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Mediterranean rivers: Consequences of water scarcity on benthic algal chlorophyll a content

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ABSTRACT

Mediterranean rivers are subjected to strong seasonality with drought during the hot season and extreme flows in autumn-winter. In particular, drought episodes and water scarcity alter the river morphology, with repercussions on primary production and the trophic chain. In this paper, we aimed at analysing the different responses in terms of chlorophyll *a* content of the three main photosynthetic groups composing stream periphyton, namely diatoms, cyanobacteria and green algae. This work was conducted in the Ligurian Alps (NW-Italy) on five oligotrophic streams (Argentina, Impero, Merula, Quiliano, and Vallecrosia), similar in terms of physico-chemical parameters. We measured chlorophyll *a* content of diatoms, cyanobacteria and green algae by means of an in situ fluorimetric probe (BenthoTorch®). Data were collected from April to October 2014 in: i) impacted sites, where the water scarcity was exacerbated by human pressure; ii) control sites. We applied Generalized Linear Mixed Models to investigate the response of total chlorophyll *a* and its relative proportions among the three algal groups in relation to the following environmental predictors: water depth, flow velocity, canopy shading, microhabitat isolation, sampling season, dissolved oxygen, temperature, pH, nutrients, and macrophyte coverage. Results showed an opposite response of diatoms and green algae. Diatoms were favoured in the control sites and under moderate flow conditions, while the probability of green algae presence was higher in the impacted sites and during the drought season. Cyanobacteria showed a response similar to green algae, preferring warm, isolated pools typical of the drought period. Diatoms proved to be the most sensitive to drought. More specifically, we found out that percentages of diatoms below 51% with respect to total benthic chlorophyll *a* indicate high hydrological disturbance. This study provides the first evidence that the proportion of chlorophyll *a* produced by diatoms can be a suitable indicator for monitoring programs aiming at determining the effects of water scarcity on river ecosystems.

Key word: Diatoms; algal biomass; BenthoTorch®; biomonitoring; drought; GLMM.

INTRODUCTION

Hydrological disturbance affects river physicochemical and morphological features, when hydrological seasonality co-occurs with anthropogenic modifications (*e.g.,* eutrophication, habitat alteration). The intermittency in water flow, defined by McDonough *et al.* (2011) as the lack of flowing surface during some portion of the year, may cause a decline in discharge or even a total drying of the river channel. Therefore, a strong alteration of the underlying structure of freshwater foodweb (Barthès *et al.*, 2015), with consequences on water quality and morphological features, is expected (Stevenson, 1996; Boix *et al.,* 2010). Drying up of the riverbed causes fragmentation of longitudinal, lateral and vertical connectivity, while deepest pools may persist and become isolated from the main course. As a result, Mediterranean streams are characterized by marked spatial and temporal heterogeneity (Lake, 2000), which may cause severe consequences on structure and functionality of biotic communities, including autotrophic organisms. Indeed, the Mediterranean climate is characterized by seasonality and variability of rainfalls, with dry summers and rainy autumns and winters. As a consequence, Mediterranean rivers experience recurring hydrological disturbances since extreme episodes (*e.g.,* floods, droughts) are part of their cyclic temporal pattern, with droughts that develop continuously and gradually over summer, followed by sudden floods in autumn (Gasith and Resh, 1999; Sabater *et al.*, 2006).

Benthic algae and cyanobacteria represent the most important primary producers in riverine ecosystems (Vannote *et al.*, 1980). They significantly contribute to the hydrological, physical and biogeochemical processes in running waters, playing an important role in providing habitats for small invertebrates and participating in the transformation of dissolved organic matter (Barthès *et al.*, 2015). Water scarcity and drought represent major constraints for biofilm in an increasing number of aquatic ecosystems, becoming a central concern in a context of climate change (Barthès *et al.*, 2015). Several works demonstrated that the hydrological disturbance could directly or indirectly alter the biofilm species composition (Boix *et al.*, 2010; Tornés and Rhuì, 2013). Cyanobacteria are considered as better adapted to desiccation than diatoms notably thanks to the production

