IUSS Scuola Universitaria Superiore Pavia

Università degli Studi di Messina

THE BIVIERE OF LENTINI LAKE:
A MODEL OF RESILIENCE TO CLIMATE CHANGE AND ECO-SUSTAINABLE DEVELOPMENT

A Thesis Submitted in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy in

Sustainable Development and Climate change

Doctoral Programme of National Interest

PhD SDC
SUSTAINABLE DEVELOPMENT
AND CLIMATE CHANGE

In the Curriculum
HEALTH AND ECOSYSTEMS

Giovanni Brancato

07, 2026

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Giovanni Brancato

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Co-Supervisor: Prof. Carmelo Iaria

ABSTRACT

Freshwater reservoirs in Mediterranean regions represent complex and highly dynamic socio-ecological systems, shaped by the continuous interaction between hydrological variability, climatic seasonality, biological communities, and multiple forms of anthropogenic pressure. Unlike natural lakes, artificial reservoirs often display pronounced environmental fluctuations related to water-level changes, nutrient inputs, sediment resuspension, altered residence times, and management practices. These factors may influence water quality, microbial dynamics, trophic status, benthic community structure, fish health, and the distribution of aquatic and semi-aquatic bird species. In this context, integrated ecosystem-level investigations are essential to understand how abiotic conditions and biological components interact across different trophic levels, particularly in Mediterranean wetlands and reservoirs increasingly exposed to climate-driven instability and human-mediated environmental change.

Lake Biviere di Lentini, located in southeastern Sicily, is one of the largest lowland artificial lakes in Europe and represents a site of considerable ecological, hydrological, and conservation relevance. The lake plays an important role in regional water management and provides suitable habitats for a wide range of aquatic organisms, including fish, benthic macroinvertebrates, microbial communities, and numerous resident and migratory bird species. Despite its importance as a biodiversity reservoir and as a functional component of the surrounding landscape, comprehensive multidisciplinary assessments of its ecological status remain limited. In particular, few studies have simultaneously considered environme

ntal quality, microbial contamination, benthic assemblages, fish community composition, fish health, parasitological risks, and avifaunal dynamics within a unified monitoring framework.

The present study aimed to provide an integrated evaluation of Lake Biviere di Lentini by combining multiple analytical approaches across abiotic and biotic compartments. To this end, physico-chemical characterization of water was performed together with microbiological analyses of water and sediments, macrozoobenthic surveys, fish community assessment, pathological and parasitological examination of fish, and multi-seasonal monitoring of avifauna. This multidisciplinary design was intended to establish an ecosystem-level baseline for the lake and to explore possible relationships among water quality, microbial communities, benthic organisms, fish populations, and piscivorous birds. Particular attention was given to the identification of potential indicators of environmental imbalance, trophic fluctuations, biological interactions, and health-related concerns relevant to both wildlife conservation and ecosystem management.

Physico-chemical analyses showed that the lake generally maintained stable water quality conditions, with no evidence of severe or persistent chemical contamination during the investigated period. Trace metals and organic pollutants were detected at low concentrations or remained below detection limits, suggesting that direct anthropogenic contamination from these sources was limited. These findings indicate that, from a chemical perspective, Lake Biviere di Lentini did not show signs of marked degradation. However, the evaluation of trophic indicators revealed a more dynamic scenario. Chlorophyll-*a* concentrations exhibited pronounced seasonal variability, with extremely high values recorded during spring, consistent with transient eutrophic conditions and intense phytoplankton development. By contrast, chlorophyll-*a* values were negligible in autumn, suggesting a rapid decline in phytoplankton biomass and a strong seasonal regulation of primary productivity. This marked fluctuation highlights the ecological sensitivity of the reservoir and suggests that trophic conditions may be strongly influenced by seasonal hydrological and climatic factors, including temperature, nutrient availability, water residence time, and biological grazing pressure.

Microbiological analyses further contributed to the characterization of the lake ecosystem by documenting the presence of fecal indicator bacteria and opportunistic microbial taxa in both water and sediment samples. The detection of these microorganisms suggests the occurrence of localized or intermittent inputs of organic matter and microbial contamination, potentially associated with wildlife, surrounding land use, or hydrological runoff. At the same time, the consistent absence of *Salmonella* spp. represents a relevant finding, indicating that this pathogen was not circulating in the analyzed matrices during the study period. The combined analysis of water and sediments proved particularly informative, as sediments may act as both reservoirs and secondary sources of microbial communities, contributing to the persistence and redistribution of microorganisms within aquatic ecosystems. These results underline the importance of including microbiological parameters in long-term monitoring programs, especially in reservoirs that support abundant wildlife populations and are subjected to seasonal environmental variability.

Fish surveys revealed a diversified assemblage, mainly dominated by cyprinid species, reflecting the ecological features of a lowland freshwater reservoir.

Health assessment of the examined fish did not reveal macroscopic or histopathological lesions attributable to active infectious diseases, suggesting the absence of overt disease outbreaks at the time of sampling. Nevertheless, the isolation of *Aeromonas veronii* from clinically healthy individuals indicates the asymptomatic circulation of potentially pathogenic bacteria within the fish community. This finding is ecologically relevant, as opportunistic bacteria such as *Aeromonas* spp. may persist in aquatic environments and become pathogenic under stressful conditions, including poor water quality, high organic load, temperature fluctuations, increased stocking density, or altered host immune status. Therefore, the presence of *A. veronii* should not be interpreted as evidence of active disease, but rather as an indicator of the need for continuous fish health surveillance in environmentally variable systems.

Parasitological investigations provided additional information on the ecological interactions occurring within the reservoir. The detection of *Contracaecum rudolphii* third-stage larvae in two *Cyprinus carpio* specimens is of particular interest, as this anisakid nematode involves fish as intermediate or paratenic hosts and piscivorous birds as definitive hosts. Its occurrence in the fish community is therefore consistent with the ecological role of Lake Biviere di Lentini as an important feeding and resting site for piscivorous avifauna. The presence of *C. rudolphii* larvae highlights the existence of cross-trophic host-parasite interactions linking fish and birds and confirms the importance of integrating parasitological surveillance into broader ecosystem monitoring. Although the larvae were detected in a limited number of specimens, their zoonotic relevance and ecological significance support the need for further investigation, particularly in relation to fish species composition, feeding habits, bird abundance, and seasonal patterns of parasite transmission.

Macrozoobenthic communities were characterized by the dominance of tolerant taxa, particularly Chironomidae, which are commonly associated with unstable environmental conditions, organic enrichment, fine sediments, and fluctuations in oxygen availability. The structure of the benthic assemblage suggests that, despite the absence of severe chemical contamination, the sedimentary compartment may experience ecological stress linked to trophic instability, organic matter accumulation, or periodic hypoxic conditions. Since benthic macroinvertebrates integrate environmental conditions over time and represent a key trophic resource for fish and birds, their community composition provides valuable information on the functional status of the reservoir. The predominance of tolerant organisms may therefore reflect the combined effects of seasonal eutrophication, sediment dynamics, and habitat heterogeneity, reinforcing the need for continuous monitoring of benthic ecological quality.

Avifauna monitoring confirmed Lake Biviere di Lentini as a major biodiversity hotspot within the Mediterranean context. The lake supported abundant and diversified bird communities, including migratory, resident, aquatic, and piscivorous species. This finding underlines the strategic importance of the reservoir as a feeding, resting, and stopover area along migratory routes, as well as its role in maintaining regional ecological connectivity. The presence of numerous piscivorous birds is particularly relevant in light of the parasitological findings, as these species may contribute to the maintenance of anisakid life cycles and to the circulation of parasites across aquatic food webs. At the same time, birds may influence nutrient dynamics through guano deposition, contribute to microbial inputs, and interact with fish populations through predation. Avifaunal communities therefore represent not only a conservation target, but also an active ecological component capable of influencing trophic, microbiological, and parasitological processes within the lake.

Taken together, the results portray Lake Biviere di Lentini as a biologically rich but environmentally variable reservoir, in which apparently stable chemical conditions coexist with marked seasonal trophic fluctuations, microbial circulation, tolerant benthic assemblages, diversified fish populations, and intense avifaunal use. The lake does not appear to be affected by severe chemical pollution, but its ecological functioning is strongly influenced by seasonal dynamics and by interactions among different biological compartments. The high spring chlorophyll-*a* values, the presence of opportunistic bacteria, the dominance of tolerant macrozoobenthic taxa, and the detection of anisakid larvae collectively suggest that ecosystem health cannot be adequately assessed through single-parameter approaches. Instead, the ecological status of Mediterranean reservoirs should be interpreted through integrated frameworks capable of linking water quality, sediment conditions, microbial communities, aquatic fauna, parasite circulation, and bird ecology.

In conclusion, this study provides a comprehensive ecosystem-level baseline for Lake Biviere di Lentini and demonstrates the value of multidisciplinary monitoring in artificial Mediterranean freshwater systems. By integrating physico-chemical, microbiological, macrozoobenthic, ichthyological, pathological, parasitological, and ornithological data, the research offers a broader understanding of the ecological processes shaping the reservoir. The findings support the implementation of long-term monitoring programs aimed at detecting environmental change, identifying early warning indicators of ecological imbalance, and informing integrated management strategies. Such approaches are particularly important for Mediterranean reservoirs, where climate change, hydrological variability, eutrophication risk, land-use pressure, and biodiversity conservation needs increasingly converge. Lake Biviere di Lentini therefore emerges as both a valuable ecological resource and a sensitive environmental system, requiring adaptive, multidisciplinary, and conservation-oriented management.

ACKNOWLEDGEMENTS

The accomplishment of this thesis work was made possible due to the participation of several co-workers, and different research teams and laboratories. First of all, I would like to thank my supervisor, Professor Fabio Marino, for all the teachings, support and his huge contribution for driving me every day on this path and, moreover, co-director, actor and first supporter of my scientific documentary presented alongside the project Laghi Vivi 2.0. My sincere thanks also to the Coordinators Professor Mario Martina and Professor Roberto Buizza and the PhD CU6 Coordinator Professor Marina Maria Boido of the PhD Sustainable Development and Climate change course of the IUSS Pavia, for help and guidance. The support and contribution of research teams of the University of Messina: veterinary pathology in the person of Prof. Carmelo Iaria (co-supervisor of my thesis), Professor Giovanni Lanteri, Dr. Fabiano Capparucci, Dr. Jessica Abbate, Dr. Sabrina Natale, Dr. Maria Giovanna Piro and Dr. Rosa Falleti; veterinary parasitology in the person of Prof. Gabriella Gaglio and Dr. Giovanni De Benedetto; marine ecology in the person of Professor Nunziacarla Spanò, Prof. Serena Savoca and Dr. Claudio Gervasi; zoology in the person of Prof. Gioele Capillo; pharmaceutical biology in the person of Prof. Antonella Smeriglio and Professor Domenico Trombetta. Their support in all kinds of daily laboratory activities and sampling procedures, was essential to the success of this thesis and, moreover, for the realization of the scientific documentary.

I would like to extend my deepest gratitude to all the others “actors”, and obviously high-level professional figures, involved in the scientific documentary, in order of appearance: Ing. Salvatore Lombardo as Project manager of Laghi Vivi 2.0, Dr Iole Rizza of the Libero Consorzio Comunale di Siracusa- 10th sector Land and Enviroment, Dr Alberto Pulizzi of Regione Sicilia- Dipartimento Regionale della Pesca Mediterranea, Ing. Mario Barbagallo of Regione Sicilia- Dipartimento Regionale dell’Acqua e dei Rifiuti and Ing. Giuseppe Rannisi LIPU Manager of Catania section. Moreover I will also thank Macrostigma International Association for fish sampling and professionality.

I sincerely thank the Reviewers Professor Gionata De Vico and Professor Maria Violetta Brundo for their interest, expertise contribution and constructive criticism.

Finally, and not least, my wife and my son for their patience and support during the doctoral years. Also, I wish to express my gratitude to my mother and other family members and friends for their encouragement. A thought goes out to my late father, who is certainly cheering for me.

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1 INTRODUCTION

1.1 FRESHWATER ECOSYSTEMS UNDER GLOBAL ENVIRONMENTAL CHANGE

Freshwater ecosystems are among the most valuable, productive, and threatened components of the biosphere. Although lakes, rivers, reservoirs, ponds, streams, wetlands, and groundwater-dependent habitats cover only a limited fraction of the Earth's surface, they support a disproportionately high share of global biodiversity and provide essential ecological functions and ecosystem services (Dudgeon et al., 2006; Strayer and Dudgeon, 2010; Tickner et al., 2020; Sayer et al., 2025). Freshwater environments sustain highly diverse communities of microorganisms, macrophytes, phytoplankton, zooplankton, benthic invertebrates, fish, amphibians, reptiles, birds, and mammals, including many endemic and habitat-specialist taxa. Their ecological importance is therefore not confined to the aquatic compartment, but extends to riparian zones, wetlands, floodplains, terrestrial food webs, and migratory corridors (Dudgeon et al., 2006; Reid et al., 2019).

Freshwater ecosystems also provide key ecosystem services for human societies, including drinking-water supply, irrigation, fisheries, aquaculture, flood regulation, nutrient retention, water purification, carbon storage, sediment trapping, recreational opportunities, and cultural values (Vörösmarty et al., 2010; Tickner et al., 2020). At the same time, they are highly vulnerable to anthropogenic disturbance because they are

strongly influenced by their surrounding catchments and integrate pressures arising from land use, agriculture, urbanization, industrial discharge, water abstraction, hydrological modification, and biological invasions (Dudgeon et al., 2006; Vörösmarty et al., 2010; Reid et al., 2019). Consequently, freshwater ecosystem health cannot be adequately interpreted only through water chemistry, but requires a multidimensional evaluation of physical, chemical, biological, microbiological, and ecological indicators.

The current global decline of freshwater biodiversity is driven by multiple and often interacting stressors, including habitat degradation, hydrological alteration, pollution, eutrophication, flow regulation, invasive alien species, overexploitation, climate change, sediment modification, and loss of ecological connectivity (Dudgeon et al., 2006; Vörösmarty et al., 2010; Reid et al., 2019; Tickner et al., 2020). These pressures can disrupt the relationships among ecosystem components and produce cascading consequences across trophic levels. Altered nutrient inputs may promote excessive primary production and algal blooms; organic enrichment may increase microbial activity and oxygen consumption; sediment degradation may affect benthic assemblages; and changes in fish communities may influence both lower trophic levels and piscivorous predators (Carpenter et al., 1998; Smith et al., 1999; Scheffer, 2004).

Such vulnerabilities are particularly pronounced in Mediterranean regions, where freshwater ecosystems are naturally shaped by strong seasonality and are increasingly exposed to rising temperatures, recurrent droughts, irregular precipitation, high evaporation, and growing water demand (Cramer et al., 2018; Tramblay and Llasat,

2020; Kharitonov et al., 2021; Rani et al., 2024). In these areas, climate-driven hydrological variability may reduce water availability, concentrate nutrients and contaminants, alter residence time, intensify eutrophication, and increase the frequency of hypoxic events. Therefore, Mediterranean lakes, wetlands, and reservoirs represent sensitive systems in which ecological functioning may change rapidly in response to seasonal and interannual variability.

1.2 SHALLOW LAKES, RESERVOIRS, AND ECOSYSTEM FUNCTIONING

Lakes and reservoirs are lentic ecosystems whose ecological functioning depends on the interaction among basin morphology, hydrological regime, water residence time, stratification, sediment characteristics, nutrient availability, light penetration, temperature, oxygen dynamics, and biological communities (Wetzel, 2001; Kalff, 2002; Scheffer, 2004). In shallow lakes and lowland reservoirs, the close connection between the water column and the sediment compartment is particularly important. Because the water column is relatively shallow, wind-driven mixing, sediment resuspension, thermal fluctuations, and rapid changes in oxygen availability can strongly influence nutrient cycling, turbidity, phytoplankton development, benthic habitats, and microbial processes (Wetzel, 2001; Scheffer, 2004; Søndergaard et al., 2003).

Artificial lakes and reservoirs represent a distinctive subset of inland water systems. Although originally created for irrigation, water storage, industrial use, hydroelectric production, or flood regulation, many reservoirs progressively evolve into ecologically complex systems that support diverse biological communities and contribute to regional biodiversity (Thornton et al., 1990; Straškraba and Tundisi, 1999; Antonietta Mariani et al., 2015; Cantonati et al., 2020). Their functioning is shaped not only by natural ecological processes, but also by management practices, water-level regulation, abstraction regimes, catchment inputs, and artificial hydrological connectivity (Thornton et al., 1990; Straškraba and Tundisi, 1999; Pelletier et al., 2020). Reservoir ecosystems are often characterized by pronounced spatial and temporal heterogeneity. Water-level fluctuations may affect littoral habitats, macrophyte distribution, sediment exposure, fish spawning areas, benthic communities, nutrient release, and bird feeding grounds (Wantzen et al., 2008; Zohary and Ostrovsky, 2011).

In Mediterranean reservoirs, such fluctuations may be intensified by seasonal drought, irrigation demand, high evaporation, and irregular precipitation. As a result, artificial lakes in Mediterranean regions should be interpreted as dynamic socio-ecological systems rather than static water bodies, because their ecological conditions are determined by the combined effects of hydrology, management, climate, and biological interactions (Cantonati et al., 2020; Palma et al., 2021; Santos et al., 2018).

Despite their ecological and management importance, Mediterranean artificial lakes remain relatively understudied, particularly in terms of integrated ecosystem

health assessments that simultaneously consider water, sediments, microorganisms, macrozoobenthos, fish, parasites, and avifauna (Palma et al., 2021; Santos et al., 2018; Hupało et al., 2022). This represents an important knowledge gap because Mediterranean reservoirs may act at the same time as water-management infrastructures, biodiversity refugia, ecological corridors, and interfaces among aquatic, terrestrial, and avian communities.

1.3 MICROBIAL COMMUNITIES AND THE MICROBIAL LOOP

Microbial communities are fundamental drivers of aquatic ecosystem functioning. Bacteria, archaea, fungi, protists, and microbial eukaryotes contribute to organic matter degradation, nutrient regeneration, primary production, nitrogen cycling, sulphur cycling, methane production and oxidation, and the transformation of dissolved and particulate organic matter (Azam et al., 1983; Cotner and Biddanda, 2002; Pomeroy et al., 2007). Through the microbial loop, dissolved organic matter released by phytoplankton, macrophytes, animals, and allochthonous inputs is incorporated into bacterial biomass and subsequently transferred to higher trophic levels through protozoa, microzooplankton, and benthic consumers (Azam et al., 1983; Pomeroy et al., 2007).

In shallow lakes and reservoirs, microbial processes are particularly relevant at the water–sediment interface, where they regulate organic matter decomposition, nutrient release, redox-dependent reactions, and the recycling of biologically available elements (Wetzel, 2001; Søndergaard et al., 2003; Forsberg, 1989). Sediments may act both as sinks and sources of nutrients, pollutants, and microorganisms. Under oxic conditions, sediments may retain phosphorus and support aerobic mineralization, whereas under hypoxic or anoxic conditions, internal nutrient loading and anaerobic microbial processes may be enhanced (Søndergaard et al., 2003; Smolders et al., 2006). These mechanisms are particularly relevant in shallow reservoirs exposed to high organic matter input, seasonal warming, and water-level fluctuations.

Microbiological monitoring also provides important information on ecosystem health and contamination processes. Fecal indicator bacteria, including total coliforms, *Escherichia coli*, and enterococci, are widely used to assess the possible input of fecal material from human, livestock, wildlife, or runoff sources (Edberg et al., 2000; Rochelle-Newall et al., 2015). Their presence does not necessarily indicate the occurrence of specific pathogens, but it suggests potential sanitary pressure and the need for broader ecological interpretation. In reservoirs supporting large bird populations, microbial inputs may derive from bird guano, fish communities, surrounding agricultural areas, sediment resuspension, and surface runoff (Kirschner et al., 2004; Gwiazda, 2014).

