

1-1-2015

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GALLO, LUANA; BATTEGAZZORE, MAURIZIO; CORAPI, ANNA; and LUCADAMO, LUCIO (2015) "Changes in epilithic diatom communities and periphytic biomass downstream of a reservoir on a Mediterranean river (Calabria region, S Italy)," *Turkish Journal of Botany*: Vol. 39: No. 3, Article 18. <https://doi.org/10.3906/bot-1403-34>

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## Changes in epilithic diatom communities and periphytic biomass downstream of a reservoir on a Mediterranean river (Calabria region, S Italy)

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Received: 14.03.2014 • Accepted: 12.12.2014 • Published Online: 04.05.2015 • Printed: 29.05.2015

**Abstract:** Diatom community structure and morphotype classification were studied and periphyton biomass, coarse particulate organic matter (CPOM), hydrogeomorphological parameters, and nutrient concentrations were measured at 4 stations downstream of the Cecita dam (Mucone River) and at 3 control sites located on different tributaries of the Mucone River in May, August, and November 2005. The lowest values of current velocities and the highest amounts of sand deposition and CPOM accumulation were detected at the stations closest to the dam. Downstream, the confluence with the first permanent tributary (Cerreto River) of the Mucone showed a “rhythral” morphology, similar to that of the control sites. Multivariate analysis showed that the community was constantly segregated into 2 groups: G1 (stations upstream of the Cerreto inflow), dominated by early colonizers, adapted to both high and low current velocity and tolerating burial and light deficiencies; and G2 (all other stations), where the prevailing taxa were species typical of stable environments with high values of flow and abundance of coarse substrates. Genus-based morphotype classification failed to detect any differences between the 2 groups. Biomass levels were higher at station G2 than G1, while accumulation of CPOM downstream of the reservoir promoted fine particulate organic matter production and a switch from autotroph to heterotroph dominated biofilms.

**Key words:** River regulation, hydrology, geofluvial morphology, diatom communities, epilithic algal biomass, autotrophic index

### 1. Introduction

River impoundment and flow regulation are among the most pervasive human effects on running water ecosystems due to the multiple negative effects on hydrochemical, geomorphological, and biological components (Nichols et al., 2006). Benthic diatoms have been extensively used in monitoring mostly organic and trophic pollution (Solak and Ács, 2011). However, there is increasing evidence of their effectiveness in detecting physical disturbances of streams and rivers (Cortez et al., 2012) coherently with the differential behavior and adaptations that diatoms show towards processes such as current drag, shear stress (Stevenson, 1996), fine sediment deposition (Fore, 2010), and desiccation (van Dam et al., 1994). Reviews of the most common responses of diatom communities to flow abstraction and regulation indicated that they consisted mostly of dominance of aerophilic taxa, occurrence of long filamentous mats, increase in relative abundance of motile diatom taxa (Bradley et al., 2012), development of heterotrophic biofilm, change in species composition, and increase in periphyton biomass (Smolar-Žvanut and Mikoš, 2014). Moreover, some authors suggested the adoption of a genus-based community analysis (Growth, 1999) or

the evaluation, at genus level, of the number of motile taxa (Bahls, 1993) to detect river alterations associated to flow regulation and siltation, because such a taxonomic identification level can be particularly effective when rapid analysis is required for biomonitoring purposes. Recently, Passy (2007) and Rimet and Bouchez (2012) proposed a genus-level classification of diatoms resulting in 3 morphoecological guilds depending on their differential response to nutrient and physical disturbance gradients: low-profile (LP) taxa, including genera experiencing resource limitations while resistant to physical stress due to their adnate, prostrate, and erect morphologies; high-profile (HP) taxa, which include large and colony-forming organisms, not suffering from resource limitation but prone to grazing activity and physical disturbance; and motile (M) taxa, colonizing highly polluted and/or unstable environments.

The Mucone River is the main tributary of the Crati River, the most important watercourse of the Calabria region. It springs from Serra Stella, in the Sila Grande, and joins the Crati after approximately 43 km. The basin covers an area of 151,334 km<sup>2</sup> and a perimeter measuring 83,719 km. Geological substrates consist mainly of granites and

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granodiorites, phyllites, marbles, quartzites, acid granulates, and gneiss granites. Half of the basin area is dedicated to agriculture; in particular, the upper part is dedicated to the cultivation of potatoes and sowable crops, and the lower part to vineyards, olive groves, and other orchards. The climate is typically Mediterranean, with relatively cold and rainy autumns and winters, cool springs with less intense rainfalls, and hot dry summers. Mucone damming dates back to the mid-1950s and consists of the construction of the Cecita reservoir, with a capacity of  $108 \times 10^6 \text{ m}^3$ , which is used for irrigation and hydroelectric power purposes. Releases take place downstream of the generators, which are located at altitudes of 480 and 198 m above sea level, respectively. An extremely reduced amount of water is released directly downstream of the dam resulting in a strong flow regulation, while larger releases occur twice a year to check the functioning of the floodgates. The first permanent tributary that joins the Mucone downstream of the dam is the Cerreto River, which enters the main course 3.2 km from the reservoir. Riparian vegetation consists primarily of alder trees (*Alnus glutinosa*) that in proximity of the dam appear to suffer a moderate thinning out.

