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A century of research on micro-organisms from the inland waters of the largest Mediterranean island

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ABSTRACT

The first studies on the micro-organisms inhabiting Sicilian inland waters date back to the middle of the XIX century. However, these were based on single samples and mainly addressed at compiling faunistic and floristic inventories. It was in the first decades of the XX century that the first methodical studies were performed, which focussed on assessing microbial diversity in saline and hypersaline inland waters. Studies on plankton dynamics in ponds and reservoirs of the island started at the beginning of the 1980s and, since the end of the 1990s, temporary waters have also been intensively sampled, especially as regards phytoplankton and micro-crustaceans. These intensified sampling efforts contributed to increasing our knowledge of the composition, structure and functioning of the planktic compartment. On the contrary, studies on benthic microflora and fauna are still numerically scarce and mostly based on occasional collections. Also, running waters have received little attention and the methodical analysis of their micro-organisms is still in its infancy.

INTRODUCTION

Limnology, as a scientific discipline, was established by François-Alphonse Forel in his chief work "Le Léman: Monographie limnologique", published in three volumes between 1892 and 1904 (Warwick and Bertola, 2014). This new branch of natural sciences was intended by his founder as "the oceanography of lakes" and represented a holistic approach to the study of biological, chemical, physical and sedimentary aspects of lakes. The discipline very soon attracted wide interest among scientists, and the "Societas Internationalis Limnologiae" (SIL, the International Society of Limnology) was founded one century ago, in 1922, followed in Italy by the establishment of the Associazione Italiana di Oceanologia e Limnologia (AIOL, the Italian Association of Oceanology and Limnology) in 1972. Studies on the biological aspects of Sicilian inland waters started to be published concurrently with the publication of Forel's monograph, and microscopic organisms were the protagonists of some pioneering investigations. At the end of the XIX century, it was probably the technical progress of the microscope, and the charming world that this instrument revealed, that attracted several investigators toward the study and description of this microworld. Today we know that, among aquatic organisms, microscopic ones (*i.e.*, according to Fontaneto and Brodie, 2011, in this paper we consider as "microscopic" those organisms belonging to taxa with an average body length of less than 2 mm) play an important role as ecosystem propellers, since they govern primary production, biogeochemical cycles, matter circulation, and are energy conveyors towards larger organisms (*e.g.*, Naselli-Flores and Padisák, 2022).

The first investigations on Sicilian freshwater micro-organisms were those of Antonino Borzì, who was a pioneer of algological studies, and extensively studied the microalgal flora (including cyanobacteria) of Sicily (a list of his contributions is available in Borzì, 1996; an anastatic reprint of his work "Studi Algologici", originally published in two volumes in 1883 and 1895). A few years later, Moniez (1889) reported on the microfauna of some Sicilian water bodies and provided the first description of the zooplankton of the "Biviere di Lentini", a lake reclaimed in the 1920s to fight malaria (Consoli, 1928). In Moniez's paper, the following recommendation appears: "Nous ne saurions trop recommander aux lecteurs de la Feuille de recueillir avec soin dans les étangs, les lacs et les cours d'eau, les petits animaux et spécialement les Crustacés entomostracés, surtout dans les régions montagneuses et méridionales"; the sentence gives us an idea of the urgency and interest, already felt in the second half of the nineteenth century, to investigate the diversity of micro-organisms that populated inland waters in remote and isolated areas. After Moniez's (1889) work, it took more than 35 years for a second short report on the zooplankton of an alkaline lake (the "Bagno di Venere" or "Specchio dell'Acqua") in Pantelleria, a volcanic island in the Sicilian Channel halfway between Africa and Europe (Brehm, 1926). This was followed, a few years later, by an accurate morphological description of a population of *Arctodiaptomus salinus* (Daday, 1885) in the endorheic, saline Lake Pergusa (Baldi, 1929). It is worth underlining that the author of this work was Edgardo Baldi, the first director of the "Istituto Italiano di Idrobiologia Dr. Marco De Marchi" and one of the most renowned limnologists of all time (see https://limnology.org/notable-limnologists/edgardo-baldi/). Regardless of this paucity of previous research, limnobiological studies in Sicily are in some way related to one of the finest scientists of the last century who tightly linked his name to limnology: George Evelyn Hutchinson. Hutchinson was in Palermo at the end of the '50s of the last century, and during his stay, he visited the sanctuary of Santa Rosalia, where he was attracted