The occurrence of opportunistic bacteria in aquatic environments should also be interpreted within an ecological framework. Genera such as *Aeromonas*, *Pseudomonas*,

and *Vibrio* may be naturally present in water and sediments, but some species can behave as opportunistic pathogens under stressful environmental conditions (Austin and Austin, 2016; Janda and Abbott, 2010). In fish, *Aeromonas* spp. are often associated with stress-related infections, particularly when poor water quality, high organic load, temperature fluctuations, or immune suppression compromise host resistance (Noga, 2010; Austin and Austin, 2016). Therefore, the isolation of opportunistic bacteria from clinically healthy fish may indicate asymptomatic circulation or environmental persistence rather than active disease, but it remains relevant for long-term fish health surveillance.

1.4 PHYSICOCHEMICAL INDICATORS AND TROPHIC DYNAMICS

Physicochemical parameters are essential for evaluating the ecological condition of aquatic systems. Temperature, dissolved oxygen, pH, conductivity, turbidity, nutrients, organic matter, trace metals, and organic pollutants influence biological communities and regulate ecosystem processes (Wetzel, 2001; Kalff, 2002). In reservoirs, these variables may vary spatially and seasonally according to hydrological inputs, water residence time, stratification, biological productivity, sediment–water exchange, and anthropogenic pressures from the surrounding catchment (Thornton et al., 1990; Straškraba and Tundisi, 1999).

Dissolved oxygen is particularly important because it affects fish survival, benthic community composition, microbial metabolism, nutrient cycling, and redox-dependent chemical transformations (Wetzel, 2001; Kalff, 2002). Hypoxia may result from high organic matter degradation, eutrophication, thermal stratification, sediment oxygen demand, or elevated temperatures. Oxygen depletion may reduce habitat suitability for sensitive taxa, favour tolerant benthic organisms, enhance phosphorus release from sediments, and shift microbial metabolism toward anaerobic pathways (Søndergaard et al., 2003; Smolders et al., 2006).

Nutrient enrichment is one of the most common drivers of ecological alteration in lakes and reservoirs. Increased nitrogen and phosphorus availability may stimulate phytoplankton growth, increase chlorophyll-*a* concentrations, reduce water transparency, promote harmful algal blooms, and contribute to hypoxia following biomass decomposition (Carpenter et al., 1998; Smith et al., 1999; Schindler, 2006). Chlorophyll-*a* is widely used as a proxy for phytoplankton biomass and trophic status, but its interpretation requires consideration of seasonality, hydrological conditions, grazing pressure, light availability, nutrient limitation, and residence time (Carlson, 1977; OECD, 1982; Scheffer, 2004).

In Mediterranean shallow reservoirs, trophic dynamics may be particularly variable. High chlorophyll-*a* concentrations may indicate transient eutrophic conditions or seasonal phytoplankton blooms rather than permanent degradation, whereas low values may reflect nutrient limitation, grazing, mixing, light limitation, or seasonal

collapse of phytoplankton communities (Scheffer, 2004; Søndergaard et al., 2003). For this reason, trophic status should be interpreted together with other environmental and biological indicators, including oxygen dynamics, nutrient concentrations, microbiological data, macrozoobenthic assemblages, fish health, and avifaunal presence.

Trace metals and organic pollutants provide information on chemical contamination. Their presence may reflect agricultural runoff, urban discharge, industrial activity, atmospheric deposition, or historical accumulation in sediments (Förstner and Wittmann, 1981; Chapman, 1996). However, the absence or low concentration of chemical contaminants does not necessarily correspond to optimal ecosystem status, because ecological imbalance may also result from eutrophication, hydrological instability, organic enrichment, sediment stress, invasive species, or altered trophic interactions (Dudgeon et al., 2006; Reid et al., 2019).

1.5 MACROZOOBENTHIC COMMUNITIES AS ECOLOGICAL INDICATORS

Macrozoobenthic communities are key components of freshwater ecosystems. They include aquatic insects, oligochaetes, molluscs, crustaceans, leeches, and other benthic invertebrates inhabiting sediments, macrophytes, and littoral habitats. These organisms contribute to organic matter decomposition, sediment bioturbation, nutrient

cycling, algal grazing, and energy transfer to fish and waterbirds (Rosenberg and Resh, 1993; Merritt et al., 2008). Because they integrate environmental conditions over time and include taxa with different tolerance levels, macrozoobenthic communities are widely used as bioindicators of ecological quality (Rosenberg and Resh, 1993; Bonada et al., 2006; Birk et al., 2012).

Sensitive taxa, including many Ephemeroptera, Plecoptera, and Trichoptera, are generally associated with well-oxygenated and less disturbed environments, whereas tolerant groups such as Chironomidae and Oligochaeta may dominate in organically enriched, low-oxygen, or unstable sedimentary conditions (Rosenberg and Resh, 1993; Armitage et al., 1995; Bonada et al., 2006). However, in shallow lentic systems and artificial reservoirs, the interpretation of benthic assemblages must consider natural habitat features, sediment type, water-level fluctuation, macrophyte cover, and seasonal oxygen dynamics (Merritt et al., 2008; Birk et al., 2012).

Chironomidae are among the most common and ecologically versatile freshwater macroinvertebrates. Their larvae occupy a wide range of habitats and many taxa tolerate organic enrichment, low oxygen availability, fine sediments, and fluctuating environmental conditions (Armitage et al., 1995; Merritt et al., 2008). A community dominated by Chironomidae does not automatically indicate severe degradation, particularly in productive lowland reservoirs, but it may suggest ecological stress when associated with reduced diversity, dominance of tolerant taxa, organic matter

accumulation, and unstable oxygenation (Rosenberg and Resh, 1993; Armitage et al., 1995).

In Mediterranean artificial lakes, macrozoobenthic communities may be influenced by water-level fluctuations, sediment exposure, habitat simplification, resuspension, predation, eutrophication, and seasonal changes in oxygen availability (Bonada et al., 2006; Birk et al., 2012). Their assessment is therefore useful not only for evaluating ecological quality, but also for understanding benthic food availability for fish and waterbirds and for linking sedimentary processes with higher trophic levels.

1.6 FISH COMMUNITIES AND FISH HEALTH IN RESERVOIR ECOSYSTEMS

Fish communities occupy a central position in freshwater food webs. They integrate ecological processes occurring at lower trophic levels and influence zooplankton, benthic invertebrates, macrophytes, nutrient cycling, and avifaunal feeding dynamics (Wootton, 1998; Winemiller and Jepsen, 1998; Jeppesen et al., 2010). In reservoirs, fish assemblages are shaped by habitat structure, water quality, trophic state, hydrological connectivity, stocking practices, fishing pressure, invasive species, predation, and water-level management (Thornton et al., 1990; Kubečka, 1993; Jeppesen et al., 2010).

Lowland artificial lakes often support cyprinid-dominated communities, particularly where waters are warm, productive, turbid, and characterized by soft sediments and abundant detrital resources (Kubečka, 1993; Jeppesen et al., 2010). Benthivorous and omnivorous cyprinids, such as common carp, may influence reservoir functioning through sediment disturbance, nutrient recycling, turbidity increase, and macrophyte reduction (Breukelaar et al., 1994; Zambrano and Hinojosa, 1999). At the same time, these species represent important prey for piscivorous birds and may serve as intermediate or paratenic hosts for parasites with complex life cycles.

Fish health assessment is an essential component of ecosystem monitoring because fish are exposed to waterborne contaminants, microbial communities, parasites, nutritional variation, temperature fluctuations, oxygen stress, and habitat changes (Noga, 2010; Woo and Bruno, 2011; Austin and Austin, 2016). Macroscopic examination, bacteriological analysis, parasitological evaluation, and histopathology provide complementary information on the health status of fish populations. The absence of gross or histopathological lesions attributable to active infectious disease may indicate the absence of overt disease outbreaks at the time of sampling, but it does not exclude subclinical infections, asymptomatic carriage, or environmental stress.

Aeromonas veronii is an aquatic bacterium frequently associated with freshwater environments and fish hosts. It may behave as an opportunistic pathogen, causing disease mainly under conditions of environmental stress or impaired host resistance (Janda and Abbott, 2010; Austin and Austin, 2016; Li et al., 2020). Its isolation from

clinically healthy fish should therefore be interpreted cautiously. Rather than indicating active disease, it may reflect asymptomatic circulation within the fish community or environmental persistence in water and sediments. Nevertheless, its detection is relevant for ecosystem health assessment because opportunistic bacteria may become pathogenic when environmental conditions deteriorate.

1.7 PARASITES AS INDICATORS OF ECOLOGICAL INTERACTIONS

Parasites are increasingly recognized as informative components of ecosystem assessment. They are not only pathological agents, but also ecological indicators capable of reflecting host distribution, food-web structure, trophic connectivity, biodiversity, and environmental change (MacKenzie, 1999; Sures, 2004; Marcogliese, 2005; Lafferty et al., 2008). Many parasites have complex life cycles involving multiple hosts from different trophic levels, and their presence may therefore indicate the persistence of specific ecological interactions, such as predator–prey relationships and the availability of suitable intermediate and definitive hosts.

In freshwater ecosystems, anisakid nematodes of the genus *Contracaecum* are particularly relevant because their life cycles connect aquatic invertebrates or fish with piscivorous birds (Anderson, 2000; Moravec, 2013). Adult nematodes occur in the digestive tract of fish-eating birds, which act as definitive hosts. Eggs are released into

the aquatic environment through bird feces, and larval stages develop through intermediate or paratenic hosts, including aquatic invertebrates and fish. Fish may harbour third-stage larvae, commonly located in the visceral cavity, on serosal surfaces, or in internal organs. Piscivorous birds become infected by consuming parasitized fish, thereby completing the parasite life cycle (Anderson, 2000; Moravec, 2013).

The *Contracaecum rudolphii* complex is frequently associated with cormorants and other fish-eating birds and has been reported in several freshwater systems where piscivorous birds are abundant (Mattiucci and Nascetti, 2008; Amor et al., 2020). In Italy, common carp, *Cyprinus carpio*, has been described as an intermediate or paratenic host for *Contracaecum* spp. in lakes characterized by high densities of fish-eating birds, including Lakes Trasimeno and Garda (Branciari et al., 2016; Menconi et al., 2023). These nematodes, primarily belonging to the *C. rudolphii* complex, typically occur as third-stage larvae within the visceral cavity and on serosal surfaces of common carp (Cammilleri et al., 2023).

From a veterinary and public-health perspective, anisakid larvae are relevant because some species have zoonotic potential. Although human infection by *Contracaecum* is rarer than infection by *Anisakis*, the consumption of raw, marinated, smoked, or undercooked fish products may represent a potential risk when viable larvae are present (Audicana and Kennedy, 2008; EFSA BIOHAZ Panel et al., 2010; EFSA BIOHAZ Panel et al., 2024). The actual risk depends on parasite species, larval viability, localization in edible tissues, fish-processing methods, and culinary habits. Therefore,

parasitological surveillance contributes not only to fish health assessment, but also to the evaluation of ecological links among fish populations, piscivorous birds, and potential food-safety concerns. Similarly, trematodes could be transmitted from fish to avian species as well as, even if rarely, human beings (Gaglio et al., 2016).

Parasites can also act as sentinels of environmental change. Alterations in host abundance, fish community structure, bird migration, trophic relationships, temperature, and habitat availability may influence parasite transmission dynamics (Marcogliese, 2005; Lafferty et al., 2008). In reservoirs supporting large populations of piscivorous birds, the occurrence of *Contracaecum* larvae in fish may therefore reflect cross-trophic ecological connectivity among aquatic habitats, fish communities, and avifauna. For this reason, parasitological data should be integrated into multidisciplinary ecosystem monitoring, particularly in wetlands and reservoirs where birds and fish are strongly interconnected.

1.8 AVIFAUNA AND CROSS-TROPHIC PROCESSES IN MEDITERRANEAN WETLANDS

Bird communities are among the most visible and ecologically significant components of wetland and reservoir ecosystems. Mediterranean wetlands are strategically important for resident species, wintering populations, and migratory birds moving between Europe and Africa (Finlayson et al., 2018; Green and Elmberg, 2014).

Artificial reservoirs may provide feeding, resting, nesting, and stopover habitats, especially where natural wetlands have been degraded, drained, or fragmented. In this sense, reservoirs can function as substitute or complementary habitats within regional ecological networks.

Waterbirds actively influence ecosystem processes. They may contribute to nutrient cycling through guano deposition, redistribute propagules and microorganisms, affect macrophyte and invertebrate communities, and influence fish populations through predation (Green and Elmberg, 2014; Gwiazda, 2014; Mukherjee et al., 2024). Piscivorous birds, such as cormorants, herons, and egrets, may directly affect fish community structure and biomass through feeding activity. At the same time, they may serve as definitive hosts for helminths, including anisakid nematodes, thereby connecting avian ecology with fish parasitology and aquatic food-web dynamics (Anderson, 2000; Moravec, 2013; Amor et al., 2020).

The presence of abundant avifauna may also influence microbiological conditions in water and sediments. Bird feces can introduce fecal indicator bacteria and other microorganisms into aquatic environments, particularly in shallow lakes, wetlands, and bird-influenced reservoirs (Kirschner et al., 2004; Gwiazda, 2014). This does not necessarily imply anthropogenic contamination, because wildlife may represent a natural source of microbial loading. However, where reservoirs are located within agricultural or human-influenced landscapes, distinguishing among wildlife, livestock,

runoff, and human sources may be difficult without detailed microbial source tracking (Rochelle-Newall et al., 2015).

Multi-seasonal avifauna monitoring is therefore essential for interpreting ecological processes in Mediterranean reservoirs. Bird abundance and species composition may change according to water level, food availability, vegetation structure, disturbance, migration phenology, and seasonal climate conditions (Green and Elmberg, 2014; Finlayson et al., 2018). Integrating ornithological data with aquatic monitoring can clarify interactions among nutrient inputs, microbial circulation, fish predation, parasite transmission, and biodiversity conservation.

1.9 LAKE BIVIERE DI LENTINI AS A CASE STUDY

Lake Biviere di Lentini, located in south-eastern Sicily, represents a particularly relevant case study for integrated ecosystem assessment. It is one of the largest artificial lowland lakes in Europe and one of the most important freshwater basins in Sicily. Its geographical position, hydrological function, and ecological characteristics make it a strategic system within the regional landscape. The lake was created and managed primarily as a water resource, but it has progressively acquired ecological relevance by supporting aquatic habitats, microbial assemblages, macrozoobenthic communities, fish populations, and abundant avifauna.

The importance of Lake Biviere di Lentini is linked to its dual role as a hydrological infrastructure and biodiversity reservoir. On the one hand, it contributes to water storage and regional water management. On the other hand, it provides habitats for a wide range of aquatic and semi-aquatic organisms and functions as an ecological interface among aquatic, terrestrial, and avian communities. This duality is typical of many Mediterranean artificial reservoirs, where water-management objectives and conservation needs coexist and sometimes interact in complex ways (Cantonati et al., 2020; Palma et al., 2021; Santos et al., 2018).

The lake supports several biological compartments that are closely interconnected. Microbial communities contribute to organic matter degradation and nutrient cycling in water and sediments. Macrozoobenthic organisms reflect sedimentary conditions and provide food for fish and birds. Fish communities occupy an intermediate trophic position and may act both as consumers of benthic and planktonic resources and as prey for piscivorous birds. Parasites with complex life cycles may connect fish and avifauna. Birds may contribute to nutrient inputs, microbial circulation, predation pressure, and parasite transmission. Therefore, Lake Biviere di Lentini should be interpreted as a cross-trophic system rather than as a simple artificial water basin.

Despite its ecological relevance, current knowledge of the ecosystem status of Lake Biviere di Lentini remains fragmented. Available information has often focused on specific components, such as avifauna, habitat value, or general environmental characteristics, whereas fewer studies have adopted a comprehensive multidisciplinary

perspective. In particular, the relationships among physicochemical conditions, microbiological indicators, sediment quality, macrozoobenthic communities, fish assemblages, fish health, parasitological findings, and avifaunal dynamics remain insufficiently characterized. This knowledge gap limits the ability to evaluate ecosystem health, identify early signs of ecological imbalance, and support integrated management strategies.

1.10 NEED FOR AN INTEGRATED ECOSYSTEM HEALTH APPROACH

Ecosystem health is a multidimensional concept that cannot be reduced to the absence of chemical pollution or visible disease. A reservoir may show low concentrations of trace metals and organic contaminants while still experiencing trophic instability, microbial circulation, hypoxia, sediment stress, loss of sensitive taxa, altered fish communities, or parasite transmission linked to changes in food-web structure (Rapport et al., 1998; Dudgeon et al., 2006; Reid et al., 2019). Conversely, the presence of certain microorganisms or parasites may not necessarily indicate degradation, but may reflect natural ecological processes in biologically rich systems. Interpretation therefore requires an integrated approach.

Multidisciplinary monitoring is particularly important in Mediterranean artificial reservoirs because these systems are shaped by the combined effects of hydrological

management, climate variability, biological interactions, and anthropogenic pressures (Cantonati et al., 2020; Palma et al., 2021; Santos et al., 2018). Physicochemical parameters provide information on water quality and trophic status. Microbiological analyses help identify fecal indicators, opportunistic bacteria, and microbial contamination patterns. Sediment analyses contribute to understanding microbial persistence and biogeochemical processes. Macrozoobenthic surveys reflect longer-term ecological conditions and sediment quality. Fish community assessments provide information on trophic structure, biodiversity, and conservation value. Fish health and parasitological analyses reveal potential pathological, ecological, and zoonotic concerns. Avifauna monitoring contextualizes higher trophic interactions, migratory relevance, nutrient inputs, and parasite life cycles.

Such an integrated framework is particularly suitable for systems like Lake Biviere di Lentini, where microbial, benthic, fish, parasitic, and avian components are likely to interact. Seasonal trophic fluctuations may influence microbial communities and oxygen dynamics. Benthic conditions may affect fish feeding resources. Fish populations may support piscivorous birds and participate in parasite transmission. Birds may contribute to nutrient and microbial inputs while also acting as definitive hosts for parasites. These processes cannot be fully understood when each compartment is studied separately.

2 AIM AND RATIONALE OF THE STUDY

In this context, the present study aimed to provide an integrated environmental and ecosystem health assessment of Lake Biviere di Lentini through a multidisciplinary monitoring approach. The investigation combined physicochemical characterization of water and sediments, microbiological analyses, macrozoobenthic surveys, fish community assessment, fish health evaluation, parasitological examination, and multi-seasonal avifauna monitoring.

The specific objectives were to:

i) characterize the main physicochemical conditions of the lake and evaluate the occurrence of chemical contamination and seasonal trophic variability;

ii) assess microbiological quality in water and sediments, with particular attention to fecal indicator bacteria, opportunistic microorganisms, and selected pathogens;

iii) describe macrozoobenthic community composition as an indicator of sedimentary and ecological conditions;

iv) characterize the fish assemblage and evaluate the health status of sampled fish through pathological, bacteriological, and parasitological analyses;

v) investigate the occurrence of zoonotically relevant anisakid larvae and their possible ecological significance in relation to fish–bird interactions;

vi) document avifaunal diversity and seasonal patterns, with particular attention to migratory and piscivorous species;

vii) integrate abiotic and biotic data to identify potential indicators of ecological imbalance and cross-trophic processes within the reservoir.

