



Photoautotrophs and macroinvertebrate trophic relations in calcareous semiarid streams: The role of Cyanobacteria



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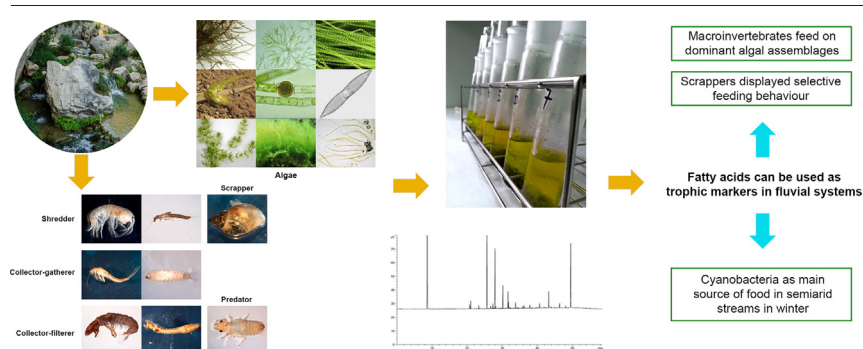
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HIGHLIGHTS

- Trophic relationships can be established by fatty acids composition.
- Most macroinvertebrate functional groups feed on dominant photoautotrophs.
- Cyanobacteria are the main source of fatty acids for macroinvertebrates in winter.
- Scrapers select Chlorophyta.
- Fatty acids can be used in biomonitoring of semiarid fluvial systems.

GRAPHICAL ABSTRACT



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ABSTRACT

Photoautotrophs and macroinvertebrate trophic relations in Mediterranean streams, especially from semiarid areas, are still poorly known, as is the role of Cyanobacteria, which is the most frequently dominant photoautotroph. To investigate the role of Cyanobacteria as a food resource in these systems, the fatty acid composition of primary and secondary producers was investigated in two streams on a semiarid climatic gradient between 200 and 500 mm of rainfall in SE Spain. Fatty acid composition of photoautotrophs and macroinvertebrates differed among streams in summer and among seasons in each stream. Fatty acid fingerprints show that macroinvertebrates usually fed on the dominant photoautotroph assemblage and that Cyanobacteria represent the main food for all the feeding groups in the Alhárabe stream in winter although filamentous green algae were preferred in summer. Only scrapers consuming Chlorophyta displayed a selective feeding behaviour. The results show the importance of cyanobacteria as food for all collected macroinvertebrates in winter in two semiarid streams and confirm that fatty acids can be used as temporal and spatial markers in fluvial systems.

1. Introduction

The importance of algae in supporting animal production in streams with high irradiance is generally accepted (Bunn et al., 2006). Benthic autotrophic communities change seasonally, which reflects environmental variations throughout the year, have a lower C:N ratio, and are more valuable food for macroinvertebrates than allochthonous organic matter (Sternler and Elser, 2002). However, no agreement has been reached on

the preferred or more suitable algae species for macroinvertebrate development (Guo et al., 2018).

Although some freshwater macroinvertebrates may have preferences for special food resources (Guo et al., 2018), they are mostly considered omnivorous or generalists for showing great plasticity to adapt their nutritional needs to food availability in the environment (Cummins, 1973; Cummins and Klug, 1979; Krenn, 2019). The nutritional requirements of these macroinvertebrates can also change ontogenetically and may be different in each stage of their development (Wotton et al., 1993; Fenoglio et al., 2014). These requirements may also adapt to seasonal availability (Guo et al., 2018). In fact, carnivorous or vegetarian behaviours in the

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last nymphal stages have been observed in some detritivorous and predator species, respectively (Anderson, 1976; Wotton et al., 1993; López-Rodríguez et al., 2009).

Food sources in freshwater ecosystems may have a diverse origin, size and texture, although algae predominate in rock coatings and allochthonous debris in coarse particulate organic matter (Cummins and Klug, 1979). Macroinvertebrates' food intake seems more related to their capability to collect food than to their nutritional composition (Cummins, 1973). Functional feeding groups (FFGs) sort freshwater macroinvertebrates according to their morpho-behavioural adaptations to feed on a particular food source (Krenn, 2019), and is a determinant of physical food properties (Cummins, 1973; Wallace and Webster, 1996). This way of grouping macroinvertebrates may be more effective than a simple assemblage of species to understand or assess the biological interrelations of stream ecosystems (Wallace and Webster, 1996; Rawer-Jost et al., 2000), such as quality of food sources based on fatty acids (FAs) composition (Guo et al., 2018).

The energy obtained from diet does not always come from the commonest ingested food (March et al., 2001; Hollows et al., 2002; March and Pringle, 2003). In fact, Mayer and Likens (1987) report that algae are responsible for 70% of the energy needs of the caddisfly *Neophylax aniqua* (Trichoptera), even when algal production represents less than 2% of all energy inputs. These minority food sources are considered quality food sources and, according to Guo et al. (2018), FAs composition is used for this quality consideration. Food quality influences physiology, ecological interactions, and the evolution of aquatic consumers, particularly its contents in FAs because they are not only structural elements of cell membranes, but also energy storage molecules and precursors of signalling hormones (Martin-Creuzburg et al., 2012). The enzymes needed to synthesise long-chained polyunsaturated fatty acids (PUFAs) are largely restricted to algae (Iverson, 2009) and metazoans must obtain them from diet: eicosapentaenoic acid (EPA, 20:5-n3), docosahexaenoic acid (DHA, 22:6-n3) or their precursor α -linolenic acid (ALA, 18:3-n3) (Parrish, 2009). Moreover, FAs composition permits allochthonous to be discriminated from autochthonous foods, which helps to disentangle complex aquatic food webs (Müller-Navarra et al., 2000).

