; Webster *et al*., 2008). Regarding phytoplankton composition, several studies showed that CDOM can benefit motile and mixotrophic species (Bergström *et al*., 2003; Joniak, 2007) or algae able to absorb the red/orange light which dominates in humic environments (Jones, 1998).

CDOM variability and its controlling factors from the main channel to the floodplain water bodies, as well as CDOM relations with phytoplankton assemblage, were examined in the Middle Paraná River system. The concentration and MW of CDOM were spectrophotometrically estimated during a low water period. It was hypothesized that: (1) hydrological connectivity of floodplain water bodies influences the spatial variability of the concentration and MW of CDOM; and (2) spatial variability of CDOM affects phytoplankton development. It was therefore predicted that: (1) The concentration and MW of CDOM increase in a connectivity gradient, from the main channel to the more isolated floodplain lakes; and (2) CDOM contributes to explaining the spatial variability of phytoplankton assemblage.

METHODS

Study area

The Paraná River is one of the largest rivers in South America, with a basin area of $2.6 \times 10^6\,km^2$, a length of 4400 km, and an average discharge volume to the sea of $470\,km^3$ per year (Drago, 2007). The river is divided into Upper, Middle, and Lower sections. The Middle Paraná River (Argentina) begins approximately 1000 km upstream of the river mouth. This section extends from its convergence with the Paraguay River (27°29′S; 58°50′W) up to Diamante City (32°4′S; 60°39′W). It has a main channel of variable width (0.4–8 km) and a large floodplain (6–40 km wide, $13\,000\,km^2$ area) along its right bank that encompasses a high number of temporary and permanent streams and lakes.

The study area was located within the Middle Paraná River system near Santa Fe City (31°38′S; 60°42′W). Sampling sites were chosen to reflect different degrees of hydrological connectivity from the main channel to the floodplain, including three lotic environments and three floodplain lakes (Figure 1). To assess the connectivity of each sampling site, its topographic position and kind of connection to the fluvial system were considered. The lotic environments were the Middle Paraná River main channel (Lo1); the Colastiné River (Lo2), a large secondary channel of 39 km in length (Iriondo, 2007) that is directly connected to Lo1; and the Miní Stream (Lo3), a small secondary channel connected to Lo1 and to several floodplain water bodies. The lakes sampled were the Miní Lake (La1) directly and permanently connected to Lo3 and to Lo1 by a 0.65-km-long channel; the Irupé Lake (La2) indirectly connected to the fluvial system by Lo3 and isolated during periods of extreme drought (connected during the whole sampling period), and El Mirador Lake (La3) isolated from the fluvial system and mainly fed by groundwater. The vegetation corresponding to La1 during the sampling period was restricted to marshy species. La2 presented stands of *Ludwigia peploides* (Kunth) P.H. Raven, *Eichhornia crassipes* (Mart.) Solms, *Eichhornia azurea* (Sw.) Kunth, *Nymphoides indica* (L.) Kuntze, and *Myriophyllum aquaticum* (Vell.) Verdc; whereas vegetation in La3 was characterized by *Salvinia* sp., *Pistia stratiotes* L., and *Lemna* sp. The sampling sites included the main kinds of aquatic environments of the system and were considered in a decreasing hydrological connectivity degree as follows: Lo1, Lo2, Lo3, La1, La2, and La3.

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Samplings and laboratory analyses

Monthly samplings were performed during a low water phase, between September and December 2010 in the morning hours. Samples were collected in the centre of the lotic environments, in the pelagic zone of the lakes, and in the littoral zone of La2 and La3 (La2′ and La3′,

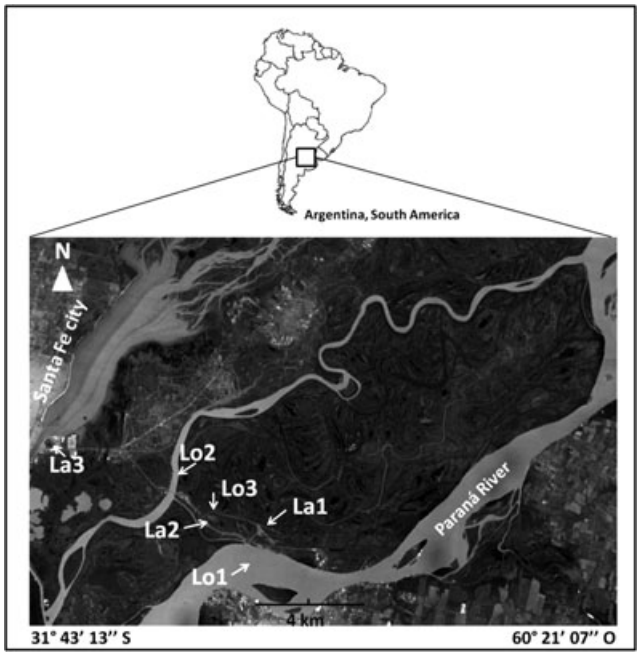

Figure 1. Study area and location of sampling sites (with arrows). Lo1: main channel; Lo2: large secondary channel; Lo3: small secondary channel; La1: directly connected lake; La2: indirectly connected lake; La3: isolated lake.

respectively) (n = 32) to assess possible spatial differences within these water bodies.

Depth (Z_d, ultrasonic probe), temperature (thermometer), transparency (Secchi disc, SD), water current velocity in lotic environments (current meter AOTT C20), DO, pH, and conductivity (HANNA portable water checkers) were measured *in situ*. Depth of the euphotic zone (Z_{eu}) was estimated using the index proposed for turbid waters ($Z_{eu} = SD \times 3.5$) (Koenings and Edmundson, 1991), and Z_d:Z_{eu} was calculated as a measure of light availability in the water column. Water level at the Paraná Harbour Gauge was obtained from *Centro de Informaciones Meteorológicas* (*UNL*).