By adopting a holistic perspective, this work aims to provide a baseline framework for future monitoring and management of Lake Biviere di Lentini and, more broadly, of Mediterranean artificial reservoirs. The study contributes to filling a relevant knowledge gap by linking environmental quality, microbial dynamics, benthic assemblages, fish health, parasitological findings, and avifaunal ecology within a unified ecosystem-level assessment. Such an approach is essential for detecting early signs of environmental change, supporting biodiversity conservation, evaluating potential public-health implications, and informing adaptive management strategies in freshwater reservoirs exposed to increasing climatic and anthropogenic pressures.

3 MATERIALS AND METHODS

3.1 STUDY AREA: LAKE BIVIERE DI LENTINI

Lake Lentini, also known as Lake Biviere di Lentini or simply Biviere di Lentini, is an artificial freshwater reservoir located in south-eastern Sicily, within the administrative territory of the municipality of Lentini, in the province of Syracuse, close to the border with the province of Catania. The lake lies in a strategic geographical position between the northern margin of the Hyblaean Plateau and the Catania plain, at approximately 37°19'23" N and 14°57'05" E (Fig. 3.1.1). Due to its extension, hydrological function, and ecological relevance, Lake Biviere di Lentini represents one of the most important inland water bodies in Sicily and one of the largest lowland artificial reservoirs in Europe.

Fig.3.1.1 *The investigation site Lake Biviere di Lentini (Italy,Sicily) – [by URL 1]*



The name “Biviere” has historical and linguistic significance. It is generally linked to the Latin term vivarium, used to indicate an enclosed area intended for the maintenance or breeding of fish. In Sicily, the term progressively entered local usage as “Bliveri ri Lentini”, referring to a marshy or semi-lacustrine area traditionally associated with fishing and wetland resources. This etymological background reflects the long-standing relationship between the local population and the wetland environment, which historically functioned not only as a water body but also as a productive landscape for fishing, agriculture, and local subsistence activities.

The present reservoir has an elongated and irregular morphology. Under ordinary operating conditions, the lake extends for approximately 4.5 km along its major axis and about 3 km along its minor axis, with a perimeter of approximately 12 km. The surface area is approximately 9.2 km², corresponding to about 920 hectares, although the flooded area may vary according to seasonal water availability, hydrological management, and reservoir filling level (Fig. 3.1.2).

Fig.3.1.2 *Lake Biviere di Lentini in November 2023*



The maximum depth is approximately 20 m, making the reconstructed reservoir smaller in surface area but deeper than the historical wetland system. This morphological configuration is relevant from an ecological perspective because depth, water residence time, shoreline development, and water-level fluctuations influence thermal conditions, oxygen dynamics, sediment–water exchanges, biological productivity, and habitat availability for fish, macroinvertebrates, and waterbirds.

From a hydrographic perspective, the reservoir occupies a natural depression located between the last northern reliefs of the Hyblaean Mountains and the Catania plain. This area represents a transition zone between upland carbonate-volcanic landscapes and lowland alluvial environments. The basin is therefore influenced by both geological and hydrological processes typical of the north-eastern Hyblaean sector and by the broader drainage network connected to the Catania plain and the San Leonardo river system. The lake is an off-stream artificial reservoir, meaning that its hydrological functioning is not exclusively dependent on a single natural riverbed, but is regulated through artificial conveyance, controlled inflows, and hydraulic management structures.

The geological setting of the area is typical of the north-eastern Hyblaean sector, which is characterized by the coexistence of sedimentary and volcanic formations. Over geological time, repeated phases of submarine and subaerial volcanism occurred in association with carbonate and marine sedimentation. In the area surrounding the reservoir, the main outcropping formations include recent and terraced alluvial deposits and grey-blue silty-marly clays, particularly evident in the hilly sectors bordering the

basin. The substratum of the reservoir area has been described as being largely composed of Lower Pleistocene marine clay deposits, locally reaching considerable thickness. These geological characteristics are relevant for the stability, drainage, and hydrological behaviour of the reservoir, as well as for sediment composition, turbidity, and the potential development of fine-grained depositional environments along the lake bottom and margins (Baldovin et al., 2012).

The historical evolution of Lake Biviere di Lentini is essential for understanding its present ecological condition. The original Biviere was a natural or semi-natural wetland system that developed within the Lentini depression and was historically used for fishing and other local activities.For centuries, the lake and its surrounding marshes represented an important environmental and economic resource. However, the same wetland conditions also favoured the spread of malaria, particularly during the warmer months, when stagnant waters and marshy habitats promoted the proliferation of mosquito vectors. As a consequence, from the end of the nineteenth century onwards, several reclamation projects were proposed in order to improve public health conditions and recover land for agriculture.

The drainage of the ancient lake began in the first half of the twentieth century, particularly around the 1930s, and reclamation works continued for several decades. These interventions led to the disappearance of the historical wetland, together with the loss of the associated aquatic vegetation, fish communities, bird fauna, and marshland habitats. The reclamation of the Biviere therefore represents a significant

example of the transformation of Mediterranean wetlands during the twentieth century, when many marshy areas were drained for sanitary, agricultural, and socio-economic reasons. This historical phase is important because it interrupted the continuity of the original wetland ecosystem and profoundly modified the hydrology, land use, and ecological structure of the area.

At the end of the 1970s, the reconstruction of Lake Biviere di Lentini was planned as part of a broader strategy for water storage and regional development. The new reservoir was conceived primarily for agricultural and industrial water supply, with additional strategic value for the provinces of Catania and Syracuse. The project was funded through the Cassa per il Mezzogiorno and involved the construction of a new artificial basin within the area historically occupied by the ancient lake. The reconstructed reservoir was designed to be smaller than the former wetland in terms of surface extension, but deeper and more suitable for controlled water storage. The works required the construction of a long perimeter embankment, hydraulic regulation structures, drainage systems, and water conveyance infrastructure.

The reservoir was completed and progressively filled between the late twentieth century and the beginning of the twenty-first century. After a long and complex construction history, including technical and administrative controversies, the basin became functional as a large multipurpose water reservoir. Its maximum storage capacity is generally reported as approximately 127 million m^3 of usable water, while official reservoir documentation may distinguish between usable volume, total

impoundment capacity, authorized filling levels, and operational volumes. This distinction is important because the actual volume of water stored in the reservoir varies over time according to rainfall, drought conditions, water demand, hydraulic restrictions, sedimentation, and management decisions.

The hydraulic supply of Lake Biviere di Lentini is based on a regulated system of natural and artificial inflows. As an off-stream reservoir, it receives water mainly through conveyance infrastructure connected to the Simeto River, particularly through the Ponte Barca–Lentini adductor system. Additional inputs are associated with local streams and drainage channels, including the Zena, Barbajanni, Trigona, and Cave streams, which are part of the hydrographic network converging towards the San Leonardo River. These inflows contribute to the reservoir water balance, although their relative importance may vary seasonally and according to hydrological management. The connection between the reservoir and surrounding drainage systems also has ecological implications, since inflowing waters may transport suspended solids, nutrients, organic matter, microorganisms, and biological propagules from the catchment.

Since 2010, the lake has been managed by the Dipartimento Regionale dell'Acqua e dei Rifiuti of the Regione Siciliana. Water regulation occurs through a system of hydraulic structures, including two main towers. The northern tower receives water from the Simeto system and is primarily connected to water distribution for industrial and agricultural uses in the Catania area (Fig. 3.1.3).

Fig. 3.1.3 *Torre Nord (Northern Tower)*

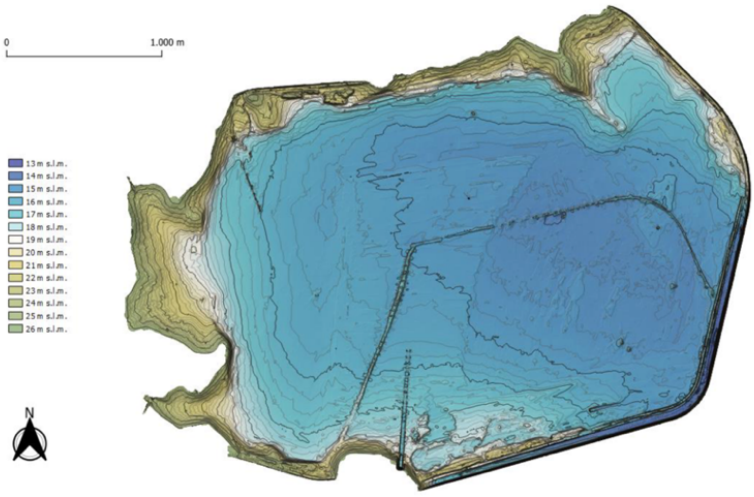


The southern tower is not currently associated with incoming water supply but is designed for possible distribution towards industrial and agricultural areas of the province of Syracuse. The presence of these infrastructures confirms the strategic role of the reservoir in regional water management and highlights its artificial and regulated nature.

In addition to the intake and outlet towers, the reservoir is equipped with a bottom outlet system connected to an approximately 2.5 km canal, which links the bottom drainage structure to the Trigona stream. The Trigona, together with the other local streams, contributes to the formation of the San Leonardo River system. This hydraulic connection is relevant not only from an engineering perspective but also from an ecological point of view, because bottom outlets and drainage systems may influence water exchange, sediment transport, downstream flow, and the movement of organic

and inorganic materials between the reservoir and the surrounding hydrographic network (Fig. 3.1.4, https://www.regione.sicilia.it/sites/default/files/2023- 12/PdGI_ progetto%20gestione%20%28aggiornato%29.pdf).

Fig.3.1.4 *Bathymetric survey and bottom drainage of the lake Biviere [URL 2].*



The maximum authorized water level of the reservoir has been reported as approximately 31.5 m above sea level, corresponding to the highest storage capacity. However, current operational constraints generally allow filling up to approximately 28 m above sea level, corresponding to a stored volume of about 100 million m^3. This volume is considered sufficient to guarantee water availability even during prolonged periods of reduced inflow, potentially covering up to two consecutive years of interruption or severe reduction in new water inputs. Such storage capacity is

particularly relevant in the Mediterranean context, where recurrent droughts, irregular precipitation, and increasing agricultural demand make water reservoirs essential components of regional adaptation strategies.

Although Lake Biviere di Lentini was reconstructed primarily for water storage, irrigation, and industrial supply, it has progressively regained considerable ecological value. The presence of a large permanent water body within a Mediterranean lowland landscape has favoured the establishment of aquatic and riparian habitats suitable for microorganisms, planktonic communities, benthic macroinvertebrates, fish, amphibians, reptiles, and birds. In particular, the lake has become an important resting, feeding, and nesting site for resident and migratory avifauna. Its ecological role is especially relevant for waterbirds and piscivorous species, which may interact with fish populations and contribute to cross-trophic ecological processes, including parasite transmission and nutrient redistribution.

The artificial origin of the reservoir, its hydrological regulation, its seasonal water-level variability, and its high ecological value make Lake Biviere di Lentini an appropriate case study for integrated ecosystem monitoring. The coexistence of water-management functions and biodiversity-supporting habitats creates a complex socio-ecological system in which physical, chemical, microbiological, biological, and health-related processes are closely interconnected. For this reason, the present study considered the lake not merely as a water storage infrastructure, but as a dynamic Mediterranean reservoir ecosystem requiring a multidisciplinary assessment.

In the framework of this thesis, Lake Biviere di Lentini was selected as a model site to evaluate the interactions among abiotic environmental conditions and biological compartments. Its large surface area, regulated hydrology, historical wetland origin, importance for fish and avifauna, and potential sensitivity to seasonal trophic fluctuations provide a suitable context for integrated monitoring. The study area therefore allowed the simultaneous investigation of physicochemical water characteristics, microbiological quality of water and sediments, macrozoobenthic assemblages, fish community composition, fish health status, parasitological findings, and avifaunal dynamics. This approach was intended to establish a baseline for future monitoring and to support the development of integrated management strategies for artificial Mediterranean freshwater reservoirs

3.2 WATER AND SEDIMENT SAMPLING

Water and sediment sampling was carried out during two monitoring campaigns performed in March and November 2023. These two sampling periods were selected in order to obtain information on the environmental conditions of Lake Biviere di Lentini under different seasonal scenarios. In Mediterranean freshwater reservoirs, seasonal variability may strongly influence water temperature, oxygen availability, biological productivity, microbial abundance, sediment–water interactions, and the concentration of suspended or dissolved substances. For this reason, the inclusion of two temporally distinct sampling campaigns allowed a preliminary evaluation of possible seasonal differences in the physicochemical, chemical, and microbiological characteristics of the lake.

Sampling procedures were carried out in accordance with the general principles established by Italian Legislative Decree 152/2006 and subsequent amendments, concerning environmental protection and water quality assessment. Water samples intended for chemical and microbiological analyses were collected and handled following standardized procedures aimed at ensuring sample representativeness, preventing contamination, and minimizing alterations during transport and storage. Additional methodological references included the APAT–IRSA/CNR 29/2003 analytical protocols and the EPA Clean Water Act Chemical Test Methods, selected according to the specific parameters investigated.

A single sampling station, named ST_C (Figures 3.2.1 and 3.2.2), was identified in the central area of the lake, at the geographical coordinates 37°19’19.5” N, 14°56’55.8” E.

The central portion of the reservoir was selected because it is representative of the open-water compartment and is less directly influenced by immediate shoreline effects, littoral vegetation, localized runoff, or marginal sediment disturbance. This site was therefore considered suitable for obtaining an integrated overview of the general environmental conditions of the lake, particularly in relation to the water column and the underlying sediment compartment.

Fig.3.2.1 *Sampling station ST_C [T*he map was generated using QGIS software*]*



Fig.3.2.2 *Sampling procedures in ST_C*



Water samples were collected at three depths: surface, mid-depth, and bottom. This vertical sampling strategy was adopted to account for possible depth-related variation in water quality. In lentic and reservoir ecosystems, physicochemical parameters and microbial distribution may differ along the water column as a consequence of thermal gradients, oxygen stratification, redox conditions, suspended particulate matter, biological activity, and interactions with bottom sediments. The inclusion of bottom-water samples was considered particularly relevant because the water–sediment interface may influence nutrient release, microbial persistence, organic matter degradation, and the resuspension of particulate material.

For each sampling event, approximately 5 L of water were collected using a Niskin bottle. This device allowed the collection of discrete samples from selected depths, reducing the risk of mixing among different water layers and improving the reliability of vertical characterization. After collection, water samples were transferred into appropriate containers according to the subsequent analytical procedures. Samples intended for laboratory chemical analyses were subdivided into 1 L polyethylene bottles, whereas samples for microbiological analyses were collected and handled separately in order to avoid cross-contamination.

Physicochemical parameters that are particularly sensitive to environmental changes were measured directly in the field. In situ measurements were performed immediately at the sampling site in order to obtain values representative of the actual conditions of the lake at the time of sampling and to avoid changes associated with storage, transport, temperature variation, gas exchange, or biological activity. The following parameters were measured using a portable multiparameter probe (HI9829, Hanna Instruments, Italy): water temperature (°C), pH, dissolved oxygen (DO, mg/L), electrical conductivity (µS/cm), redox potential (mV), and turbidity (NTU).

Water temperature was recorded because it is one of the main drivers of physical, chemical, and biological processes in freshwater ecosystems. Temperature may influence oxygen solubility, microbial metabolism, organic matter degradation, phytoplankton growth, fish physiology, and the seasonal dynamics of biological communities. The measurement of pH provided information on the acid–base

conditions of the water column, which may affect nutrient availability, metal speciation, microbial processes, and aquatic organism health. Dissolved oxygen was included as a key indicator of ecosystem functioning, since oxygen availability is essential for fish, aerobic microorganisms, and most benthic organisms, and may also influence redox-dependent reactions at the water–sediment interface.

Electrical conductivity was measured as an indicator of the concentration of dissolved ions and general mineralization of the water. This parameter may reflect the influence of geological substratum, inflowing waters, evaporation, agricultural runoff, and seasonal concentration effects. Redox potential was recorded to provide additional information on the oxidation–reduction conditions of the water column, particularly in relation to oxygen availability and possible biogeochemical processes involving organic matter degradation, nutrient cycling, and sediment interactions. Turbidity, expressed in nephelometric turbidity units (NTU), was measured to evaluate the presence of suspended particles in the water column. In reservoirs, turbidity may be influenced by phytoplankton biomass, sediment resuspension, wind-driven mixing, runoff inputs, organic particles, and the activity of benthivorous fish.

Water samples intended for laboratory analyses were transported under controlled conditions and processed according to the requirements of each analytical determination. The methods used for the analysis of chemical and physicochemical parameters were selected on the basis of the target analytes and are reported in detail in the following section. These analyses were aimed at characterizing the general

chemical quality of the lake water and assessing the presence of selected compounds or elements potentially relevant to environmental monitoring.

For microbiological analyses, an additional 2 L of water were collected during each sampling campaign. Microbiological samples were managed separately from the chemical samples in order to preserve their integrity and avoid contamination. All samples were stored under refrigerated conditions and transported to the laboratory at approximately 4 °C. Refrigerated transport was adopted to limit microbial growth or die-off during transfer and to preserve the microbiological characteristics of the samples as closely as possible to those present at the time of collection. Samples were processed after arrival at the laboratory according to the microbiological procedures described in the dedicated methodological section.

Sediment sampling was performed at the same central station used for water collection. Sediment samples were collected using an Ekman bottom grab with a sampling area of 225 cm^2. This instrument was selected because it allows the collection of superficial bottom sediment from soft substrates, which are commonly present in lentic and reservoir environments. The superficial sediment layer is particularly important in environmental assessment because it represents the zone most directly involved in sediment–water exchanges, organic matter accumulation, microbial activity, nutrient release, and the deposition of suspended material.

After retrieval, sediment samples were carefully transferred into 1 L glass bottles for subsequent laboratory analyses. Glass containers were used because they are

suitable for preserving sediment samples and reducing possible interactions between the sample matrix and the container material. Sediment samples were stored under refrigerated conditions and transported to the laboratory together with the water samples. The inclusion of sediment in the sampling design was considered essential because sediments may act both as sinks and as secondary sources of nutrients, microorganisms, organic matter, and contaminants. In shallow and artificial reservoirs, the sediment compartment may strongly influence the overlying water column, especially under conditions of resuspension, oxygen depletion, or seasonal changes in redox conditions.

Overall, the combined collection of water samples at different depths and superficial sediment samples allowed the characterization of both the pelagic and benthic compartments of Lake Biviere di Lentini. This sampling design was consistent with the multidisciplinary aim of the study, which sought to integrate physicochemical, chemical, microbiological, and ecological information in order to provide a more complete assessment of the environmental status of the reservoir.

3.3 CHEMICAL ANALYSES

Chemical analyses were performed to characterize the main physicochemical and chemical features of the water collected from Lake Biviere di Lentini and to evaluate parameters relevant to trophic status, organic load, ionic composition, and the possible occurrence of chemical contamination. The analytical plan included the determination of chlorophyll-*a*, chemical oxygen demand, total dissolved solids, acidity, alkalinity, ammoniacal nitrogen, major anions and cations, volatile and semi-volatile organic compounds, organochlorine pesticides, and trace metals.

Analyses were conducted according to standardized and validated procedures selected on the basis of the target analytes and analytical requirements. When applicable, methods followed the APAT–IRSA/CNR 29/2003 protocols and the corresponding EPA Clean Water Act Chemical Test Methods. Samples were processed under conditions suitable to preserve analyte stability, minimize contamination, and ensure analytical reproducibility. Field and laboratory procedures were carried out using appropriate containers, clean laboratory materials, calibrated instruments, reagent blanks, standard solutions, and replicate determinations, according to the specific requirements of each analytical method.

The inclusion of these parameters allowed the assessment of both general water quality and specific chemical indicators. Chlorophyll-*a* was used as an indicator of phytoplankton biomass and trophic status. Chemical oxygen demand provided information on the oxidizable organic and inorganic load present in the water. Total

dissolved solids, acidity, alkalinity, ammoniacal nitrogen, and major ions contributed to the characterization of the mineral and nutrient composition of the reservoir. The analysis of organic pollutants and trace metals was performed to evaluate the possible presence of contaminants of environmental relevance.

