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STABLE ISOTOPE VALUES OF THE SEAGRASS *POSIDONIA OCEANICA* IN PANAREA HYDROTHERMAL VENTS

ISOTOPI STABILI DELLA FANEROGAMA MARINA POSIDONIA OCEANICA *NEI SISTEMI IDROTERMALI DI PANAREA*

Abstract - *The hydrothermal area of Panarea (Aeolian Islands) represents a valuable natural laboratory to study the effects of ocean acidification (OA) on* Posidonia oceanica *(L.) Delile, that populates these shallow vents expressing unique plant features. Stable isotopes of carbon, nitrogen and sulphur (* $\delta^{13}C$*,* $\delta^{15}N$*,* $\delta^{34}S$*), powerful indicators of the origin and dynamics of these elements widely applied to* P. oceanica*, were measured in leaves collected in May 2017 and September 2018 in three vent systems and two control areas off Panarea.* $\delta^{13}C$ *results show that hydrothermalism is still active in the area, and that* P. oceanica *can record changes occurring in the surrounding environment. The coupling with* $\delta^{15}N$ *provides useful information about the physiology of the plant under OA. The preliminary* $\delta^{34}S$ *results encourage further investigation of this element cycle, to understand the dynamics and origin of sulphur in the hydrothermal areas.*

Key-words*: Aeolian Archipelago,* CO_2 *emissions,* $\delta^{13}C$*,* $\delta^{15}N$*,* $\delta^{34}S$

Introduction - Hydrothermal vents are extreme habitats where high levels of CO_2, toxic gases (*e.g.*, H_2S), and the potential excess of metal ions may pose a physiological challenge to the survival and growth of marine organisms. The decrease of pH and the alteration of carbonate chemistry, leading to seawater acidification, is one of the best-known stressors occurring in these altered systems. Indeed, the use of areas naturally affected by CO_2 release, such as volcanic vents, as natural laboratories is known to provide important insights for ocean acidification (OA) research, as laboratory/mesocosm experiments are limited due to their small scale and basic conditions, although input of other toxics can bias the effect of OA. The area of Panarea Island (Aeolian Archipelago), one of the largest hydrothermal fields in the Mediterranean Sea, is considered a valid natural laboratory due to the widespread presence of CO_2 in the hydrothermal fluids of its various vent's systems (Saidi *et al.*, 2023). Many of these shallow vents host extensive settlements of the seagrass *Posidonia oceanica* (L.) Delile, where unique plant characteristics occur, such as reduced plant size, lack of a seasonal growth cycle, and a poor epiphytic leaf community (Gambi *et al.*, 2023). Therefore, *P. oceanica*, occurring also in other vents (*e.g.,* Ischia vents, Scartazza *et al.*, 2017; Gambi *et al.*, 2023 and literature herein), represents a good model for studying the effects of OA in Panarea vents. Stable isotopes, a widely used method and a powerful tool for detecting the origin of carbon and nitrogen and their dynamics, have been widely applied to *P. oceanica* (Vizzini *et al.*, 2010). Here, we measured the patterns of $\delta^{13}C$ and $\delta^{15}N$ as well as $\delta^{34}S$ in leaves of *P. oceanica* shoots collected in May 2017 and September 2018 in three vent systems (Bottaro, Campo 21, Hot-Cold) and two control areas (Raja and Formiche) off Panarea (Fig. 1) in order to investigate the possibility of tracing the response of the plant in complex environments where high CO_2 concentration co-occurs with H_2S.

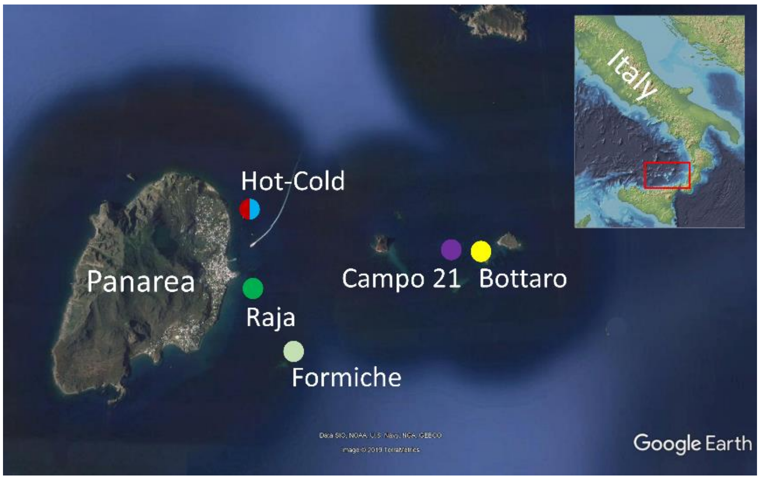

Fig. 1 - Map of the study area and sampling stations. Hot-Cold, Bottaro and Campo 21 stations are the sites interested by CO_2 emissions, while Raja and Formiche are the control stations.
Mappa dell'area di studio e stazioni di campionamento. Le stazioni Hot-Cold, Bottaro e Campo 21 sono caratterizzate da emissioni di CO_2, mentre Raja e Formiche sono le stazioni di controllo.

