NOTA/ NOTE

PRELIMINARY STUDY ON THE EFFECT OF NATURAL ACI-DIFICATION ON MEIOFAUNAL COMMUNITIES IN SANDY SUBSTRATES

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Abstract

In recent years, research on ocean acidification in $\rm CO_2$ vents has increased, but very few have considered the meiofaunal communities living in sandy substrates, despite their importance as bioindicators. Therefore, the aim of this work is to carry out a preliminary study of the meiofauna associated with sandy substrates in the acidified system of La Palma, Canary Islands, which includes coastal lagoons with very extreme environments. The results obtained based on abundance confirm a significant change in the meiofaunal communities in these coastal lagoons. We found an increase in bioindicator taxa of altered environments: oligochaetes, nematodes, copepods and, to a lesser extent, ostracods and acarids. While xenacelomorphs are the most affected by acidification as they are the only group with a decrease in abundance. All this indicates an alteration in the composition of the meiofaunal communities due to the extreme acidification in the coastal environment of La Palma.

Keywords: Ocean acidification, CO₂ vent, Echentive lagoons, bioindicators.

ESTUDIO PRELIMINAR SOBRE EL EFECTO DE LA ACIDIFICACIÓN NATURAL EN LAS CO-MUNIDADES MEIOFAUNALES DE SUSTRATOS ARENOSOS

Resumen

Muy pocas investigaciones realizadas en afloramientos de CO_2 han tenido en cuenta las comunidades meiofaunales de los sustratos arenosos, a pesar de su importancia como bioindicadoras. Por ello, este trabajo tiene como objetivo realizar un estudio preliminar de la meiofauna asociada a los ambientes arenosos en el sistema acidificado de La Palma, islas Canarias, donde se incluyen unas lagunas costeras con ambientes muy extremos. Los resultados obtenidos confirman un cambio significativo en las comunidades meiofaunales en estas lagunas costeras. Encontramos un aumento de taxones bioindicadores de ambientes alterados: oligoquetos, nemátodos, copépodos y, en menor medida, ostrácodos y ácaros. Mientras que los xenacelomorfos son los más afectados por la acidificación ya que es el único grupo con una disminución de su abundancia. Todo ello nos indica una clara alteración en la composición de las comunidades meiofaunales debido a la extrema acidificación en el ambiente costero de La Palma.

Palabras clave: acidificación oceánica, afloramiento de CO₂, lagunas de Echentive, bioindicadores. In recent years, much research has focused on CO_2 vents as a natural laboratory for the study of ocean acidification (OA). These studies have been mainly focusing on rocky bottom communities (González-Delgado and Hernández 2018; Foo *et al.* 2018). However, there is a lack of studies focusing on other types of substrates like sandy environments. The only example recently published is the work made by Fanelli *et al.* (2022) which considers the role played by meiofauna as bioindicators and bioturbators of sediments (Kennedy-Jacoby 1999). The meiofauna has an important ecological role at the marine communities due to the close relationship and influence on other trophic levels (Danovaro *et al.* 2007). Therefore, the study of these organisms in acidified ecosystems is essential to determine the possible indirect effects of OA.

The CO₂ vent off Punta de Fuencaliente, located in the south coast of the island of La Palma (Canary Islands, Spain) has proven to be a valuable site for studying the long-term effects of acidification on key species in these ecosystems, as well as on entire rocky benthic communities. This CO₂ vent is characterized by shallow rocky and sandy substrates inhabited by species from subtropical regions. It is originated by a discharge of groundwater naturally acidified by the island's volcanic activity. The discharge of acidified groundwater into the sea occurs during low tide, which is when the lowest acidification values are observed (González-Delgado et al. 2021). Moreover, it does not only affect the beaches, but also some coastal lagoons. These lagoons, called Echentive lagoons, present an extreme environment (Sangil et al. 2008), due to an increased influx of brackish and acidified groundwater that confers a much higher input of CO₂ and alkaline substances (González-Delgado et al. 2021). So far, this natural laboratory has shown that prolonged acidification has a negative impact on the integrity of the shells of the mollusc *Phorcus sauciatus* (Viotti et al. 2019), and results in smaller individuals in populations of the sea urchin Arbacia lixula, with their shells being more robust (Sosa-Navarro 2021). Furthermore, the most recent research published by González-Delgado et al. (2023) shows that under acidification, the entire benthic community undergoes miniaturisation and homogenisation, with small and fast-growing organisms dominating.