3.3.1 Chlorophyll-*a* determination

Chlorophyll-*a* concentration was determined according to APAT–IRSA/CNR 29/2003, Method 9020. This parameter was included because chlorophyll-*a* represents one of the most widely used indicators of phytoplankton biomass and provides useful information on the trophic status and primary productivity of freshwater ecosystems. In lentic systems and reservoirs, chlorophyll-*a* may show marked seasonal variability in relation to nutrient availability, temperature, light conditions, water residence time, phytoplankton growth, and grazing pressure.

Water samples intended for chlorophyll-*a* determination were collected in dark bottles in order to reduce light-induced pigment degradation. After collection, samples were stored under refrigerated conditions at approximately 4 °C and processed within 8 h from sampling. The use of low temperature and dark storage was adopted to preserve pigment stability and to limit biological and photochemical alteration before laboratory analysis.

In the laboratory, water samples were filtered through glass fibre filters with a pore size of 0.7 µm and a diameter of 47 mm. Filtration was performed under gentle

vacuum in order to retain suspended phytoplanktonic material while minimizing mechanical damage to cells and pigment loss. After filtration, filters were gently drained to remove excess water and transferred to extraction vessels.

Pigments retained on the filters were extracted using an acetone/Milli-Q water solution at a ratio of 90:10 (v/v). Extraction was performed under cold conditions to limit pigment degradation. The extract was brought to a final volume of 10 mL, stored for 2 h at 4 °C in the dark, and subsequently clarified by filtration before spectrophotometric reading.

Absorbance was measured at 750 nm and 664 nm before acidification, and at 750 nm and 665 nm after acidification with hydrochloric acid. The readings at 750 nm were used to correct for turbidity and background absorbance, while the acidification step allowed correction for pheopigments. Chlorophyll-*a* concentration was calculated according to the standard equations provided by the reference method and expressed as mg m^{-3}. This unit is equivalent to µg L^{-1} and is commonly used for the evaluation of phytoplankton biomass in aquatic systems.

3.3.2 Chemical oxygen demand determination

Chemical oxygen demand, hereafter COD, was determined using the dichromate reflux method according to APAT–IRSA/CNR 29/2003, Method 5130. COD was included as an indicator of the amount of oxygen required to chemically oxidize organic and inorganic oxidizable substances present in the water sample. This parameter provides

information on the potential organic load of the aquatic system and can be useful for evaluating the influence of organic matter, runoff, biological productivity, and decomposition processes.

For each analysis, 50 mL of water sample were treated with potassium dichromate under strongly acidic conditions using concentrated sulphuric acid. Silver sulphate was added as a catalyst to promote the oxidation of organic compounds, while mercury(II) sulphate was used to complex chloride ions and reduce chloride-related analytical interference. The reaction mixture was subjected to reflux for 2 h, allowing the oxidation of the chemically oxidizable fraction of the sample.

After digestion, the mixture was allowed to cool and was diluted with Milli-Q water. The excess dichromate remaining after oxidation was titrated with standardized ammonium iron(II) sulphate solution using phenanthroline as redox indicator. A reagent blank was processed in parallel under the same analytical conditions in order to correct for reagent consumption not attributable to the sample.

COD values were calculated from the difference between the titration volume of the blank and that of the sample and were expressed as mg O_2 L^{-1}. This expression represents the oxygen equivalent required for chemical oxidation. The use of blanks and standardized titrant solutions allowed the control of analytical accuracy and reproducibility.

3.3.3 Total dissolved solids determination

Total dissolved solids, hereafter TDS, were determined gravimetrically. TDS represents the total amount of dissolved inorganic and organic substances remaining in solution after filtration and subsequent evaporation of the filtrate. This parameter contributes to the characterization of water mineralization and may reflect the influence of geological substratum, evaporation, inflowing waters, agricultural runoff, and seasonal concentration processes.

Before analysis, water samples were thoroughly mixed to ensure homogeneity. Samples were filtered under vacuum in order to remove suspended particulate matter. The filtrate was then collected in a previously dried and pre-weighed evaporation capsule. The filtered sample was evaporated to dryness and subsequently dried in an oven at 105 °C until constant weight was reached.

The concentration of total dissolved solids was calculated from the difference between the final weight and the initial weight of the capsule, taking into account the volume of sample analyzed. Results were expressed as mg L^{-1}. Constant-weight drying was used to ensure that the measured residue corresponded to stable dissolved material and was not influenced by residual moisture.

3.3.4 Acidity and alkalinity

Acidity and alkalinity were determined by titrimetric methods according to the IRSA-CNR/APAT standard method 2010. These parameters were included because they

provide information on the acid-neutralizing and base-neutralizing capacity of the water, which are important for evaluating buffering capacity, carbonate equilibrium, pH stability, and the potential response of the lake to external inputs or biological processes.

Water samples were analyzed immediately after collection whenever possible, in order to minimize changes in dissolved carbon dioxide, pH, and carbonate equilibria. Measurements were performed in triplicate (n = 3) to improve analytical reliability and to evaluate repeatability.

For alkalinity determination, a fixed volume of 100 mL of water sample was titrated with standardized hydrochloric acid, 0.02 N. Phenolphthalein was used to identify the endpoint corresponding to pH 8.3, allowing the determination of phenolphthalein alkalinity. Titration was then continued using methyl orange to identify the endpoint corresponding to approximately pH 4.5, allowing the determination of total alkalinity. Total alkalinity was therefore related mainly to the presence of carbonate, bicarbonate, and hydroxide species.

Acidity was determined by titration with standardized sodium hydroxide, 0.02 N. The titration was conducted to pH 4.5 and, when required, extended to pH 8.3, depending on the analytical objective and the characteristics of the sample. For turbid or colored samples, visual endpoints may be difficult to identify accurately. In these cases, endpoints were verified potentiometrically using a calibrated pH meter with an accuracy of ±0.01 pH units, identifying the inflection point of the titration curve.

Quality assurance procedures included calibration and standardization of titrants, analysis of reagent blanks, and replicate measurements. Analytical repeatability was considered acceptable when the relative standard deviation was below 5%. Results were expressed as mg L^{-1} $CaCO_3$, allowing comparison with standard water-quality classifications and buffering-capacity assessments.

3.3.5 Ammoniacal nitrogen determination

Ammoniacal nitrogen, expressed as NH_4^+–N, was determined spectrophotometrically using the indophenol blue method according to the IRSA-CNR/APAT standard method 4030. Ammoniacal nitrogen was included because it represents an important form of inorganic nitrogen in freshwater systems and may derive from organic matter decomposition, excretion by aquatic organisms, sediment release, agricultural inputs, wastewater contamination, or microbial transformations. Its concentration is ecologically relevant because ammonia and ammonium are involved in nitrogen cycling and may influence algal productivity and aquatic organism health.

Water samples were analyzed immediately after collection or stored at 4 °C and analyzed within 24 h. All measurements were performed in triplicate (n = 3). This procedure was adopted to limit changes in nitrogen speciation and microbial transformation that could occur during prolonged storage.

The method is based on the reaction of ammonium ions with phenol and hypochlorite in an alkaline medium, in the presence of sodium nitroprusside as catalyst,

forming an intensely colored indophenol blue compound. After reagent addition, samples were incubated at room temperature for color development. Absorbance was measured at 640–660 nm using a UV–Vis spectrophotometer against reagent blanks.

Calibration curves were prepared using ammonium standard solutions at known concentrations. Quality assurance procedures included the analysis of reagent blanks, verification of calibration curves, and replicate analyses. Analytical repeatability was considered acceptable when the relative standard deviation was below 5%. Results were expressed as mg L^{-1} NH_4^+–N.

3.3.6 Ion chromatography for major anions and cations

Major anions and cations were determined by high-performance ion chromatography, HPIC. This analysis was performed to characterize the ionic composition of lake water and to evaluate the concentration of major dissolved species relevant to mineralization, nutrient dynamics, and possible external inputs. The determination of major ions is particularly useful in reservoirs because ionic composition may be influenced by geological substratum, evaporation, inflowing waters, agricultural drainage, and hydrological management.

Analyses were performed using a Dionex DX-LAN 500 system equipped with a quaternary gradient pump, conductivity detector, and electrolytic self-regenerating suppressor. Cations, including Na^+, K^+, Mg^{2+}, and Ca^{2+}, were separated using an IonPac CS10 column set with 25 mM HCl as eluent. Anions, including F^-, Cl^-, NO_2^-, SO_4^{2-}, NO_3^-,

and PO_4^{3-}, were analyzed using an IonPac AS11HC column set with 10 mM NaOH as eluent.

The flow rate was set at 1.0 mL min^{-1}, and the injection volume was 25 µL. Prior to analysis, water samples were filtered through 0.45 µm filters to remove suspended particles that could interfere with chromatographic separation or damage the analytical columns. Samples were then diluted 1:1 with HPLC-grade water when required by the analytical range of the method.

Calibration standards were prepared from analytical-grade reagents. Calibration curves were obtained for each ion and showed linearity with correlation coefficients greater than 0.998. All measurements were performed in triplicate (n = 3). The use of calibration standards, sample filtration, dilution, conductivity detection, and replicate analyses ensured reliable quantification of the selected ionic species.

3.3.7 Determination of volatile organic compounds, semi-volatile organic compounds, and organochlorine pesticides

The occurrence of selected organic contaminants was investigated through the determination of volatile organic compounds, semi-volatile organic compounds, and organochlorine pesticides. These analyses were included to evaluate the possible presence of organic pollutants of environmental concern and to assess potential anthropogenic pressures affecting the lake.

Volatile organic compounds, hereafter VOCs, including chloroform, 1,2-dichloroethane, carbon tetrachloride, and trichloroethylene, were determined by purge-and-trap gas chromatography–mass spectrometry, P&T-GC–MS, according to EPA Methods 5030C and 8260C. Purge-and-trap extraction was used because VOCs are characterized by high volatility and require analytical procedures capable of transferring analytes from the aqueous matrix to the gas phase while minimizing losses. The GC–MS system allowed chromatographic separation and mass-spectrometric identification and quantification of the target compounds.

Semi-volatile chlorinated compounds, hereafter SVOCs, including hexachlorobutadiene and chlorobenzenes such as 1,2,4-trichlorobenzene, were analyzed by gas chromatography–mass spectrometry, GC–MS, following appropriate solvent extraction procedures. These compounds were investigated because of their environmental persistence and potential relevance as indicators of industrial or historical contamination. Solvent extraction was used to concentrate the analytes from the aqueous matrix before chromatographic analysis.

Organochlorine pesticides, hereafter OCPs, including aldrin, dieldrin, endrin, isodrin, dichlorodiphenyltrichloroethane, DDT, hexachlorocyclohexane, HCH, and hexachlorobenzene, HCB, were determined by gas chromatography with electron capture detection, GC-ECD, according to EPA Method 8081B after sample extraction. GC-ECD was selected because of its high sensitivity for halogenated compounds, making it suitable for the detection of chlorinated pesticides even at low concentrations.

Quality-control procedures included procedural blanks and spiked samples, processed under the same analytical conditions as the environmental samples. Analyses were performed in triplicate (n = 3) to evaluate repeatability. The combined determination of VOCs, SVOCs, and OCPs provided information on the possible presence of selected organic contaminants in the water column and contributed to the broader chemical characterization of the reservoir.

3.3.8 Trace metal determination

Trace metals in water samples were determined according to APAT–IRSA/CNR 29/2003 official methods. The analysis focused on metals of environmental relevance, including cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), copper (Cu), zinc (Zn), and mercury (Hg). These elements were selected because they may derive from natural geological sources or anthropogenic inputs and may be relevant for aquatic ecosystem health even at low concentrations, depending on their bioavailability, persistence, and potential toxicity.

Elemental analysis of Cd, Cr, Ni, Pb, Cu, and Zn was performed by graphite furnace atomic absorption spectrometry, GF-AAS, using a Varian SpectrAA 220 equipped with GTA110 and Zeeman background correction. GF-AAS was used because of its sensitivity for trace-level metal determination in aqueous matrices. Zeeman correction allowed compensation for non-specific background absorption and matrix-related interferences, improving analytical accuracy.

Mercury was determined by cold vapour atomic absorption spectrometry, CV-AAS. This technique was selected because mercury can be efficiently reduced to elemental mercury vapour and measured with high sensitivity by atomic absorption. The method is particularly suitable for mercury determination in environmental water samples.

All samples were handled using acid-cleaned glassware previously treated with 0.1% HNO_3 in order to minimize contamination during laboratory procedures. Quality-assurance measures included the use of procedural blanks, clean laboratory materials, calibrated instrumentation, and adherence to good laboratory practices. These precautions were adopted to reduce the risk of external contamination and to ensure reliable determination of trace metals in the analyzed water samples.

3.4 MICROBIOLOGICAL INDICATORS OF WATER QUALITY

Microbiological analyses were performed to evaluate the sanitary status of Lake Biviere di Lentini and to investigate the occurrence of selected bacterial indicators and potentially pathogenic microorganisms in the water column and sediment compartment. The analytical approach was based on the detection and enumeration of microorganisms commonly used in environmental monitoring, including total coliforms, *Escherichia coli*, enterococci, sulphite-reducing Clostridia spores, *Vibrio* spp., and

Salmonella spp. These microbial groups were selected because they provide complementary information on fecal contamination, environmental persistence, sediment-associated microbial reservoirs, and the possible presence of bacteria of sanitary or ecological relevance.

The microbiological analyses were carried out using the membrane filtration technique, in accordance with standardized procedures for environmental microbiology and with reference to the APAT Manual 46/2007, ISPRA and ICRAM guidelines, and the indications provided by the competent national environmental authorities. The use of membrane filtration allowed the concentration of microorganisms from defined volumes of water and their subsequent cultivation on selective or differential media. This method is particularly suitable for the analysis of environmental waters, as it permits the quantitative expression of results as colony-forming units per unit volume of sample.

Sampling was performed during two different periods of the year, March and November 2023, in accordance with the water and sediment sampling design described above. These two sampling campaigns allowed the evaluation of microbiological conditions under different seasonal scenarios, considering that microbial abundance and composition in freshwater reservoirs may be influenced by temperature, rainfall, runoff, wildlife presence, organic matter input, water residence time, and sediment resuspension.

For each sampling campaign, microbiological analyses were performed on water collected at three depths: surface, mid-depth, and bottom. The inclusion of different depths was intended to evaluate possible vertical variation in microbial indicators within the water column. Surface waters may be more directly influenced by atmospheric inputs, bird activity, and superficial runoff, whereas bottom waters may reflect sediment–water interactions, organic matter accumulation, resuspension processes, and lower oxygen availability. This vertical approach was therefore considered appropriate for a reservoir in which microbial communities may be distributed unevenly in relation to hydrological and trophic conditions.

For each depth, three independent aliquots of 100 mL were filtered and analysed separately. Filtration was carried out using sterile membrane filters with a pore size of 0.45 µm, which is commonly used for retaining bacterial cells from water samples. After filtration, membranes were carefully transferred onto Petri dishes containing selective or differential culture media specific for the microbial group under investigation. All procedures were performed under aseptic conditions in order to avoid external contamination during sample handling and processing.

For the simultaneous detection and enumeration of total coliforms and *Escherichia coli*, membranes were placed on Chromogenic Coliform Agar, CCA. This medium allows the differentiation of coliform bacteria and *E. coli* on the basis of enzymatic activity and colony colour development. Total coliforms were included as

general indicators of microbial contamination and environmental hygiene, whereas *E. coli* was considered a more specific indicator of recent fecal contamination.

For the detection of Vibrio spp., membranes were placed on Thiosulfate Citrate Bile Sucrose agar, TCBS. This selective medium was used to investigate the occurrence of bacteria belonging to the genus *Vibrio*, which includes species naturally associated with aquatic environments as well as species of potential sanitary or ecological relevance. Although *Vibrio* spp. are more commonly associated with marine and brackish environments, their investigation may be informative in reservoir ecosystems where temperature, organic matter, ionic composition, and environmental variability can influence the distribution of opportunistic aquatic bacteria.

For the detection and enumeration of enterococci, membranes were placed on Slanetz and Bartley agar. Enterococci were included because they are widely used as indicators of fecal contamination and may show greater environmental persistence than *E. coli* under certain conditions. Their presence may reflect inputs from humans, livestock, wildlife, or birds, and is therefore useful for interpreting microbial pressure in reservoirs with abundant avifauna and surrounding land-use influence.

Plates for total coliforms, *E. coli*, *Vibrio* spp., and enterococci were incubated at 37 °C for 24–48 h, according to the growth requirements of the target microorganisms and the indications of the culture media used. After incubation, colonies showing typical morphology and colour on each selective medium were counted. Results were

expressed as colony-forming units per 100 mL, CFU 100 mL^{-1}, for each depth and sampling period.

The detection of sulphite-reducing Clostridia spores was performed using membrane filtration after heat treatment of the samples. This analysis was included because sulphite-reducing anaerobic spore-forming bacteria are indicators of fecal or environmental contamination with high persistence. Their spores are resistant to adverse environmental conditions and may persist in water and sediments for longer periods than vegetative bacterial cells. For this reason, their detection can provide useful information on historical or persistent contamination and on the role of sediments as microbial reservoirs.

In the first phase of the analysis, water samples were subjected to heat treatment at 70 °C in order to select for spores by reducing the presence of vegetative bacterial cells. Subsequently, three aliquots were filtered for each sample through 0.45 µm membranes. The membranes were then placed on SPS agar, a selective medium for sulphite-reducing Clostridia, and incubated at 37 °C under anaerobic conditions for 24 h. After incubation, typical colonies were counted, and results were expressed as CFU 100 mL^{-1}.

For sediment samples, the presence of sulphite-reducing Clostridia spores was investigated using 5 g aliquots of sediment. This matrix was included because sediments can accumulate spores and other persistent microorganisms and may act as a secondary source of microbial contamination to the overlying water column, particularly during

resuspension events or under changing redox conditions. Results for sediment samples were expressed as CFU g^{-1} of sediment.

The detection of *Salmonella* spp. was performed using membrane filtration followed by selective enrichment and culture, according to Method 7080. This analysis was included because *Salmonella* spp. are enteric pathogens of sanitary relevance and their presence in environmental waters may indicate fecal contamination from human or animal sources. Unlike the other bacterial indicators, *Salmonella* detection was performed as a qualitative analysis, and results were expressed as presence or absence in the analysed sample volume.

For water samples, 1 L of water was filtered through membranes with a pore size of 0.45 µm. The membranes were then transferred to 100 mL of buffered peptone water for pre-enrichment and incubated at 37 °C for 24 h. The pre-enrichment step was used to promote the recovery of stressed or low-abundance *Salmonella* potentially present in the environmental samples.

After pre-enrichment, three aliquots of 100 µL were inoculated into tubes containing Rappaport–Vassiliadis enrichment broth, RVS, and incubated at 44 °C for 24 h. Selective enrichment was followed by streaking onto MacConkey agar plates using a sterile loop. Plates were incubated at 37 °C for 24 h. Suspected colonies were subjected to biochemical identification using the API 20E system, bioMérieux, according to the manufacturer's instructions.

For sediment samples, 27 g of sediment were suspended in 300 mL of physiological solution. After sedimentation of the coarser particulate fraction, the supernatant was processed following the same procedure described for water samples, including filtration, pre-enrichment, selective enrichment, plating, and biochemical identification. Results for *Salmonella* spp. in sediment were expressed as presence or absence in the processed sediment aliquot, rather than as colony counts, because the adopted enrichment-based method is qualitative.