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of mucilage (Romaní *et al.*, 2012). Variation of flow also causes changes in photosynthetic pigments (*i.e.,* the production of protective carotenoids), and occurrence of cell resistance structures (*i.e.,* spores; Timoner *et al.*, 2014). High nutrient concentration and light intensity may cause an increase in biomass during stable hydrological regimes (von Schiller *et al.*, 2008). However, in Mediterranean rivers, biomass of photosynthetic organisms is highly related to seasonal variations in river discharge, which can strongly affect water temperature, light and nutrient availability (Guasch *et al.*, 1995). Moreover, according to Dallas (2013), marked spatial heterogeneity may contribute to local differentiation of river biotic communities. Concerning diatoms, Smucker and Vis (2010) observed differences in terms of species composition between different microhabitats. As a consequence, we may expect differences also in terms of algal biomass among microhabitats, especially in Mediterranean rivers, where during summer spatial heterogeneity is exacerbated. Several works examined the physiological response of photosynthetic organisms to desiccation (Caramujo *et al.*, 2008; Timoner *et al.*, 2014), or focused on the response of algal biomass to variation of light and nutrients in Mediterranean streams (Sabater *et al.*, 2000, 2011; Veerart *et al.*, 2008; Tornés and Sabater, 2010). However, in very few cases a comprehensive examination of factors affecting the algal biomass of phototrophic communities in Mediterranean streams was performed (Riseng *et al.*, 2004; Sabater *et al.*, 2008; Urrea-Clos *et al.,* 2014). Moreover, a specific analysis on the different response of the main groups composing photobiota in streams is still missing. Thus, it is presently unclear how environmental parameters affected by hydrological variability induce significant variations on primary production, especially on the relative proportion of the three main groups that constitute the autotrophic biofilm, namely diatoms, cyanobacteria and green algae.

In this study, we aim at analysing the different response to environmental parameters, in terms of benthic chlorophyll *a* concentration (chl a), assumed as a proxy of algal biomass. Our hypotheses are: i) hydrological variability influences water quality and plays a main role in determining the biomass of diatoms, cyanobacteria and green algae in Mediterranean streams during the dry season; ii) biomass of diatoms, cyanobacteria and green algae show different responses to local variations of environmental parameters so the relative proportions of the three groups can be altered. We evaluated the chl a of the three main photosynthetic groups of stream periphyton under different levels of hydrological disturbance. In particular, we applied regression models to investigate their relationship with environmental features during flow intermittency in Mediterranean streams.

METHODS

Site description

This study was conducted in five streams of Liguria (NW-Italy), belonging to the same HER (122, Ligurian Alps) in the Mediterranean region. All five study streams are comparable in terms of geology (mostly calcareous), climate and altitude, substratum size (mainly cobbles and pebbles) and water quality. We selected sites classified at least as "good" (DM 260/2010), thus guaranteeing low interference on algal biomass data. All streams are permanent in the upper part of their course, but become temporary next to the mouth in the Ligurian Sea (Fig. 1).

Sampling design

We performed eight sampling campaigns from April to October 2014: the first one during spring (04/17), with moderate flow; the other seven campaigns were performed approximately every 15-20 days from the end of June to the end of October, before the first flood event and covering the entire drought period (summer: 06/30, 07/22, 08/05, 08/28; autumn: 09/24, 10/08 and 10/28). We selected 2 sampling sections for each stream (Fig. 1), one exposed to high hydromorphological disturbance (Impacted Section, IS) and the other acting as a control (Control Section, CS). The ISs were located downstream, in urban areas, characterized by intermittent water, which dried out during the summer, with only some deep isolated pools persisting in the dry riverbed. The CSs were located upstream, in natural areas, characterized by permanent water according to historical data, where we observed just a natural flow reduction.

In each section, we identified five sampling plots (microhabitats) representing the highest possible heterogeneity in terms of flow velocity, water depth, canopy shading, macrophyte coverage and isolation from the main river course. We selected such different microhabitats in order to detect differences in the response of photosynthetic organisms to spatial heterogeneity typical of Mediterranean rivers (Tornés and Sabater, 2010). Water was always present at the sampling moment in all microhabitats, even in small amount, also during water scarcity, when the main channel of the study streams was dry.

Data collection

In each sampling section, two types of water quality parameters were measured: i) physical and chemical parameters*:* water dissolved oxygen (DO), pH, temperature and conductivity were measured with a multiparametric probe (Hydrolab mod. Quanta), while suspended sediments (TSS) were determined by gravimetry following the Italian standard methods (APAT-IRSA CNR, 2003);

ii) nutrients: soluble reactive phosphorous (SRP) and nitrates were determined with a LASA 100 spectrophotometer according to APAT-IRSA (2003).