Subsurface water samples were collected with 2-l bottles for physical and chemical analyses. Turbidity (formazin turbidity units, FTU) was spectrophotometrically estimated at 450 nm using a HACH DR 2000 spectrophotometer. Nutrients and CDOM were analysed from water filtered through Whatman GF/F glass-fibre filters. Soluble reactive phosphorus (SRP) was assessed by the ascorbic acid method (Murphy and Riley, 1962 in APHA, 1992) at 880 nm, and N-NO_3^- by reduction with metallic cadmium and subsequent colorimetric determination of N-NO_2^- at 400 nm using HACH® reagents.

CDOM was optically assessed at 250 and 365 nm using a HACH DR5000 ultraviolet–visible spectrophotometer and a quartz cuvette with 1-cm path length. The absorption coefficients were calculated following Kirk (1994):

$$A\lambda = 2.303 D\lambda / r$$

where $A\lambda$ (m^{-1}) is the CDOM absorption coefficient at wavelength λ, $D\lambda$ is the optical density at wavelength λ, and r is the cuvette path length in m. A_{250} and A_{365} are estimators of low-MW-CDOM and high-MW-CDOM, respectively (Stewart and Wetzel, 1981; Mostofa *et al.*, 2013). E_2:E_3 (A_{250}:A_{365} ratio), which is inversely proportional to MW of CDOM (Helms *et al.*, 2008), was calculated.

Chlorophyll-*a* (Chl-*a*, estimator of phytoplankton biomass) and pheophytin-*a* (Pheo-*a*, the main Chl-*a* degradation product) were extracted from the GF/F filters with acetone (90%) through maceration in a glass grinder and storage at 4 °C for 6 to 12 h in darkness. The extracts were clarified and measured with a spectrophotometer at 664 and 750 nm, and at 665 and 750 nm after acidification with HCl 0.1N (Lorenzen, 1967 in APHA, 1992). Pheo-*a*:Chl-*a* ratio, which increases in senescent phytoplankton communities (Lorenzen, 1965), was used as an indicator of algal physiological state.

Phytoplankton samples were collected with 125-ml flasks and fixed *in situ* with acidified lugol solution (1%). Quantitative analysis was performed according to Utermöhl (1958), and density was expressed as ind ml^{-1}. Taxonomic identification of phytoplankton was made up to the minimum possible taxonomic level using specific keys and bibliography.

Statistical analyses

Differences among environments with respect to limnological variables were assessed with the Kruskal–Wallis test and Dunn's post test for multiple comparisons using PAST software (Hammer *et al.*, 2001). To assess the spatial variability of CDOM and its explanatory variables, a redundancy analysis (RDA) was performed because Detrended Correspondence Analysis (DCA) showed that the gradient length of the response data did not exceed 3 standard deviations (ter Braak and Smilauer, 2002). Hellinger transformation was performed to A_{250}, A_{365}, and E_2:E_3 (response variables), as well as to the other limnologic variables (explanatory variables).

The explanation of phytoplankton species density by means of limnological variables was analysed using a canonical correspondence analysis (CCA), because the gradient length of species abundance exceeded 5 standard deviations in the DCA analysis. The measured limnological variables (explanatory variables) and phytoplankton species with contribution to total density higher than 3% (response variables) were included in the analysis and logarithmically transformed. A partial CCA (pCCA) was performed afterwards to assess the percentage of explanation of phytoplankton species density for physical variables

(turbidity and conductivity), nutrients (SRP and N-NO_3^-), and CDOM (A_{250} and A_{365}), as well as for the combinations of the three categories of explanatory variables.

The more significant subsets of environmental variables for the RDA and CCA were checked with Monte Carlo test under unrestricted model of 999 permutations, being p-value adjusted with Bonferroni correction. The multivariate analyses were performed using the CANOCO 5.0 software.

RESULTS

CDOM relations with limnological variables in the spatial gradient

Limnological variables varied along the hydrological connectivity gradient (Table I) showing significant differences among environments (Table II). Conductivity and Chl-*a* increased from the main channel to the most isolated floodplain lake, being significantly higher at La3 ($P < 0.05$), while depth, Z_d:Z_{eu} ratio, N-NO_3^-, Pheo-*a*: Chl-*a* ratio, and water current velocity of lotic environments increased in sites with higher hydrological connectivity ($P < 0.05$). pH was circumneutral, and DO was near saturation at all sites except at the isolated La3, where water was alkaline ($P < 0.05$) and values of DO were oversaturated. A_{250} increased from the main channel towards the isolated sites (Figure 2a), being significantly higher in La2 and La3 lakes ($P < 0.05$). In addition, La2 showed the highest A_{365} (Figure 2b) and La3 the highest E_2:E_3 (Figure 2c) ($P < 0.05$).

RDA accounted for 76% of the cumulative variance that explained CDOM variations (79% the first axis and 21% the second axis of the explained fitted variation), being conductivity (F = 61.4, $P = 0.009$) and turbidity (F = 17.9, $P = 0.01$) the significant explanatory variables. Samples were arranged into three groups reflecting the hydrological connectivity degree of the environments (Figure 3). The first group included the samples of the main channel (Lo1) and its directly connected environments (Lo2, Lo3, and La1), and was characterized by low A_{250} and high A_{365} associated with low conductivity. The second group was formed by the indirectly connected lake (La2), and had high A_{365} and low E_2:E_3 linked to high turbidity. Finally, the third group was represented by the isolated environment (La3) and was characterized by high A_{250} and low A_{365} associated with high conductivity.

Influence of CDOM and limnological variables on phytoplankton assemblage

Total phytoplankton density increased from the main channel to the most isolated lake (Figure 4a). Phytoplankton was composed of 199 taxa that belong to Cyanobacteria, Bacillariophyceae, Chlorophyceae, Zygnematophyceae, Cryptophyceae, Chrysophyceae, Dinophyceae, and Euglenophyceae. Figure 4b shows the relative importance of these phytoplankton groups in each environment.