Positive and negative controls were included in each analytical session in order to verify the performance of culture media, incubation conditions, enrichment procedures, and biochemical identification systems. Procedural blanks were used to monitor possible contamination during filtration and handling. Replicate analyses were performed for the quantitative microbiological indicators, and results were reported using the appropriate units according to the matrix analysed: CFU 100 mL^{-1} for water samples and CFU g^{-1} for sediment samples when applicable.

Overall, the microbiological analytical design allowed the assessment of both recent and persistent indicators of contamination in Lake Biviere di Lentini. The combined analysis of water samples from different depths and sediment samples provided information on the microbial status of the water column, the possible role of the sediment compartment as a reservoir of persistent microorganisms, and the occurrence of selected bacteria of sanitary and ecological relevance within the reservoir ecosystem.

3.5 BACTERIOLOGICAL INVESTIGATION

Bacteriological screening was performed on a subset of six specimens of common carp, *Cyprinus carpio*, selected from the fish collected in Lake Biviere di Lentini. The examined fish had a body weight ranging from approximately 1000 to 1500 g. This subset was selected in order to investigate the possible presence of cultivable bacteria in internal organs and to assess whether apparently healthy fish could harbour potentially pathogenic or opportunistic bacterial taxa. Culture-based bacteriological examination remains a widely used approach in fish health assessment, particularly for the detection of systemic bacterial infections and the isolation of viable bacterial agents from internal organs (Noga, 2010; Austin and Austin, 2016; Woo and Bruno, 2011).

After sampling, fish were maintained under refrigerated conditions and transported to the laboratory facilities of the University of Messina, where they were subjected to necropsy. Refrigerated transport was adopted to reduce post-mortem autolysis and uncontrolled bacterial proliferation before diagnostic examination. All specimens were processed as soon as possible after arrival at the laboratory in order to preserve the reliability of bacteriological results and to minimize the risk of post-mortem contamination or overgrowth by environmental bacteria (Noga, 2010; Austin and Austin, 2016).

Before bacteriological sampling, each fish was externally examined and then dissected under controlled laboratory conditions. Particular attention was paid to

minimizing contamination from the skin, mucus, intestinal content, sampling instruments, or surrounding environment. For this reason, internal sampling was performed after disinfection of the external surface and opening of the body cavity using sterile instruments. Aseptic sampling is essential in fish bacteriology because many aquatic bacteria are also present on the skin, gills, mucus, and intestinal tract and may contaminate internal organs during necropsy if appropriate precautions are not adopted (Noga, 2010; Austin and Austin, 2016).

Samples were aseptically collected from the cranial kidney and caudal kidney. These anatomical sites were selected because the kidney is one of the main target organs for bacteriological investigation in fish. In teleosts, the kidney has both excretory and haematopoietic functions and is commonly involved in systemic bacterial infections. Therefore, the detection of bacteria in kidney tissue may provide useful information on possible systemic colonization or infection. Sampling of the anterior or caudal kidney is commonly recommended in fish bacteriology for the investigation of systemic bacterial diseases, and kidney swabs are routinely inoculated onto general-purpose media such as tryptic soy agar or related media (Noga, 2010; Austin and Austin, 2016; WorldFish, 2021).

Collected samples were inoculated onto Blood Agar, BA, and Tryptic Soy Agar, TSA. Blood Agar was used as an enriched medium suitable for the growth of a wide range of bacteria and for the preliminary evaluation of colony morphology and haemolytic activity. Haemolysis, when present, may provide useful preliminary information on

bacterial behaviour, although it is not sufficient for species identification. Tryptic Soy Agar was used as a general-purpose medium supporting the growth of several heterotrophic bacterial taxa commonly isolated from fish and aquatic environments. The use of both media allowed a wider screening of cultivable bacteria potentially associated with the sampled tissues (Noga, 2010; Austin and Austin, 2016; WorldFish, 2021).

Inoculated plates were incubated at 30 °C for 24–48 h. This incubation temperature was selected as appropriate for the recovery of mesophilic and aquatic bacteria commonly associated with freshwater fish. Incubation conditions in fish bacteriology are usually adapted to the ecology of the host species, the water temperature, and the expected bacterial taxa, since several fish-associated bacteria grow optimally at temperatures lower than those routinely used for mammalian clinical microbiology (Noga, 2010; Austin and Austin, 2016). Plates were checked after incubation for bacterial growth. Colony morphology, including size, shape, colour, margin, elevation, surface appearance, and haemolytic pattern on Blood Agar when present, was recorded as part of the preliminary characterization.

After incubation, representative bacterial colonies were selected from primary culture plates and subcultured when necessary to obtain pure cultures. Isolation of pure colonies was essential to ensure the reliability of the subsequent identification procedures. Gram staining was then performed on bacterial isolates in order to evaluate cell morphology, cellular arrangement, and Gram reaction. This preliminary microscopic

examination provided basic information useful for orienting the interpretation of bacterial isolates and for distinguishing major bacterial groups before instrumental identification. However, colony morphology and Gram staining alone are not sufficient for accurate species-level identification of many aquatic bacteria, because several fish-associated taxa may show overlapping phenotypic characteristics (Austin and Austin, 2016; Janda and Abbott, 2010).

Species-level identification of bacterial isolates was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF MS. Pure bacterial colonies were subjected to MALDI-TOF MS analysis according to the manufacturer's instructions and laboratory procedures. This technique is based on the generation of protein mass spectral profiles, mainly derived from abundant ribosomal proteins, which are compared with reference spectra included in the instrument database. MALDI-TOF MS has become a rapid and widely used method for routine bacterial identification in diagnostic microbiology, as it allows fast comparison of spectral profiles with reference databases and may reduce the time required for identification compared with conventional biochemical methods (Seng et al., 2009; Clark et al., 2013; Croxatto et al., 2012).

The use of MALDI-TOF MS was particularly appropriate in this study because fish-associated and environmental bacterial isolates may be difficult to identify accurately using only conventional phenotypic procedures. MALDI-TOF MS has been successfully applied to the identification of several bacterial fish pathogens and has been proposed

as a useful diagnostic tool in aquatic animal health, including for isolates belonging to genera of veterinary relevance such as *Aeromonas, Streptococcus, Lactococcus, Vibrio*, and other fish-associated bacteria (Assis et al., 2017; Pérez-Sancho et al., 2018; Çağatay et al., 2024). Nevertheless, MALDI-TOF MS identification may be influenced by the quality and completeness of the reference database, and some aquatic or environmental taxa may be underrepresented. For this reason, identification results were interpreted according to the reliability scores provided by the instrument software and with consideration of the ecological origin of the isolates (Seng et al., 2009; Pérez-Sancho et al., 2018).

Only isolates obtained in pure culture and producing acceptable identification scores according to the instrument software were considered for species-level reporting. When identification scores were below the threshold required for reliable species assignment, results were interpreted cautiously and, when appropriate, limited to genus level. This approach was adopted to avoid overinterpretation of uncertain identifications, particularly for environmental or aquatic bacteria that may be underrepresented in commercial MALDI-TOF MS databases (Croxatto et al., 2012; Pérez-Sancho et al., 2018).

The bacteriological investigation was interpreted together with necropsy findings, histological observations, and the general health status of the sampled fish. This integrated approach was important because the isolation of bacteria from internal organs does not always indicate overt disease, especially in wild or free-living fish. Some

bacterial taxa may behave as opportunistic microorganisms and may be isolated from clinically healthy specimens without associated gross or microscopic lesions. For instance, several *Aeromonas* species are common in aquatic environments and may act as opportunistic pathogens under stressful conditions, including poor water quality, temperature fluctuations, high organic load, or impaired host resistance (Janda and Abbott, 2010; Noga, 2010; Austin and Austin, 2016). Therefore, bacteriological results were evaluated in relation to the presence or absence of pathological changes and to the environmental context of Lake Biviere di Lentini.

Overall, bacteriological screening was included to assess the occurrence of cultivable bacteria in internal tissues of *C. carpio* and to identify potentially pathogenic or opportunistic bacterial species circulating within the fish community. The integration of culture-based methods, Gram staining, and MALDI-TOF MS identification contributed to the fish health assessment and provided additional information on microbial interactions between the aquatic environment and resident fish populations.

3.6 PARASITOLOGICAL EXAMINATION

Parasitological examination was performed on all fish included in the health assessment in order to detect the presence of ectoparasites and endoparasites and to evaluate possible parasite-associated lesions. The procedure combined external

inspection, gill biopsy, coelomic cavity examination, gastrointestinal tract analysis, parasite recovery, fixation, clearing, and morphological identification. This integrated approach was adopted because fish parasites may occur on external surfaces, gills, internal organ serosae, within the gastrointestinal lumen, or encysted in visceral tissues, and therefore require a systematic examination of multiple anatomical compartments (Eiras et al., 2006; Noga, 2010; Woo and Bruno, 2011; Moravec, 2013).

Each fish underwent a careful external macroscopic evaluation before necropsy. The skin, fins, eyes, oral cavity, opercular region, and gills were inspected for visible parasites and for lesions potentially attributable to parasitic infestation, including abnormal mucus production, epithelial erosions, haemorrhages, discoloration, nodules, cysts, or focal tissue reactions. External examination was performed as a first screening step because several ectoparasites and parasite-associated lesions may be detected directly on the body surface or gill apparatus during gross inspection (Eiras et al., 2006; Noga, 2010; Woo and Bruno, 2011).

The gills were examined with particular attention, as they represent one of the most common target sites for ectoparasites in freshwater fish. Gill arches were removed and inspected macroscopically and under a Zeiss SteREO Discovery.V12 stereomicroscope. The use of stereomicroscopy allowed the detection of small parasites, cysts, focal lesions, or subtle tissue alterations that may not be clearly visible during routine gross examination. In addition, gill biopsies and wet mounts were prepared to detect microscopic ectoparasites, including monogenean flukes, ciliates,

and other small parasitic organisms. Fresh gill material was mounted on glass slides and observed using a light microscope, allowing the evaluation of motile or attached parasitic forms before fixation-related artefacts could occur (Eiras et al., 2006; Noga, 2010).

After external and branchial examination, the coelomic cavity was opened and internal organs were inspected. The serosal surfaces of the liver, spleen, kidney, gonads, swim bladder, mesenteries, and gastrointestinal tract were examined macroscopically and under stereomicroscopy. This step was included to detect free, attached, or encysted endoparasites, particularly helminth larvae that may occur on visceral surfaces or within the coelomic cavity. The inspection of internal organ serosae is particularly relevant for anisakid nematodes and other helminths, which may be found attached to the viscera, embedded in the intestinal wall, or encysted within surrounding tissues (Anderson, 2000; Moravec, 2013).

To perform a comprehensive helminth detection, the gastrointestinal tract was removed and processed separately. The stomach and intestine were examined as distinct anatomical segments according to the procedure described by De Benedetto et al. (2023). Segmental processing of the gastrointestinal tract allowed a more precise localization of parasitic stages and reduced the risk of overlooking parasites present in specific digestive compartments. Each segment was opened longitudinally and examined under stereomicroscopic observation. The luminal contents, mucosal surface, intestinal wall, and serosal surface were carefully inspected for adult helminths, larval

nematodes, cysts, embedded parasites, and parasite-associated lesions. Similar approaches are commonly recommended for the recovery of helminths from the digestive tract of fish and other vertebrate hosts (Eiras et al., 2006; Justine et al., 2012; Moravec, 2013).

All collected parasites were gently removed using fine forceps or suitable dissecting instruments. Parasite specimens were rinsed in freshwater to remove mucus, host debris, intestinal content, and other adherent material. Rinsing before fixation was performed to improve the subsequent visualization of morphological structures and to reduce the presence of artefacts during microscopic examination. After rinsing, parasites were fixed and preserved in 70% ethanol, a commonly used preservative for helminth specimens, particularly when subsequent morphological observation or additional analyses may be required (Eiras et al., 2006; Justine et al., 2012; Moravec, 2013).

After fixation, selected parasite specimens were cleared in glycerin for 24 h and mounted on glass slides for morphological identification. Clearing in glycerin was performed to increase tissue transparency and allow the visualization of internal structures useful for taxonomic identification, including the digestive tract, anterior and posterior extremities, and other diagnostic characters. Clearing and mounting procedures were carried out according to standard approaches used in helminth morphology and fish nematode studies (Moravec, 2013; Abdullah et al., 2021).

Morphological identification was performed using recent taxonomic keys and descriptions, with particular reference to Abdullah et al. (2021) for anisakid nematode larvae and diagnostic features of *Contracaecum* larvae. For nematode larvae, particular attention was paid to body shape, cuticular features, anterior extremity, boring tooth or larval tooth when visible, oesophagus, intestinal caecum, ventricular appendix, posterior end, and general larval organization. These characters were evaluated to support the recognition of anisakid larvae and their attribution to the genus *Contracaecum* when compatible with the observed morphology (Anderson, 2000; Moravec, 2013; Abdullah et al., 2021).

Morphological observations were conducted using an Axioskop 2 plus light microscope. Representative images were acquired using an Axiocam Mrc digital camera connected to the microscope and managed through the Axiovision imaging system. Digital imaging was used to document diagnostic morphological features, support taxonomic interpretation, and provide illustrative material for the thesis. Photographic documentation is also important for traceability and for subsequent comparison with taxonomic descriptions or reference material (Moravec, 2013).

Because species-level identification of anisakid larvae based exclusively on morphology may be difficult, especially within species complexes such as the *Contracaecum rudolphii* complex, taxonomic assignment was interpreted cautiously when molecular confirmation was not available. Morphological examination was therefore considered appropriate for the recognition of anisakid larvae and for

presumptive genus-level or complex-level identification, while definitive species-level attribution was considered more reliable when supported by molecular methods, as recommended in studies on *Contracaecum* larvae (Mattiucci and Nascetti, 2008; Abdullah et al., 2021).

Overall, the parasitological examination combined macroscopic inspection, stereomicroscopy, gill wet-mount observation, gastrointestinal tract dissection, parasite recovery, ethanol fixation, glycerin clearing, and light-microscopy-based morphological identification. This methodological approach allowed the detection and characterization of visible ectoparasites, gastrointestinal helminths, encysted parasitic stages, and anisakid nematode larvae, contributing to the integrated assessment of fish health and host–parasite interactions in Lake Biviere di Lentini.

3.7 FISH SAMPLING AND HEALTH ASSESSMENT

Fish sampling was carried out in August 2023 in Lake Biviere di Lentini. This period was selected to investigate the fish community during the summer season, when Mediterranean reservoirs may experience high water temperatures, increased biological activity, possible reduction in oxygen availability, and greater environmental stress for aquatic organisms. These conditions may influence fish physiology, host–

microorganism interactions, parasite detectability, and the potential expression of subclinical or overt pathological conditions.

Sampling was performed by a private company Macrostigma International Association at two sites within the lake (Fig. 3.7.1).: ST_TG, located in the watchtower area at 37°18’48.0” N, 14°56’46.0” E (Fig. 3.7.2) and ST_TA, located near the water lift tower at 37°19’52.2” N, 14°56’09.3” E (Fig. 3.7.3).

Fig.3.7.1 *Fish sampling sites ST_TG and ST_TA [by QGIS Software]*

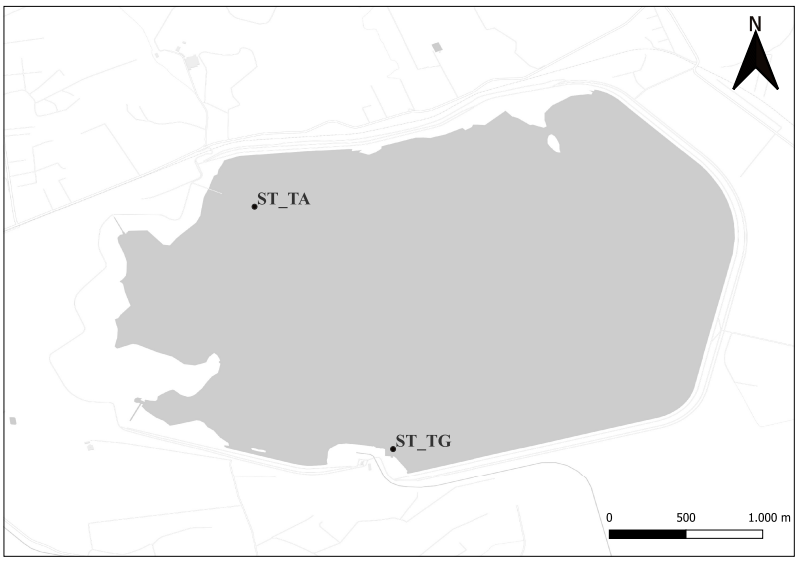


Fig. 3.7.2 *ST_TG sampling site near the watchtower*



Fig. 3.7.3 *ST_TA sampling site near the Northern water lift tower*



The selection of two sampling sites allowed the inclusion of fish from different sectors of the reservoir and provided a preliminary representation of the health status

of common carp inhabiting the lake. The two areas were chosen to include zones differing in their position within the reservoir and in their proximity to relevant structural and hydrological features.

A total of 20 common carp, *Cyprinus carpio*, were provided by the fishing authorized company Macrostigma International Association (Fig.3.7.4).

Fig.3.7.4 *A specimen of* Cyprinus carpio *caught by Macrostigma*



This species was selected because it is one of the dominant and ecologically relevant fish species in the reservoir and is commonly associated with lowland freshwater systems. Common carp is also of interest in ecosystem health studies because of its benthivorous and omnivorous feeding habits, its close interaction with sediments, and its potential role as an intermediate or paratenic host for parasites

transmitted through aquatic food webs. Its biology therefore makes it particularly suitable for investigating links among sediment conditions, fish health, microbial circulation, and parasite transmission.

After capture, fish were humanely euthanized by immersion in an overdose of tricaine methanesulfonate, MS-222. The euthanasia solution was prepared at a concentration of 500 mg L^{-1} MS-222, corresponding to the upper range of the commonly recommended euthanasia dose for fish, generally reported as 250–500 mg L^{-1} or approximately 5–10 times the anaesthetic dose (AVMA, 2020; Lavalle et al., 2025). The use of MS-222 by immersion is widely accepted for fish euthanasia when applied at overdose concentrations and under appropriate welfare conditions (AVMA, 2020; Popovic et al., 2012).

Because MS-222 produces an acidic solution when dissolved in water, the euthanasia bath was buffered before use. Sodium bicarbonate, $NaHCO_3$, was added gradually to the MS-222 solution until an approximately neutral pH was reached. The final pH was checked before fish immersion and maintained within the range of pH 7.0–7.5, as recommended for buffered MS-222 solutions used in aquatic animals (AVMA, 2020; Popovic et al., 2012). Buffering was performed to reduce the possible irritant or aversive effects associated with acidic, unbuffered MS-222 and to ensure that euthanasia was carried out under appropriate animal-welfare conditions.

Fish were maintained in the buffered MS-222 overdose solution until complete loss of equilibrium, absence of response to external stimuli, and cessation of opercular

movements were observed. Specimens were kept immersed for an additional confirmation period after the cessation of opercular activity, in order to ensure irreversible loss of vital functions before necropsy. Death was confirmed before further handling. The procedure was performed in accordance with current animal welfare regulations and with internationally accepted recommendations for the use of buffered MS-222 as a euthanasia agent in fish (AVMA, 2020; Popovic et al., 2012; Lavalle et al., 2025).

Following confirmation of death, fish were stored under refrigerated conditions at approximately 4 °C and transported to the necropsy room of the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy. Refrigerated transport was used to slow post-mortem autolysis and bacterial overgrowth, thereby preserving tissues and organs for pathological, microbiological, and parasitological analyses. All specimens were processed as soon as possible after arrival at the laboratory.