Hence, the aim of this study was to conduct a preliminary exploration of the composition of the meiofauna associated with the intertidal and subtidal sandy substrates affected by acidified system off Punta de Fuencaliente (Figure 1A).

Samples were collected during low tide at four points around this acidified system of Fuencaliente (Figure 1A): (1) Echentive beach, whose pH values are normal; (2) Playa del Faro beach and (3) Los Barqueros beach, whose low pH values fluctuate between 7.8 and 7.2 units, depending on the tide; and (4) Echentive lagoons, whose chemical conditions are considered extreme, with pH fluctuations

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ples). The sampling had approximately trom at their deepthe lagoon's sandy 4 μm sieve and the o the lowest possi-Z4) and preserved munities, a one-way as performed. The intertidal samples

between 7.6 and 7.4 units and very high alkalinity values (of more than 9000 μmol kg-1) (González-Delgado *et al.* 2021).

Three replicates were collected for each sampling point using a 0.25 litre cylindrical plastic container, and at different depths in the case of beaches: around 2 metres deep (subtidal samples) and at the shore (intertidal samples). The sampling points were chosen at random, considering that the sediment had approximately the same granulometry. The Echentive lagoons have muddy bottom at their deepest part (Sangil *et al.* 2008), therefore only shallow samples of the lagoon's sandy bottom were collected. Sand samples were passed through a 64 μ m sieve and the meiofaunal fraction was separated. Specimens were identified to the lowest possible taxonomic level using a binocular stereomicroscope (Leica EZ4) and preserved in 70 % ethanol.

To assess differences in abundance of meiofaunal communities, a one-way multivariate analysis with permutations (PERMANOVA) was performed. The fixed factor of the analysis was "pH level" with three levels for intertidal samples ("extreme", "low", and "normal") and two levels for subtidal samples ("low" and "normal"). Abundance data were square-root transformed and then the Bray-Curtis distance was applied. When insufficient permutations were obtained for a valid test, p-values were corrected using the Monte Carlo method (Anderson and Robinson 2003). Significant terms (p-value < 0.05) of the models were analysed *a posteriori* by pairwise tests. Finally, a shade-plot representing the abundance of taxonomic orders per sampling point was produced. The statistical package PRIMER 6 and PERMANOVA + v.1.0.1 (Anderson 2001) was used for all analyses.

A total of 3,680 specimens were found, belonging to 30 taxa, of which only 20 were identified to species level (Table 1). Statistical analyses revealed that the only significant differences were found in the intertidal samples (SS = 9,242.3; MS = 4,621.2; pseudo-F = 7.268; P = 0.001) between the extreme environment (Echentive lagoons) and the rest ("extreme", "low": t = 3.2478; P = 0.003. "extreme", "normal": t = 3.500; P = 0.011. "low", "normal": t = 1.322; P = 0.186). Furthermore, the shade-plot shows that the extreme environment had by far the highest mean abundance of taxa (Figure 1E). The most abundant taxa in extreme environment were the oligochaetes, specifically the genus *Grania* of the family Enchytraeidae and the family Naididae. Additionally, we also found high abundances of nematodes Chromadorea and Enoplea, and arthropods (of the subclass Acari, and of the classes Ostracoda and Copepoda).

All these taxa are considered bioindicators of extreme environments. For example, oligochaetes from family Naididae are often associated with heavily contaminated sediments due to anthropogenic activities (Vivien *et al.* 2020). Nematodes and copepods have also long been considered bioindicators of extreme environments, as they are often the two most abundant phyla of meiofauna found in association with most habitat disturbances (Kennedy-Jacoby 1999). Hence, it has been confirmed that the discharge of acidic water into the Echentive lagoons does impact the species composition of the meiofaunal communities. Similar results have been obtained in the Panarea hydrothermal vent, where nematodes, followed by copepods, dominate in all areas close to the vent (Fanelli *et al.* 2022). In addition, these

TABLE 1. TAXONOMIC LIST OF THE MEIOFAUNA SPECIES FOUND. UNDERLINED ARE THE SPECIES THAT WERE ONLY FOUND IN THE ECHENTIVE LAGOONS (EXTREME AMBIENT).