Samples in the CCA were sorted similarly to the RDA ordination (Figure 5). The first two axes explained 46.7% of the phytoplankton species variability. The significant explanatory variables were conductivity ($F = 16.7$, $P = 0.009$), turbidity ($F = 4.0$, $P = 0.009$), and A_{365} ($F = 3.0$, $P = 0.009$). Diatoms and flagellates belonging to Chlorophyceae, Cryptophyceae, and Chrysophyceae were associated with lotic environments and water bodies connected (directly or indirectly) with the fluvial system, and negatively correlated with conductivity (Figure 5). Centric diatoms (*Melosira varians*, *Skeletonema potamos*, *Aulacoseira italic*, and *A. granulata* var. *angustissima*), cryptomonadals (*Cryptomonas*

Table I. Mean values of limnological variables at the Middle Paraná environments sampled during a low water phase (September–December 2010). Standard deviation is in parentheses.

	Lo1	Lo2	Lo3	La1	La2	La2′	La3	La3′
Current velocity ($m\,s^{-1}$)	1.1 (0.1)	0.7 (0.0)	0.3 (0.1)					
Depth (m)	6.1 (1.4)	12.5 (0.8)	2.2 (0.5)	1.5 (0.7)	2.0 (0.5)	0.5 (0.0)	1.3 (0.2)	0.6 (0.2)
Temperature (°C)	20.9 (3.6)	21.0 (4.6)	20.4 (4.4)	20.4 (3.4)	20.3 (4.3)	20.4 (4.8)	18.7 (4.3)	18.5 (4.5)
Secchi disc (cm)	35.8 (5.4)	34.3 (3.8)	29.0 (4.0)	33.5 (6.2)	25.0 (7.9)	24.3 (6.5)	34.0 (17.2)	30.6 (14.3)
Turbidity (FTU)	38.0 (5.4)	46.0 (10.1)	38.0 (6.1)	44.5 (9.3)	87.0 (39.6)	94.0 (38.9)	53.5 (49.2)	53.5 (47.1)
Z_d:Z_{eu}	4.9 (0.7)	10.5 (1.4)	2.2 (0.4)	1.4 (0.3)	2.4 (0.6)	1.0 (0.1)	1.4 (0.7)	1.2 (0.4)
Conductivity ($\mu S\,cm^{-1}$)	60.0 (10.7)	66.1 (1.2)	64.8 (1.1)	69.1 (2.9)	71.5 (6.4)	71.5 (6.3)	1342.5 (222.6)	1344.8 (230.2)
pH	7.3 (0.3)	7.5 (0.2)	7.1 (0.2)	7.4 (0.2)	7.2 (0.2)	7.3 (0.3)	8.4 (0.3)	8.4 (0.3)
DO ($mg\,l^{-1}$)	8.7 (1.0)	8.0 (1.1)	7.9 (1.7)	8.2 (0.8)	8.2 (2.2)	8.0 (1.6)	9.6 (1.5)	9.9 (1.6)
N-NO_3^- ($\mu g\,l^{-1}$)	525 (50)	533 (58)	550 (58)	525 (236)	475 (206)	375 (150)	350 (58)	400 (81)
SRP ($\mu g\,l^{-1}$)	19 (7)	26 (12)	28 (18)	23 (9)	22 (7)	26 (11)	67 (65)	71 (69)
Chl-*a* ($\mu g\,l^{-1}$)	3.4 (2.5)	3.2 (0.9)	5.3 (1.5)	5.8 (1.5)	9.5 (3.2)	9.7 (2.3)	67.2 (48.8)	76.8 (64.2)
Pheo-*a*:Chl-*a*	0.4 (0.1)	0.4 (0.2)	0.3 (0.1)	0.2 (0.1)	0.3 (0.1)	0.3 (0.1)	0.1 (0.2)	0.1 (0.1)

Lo1, main channel; Lo2, large secondary channel; Lo3, small secondary channel; La1, directly connected lake; La2 and La2′, pelagic and littoral zones of indirectly connected lake, respectively; La3 and La3′, pelagic and littoral zones of isolated lake, respectively.

Table II. Differences among Middle Paraná environments sampled during a low water phase (September–December 2010) according to Kruskal–Wallis test. Only statistically significant differences ($P < 0.05$) are shown. The site abbreviations are the same as in Table I.

	Lo1	Lo2	Lo3	La1	La2	La2′	La3	La3′
Water current velocity ($H = 9.85$; $P = 0.0073$)								
Lo1		0.020	0.028					
Lo2			0.020					
Depth ($H = 28.13$; $P = 0.0002$)								
Lo1		0.030	0.030	0.030	0.029	0.027	0.029	0.030
Lo2			0.030	0.030	0.029	0.027	0.029	0.030
Lo3						0.027		0.030
La1						0.027		
La2						0.026		0.029
La3						0.026		0.029
Z_d:Z_{eu} ($H = 25.40$; $P = 0.0006$)								
Lo1		0.030	0.030	0.030	0.030	0.026	0.029	0.027
Lo2			0.030	0.030	0.030	0.026	0.029	0.027
Lo3						0.026		
La2						0.026		0.027
Conductivity ($H = 22.14$; $P = 0.0024$)								
La1			0.030					
La3	0.030	0.030	0.030	0.030	0.030	0.030		
La3′	0.030	0.030	0.030	0.030	0.030	0.030		
pH ($H = 21.52$; $P = 0.0031$)								
Lo3		0.028						
La3	0.029	0.028	0.028	0.029	0.028	0.029		
La3′	0.030	0.029	0.029	0.030	0.029	0.030		
A_{250} ($H = 24.18$; $P = 0.0011$)								
La2	0.030	0.029	0.030	0.030				
La2′	0.030	0.029	0.030	0.030				
La3	0.029	0.028	0.029	0.029				
La3′	0.030	0.029	0.030	0.030				
A_{365} ($H = 19.36$; $P = 0.0071$)								
La2	0.030	0.029	0.029				0.030	0.030
La2′	0.029	0.028	0.028	0.029			0.029	0.029
E_2:E_3 ($H = 21.92$; $P = 0.0026$)								
La3	0.030	0.030	0.030	0.030	0.030	0.029		
La3′	0.030	0.030	0.030	0.030	0.030	0.029		
Chl-a ($H = 24.58$; $P = 0.0009$)								
La3	0.030	0.030	0.030	0.030	0.030	0.030		
La3′	0.030	0.030	0.030	0.030	0.030	0.030		
Pheo-a:*Chl-a* ($H = 14.46$; $P = 0.0436$)								
La3′	0.040	0.036	0.026					