Each specimen was individually identified before examination. Biometric data were recorded for all fish. Body weight was measured using a digital scale (PBA220, Mettler Toledo) with an accuracy of 1 g, while total length was measured using an ichthyometer with an accuracy of 0.1 cm. The collection of biometric data allowed the characterization of the sampled individuals and provided useful contextual information for interpreting health status, body condition, and parasitological findings.

Before necropsy, each fish underwent a complete external examination. The skin, scales, fins, eyes, mouth, opercular region, gills, and body surface were inspected for the presence of gross abnormalities. Particular attention was paid to external lesions, ulcers, erosions, haemorrhages, fin damage, abnormal pigmentation, excessive mucus production, ectoparasites, deformities, traumatic injuries, or other visible signs potentially compatible with infectious, parasitic, environmental, or mechanical causes. External examination represented the first step of the health assessment and provided information on possible clinical or subclinical conditions affecting the sampled fish.

Before opening the body cavity, the external surface of each fish was disinfected with ethanol in order to reduce the risk of contamination during internal sampling, particularly for microbiological analyses. After disinfection, the abdominal cavity was opened using sterile instruments and a systematic necropsy examination was performed. The coelomic cavity was inspected for the presence of abnormal fluid, fibrin, adhesions, haemorrhages, parasites, organ displacement, or other gross pathological changes. When present, coelomic fluid was evaluated macroscopically for quantity, colour, transparency, and consistency.

Internal organs were examined in situ and then sampled according to the requirements of the different diagnostic procedures. The liver, spleen, kidney, gastrointestinal tract, gonads, and other visible organs were inspected for gross lesions, including enlargement, discoloration, nodules, congestion, haemorrhages, necrotic areas, abnormal consistency, or parasitic forms. This macroscopic evaluation was aimed

at detecting possible evidence of systemic disease, localized lesions, organ-specific abnormalities, or parasitic infections.

Kidney samples were collected for microbiological analyses. The kidney was selected because it is commonly used in fish bacteriology as a target organ for the detection of systemic bacterial infections. Sampling was performed after external disinfection and opening of the body cavity in order to reduce contamination from the skin, mucus, intestinal contents, or surrounding environment. The bacteriological investigation was intended to identify cultivable bacteria potentially associated with fish tissues and to assess whether clinically healthy or apparently normal fish harboured opportunistic or pathogenic bacterial taxa.

Representative samples of internal organs were collected for histological examination. Histology was included to evaluate tissue architecture and to detect microscopic lesions not visible during gross examination. This approach allowed the assessment of possible inflammatory, degenerative, circulatory, necrotic, proliferative, or parasitic lesions affecting internal organs. The integration of gross pathology and histology provided a more reliable evaluation of the health status of the sampled fish.

Parasitological examination was also performed on the collected fish. The coelomic cavity, visceral surfaces, and internal organs were inspected for the presence of helminths or other parasites. Particular attention was given to larval nematodes potentially belonging to anisakid taxa, because these parasites may connect fish populations with piscivorous birds and may provide information on cross-trophic

ecological interactions within the reservoir. Parasitological findings were therefore interpreted not only in relation to fish health, but also as possible indicators of food-web structure and host–parasite dynamics in Lake Biviere di Lentini.

Overall, the fish health assessment combined biometric measurements, external examination, necropsy, bacteriological sampling, histological analysis, and parasitological evaluation. This integrated approach was adopted to obtain a comprehensive characterization of the sampled common carp and to investigate potential links among environmental conditions, microbial circulation, fish health, and parasite transmission within the reservoir ecosystem.

3.8 ICHTHYOFAUNAL SURVEY

An extended ichthyofaunal survey was carried out in Lake Biviere di Lentini by the Macrostigma International Association in order to document the composition of the fish assemblage inhabiting the reservoir. The survey was designed to complement the fish health assessment described above by providing broader information on the ichthyofauna of the lake, including the occurrence of different species, their relative representation in the sampled areas, and their ecological relevance within the reservoir ecosystem.

The use of an extended ichthyofaunal survey was considered important because fish communities represent a central component of freshwater food webs and provide useful information on ecosystem structure, habitat suitability, trophic interactions, and ecological connectivity. In artificial Mediterranean reservoirs, fish assemblages may be influenced by hydrological management, water-level fluctuations, habitat heterogeneity, trophic status, stocking history, species introductions, predation pressure, and the availability of littoral and benthic habitats. For this reason, the survey was conducted using a combination of passive and active fishing methods, in order to increase the probability of detecting species with different ecological habits, body sizes, swimming abilities, and habitat preferences.

Several fishing gears were used during the survey, including trammel nets with a mesh size of 12 mm, invisible monofilament nets with a mesh size of 10 mm, Bertovelli half-moon nets with a mesh size of 6 mm, sinking pots with a mesh size of 4 mm, baited cages, and electrofishing in shallow vegetated areas. The combination of these methods allowed the investigation of different ecological compartments of the lake. Passive nets and traps were suitable for capturing fish moving through open-water, littoral, or benthic habitats, whereas electrofishing was used specifically in shallow vegetated zones, where conventional nets may be less effective and where juvenile, small-bodied, or habitat-associated species may occur.

The use of multiple gears was adopted to reduce the selectivity associated with any single capture method. In fish community studies, each fishing technique tends to

favour the capture of certain species or size classes. For example, gillnets and trammel nets are generally more effective for mobile fish of medium or large size, while pots and cages may be more suitable for benthic or shelter-seeking species. Electrofishing, when applied in shallow and vegetated areas, can improve the detection of species associated with marginal habitats. Therefore, the combined approach provided a more comprehensive representation of the fish assemblage of Lake Biviere di Lentini.

Captured fish not intended for diagnostic analyses were handled carefully in order to minimize stress and physical injury. Each specimen was identified at the lowest possible taxonomic level, measured, photographed or otherwise documented when required, and released immediately after data collection. The rapid release of non-diagnostic specimens was adopted to reduce the impact of the survey on the resident fish community and to ensure that the ichthyofaunal assessment was conducted according to a low-impact monitoring approach.

Field activities were carried out in compliance with animal welfare, operator safety, and biosecurity standards. Fish handling was minimized, and all procedures were performed by trained personnel. Particular attention was paid to the prevention of accidental pathogen transfer among sampling areas or between different aquatic environments. For this reason, fishing equipment and field materials were disinfected using a diluted chlorine solution after use. This biosecurity measure was adopted to reduce the risk of spreading microorganisms, parasites, or other biological agents through nets, traps, containers, boots, or other sampling equipment.

Overall, the ichthyofaunal survey provided complementary information to the pathological and parasitological assessment of common carp. While diagnostic sampling focused on the health status of selected fish specimens, the broader survey allowed the characterization of the fish community as a biological component of the lake ecosystem. The integration of ichthyofaunal data with physicochemical, microbiological, macrozoobenthic, parasitological, and avifaunal information contributed to a more complete interpretation of Lake Biviere di Lentini as a dynamic artificial freshwater reservoir.

3.9 HISTOLOGICAL EXAMINATION

Because of its abundance within Lake Biviere di Lentini, its ecological relevance, and its close interaction with the benthic compartment, common carp, *Cyprinus carpio*, was selected as the target species for histopathological and parasitological evaluation

This species is particularly suitable for health assessment in reservoir ecosystems because of its benthivorous and omnivorous feeding behaviour, its long life, its frequent contact with sediments, and its ability to reflect environmental conditions affecting both the water column and the bottom compartment. In addition, common carp may act as a host for several parasitic organisms and may be exposed to opportunistic bacteria,

organic enrichment, oxygen fluctuations, and other environmental stressors typical of shallow and artificial freshwater systems.

Histological examination was included in the diagnostic workflow as a complementary tool to gross necropsy, bacteriological analysis, and parasitological inspection. While macroscopic examination allows the identification of visible lesions, organ enlargement, discoloration, haemorrhages, external damage, or parasites, histology provides a more detailed evaluation of tissue architecture and cellular morphology. This approach is particularly important when fish appear clinically healthy or do not show evident gross lesions, as microscopic changes may reveal subclinical inflammatory, degenerative, circulatory, proliferative, necrotic, or parasite-associated alterations. Therefore, histopathology was used to obtain a more complete assessment of the health status of the sampled fish and to identify possible tissue-level responses to environmental or biological stressors.

Representative tissue samples were collected during necropsy from the main internal organs. These included organs commonly evaluated in fish pathology, such as gills, liver, kidney, spleen, gastrointestinal tract, gonads, and other tissues showing gross abnormalities when present. The selection of multiple organs allowed a broad assessment of systemic and organ-specific conditions. Particular attention was given to organs involved in respiration, metabolism, detoxification, osmoregulation, immune response, digestion, and reproduction, as these tissues may respond differently to environmental stress, infectious agents, parasitic infections, or changes in water quality.

Immediately after collection, tissue samples were gently rinsed in physiological saline to remove blood, mucus, debris, intestinal content, or free coelomic material. This step was performed carefully in order to avoid mechanical damage to delicate tissues and to preserve the relationship between lesions and surrounding structures. Samples were then fixed in 10% neutral buffered formalin using a tissue-to-fixative ratio of at least 1:10. Adequate fixation is a critical step in histological processing because it preserves tissue morphology, prevents autolysis, stabilizes cellular and extracellular components, and reduces post-mortem degradation.

The use of neutral buffered formalin was chosen because it is widely employed for routine histopathological examination and provides good preservation of general tissue architecture. The 1:10 tissue-to-fixative ratio was adopted to ensure adequate penetration of the fixative into the samples. Tissue fragments were kept as thin as possible and excessive tissue thickness was avoided, since large fragments may result in incomplete fixation, poor paraffin infiltration, sectioning artefacts, and unreliable microscopic interpretation. Fixation was carried out at room temperature for 24–48 h, depending on the thickness and consistency of the tissue samples.

After fixation, samples were washed in running tap water to remove residual formalin and to reduce the presence of fixative residues that could interfere with subsequent processing or staining. Tissues were then processed according to standard histological procedures. Briefly, samples were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Dehydration allowed the gradual

removal of water from the tissues, while clearing in xylene made the samples compatible with paraffin infiltration. Paraffin embedding provided structural support for the preparation of thin sections suitable for microscopic analysis.

Paraffin-embedded tissues were sectioned at 3 µm using a rotary microtome. The use of thin sections allowed detailed visualization of cellular morphology and tissue organization. Sections were carefully mounted on glass slides and dried before staining, following the procedures previously described by Iaria et al. (2021). During sectioning and slide preparation, attention was paid to minimizing compression, folds, tearing, and detachment artefacts, particularly in fragile tissues such as gills, intestine, and gonads.

Routine histological evaluation was performed on sections stained with haematoxylin and eosin, H&E. This staining method was used as the primary technique for general microscopic assessment. Haematoxylin stains nuclei and basophilic structures, while eosin highlights cytoplasmic and extracellular components, allowing the evaluation of tissue architecture, cellular detail, vascular changes, inflammatory infiltrates, degenerative processes, necrosis, and parasitic structures. H&E staining was therefore suitable for detecting both general pathological changes and organ-specific alterations.

Histological sections were examined systematically under light microscopy. For each organ, normal tissue architecture was first evaluated, followed by assessment of possible pathological findings. In the gills, attention was paid to epithelial lifting, lamellar fusion, hyperplasia, congestion, haemorrhage, inflammatory cells, mucus cell changes,

parasites, and possible circulatory disturbances. In the liver, hepatocyte organization, cytoplasmic vacuolization, nuclear morphology, sinusoidal architecture, vascular congestion, melanomacrophage centres, inflammatory infiltrates, necrosis, and degenerative changes were evaluated. In the kidney, both haematopoietic and renal components were examined, with attention to tubular degeneration, interstitial inflammation, glomerular changes, haemopoietic tissue organization, melanomacrophage centres, and bacterial or parasitic-associated lesions.

The spleen was examined for changes in lymphoid and haematopoietic tissue organization, congestion, melanomacrophage centres, inflammatory reactions, and possible evidence of systemic response. The gastrointestinal tract was evaluated for mucosal integrity, epithelial morphology, goblet cell distribution, inflammatory infiltrates, parasites, necrosis, and alterations of the muscular or serosal layers. Gonadal tissue was examined to assess general reproductive tissue organization and to identify possible inflammatory, degenerative, or parasitic changes. When parasites or suspected parasitic structures were observed, their location, associated tissue reaction, and relationship with the surrounding organs were described.

When required, special stains were applied to provide additional information on selected tissue components or pathological processes. Periodic Acid–Schiff, PAS, staining was used to highlight carbohydrates, glycogen, basement membranes, neutral mucopolysaccharides, and PAS-positive structures. This stain was particularly useful for evaluating mucosal tissues, basement membrane integrity, and selected infectious or

degenerative processes. Masson's trichrome staining was used to highlight collagen deposition and connective tissue organization, especially when fibrosis, capsule formation, chronic inflammation, or stromal remodelling were suspected. Alcian Blue staining was used to identify acidic mucopolysaccharides and mucin-producing cells, particularly in mucosal epithelia, gill tissues, and intestinal sections where mucus production or mucous cell distribution required further characterization.

The use of special stains was not applied indiscriminately to all samples, but rather selected according to the microscopic findings observed in routine H&E sections. This approach allowed a targeted histochemical characterization of specific alterations while maintaining H&E staining as the reference method for general histopathological evaluation. The combination of routine and special stains improved the ability to distinguish different tissue components and to interpret selected lesions more accurately.

Microscopic findings were described qualitatively. The evaluation considered the type of lesion, affected organ, anatomical distribution, severity when appreciable, and association with inflammatory, degenerative, vascular, necrotic, proliferative, or parasitic processes. Particular attention was given to distinguishing true pathological findings from post-mortem changes, fixation artefacts, tissue handling artefacts, and sectioning-related alterations. This distinction was considered essential because fish tissues may undergo rapid autolysis after death, especially under warm environmental

conditions, and inappropriate interpretation of artefacts could lead to overestimation of pathological changes.

Representative microscopic fields were selected and acquired using a digital imaging system connected to the light microscope. Digital images were used to document normal histological features, relevant lesions, parasite-associated findings, inflammatory reactions, or other microscopic changes of interest. Image acquisition also allowed the preparation of illustrative material for the thesis and supported the integration of histological observations with gross pathology, microbiology, and parasitology.

Histological results were interpreted together with the other diagnostic and environmental data collected during the study. In particular, microscopic findings were compared with external examination, necropsy observations, bacteriological results, parasitological data, and the general environmental context of the reservoir. This integrated interpretation was important because tissue alterations in wild or free-living fish may have multifactorial origins and may reflect the combined effects of environmental variability, opportunistic microorganisms, parasite exposure, nutritional status, age, reproductive condition, and seasonal stress.

Overall, histological examination represented a key component of the fish health assessment performed in Lake Biviere di Lentini. By allowing the detection of microscopic lesions and tissue-level responses, this method contributed to a more reliable evaluation of the health status of *C. carpio* specimens. Within the broader

framework of the thesis, histopathology provided essential information for linking fish health to environmental conditions, microbial circulation, parasitological findings, and ecosystem-level processes occurring in the reservoir.

3.10 AVIFAUNA MONITORING

The assessment of avifaunal biodiversity in Lake Biviere di Lentini was carried out between 2022 and 2024 through repeated field surveys performed at approximately four-month intervals. This temporal design was adopted to cover different seasonal periods and to obtain information on the bird communities using the lake during different phases of the annual cycle. In Mediterranean wetlands and artificial reservoirs, bird assemblages may vary markedly according to migration phenology, breeding activity, wintering dynamics, water level, food availability, vegetation cover, disturbance, and hydrological conditions. For this reason, repeated surveys distributed across multiple seasons are essential to document both resident and migratory components of the avifauna.

Avifauna monitoring was designed as an integrated survey combining direct visual census, drone-assisted observation, and fixed-camera documentation. The combination of these methods was adopted to improve the detectability of bird species across different areas of the lake and to reduce the limitations associated with any single

monitoring technique. Visual census methods represent a standard approach in bird monitoring and are widely used for estimating species richness, abundance, distribution, and habitat use in wetland ecosystems (Bibby et al., 2000; Sutherland, 2006; Gregory et al., 2004). In addition, the use of repeated counts is consistent with the principles of waterbird monitoring adopted in long-term census programs, such as the International Waterbird Census, where standardized site-based counts are used to document waterbird occurrence, abundance, and population trends (Wetlands International, 2010; Wetlands International, 2018).

During each survey, bird communities were monitored through visual census surveys performed from suitable observation points located around the lake. Observations were conducted using binoculars and/or spotting scopes when required by distance, species size, and visibility conditions. The use of fixed or repeated observation points allowed wide coverage of the open-water areas, shoreline sectors, vegetated margins, mudflats, embankments, and other habitats potentially used by aquatic and semi-aquatic birds. Particular attention was given to waterbirds, waders, raptors, passerines associated with wetland vegetation, and piscivorous species, given their ecological relevance in reservoir food webs and their potential interactions with fish populations.

Species were identified in the field at the lowest possible taxonomic level, generally at species level, according to standard ornithological identification criteria. For each taxon, the number of individuals observed was recorded whenever possible. When

birds occurred in large aggregations, counts were performed using direct enumeration for small groups and block-counting or estimation procedures for larger flocks, according to standard waterbird census recommendations (Bibby et al., 2000; Wetlands International, 2010; Wetlands International, 2018). Repeated scanning of the same sector was avoided as far as possible in order to reduce the risk of double counting, especially for highly mobile species moving across the lake surface.

Birds were recorded according to their occurrence within the monitored area and, when possible, according to their main ecological or behavioural category. Particular attention was given to the distinction among resting, feeding, flying, roosting, breeding, and transit individuals. This information was considered useful for interpreting the functional role of the lake as a feeding site, stopover area, wintering site, breeding habitat, or temporary refuge for migratory species. In the case of piscivorous birds, such as cormorants, herons, egrets, and other fish-eating taxa, their presence was also considered relevant for the interpretation of fish–bird interactions and parasite transmission dynamics.

The visual census was supported by a light unmanned aerial vehicle, light-UAV (under 250g), equipped with a 4k resolution camera (DJI Mini3 pro, 249g of weight). Drone-assisted monitoring was used as a complementary method to obtain aerial imagery of selected lake sectors, especially areas that were difficult to observe completely from ground-based vantage points. UAV imagery can be useful in wetland and waterbird surveys because it may improve spatial coverage, allow documentation

of large aggregations, and support post-survey verification of counts and spatial distribution patterns (Chabot and Bird, 2015; Hodgson et al., 2018; McEvoy et al., 2016). However, drone-based monitoring was used as a support tool rather than as a replacement for field identification, because detectability and taxonomic resolution may vary according to flight altitude, image quality, bird size, plumage, behaviour, habitat complexity, and species similarity.

Light surveys were carried out with attention to minimizing disturbance to birds and other wildlife. Flights were planned to avoid unnecessary disturbance of resting, feeding, or nesting birds, and UAV observations were integrated with ground-based visual monitoring to detect possible behavioural responses. The use of UAVs in bird monitoring requires careful consideration of flight height, distance from birds, noise, approach direction, species sensitivity, and breeding or roosting status, because disturbance responses may vary among taxa and ecological contexts (Chabot and Bird, 2015; Hodgson et al., 2018; Lyons et al., 2018). For this reason, drone observations were interpreted in combination with visual census data and were used primarily to improve documentation and spatial coverage.

In addition to direct observations and drone-assisted surveys, avifaunal monitoring was supported by three fixed cameras distributed across the lake area. These cameras, a Panasonic Lumix s5II equipped with a Sigma 100-400 mm lens, a Sony α7sIII with a Samyang 35-150 mm lens and a Sony α7IV with a 24-105 mm and 16-35 mm native lenses were used to document bird presence and activity in selected sectors of

the reservoir and to complement field observations. Fixed-camera monitoring can provide useful information on species occurrence, temporal patterns of habitat use, flock presence, and behaviour, especially in areas that are difficult to observe continuously during field visits. The camera data were used as supplementary evidence to support species detection, improve documentation of avifaunal presence, and assist in the interpretation of seasonal patterns.