Phylum Xenacoelomorpha

Class Acoelomorpha, Order Acoela

Phylum Echinodermata

Class Ophiuroidea, Order Amohilepidida, Family Amphiuridae, *Amphipholis squamata* (Delle Chiaje, 1828)

Phylum Annelida

Class Clitellata Subclass Oligochaeta Order Enchytraeida, Family Enchytraeidae, Grania sp. Order Tubificida Family Naididae Subfamily Phallodrilinae Class Polychaeta Family Saccocirridae, Saccocirrus papillocercus Bobretzky, 1872 Subclass Errantia Order Amphinomida Family Amphinomidae, Eurythoe complanata (Pallas, 1766) Order Phyllodocida Family Nereididae, Platynereis nunenzi Teixeira et al., 2020 Family Syllidae, Brania arminii (Langerhans, 1881) Exogone breviantennata Hartmann-Schröder, 1959 Prosphaerosyllis campoyi (San Martín, Acero, Contonente and Gómez, 1982) Syllis armillaris (O.F. Müller, 1776) Syllis cf. gerlachi (Hartmann-Schröder, 1960) Subclass Sedentaria Order Sabellida Family Fabriciidae, Fabricia stellaris (Müller, 1774) Pseudofabriciola sp. Family Sabellidae Amphiglena mediterranea (Leydig, 1851) Jasmineira sp. Order Scolecida Family Capitellidae

Capitella capitata (Fabricius, 1780)

Phylum Mollusca

Class Gastropoda Subclass Caenogastropoda Order Littorinimorpha Family Caecidae *Caecum* sp. Family Eulimidae *Discaclis* sp. Family Rissoidae Subclass Vetigastropoda Order Trochida Family Skeneidae

Phylum Nemertea

Phylum Platyhelminthes

Phylum Nematoda Subclass Adenophorea		
Phylum Artropoda		
Class Arachnida		
Subclass Acari		
Order Mesostigmata		
Family Cyrtolaelapid	2	
Order Trombidiformes		
Family Rhagidiidae		
Class Ostracoda		
Class Copepoda		
Class Malacostraca		
Subclass Eumalacostraca		
Order Isopoda		
Family Idoteidae		

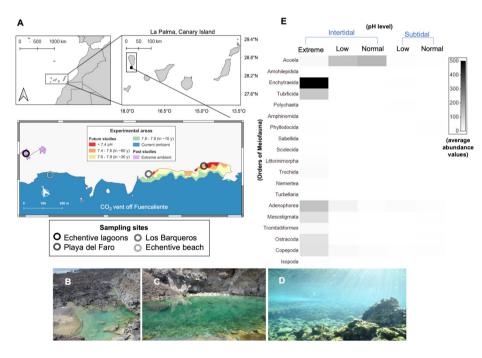


Figure 1. (A) Location of the CO_2 vent of Fuencaliente with the sampled sites marked with circles of different shades of grey. In colour you can see the pH levels found in the area during low tide.

This colour gradient has the most extreme environment marked in purple, and then goes from red (low pH) to blue (normal pH). The map was obtained and adapted from González-Delgado *et al.* (2021). (**B**, **C** and **D**) Images of the Echentive lagoons (extreme environment). (**E**) Shade-plot showing the mean abundance by pH level of each identified order of the meiofaunal community.

authors also observed an increase in ostracod and acarid species, as in our study, indicating a high availability of organic matter (Tangherlini *et al.* 2021).

The only group that showed a gradient of decline as extreme conditions increased was the phylum Xenacelomorpha (specifically the order Acoela). Previously considered as flatworms, this group is one of the least studied of the meiofauna due to the lack of experts who can identify them taxonomically (Zeppilli *et al.* 2018). Therefore, we do not have much information on their behaviour in disturbed environments. This work may be the first to record negative effects of acidification on species of the order Acoela. This preliminary study indicates a clear alteration in the composition of meiofaunal communities caused by the extreme acidification of seawater in the coastal environment of La Palma.