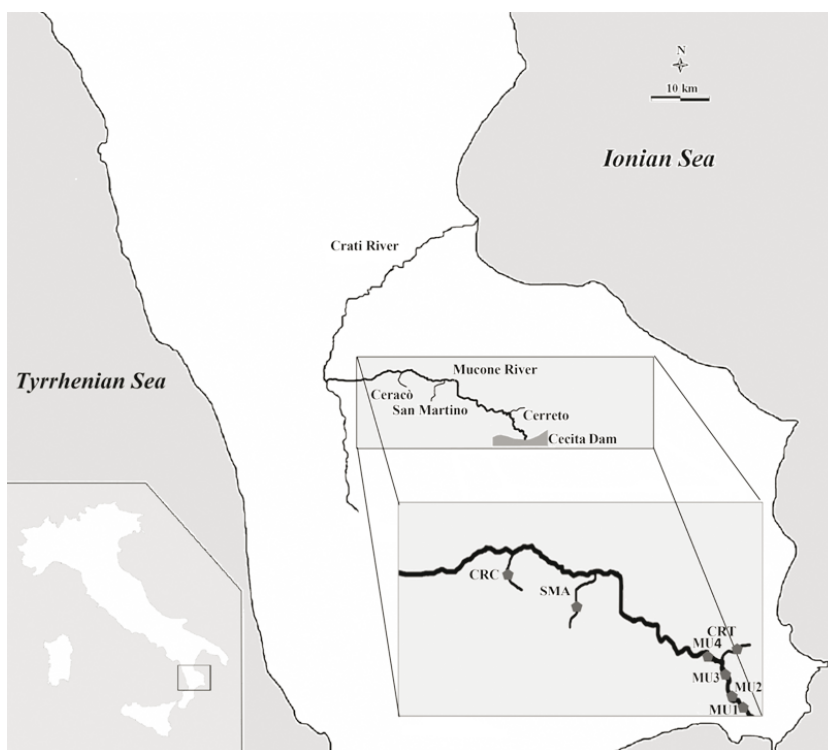
The aims of this work were the following: a) evaluation of the impact of river regulation through a comparison of river sites of the (regulated) downstream reservoir and upstream confluence of the first permanent tributary with the control sites, b) evaluation of river recovery through

a comparison of the abovementioned sites (regulated and control) with a site located downstream of the inflow of the first permanent tributary, and c) evaluation of the effectiveness of a genus-based morphotype index in detecting hydrogeomorphological differences between regulated and unregulated (control + site downstream of the Cerreto inflow) river segments.

The experimental design consisted of 4 stations located downstream of a multiple-use reservoir, one of which was approximately 700 m below the inflow of the first permanent tributary, and 3 control sites, each located on a different tributary, to be sampled in May, August, and November 2005. Diatom community structure, morphoecological guilds, periphyton biomass, and hydrochemical and geomorphological parameters were evaluated on each sampling date to test the abovementioned hypotheses.

## 2. Materials and methods

Four stations were located downstream of the Cecita dam. Another 3 stations, designated as control sites, were located on different tributaries of the Mucone: the Cerreto, S Martino, and Ceracò rivers (Figure 1). The stations on the tributaries were considered as control sites because of their similar altitude and rhythron morphologies compared to the Mucone downstream of the reservoir and the lack of human pressures. Table 1 gives the geographical descriptors of these stations.



**Figure 1.** Mucone River downstream of the Cecita dam and the affluents Cerreto, Ceracò, and S Martino. Monitoring sites are denoted by the following codes: MU: Mucone, CRT: Cerreto, SMA: S Martino, CRC: Ceracò.

**Table 1.** Altitude and geographical coordinates of the monitoring sites. MU: Mucone, MU1 = 0.6 km, MU2 = 0.9 km, MU3 = 3.1 km, MU4 = 4 km. CRT: Cerreto, SMA: S Martino, CRC: Ceracò.

Stations	MU1	MU2	MU3	MU4	CRT	SMA	CRC
Altitude (m a.s.l.)	1097	1077	977	960	977	905	920
Geographical coordinates	39°24'19.8"N 16°32'03.0"E	39°24'14.3"N 16°31'59.9"E	39°24'58.7"N 16°31'16.4"E	39°25'16.3"N 16°31'02.7"E	39°24'43.6"N 16°31'08.3"E	39°26'33"N 16°24'33"E	39°25'51"N 16°22'16"E

All sample collections and field measurements were conducted in riffle environments. Current velocity was measured at a height of 3 cm from the riverbed in 3 different riffles, using a General Oceanics flowmeter (Mod. 2030R, General Oceanics, USA). Water samples were collected in 2-L bottles and returned to the laboratory for measuring nitrate and phosphate concentrations (APAT-CNR-IRSA, 2003). Temperature was measured with a Hanna Instruments portable probe (Mod. HI 991300, Hanna Instruments, USA). Light intensity was measured using a portable Instruments and Systems light meter (Mod. LX07, Instruments and Systems, Italy). Both substrate and woody debris were sampled and processed according to Lucadamo et al. (2012) for the evaluation of substrate typology representation (%) and the amount of coarse particulate organic matter (CPOM) (g) per surface unit (m<sup>2</sup>), respectively. Epilithic diatoms were scraped from 5 cobbles (each from a different riffle) using a hard toothbrush (Kelly et al., 1998; European Committee for Standardization, 2003; European Committee for Standardization, 2004). They were then placed in 50-mL plastic containers and preserved with 2 mL of formalin. Organic matter was eliminated by hydrogen peroxide digestion through repeated rinses with distilled water and mounted in Naphrax resin. Diatom community analysis was undertaken with an Axioscope-Zeiss light microscope (Carl Zeiss, Germany) at 1000× magnification. For each sample, 400–500 individuals were identified. Taxonomical identification, at least to the species level whenever possible, was made with the use of appropriate keys (Lange-Bertalot and Metzeltin, 1996; Lange-Bertalot, 2003). Morphotype classification of diatoms and the estimation of their representation were performed according to Passy (2007) and Rimet and Bouchez (2012). Periphyton biomass evaluation was performed by removing one more cobble from the same 5 riffles sampled for diatom community investigation and placing it in a 5-L dark plastic bottle containing river water. Once in the laboratory, epilithic chlorophyll *a* was measured according to Steinmann and Lamberti (1996). Multivariate analyses (Bray–Curtis, cluster analysis, and multiresponse permutation procedure) were performed on log ( $x + 1$ ) transformation of relative abundance of diatoms. Endpoints in Bray–Curtis analysis were selected according to the variance-regression