In each microhabitat, we measured water depth and flow velocity with a current meter (Hydro-bios Kiel). We also visually evaluated if the microhabitat was shaded or not and if it was isolated or connected with the main course. In isolated pools, we took measures of both physical and chemical parameters and nutrients to detect possible differences with the main course. For each microhabitat we took three measures of epilithic chl a of diatoms, cyanobacteria and green algae with the BenthoTorch®, developed by BBE Moldaenke GmbH (Schwentinental, Germany). BenthoTorch® is a Pulse Amplitude Modulated (PAM) fluorimeter emitting light pulses at three different wavelengths (470, 525 and 610 nm), recording the response of cyanobacteria, diatoms and green algae at 690 nm wavelength (Kahlert and McKie, 2014). We then selected the median value of chl a concentration for each autotrophic group and we calculated their proportion with respect to the total chl a concentration.

Statistical analyses

We firstly performed data exploration in accordance with Zuur *et al.* (2009, 2010). We used Cleveland dotplots and boxplots to assess the presence of extreme values and avoid unusual observations to exert an undue influence on estimated parameters (Zuur *et al.*, 2009). We then evaluated multicollinearity among predictors using Pearson correlation test and variance inflation factors (VIFs). Variables highly correlated (R^2 correlation value >0.05 and VIF >2) were excluded to avoid confounding effects and model overfitting (Zuur *et al.*, 2009). Given the high number of zeros, we transformed the flow velocity into a categorical variable (0 m s^{-1} = standing water; >0 m s^{-1} = flowing water). In accordance with the results obtained from these analyses, we selected the following predictive variables: i) continuous variables: water depth, DO, temperature, pH, SRP, nitrates, percentage of macrophyte coverage; ii) categorical variables: sampling section, sampling date, flow velocity, isolation and canopy shading. We considered the sampling date as a proxy of the hydrological disturbance, since we observed a progressive and

