

The **Adriatic Sea** has been interested by a **complicated history of transgressions and regressions** of the water level, repeatedly connecting or isolating the Western Balkans and Apulia (Italy), promoting respectively the linkage and segregation of populations and species (Fig. 1). The temporary ponds (TPs) of these regions are an example of a remote environment

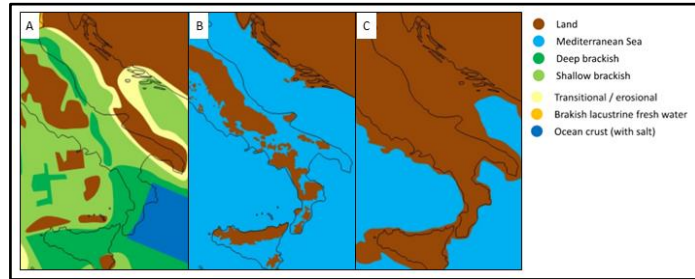


Fig.1. Paleo reconstruction of Adriatic Sea and study area. A: latest Miocene (6.0–5.6 Ma); B: Pliocene (5.3–2.6 Ma); C: Pleistocene: Last Glacial Maximum (21 ka). Maps adapted from Schmitt et al. 2021.

not naturally connected to other freshwater basins, but that may have been colonized in the past, but also the present, through the passive transport by vertebrates (e.g., birds). Moreover, TPs are home to several endemic and highly specialized taxa and are endangered by climate change and human activities. This project will investigate the effects of historical and contemporary drivers on the spatial biodiversity patterns and functioning of TPs in two regions (Apulia and the Western Balkans) threatened by anthropogenic pressure. The following working hypotheses will be tested within the project: **H1)** Reiterative paleo-connections between Apulia and the Balkans shaped the invertebrate communities of TPs; **H2)** Vertebrates play a key role connecting TPs locally and regionally; **H3)** Climate change and other anthropogenic pressures affect the TPs communities; **H4)** Temperature alters the hatching and the functioning of dormant communities in TPs.

With our integrated approach we aim to achieve the following: 1) **model the anthropogenic, environmental and climatic conditions driving the biodiversity of TPs** (see **H3**), 2) **assess the effects of climate change on life history traits** (see **H3** and **H4**), 3) **test the genes expression of the community under different climates** (see **H4**), and 4) **unravel past and present connections between these regions** (see **H1** and **H2**). Firstly, we will **develop barcode libraries** for the TPs communities, which will provide an important **baseline** for future works, and a comparison point with other regions. Additionally, the use of **advanced microscopy** will complement molecular data with high-resolution imaging. In fact, through the study of **traces of genetic material** left behind by shedding of cells, feces, mucus, body fluids, gametes and other propagules, or dead/decomposing organisms (environmental DNA - **eDNA**), we will be able to obtain information about **community composition**, without the need of a direct collection of the organisms. The use of eDNA has increased in biodiversity monitoring and is often more affordable and logistically more feasible than conventional counterparts, having **enormous potential** to enable **ecological studies at greater temporal and spatial scales**. Less common is the use of the environmental RNA (**eRNA**), representing the **expressed portion of genes of a community**. This method poses a unique chance to study not only the identity of the organisms, but also their **biological functions** taking place in a certain moment. For example, analyses of the **metatranscriptome of a community** make it possible to identify **genes expressed in presence of a thermic stress**, allowing the study of a response to such stress at a community rather than at individual level. This will allow the **development of stress-related markers** for the whole **eukaryotic community**. Integrating **morphological, molecular, and functional** data about the invertebrate communities of the TPs, we will be able to contribute important information for their **management and protection**, since the data obtained will be made available for public use. Moreover, by coupling sampling and experiments, we will also **test possible patterns** that might be pointed out from the field observations/data along the large and diverse geographic area we will explore.