spp.), and volvocaleans (*Spermatozopsis exultans* and *Chlamydomonas* sp.) were better represented at the main channel and directly connected environments, associated with high depth. Other flagellates (Chrysophyta n.i., *Chroomonas acuta*, *Plagioselmis nannoplanctica*, and a volvocalean species) were associated with La2 (indirectly connected lake), linked to high turbidity and A_{365}. Phytoplankton species in the isolated La3 were represented by Cyanobacteria (*Dolichospermum* sp. and *Coelomorum* sp.), chlorococcaleans (*Scenedesmus ecornis*, *Chlorella* sp., and *Monoraphidium* spp.), diatoms (*Cyclotella meneghiniana*, *Navicula* sp. and *Nitszchia* sp.), and few species belonging to Zygnematophyceae, Euglenophyceae, and Dinophyceae (*Closterium acutum*, *Trachelomonas curta*, and *Peridinium* sp. respectively), linked to high conductivity and A_{250}.

The pCCA reflected that a low percentage (<7% each group) of phytoplankton structure was explained by the pure effect of physical variables (turbidity and conductivity), nutrients (SRP and N-NO_3^-), and CDOM (A_{250} and A_{365}). Only the combined effect of physical variables + CDOM showed a high explanation percentage (54.3%) (Table III). T3

DISCUSSION

CDOM and associated limnological variables

The hydrological connectivity of water bodies influences the concentration and molecular weight (MW) of CDOM in a variable way according to the magnitude of several factors. These factors include: evapoconcentration, dilution

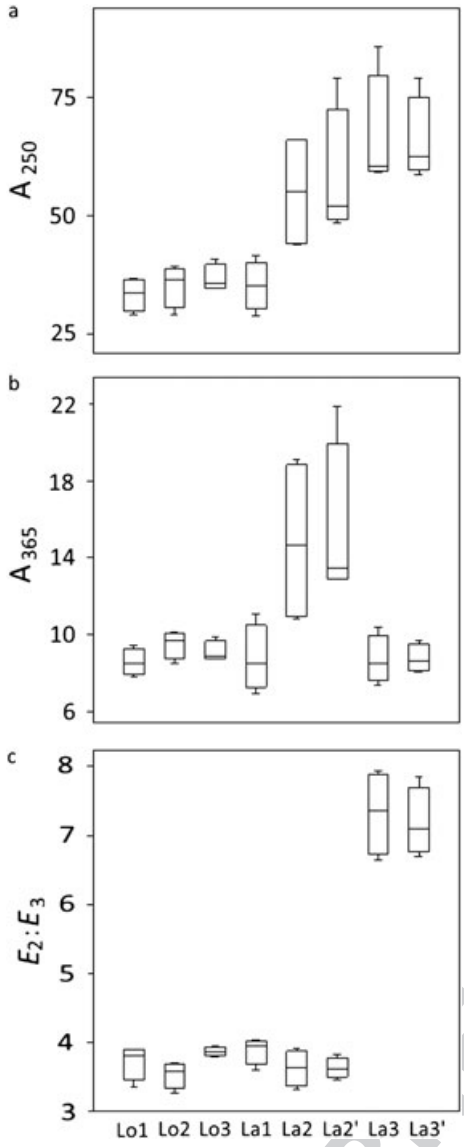

Figure 2. Box plot of A250 (a), A365 (b), and E2:E3 (c) in a decreasing hydrological connectivity degree from the main channel of the Middle Paraná River to the more isolated lake during a low water phase (September–December 2010). The site abbreviations (x axis) are the same than in Table I.

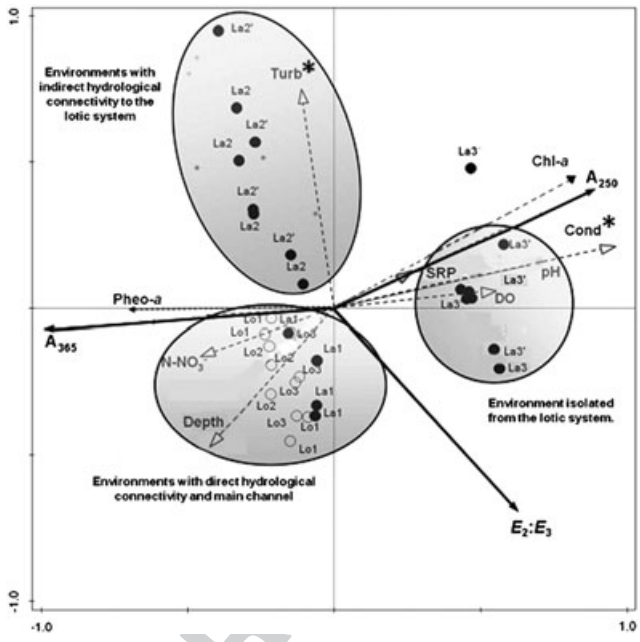

Figure 3. RDA plot. Dependant variables (A250, A365, and E2:E3) are represented with solid black arrows, and explanatory variables are represented with dotted grey arrows. Significant explanatory variables are indicated with an asterisk, lake samples are represented with filled circles, and lotic environment samples are represented with empty circles. The site abbreviations are the same than in Table I.

by water inflow, exports and inputs of CDOM, and water residence time that affects CDOM transformations (Mladenov *et al*., 2005; Peduzzi *et al*., 2008; Cawley *et al*., 2012; Sieczko and Peduzzi, 2014). As in other studies (Mladenov *et al*., op. cit.; Cawley *et al*., op. cit.), CDOM concentration was negatively associated with hydrological connectivity in the spatial gradient of the Middle Paraná River. The indirectly connected lake (La2) was associated with high-MW-CDOM but, contrary to our expectations, MW of CDOM was low in the isolated lake (La3). The studies mentioned above recorded high-MW-CDOM in sites with low hydrological connectivity which were periodically flooded by the connection with the lotic system. This type of connectivity determined a high input of allochthonous CDOM, unlike what seems to occur in an isolated lake.