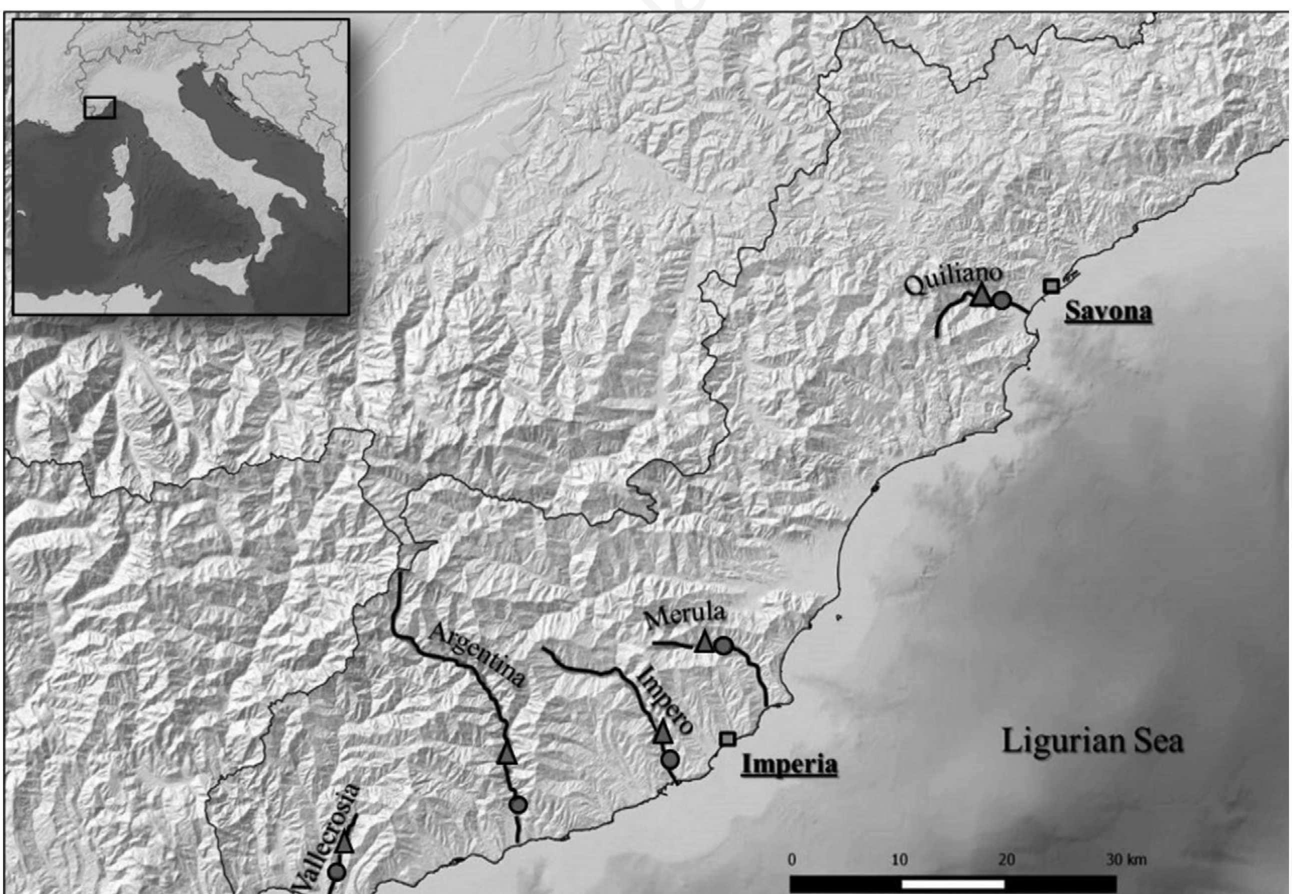

Fig. 1. Map of the five study streams and relative sampling sections. Diamonds, control sections (CSs); circles, impacted sections (ISs).

gradual reduction of water along the sampling period, together with the fragmentation in isolated pools. We tested the predictor variables and potential interactions against total chl a and relative proportions of diatoms, cyanobacteria and green algae, via Generalized Linear Mixed Models (GLMMs, in accordance with Zuur *et al.*, 2009) in R environment (R Core Team, 2014). Given the high number of zeros, green algae data were transformed into presence/absence data to obtain a more balanced dataset.

Given the spatial dependence of the data (two sections in each river), we applied the mixed procedure to include a grouping variable (river) as a random factor in order to account for the variation it introduced in our samples, rather than to test for its direct effect on the dependent variables. For the total chl a model, we assumed a gamma distribution (link function: log) which allowed us to deal with strictly positive variables, (Zuur *et al.*, 2009). For the relative proportions of the three photosynthetic groups, models were fitted with a binomial distribution (link function: log) which is able to deal with both presence/absence data (Bernoulli distribution) and proportional data (strictly binomial distribution) as recommended in Zuur *et al.*, (2009). In order to identify the best hypothesis supported by observations, we applied model selection (Johnson and Omland, 2004). We performed a backward elimination, progressively excluding variables according to AIC values (Zuur *et al.*, 2009). Variables not contributing to the fit of the model (*i.e.,* variables increasing the AIC value) were progressively dropped from the models thus avoiding overfitting (Hawkins, 2004). GLMMs were fitted via the lme4 R package (Bates *et al.*, 2014, version 1.0-6). We finally checked the correlation between the three photosynthetic groups with the Pearson correlation test.

RESULTS

Algal biomass and environmental factors

Flow velocity was higher in the CSs and presented lower values in summer and autumn during the drought season (lowest observed values 0.04 m s^{-1} in CSs and 0.00 in ISs), while no particular trend was observed for water depth (Tabs. 1 and 2). Conductivity showed an increasing trend from spring to autumn with higher values in the ISs (up to 777 μS cm^{-1}), while DO and nutrients decreased. TSS presented very low values; the only peaks (171 mg L^{-1}) were in ISs and during summer. pH was alkaline in both CSs and ISs and remained almost constant during all the sampling period. Temperature varied in accordance with the

Tab. 1. Summary of environmental parameters, in control and impacted sections. Data are expressed as mean and standard deviation of all samples.

Environmental parameters	Control sections	Impacted sections
FV (m s^{-1})	0.12 (±0.22)	0.07 (±0.16)
Water depth (cm)	28 (±18)	21 (±16)
Cond (μS cm^{-1})	401 (±159)	434 (±153)
DO (mg L^{-1})	9.3 (±1.2)	9.4 (±2.75)
Temperature (°C)	18.3 (±3.78)	19.9 (±4.15)
pH	8.53 (±0.46)	8.46 (±0.46)
TSS (mg L^{-1})	2.83 (±3.44)	8.20 (±28.34)
SRP (mg L^{-1})	0.009 (±0.013)	0.019 (±0.045)
N-NO_3 (mg L^{-1})	0.433 (±0.258)	0.675 (±0.868)
% Macrophytes	58 (±36)	65 (±34)

FV, flow velocity; Cond, conductivity; DO, dissolved oxygen; TSS, total suspended solids; SRP, soluble reactive phosphorus; N-NO_3, nitrates.

Tab. 2. Summary of environmental parameters (continuous variables), along the three seasons (spring, sampling campaign 04/17/14; summer, sampling campaigns 06/30/14, 07/22/14, 08/05/14 and 08/28/14; autumn, sampling campaigns 09/24/14, 10/08/14 and 10/28/14). Data are expressed as mean and standard deviation for each period considered.