procedure to avoid their sensitivity to outliers (McCune and Grace, 2002). Student t-tests were performed on log ( $x + 1$ ) transformation of relative abundance of diatom species and log ( $x$ ) transformation of representations of morphoecological types, respectively. Exponential transformation ( $e^{0.025}$ ) was used to perform ANOVA with post hoc multiple comparison (Tukey tests) on hydrogeomorphological parameters, epilithic chlorophyll *a*, autotrophic index, and CPOM amounts. Nonparametric analysis (Spearman correlation coefficients) was performed on ranked data of the chemical and physical variables and epibenthic algae parameters (relative abundance of diatoms, chlorophyll *a* concentration, and autotrophic index). Univariate and bivariate analyses were performed using Minitab Release 13.2 (Minitab, USA), while multivariate analyses were performed with PC-ORD4 software (MJM Software Design, USA).

### 3. Results

The results for the Ceracò station in November are missing, because it was not possible to reach the monitoring site due to the high amount of snow.

Table 2 shows the results for nutrients and hydrogeomorphological parameters.

The highest concentration values of NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> were measured at the S Martino and MU3 stations, respectively. Both anions displayed comparable levels in May and August, while in November nitrate significantly increased and phosphate decreased.

In August, stations MU1 and MU2 constantly showed the lowest values of current velocity as well as the strongest decrease (-60% and -66%), followed by a slight recovery. The other stations displayed the same trend of reduction in August and recovery in November, except for MU3, which showed a further, very weak decrease (-2.77%) in the third sampling campaign. In May, the only statistically significant difference was between MU2 and Ceracò, which, on this occasion, showed the highest values of current velocity of the entire study. In August and November, MU1 and MU2 differed significantly from all the other stations. Sand % constantly increased at the MU2 station, while at MU1 the August–November variation was very slight (1.63%). The other stations constantly showed different trends and never reached an appreciable amount of sand content, except

**Table 2.** Nutrient concentrations and values of hydrogeomorphological parameters measured at monitoring sites in the 3 sampling campaigns. \*November samplings at the Cerreto site are absent because access to the site was not possible due to high amounts of snowfall.

Stations		NO <sub>3</sub> <sup>-</sup> (mg/L)	PO <sub>4</sub> <sup>3-</sup> (mg/L)	Current velocity	% Sand (S)	% Pebble (P) + cobble (C)	S/P + C
MU1	May	0.123	0.006	0.46	13.718	67.657	0.928
	August	0.066	0.008	0.184	32.26	42.508	0.816
	November	0.249	0.005	0.202	32.787	39.879	1.051
MU2	May	0.164	0.010	0.404	14.151	73.282	0.196
	August	0.047	0.009	0.136	29.858	47.427	1.229
	November	0.205	0.005	0.207	46.33	14.894	7.931
MU3	May	0.194	0.017	0.728	14.614	70.939	0.246
	August	0.179	0.016	0.512	11.318	68.29	0.168
	November	0.815	0.009	0.498	11.415	61.871	0.236
MU4	May	0.152	0.015	0.651	7.465	76.949	0.105
	August	0.121	0.008	0.553	7.532	67.559	0.151
	November	0.551	0.006	0.71	4.806	81.558	0.069
CRT	May	0.102	0.012	0.977	5.859	85.477	0.072
	August	0.128	0.012	0.471	8.574	74.501	0.124
	November	0.223	0.006	0.598	3.522	83.284	0.044
SMA	May	0.671	0.013	0.794	9.16	86.052	0.107
	August	0.590	0.012	0.543	10.349	66.205	0.16
	November	1.378	0.009	0.808	11.244	65.481	0.202
CRC	May	0.078	0.016	1.19	18.935	83.53	0.224
	August	0.109	0.012	0.587	20.906	59.927	0.291
	November*	---	---	---	---	---	---

Ceracò. MU1 and MU2 displayed significant difference with MU4 in August and MU4 and Cerreto in November. The highest values of S/P + C (which can be considered an estimation of burial of coarse substrates) were measured at the MU1 and MU2 stations in November, with the latter resulting in a significant difference from the other stations. MU4, Ceracò, and S Martino peaked in August when MU3 showed the lowest S/P + C value. The percentage of pebble + cobble fraction resulted in an opposite trend to that of % sand at MU1 and MU2. On the other hand, the representation of coarse substrates varied in a comparable way in the cases of MU4 and Cerreto (lowest value in August), but were different for MU3 and S Martino (Table 2). Only MU2 in November differed significantly from the other stations.

Thirty-seven genera and 97 species were identified and are listed in Table 3. Relative abundance and, for 86 species, groupings into morphoecological guilds (Passy, 2007; Domaizon, 2012) are also shown. Thirty species were attributed to the low-profile guild (LPG), 21 to the high-profile guild (HPG), and 35 to the motile guild (MG).

However, when calculated as % of organisms, the first guild was the most represented (66.98%), followed by the MG (21.19%) and HPG (11.83%).

Figures 2 and 3 show the ordination diagram and dendrogram resulting from the application of Bray–Curtis and cluster analyses on the dataset “diatoms × samples”, respectively (including only taxa with a representation higher than 2%).

The first 2 axes of Bray–Curtis ordination extracted 34.66% and 17.99% of the original distance matrix, respectively. The strongest segregation between samples took place along the first axis, where all the samples of the stations situated upstream of the inflow of the Cerreto stream clearly separated from the other samples, namely the MU4 station (downstream of the Cerreto inflow) and the 3 control sites. Ordination along the second axis separated the August Ceracò sample and, to some extent, the MU1 sample from the remaining ones. Stations did not show any clear segregation according to sampling dates; in fact, the main ordination criterion seemed to be their spatial placement.