As in the Danube (Mladenov *et al*., 2005) and the Okavango (Cawley *et al*., 2012) rivers, CDOM was significantly explained by conductivity, which was positively associated with A_{250} (RDA). Conductivity is a measure of ion concentration that responds to the dilution–concentration processes. It constitutes a good indicator of the degree of hydrological connectivity and tends to increase from the main channel to the floodplain water bodies (Unrein, 2002; Mladenov *et al*., op. cit.). The positive relation between conductivity and CDOM concentration indicates that input and evapoconcentration of CDOM override its dilution and degradation in more isolated water bodies. In contrast, the low CDOM concentration registered in the more connected water bodies (Figure 2) may be because of the inflow of lotic water that dilutes CDOM mainly produced within the floodplain (Depetris and Kempe, 1993).

The low MW of CDOM observed in the isolated lake can be associated with several factors linked to its low hydrological connectivity. First, the high conductivity of the aqueous medium is indicative of high ionic strength (Alva *et al*., 1991), which favours the reduction in the MW of CDOM (De Haan *et al*., 1987). Second, the absence of surface hydrological connection implies a high water residence time, which is also associated with a reduction

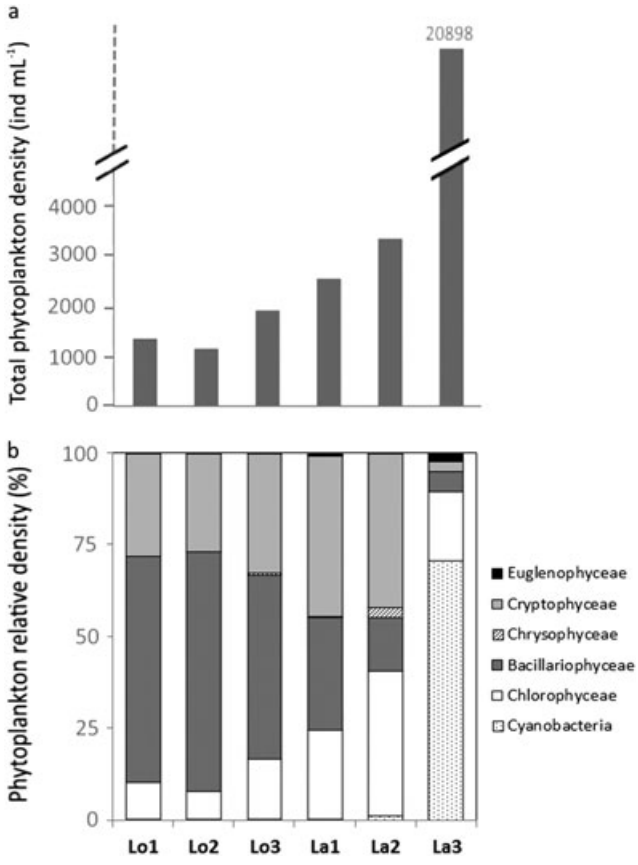

Figure 4. Total phytoplankton density (median) (a), and relative abundance of phytoplankton taxonomical groups (b) in a decreasing hydrological connectivity degree from the main channel of the Middle Paraná River to the more isolated lake during a low water phase (September–December 2010). The site abbreviations are the same than in Table I.

in the MW of CDOM by degradation over long periods of time (Curtis, 1998; Mazzuoli *et al*., 2005). Third, more isolated lakes are characterized by high transmission of sunlight (Z_d:Z_{eu} close to 1 at La3) increasing the degradation of CDOM through photobleaching, which is accompanied by a reduction in the MW of CDOM (Osburn *et al*., 2009). Fourth, isolation improves phytoplankton growth, which is shown at La3 by high Chl-*a* and low Pheo-*a*:Chl-*a* ratio; the alkaline conditions of the water because of the photosynthetic activity of phytoplankton increase the susceptibility of CDOM to photobleaching (Reche *et al*., 1999). Fifth, isolation increases the production of autochthonous CDOM with low MW because of development of phytoplankton and bacteria (Sundh and Bell, 1992; Peduzzi *et al*., 2008; Teixeira *et al*., 2011a, 2011b). Finally, groundwater hydrological connection reduces the input of high-MW-CDOM because this fraction is preferentially sorbed and retained by soil particles (Kaiser *et al*., 2002).

Sediment resuspension affects the chemical conditions of shallow lakes by translocation of substances from the bottom to the water column (Hamilton and Lewis, 1990). The turbulence generated by the wind increases sediment stirring during low water periods, which is evidenced by increments in turbidity (Izaguirre *et al*., 2001; Maine *et al*., 2004). The significant explanation of CDOM by turbidity can be linked to the sediment resuspension occurring at the indirectly connected lake (La2). Associations of this turbid lake with high-MW-CDOM can have two non-exclusive explanations. On the one hand, high-MW-CDOM is more hydrophobic than low-MW-CDOM, and therefore has a greater tendency to be retained in sediments (Davis and Gloor, 1981; Luider *et al*., 2003; Tipping, 2004). On the other hand, high concentration of suspended particles prevents photobleaching of CDOM, which leads to a marked decrease in the MW of CDOM in clear water (Osburn *et al*., 2009).