Although the synthesis of essential FAs (EFAs) has been reported in some invertebrate species under special conditions (Cripps et al., 1986; Kabeya et al., 2018; Malcicka et al., 2018; Monroig and Kabeya, 2018), invertebrates must fulfil their FAs requirements through their diet because they cannot synthesise them to meet physiological demands (Arts et al., 2001; Kabeya et al., 2018). PUFAs, in particular EPA, seem ubiquitous among aquatic insects in temperate streams (Sushchik et al., 2003) and several pieces of evidence indicate that invertebrates obtain n3 PUFAs primarily from algae in marine and lacustrine systems (Ahlgren et al., 1992; Guo et al., 2017, 2022).

The distribution patterns of FAs are considered chemotaxonomic markers at the algae high rank taxa level and vastly vary at the species level (Lang et al., 2011). Several FAs have been suggested as biomarkers for various freshwater organisms: Vaccenic acid (VA, 18:1-n7) and γ -linolenic (GLA, 18:3-n6) for cyanobacteria, although the former seems to also mark sulphur-oxidising bacteria (Fredrickson et al., 1986; Napolitano, 1999; Yang et al., 2016). Both the palmitoleic (16:1-n7) to palmitic acid ratio (16:0) and palmitoleic to EPA ratios have been proposed as diatom biomarkers (Miyazaki, 1983; Parrish et al., 1995), with the DHA to EPA ratio for dinoflagellates (Budge and Parrish, 1998). The n3/total n6 PUFAs total ratio has been proposed as a marker for terrestrial (<1.0) and aquatic (>1.0) matters (Pollero et al., 1981); FAs have been put forward as both a fingerprint of biofilm stream communities and a proxy of water quality (DeForest et al., 2016).

Macroinvertebrates are a very diverse component of waterbodies from semi-arid and arid regions (Mabidi et al., 2017) and they represent an important source of food for fishes in these systems and elsewhere (Wallace and Webster, 1996). However, in some of the freshwater systems of these regions, the macroinvertebrates can sometimes constitute the higher aquatic trophic level, being essential for the maintenance and resilience of the food web structure of ecosystem (Sroczynska et al., 2020), or

for the growth and development of endangered species with a very restricted distribution (Doadrio, 2002), such as the fish *Aphanius iberus* (Valenciennes, 1846) in the semi-arid areas of southeast Spain (Oliva-Paterna et al., 2022).

Cyanobacteria are a dominant constituent of benthic photoautotrophs communities in several different habitats that range from rivers to marshes (Janousek et al., 2007), especially in headwaters of calcareous streams. Here they exhibit high species richness and produce a large biomass all year long, as they do in the Alhárabe and Chícamo streams in the south-eastern part of the Iberian Peninsula (Aboal, 1989; Aboal et al., 2005, 2014a, 2014b; Perona et al., 2003; Sabater, 1989). Cyanobacteria are considered unpalatable for most herbivores given their potential toxicity or lack of essential FAs (O'Neil, 1999; Guo et al., 2022). However, an increasing number of field and experimental data apparently show that this group may constitute an important food resource for different groups of aquatic invertebrates (Cook et al., 2004). Some mayflies, such as *Hexagenia* spp., may feed on toxic species without lethality or growth impairments (Shahmohamadloo et al., 2020), some mollusk have a high tolerance to cyanotoxins (Pham et al., 2015) or toxic species such as *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault represents a valuable food resource for different aquatic consumers (Berezina et al., 2021).

We aimed to explore the value of semiarid stream Cyanobacteria assemblages and other photoautotrophs as food for freshwater macroinvertebrates, sorted as functional feeding groups, based on their FAs composition.

2. Material and methods

2.1. Study area

Two tributary streams of the Segura River (SE Spain) were selected: the Alhárabe and Chícamo (Fig. 1). Alhárabe flows through the forested and mountainous NW area of the Murcia Region on calcareous rocks. Chícamo runs over conglomerates, siltstones, clay and sandstone in an area with poorly developed riverine vegetation (mainly helophytes) from the north-eastern part of the region. Both basins are on a gradient of rainfall from 200 mm to more than 500 mm in Chícamo and Alhárabe, respectively, and both are included within ranges of semiarid streams (UNESCO, 1979). The environmental characterisation of the two sampling sites appears in Table 1.

2.2. Sampling

Seasonal sampling was carried out in the two studied streams: the Alhárabe samples were taken in winter, spring and summer, and the Chícamo samples were collected only in summer and winter because assemblages are more constant throughout the year (Gonzalez-Silvera et al., 2017). All the different photoautotroph growths were collected with a knife along a 100 m-long transect (Gómez et al., 2009), put in plastic vials and frozen separately with liquid nitrogen in the field. A subsample of each species was separated and maintained under dark and cold conditions before reaching the laboratory, where subsamples were studied alive and then preserved with 4% formaline.