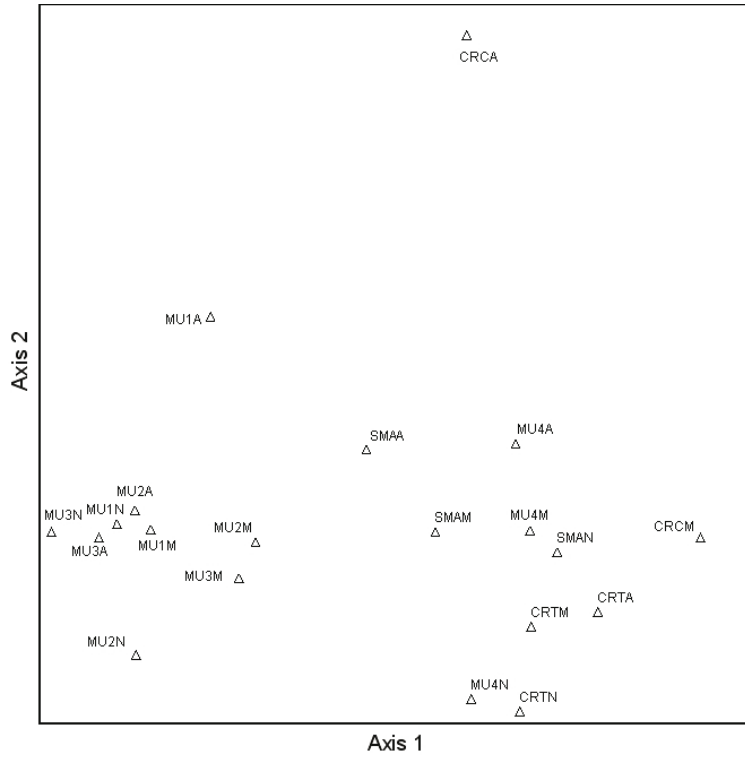
**Table 3.** Taxonomic list of diatoms collected during the 3 sampling campaigns (M: May, A: August, N: November 2005) and relative attribution to morphoecological guilds (G). LP: low-profile type, HP: high-profile type, M: motile type, CRT: Cerreto, SMA: S Martino, CRC: Ceracò.

Species	G	MU1M	MU1A	MU1N	MU2M	MU2A	MU2N	MU3M	MU3A	MU3N	MU4M	MU4A	MU4N	CRTM	CRTA	CRTN	SMAM	SMAA	SMAN	CRM	CRC	CRA
<i>Achnanthes laevis</i> Oestrup var. <i>quadriarea</i> (Oestrup) Lange-Bertalot	LP	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. parvula</i> Kützing	LP	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. stollia</i> (Kraske) Kraske	LP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Achnanthisium daonense</i> (Lange-Bertalot) Lange-Bertalot Monnier & Ector	LP	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. minutissimum</i> (Kützing) Czarnecki	LP	179	217	125	101	67	108	156	92	137	136	89	81	46	117	9	51	16	8	57	66	66
<i>A. pyrenaicum</i> (Hustedt) Kobayasi	LP	0	0	0	1	0	2	0	0	7	87	38	101	64	33	78	21	31	79	3	0	0
<i>A. subatomoides</i> (Hustedt) Monnier, Lange-Bertalot & Ector	LP	0	0	11	1	16	0	3	0	10	0	0	0	0	0	1	0	0	0	0	0	0
<i>Adlafia bryophila</i> (Petersen) Moser Lange-Bertalot & Metzeltin	M	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. langebertalotii</i> Monnier & Ector	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	180	66	0	14	14
<i>Amphora inarionis</i> Krammer	LP	3	3	0	0	0	0	0	2	0	7	6	4	1	1	4	20	10	18	23	0	23
<i>A. montana</i> Kraske	LP	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. pediculus</i> (Kützing) Grunow	LP	3	0	2	0	0	6	2	0	0	0	21	0	3	0	1	6	11	5	0	7	7
<i>Cocconeis disculus</i> (Schumann) Cleve in Cleve & Jentzsch	LP	0	0	0	6	0	2	21	44	5	2	2	4	0	1	5	0	10	0	0	0	0
<i>C. egyptia</i> Ehrenberg	LP	81	65	37	7	88	45	7	127	82	7	17	27	37	5	30	73	14	6	0	2	2
<i>C. placentalis</i> Ehrenberg var. <i>lineata</i> (Ehr.) Van Heurck	LP	19	0	16	0	3	27	28	47	51	0	0	22	13	1	16	8	7	16	0	0	0
<i>C. placentalis</i> Ehrenberg var. <i>pseudolunata</i> Gattler	LP	0	7	3	0	0	0	0	2	2	2	21	25	108	8	201	35	13	60	61	19	19
<i>Cymbella affinis</i> Kützing var. <i>proera</i> Krammer	LP	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diadsmis contenta</i> (Grunow ex V. Heurck) Mann	HP	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>D. perpusilla</i> (Grunow) D.G. Mann in Round et al.	HP	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diatoma hyemalis</i> (Roth) Heiberg var. <i>hyemalis</i>	HP	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
<i>D. mesodon</i> (Ehrenberg) Kützing	HP	0	0	0	0	0	0	0	0	0	0	15	1	0	3	1	0	2	0	3	3	3
<i>Diploneis oblongella</i> (Näegeli) Cleve-Euler	LP	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>D. puella</i> (Schumann) Cleve	LP	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula tenuis</i> Kützing	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Encyonema mesianum</i> (Cholnoky) D.G. Mann	LP	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>E. minutum</i> (Hilse in Rabh.) D.G. Mann	LP	0	0	0	3	15	0	4	1	0	0	3	0	0	14	0	6	3	6	0	0	0
<i>E. retchardii</i> (Krammer) D.G. Mann	LP	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0
<i>E. silisianum</i> (Bleisch in Rabh.) D.G. Mann	LP	0	0	0	4	4	0	1	0	0	0	0	0	0	3	0	0	0	1	0	0	0
<i>Eucoconeis flexella</i> (Kützing) Brun	LP	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>E. laevis</i> (Oestrup) Lange-Bertalot	LP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