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by a nearby small temporary pond. This became the scene of one of his most famous papers (Hutchinson, 1959), and probably inspired another seminal paper addressed at solving the so-called "paradox of the plankton" (Hutchinson, 1961). In addition, part of the second volume of Hutchinson's monumental work "A Treatise on Limnology" (Hutchinson, 1967), which deals with the analysis of limnoplankton, was written while he was a guest at the Institute of Zoology of the University of Palermo.

More than one century has passed since the first studies on micro-organisms inhabiting Sicilian inland waters, and our knowledge of their diversity and distribution has greatly increased. Updated checklists for some taxa (*e.g.*, rotifers, turbellarians, some crustacean groups) have been recently published in the frame of the new checklist of the Italian fauna (Bologna *et al.*, 2022), and the occurrence localities of some taxa are reported in the frame of the project CkMap (Ruffo and Stoch, 2005). However, many gaps still exist, and the complexity related to the analysis of distribution patterns of these organisms is further enhanced by the ongoing climate change; according to Woolway and Maberly (2020), the velocity of climate change could increase more rapidly than the dispersal velocity of most aquatic species, which are particularly vulnerable to increasing water temperature. This pattern, if confirmed, would reduce the species' ability to disperse in cooler areas, and thus increase the risk of extinction.

As mentioned above, the study of Sicilian inland waters is closely related to some of the finest scientists of the last century, who tightly linked their names to limnology and ecology. To pay tribute to these scientists, to celebrate the 50^{th} anniversary of the foundation of the Italian Association of Limnology and Oceanography (AIOL), and to set a baseline as regards the knowledge available to date, in this paper we try to: i) briefly summarise the work carried out on the micro-organisms of the Sicilian inland waters in the last century, and, ii) outline the knowledge gaps that still need to be filled.

Sicily and its inland waters

Studying aquatic micro-organisms inhabiting the inland waters of an island located in the middle of the Mediterranean Sea and characterised by a semi-arid climate could appear, at first sight, somewhat misplaced. However, from a geographical point of view, Sicily is part of the southern European border, and an important biogeographical crossroad between subtropical and temperate areas, and the Eastern and Western part of the Mediterranean Basin; accordingly, the biological study of Sicilian inland waters might allow to better understand the dispersal and distribution patterns of several Mediterranean species (*e.g.*, Marrone *et al.*, 2009).