Macroinvertebrates were collected through qualitative sampling following the same seasonal cadence as for algae. The samples were obtained by means of a 250 μ m mesh kick-net that was applied in the different stream habitats. Only were selected those groups of macroinvertebrates that, due to their size or abundance, allowed them to meet the biomass requirements necessary to carry out the fatty acid analyses, as well as to represent the general categories of FFGs defined by Cummins (1973). To facilitate this selection, the samples were washed and sieved with a 500 μ m mesh to remove silt, then placed on a tray to pick up the main macroinvertebrates group. The selected macroinvertebrates were placed on a Petri dish with distilled water for 1 h to empty their guts. Subsequently, these organisms were classified and stored in tubes by families, proceeding to freeze them in situ in liquid nitrogen. The biomass of each tube was sufficient to be able to carry out three independent replicates of fatty acid analyzes per family of



Fig. 1. Map of the study area, located in the South-East of Iberian Peninsula. The studied transects of Alhárabe and Chícamo Rivers (tributaries of Segura River) are indicated with a wider line.

macroinvertebrates. Additionally, a subsample of each macroinvertebrate group was preserved in 70% ethanol for taxonomy purposes and FFG assignments.”

2.3. Algae and macroinvertebrate taxonomic identification

The taxonomic identification of photoautotrophs was performed to reach the lowest possible level: genus or species (see Table 2). Macroinvertebrates were taxonomically identified up to the family level to be assigned to a FFG (Table 3) following Cummins (1973), Oscoz et al. (2011) and Ramírez and Gutiérrez-Fonseca (2014).

Table 1

The main environmental characteristics of stream sampling sites. Mean, minimum and maximum values are presented.

Environmental variables	Sampling points	
	Alhárabe	Chícamo
Coordinates	38°12'50"N 1°57'46"W	37°49'N, 0°47'W
Altitude (m)	900	280
Orientation	NW	SE
Mean flow ($L s^{-1}$)	29.4 (8.5–40.8)	17.7 (9.0–29.3)
Maximum depth (cm)	15 (5.0–25.0)	10.0 (2.0–15.0)
Mean rainfall (mm)	521.2 (450–600)	200 (100–250)
Mean temperature (°C)	16.2 (8.5–20.6)	20 (11.9–22.3)
Total P ($\mu g L^{-1}$)	34.1 (10.5–45.2)	0.10 (0.02–0.25)
DIN ($mg L^{-1}$)	11.9 (8.5–13.5)	4.7 (1.7–6.2)
pH	7.8 (7.7–7.9)	8.3 (8.1–8.5)
Conductivity ($\mu S cm^{-1}$)	650 (630–900)	2877 (2640–3100)
Substrate particle size	Heterogeneous (From boulder to silt)	Homogeneous (bedrock, sand and silt)
Lithology	Limestone	Conglomerates, sandstone, siltstone
Canopy cover (%)	50	5

Table 2

Seasonal composition of algal communities in the Alhárabe and Chícamo (coverage scale: 1 = 1–10%, 2 = 11–20%, 3 = 21–50%, 4 = 51–75%, 5 = 76–100%).

Algae taxa	Streams				
	Alhárabe		Chícamo		
	WI	SP	SU	WI	SU
Cyanophyceae					
<i>Calothrix parietina</i> Thuret ex Bornet & Flahault					1
<i>Dichothrix gypsophila</i> Bornet & Flahault			1		
<i>Geitlerinema splendidum</i> (Greville ex Gomont) Anagnostidis					1
<i>Leptolyngbya</i> sp.			1		1
<i>Rivularia bioleptiana</i> Meneghini ex Bornet & Flahault	3	4		3	3
<i>Scytonema mycochrous</i> C. Agardh ex Bornet & Flahault			1		1
<i>Tolypothrix distorta</i> Kützing ex Bornet & Flahault		1			
Rhodophyceae					
<i>Batrachospermum gelatinosum</i> (L.) De Candolle		1			
<i>Chrootheca lobata</i> Aboal, Whitton, Chapuis, Sánchez y Necchi				3	3
<i>Kyliniella latvica</i> Skuja	1	1			
Chlorophyceae					
<i>Bulbochaete</i> sp.		1			
<i>Chaetophora elegans</i> (Roth) C. A. Agardh		1			
<i>Draparnaldia glomerata</i> (Vaucher) C. Agardh			1		
<i>Gongrosira</i> sp.					2
<i>Oedogonium</i> sp.			2		
Ulvophyceae					
<i>Gladophora glomerata</i> (L.) Kützing			2		2
Streptophyta, Charophyceae					
<i>Chara crassicaulis</i> J.C. Schleicher				1	
<i>Spirogyra</i> sp.		3	3		2
<i>Zygnema</i> sp.				3	
Bacillariophyta					
<i>Cocconeis pediculus</i> Ehrenberg				1	
<i>Diatoma elongatum</i> (Lyngby) C. Agardh					
<i>Ulnaria biceps</i> (Kützing) Compère				1	
<i>Gomphonema</i> sp.		2			
<i>Cymbella</i> sp.				2	

Table 3

Macroinvertebrates associated with functional feeding groups in the studied streams. Only presence is indicated (¹ the sample of autumn in the Chicamo was broken down.² Samples did not reach the necessary weight for the FAs analysis).