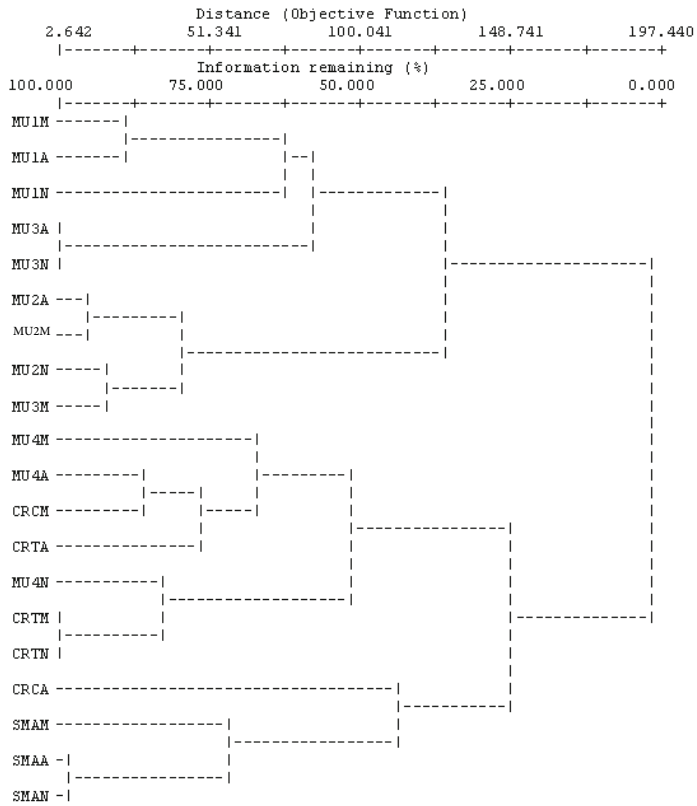








**Figure 2.** Ordination diagram that resulted by performing Bray–Curtis analysis on the dataset: sampling dates × diatom species with a representation higher than 2%. M: May, A: August, N: November.



**Figure 3.** Dendrogram that resulted by performing cluster analysis on the dataset: sampling dates × diatom species with a representation higher than 2%. M: May, A: August, N: November.

Percent chaining associated to the cluster analysis dendrogram was relatively low (1.65%), supporting the idea that cluster components had a good degree of similarity. The classification analysis gave a result that highly overlapped with the result of the Bray–Curtis analysis. In fact, the Mucone stations placed upstream of the Cerreto tributary were segregated once more from the MU4 station and the control sites.

Multiresponse permutation procedure (MRPP) was performed to test the null hypothesis of no dissimilarity between the 2 groups emerging from the application of the Bray–Curtis and cluster analyses. The first was assigned the code G1 and the second the code G2. MRPP resulted in such a value of T (-8.6691) that we could refute the null hypothesis with an extremely low probability of error (P = 0.00000132). Moreover, the A value (the chance corrected within group agreement) was 0.0946, in agreement with the idea that the observed effect (independently of the size of the samples) had an appreciable size.

For each of the 2 groups, the averages of the relative abundance of the 15 diatomic taxa (>2%) and representation of ecological morphotypes were calculated and compared to test the difference between G1 and G2. *Sellaphora stroemi* and *Adlafia langebertalotii* (a recently described species; see Monnier et al., 2012) were never detected in the G1 stations; neither did they seem to characterize the G2 community. In fact, the former was detected only at the Ceracò station in August, and the latter at the S Martino station in all samples and at the Ceracò station in August. Relative abundances

of *Achnantheidium minutissimum*, *Cocconeis euglypta*, and *Sellaphora minima* were significantly higher (P < 0.05) in G1 than in G2, while the reverse was true for *Achnantheidium pyrenaicum*, *Cocconeis placentula* var. *pseudolineata*, and *Navicula lanceolata* (P < 0.05).

No statistically significant differences were detected between the representation of the morphoecological guilds of G1 and G2.

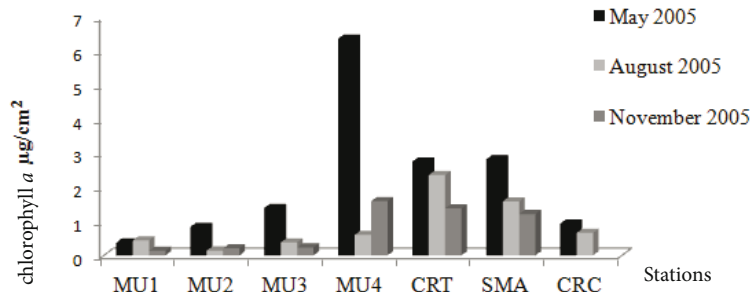
Table 4 shows the light intensity measured in May and August. November data are missing due to malfunctioning of the luximeter. The highest values were registered in May, before canopy closure, except for MU1 and MU2. At these stations, especially MU1, riparian vegetation showed a thinning out as a consequence of clear cutting, and so a higher amount of light reached the riverbed in August than in May.

Figure 4 and Table 5 display the concentration of epilithic chlorophyll *a* and the autotrophic index with the amount of CPOM collected, respectively.

The highest levels of chlorophyll were measured in May at all stations with M4 showing the highest value (P < 0.0005). The stations located upstream of the Cerreto inflow (G1 group) showed constantly low values of chlorophyll *a* in August and November. Appreciable levels of chlorophyll *a* were detected at the Cerreto station in August (significantly different from all the other stations except for S Martino; P < 0.0005), while the MU4, S Martino, and Cerreto stations differed significantly from the MU1, MU2, and MU3 stations in November.