The integration of visual census, UAV imagery, and fixed-camera documentation allowed a broader assessment of bird biodiversity in Lake Biviere di Lentini. Direct observations provided taxonomic identification and field-based abundance estimates, UAV imagery improved spatial documentation of selected areas, and fixed cameras contributed additional temporal information from specific sectors of the lake. Together, these methods increased the robustness of the monitoring approach and allowed the evaluation of avifaunal diversity across multiple seasons.

The survey focused on documenting the composition of the bird community and identifying the main ecological groups using the reservoir. Particular attention was paid to resident and migratory waterbirds, wintering species, breeding or potentially breeding species, waders, raptors, and piscivorous birds. The latter group was considered especially relevant within the broader aims of this thesis because piscivorous birds may influence fish communities through predation and may act as definitive hosts for helminths with indirect life cycles, including anisakid nematodes of the genus *Contracaecum*. Therefore, avifauna monitoring was integrated with fish, parasitological,

microbiological, and environmental data to support a cross-trophic interpretation of the lake ecosystem.

All field activities were conducted with attention to minimizing disturbance to wildlife and avoiding unnecessary interference with bird behaviour. Observers maintained an appropriate distance from sensitive areas when possible, and monitoring was conducted using non-invasive observation methods. The use of optical instruments, remote imagery, and fixed cameras allowed the collection of ecological information while reducing direct contact with animals and habitats.

Data obtained from the avifauna monitoring were organized by survey period and taxonomic group. Species richness, occurrence, and relative abundance patterns were evaluated descriptively in relation to season and habitat use. The multi-seasonal monitoring design allowed the identification of recurrent species, seasonal visitors, migratory taxa, and ecologically relevant groups. Within the integrated framework of the study, avifaunal data were used to contextualize the ecological role of Lake Biviere di Lentini as a biodiversity hotspot and to support the interpretation of fish–bird interactions, nutrient inputs, microbial circulation, and parasite transmission pathways within the reservoir.

3.11 MACROZOOBENTHIC SURVEY

The census of the macrozoobenthic fauna was carried out using the bucket technique, using a Van Veen type bucket, a 5 liters bucket with an opening of 0.053 m^2. This type of bucket allows you to take a surface sample approximately covering the first 5-10 cm of thickness. The bucket used has a door opening system that allows the collection of undisturbed sediment before extrusion from the bucket itself.

The activities involved the collection of samples of surface sediment from the lake bed. For macrozoobenthos analyses 5 sampling station have been identified randomly by scubadivers in a 500m^2 area in correspondence of ST_TA (fig. 3.7.1), 10 samples of mobile bottom were taken (two sampling replicates for each sampling station). Prior to the work, a safety and functioning check was carried out on the existing equipment and consumables. Once the canoe was positioned at the sampling point, the weather conditions, the operators, the transparency of the water and the possible presence of surface obstacles that could affect the success of the operations were recorded. Subsequently, the sampling was carried out.

The sediments, as previously described, were collected using the bucket shown in the figure, to allow the recovery of a sufficient volume for subsequent analyses.

Each sample was taken for the different analyzes and stored in special labeled and dated containers.

After collection, the sediment was subjected to sieving for the separation of the benthos organisms thanks to washing with lake water in the necessary volumes. The

organisms and thanatocoenosis thus collected, and separated from the matrix on sieves with 0.5 mm diameter mesh, were recovered from the sieves and transferred into containers suitable for the volume of the sample cleaned of excess matrix. A 70% ethanol solution was used to fix the samples in situ. As per the methodological sheets of the monitoring programs for the Marine Strategy (Art. 11, Legislative Decree 190/2010), the following additives were added: propylene phenoxetol and propylene glycerol (at 3%). Ethyl alcohol is widely recognized as a valid alternative option to formaldehyde for short storage times also by the ISO reference standards for taxonomic investigations. As regards the sampling methodology and the analytical methods applied, reference was made to current legislation, to the APAT 46/2007 manual, to the ISPRA and ICRAM manuals and to the indications of the Ministry of Ecological Transition.

The samples, appropriately fixed on board through the use of a 70% ethanol-based solution added with propylene phenoxetol and propylene glycerol (at 3%), were delivered to the laboratories of the University of Messina within 12 hours from sampling, where they were processed within 240 hours (10 days) of receipt.

The macrozoobenthos samples were treated in the laboratory according to the following procedure:

- sorting and subdivision of organisms by large taxa;
- systematic determination down to the lowest taxonomic level;
- annotation of relative abundance counts for each taxon. Based on the taxonomic determinations carried out, a complete list of species was drawn up.

Once they arrived in the laboratory, an initial sorting was carried out on the samples in order to divide the macrozoobenthic organisms by main taxa. The washing and sorting phase of the sample was carried out in the laboratory using running water and a 500 µm mesh sieve, in order to recover all the material retained by the sieve used in the field and at the same time allow the removal of the sediment still present. After extensive washing, the material retained by the sieve was examined under the stereo microscope in small aliquots using a Petri dish as a container. All organisms and fragments were collected with the use of tweezers, divided into the different taxa (if present) and subsequently preserved in the above-described 70% ethanol-based solution until systematic identification.

Each specimen was identified at the lowest possible taxonomic level (LPT=Lowest Possible Taxon) which does not always coincide with the species level. The conservation status and maturity of the individuals present in the samples were the factors that mainly influenced the LPT.

For some specimens, where necessary, taxonomic determination was achieved using an optical microscope with 10x, 20x and 40x magnifications.

The list of species was drawn up by comparing the results with what was proposed in the Italian aquatic fauna checklist.

4 RESULTS

4.1 PHYSICOCHEMICAL CHARACTERIZATION AND WATER QUALITY ASSESSMENT

The physicochemical parameters measured at the ST_C sampling site (Fig. 3.2.1) of Lake Biviere di Lentini are reported in Tables 4.1.1–3. Data include measurements obtained from three water-column layers, namely surface, mid-depth, and bottom waters, during the two sampling campaigns carried out in March and November 2023. Overall, the results showed that the lake maintained generally stable physicochemical conditions across the investigated depths, although several parameters displayed clear seasonal variation between the two sampling periods.

Table 4.1.1. Comparison of chemical parameters in surface water samples collected from Lake Biviere di Lentini, at ST_C sampling site, during two sampling campaigns: Sampling 1 (S1, March 30, 2023) and Sampling 2 (S2, November 22, 2023). Sampling was performed using a Niskin bottle in accordance with D.Lgs. 152/99. Data are reported as mean ± standard deviation (n = 3).

Parameter	Unit	S1	S2	RL[a]	LOD[b]
Recorded in situ					
Redox potential	mV	75	99	–	–
Electrical conductivity	µS/cm	488	895	–	–
pH	–	8.90	8.70	–	–
Dissolved oxygen (DO)	$\%O_2$	98	95	–	–
Temperature	°C	17.11	19.22	–	–
Turbidity	cm	60	60	–	–
General physicochemical and trophic parameters					
Chemical Oxigen Demand (COD)	mg/L O_2	21.34 ± 2.10	12.27 ± 1.80	–	–
Chlorophyll-*a*	µg/L	168.28 ± 5.25	0.02 ± 0.00	> 25	1.0
Alkalinity	meq/L	5.10 ± 0.27	0.46 ± 0.017	–	0.2
Acidity	meq/L	0.60 ± 0.07	2.30 ± 0.14	–	0.2
Total Dissolved Solids (TDS)	mg/L	661 ± 3.25	305 ± 2.05	–	0.1
Trace metals					
Cadmium (Cd)	µg/L	0.021 ± 0.00	< LOD	–	0.01
Chromium (Total Cr)	µg/L	< LOD	0.40 ± 0.05	–	0.2
Mercury (Hg)	µg/L	0.95 ± 0.01	0.62 ± 0.02	–	0.1

Nickel (Ni)	µg/L	< LOD	0.36 ± 0.01	–	0.3
Lead (Pb)	µg/L	0.12 ± 0.01	< LOD	–	0.05
Copper (Cu)	µg/L	1.81 ± 0.14	< LOD	–	0.5
Zinc (Zn)	µg/L	4.19 ± 0.02	< LOD	–	1.0
Nutrient-related					
Ammonium nitrogen (NH_4^+)	mg/L	0.33 ± 0.02	0.45 ± 0.02	–	0.1
Major cations					
Calcium (Ca)	mg/L	36.22 ± 3.15	35.40 ± 2.00	–	0.05
Magnesium (Mg)	mg/L	46.17 ± 2.08	47.70 ± 2.30	–	0.05
Sodium (Na)	mg/L	121.35 ± 3.19	127.40 ± 3.20	–	0.05
Potassium (K)	mg/L	121.08 ± 5.54	122.00 ± 1.05	–	0.05
Anions					
Chloride (Cl^-)	mg/L	102.11 ± 3.10	99.50 ± 3.10	–	0.01
Fluoride (F^-)	mg/L	0.50 ± 0.01	0.47 ± 0.12	–	0.01
Nitrate (NO_3^-)	mg/L	0.23 ± 0.04	0.18 ± 0.02	–	0.01
Nitrite (NO_2^-)	mg/L	< LOD	< LOD	–	0.01
Sulfate (SO_4^{2-})	mg/L	114.28 ± 2.05	110.05 ± 3.30	–	0.01
Phosphate (PO_4^{3-})	mg/L	< LOD	< LOD		0.01
Chlorinated aliphatic compounds (including carcinogenic substances)					
Chloroform	µg/L	< LOD	< LOD	–	0.03
1,2-Dichloroethane	µg/L	< LOD	< LOD	–	0.02
Carbon tetrachloride	µg/L	< LOD	< LOD	–	0.07
Trichloroethylene	µg/L	< LOD	< LOD	–	0.04
Hexachlorobutadiene (HCBD)	µg/L	< LOD	< LOD	–	0.0001
Chlorobenzenes					
1,2,4-Triclorobenzene	µg/L	< LOD	< LOD	–	0.2
Organochlorine pesticides					
Aldrin	µg/L	< LOD	< LOD	–	0.03
Dieldrin	µg/L	< LOD	< LOD	–	0.06
Endrin	µg/L	< LOD	< LOD	–	0.02
Isodrin	µg/L	< LOD	< LOD	–	0.08
Dichlorodiphenyltrichloroethane (DDT)	µg/L	< LOD	< LOD	–	0.0005
Hexachlorobenzene (HCB)	µg/L	< LOD	< LOD	–	0.005
Hexachlorocyclohexane (HCH)	µg/L	< LOD	< LOD	–	0.03

[a]RF, Reference limits in accordance with D.Lgs. 152/2006 (Annex 1, Table 1); [b]LOD, limit of quantification.

Table 4.1.2. Comparison of chemical parameters in mid-depth water samples collected from Lake Biviere di Lentini, at ST_C sampling site, during two sampling campaigns: Sampling 1 (March 30, 2023) and Sampling 2 (November 22, 2023). Sampling was performed using a Niskin bottle in accordance with D.Lgs. 152/99. Data are reported as mean ± standard deviation (n = 3).

Parameter	Unit	S1	S2	RL[a]	LOD[b]
Recorded in situ					
Redox potential	mV	83	99	–	–
Electrical conductivity	µS/cm	561	887	–	–
pH	–	8.75	8.73	–	–
Dissolved oxygen (DO)	$\%O_2$	89	89	–	–
Temperature	°C	15.20	19.09	–	–
Turbidity	cm	60	60	–	–

General physicochemical and trophic parameters					
Chemical Oxigen Demand (COD)	mg/L O_2	7.60 ± 1.85	17.51 ± 1.55	–	–
Chlorophyll-*a*	µg/L	24.85 ± 3.15	0,09 ± 0.00	> 25	1.0
Alkalinity	meq/L	5.10 ± 0.30	0.66 ± 0.03	–	0.2
Acidity	meq/L	0.3 ± 0.01	2.0 ± 0.02	–	0.2
Total Dissolved Solids (TDS)	mg/L	610 ± 4.05	537 ± 3.23	–	0.1
Trace metals					
Cadmium (Cd)	µg/L	0.015 ± 0.00	< LOD	–	0.01
Chromium (Total Cr)	µg/L	< LOD	0.38 ± 0.05	–	0.2
Mercury (Hg)	µg/L	0.83 ± 0.01	0.58 ± 0.03	–	0.1
Nickel (Ni)	µg/L	< LOD	< LOD	–	0.3
Lead (Pb)	µg/L	0.05 ±0.00	< LOD	–	0.05
Copper (Cu)	µg/L	4.26 ± 0.18	< LOD	–	0.5
Zinc (Zn)	µg/L	3.52 ± 0.04	< LOD	–	1.0
Nutrient-related					
Ammonium nitrogen (NH_4^+)	mg/L	0.34 ± 0.02	0.81 ± 0.04	–	0.1
Major cations					
Calcium (Ca)	mg/L	35.12 ± 2.05	36.40 ± 2.10	–	0.05
Magnesium (Mg)	mg/L	46.28 ± 1.24	48.50 ± 1.30	–	0.05
Sodium (Na)	mg/L	119.05 ± 1.08	128.30 ± 3.02	–	0.05
Potassium (K)	mg/L	121.44 ± 4.03	123.00 ± 1.02	–	0.05
Anions					
Chloride (Cl^-)	mg/L	106.40 ± 4.20	107.20 ± 4.10	–	0.01
Fluoride (F^-)	mg/L	0.40 ± 0.01	0.38 ± 0.11	–	0.01
Nitrate (NO_3^-)	mg/L	0.23 ± 0.02	0.22 ± 0.01	–	0.01
Nitrite (NO_2^-)	mg/L	< LOD	< LOD	–	0.01
Sulfate (SO_4^{2-})	mg/L	118.00 ± 4.12	119.00 ± 3.25	–	0.01
Phosphate (PO_4^{3-})	mg/L	< LOD	< LOD	–	0.01
Chlorinated aliphatic compounds (including carcinogenic substances)					
Chloroform	µg/L	< LOD	< LOD	–	0.03
1,2-Dichloroethane	µg/L	< LOD	< LOD	–	0.02
Carbon tetrachloride	µg/L	< LOD	< LOD	–	0.07
Trichloroethylene	µg/L	< LOD	< LOD	–	0.04
Hexachlorobutadiene (HCBD)	µg/L	< LOD	< LOD	–	0.0001
Chlorobenzenes					
1,2,4-Triclorobenzene	µg/L	< LOD	< LOD	–	0.2
Organochlorine pesticides					
Aldrin	µg/L	< LOD	< LOD	–	0.03
Dieldrin	µg/L	< LOD	< LOD	–	0.06
Endrin	µg/L	< LOD	< LOD	–	0.02
Isodrin	µg/L	< LOD	< LOD	–	0.08
Dichlorodiphenyltrichloroethane (DDT)	µg/L	< LOD	< LOD	–	0.0005
Hexachlorobenzene (HCB)	µg/L	< LOD	< LOD	–	0.005
Hexachlorocyclohexane (HCH)	µg/L	< LOD	< LOD	–	0.03

[a]RF, Reference limits in accordance with D.Lgs. 152/2006 (Annex 1, Table 1); [b]LOD, limit of quantification.

Table 4.1.3. Comparison of chemical parameters in bottom water samples collected from Lake Biviere di Lentini, at ST_C sampling site, during two sampling campaigns: Sampling 1 (March 30, 2023) and Sampling 2 (November 22, 2023). Sampling was performed using a Niskin bottle in accordance with D.Lgs. 152/99. Data are reported as mean ± standard deviation (n = 3).

Parameter	Unit	S1	S2	RL[a]	LOD[b]
Recorded in situ					
Redox potential	mV	54.80	81.4	–	–
Electrical conductivity	µS/cm	591	788	–	–
pH	–	8.31	8.43	–	–
Dissolved oxygen (DO)	$\%O_2$	88	88	–	–
Temperature	°C	15.11	19.08	–	–
Turbidity	cm	60	60	–	–
General physicochemical and trophic parameters					
Chemical Oxigen Demand (COD)	mg/L O_2	1.69 ± 0.55	13.79 ± 1.75	–	–
Chlorophyll-*a*	µg/L	40.86 ± 4.65	0.05 ± 0.00	> 25	1.0
Alkalinity	meq/L	5.10 ± 0.2	0.66 ± 0.10	–	0.2
Acidity	meq/L	0.3 ± 0.01	2.3 ± 0.01	–	0.2
Total Dissolved Solids (TDS)	mg/L	374 ± 2.35	546 ± 3.35	–	0.1
Trace metals					
Cadmium (Cd)	µg/L	< LOD	0.011 ±0.00	–	0.01
Chromium (Total Cr)	µg/L	< LOD	0.52 ± 0.02	–	0.2
Mercury (Hg)	µg/L	1.20 ± 0.05	0.54 ± 0.03	–	0.1
Nickel (Ni)	µg/L	< LOD	0.54 ± 0.04	–	0.3
Lead (Pb)	µg/L	< LOD	< LOD	–	0.05
Copper (Cu)	µg/L	0.86 ± 0.08	2.57 ± 0.12	–	0.5
Zinc (Zn)	µg/L	3.2 ± 0.09	3.81 ± 0.12	–	1.0
Nutrient-related					
Ammonium nitrogen (NH_4^+)	mg/L	0.39 ± 0.02	0.21 ± 0.01	–	0.1
Major cations					
Calcium (Ca)	mg/L	34.38 ± 1.22	35.80 ± 1.50	–	0.05
Magnesium (Mg)	mg/L	45.02 ± 1.06	47.90 ± 1.25	–	0.05
Sodium (Na)	mg/L	120.17 ± 1.35	127.50 ± 2.03	–	0.05
Potassium (K)	mg/L	120.45 ± 3.04	122.00 ± 1.17	–	0.05
Anions					
Chloride (Cl^-)	mg/L	105.08 ± 2.15	98.05 ± 1.20	–	0.01
Fluoride (F^-)	mg/L	0.40 ± 0.01	0.41 ± 0.11	–	0.01
Nitrate (NO_3^-)	mg/L	0.21 ± 0.04	0.22 ± 0.03	–	0.01
Nitrite (NO_2^-)	mg/L	< LOD	< LOD	–	0.01
Sulfate (SO_4^{2-})	mg/L	117.21 ± 3.18	119.03 ± 3.20	–	0.01
Phosphate (PO_4^{3-})	mg/L	< LOD	< LOD	–	0.01
Chlorinated aliphatic compounds (including carcinogenic substances)					
Chloroform	µg/L	< LOD	< LOD	–	0.03
1,2-Dichloroethane	µg/L	< LOD	< LOD	–	0.02
Carbon tetrachloride	µg/L	< LOD	< LOD	–	0.07
Trichloroethylene	µg/L	< LOD	< LOD	–	0.04
Hexachlorobutadiene (HCBD)	µg/L	< LOD	< LOD	–	0.0001
Chlorobenzenes					
1,2,4-Triclorobenzene	µg/L	< LOD	< LOD	–	0.2

Organochlorine pesticides					
Aldrin	µg/L	< LOD	< LOD	–	0.03
Dieldrin	µg/L	< LOD	< LOD	–	0.06
Endrin	µg/L	< LOD	< LOD	–	0.02
Isodrin	µg/L	< LOD	< LOD	–	0.08
Dichlorodiphenyltrichloroethane (DDT)	µg/L	< LOD	< LOD	–	0.0005
Hexachlorobenzene (HCB)	µg/L	< LOD	< LOD	–	0.005
Hexachlorocyclohexane (HCH)	µg/L	< LOD	< LOD	–	0.03

[a]RF, Reference limits in accordance with D.Lgs. 152/2006 (Annex 1, Table 1); [b]LOD, limit of quantification.