Functional feeding groups/macroinvertebrate taxa	Streams				
	Alhárabe			Chicamo	
	WI	SP	SU	WI	SU
Scrapers					
Mollusca, Gasteropoda, Lymnaeidae		X	X		
Mollusca, Gasteropoda, Neritidae				X	
Mollusca, Gasteropoda, Melanopsidae				X	
Shredders					
Insecta, Trichoptera, Limnephilidae	X ²				
Insecta, Plecoptera, Leuctridae	X		X		
Insecta, Plecoptera, Nemouridae	X ²				
Insecta, Plecoptera, Capniidae	X ²				
Insecta, Coleoptera, Driopidae			X		
Crustacea, Amphipoda, Gammaridae				X	X
Collector-gatherers					
Insecta, Ephemeroptera, Baetidae	X	X	X	X ¹	X
Insecta, Diptera, Stratiomyidae	X	X	X		
Collector-filterers					
Insecta, Diptera, Simuliidae			X		
Insecta, Trichoptera, Hydropsychidae	X	X	X		
Insecta, Trichoptera, Philopotamidae		X ²			
Predators					
Insecta, Diptera, Athericidae		X ²			
Insecta, Odonata, Aeshnidae		X	X		
Insecta, Odonata, Calopterygidae			X		
Insecta, Odonata, Gomphidae			X ²		
Insecta, Trichoptera, Rhyacophilidae				X ²	

2.4. Lipid extraction and FA identification and quantification

All the collected macroinvertebrate and photoautotrophs samples were divided into three subsamples, which were separately analysed. Total lipids were extracted from 1 to 2 g of material by homogenising in 20 mL of chloroform/methanol (2:1 v/v) in a tissue disrupter (IKA ULTRA-TURRAX T 25 digital, IKA-WERKE, Staufen, Germany). Total lipids were prepared according to the method of Folch et al. (1957). Non-lipid impurities were removed by washing with 0.88% KCl. Lipid weight was determined gravimetrically after evaporating the solvent under a nitrogen stream and overnight vacuum drying. FA methyl esters (FAME) were prepared by the acid-catalysed transesterification of total lipids following the method of Christie (2003). The total lipid samples were *trans*-methylated overnight in 2 mL of 1% sulphuric acid in methanol (plus 1 mL of toluene to dissolve neutral lipids) at 50 °C. Methyl esters were extracted twice in 5 mL of hexane-diethyl ether (1:1, v/v) after neutralisation with 2 mL of 2% KHCO₃, dried under a nitrogen stream and redissolved in 1 mL of isohexane. FAME were separated and quantified by gas-liquid chromatography in an SPTM 2560 flexible fused silica capillary column (length 100, internal diameter 0.25 mm, film thickness 0.20 mm SUPELCO) in a Hewlett-Packard 5890 gas chromatograph. The 140 °C oven temperature was initially increased at a rate of 3 °C/min-1 to 230 °C, followed by 2 °C/min-1, and then to 240 °C and held for 12 min. The injector and the flame ionization detector set were at 260 °C. Helium was used as the carrier gas at 300 kPa pressure and peaks were identified by comparing their retention times with appropriate FAME standards from Sigma Chemical Company (St. Louis, MO, USA). The data for the individual components were expressed as a percentage of total content.

2.5. Statistical analysis

The multivariate analyses were performed with the normalised FA data from both algae and macroinvertebrates, and a ranked triangular similarity matrix was constructed using Euclidean distances. A two-way PERMANOVA crossed design was performed (9999 permutations) to test

any significant differences in the FA patterns between sites and seasons. Significant similarities in the FA compositions between the algae and macroinvertebrate taxa were explored to test the trophic relation by means of a pairwise comparison using Monte Carlo *p*-values due to the restricted number of possible permutations. These statistical procedures were implemented with the PRIMER 6 & PERMANOVA + v.1.0.2 software package (Anderson et al., 2008).

3. Results

3.1. Photoautotroph assemblages

Photoautotroph assemblages were more diverse in Alhárabe than in Chicamo. A clear seasonal pattern was observed in Alhárabe, while constancy of populations was remarkable in Chicamo all year long (Table 2). In both cases, the cyanobacteria colonies occupied an important proportion of the available substrata, particularly *Rivularia biolettiana* Meneghini ex Bornet & Flahault, which was permanently present in both streams. Green algae (Charophyceae, Chlorophyceae and Ulvophyceae), red algae (Rhodophyta) and diatoms (Bacillariophyta) completed both the Alhárabe and Chicamo algae assemblages (Table 2). Green algae formed extensive patches in summer, especially in Alhárabe, when filamentous Charophyceae (*Spirogyra*, *Zygnema*) and Chlorophyceae (*Oedogonium*) bloomed. Rhodophytes were always scarce in Alhárabe, with *Batrachospermum gelatinosum* growing in summer and *Kyliniella latvica* in winter. In Chicamo however, the presence of *Chrootheca lobata* was constant. Diatoms were important as epiphytes on most filaments and colonies and formed brownish mucilaginous aggregates in winter in Chicamo and in spring in Alhárabe.

3.2. Functional feeding groups assemblages

The general category of FFGs defined by Cummins (1973) (Table 1) based on feeding mechanisms of macroinvertebrates and general size range of food particle that can eat: shredders, collectors, scrapers and predators, were represented in both streams (Table 3). Unfortunately, not enough biomass was collected from them all for our study goals (Table 3): since taxa occurrence and biomass were conditioned by the environmental conditions of each stream and their seasonal variability. Additionally, the subdivision of collectors in gatherers and filterers, which attends to the type of food deposit that exploit in the ecosystem, was also defined in both rivers, although in Chicamo no family of macroinvertebrates representing collector filterers was found in any seasons (Table 3).