Relation of CDOM with phytoplankton structure

In subtropical floodplain fluvial systems, physical variables (especially light availability and hydrological conditions) are important factors regulating the structure of phytoplankton, whereas nutrients play a secondary role (Zalocar de Domitrovic *et al*., 2007; Devercelli *et al*., 2014). The relative importance of CDOM as another controlling variable of phytoplankton structure has not been explored in these systems. In boreal and temperate regions, however, several studies found that CDOM is associated with high densities of mixotrophic flagellates or algae with pigments that absorb red/orange light (Jones, 1998; Bergström *et al*., 2003; Joniak, 2007). Our results show that high-MW-CDOM significantly explained phytoplankton structure in the hydrological connectivity gradient, as well as the known physical variables (turbidity and conductivity). Furthermore, the variation partitioning analysis revealed that the combination of physical factors and CDOM (A_{250} and A_{365}) explained the most important fraction of the spatial variability of phytoplankton structure.

Phytoplankton structure in the main channel and directly connected environments (with the lowest conductivity values) was characterized by species well adapted to lotic, turbid, and deep environments. Diatoms, which are able to survive under high turbulence and poor light condition (Round *et al*., 1990), were the most abundant group. Chrysophyta and Chlorophyta were the subdominant groups because they are physiologically adapted to light limitation and can offset the effect of the short water residence time through a high reproduction rate (Reynolds, 1994; Reynolds and Descy, 1996).

The indirectly connected lake (La2) was linked to flagellated algae such as volvocaleans, cryptophytes, and chrysophytes, which are adapted to environments with low availability of light and P (Steinberg, 2003). These conditions are frequent in coloured and turbid water bodies as La2, where

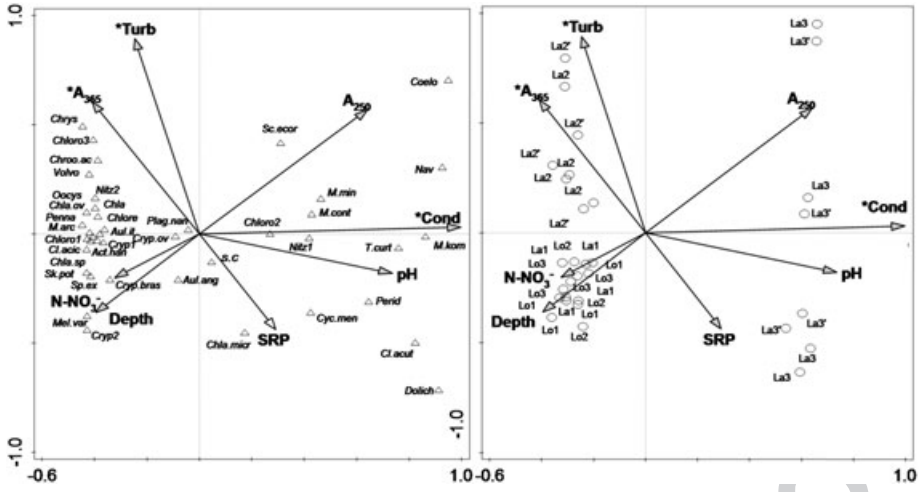

Figure 5. CCA plot. Phytoplankton species (dependant variables) are represented with triangles, and explanatory variables with arrows. Significant explanatory variables are indicated with an asterisk, lake samples are represented with filled circles, and lotic environment samples are represented with empty circles. The site abbreviations are the same than in Table I. Species key: Act.han: Actinastrum hantzschii Wol.; Aul.ang: Aulacoseira granulata var. angustissima (O.Müller) Sim.; Aul.it: A. italica (Ehr.) Sim.; Chla: Chlamydomonas sp.; Chla.micr: C. microsphaera Pasch. & Jahoda; Chla.ov: C. ovata Dang.; Chlore: Chlorella sp.; Chloro1, Chloro2, Chloro3: chlorococcalean species; Chroo.ac: Chroomonas acuta Uterm.; Chrys: a chrysophyte species; Cl.acic: Closteriopsis acicularis Belch. & Swale; Cl.acut: Closterium acutum Breb.; Coelo: Coelomorum sp.; Cryp.bras: Cryptomonas brasiliensis Castro, Bicudo & Bicudo; Cryp.ov: Cryptomonas ovata Ehr.; Cryp: Cryptomonas spp.; Cyc.men: Cyclotella meneghiniana Kütz; Dolich: Dolichospermum sp.; M.arc: Monoraphidium arcuatum (Kors.) Hind.; M.cont: Monoraphidium contortum (Thur.) Kom.- Legn.; M.kom: Monoraphidium komarkovae Nygaard; M.min: Monoraphidium minutum (Näg.) Kom.-Legn.; Mel.var: Melosira varians C. Agardh; Nav: Navicula sp.; Nitz1, Nitz2: Nitzschia species; Oocys: Oocystis sp.; Penna: pennate diatom; Perid: Peridinium sp.; Plag.nan: Plagioselmis nannoplanctica (Sk.)Nov. Luc. & Morr.; S.C.: small centric diatoms; Sc.ecor: Scenedesmus ecornis (Ehr.) Chod.; Sk.pot: Skeletonema potamos (Weber) Hasle; Sp.ex.: Spermatozopsis exultans Korsch.; T. curt: Trachelomonas curta Da Cunha; Volvo: a volvocalean species.

Table III. Explanation of phytoplankton species in Middle Paraná environments during a low water phase (September–December 2010) for three categories of explanatory variables and combinations of them based on partial CCA.

