JOURNAL OF LIMNOLOGY

DOI: 10.4081/jlimnol.2015.1268

Carbon stable isotopes in charophyte organic matter

Carbon stable isotope composition of charophyte organic matter in a small and shallow Spanish water body as a baseline for future trophic studies

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Supplementary Tab. 1. Details of the sampling procedure for macrophytes, water and sediments in the studied pond. *C. hispida*, the dominant species, was sampled throughout the study period, while the other species were sampled only when they occurred in the pond. 6→3, 6 complete individuals taken to produce 3 replicates (see text for details).

Site	Water depth (cm)	Macrophytes			Water		Sediment		Sampling period (number of occasions)
		Species	Analysed variables	Replicates / sampling	Analysed variables	Replicates / sampling	Analysed variables	Replicates / sampling	
1	30	*Cha_ his*	δ^{13}C, %C, %N	6→3	pH, alk	1	δ^{13}C, %C, %N	1	05/09-06/10 (2)
2	110			6→3	pH, alk	1	δ^{13}C, %C, %N	1	04/09-03/10 (14)
3	100			6→3	pH, alk	1	δ^{13}C, %C, %N	1	05/09-03/10 (11)
4	130			6→3	pH, alk	1	δ^{13}C, %C, %N	1	07/09-03/10 (9)
5	20	*Nit_ hya*	δ^{13}C, %C, %N	6→3	pH, alk	1	δ^{13}C, %C, %N	1	05/09-08/09 (4)
6	60	*Tol_glo*	δ^{13}C, %C, %N	6→3	-	-	-	-	01/10-03/10 (3)
7	100	*Myr_ spi*	δ^{13}C, %C, %N	6→3	-	-	-	-	05/09-06/09 (2)
8	200	-	-	-	Ox, Cond, TP, SRP, TN, COD, Chl *a*	1	-	-	04/09-03/10 (13)

Cha_his, Chara hispida; Nit_hya, Nitella hyalina; Tol_glo, Tolypella glomerata; Myr_spi, Myriophyllum spicatum; alk, alkalinity; Ox, oxygen; Cond, conductivity-salinity; TP, total phosphorus; TN, total nitrogen; SRP, soluble reactive phosphorus; COD, chemical oxygen demand; Chl *a*, sestonic chlorophyll *a*.

Supplementary Tab. 2. Results of the ANOVA tests applied on the $\delta^{13}C$, %C and % N data of macrophytes, water and sediment.

Comparison		Test	Inter-group d.f.	Intra-group d.f.	F			P-value		
Inter-sites[1]										
Alkalinity		One-way ANOVA	2	21	1.1			0.360		
pH		Kruskal-Wallis						0.977		
Sediment $\delta^{13}C$		One-way ANOVA	2	30	4.9			0.014		
		Post-hoc Bonferroni								
		Site 2/site 3						0.442		
		Site 2/site 4						0.011		
		Site 3/site 4						0.315		
Inter-species										
Chara/Nitella/Myriop.	$\delta^{13}C$					36.7			<0.001	
	%C	One-way ANOVA	2	23		15.1			<0.001	
	%N					2.6			0.100	
		Post-hoc Bonferroni						$\delta^{13}C$	%C	
		Chara/Nitella						<0.001	0.501	
		Chara/Myriop.						<0.001	<0.001	
		Nitella/Myriop.						<0.001	<0.001	
Chara/Nitella		Two-way ANOVA	1	25	$\delta^{13}C$	%C	%N	$\delta^{13}C$	%C	
		Species			9.0	10.7	-	<0.001	0.003	
		Time			12.1	3.3	-	<0.001	0.053	
		Species x time			13.0	0.4	-	<0.001	0.671	
Chara/Tolypella		Two-way ANOVA	1	25	$\delta^{13}C$	%C	%N	$\delta^{13}C$	%C	%N
		Species			10.9	1.1	4.9	0.003	0.295	0.036
		Time			16.5	1.1	5.5	<0.001	0.354	0.011
		Species x time			4.3	0.03	1.4	0.024	0.968	0.264
In *Chara hispida*		Two-way robust ANOVA	2	381				$\delta^{13}C$	%C	%N
		Site 2/site 3						0.682	0.835	0.529
Inter-sites[1], inter-parts[2]		Site 2/site 4						0.002	0.230	0.771
*non-significant interactions		Apex/internodes						0.108	0.001	0.064
		Apex/rhizoid						0.004	0.053	0.029
	$\delta^{13}C$					0.2			0.654	
Oogon.+anther.+oospor. *vs* the rest	%C	One-way ANOVA	1	176		44.3			<0.001	
	%N					8.2			0.005	

Comparison		Test	Inter-group d.f.	Intra-group d.f.	F	P-value
Seasonality: Spring-Summer / Autumn-Winter	$\delta^{13}C$	One-way ANOVA	1	124	116.6	<0.001
	%C		1	131	0.02	0.879
	%N		1	102	11.0	0.001
Inter-parts in *Nitella*	$\delta^{13}C$	One-way ANOVA	2	30	5.7	0.008
	%C				4.7	0.017
	%N				5.0	0.018
		Post-hoc Bonferroni				$\delta^{13}C$ / %C / %N
		Apex/internodes				0.074 / 0.733 / 0.024
		Apex/rhizoids				0.472 / 0.014 / 0.229
		Internodes/rhizoids				0.008 / 0.222 / 0.328
Inter-parts in *Tolypella*	$\delta^{13}C$	One-way ANOVA	2	25	4.3	0.025
	%C				3.5	0.046
	%N				2.0	0.159
		Post-hoc Bonferroni				$\delta^{13}C$ / %C
		Apex/internodes				0.233 / 0.342
		Apex/rhizoids				0.474 / 0.042
		Internodes/rhizoids				0.024 / 0.301

d.f.: degrees of freedom. Inter-sites[1]: considering sites 2-4 for sediment and *C. hispida*; inter-parts[2]: considering apical nodes, internodes and rhizoids for *C. hispida*.

Supplementary Tab. 3. Results of Pearson correlation analyses applied between the *C. hispida* $\delta^{13}C$, %C and % N data and the limnological variables, and between *C. hispida* $\delta^{13}C$, %C and % N values and sediment ones.

Comparison		n	r	P-value
***C. hispida* and limnological variables**				
Temperature	$\delta^{13}C$	13	0.56	0.047
pH			-0.78	0.002
Sestonic Chl *a*			-0.69	0.010
Chemical oxyg. demand			-0.63	0.022
Temperature	%N		-0.81	0.001
pH			0.74	0.004
***C. hispida* and sediment**				
Overall*	$\delta^{13}C$	33	-0.15	0.405
Site 2 sedim./rhizoids		12	0.62	0.031
Overall*	%C	33	-0.65	0.723
Overall*	%N	28	-0.27	0.166

*Including all sediment sites and all parts of *C. hispida*.