3.3. The FA composition of algae

Of all the saturated FAs (SFAs), palmitic, followed by myristic acid (14:0), had the highest proportion in Cyanobacteria and all the algae groups (Supplementary Table 1), especially in rodophytes, and except for Ulvophyceae and Chlorophyceae. Palmitoleic acid represented the highest proportion of the mono-unsaturated FAs (MUFA) in all the studied photoautotrophs, except for Ulvophyceae, in which oleic acid (18:1-n9) predominated. Linoleic acid (LA) was the predominant n6 PUFA in all photoautotrophs, especially in Cyanobacteria and Charophyceae, while the proportion of arachidonic acid (ARA, 20:4-n6) was higher in diatoms than in all the other groups (Fig. 2A). ALA was the predominant n3 PUFA in Cyanobacteria, Charophyceae and Ulvophyceae. GLA came in the highest proportion in the chlorophytes epiphyted with diatoms. Diatoms produced the highest EPA proportion, followed by Rhodophyceae. DHA was detected in all the tested algae (except for *Chaetophora elegans*, Chlorophyceae) and obtained the highest values in diatoms, Chlorophyceae and Rhodophyta. The n3/n6 PUFA ratio was always higher than 1, was higher than 3 in Rhodophyceae, Chlorophyceae, Ulvophyceae and Charophyceae, and remained relatively similar in all the groups except for cyanobacteria where it took a lower value (Fig. 2A). There were, however, major differences in the highly unsaturated FAs (HUFA) in diatoms and rhodophytes because they presented the highest concentrations, while Cyanobacteria the lowest. Green algae had intermediate values,

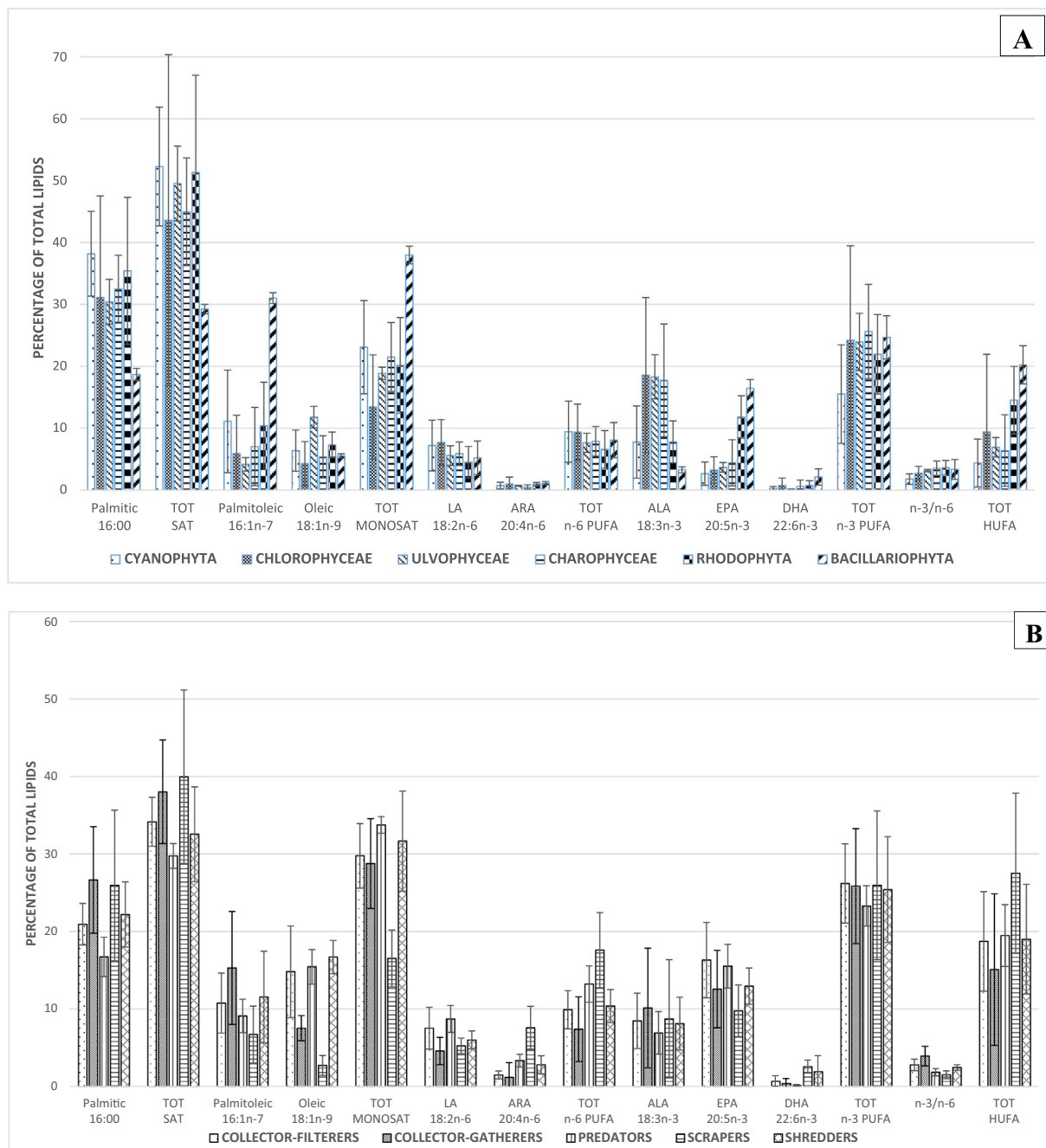


Fig. 2. Mean and standard deviation of the FA composition of the different taxonomic groups of algae (A) and the FFGs (B) collected (SFA = total saturated FA, MUFA = total monosaturated FA, n-6 PUFA = total n-6 PUFA, n3 PUFA = total n-3 PUFA, HUFA = total highly unsaturated FA).

which increased from Ulvophyceae to Charophyceae and Chlorophyceae (Fig. 2A).