Environmental parameters	Spring	Summer	Autumn
Flow velocity (m s^{-1})	0.16 (±0.25)	0.07 (±0.18)	0.09 (±0.18)
Water depth (cm)	22 (±17)	24 (±16)	25 (±18)
Conductivity (μS cm^{-1})	391 (±145)	393 (±163)	442 (±175)
Dissolved oxygen (mg L^{-1})	10.3 (±0.73)	8.95 (±2.1)	9.2 (±2.2)
Temperature (°C)	15.4 (±3.40)	22.3 (±2.37)	17.7 (±2.66)
pH	8.79 (±0.24)	8.68 (±0.97)	8.35 (±0.35)
TSS (mg L^{-1})	1.54 (±2.05)	7.32 (±26.65)	2.31 (±2.10)
SRP (mg L^{-1})	0.017 (±0.011)	0.010 (±0.013)	0.019 (±0.053)
N-NO_3 (mg L^{-1})	0.971 (±0.414)	0.420 (±0.205)	0.521 (±0.972)
% Macrophytes	35 (±32)	57 (±38)	69 (±31)

TSS, total suspended solids; SRP, soluble reactive phosphorus; N-NO_3, nitrates.

season with slight difference between the CSs and ISs. Total chl a showed higher values in the CSs (up to 31.4 μg cm^{-2}), with lowest values during summer (Fig. 2a). Diatoms were always the most abundant group, with higher values in CSs (up to 30.3 μg cm^{-2}), but their proportion progressively decreased in summer and autumn with respect to spring (Fig. 2b). On the contrary, cyanobacteria and green algae showed lower values but their proportions were higher in ISs and increased from spring to autumn (Fig. 2 c,d). The highest total chl a values were observed in September, which corresponded to a sharp increase in diatom primary production and a consequent reduction in proportion of green algae (Fig. 2c).

Statistical models

Results obtained from the final selected models showed clear differences between the three photosynthetic groups and total chl a in terms of environmental preferences. Total chl a was positively affected by the flow velocity and season; actually, a significant increase at the beginning of autumn was observed (Tab. 3 and Fig. 2a). According to the final selected model, diatoms proved to be favoured in environmental conditions of moderate flow periods. Indeed, their relative proportion was higher in microhabitats with flowing water and it was positively influenced by the oxygen concentration. The negative effect of drought on diatoms was revealed by the significant lower proportion in the ISs, and their decrease during the drought period (Tab. 3 and Fig. 2b). In particular, their proportion significantly decreased at the end of June and especially at the beginning of August. Cyanobacteria proved to be not so influenced by the drought, since the variables chosen as drought proxy (section and sampling date) were both excluded from the final model. However, their relative proportion was positively affected by the temperature, while the positive effect of isolation and the negative effect of depth were nearly significant, thus indicating a preference for warm, isolated shallow pools typical of the drought period. On average ISs showed a slight increase of cyanobacteria compared to CSs and a marked variability (Tab. 3 and Fig. 2c). Green algae showed an opposite trend with respect to diatoms, being

Tab. 3. For each dependent variable (total chl a; diatoms; cyanobacteria; green algae) the final selected model, estimated parameters (β-Estimate), standard errors (SE), t (or z) statistics and P values for each significant covariate are reported. For categorical variable, the reported values are referred to: section, control; date, 04/17/2014; flow velocity, class 0 (v = 0 m s^{-1}); isolation, connected.

Final selected model				
		Total chl a ~ velocity + date + (1|river)		
Variable	**β-Estimate**	**SE**	**t**	**P value**
Flow velocity (>0 m s^{-1})	0.349	0.092	3.806	0.0001
Date (24/09/2014)	0.613	0.176	3.484	0.0005
		Diatoms ~ section + velocity + oxygen + date + (1|river)		
Variable	**β-Estimate**	**SE**	**z**	**P value**
Section (impacted)	-1.675	0.385	-4.352	<0.0001
Flow velocity (>0 m s^{-1})	1.084	0.457	2.373	0.0176
Oxygen	0.174	0.080	2.179	0.0293
Date (30/06/2014)	-1.678	0.830	-2.021	0.0433
Date (05/08/2014)	-2.514	0.832	-3.022	0.0025
		Cyanobacteria ~ temperature + isolation + depth + (1|river)		
Variable	**β-Estimate**	**SE**	**z**	**P value**
Temperature	0.235	0.095	2.463	0.0138
Isolation (isolated)	1.287	0.705	1.826	0.0678
Water depth	-0.065	0.035	-1.893	0.0583
		Green algae ~ section + velocity + oxygen + macrophytes + depth + date + (1|river)		
Variable	**β-Estimate**	**SE**	**z**	**P value**
Section (impacted)	-1.223	0.353	3.463	0.0005
Flow velocity (>0 m s^{-1})	-2.173	0.543	-4.001	<0.0001
Oxygen	-0.252	0.091	-2.765	0.0057
Macrophyte	0.010	0.006	1.732	0.0833
Water depth	0.179	0.010	1.745	0.0810
Date (05/08/2014)	2.723	0.775	3.516	0.0004