Sicily is the largest island (~25,700 km^2) of the Mediterranean Sea. Sicilian physiography is dominated by a hilly landscape (61%) and some mountain ranges (25%, including Mount Etna, the highest European volcano), with the remaining surface (14%) characterized by lowlands. The climate is typically Mediterranean, with an out-of-phase seasonality of rainfall and air temperature that determines the alternance of rainy and cool (winter) and dry and warm (summer) periods. According to the precipitation patterns in the different parts of the island, the climate ranges from semiarid to temperate-dry, with temperate-humid and humid zones limited to the highest mountain ranges (Naselli-Flores, 2010). Due to its insular and climatic conditions, Sicily is characterized by a drainage network formed by numerous short, torrent-like, temporary rivers, and a few permanent rivers with a discharge greatly varying between the rainy and the dry periods. A few small, permanent water bodies, and thousands of Mediterranean temporary ponds are scattered throughout the island (Naselli-Flores and Marrone, 2019). In addition, several thousands of small agriculture reservoirs have been built in the hilly and lowland parts of the islands in the last 70 years, along with about 30 dam reservoirs with stored volumes ranging between 5 and 150 10^6 m^3 (Naselli-Flores and Lugliè, 2014). The geology of the island strongly conditions the chemical and physical characteristics of these water bodies. An extremely simplified geological division of the island results in two large zones. The northern zone consists of numerous stratigraphic structural units in overthrust, mainly dolomitic and carbonate rocks, and metamorphic rocks. The southern zone is for the most part comprised of carbonate rocks which, in the central part of the island, after the tectonic phase of the Middle Miocene, were covered by sediments and evaporites, with widespread outcrops of sulphate and gypsum rocks. As a consequence, the ponds and wetlands located in the southern zone often have high conductivity values, generally above 2,000 $\mu S\ cm^{-1}$ (athalassohaline waters). In addition, some saline and brackish lakes and wetlands are located in the plains distributed along the coasts, influenced by the nearby seawater (thalassohaline waters). Therefore, the permanent freshwaters are mostly located in the northern mountain chain, at altitudes higher than 900 m a.s.l., and on the Hyblean plateau in the south-eastern part of the island. On the whole, a great variety of inland waters are present on the island, from small rockpools to large dam reservoirs, and they include also some relict aquatic environments such as the small, relict peat bogs located in the northern mountain range.

The current state of knowledge on micro-organisms of Sicilian inland waters

In the following paragraphs, we review the state-of-the-art of some groups of micro-organisms inhabiting a variety of Sicilian continental aquatic ecosystems. The review also includes micro-organisms forming large colonies, such as those belonging to the groups of bryozoans and sponges. The papers were collected by searching the internet (Google) and using the keywords “Sicilian freshwaters” and “Sicilian inland waters”, and both the common and taxonomic names of the group to which micro-organisms belong (*e.g.*, “water mites in Sicilian inland waters” and “Hydrachnidia in Sicilian freshwaters”).

Figure 1 shows the trend in the number of publications on the micro-organisms of Sicilian inland waters over the course of more than a century. Until the end of the 1970s, most of the contributions were directed to the study of the microbial community of saline and hypersaline lakes. Conversely, freshwater micro-organisms have been intensively studied in the last 30 years, especially as regards