Diatoms presented the highest mean proportion of ARA, EPA and DHA, a relatively low LA proportion and the lowest ALA proportion. Rhodophyta had a fairly high proportion of all the considered EFAs (Fig. 2A). Diatom markers were present in all the algal assemblages and represented an important fraction of FAs, especially in filamentous green algae. The *Oedogonium* with *Ulnaria biceps* assemblages may reach 15.7% myristic acid, while palmitoleic acid might represent 31.63% of benthic diatom mats and 21.73% of epiphyted *Oedogonium* assemblages. n6 PUFA may reach 27.04% in *Rivularia* colonies and 14.71% in *Chara* with *Ulnaria*, while EPA might represent up to 16.9% of the FA contents of *Oedogonium* with *Ulnaria*.

Differences were also detected among taxa at the species level. Palmitoleic acid was the predominant MUFA in cyanobacteria, except for *Rivularia bioislettiana* in which oleic acid dominated, while EPA doubled the proportion of ALA in *Geitlerinema splendidum* and represented 76% of the total n3 PUFA in *Scytonema myochrous*. The mixed *Zygnema* and *Spirogyra* assemblages had higher EPA and ALA proportions than *Spirogyra* alone. Oleic acid, but not palmitoleic acid, was predominant in *Chaetophora elegans*, which had the highest LA proportion of all the chlorophytes. EPA predominated over ALA only in *Oedogonium*, and ALA did so over EPA in *Chaetophora elegans*, *Draparnaldia glomerata* and *Gongrosira* (Supplementary Table 1, Fig. 2A).

Bacterial markers, particularly VA, remained low in all the assemblages, with only 10% exceptionally in the *Spirogyra* assemblages in summer.

3.4. FAs composition of FFGs

FFGs showed a similar composition of FAs, except for scrapers, with SFA, MUFA and PUFA percentages each representing approximately one third of the total (Fig. 2B and Supplementary Table 2). In scrapers, SFA and PUFA represented almost 84% of the total FAs (43.54% and 39.96%, respectively), while the percentages of MUFA were the lowest of all the FFGs. In relative terms, PUFA were the main component of the total FAs in all cases, except for collector-gatherers in which SFA were higher. MUFA were the minority fraction of the total FAs in all the FFGs, except in predators whose SFA fraction was lower (Fig. 2B and Supplementary Table 2). As regards the total amount of PUFA, n3 was clearly more abundant than n6 in all the FFGs (Fig. 2B), sometimes with a 70%/30% ratio, such as collector-gatherers, collector-filterers and shredders (Supplementary Table 2). It is also noteworthy that the n3 PUFA proportion in the different FFGs was more homogeneous than that of n6 PUFA (Fig. 2B). In fact, scrapers had the higher n6 PUFA proportion of all the FFGs, which almost doubled the values of collector-gatherers, collector-filterers and shredders (Supplementary Table 2). Scrapers by far had the largest amount of HUFAs (Fig. 2B). Finally, the n3/n6 ratio confirmed the predilection of all the FFGs, especially collector-gatherers, for autochthonous food sources.

The most abundant SFA in FFGs was palmitic (Fig. 2B) and represented up to 25% of the total FAs in scrapers and collector-gatherers (Fig. 2B and Supplementary Table 2). Palmitoleic and oleic were the most abundant MUFA in all the FFGs, except for scrapers (Fig. 2B), in which VA was more abundant than oleic. The amount of VA was also notable in predators and collector-gatherers (Supplementary Table 2). LA, followed by ARA, was the most abundant n6 PUFA in all the FFGs, except for scrapers which were the exact opposite: the amount of ARA doubled that of the other FFGs (Fig. 2B). Predators, collector-filterers and shredders showed the highest LA proportion, while collector-gatherers had the smallest amount of examined n6 PUFA. The minority n6 PUFA were only well represented in scrapers, especially for EPA (20:2-n6) and DTA (22:4-n6) (Supplementary Table 2).

EPA was the most abundant for n3-PUFA, particularly in collector-filterers and predators, followed by ALA. Predators obtained the lowest percentage of this FA (Fig. 2B and Supplementary Table 2). Of the minority n3 PUFA, it is worth highlighting that percentages were around 2% for stearidonic acid (18:4-n3) in collector-gatherers, scrapers and shredders, and docosapentaenoic acid (DPA, 22:5-n3) in scrapers (Supplementary Table 2). Interestingly, DHA obtained a very low proportion in all the FFGs and came close to 2% in scrapers and shredders (Fig. 2B).

3.5. FAs of algae, FFGs and trophic relations

The PERMANOVA results (Table 4) revealed significant differences in the FA fingerprints among the photoautotrophs taxonomic groups (Supplementary Table 1), and also in macroinvertebrates and photoautotrophs between streams and seasons. FAs composition was significantly different in both streams in summer, but not in winter (*pairwise a*, Table 4). However, FAs composition was significantly different between winter and summer in both streams if taken separately. In Alhárabe, differences were also found between summer and spring, but not between winter and spring (*pairwise b*, Table 4).