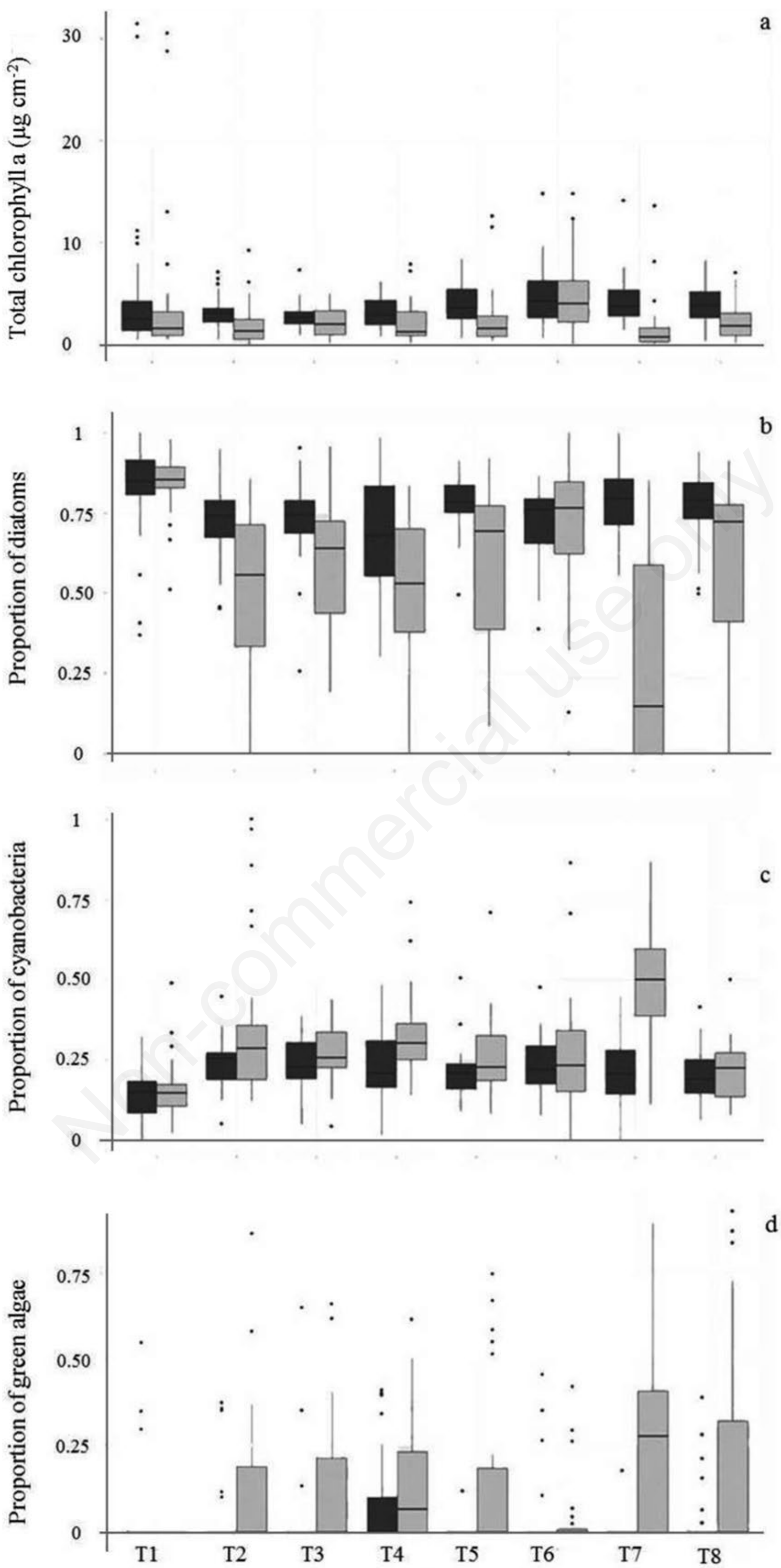

Fig. 2. Boxplots of total chlorophyll a (a) and relative proportions of diatoms (b), cyanobacteria (c) and green algae (d) in control (black) and impacted sites (grey). T1, 04/17/2014; T2, 06/30/2014; T3, 07/22/2014; T4, 08/05/2014; T5, 08/28/2014; T6, 09/24/2014; T7, 10/08/2014; T8, 10/28/2014.