**Table 4.** Light intensity values (lx) measured in the sampling stations. The November data are missing due to malfunctioning of the luximeter.

	MU1	MU2	MU3	MU4	CRT	SMA	CRC
May	705	905	1579	2418	2372	1860	5069
August	3466	1046	1148	579	509	373	2056
November	---	---	---	---	---	---	---



**Figure 4.** Average values of the concentrations of epilithic chlorophyll *a* measured at the monitoring sites on each of the sampling dates.

**Table 5.** Average values of the autotrophic index and amount of CPOM measured at the monitoring sites on each of the sampling dates.

		MU1	MU2	MU3	MU4	CRT	SMA	CRC
Autotrophic index	May	0.00503	0.00597	0.01617	0.02579	0.01515	0.01093	0.01240
	August	0.00299	0.00111	0.00476	0.00556	0.01552	0.01209	0.00754
	November	0.00196	0.00252	0.00403	0.01068	0.01642	0.00982	---
CPOM	May	32.4083	26.4139	0.63096	0.13310	0.79081	0.98479	0.67278
	August	12.4337	10.6696	1.28366	5.12366	2.43436	4.04151	2.74976
	November	22.9575	14.3222	9.31261	23.8353	15.5737	17.3206	---

MU1, MU2, and MU3 showed very low values of autotrophic index, except for MU3 in May. However, only the first two (MU1 and MU2) differed significantly from MU4 and the control sites in May ( $P < 0.0005$ ) and August, while the only significant difference in November was between MU2 and Cerreto ( $P < 0.0005$ ).

The highest amounts of CPOM were collected in MU1 and MU2 in May, when all their comparisons with other stations resulted in significant differences ( $P < 0.0005$ ). In August, a marked reduction of CPOM in MU1 and MU2 was evident and showed a significantly different trend compared to the MU3 and Cerreto stations ( $P < 0.01$ ). In November, all stations showed an appreciable and comparable amount of CPOM confirmed by a negative Tukey test ( $P > 0.05$ ).

Table 6 illustrates the results of correlation analysis between relative abundance of diatom species with a representation higher than 2% and hydrochemical and geomorphological parameters, CPOM, and temperature. Table 7 shows the results of correlation analysis between chlorophyll *a* and autotrophic index and the same abiotic parameters. The abundance of all the species that displayed significant differences between the 2 groups that emerged from multivariate analysis showed statistically significant correlations with geomorphological variables, except for *Cymbella euglypta*, *Achnanthydium pyrenaicum*, *Cocconeis placentula* var. *pseudolineata*, and *Navicula lanceolata* all negatively correlated with sand percentage and burial of coarse substrate, while only *Achnanthydium pyrenaicum* and *Cymbella euglypta* var. *pseudolineata* showed a positive correlation with current velocity. Among the characteristic species of group G1, *Sellaphora minima* displayed negative correlation with current velocity and coarse substrate and positive correlation with sand percentage and the ratio S/P + C. *Achnanthydium minutissimum* correlated negatively only with current velocity. Both epilithic chlorophyll *a* and autotrophic index (Table 6) negatively correlated with sand %, burial of coarse substrate, and CPOM and positively correlated with pebble % + cobble % and current velocity.

Only the autotrophic index showed a positive correlation with phosphate.

#### 4. Discussion

During the experimental period, nitrate levels probably depended mainly on the breakdown of organic matter (CPOM) deposited in the riverbed. In May, the  $\text{NO}_3^-$  concentrations were quite similar in all stations despite the higher amount of CPOM detected at MU1 and MU2. However, the litter present in spring mostly consists of nitrogen compounds linked to lignin and cellulose (Allan and Castillo, 2007), recalcitrant to decomposition and rich in scarcely soluble proteins (Bärlocher, 1983), thus determining a poorly efficient nitrogen mineralization process. In August, the increase in average temperature stimulates microbial reproduction and metabolism (Abelho et al., 2005), which causes both nitrogen incorporation in microbial biomass and a reduction in water nitrate concentration. In November, the increase in CPOM due to litter deposition coincides with a general increase in  $\text{NO}_3^-$  levels, suggesting that the accumulation and decomposition of biodegradable organic matter results in an excess of mineral nitrogen in relation to microbial requests (Zeller et al., 2001). Due to the prevailing igneous and metamorphic nature of the rocks of the Mucone watershed,  $\text{PO}_4^{3-}$  concentrations are low. The lowest phosphate concentrations in the Mucone watershed were detected in November, coinciding with the highest amount of rainfall during the study period, in agreement with the hypothesis that the run-off taking place on scarcely erodible rocks results in the dilution of river network phosphate concentration (Lewis and Saunders, 1990).

Despite flow standardization, it was only since August that the differences in current velocity between the stations closest to the dam and the other monitoring sites were highest. The prolonged lack of rainfall together with flow abatement resulted in a marked drop of current velocity and sand deposition. Interestingly, at the second site downstream of the dam (900 m), an increasing amount

**Table 6.** Correlation analysis (Spearman) between variation of relative abundance of diatom species with a representation higher than 2% and hydrochemical and geomorphological parameters (ADLB: *Adlafia langebaltotii*; ADMI: *Achnanthydium minutissimum*; ADPY: *Achnanthydium pyrenaicum*; CEUG: *Cocconeis euglypta*; CPLI: *Cocconeis placentula* var. *lineata*; CPPL: *Cocconeis placentula* var. *pseudo lineata*; FPRU: *Fragilaria pararumpens*; GPUM: *Gomphonema pumilum*; NGRE: *Navicula gregaria*; NLAN: *Navicula lanceolata*; PTLA: *Planorhynchium lanceolatum*; RABB: *Roicosphenia abbreviata*; RSIN: *Reimeria sinuata*; SEMN: *Sellaphora minima*; SSTM: *Sellaphora stroemi*).