Materials and methods – During the survey conducted in May 2017, two stations with different degrees of hydrothermal activity were selected as impacted sites (Fig. 1): Bottaro, a coarse sediment station characterised by gaseous CO_2 emissions, and Hot-Cold, a sandy station characterised by a very heterogeneous sediment temperature (Hot: up to <50°C; Cold: ~20°C, the two stations are about 1 m apart). Control stations (Raja and Formiche, Fig. 1) were established to account for the natural variability of such a complex environment. During the survey conducted in September 2018 only Raja was sampled as control station, and, in addition to Bottaro and Hot-Cold, another CO_2 impacted station (Campo 21) was selected as it was characterised by strong gaseous CO_2 emissions (Fig. 1). During the survey conducted in May 2017, a single spatial replicate of *P. oceanica* was sampled at each station where 3 shoots were sampled and 3 adult leaves (older, externalmost on the shoot) were selected from each shoot. In September 2018, 3 spatial replicates of *P. oceanica* were collected at each station. From each replicate, 4 shoots were selected and one adult and one intermediate (younger, internalmost on the shoot) leaf were collected from each shoot. After removing the non-photosynthesising part (brown tissues), the leaves were freeze-dried and homogenised using an agate mortar. The stable isotope composition of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) was determined on samples from both surveys, while the isotopic composition of sulphur ($\delta^{34}S$) was determined only in samples collected in September 2018. Carbon, nitrogen and sulphur isotopic composition was analysed simultaneously on an IsoPrime100 – Vario PYRO Cube (OH/CNS Pyrolyser/Elemental Analyzer) (IsoPrime, Cheadle, Hulme, UK) using the procedure described in Hamzić Gregorčič *et al.* (2020).

Results - The carbon isotopic composition ($\delta^{13}C$) of *P. oceanica* leaves (Fig. 2a) was significantly lower ($p \leq 0.01$) in September 2018 (−17.99 ± 1.67 ‰) than in May 2017 (−15.38 ± 1.53 ‰), even when excluding the extremely low values measured at Campo 21 station (−27.02 ± 2.20 ‰). This difference was particularly significant ($p \leq 0.01$) when only Bottaro and Hot stations were considered (May 2017: −14.13 ± 0.80 ‰; September 2018: −18.77 ± 1.25 ‰). In the September 2018 survey, differences, although not significant, were recorded between intermediate and adult leaves, the latter showing slightly lower values. The differences between the two surveys could be explained by the seasonal cycle of the plant. From April to June, *P. oceanica* growth reaches the highest rates, therefore the carbon demand increases and favours lower discrimination against heavy isotope, resulting in ^{13}C enrichment and consequently in higher $\delta^{13}C$ values. On the other hand, autumn and winter are characterised by a typical decrease of leaf growth, so that lower $\delta^{13}C$ values can be expected. In addition, Bottaro and Hot-Cold stations are characterised by CO_2 emissions that increase carbon availability to the plant, favouring higher photosynthetic activity. This increased photosynthetic CO_2 uptake results in the higher ($p \leq 0.05$) $\delta^{13}C$ values in Bottaro and Hot than in the control stations (−15.91 ± 1.30) measured in May 2017. The very low $\delta^{13}C$ values measured in *P. oceanica* leaves collected in Campo 21 in September 2018 are comparable to the data reported by Vizzini *et al.* (2010) after the strong degassing event occurred in 2002 at Bottaro, indicating that intense hydrothermal activity still occurs. On the other hand, $\delta^{13}C$ values measured at Bottaro (−18.62 ± 1.02 ‰) were higher than at Campo 21 and more similar to the control station Raja (−16.04 ± 1.05 ‰), confirming that a state of equilibrium was reached after the gas outburst and crater formation (Noè *et al.*, 2020). Except for the high values measured at Campo 21 station, no difference was observed in nitrogen isotopic composition ($\delta^{15}N$) between the two sampling campaigns (Fig. 2a). The high $\delta^{15}N$ values at Campo 21 (2.69 ± 0.39 ‰) also indicate the strong hydrothermal activity at this site. As previously observed at other hydrothermal areas (Scartazza *et al.*, 2017), $\delta^{15}N$ values appear to increase at sites characterised by CO_2 emissions, similar to Campo 21.