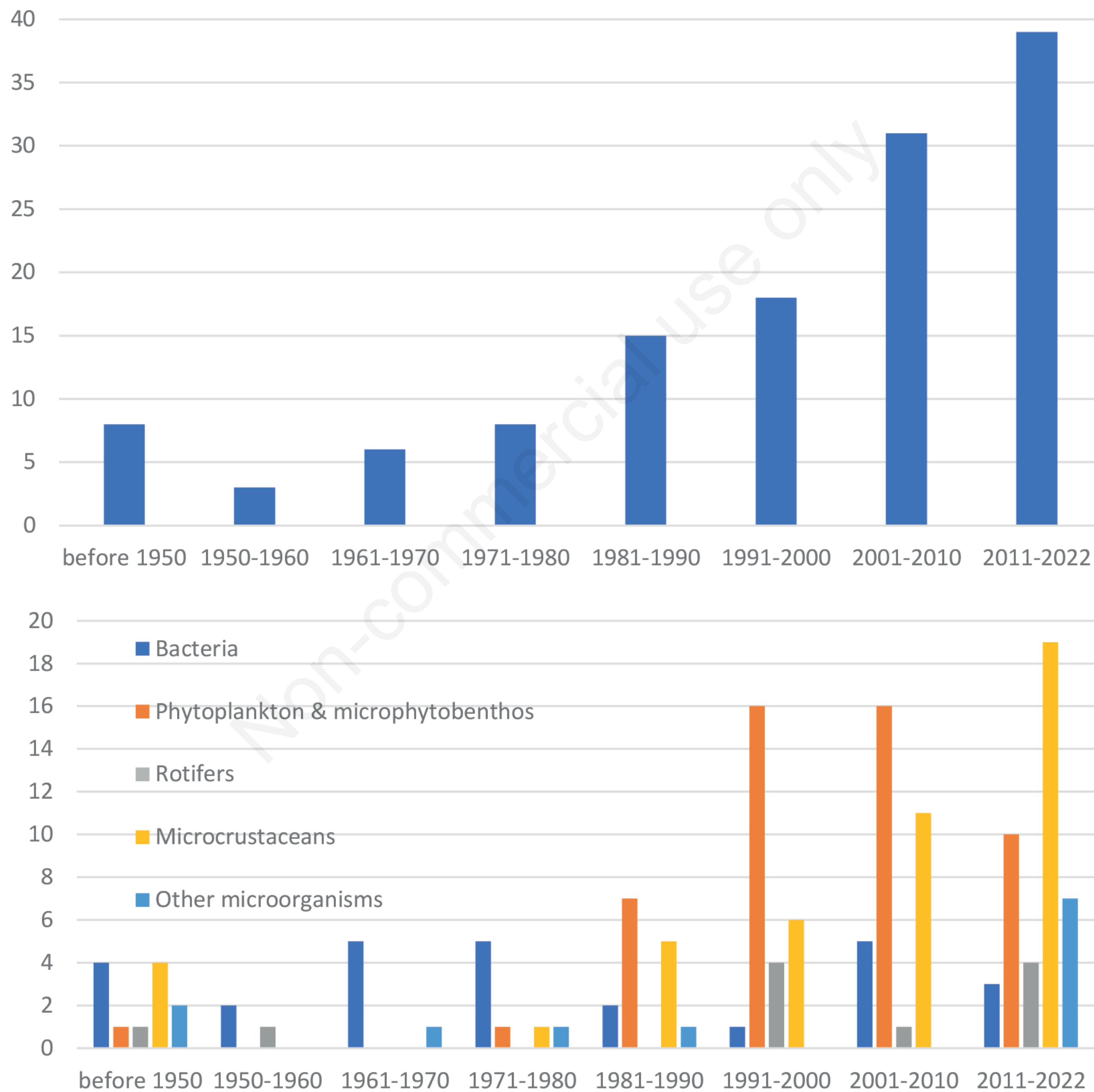

Figure 1. Upper panel, the temporal trend of the number of publications on micro-organisms in Sicilian inland waters. Lower panel, the number of papers containing information on the different groups of micro-organisms. The total number of papers per decade in the upper panel can be higher than the total number of papers appearing in the lower panel since several papers contain information on more than one biological group of micro-organisms.

phytoplankton and microcrustaceans. The publications addressed at this latter group have been continuously increasing in the analyzed period.

Bacteria and Archaea

Both endorheic and coastal saline lakes have been repeatedly investigated for their bacterial communities since the beginning of the 1930s. Lake Pergusa is the largest Sicilian natural lake, and is located on the evaporite outcrops covering the center of the island. It was an early subject of research due to its cyclic reddish water blooms, due to purple sulphur bacteria that periodically bloomed in its hypersaline waters (Brunelli and Maldura, 1929; Forti, 1932; 1933a; 1933b). Further investigations on this phenomenon were carried out in the late 1970s (Faranda *et al.*, 1977; Genovese *et al.*, 1977; Bruni and Pulicanò, 1978) and at the beginning of this century (De Francesco *et al.*, 2002). Urbanization and increased water demand for agriculture in the surroundings of the lake promoted intensive groundwater extraction (Canova *et al.*, 2011), which caused a strong reduction in the water level at the beginning of this century, threatening its existence. To counteract this tendency, since 2005, the lake has been refilled using fresh waters from dam-reservoirs with much lower conductivity values (Lake Ancipa, ~200 µS cm^{-1}). This induced a quite drastic rearrangement of the biota in Lake Pergusa as discussed in Barone *et al*. (2008) and Naselli-Flores *et al*. (2016), and caused the disappearance of the purple sulphur bacteria cyclic blooms.

Lake Faro, a meromictic and quite deep coastal lake was the theatre of several studies on its peculiar bacterial community. Since the second half of the 1950s, and for more than half a century, the lake has attracted the attention of scientists due to the stable presence of hydrogen sulphide in the monimolimnion and of a layer of red water at depths between 9 and 12 meters (Genovese, 1957; 1961; 1963), caused by phototrophic sulphur bacteria (Genovese *et al*., 1958; 1962; Genovese and Machi, 1963; Trüper and Genovese 1968; Sorokin and Donato 1975; Bruni *et al*., 1978; Acosta-Pomar *et al*., 1988; Maugeri *et al*., 2001; Saccà *et al*., 2008; Gugliandolo *et al*., 2011; Lentini *et al*., 2012).

Isolated investigations on the bacterial community of Tindari ponds (Zaccone *et al*., 2000; Caruso *et al*., 2005) and River Alcantara (Guglielmo *et al*., 2013) were carried out in the first years of this century.

Besides the pioneering work carried out by Antonino Borzì on benthic cyanobacteria (see Borzì, 1996, for a detailed list of papers), no additional information is available in the literature on these micro-organisms other than the recent paper by Russo *et al*. (2021), who studied the diversity of cyanobacteria colonizing the surface of an urban fountain in Palermo by combining microscopic analysis and molecular tools. Conversely, the distribution and abundance of planktic cyanobacteria in Sicilian water bodies (Barone and Naselli-Flores, 1989) was first systematically assessed in a limnological investigation aimed at evaluating the trophic state of all the Sicilian lakes and reservoirs with a surface area >0.2 km^2 (Calvo *et al*., 1993). Since cyanobacteria are commonly found in the plankton of lakes and ponds, information on this group of micro-organisms is available in the papers on phytoplankton of Sicilian inland waters (see next paragraph).

Phytoplankton and microbenthic algae

Faranda (1977) compiled the first inventory of the Sicilian water bodies suitable for aquaculture and investigated their main chemical, physical and biological (fish) characteristics. The paper also contains some information on phyto- and zooplankton, but it is largely incomplete, since this information is only available for 7 out of the 22 studied water bodies, and just a few species are listed.

Phytoplankton, including the "pico" fraction, in a complex system of brackish and saline inland waters (Capo Peloro Lagoon system, which also includes Lake Faro) was investigated in detail, over a multi-year period, by Platt *et al*. (1985); Bruni *et al*. (1990); Giuffrè (1991; 2011); Giacobbe and Maimone (1994); Gangemi (2000); Gangemi *et al*. (2006); Giuffrè and Pezzani (2005).

The first studies on the diversity, abundance, and annual dynamics of freshwater phytoplankton in different Sicilian man-made lakes are those by Barone (1983; 1985) and Barone *et al.* (1989). These works were prodromal for a more comprehensive investigation of plankton from Sicilian reservoirs (Calvo *et al*., 1993; Barone and Naselli-Flores, 1990; Barone *et al*., 1991), and entailed a greater effort in the analysis of phytoplankton assemblages in Sicilian permanent waters, based on weekly samplings, which followed the phytoplankton generation times (Barone and Naselli-Flores, 1994; 1995; Naselli-Flores and Barone, 1994; 1995; 1998; 2003). This sampling frequency, carried out for almost twenty years in the dam-reservoir "Lake Arancio" (Naselli-Flores, 2014), allowed to clarify several ecological aspects of the phytoplankton dynamics in the Mediterranean dam-reservoirs, and to establish a link between phytoplankton dynamics and the peculiar morphological and hydraulic characteristics of these water bodies (Naselli-Flores, 1996; 1998; 1999; 2000; 2003; 2010; Naselli-Flores and Barone, 2007; 2011). Furthermore, the importance of the major role exerted on plankton dynamics by the "filling-emptying" dynamics in Mediterranean reservoir, compared to the "stratification-circulation" paradigm in temperate lakes (*e.g.*, Sommer, 1987) was highlighted (Naselli-Flores, 2003), as well as the usefulness of a functional classification of planktic organisms based on traits (Barone and Naselli-Flores, 1990; Naselli-Flores and Barone, 2000). The "filling-emptying" dynamics in Sicilian reservoirs also resulted in favouring the growth of toxic cyanobacteria like *Microcystis* spp., with the formation of long-lasting blooms in those years characterized by wider fluctuations in the water level (Naselli-Flores *et al*., 2007). A partial inventory of freshwater phytoplankton (including cyanobacteria) in Sicilian inland water >0.2 km^2 can be found in Barone (2003).

Phytoplankton studies in permanent and temporary natural ponds in Sicily were mainly addressed at understanding the effects of climate change on phytoplankton composition and dynamics (Barone *et al*., 2010), how regional and local factors govern the phytoplankton assembly (Naselli-Flores and Barone, 2005; 2012; Jeppesen *et al*., 2015) and how passive dispersal can influence phytoplankton distribution in natural and temporary waters (Incagnone *et al*., 2015; Naselli-Flores *et al*., 2016). Factors influencing the seasonal distribution of mixotrophic phytoplankton were also studied over a multi-year (6.5) period (Naselli-Flores and Barone, 2019). Moreover, Sicilian permanent and temporary ponds host a peculiar microflora and several rare species (Naselli-Flores and Barone, 2000; 2012; Barone *et al*., 2006; Madonia *et al*., 2006). In particular, the ponds located on the northern mountain range host a relict microflora typical of more temperate aquatic ecosystems (Barone *et al*., 1989; Barone, 2003). A complete account of the biodiversity of these ponds deserves further investigations, as shown in a recent paper by Pang and Van de Vijver (2021), who investigated the chrysophycean stomatocyst flora in some small pools on the Hyblean Mountains in south-eastern Sicily.

Microbenthic eukaryotic algae have seldom been investigated in Sicilian inland waters and, apart from an in-depth study on the benthic diatoms of the Simeto hydrographic basin (Finocchiaro *et al*., 2009; 2011), the most extensive river network of the island, only a few further contributions are available on benthic diatoms in a coastal meromictic lake, and in freshwater and saline rivers (Giuffrè, 2000; Mannino, 2001; 2007; Campisi *et al*., 2020).

Rotifers

Free swimming rotifers of the class Monogononta commonly inhabit freshwaters and represent an important component of the zooplankton. Moniez (1889) was the first who studied rotifers in Sicilian inland waters: in his paper this author writes "On n'a encore rien publié, que je sache, sur la faune des eaux douces de la Sicile". This first contribution provided a list of eight species from a shallow lake in the eastern part of the island, the Biviere di Lentini. The original water body was drained at the end of the 1920s to fight malaria (Consoli, 1928) but at the end of the last century, a new dam-reservoir with the same name was built in a nearby location. Among the eight species found by Moniez in the "old" Biviere di Lentini, *Anuraea longispina* Kellicot, 1879, is listed. This species, whose accepted name is *Kellicottia longispina* (Kellicot, 1879), is considered a cold stenothermic taxon (Jersabek and Bolortsetseg, 2010) and thus represent an unusual record for the island. However, Naselli-Flores (unpublished) in 1987 found a single specimen of this species in a single zooplankton winter sample collected in the Biviere di Cesarò, a Sicilian mountain pond located at 1274 m a.s.l. (Naselli-Flores, unpublished). Due to the occasional nature of the finding, it was decided not to include the record in a paper on the zooplankton of the Biviere di Cesarò (cf. Naselli-Flores and Barone, 1991) and no further records for this species are to date known for the island. The "unusual" report of *Kellicottia longispina* was also noted by Berzins (1954), who, in a fruitless attempt to find it, collected and examined some samples in two water bodies which were located close to the, at that time disappeared, Biviere di Lentini. In his paper, Berzins accurately listed and described 37 species of rotifers (mainly belonging to the class Monogononta) along with six species of cyclopoid copepods and eight cladocerans.

After the publication of the paper by Berzins, the monogonont fauna in Sicilian inland waters was seldom investigated, and no information is available on bdelloid rotifers. A further list of rotifers, collected in a series of seasonal surveys on the plankton of Sicilian lakes and reservoirs, was published almost 40 years after the paper by Berzins (Calvo *et al*., 1993), whereas the first data on rotifers in lotic ecosystems were recently provided by Guglielmo *et al*. (2013) and Rodriguez *et al.* (2013) who studied the rotifer assemblage of River Alcantara.

An extensive review of rotifers occurring in Sicilian inland and marine waters, including geo-referenced occurrence localities, is reported in Ferrari *et al.* (2023).

Microcrustaceans

Information on microcrustaceans in Sicilian inland waters is scattered in several papers. These include the first observations by Moniez (1889) on the zooplankton of the Biviere di Lentini, and by Brehm (1926) and Baldi (1929) on the calanoid copepod *Arctodiaptomus salinus* (Daday, 1885). Cannicci (1939) reports on the plankton collected in a small, hypersaline pond on the island of Salina (Aeolian Archipelago) and lists, among others, the occurrence of a harpacticoid copepod, *Mesochra aestuarii* Gurney, 1921.

The first inventory of microcrustaceans from Sicilian freshwaters was provided by Margaritora *et al*. (1982), who studied in detail the entomostracan fauna collected in some dam-reservoirs, and in several permanent and temporary ponds. In the same period, the copepods of Sicilian groundwaters were investigated in detail (*e.g.*, Pesce, 1988, 1994; Pesce and Galassi, 1987; Pesce *et al*., 1987, 1988). These sampling efforts were further integrated by a study, carried out with seasonal frequency, on the trophic state of all Sicilian lakes and reservoirs with a surface area larger than 0.2 km^2 (Calvo *et al*., 1993). In that occasion, crustacean zooplankton was also collected and identified and further records were added to the knowledge on the distribution and diversity of microcrustacean fauna in Sicilian inland waters (Naselli-Flores *et al*., 1998; Alfonso *et al*., 2010). Moreover, long-term weekly samplings allowed to clarify the ecological relationships between seasonal water-level fluctuations and cladoceran dynamics in a Mediterranean dam-reservoir (Naselli-Flores and Barone, 1997).

However, the picture was far from exhaustive as demonstrated by the numerous new records of microcrustaceans (calanoid, cyclopoid, and harpacticoid copepods, cladocerans and ostracods) obtained in the new century through an intensified sampling activity in Sicilian groundwaters (*e.g*., Cottarelli *et al*., 2012; Bruno *et al*., 2018), and in some hundreds of temporary ponds (*e.g*., Marrone, 2003, 2006; Marrone and Naselli-Flores, 2004, 2005; Marrone and Vecchioni, 2020; Marrone *et al*., 2005; 2006a, b, 2020; Castelli *et al*., 2006; Pieri *et al*., 2006, 2020; Vecchioni *et al*., 2019a, 2021a; Marchese *et al*. 2022). These studies have contributed to increasing knowledge of the biodiversity of Sicilian inland waters, making the island one of the best-studied territories in the Mediterranean Basin. Furthermore, they allowed: to clarify taxonomic problems and biogeographical issues related to micro-crustaceans distribution in the Mediterranean inland waters (Marrone *et al*., 2009; 2017; Mazzini *et al*., 2017; Alfonso *et al*., 2021; Vecchioni *et al*., 2021b; Kotov *et al*., 2022); to better understand speciation in copepods (Marrone *et al*., 2010; 2013; 2016); to analyze species extinction, coexistence and replacement (Vecchioni *et al*., 2019b); and to account for the establishment of non-indigenous species (Marrone and Naselli-Flores, 2015; Naselli-Flores and Marrone, 2019).