The pairwise comparison showed that the photoautotrophic taxonomic groups' fingerprints were significantly different per season in each stream, and the fingerprints of Charophyceae, Cyanobacteria, Ulvophyceae and Chlorophyceae also differed between streams. The cyanobacteria assemblages were significantly distinct in both streams, with Alhárabe much more diverse especially in summer (Table 2). The only species in common in both streams was *Rivularia biolettiana*, whose FAs composition changed from winter to summer, and the main drivers of change were DPA, GLA, ARA, DHA, gondoic acid (20:1n9), stearic acid (18:0) and ARA (20:0).

All the studied macroinvertebrate groups had an n3/n6 ratio above 1 and the presence of DHA was detected (Fig. 2B and Supplementary Table 2). These findings showed that they fed on sources of living or dead

Table 4

Results of the PERMANOVA tests (main and pairwise) with the algae and macroinvertebrate FAs from both streams.

PERMANOVA main test	SS	MS	Pseudo-F	P(perm)
Site	129,89	129,89	3,51	0,00
Season	565,54	282,77	7,65	0,00
Site x Season	100,98	100,98	2,73	0,01
<i>Pairwise: Alharabe (summer)</i>				
Groups comparison	t	P(MC)		
Charophyceae x Collector Gatherers	1.17	0.23		
Chlorophyceae + Rhodophyta x Collector-gatherers	1.68	0.05		
<i>Pairwise: Alharabe (spring)</i>				
Groups comparison	t	P(MC)		
Cyanophyceae x Collector-gatherers	1.34	0.13		
Cyanophyceae x Collector-filterers	1.52	0.08		
Chlorophyceae x Collector-gatherers	1.36	0.15		
Chlorophyceae x Collector-filterers	1.53	0.16		
Chlorophyceae x Predators	1.85	0.06		
Chlorophyceae x Scraper	1.48	0.21		
Charophyceae x Collector-gatherers	0.97	0.43		
Charophyceae x Collector-filterers	2.38	0.18		
Charophyceae x Predators	2.02	0.10		
Bacillariophyta x Collector-gatherers	1.27	0.21		
Bacillariophyta x Scraper	2.76	0.05		
Rhodophyta x Collector-gatherers	1.45	0.14		
Rhodophyta x Collector-filterers	3.68	0.12		
<i>Pair-wise: Alharabe (winter)</i>				
Groups comparison	t	P(MC)		
Cyanophyceae x Collector-gatherers	1.50	0.20		
Cyanophyceae x Collector-filterers	2.55	0.17		
Cyanophyceae x Shredders	2.25	0.19		

organic matter from autochthonous primary producers or on secondary producers with predators.

In Alhárabe, collector-filterers and collector-gatherers had similar fingerprints in summer to the mixed populations of Zygnemataceae and *Oedogonium*. This implies that these invertebrates preferred food sources from filamentous masses composed of *Spirogyra*, *Zygnema* and *Oedogonium*. In winter, collector-gatherers, collector-filterers and shredders had similar fingerprints to Cyanobacteria, which indicates the relation between these trophic groups and the food sources of Cyanobacteria. In spring, all photoautotrophs fingerprints showed significant differences: collector-gatherers also had a similar fingerprint to Chlorophyceae, Charophyceae, Cyanobacteria and diatoms; collector-filterers and predators resembled Chlorophyceae, Charophyceae, Cyanobacteria and Rhodophyta; the fingerprints of scrapers were similar to those of Chlorophyceae, and predators resembled collector-gatherers and collector-filterers, but not scrapers. However, in Chícamo all FA fingerprints were significantly different.

4. Discussion

FAs composition varied among taxonomic groups between both seasons and streams. What these findings evidence is the interest of these compounds in biomonitoring and the search for biomarkers (Descroix et al., 2010; Dalu et al., 2016; DeForest et al., 2016). The effect of environmental variables, such as temperature, conductivity and UV radiation, on FA synthesis has been formerly reported (Aboal et al., 2014a, 2014b; Gonzalez-Silvera et al., 2017; Guo et al., 2016) by showing that FAs can be used as indicators of environmental variations and climate change. The presented data showed that palmitoleic acid and EPA were abundant on an annual basis in both producers and consumers, and in not only tropical streams (Lau et al., 2009), but also in temperate semiarid streams.

Cyanobacteria had a relatively low ARA proportion and the second lowest DHA proportion, but the highest LA proportion and total n6 PUFAs of all the photoautotrophs in our samples. Hence, they seem to sustain the food web of rivers in winter when they clearly predominate among primary

producers. Some authors (Martin-Creuzburg et al., 2012) report how some cyanobacteria do not produce essential amino acids for macroinvertebrates, which represents poor-quality food (Groendahl and Fink, 2017; Guo et al., 2022), or do not provide enough food quality to sustain mussel growth (Bartsch et al., 2017). However, our data contradict this scenario. Cyanobacteria are a diversified group in both taxonomic and environmental terms, and generalisations will be very difficult to make until much more work has been done. Macroinvertebrates' demands are probably lower in winter and can be fulfilled by food sources from cyanobacteria. However, most macroinvertebrates select food sources from other photoautotrophs during other seasons, when the biodiversity of phytobenthic communities increases they feed on them especially during reproductive periods when lack of some PUFAs may be critical (Stanley-Samuels, 1994). The importance of cyanobacteria in coast marsh food webs has already been demonstrated (Currin et al., 2011), and is very likely the same in other habitats. Some cyanobacteria may produce toxins. However, as toxicity effects have been much more widely studied in humans than in aquatic animals, tolerance to toxicity cannot be ruled out (Gustafsson and Hansson, 2004; Gustafsson et al., 2005; Guo and Xie, 2006), and detailed studies are worthwhile.