Fraction	Adjusted variation	% explained	% of total variation
A	0.043	6.7	3.2
B	0.040	6.2	2.9
C	0.040	6.4	3.0
D	0.044	7.0	3.3
E	0.020	3.1	1.5
F	0.344	54.3	25.7
G	0.103	16.3	7.7
Total explanation	0.634	100.0	47.2
Variation total	1.341	100.0	30.0

A: physical variables (turbidity and conductivity); B: nutrients (SRP and $N\text{-}NO_3^-$); C: CDOM (A_{250} and A_{365}); D: combination of A and B; E: combination of B and C; F: combination of A and C; G: combination of A, B, and C.

suspended particles and CDOM reduce the photic zone because of light scattering and absorption (Costa *et al*., 2013). In addition, high-MW-CDOM (generally humic substances) forms complexes with PO_4^{3-} influencing its bioavailability (Jones, 1998). Under such conditions, flagellate algae have competitive advantages because they can migrate to the surface layers with better light climate and high PO_4^{3-} bioavailability because of photochemical breakdown of humic substances–PO_4^{3-} complexes (Boavida and Wetzel, 1998; Bastidas Navarro and Modenutti, 2010). Moreover, Chrysophyta and Cryptophyta can combine phototrophy with phagotrophic heterotrophy (Callieri *et al*., 2006; Modenutti, 2014). They can ingest bacteria, which are abundant in humic lakes and are more effective than phytoplankton to accumulate P (Jansson *et al*., 1996; Vadstein *et al*., 2003). This ability allows phagotrophic phytoplankton to succeed under poor light conditions and/or low bioavailability of inorganic P (Pålsson and Granéli, 2004; Alves-de-Souza *et al*., 2006; Reynolds, 2006). In addition, cryptophytes contain biliproteins capable of absorbing the red wavelength radiation

prevailing over shorter wavelength radiation in humic environments (Kirk, 1994; Rodríguez and Pizarro, 2007).

The isolated lake (La3, with the highest values of conductivity and A_{250}) was linked to Cyanobacteria, which is favoured by the stability of the water column and the alkaline conditions of the lake (Unrein *et al*., 2010; Mihaljević and Stević, 2011). Though Euglenophyta showed low densities, it was only present in the isolated lake where a higher concentration of CDOM could favour these mixotrophic organisms (Reynolds, 2006; Zalocar de Domitrovich *et al*., 2007). Similarly the phagotrophic dinoflagellate *Peridinium* sp. (Pålsson and Granéli, 2003) could have been favoured in La3 by the high concentration of CDOM, which improves the growth of bacteria. On the other hand, the strong association of A_{250} with *Coelomorum* sp. possibly reflects the contribution of CDOM by this abundant cyanobacteria, because phytoplankton can provide significant amounts of CDOM through exudation or cell lysis when high densities are reached (Descy *et al*., 2002; Zhang *et al*., 2013).

CONCLUSION

In accordance with our expectations, the hydrological connectivity affected the spatial variability of CDOM concentration, which increased from the main channel to the more isolated floodplain lakes. Furthermore, results suggest that sediment resuspension of a shallow floodplain lake is linked to an increase in high-MW-CDOM whereas lake isolation from the river is linked to an increase in low-MW-CDOM. Finally, CDOM spatial variability was linked to phytoplankton structure, specially the high MW fraction which was associated with phytoflagellates. In this sense, the inclusion of CDOM analysis in floodplain studies can help to understand the prevailing phytoplankton assemblages.

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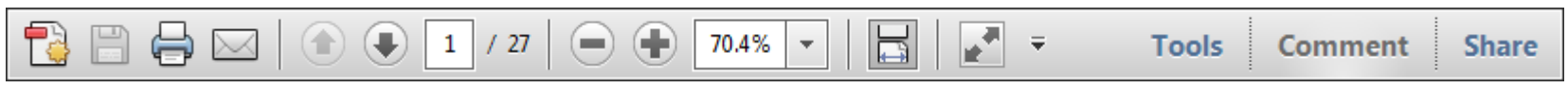

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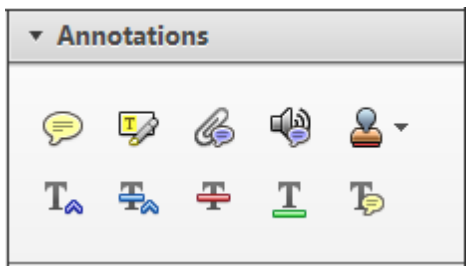

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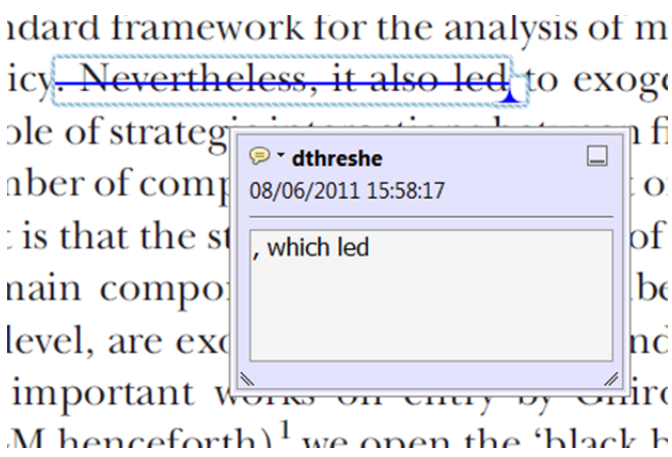
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