	% Sand		% P + C		S / P + C		Current velocity		NO <sub>3</sub> <sup>-</sup>		PO <sub>4</sub> <sup>3-</sup>		Temperature (°C)		CPOM	
	R	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
ADLB	0.112	0.639	-0.06	0.815	0.103	0.665	0.413	0.070	0.003	0.989	0.311	0.181	0.085	0.722	-0.16	0.509
ADMI	0.398	0.082	-0.35	0.126	0.382	0.097	-0.54	0.014	-0.008	0.975	-0.20	0.407	0.051	0.830	0.146	0.539
ADPY	-0.72	0.000	0.579	0.007	-0.68	0.001	0.714	0.000	0.017	0.944	0.204	0.387	-0.045	0.852	-0.35	0.134
CEUG	0.343	0.139	-0.43	0.056	0.414	0.070	-0.37	0.112	-0.09	0.698	-0.42	0.068	-0.25	0.288	0.388	0.091
CPLI	0.214	0.364	-0.18	0.455	0.255	0.278	0.013	0.957	0.269	0.252	-0.16	0.509	-0.49	0.031	0.132	0.579
CPPL	-0.62	0.004	0.422	0.064	-0.62	0.004	0.603	0.005	0.157	0.509	-0.02	0.939	-0.21	0.368	-0.12	0.615
FPRU	0.131	0.582	-0.13	0.593	0.214	0.365	-0.24	0.300	0.009	0.07	-0.02	0.929	0.189	0.424	0.216	0.361
GPUM	-0.15	0.516	0.207	0.382	-0.14	0.544	0.151	0.525	-0.38	0.101	0.416	0.068	0.406	0.076	-0.35	0.591
NGRE	0.128	0.591	0.145	0.541	0.065	0.786	0.111	0.642	0.503	0.024	0.050	0.834	-0.36	0.117	0.108	0.651
NLAN	-0.63	0.003	0.557	0.011	-0.63	0.003	0.408	0.074	0.164	0.489	0.056	0.814	-0.31	0.182	-0.19	0.424
PTLA	0.204	0.388	-0.099	0.678	0.182	0.444	-0.15	0.537	-0.39	0.090	-0.11	0.638	0.174	0.463	-0.12	0.611
RABB	-0.14	0.569	0.064	0.789	-0.06	0.791	0.056	0.813	0.044	0.852	-0.18	0.455	-0.21	0.365	0.197	0.406
RSIN	-0.17	0.464	0.281	0.230	-0.16	0.486	0.541	0.014	0.164	0.490	0.580	0.007	0.005	0.982	-0.46	0.039
SEMN	0.585	0.007	-0.61	0.004	0.633	0.003	-0.53	0.016	-0.10	0.66	-0.20	0.388	0.116	0.626	0.204	0.388
SSTM	-0.099	0.677	-0.099	0.677	-0.099	0.677	-0.02	0.934	0.259	0.271	0.119	0.616	0.298	0.201	-0.06	0.803

**Table 7.** Correlation analysis (Spearman) between variation of chlorophyll *a*, autotrophic index, and hydrochemical and geomorphological parameters.

	% Sand		% P + C		S/P + C		Current velocity		NO <sub>3</sub> <sup>-</sup>		PO <sub>4</sub> <sup>3-</sup>		Temperature (°C) CPOM			
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Chlorophyll <i>a</i>	-0.644	0.002	0.669	0.002	-0.714	0.000	0.555	0.011	0.021	0.930	0.442	0.051	0.128	0.591	-0.474	0.035
Autotrophic index	-0.686	0.001	0.774	0.000	-0.735	0.000	0.734	0.000	0.093	0.696	0.517	0.020	-0.42	0.860	-0.528	0.017

of sand was sampled from May to November, while no changes were evident between August and November at the site nearest to the reservoir. Water release from the dam, which takes place twice a year to test the floodgates, may result in the following for the segment closest to the reservoir: channel erosion, high capacity of interspaces between coarse substrates, and a longer time to fill them than in a less disturbed segment such as the MU2 station. This could also explain why a burial of coarse substrates, which was significantly higher than at the other sites, was detected in November at the MU2 station and not at MU1. Station MU3, located immediately upstream of the Cerreto inflow, seemed to occupy an intermediate position between the MU1 and MU2 stations and the MU4 and control sites. This was indicated by the % sand, which in November was not significantly different from either the upstream stations or from the MU4 and Cerreto stations. Despite the clear seasonal progression of geomorphological gradients downstream of the Cecita reservoir, the diatomic communities displayed a constant spatial segregation between the stations located upstream of the Cerreto inflow and the MU4 station, together with the control sites. The succession of flow reactivation due to winter rains, flow abatement, and sand accumulation promoted the colonization and persistence of eurivalent taxa with a high colonization rate, tolerating both high and low current velocity as well as temporary burial of coarse substrates. *Achnanthydium minutissimum* is an early pioneer taxon capable of substrate colonization both at low (Plenkovic-Moraj et al., 2008) and high (Kelly, 2002) current velocity values, tolerating physical disturbance (Walsh and Wepener, 2009), often dominating community in sandy littoral (Cremer, 2006) and able to survive and reproduce in poorly illuminated habitats (Johnson et al., 1997). *Cocconeis euglypta* is also a pioneer taxon, although colonizing later than *Achnanthydium*, which shows adaptation to both low (Martina et al., 2013) and high (Battegazzore et al., 2004) current velocities. These taxa represented about 47% of all organisms identified at stations MU1, MU2, and MU3, and their ecological needs are well matched to the prevailing environmental conditions detected at these sites. The third G1 group is

characterized by the taxon *Sellaphora minima*, a motile diatom, not only adapted to settling in unstable substrates (Spauldin et al., 2010) but also a facultative-N heterotroph (Munn et al., 2002) coherently with the high average amount of CPOM collected at the sites mainly colonized by this species. The diatom taxa mostly contributing to the segregation of groups G2 and G1, *Achnanthydium pyrenaicum*, *Cocconeis placentula* var. *pseudolineata*, and *Navicula lanceolata*, are all taxa that are well adapted to high current velocities (Battegazzore et al., 2004). They all correlated negatively with % sand and S/P + C, and two of them (*Achnanthydium pyrenaicum* and *Cocconeis placentula* var. *pseudolineata*) correlated positively with current velocity, whereas *Achnanthydium minutissimum* correlated negatively only with current velocity. *Cocconeis euglypta* did not show any significant correlation, coherently with its persistence in environments with different characteristics.