favoured in environmental conditions characterising the drought season. Indeed, their presence was favoured in the ISs (Tab. 3 and Fig. 2d) and in microhabitats with standing water and low oxygen concentrations. Their probability of presence showed a positive trend during the drought period, with a significant increase at the beginning of August (Tab. 3 and Fig. 2d). A strong negative relationship between diatoms and the other two groups was revealed by the Pearson correlation test (diatoms *vs* cyanobacteria: R = -0.59, t = -12.465, P<0.0001; diatoms *vs* green algae: R = -0.76, t = -19.844, P<0.0001), while no correlation was found between cyanobacteria and green algae (R = -0.07, t = -1.227, P=0.221).

Proportion of diatoms was then selected as an indicator of hydrological disturbance, since it proved to be the most sensitive variable to water scarcity and it was also strongly negatively correlated with relative proportions of cyanobacteria and green algae. In order to determine a threshold to distinguish between natural drought and human-induced water scarcity we measured the median between the 75th percentile in the IS dataset and the 25th percentile in the CS dataset of diatom proportion. For having a better indication, data from the first sampling campaign were excluded in this phase. According to this procedure, the final threshold was 51% (75th percentile IS dataset = 50%; 25th percentile CS dataset = 52%).

DISCUSSION

Our data demonstrate that in Mediterranean streams the effects of hydrological variability can be quantitatively evaluated in terms of chlorophyll *a* content. More specifically, we verified the hypothesis that, among periphytic primary producers, diatoms are the most affected by hydrological disturbance, as partly suggested by previous studies (Romaní *et al.*, 2012; Barthes *et al.*, 2015), but controversial in others (Caramujo *et al.*, 2008).

The hydrological alteration was highlighted by a progressive lentification and fragmentation of the riverine habitat, with the formation of isolated pools in dry stretches. As a consequence, the flow velocity diminished from spring to autumn and from upstream to downstream (Tabs. 1 and 2). As pointed out in other studies (Gasith and Resh, 1999; Lake, 2003), the reduction of water supply and the evaporative processes caused an increase of ion concentration as demonstrated by the higher values of conductivity in the IS dataset and the increasing values from April to September. Moreover, we detected a lower oxygen concentration in isolated pools. On the contrary, nutrients did not show a pattern clearly related to the progressive hydromorphological alteration. According to literature data on Mediterreanean streams (Guasch *et al.*, 1995; Sabater *et al.*, 2006), we expected an increase of nutrient concentration with the progression of the drought; on the contrary, our summer values were lower than spring ones. The loss of lateral and longitudinal connectivity, due to the progressive drought, in parallel with reduction in precipitations, may cause a reduction in nutrient supply, as pointed out by Dahm *et al.* (2003). Moreover, the growth of riparian vegetation during the summer period may also act as a buffer zone retaining nitrates, as suggested by Sabater *et al.* (2000). Thus, during droughts nutrient input is expected to originate from groundwater and reflect the regional biogeochemistry (Clifford *et al.*, 2003).

Despite SRP and nitrate show higher values in the ISs than in Cs, due to different land uses, both CSs and ISs can be classified as oligotrophic and oligosaprobious in all five study streams according to common water quality classifications (Hofmann, 1994; Van Dam *et al.*, 1994). Accordingly, the range of total chl a corresponds to those of unenriched streams (Biggs, 1996). A further confirmation of the scarce influence of land use is given by the slightly lower values of total chl a concentration in the IS than in the CS dataset. Even if anthropogenic land uses may strongly increase algal biomass growth and alter community composition of photobiota, we observed lower values in the IS dataset, probably due to the hydrological disturbance (Taylor *et al.*, 2004; Cooper *et al.,* 2013). Our results are in accordance with Proia *et al.* (2012), who affirmed that flow variability, and in general physical disturbance, may result in a weak relationship between chl a and nutrient concentrations. However, it should be pointed out that the total chl a does not give a clear response to the reduction of water supply, since no significant differences were revealed between the CSs and ISs and no significant reduction along the sampling seasons was observed (Tab. 3). In fact, we found much clearer effects in terms of relative proportion of the three main photosynthetic groups compared to total chl a.