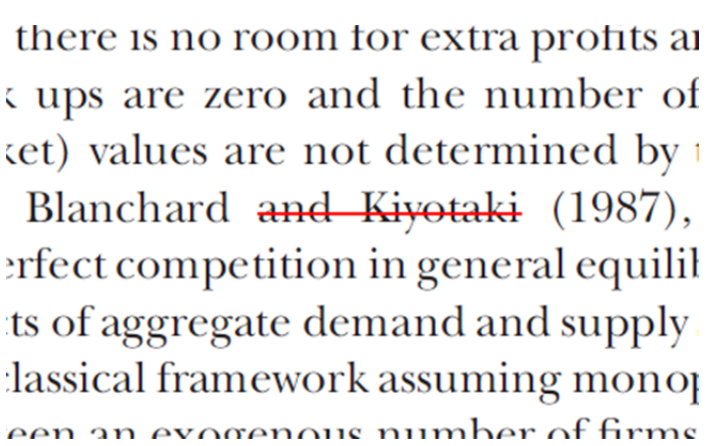


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

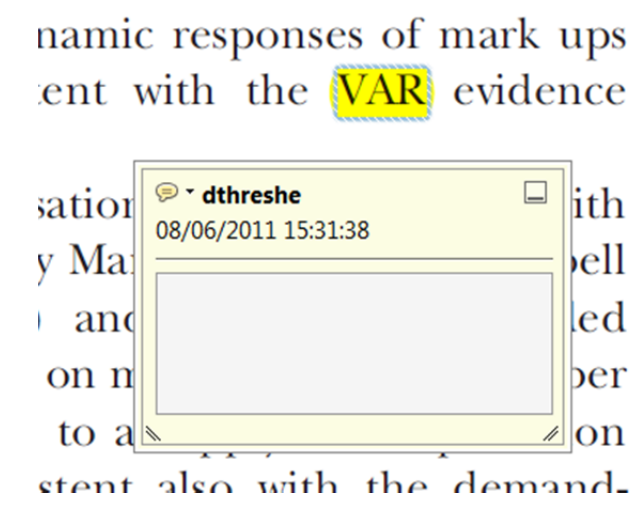


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

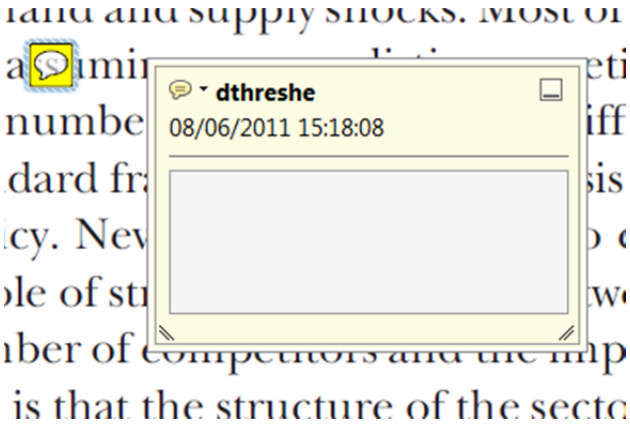


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



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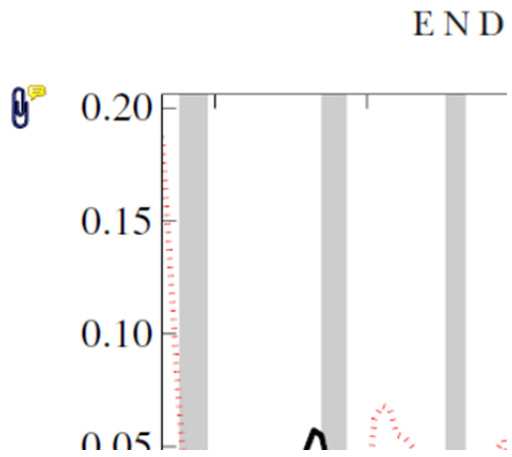
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

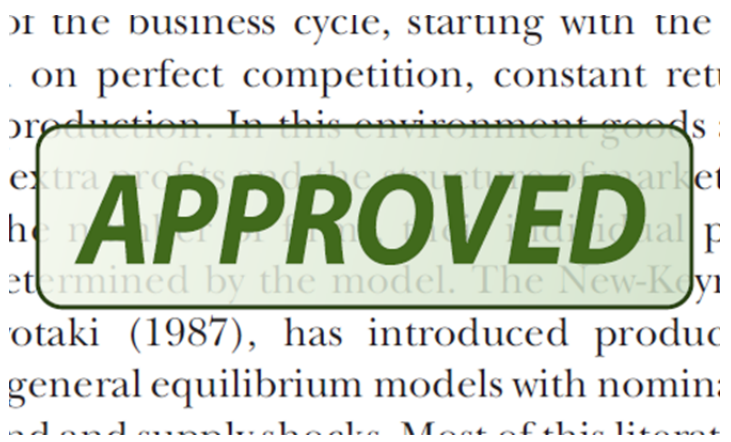


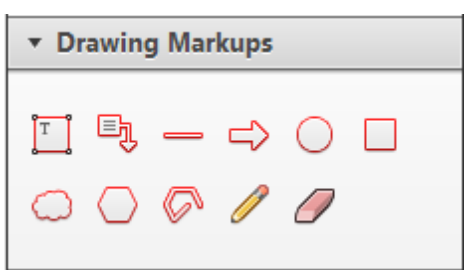
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).



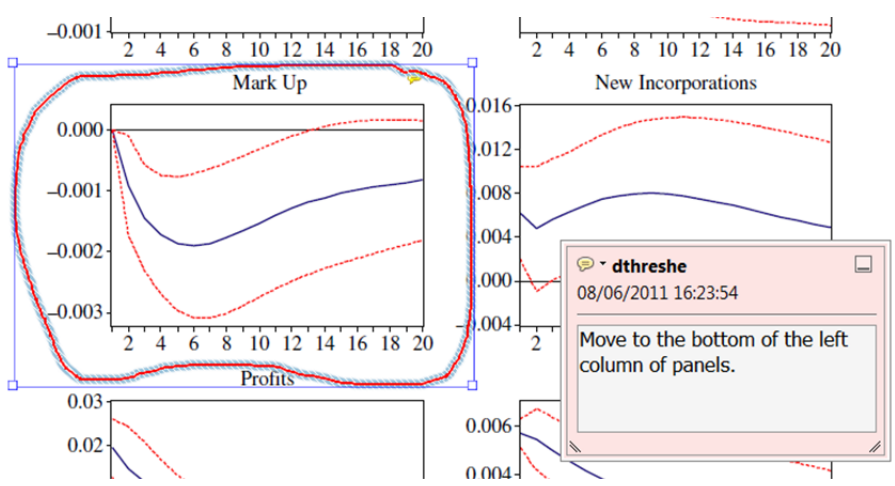


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: