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Stable isotope variability in macrophytes and sediments of two shallow lakes in Sicily: a first contribution for ecological monitoring

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Abstract

We present the first isotopic dataset for two shallow, slightly brackish Mediterranean lakes in Sicily colonised by *Chara baltica* (Lago Preola) and *Najas marina* (Lago Murana). Seasonal stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and elements concentration (C%, N%) were measured in macrophytes and sediments over one year. Lago Murana sediments were more ^{13}C-depleted than Lago Preola. Both lakes showed similar seasonality: ^{13}C-enriched and ^{15}N-depleted values in spring–summer, lower in autumn–winter. Greater seasonal variability in Preola indicates macrophyte-derived organic matter input to sediments, whereas Murana reflects run-off from surrounding pastures and vineyards. These results offer a preliminary framework for future work.

Key words: aquatic plants; charophytes; inland waters; karstic lakes; stable isotopes.

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The study of stable isotope physiology in plants is still underutilised compared to animals, although it can improve our understanding of ecological interactions and provide a tool for planning and monitoring conservation (Snyder *et al.,* 2022). Even fewer investigations using stable isotope analysis have been carried out on freshwater aquatic plants compared to terrestrial plants, partly due to a large, often unexplained variability (Chappuis *et al.,* 2017). Stable isotope analysis has been used to investigate ecological processes, particularly food webs within aquatic ecosystems: carbon isotope ratios ($\delta^{13}C$) are usually used to track the sources of energy fuelling the food webs, while nitrogen isotope ratios ($\delta^{15}N$) are used to estimate trophic levels of organisms and sources of inorganic nitrogen (Rodrigo *et al.,* 2016; Matuszak *et al.,* 2011).

This paper reports preliminary observations on the carbon and nitrogen stable isotope composition of sediments and macrophytes sampled in July and October 2018, February and June 2019 within two small shallow lakes in a protected area. Our aim was to provide a baseline for future ecophysiological studies of the two currently dominating macrophytes and to provide reference values both for studies on trophic relations within the basins and for conservation planning in the natural reserve.

Lago Preola (37° 37' 13.33" N, 12° 38' 28.18" E, ~270 hectares, maximum depth 1.5 m) and Lago Murana (37° 37' 35.77" N, 12° 38' 3.29" E, ~74 hectares, maximum depth 1 m) are two small shallow lakes, located in SW Sicily near Mazara del Vallo (Trapani, Italy), in a karstic depression within the Nature Reserve "Lago Preola e Gorghi Tondi" and a Natura 2000 site (Special Area of Conservation cod. ITA010005, Special Protection Area cod. ITA010031).

To characterise the physicochemical conditions at the sampling sites, water temperature, conductivity, salinity and total dissolved solids (TDS) were measured between 11:00 and 13:00 h at each sampling date with a waterproof portable meter (Hanna HI9835). Water pH was measured with a portable pH meter (Hanna HI8424). Lago Preola showed greater thermal extremes (29.6±0.3°C in summer; 12.4±0.0°C in winter) compared to Lago Murana (28.6±0.2°C in summer; 13.6±0.8°C in winter). Salinity, conductivity and TDS were also higher in Lago Preola (average yearly values of 2.5 PSU, 3.7 mS cm^{-1}, 1846 mg L^{-1} respectively) than in Lago Murana (average yearly values of 1.7 PSU, 2.5 mS cm^{-1}, 1258 mg L^{-1} respectively). Both lakes were alkaline, with different seasonal pH peaks: Lago Preola reached maximum pH (9.0±0.1) in summer, while in Lago Murana maximum pH (9.6±0.9) was recorded in autumn. In both lakes lowest pH values were recorded in spring and winter, ranging between 7.7 and 8.0.

Two different macrophytes were sampled from the lakes: the so-called "Mediterranean Chara" (*sensu* Blindow *et al.*, 2024) (Charophyta, Characeae) in Lago Preola and the angiosperm *Najas marina* subsp. *armata* Horn (Tracheophyta, Hydrocharitaceae) in Lago Murana. Although Mediterranean Chara has previously been identified as *Chara baltica* (Hartm.) Bruzelius by several authors, recent work indicates it is a distinct taxon with unresolved status; thus, the provisional name "Mediterranean Chara" (hereafter *Chara*) has been adopted (Blindow et al., 2024).

Najas marina L. is an angiosperm with a near-cosmopolitan distribution. The population in Lago Murana has been identified as *Najas marina* subsp. *armata* (hereafter "*Najas*"), a taxon predominantly distributed in tropical and subtropical Africa and likely a recent northward range-shifter to Sicily (Troia, 2022). Dried specimens of the two taxa have been deposited at the *Herbarium Mediterraneum Panormitanum* (PAL) of the University of Palermo.

The macrophytes and sediment from the two lakes were analysed for organic carbon (C_{org}), total nitrogen (N) and stable isotope composition. *Chara* and *Najas* samples were collected with a hook in the littoral zone at about 2 m from the shore, at a depth of approximately 0.5 m, from two different sites for each lake. Two macrophyte samples were taken per site per date. Since the size of the lakes was relatively small and preliminary tests had shown no significant differences between sites of the same lake, data from the two sites of each lake were pooled. Macrophyte samples were sealed in plastic bags and stored in a cool-box till reaching the laboratory. At the same sites, surface sediment samples (first three cm) were collected with a stainless-steel trowel and stored in 50-mL plastic tubes. In the laboratory, macrophytes were rinsed with non-chlorinated tap water to remove epiphytes. Samples were oven dried (60°C) to constant weight, ground to a fine powder with a laboratory ball mill (Retsch MM 400) and weighed into tin capsules. Subsamples were acidified with 1N HCl to remove inorganic carbon and analysed for C_{org} and $\delta^{13}C$; untreated subsamples were analysed for total N and $\delta^{15}N$. Analyses of C_{org}, total N, $\delta^{13}C$ and $\delta^{15}N$ were run with a Thermo Fisher Delta Plus XP mass spectrometer, coupled with a Thermo Fisher Flash EA 1112 elemental analyser. Analyses were conducted in duplicate, for a total of 28 samples. Isotopic values were reported in delta (δ) units referred to International Atomic Energy Agency IAEA-CH-6 and IAEA-NO-3, calibrated in turn with V-PDB and atmospheric nitrogen standards for carbon and nitrogen, respectively. Measurement uncertainty for $\delta^{13}C$ and $\delta^{15}N$ was ±0.1‰.

Results were reported as mean values ±SD. To assess seasonal variability in stable isotope values (Fig. 1A), June and July samples were grouped as spring/summer (SS) and October and February ones as autumn/winter (AW). Data were analysed using the software package SigmaPlot 16 (Systat Software, Inc., San Jose, CA, USA). Datasets were tested for normality (Shapiro-Wilk) and homogeneity of variance (Brown-Forsyth). Statistical significance was set at $p<0.05$. Seasonal variation within lakes in C_{org}, total N, $\delta^{13}C$ and $\delta^{15}N$ data of the macrophytes and the sediments was analysed using One-Way Analysis of Variance (ANOVA), followed by Fisher's LSD *post-hoc* test. Linear regression (least-squares) was used to assess relationships between $\delta^{13}C$ and water pH. Sediments from Lago Murana were depleted in $\delta^{13}C$ compared to sediments of Lago Preola. Within each basin, $\delta^{13}C$ did not differ significantly between AW and SS. In contrast, $\delta^{15}N$ of sediments showed seasonal variability, with higher values in AW. This seasonal increase was significant both for Lago Preola ($F=18.91$, df=3, $p=0.025$) and for Lago Murana ($F=26.86$, df=3, $p=0.03$). Lake sediments contain organic and inorganic matter deriving both from within the water body and from the lake catchment, so their stable C and N isotope values are influenced by sources and biological processes (Woodward *et al.,* 2012).

In macrophytes, *Chara* samples collected in AW were depleted in $\delta^{13}C$ by 3‰ compared with SS samples ($F=29.97$, df=3, $p=0.002$), following a seasonal pattern similar to that reported for *C. hispida* in Spain (Rodrigo *et al.,* 2016). The AW-SS difference in $\delta^{15}N$ was smaller (1.1‰) than the one in $\delta^{13}C$. Considerable temporal and spatial variability in $\delta^{13}C$ and $\delta^{15}N$ has been previously reported for the genus *Chara* (Matuszak *et al.*, 2011; Boon

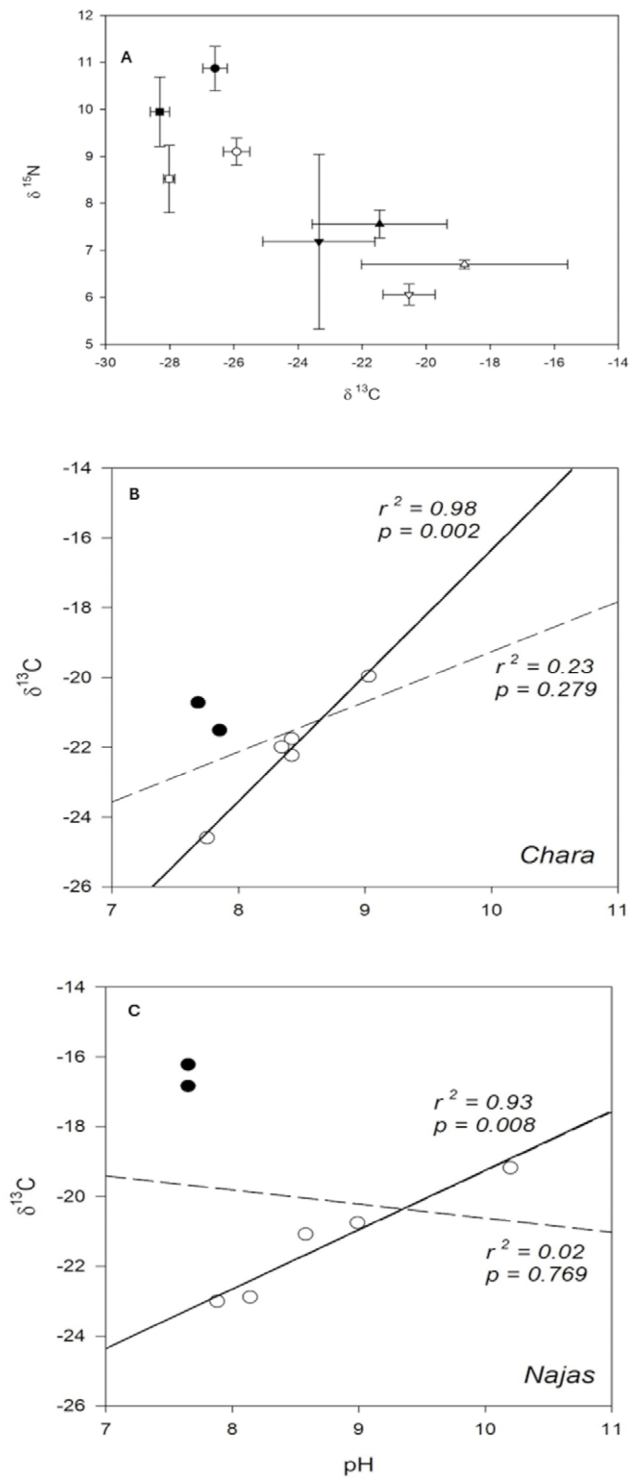

Fig. 1. A) Stable isotope ratios of carbon and nitrogen in samples of Lago Preola sediment (●), Lago Murana sediment (■), *Chara* (▼) in Lago Preola and *Najas* (▲) in Lago Murana collected in different seasons. Closed symbols refer to autumn/winter (AW), open symbols to spring/summer (SS); data are reported as mean values±SD (n=4). Carbon stable isotope values ($\delta^{13}C$) of *Chara* (B) and *Najas* (C) in relation to water basin pH. The dashed line indicates the estimated linear regression of the entire data set, while the solid regression line was estimated without including data from June sampling (closed circles). Further explanation in the text.

Tab. 1. C%, N% and C/N values in sediment and in macrophytes. Data are reported as mean values ±SD (n=4).

	C%		N%		C/N	
	SS	AW	SS	AW	SS	AW
Preola sediment	15.2±1.0	14.2±2.9	0.8±0.0	1.6±0.4	18.1±2.0	8.9±0.5
Murana sediment	13.5±0.9	16.8±0.8	1.2±0.3	1.7±0.2	12.1±2.1	10.4±1.6
Chara	20.9±2.0	19.1±0.6	1.1±0.1	1.8±0.4	19.7±2.4	10.9±2.3
Najas	28.9±2.9	24.7±3.2	2.3±0.1	2.6±0.9	12.7±1.5	10.3±2.8

AW, autumn/winter; SS, spring/summer.

and Bunn, 1994); although we observed pronounced intra-seasonal variability (greater in AW than SS), preliminary site-based analyses in Lago Preola showed no significant site effects.

Najas exhibited an AW depletion in $\delta^{13}C$ of 2‰ relative to SS, but this difference was not significant (F=24.37, df=3, p=0.076). The AW–SS difference in $\delta^{15}N$ was minor and not significant (0.9‰). Intra-seasonal variability was high for $\delta^{13}C$ in both seasons but low for $\delta^{15}N$. Variability in $\delta^{13}C$ of aquatic macrophytes may reflect both intrinsic physiological factors and extrinsic environmental drivers, whereas variability in $\delta^{15}N$, although influenced by assimilation discrimination (Evans, 2001), is primarily determined by extrinsic factors such as basin geology and land use (Chappuis et al., 2017). Our results agree with those of Chappuis *et al.* (2017), showing large variability and similar range of values as those reported for low altitude coastal basins. Asaeda *et al.* (2008) also reported generally higher values of $\delta^{13}C$ in *Najas* species than in Charophytes; for $\delta^{15}N$, they observed higher values in charophytes, a pattern we did not detect (Asaeda *et al.,* 2008). Seasonal trends in sediment and macrophytes were similar in both lakes, with more ^{13}C-enriched and ^{15}N-depleted values in summer than in AW. However, seasonal $\delta^{13}C$ shifts were larger in Lago Preola than in Lago Murana. This suggests a more substantial contribution of macrophyte-derived organic matter to sediment in Lago Preola, whereas land-derived carbon inputs may dominate in Lago Murana (Li *et al.,* 2016). This hypothesis seems to be confirmed by sediment $\delta^{15}N$ values that indeed suggest the contribution of run-off from the fields surrounding the lakes. The study area is rural, surrounded by vineyards and used periodically for sheep grazing. The increase in $\delta^{15}N$ found in AW sediment samples could be related to rainfall, that may mobilize fertiliser residues or manure into the lakes. The parallel seasonal change in $\delta^{15}N$ in sediment and in macrophytes suggests for both lakes the occurrence of ^{15}N-enriched nutrients in AW more than in SS. These observations underscore the need to account for macrophyte isotopic variability when assessing trophic relationships (Boon and Bunn, 1994).

One of the factors affecting the discrimination of macrophytes against ^{13}C is related to the pH of the water body: pH influences macrophyte discrimination against ^{13}C because higher pH shifts dissolved inorganic carbon toward HCO^{3-}, and photosynthetic uptake of greater proportions of HCO^{3-} tends to enrich $\delta^{13}C$ (Chappuis *et al.,* 2017). Positive correlations between $\delta^{13}C$ values and pH are frequently reported (Chappuis *et al.,* 2017; Pronin *et al.,* 2023). In our data, the linear relationship between basin water pH and macrophyte $\delta^{13}C$ was not significant for the full dataset but became significant after excluding June samples (Figs. 1B and 1C). One possible explanation could be that in June both *Najas* and *Chara* species start actively growing after an overwintering stage of rest. At least for seagrasses, it has been reported that young, developing leaves tend to be enriched in ^{13}C as their high productivity leads to a reduction in the discrimination against the heavy isotope ^{13}C (Vizzini *et al.,* 2003), though this analogy with the apical shoots of charophytes is not univocal (Rodrigo *et al.,* 2016).

Organic C%, N% and C/N ratio for lake sediments and macrophytes are reported in Tab. 1. The charophyte had a slightly lower organic carbon content than the angiosperm, as has been reported for other aquatic macrophytes (Rodrigo *et al.,* 2016), and seasonal changes were small for both species. Sediment C/N in Lago Preola was significantly lower in AW than in SS (F=29.51, df=3, p<0.001), whereas the decrease in Lago Murana was slight. The C/N ratio in the macrophytes followed the same trend as that of the sediment. In *Chara*, C/N values in SS were significantly higher (F=29.51, df=3, p<0.001), almost twice those measured in AW samples. In *Najas*, the pattern of the C/N values showed only a slight but not significant decrease from SS to AW. A higher C/N ratio has been related to lower palatability (Reitsema *et al.,* 2020), so it could be speculated that in water bodies where *Chara* and *Najas* should coexist, the latter could be preferred by grazers in summer. Macrophyte elemental composition likely reflects environmental nutrient availability (Close *et al.*, 2020), and the lower C/N of *Chara* in autumn–winter aligns with the sediment $\delta^{15}N$ shifts linked to catchment land use.

This one-year dataset provides a preliminary baseline for further studies of these lakes, offers initial $\delta^{13}C$ and $\delta^{15}N$ values of the dominant macrophytes crucial for interpreting their roles in food webs, and highlights the value of integrated ecosystem studies that concurrently consider producers, consumers, sediments, water chemistry, climate variables and socio-economic context.

References

Asaeda T, Yamamuro M, Siong K, Rajapakse L, Sanderson B, 2008. Nutrient sources for charophytes and *Najas marina* in Myall Lake, Australia, indicated by carbon and nitrogen stable isotope ratios. SIL Proceedings 30:401-405.

Blindow I, Becker R, Romanov R, Schubert H, van de Weyer K, 2024. Taxon of uncertain affiliation "Mediterranean Chara", pp. 1039-1049. In: H. Schubert, et al. (eds.), Charophytes of Europe. Springer.

Boon PI, Bunn SE, 1994. Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. Aquat Bot 48:99-108.

Chappuis E, Seriñà V, Martì E, Ballesteros E, Gacia E, 2017. Decrypting stable-isotope ($\delta^{13}C$ and $\delta^{15}N$) variability in aquatic plants. Freshwater Biol 144:1807–1818.

Close SL, Hacker SD, Menge BA, Chan F, Nielsen KJ, 2020. Biogeography of macrophyte elemental composition:

Spatiotemporal modification of species-level traits. Ecosystems 23:1494-1522.

Evans RD, 2001. Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–126.

Li Y, Zhang H, Tu C, Fu C, Xue Y, Luo Y, 2016. Sources and fate of organic carbon and nitrogen from land to ocean: Identified by coupling stable isotopes with C/N ratio. Estuar Coast Shelf Sci 181:114-122

Matuszak A, Voigt CC, Storch I, Bauer HG, Quillfeldt P, 2011. Depth-specific and spatiotemporal variation of $\delta^{13}C$ and $\delta^{15}N$ in Charophytes of Lake Constance: implications for food web studies. Rapid Commun Mass Spectrom 25:2089–2094.

Pronin E, Banaś K, Chmara R, Ronowski R, Merdalski M, Santoni AL, Mathieu O, 2023. Do stable carbon and nitrogen isotope values of *Nitella flexilis* differ between softwater and hardwater lakes? Aquat Sci 85:79

Reitsema RE, Preiner S, Meire P, Hein T, De Boeck G, Blust R, Schoelynck J, 2020. Implications of climate change for submerged macrophytes: effects of CO_2, flow velocity and nutrient concentration on *Berula erecta*. Aquat Ecol 54:775-793.

Rodrigo MA, García A, Chivas AR, 2016. Carbon stable isotope composition of charophyte organic matter in a small and shallow Spanish water body as a baseline for future trophic studies. J Limnol 75:1268.

Snyder KA, Robinson SA, Schmidt S, Hultine KR, 2022. Stable isotope approaches and opportunities for improving plant conservation. Conserv Physiol 10:coac056.

Troia A, 2022. The unnoticed northward expansion of *Najas marina* subsp. *armata* (Hydrocharitaceae) in the Mediterranean area: an effect of climate change? Willdenowia 52:91–101.

Vizzini S, Sarà G, Mateo MA, Mazzola A, 2003. $\delta^{13}C$ and $\delta^{15}N$ variability in *Posidonia oceanica* associated with seasonality and plant fraction. Aquat Bot 76:195-202.

Woodward CA, Potito AP, Beilman DW, 2012. Carbon and nitrogen stable isotope ratios in surface sediments from lakes of western Ireland: implications for inferring past lake productivity and nitrogen loading. J Paleolimnol 47:167-184.

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