Diatoms largely dominated the phototrophic community over the entire study period, in accordance with Graba *et al.* (2014). However, their proportion gradually decreased during the hot season, being replaced by cyanobacteria and green algae, in accordance with Romaní *et al.* (2012). In particular, as confirmed by the Pearson correlation test, we observed an opposite trend between diatoms and green algae, similarly to what observed by Luttenton and Lowe (2006) in lentic environments. These results are also consistent with those found in artificially illuminated cave environments by Piano *et al.* (2015), who observed opposite trends for diatoms and green algae chlorophyll *a* contents. As demonstrated by the results of the statistical models, hydrological disturbance seemed to have a main role in determining the relative proportion of diatoms and green algae within the periphyton. In particular, at the beginning of August a significant decrease of the relative proportion of diatoms is combined with a significant increase in green algae probability of presence (Tab. 3). In general, diatoms were favoured in the CSs, whereas green algae probability of presence was higher in the ISs. The three photosynthetic groups showed clear different responses to hydrological changes. Microhabitat characteristics, described by flow velocity and water depth, proved to have a significant effect, confirming a microscale pattern of benthic photosynthetic microorganisms (Biggs, 1996). In particular, diatoms proved again to be the photosynthetic group most negatively influenced by drought. Their relative proportion was favoured in riffles, characterized also by high DO availability, while the presence of green algae was favoured in pools with lower DO, in accordance with Stevenson (1996). Cyanobacteria seemed not be directly influenced by the water scarcity, since their proportion did not show any differences between the two sampling sections or between the sampling dates. However, an indirect effect of low water flow could be hypothesized: cyanobacteria relative proportion significantly grows in shallow isolated pools, with high temperatures. We can assume that cyanobacteria are favoured during the hot season, as generally seen in lakes and ponds (Lake, 2003). These relationships highlighted an indirect response of cyanobacteria to the water scarcity, being favoured in environmental conditions strictly linked to this phenomenon.

Some limitations of this work should be highlighted. Indeed, our results were obtained from a limited number of streams, all belonging to the same HER and all classified at least as "good" in terms of water quality, in accordance with the WFD thresholds. There is thus a call for a validation in other streams at different water quality levels and belonging to different HERs of the Mediterranean region. In particular, a gradient of anthropic disturbance should be considered in order to disentangle the effect of nutrients on the primary production of our focus groups.

CONCLUSIONS

Comparing to phytoplankton, the assessment of benthic algal biomass has always been considered more challenging. On the one hand, it is considered as essential for tracking short and long-term changes and to assess the role of benthic algae in freshwater foodwebs (Stevenson, 1996). There have always been a series of constrains related to the costs of extensive sampling surveys needed for the high spatial and temporal variability of phytobenthic community (Kahlert and McKie, 2014). In recent years, promising methods have been developed to overcome these limitations, such as instruments for *in situ* measurements of chl a specifically conceived for benthic algae. In our study, the use of an *in situ* fluorimetric probe allowed us to discriminate between the main groups composing autotrophic biofilm and to obtain a rapid assessment of its composition in terms of primary producers. In a broader context, we suggest to use such probe as an integrative tool in supporting monitoring programs. Total chl a in itself is a good indicator of human-induced water-quality degradation and should be routinely monitored as part of an effective management program (McNair and Chow-Fraser, 2003), but as a response metric it is not stressor specific as it reflects changes in concentration of nutrients, various pollutants, physical conditions and interactions of stressors (Zalack *et al.*, 2010). In our study it did not prove to clearly distinguish between the CS and the IS datasets, thus not being a useful indicator for measuring the disturbance caused by water scarcity. On the other hand, diatoms proved to be the most sensitive group to water scarcity in terms of chl a and the group with the highest representativeness. According to our data, we can conclude that within a biofilm, a diatom proportion below the threshold of 51% could be a signal of hydrological stress caused by water scarcity in nutrient unenriched Mediterranean streams. These values could be applied in the future for the environmental impact assessment of water abstraction works. In general, the decrease of diatom proportion within a biofilm can imply negative consequences in the stream ecosystem functionality since it enhances the competitiveness of filamentous green algae and cyanobacteria that are less edible for grazers, similarly to what happens in lentic and eutrophic ecosystems (Caramujo *et al.*, 2008). Understanding the response and contribution of biofilm main components is essential to evaluate the effect of flow intermittency on stream ecosystem functioning.

The ratio between diatom chl a and total chl a can be potentially included as a metric in monitoring programs of Mediterranean streams, integrating chemical parameters and biological indices commonly adopted for classifying the ecological status, but not specifically sensitive to hydrological disturbance.

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