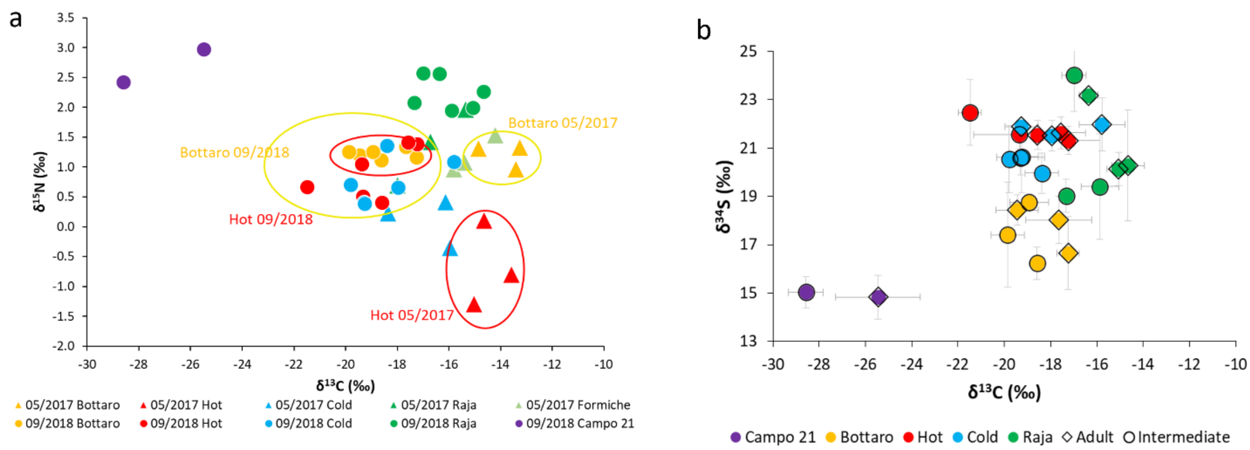

Fig. 2 – (a) Isotopic composition of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) of *P. oceanica* leaves collected during May 2017 and September 2018 surveys. (b) Isotopic composition of carbon and sulphur ($\delta^{13}C$ and $\delta^{34}S$) of *P. oceanica* leaves collected during September 2018 survey.

(a) Composizione isotopica del carbonio e dell'azoto (*δ13C and δ15N) delle foglie di* P. oceanica *prelevate durante le campagne di maggio 2017 e settembre 2018. (b) Composizione isotopica del carbonio e dello zolfo (δ13C and δ34S) delle foglie di* P. oceanica *prelevate durante la campagna di settembre 2018.*

The sulphur isotopic composition ($\delta^{34}S$; Fig. 2b) measured at control station Raja (21.00 ± 2.08 ‰) was comparable to the average $\delta^{34}S$ of seawater sulphate (ranging between 18 and 22 ‰; Böttcher *et al.,* 2007), while statistically significantly lower values ($p \leq 0.01$) were found at Bottaro and Campo 21 (19.62 ± 1.49 ‰), suggesting the presence of different sulphur sources at vent sites, as no difference was found between adult and intermediate leaves. Sulphide $\delta^{34}S$ is affected by many processes involved in the sulphur cycle, particularly at the sediment-water interface, thus it is difficult to attribute the observed differences to a specific sulphur source without data from the surrounding area.

Conclusions - The results of the two surveys confirm that *P. oceanica* is a valuable bioindicator of surrounding ecosystem conditions because it can detect both short- and long-term changes. The carbon stable isotope composition of seagrasses is a useful indicator of the uptake of various carbon sources and of changes in seawater CO_2 concentrations in hydrothermal areas. The results of the two surveys confirm that hydrothermalism is still high in the area and that *P. oceanica* can register changes in the surrounding environment, but can also recover after extreme events such as the 2002 outburst occurred in Bottaro in line with results by Noè *et al.* (2020). In addition, the coupling with stable nitrogen isotopes could provide detailed information about the physiology of the plant under ocean acidification pressure. The preliminary results of sulphur isotopes in the Panarea vents encourage a more detailed study of the cycling of this element in the area. Despite its low concentrations in Panarea hydrothermal fluids, the presence of H_2S is well documented (Italiano and Nuccio, 1991), so its isotopic composition could provide interesting information about its origin and biological utilization. Further and detailed studies are needed to investigate the carbon, nitrogen, and sulphur cycles in such a complex ecosystem and to determine how the isotopic composition of *P. oceanica* is affected by environmental changes caused by both hydrothermal activity and anthropogenic pressures. A deeper understanding of these processes would be particularly useful to better understand the impacts of climate change and ocean acidification in particular, and on the physiology of this key habitat-former species.

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