

TITLE: Breathing in the dark: interactive influence of intrinsic and extrinsic factors on stygofauna metabolic rate

APPENDIX 1 - COLLECTION SITE

Study site

The specimens of *S. bottazzii* used in this study have been collected from a cave in the locality of Sant'Isidoro, (Nardò Municipality - Apulia, Italy) (40.222 N - 17.929 E). The site is located along the wide coastal plain of the Ionian Sea, carved in Upper Pleistocene Calcarenes overlain by the Lower Pleistocene Gravina Calcarenes (Rossi, 1968; Delle Rose, 2002; Carrozzo et al., 2003). The mixing between fresh and saltwater enhances the carbonate dissolution leading to rapid geomorphological evolution of the coast (Cigna & Forti, 1986; Liso & Parise, 2023).

The about 25 km-long coastal stretch comprised between Torre Castiglione (Municipality of Porto Cesareo) to the north, and Serra Cicora (Municipality of Nardò) to the south, is characterized by numerous collapse dolines (more than 100), from the coastline to a maximum distance of about 1 km inland, aligned along the main tectonic lines (Pepe & Parise, 2014). They are locally known with the dialect term *spunnulate* or *spundulate* and extend from 5 to 6000 m², while their depth varies from 0,1 to 5,0 m (Delle Rose & Parise, 2002; Beccarisi et al, 2010; Basso et al., 2013).

Among these, Palude del Capitano (Nardò Municipality) is the largest collapse sinkhole in the area (5 to 20 times larger than the surrounding ones). The environmental conditions of these aqueous systems are substantially constant throughout the year: temperatures vary between 17 and 20 °C; salinity varies between 2‰ and 20.50‰ in the closest portion to the seaside. pH is between 7.00 and 7.65 (Denitto et al., 2006). Cave-diver surveys have highlighted the presence of further extensive systems of flooded tunnels and passages with evident phreatic morphology, several hundred meters long, which connect these systems among them and with the sea (Palmisano & Onorato, 1994; Onorato et al., 2017; Parise et al., 2017).



Figure 1: image of the buckets used for the specimens collection in our study site. Inside of the cave.



Figure2 : map showing the collection site in red.

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