Diatoms obtained the highest mean ARA, EPA and DHA proportions, relatively low LA and the lowest ALA proportion. These findings confirm that they are an excellent food source (Brett and Müller-Navarra, 1997; Müller-Navarra et al., 2000; Berenike et al., 2009; Guo et al., 2022). As all submerged substrata are usually epiphytic and they are especially abundant during some periods of the year, their presence in all diets in higher or lower proportions is probably unavoidable, and their contribution could be important, and might even be difficult to quantify. However, no significant differences were detected between filamentous green algae with and without diatoms upon sampling when the epiphytic community was not well-developed. The situation could probably change at the end of summer-beginning of autumn when green filaments turn into brown because they are covered by dense assemblages of epiphytic diatoms. A similar proportion of diatom markers has been reported by Descroix et al. (2010) in a French river, but palmitoleic/palmitic ratio may contribute to differentiate the assemblages between the Alhárabe and Chícamo. The relative importance of diatom markers in all our algal assemblages showed the importance of the epiphytism to improve food quality and to reinforce interest in diatoms (selected or consumed by chance) in the trophic webs of these aquatic systems (Guo et al., 2022).

Rhodophytes had the highest n3/n6 ratio and a high proportion of all the EFAs, which means that they can be very valuable food. Unfortunately, normally they are relatively rare and occupy a very small area in most calcareous streams/rivers. This is not the case of Chícamo, where *Chrootheca lobata* covers a high proportion of available substrata and represents an important part of food resources. The high EPA proportion in this alga has already been pointed out (Aboal et al., 2014a, 2014b). Unfortunately, no link was detected in this stream between the collected algae and macroinvertebrates. The existence of other non-detected autotrophic food sources in nearby areas, but beyond our sampling transect, cannot be ruled out. Torres-Ruiz et al. (2007) report the highest LA proportion in *Cladophora glomerata*, but our data showed that other green algae (*Draparnaldia glomerata*, *Spirogyra* sp. and *Chara crassicaulis*) had higher LA concentrations in not only these semiarid streams, but also in heterocystous cyanobacteria (*Dichothrix gypsophila*, *Scytonema myochrous*, *Rivularia bisolettiana*), diatoms and *Kylinella latvica*.

The presence of DHA, which is not synthesised by animals (Olsen, 1999), in all the assayed macroinvertebrate specimens and almost all the algae demonstrates the direct trophic relation between the two groups in the tested streams. The highest DHA levels in macroinvertebrates appeared in scrapers and shredders, whose feeding habits are linked with algae and a high proportion of this n3 PUFA, especially in scrapers and diatoms. A remarkable finding was that the macroinvertebrates that fed on other food sources available in the stream had very low DHA values, such as predators and collector filterers. Fredrickson et al. (1986) and Napolitano (1999)

consider this FA to be a biomarker for dinoflagellates, but its distribution is probably much wider than that known to date, and many more efforts are needed to acquire exact knowledge of algae biomarkers. The similarities in the fingerprints of Charophyceae, Chlorophyceae and collector-gatherers showed that these groups of algae are their main food sources in summer, perhaps due to the dominant position of these algae during that season. In winter, both groups, together with shredders, share similar fingerprints to Cyanobacteria. In spring, collector-gatherers and filterers fed on food sources from almost all the available photoautotrophs (Cyanobacteria, Chlorophyceae, Charophyceae), but scrapers selected Chlorophyceae. These fingerprints indicate the predominance of autochthonous food sources in the trophic relations of semiarid streams through either direct consumption of primary producers like scrapers or the enrichment of allochthonous sources of organic matter like shredders and collectors.

All the collected photoautotrophs could offer consumers the considered EFAs. Most invertebrates fed on the dominant resources available at the time, and only scrapers seemed to display more selective behaviour, probably because of their more restricted capacity to move and/or their feeding apparatus. FAs composition is not constant, but changes with time, habitat and land uses, even for the same species (Larson et al., 2013; Gonzalez-Silvera et al., 2017). This scenario confirms the keen interest they may show in biomonitoring as fingerprints of ecologic status.

5. Conclusion

Despite the relatively low nutritional value of cyanobacteria, compared to other algal groups like diatoms, they represent an essential food resource for all the FFGs of macroinvertebrate and may be critical for their survival in calcareous streams in winter, when other photoautotrophs are absent or are very scarce. During other seasons, the greater heterogeneity and abundance of primary producers seem to hint at macroinvertebrates' selective behaviour for higher quality food sources, and the trophic relation between scrapers and diatoms is the most prominent case.

CRedit authorship contribution statement

Category 1

Conception and design of study: M. Aboal.

Acquisition of data: M. Aboal, M.D. Belando, N. Ubero, D. Gonzalez-Silvera, J.A. López-Jiménez.

Analysis and/or interpretation of data: M. Aboal, M.D. Belando, N. Ubero, D. Gonzalez-Silvera, J.A. López-Jiménez.

Drafting the manuscript: M. Aboal, M.D. Belando, N. Ubero, D. Gonzalez-Silvera, J.A. López-Jiménez.

Revising the manuscript critically for important intellectual content: M. Aboal, N. Ubero.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.156206>.

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