Other micro-organisms

Moniez's pioneering work (Moniez, 1889) reports a number of aquatic micro-organisms (*e.g*., Protozoa, Hydrozoa, water mites) that were not further investigated or received little attention in the following studies on the biota of Sicilian inland water. Apart from some faunal and systematics works on water mites (Gerecke *et al*., 2014 and literature therein) and tardigrades (Lisi, 2015 and literature therein), knowledge of other groups of aquatic or semiaquatic micro-organisms is incomplete and fragmentary. Heterotrophic protozoa (*e.g.*, heterotrophic micro- and nanoflagellates as well as Ciliophora, both Peritrichida and Tintinnida, and Amoeba-like protozoans and testate amoebae belonging to Arcellinida) are often observed in live samples of plankton but taxonomic and ecological studies on this group of organisms for Sicilian inland waters are lacking. In the same way, freshwater turbellarian flatworms occurring in the island are poorly known (but see De Vries, 1985 and literature therein, and Lázaro and Riutort, 2013 and literature therein); Colomba and Vinciguerra (1980) provided an account of free-living freshwater nematodes in the river Anapo, whereas Vecchioni *et al*. (2021c) reported of an ectosymbiotic platyhelminth on freshwater crustaceans from an aquaculture farm. As regard freshwater hydrozoans, after the finding by Moniez (1889) of *Hydra fusca* Linnaeus, 1767 (synonym of *H. oligactis* Pallas, 1766), the only published report in the last 130 years for Sicilian inland water is related to the finding of *Hydra viridissima* Pallas, 1766 and *H. oligactis* in the river Oreto near Palermo (Riggio, 1978), and of a population of *Craspedacusta sowerbii* Lankester, 1880 in a small concrete-reservoir in the city of Palermo (Schifani *et al*., 2019). Bryozoans were reported by Riggio (1978) and studied by Taticchi *et al*. (2011) who found 7 species by sampling 4 dam-reservoirs and the river Anapo. The first record of a freshwater sponge in Sicily date back to 1961, when a population of *Ephydatia fluviatilis* (Linnaeus, 1759) was sampled in a fountain close to the Greek theatre of Syracuse (Sarà, 1961). Riggio (1978) found this species in the river Oreto, and Manconi and Pronzato (2016) reported of a population in a temporary canal in north-western Sicily. These further findings suggest that the sponge might be quite widespread on the island.

CONCLUSIONS

Although Sicily (and its surrounding archipelagos) is probably among the best studied Italian regions regarding the biodiversity of its inland waters, new and interesting information emerge whenever new environments are studied or when understudied taxonomic groups are considered. As an example, Marrone *et al*. (2022) highlighted some cases of apparent counter-intuitive distribution patterns and ecological preferences for Sicilian calanoid copepods, possibly to be ascribed to niche displacement or inaccurate taxonomy.

Dam-reservoirs, and permanent and temporary ponds have been studied over multiannual periods as regards their plankton, but the information on several groups of micro-organisms is still far from being complete. Moreover, systematic surveys aimed at assessing the biodiversity of micro-organisms in permanent and temporary rivers are highly desirable.

New methods such as the implementation of eDNA surveys can provide valuable information and represent an interesting opportunity to assess biodiversity in Sicily as revealed by a study carried out by Hupało *et al*. (2022) in a Sicilian stream. However, it should also be stressed that the method has shown some clear downsides (*e.g*., Roussel *et al*., 2015). Among these, the paucity and poorly representativeness of the available reference libraries for DNA sequences of the micro-organisms of the Mediterranean area might constitute a potential pitfall for a sound species identification and assessment, so that before successfully becoming an efficient tool to investigate and monitor aquatic species diversity, eDNA surveys must be preceded by accurate studies and characterization of the local biological diversity based on an integrative approach.

In order to get a representative and sound assessment of the biodiversity of micro-organisms inhabiting Sicilian inland water bodies, the formation and financial support of a new generation of specialists is really mandatory. As often stressed (*e.g.*, Engel *et al*., 2021), the taxonomic impediment, and the consequent "Linnean shortfall" (Brown and Lomolino, 1998), is due to a lack of taxonomists, not to a lack of available techniques!

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