A comparison of our results with the diatomic communities detected in other regulated rivers in Mediterranean climate river catchments in Europe (Comte and Cazaubon, 2002; Gallo et al., 2013), N Africa (Nehar et al., 2014), and Australia (Growthns and Growthns, 2001) showed both a moderate degree of sharing of taxa and a higher representation of species such as *Achnanthydium minutissimum* and *Cocconeis euglypta* downstream of dams than in unregulated segments. This suggests comparable patterns of colonization.

Our results do not support the use of a diatom genus-based index, such as that deriving from morphoecological groupings, for revealing geomorphological changes taking place in river segments. In fact, species taking part in the same genus (*Achnanthydium* and *Cocconeis*) showed marked differences between environments, clearly being unlike in current velocity and sand deposition preferences.

Epilithic chlorophyll showed a value range that suggested moderate nutrient enrichment (Tank and Dodds, 2003), and so biomass variation did not correlate with nitrate concentration. However, neither did phosphate concentration correlate with chlorophyll *a* variation despite its limiting levels (Bothwell, 1989),

suggesting that other factors result in an overriding effect on  $\text{PO}_4^{3-}$  limitation.

Epilithic chlorophyll correlated ( $P < 0.05$ ) positively with pebble + cobble fraction percentage and negatively with sand content and  $\text{S/P} + \text{C}$ , a result that points toward the abundance and diversity of coarse substrates as a factor promoting algal epibenthic colonization (Power and Stewart, 1987) and burial of riverbeds as a cause of depression of algal growth (Izagirre et al., 2009).

Current velocity also showed a direct correlation with epilithic chlorophyll, although the values measured during the experimental period at the control sites and MU4 sometimes indicated that it probably exceeded the stress threshold (Horner et al., 1990) in May and November, particularly at the Ceracò site, where the appreciable amount of sand could result in a strong scouring of periphytic biomass (Blinn and Cole, 1991). Lightening of the riverbed was controlled both by canopy closure–opening cycles and by sand deposition. The former was dominant in May, when the flow was still quite intense; in August, however, when the canopy closes and the flow markedly drops, the effects of the two factors were additive and depressed microalgal biomass. Light intensity was not measured in November, yet it is reasonable to suppose that solar radiation was lower than in August (Burgess, 2009), although the defoliation of the canopy makes the riverbed more exposed to the incoming light. As a consequence, while the epilithic chlorophyll remained very low at MU1 and MU2 due to substrate burial, appreciable amounts of epibenthic algal biomass were detected at the Cerreto, MU4, and S Martino stations.

The autotrophic index was calculated according to the formula proposed by Barbour et al. (1999). Interestingly, the ratio on the basis of sampling dates was always lowest at the MU1 and MU2 stations, dropping on 5 out of 6 relative sampling dates below the threshold and indicating a switch of community dominance from autotrophs to heterotrophs (Weitzel, 1979). Such a result is further supported by the negative correlation between the autotrophic index and the variation in CPOM levels. In fact, it is known that woody debris decomposition generates the production of fine particulate organic matter (FPOM) (Allan and Castillo, 2007), which in river segments with a high density

of retention structures, such as in this study, is quickly trapped by developing biofilms (Webster et al., 1999) and favors their heterotrophic components. The autotrophic index, as opposed to epilithic chlorophyll, showed a significant positive correlation with phosphate. However, this does not modify the interpretation regarding the lack of correlation between  $\text{PO}_4^{3-}$  and microalgal biomass, suggesting that only the amount of chlorophyll per unit of ash-free dry matter significantly correlates (a datum also depending on the variation of the denominator), and not its quantity per surface unit.

In conclusion, our work showed that the diatomic community is permanently dominated by early colonizer species, tolerating both low and high current velocities as well as a high percentage of sand and irradiance deficiencies in the river segment downstream of the Cecita reservoir and upstream of the inflow of the first permanent tributary. There, the interaction between flow regulation and alternation of wet (autumn–winter) and dry (spring–summer) periods, typical of Mediterranean areas, results in an exacerbation of the natural succession of hydrogeomorphological conditions, in agreement with the serial discontinuity concept of Ward and Stanford (1983). A genus-based index, such as that conceived by grouping different morphotypes and characterizing the hydrological and morphological distinctions between river segments, failed to reveal them in our study.

Marked current velocity abatement promotes CPOM accumulation and the burial of coarse substrates so that both epibenthic algal biomass is depressed and a switch of dominance from autotrophs to heterotrophs in epilithic biofilms takes place, presumably as a consequence of FPOM enrichment generated by CPOM decomposition (Sobczak, 1996). Phosphate limitation does not seem to affect chlorophyll *a* changes due to the overriding effect of hydrogeomorphological parameters. Downstream of the inflow of the first permanent tributary, the hydrogeomorphological characteristics, woody debris amounts, diatom community structure, and periphyton biomass levels of the Mucone River became similar to those of the control sites as a consequence of the improved hydrological conditions (Gloss et al., 2001).

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