Author contributions

The research design was primarily led by JCH and SGD. The sampling process was made by SGD, JN, and JCH. Laboratory work including taxonomic identification was made by SGD and JN. The statistical analysis and interpretation were performed by SGD. In the manuscript's development, including the writing of the main text, figures, and tables, SGD took the lead, with the help of JCH with the supervision of JN. The necessary funding was secured by JCH. All authors collaborated on the manuscript revision.

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REFERENCES

- ANDERSON, M. J., and ROBINSON, J. 2003. Generalized discriminant analysis based on distances. *Aust. N. Z. J. Stat.*, 45(3), 301-318.
- ANDERSON, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. *Can. J. Fish. Aquat. Sci.* 58, 626–639.
- DANOVARO, R., SCOPA, M., GAMBI, C., and FRANSCHETTI, S. 2007. Trophic importance of subtidal metazoan meiofauna: evidence from in situ exclusion experiments on soft and rocky substrates. *Mar. Bio.* 152: 339–350.
- FANELLI, E. *et al.* 2022. Effects of local acidification on benthic communities at shallow hydrothermal vents of the Aeolian Islands (Southern Tyrrhenian, Mediterranean Sea). *Biology*, 11(2), 321.
- FOO, S. A., BYRNE, M., RICEVUTO, E., and GAMBI, M. C. 2018. The carbon dioxide vents of Ischia, Italy, a natural system to assess impacts of ocean acidification on marine ecosystems: an overview of research and comparisons with other vent systems. *Oceanogr. Mar. Biol. Annu. Rev.* 56: 237-310.
- GONZÁLEZ-DELGADO, S., and HERNÁNDEZ, J. C. 2018. The importance of natural acidified systems in the study of ocean acidification: what have we learned?. *In Adv. Mar. Biol.* 80: 57-99. *Academic Press*.
- $\label{eq:González-Delgado, S., González-Santana, D., Santana-Casiano, M., González-Dávila, M., Hernández, C. A., Sangil, C., and Hernández, J. C. 2021. Chemical characterization of the Punta de Fuencaliente CO_2-enriched system (La Palma, NE Atlantic Ocean): a new natural laboratory for ocean acidification studies.$ *Biogeosci.*18(5): 1673-1687.
- GONZÁLEZ-DELGADO S., WANGENSTEEN O.S., SANGIL C, HERNÁNDEZ C. A., ALFONSO B., SOTO A. Z., PÉREZ-PORTELA R., MARIANI S., HERNÁNDEZ J. C. 2023. High taxonomic diversity and miniaturization in benthic communities under persistent natural CO₂ disturbances. *Proc. R. Soc. B* 290: 20222417. https://doi.org/10.1098/rspb.2022.2417.
- KENNEDY, A. D., and JACOBY, C. A. 1999. Biological indicators of marine environmental health: meiofauna-a neglected benthic component?. *Environ. Monit. Assess.* 54(1): 47-68.
- SOSA-NAVARRO, N. 2021. Effects of long exposure to low pH conditions on calcareous structures of the sea urchin *Arbacia lixula* (Linnaeus, 1758). Universidad de La Laguna, Spain.
- TANGHERLINI, M., CORINALDESI, C., APE, F., GRECO, S., ROMEO, T., ANDALORO, F. and DANO-VARO, R. 2021. Ocean acidification induces changes in virus–host relationships in Mediterranean benthic ecosystems. *Microorganisms* 9, 769.
- VIOTTI, S., SANGIL, C., HERNÁNDEZ, C. A., and HERNÁNDEZ, J. C. 2019. Effects of long-term exposure to reduced pH conditions on the shell and survival of an intertidal gastropod. *Mar. Environ. Res.*, 152, 104789.
- VIVIEN, R., CASADO-MARTÍNEZ, C., LAFONT, M., and FERRARI, B. J. 2020. Effect thresholds of metals in stream sediments based on *in situ* oligochaete communities. *Environments*, 7(4), 31.
- ZEPPILLI, D. *et al.* 2018. Characteristics of meiofauna in extreme marine ecosystems: a review. *Mar. Biodivers*, 48(1): 35-71.