In situ measurements showed relatively homogeneous vertical profiles for the main physicochemical parameters. The pH values were slightly alkaline throughout the water column, ranging from 8.3 to 8.9, with no evidence of marked acidification or abrupt vertical changes. Dissolved oxygen values indicated well-oxygenated conditions, with oxygen saturation ranging between 88% and 98% O_2. These values suggest that, during both sampling campaigns, the water column did not show evidence of severe oxygen depletion at the central sampling station. Electrical conductivity was moderate but showed a marked seasonal increase in November compared with March across all sampled depths, indicating a higher concentration of dissolved ions during the autumn campaign. Temperature profiles reflected the seasonal conditions recorded at the time of sampling, with higher values observed during the November campaign.

A clear seasonal pattern emerged for the general physicochemical parameters. Chemical oxygen demand, COD, showed different vertical distributions between the two sampling campaigns. In March, COD values were higher at the surface, reaching 21.34 ± 2.10 mg O_2 L^{-1}, and decreased progressively with depth. This pattern suggests that,

during the spring sampling, the oxidizable organic load was mainly associated with the upper water layer, possibly in relation to surface biological productivity and suspended organic material. In November, by contrast, higher COD values were observed at mid-depth and in the bottom layer, indicating a different vertical distribution of oxidizable material within the water column. This shift may reflect seasonal redistribution of organic matter, changes in water-column mixing, sediment–water interactions, or the settling and accumulation of particulate organic material in deeper layers.

Total dissolved solids, TDS, also showed marked seasonal and vertical variability. In March, higher concentrations were recorded at the surface and mid-depth, while in November a relative increase was observed in the deeper layer. This pattern suggests that dissolved matter dynamics varied between the two sampling periods, potentially reflecting differences in evaporation, inflow composition, biological activity, water mixing, and interactions with the sediment compartment. The combined evaluation of conductivity and TDS indicates that the ionic and dissolved-solid composition of the lake changed seasonally, although without evidence of abrupt or extreme mineralization.

Chlorophyll-*a* concentrations displayed the most pronounced seasonal contrast among the investigated parameters. In March, very high chlorophyll-*a* values were recorded, especially in the surface layer, where concentrations reached 168.28 ± 5.25 µg L^{-1}. These values were well above the reference threshold of 25 µg L^{-1} adopted for the assessment of elevated phytoplankton biomass, indicating intense algal development during the spring sampling campaign (Fig. 4.1.1).

Fig. 4.1.1 *A marbled appareance of the lake due to the spring algal bloom*



The vertical distribution of chlorophyll-*a* was consistent with a strong phytoplankton signal in the upper part of the water column. Conversely, in November, chlorophyll-*a* concentrations were negligible across all depths, indicating very low phytoplankton biomass at the time of autumn sampling. This sharp seasonal difference highlights a marked shift in trophic conditions between the two monitoring periods, with a spring phase characterized by high phytoplankton biomass and an autumn phase characterized by low primary productivity.

Acid–base-related parameters also showed seasonal differences. Alkalinity was consistently higher in March than in November across all water layers, indicating greater buffering capacity during the spring campaign. Conversely, acidity showed the opposite pattern, with higher values recorded in November. These variations suggest seasonal

changes in carbonate equilibrium and acid-neutralizing capacity, possibly related to differences in biological activity, carbon dioxide dynamics, water inputs, and the balance between photosynthetic uptake and respiratory processes. Despite these changes, pH remained within a slightly alkaline range, indicating that the lake maintained an overall buffered condition during the study period.

Trace metals were generally detected at low concentrations. In March, cadmium, lead, copper, and zinc were measurable in several samples, although at low levels. In November, most of these metals were below the analytical detection limits. Chromium and nickel showed a different pattern, being detectable mainly during the November campaign and particularly in the deeper layers. This vertical and seasonal distribution may indicate differences in metal mobility, sediment–water interactions, or the influence of suspended particulate material. Mercury was consistently detected during both sampling campaigns, with slightly higher values recorded in March. However, the measured concentrations remained low and did not indicate evident metal contamination in the analyzed samples.

Ammoniacal nitrogen concentrations were generally low, although some variability was observed among depths and sampling periods. The highest value was recorded in November at mid-depth, reaching 0.81 ± 0.04 mg L^{-1}. This localized increase may indicate temporary accumulation of reduced inorganic nitrogen, potentially associated with organic matter degradation, reduced nitrification efficiency, water-column stratification or mixing conditions, or sediment-related nitrogen release.

However, the overall concentrations remained limited and did not indicate widespread ammonium enrichment across the water column.

The analysis of major ions showed relatively stable concentrations of the main cations, including calcium, magnesium, sodium, and potassium, and of the principal anions, including chloride, nitrate, and sulphate. These parameters showed limited variation across depths and between the two sampling campaigns, indicating a relatively consistent ionic composition of the lake water. Chloride and sulphate values did not suggest major salinization processes during the investigated period. Nitrate concentrations remained low, while nitrite and phosphate were consistently below detection limits in all analyzed samples. The absence of detectable phosphate, despite the high chlorophyll-*a* values recorded in March, may reflect rapid biological uptake of available phosphorus during the spring phytoplankton bloom or concentrations below the analytical detection threshold at the time of sampling.

Organic contaminants, including selected chlorinated aliphatic compounds, chlorobenzenes, and organochlorine pesticides, were below detection limits in all analyzed samples. This finding indicates that, during the two sampling campaigns, no measurable contamination from the investigated classes of volatile organic compounds, semi-volatile chlorinated compounds, or organochlorine pesticides was detected in the water samples collected at the central station.

Overall, the physicochemical and chemical results indicate that Lake Biviere di Lentini was characterized by generally stable water-quality conditions and low

detectable chemical contamination during the study period. The main element of variability was represented by the marked seasonal change in trophic status, with very high chlorophyll-*a* concentrations and elevated phytoplankton biomass in March, followed by negligible chlorophyll-*a* values and low phytoplankton biomass in November. These results suggest that the lake is not affected by evident chemical pollution from the analyzed contaminants, but it may undergo pronounced seasonal fluctuations in primary productivity and organic matter dynamics.

4.2 MICROBIOLOGICAL ANALYSES

Microbiological analyses were performed to evaluate the sanitary and environmental microbiological status of Lake Biviere di Lentini during the two sampling campaigns. The analyses included the detection and enumeration of *Enterococci*, *Escherichia coli*, total coliforms, *Vibrio* spp., sulphite-reducing Clostridia, and *Salmonella* spp. Results were expressed as CFU/100 mL for water samples and CFU/g for sediment samples. The complete microbiological results are reported in Table 4.2.1

Table 4.2.1 Microbiological parameters measured at the central sampling site (37°19'37"N, 14°57'05"E) of Lake Biviere di Lentini in water (surface, mid-depth and bottom) and sediment samples during two sampling campaigns: S1 (March 2023) and S2 (November 2023). Results are expressed as colony-forming units (CFU/100 mL for water samples and CFU/g for sediment samples). ND = not detected. Analyses were performed according to standard APAT methods.

SURFACE WATER (CFU/100 mL)			
Parameter	**S1**	**S2**	**Method**
E. coli	2	6	APAT 7030
Total coliforms	1176	1022	APAT 7010
Vibrio spp.	372	200	APAT 7090
Enterococci	72	56	APAT 7040
Sulphite-reducing *Clostridia*	10	13	APAT 7060
Salmonella spp.	ND	ND	APAT 7080
MID-POINT WATER (CFU/100 mL)			
Parameter	**S1**	**S2**	**Method**
E. coli	0	3	APAT 7030
Coliforms tot.	2688	986	APAT 7010
Vibrio spp.	224	133	APAT 7090
Enterococci	8	38	APAT 7040
Clostridia	5	0	APAT 7060
Salmonella spp.	ND	ND	APAT 7080
BOTTOM WATER (CFU/100 mL)			
Parameter	**S1**	**S2**	**Method**
E. coli	22	28	APAT 7030
Coliforms tot.	1344	860	APAT 7010
Vibrio spp.	200	222	APAT 7090
Enterococci	48	11	APAT 7040
Clostridia	0	10	APAT 7060
Salmonella spp.	ND	ND	APAT 7080
ST1 – SEDIMENT (CFU/g)			
Parameter	**S1**	**S2**	**Method**
E. coli	0	0	APAT 7030
Coliforms tot.	76	128	APAT 7010
Vibrio spp.	96	26	APAT 7090

Enterococci	15	33	APAT 7040
Clostridia	16	20	APAT 7060
Salmonella spp.	ND	ND	APAT 7080

After incubation on Chromogenic Coliform Agar, CCA, blue colonies were considered presumptive *E. coli*, whereas red or pink colonies were counted as presumptive total coliforms. Colonies showing a dark red to brown colour on Slanetz and Bartley agar were considered presumptive Enterococci. After incubation on Thiosulfate Citrate Bile Salts Sucrose, TCBS, agar, yellow or green colonies were considered presumptive Vibrio spp. Sulphite-reducing Clostridia were enumerated after anaerobic incubation on the appropriate selective medium, while *Salmonella* spp. detection was expressed as presence or absence.

Overall, microbiological indicators were detected in both sampling campaigns, although their distribution differed according to the sampling period, water depth, and matrix analysed. Total coliforms represented the most abundant microbial group in the water column. In surface water, total coliforms were detected at 1176 CFU/100 mL in March and 1022 CFU/100 mL in November. At mid-depth, the highest value was recorded during the March campaign, with 2688 CFU/100 mL, followed by a decrease to 986 CFU/100 mL in November. Bottom water also showed detectable total coliforms, with 1344 CFU/100 mL in March and 860 CFU/100 mL in November. These results

indicate the widespread presence of coliform bacteria throughout the water column, with generally higher values during the March campaign.

By contrast, *E. coli* showed much lower values than total coliforms. In surface water, *E. coli* was detected at 2 CFU/100 mL in March and 6 CFU/100 mL in November. At mid-depth, *E. coli* was not detected in March, while 3 CFU/100 mL were recorded in November. The highest *E. coli* values were observed in bottom water, with 22 CFU/100 mL in March and 28 CFU/100 mL in November. This distribution suggests that, although indicators of recent fecal contamination were present, their levels remained relatively low compared with the broader coliform component. The higher values observed near the bottom may reflect sediment–water interactions, particle-associated bacteria, or localized accumulation in the deeper water layer.

Enterococci were detected in all water samples, but their abundance varied according to depth and season. Surface water showed values of 72 CFU/100 mL in March and 56 CFU/100 mL in November. At mid-depth, enterococci increased from 8 CFU/100 mL in March to 38 CFU/100 mL in November. In bottom water, the opposite trend was observed, with 48 CFU/100 mL in March and 11 CFU/100 mL in November. These findings indicate that enterococci were consistently present in the lake water, although without a uniform seasonal or vertical pattern.

Presumptive *Vibrio* spp. were also detected in all water layers during both sampling campaigns. In surface water, values decreased from 372 CFU/100 mL in March to 200 CFU/100 mL in November. At mid-depth, values decreased from 224 CFU/100 mL

to 133 CFU/100 mL. Conversely, bottom water showed comparable values between the two periods, with 200 CFU/100 mL in March and 222 CFU/100 mL in November. These results indicate the presence of cultivable bacteria compatible with *Vibrio* spp. throughout the water column, with higher surface and mid-depth values in March and relatively stable bottom values between the two campaigns.

Sulphite-reducing Clostridia were detected at low concentrations in water samples. Surface water showed values of 10 CFU/100 mL in March and 13 CFU/100 mL in November. At mid-depth, Clostridia were detected at 5 CFU/100 mL in March and were not detected in November. In bottom water, they were not detected in March but reached 10 CFU/100 mL in November. The presence of these spore-forming bacteria, even at low levels, suggests the occurrence of persistent microbial forms in the aquatic environment, possibly associated with organic matter, sediment particles, or previous contamination events.

Sediment samples showed a different microbiological profile compared with the water column. *E. coli* was not detected in sediment in either campaign, whereas total coliforms increased from 76 CFU/g in March to 128 CFU/g in November. Presumptive *Vibrio* spp. decreased from 96 CFU/g in March to 26 CFU/g in November. Enterococci increased from 15 CFU/g to 33 CFU/g, while sulphite-reducing Clostridia were detected in both campaigns, with 16 CFU/g in March and 20 CFU/g in November. These findings suggest that the sediment compartment may act as a reservoir for selected microbial

groups, particularly total coliforms, enterococci, and sulphite-reducing Clostridia, even in the absence of detectable *E. coli*.

Salmonella spp. were not detected in any of the analysed samples. The absence of *Salmonella* was consistent across all water layers and sediment samples in both March and November. This result indicates that, during the investigated sampling campaigns, no detectable contamination by this pathogen was observed at the central sampling station.

Overall, the microbiological results show that Lake Biviere di Lentini was characterized by the presence of fecal indicator bacteria and aquatic opportunistic microbial groups, but without detection of *Salmonella* spp. Total coliforms were the most abundant indicators in the water column, whereas *E. coli* remained comparatively low and was absent from sediment samples. The detection of enterococci, sulphite-reducing Clostridia, and presumptive *Vibrio* spp. indicates a complex microbial profile involving both water and sediment compartments. These findings support the importance of including microbiological monitoring in the integrated assessment of the reservoir, particularly considering the ecological interactions among water quality, sediments, fish, avifauna, and potential organic inputs.

4.3 BACTERIOLOGICAL INVESTIGATION ON FISH

Bacteriological investigation was performed on kidney samples collected from selected *Cyprinus carpio* specimens in order to assess the presence of cultivable bacteria in internal tissues. After incubation on Blood Agar and Tryptic Soy Agar, bacterial growth was observed in fish tissue samples. Representative colonies were isolated in pure culture and subjected to Gram staining and species-level identification by MALDI-TOF MS.

The bacteriological analyses revealed the presence of *Aeromonas veronii* in fish samples, indicating the occurrence of this bacterial species within the lake system and its association with the examined fish population. The isolates were obtained from internal tissue samples, supporting the detection of cultivable bacteria in apparently healthy *C. carpio* specimens.

No external clinical signs, gross pathological lesions, or histopathological alterations compatible with bacterial disease were observed in the examined fish. In particular, the specimens positive for *A. veronii* did not show skin ulcers, haemorrhagic lesions, fin erosion, abdominal distension, organ enlargement, necrotic foci, or microscopic evidence of septicemic or inflammatory disease.

Therefore, the detection of *A. veronii* was not associated with evidence of active bacterial disease during the sampling period. The result indicates the presence of a potentially relevant bacterial species in clinically and histologically normal fish, without macroscopic or microscopic lesions attributable to an ongoing infectious process. These

findings are considered in the Discussion in relation to the opportunistic nature of *Aeromonas* spp., the environmental characteristics of freshwater reservoirs, and the possible asymptomatic circulation of bacterial taxa within wild fish communities.

4.4 PARASITOLOGICAL EXAMINATION

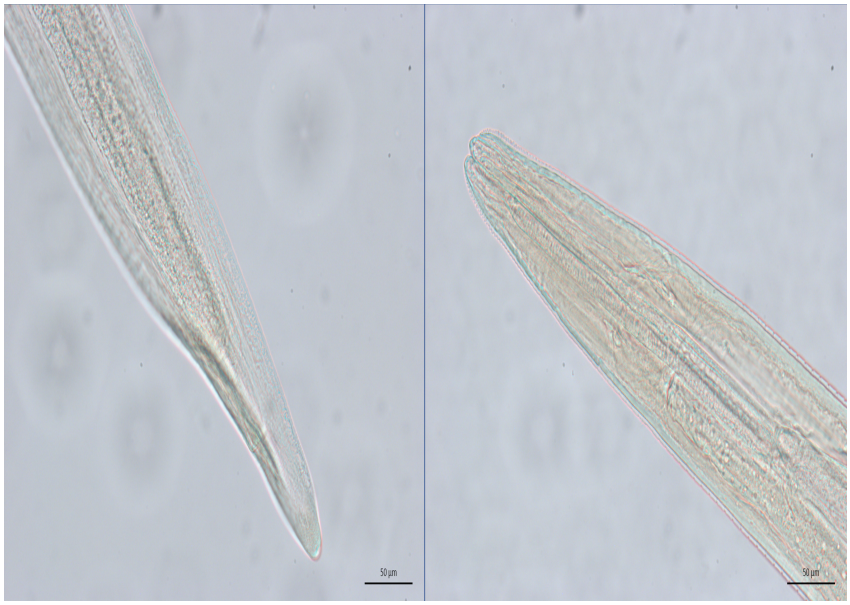
The parasitological examination included external assessment, gill inspection, coelomic cavity evaluation, and examination of the gastrointestinal tract and visceral surfaces. Overall, the external parasitological assessment performed on the examined fish did not reveal visible ectoparasitic infections. No macroscopic parasites were observed on the skin, fins, eyes, oral cavity, opercular region, or gills. Similarly, gill biopsies and microscopic observations did not show parasitic forms compatible with branchial ectoparasites, such as monogenean flukes or ciliates.

Inspection of the coelomic cavity and internal organ serosae did not reveal free endoparasites or gross lesions attributable to parasitic infestation in the majority of examined fish. No visible helminths, cysts, nodules, adhesions, haemorrhagic reactions, or parasite-associated inflammatory changes were observed during the initial macroscopic examination of the visceral organs and coelomic surfaces.

However, during the detailed evaluation of the gastrointestinal tract, nematode larvae were detected in two adult specimens of common carp*, Cyprinus carpio*, collected

during the monitoring activities. One adult specimen collected in November 2022 was positive for five nematode larvae, which were found within the intestinal lumen. A second adult *C. carpio* specimen, collected in March 2023, was positive for 15 nematode larvae, detected both within the intestinal lumen and encysted in the intestinal wall. Macroscopically, all collected larvae showed morphological features consistent with nematodes belonging to the family Anisakidae. The larvae appeared as whitish, elongated nematode forms, compatible with larval stages commonly observed in fish hosts. Subsequent morphological evaluation after fixation, clearing, and microscopic examination allowed the larvae to be morphologically identified as *Contracaecum rudolphii* L3 larvae (Fig. 4.4.1)

Fig. 4.4.1 Contracaecum rudolphii *L3 larvae*



The localization of larvae within the intestinal lumen and, in one specimen, encysted in the intestinal wall was recorded as a relevant parasitological finding. No

severe gross lesions were associated with the presence of the larvae, and no generalized parasitic burden was observed in the remaining examined specimens. The parasitological results therefore indicate the absence of widespread ectoparasitic or endoparasitic infections in the sampled fish, while documenting the occurrence of anisakid nematode larvae in selected adult *C. carpio* specimens from Lake Biviere di Lentini.

Overall, the parasitological examination showed that most examined fish were negative for visible ectoparasites and endoparasites. Nevertheless, the detection of morphologically identified *C. rudolphii* L3 larvae in two adult common carp specimens represents a relevant finding within the integrated assessment of the lake ecosystem, particularly considering the presence of piscivorous birds and the potential involvement of fish in parasite life cycles.

4.5 ICHTHYOFAUNA AND OTHER VERTEBRATE SPECIES

Sampling activities documented a diverse fish assemblage in Lake Biviere di Lentini, mainly represented by cyprinids and other freshwater species typical of Mediterranean lentic systems. The recorded taxa included *Cyprinus carpio* Linnaeus, 1758, including mirror and common carp morphotypes; *Carassius* spp.; *Mugil cephalus* Linnaeus, 1758; *Atherina boyeri* Risso, 1810; and *Rutilus* spp.

Cyprinids, particularly *Cyprinus carpio* (Fig. 4.5.1) and *Carassius* spp., were among the most frequently recorded taxa, confirming their relevance within the fish assemblage. *Atherina boyeri* was recorded in high numbers, indicating that small-bodied euryhaline species represent an important component of the fish community.

Fig. 4.5.1 *Two Common carps swimming in the surface in summer time.*



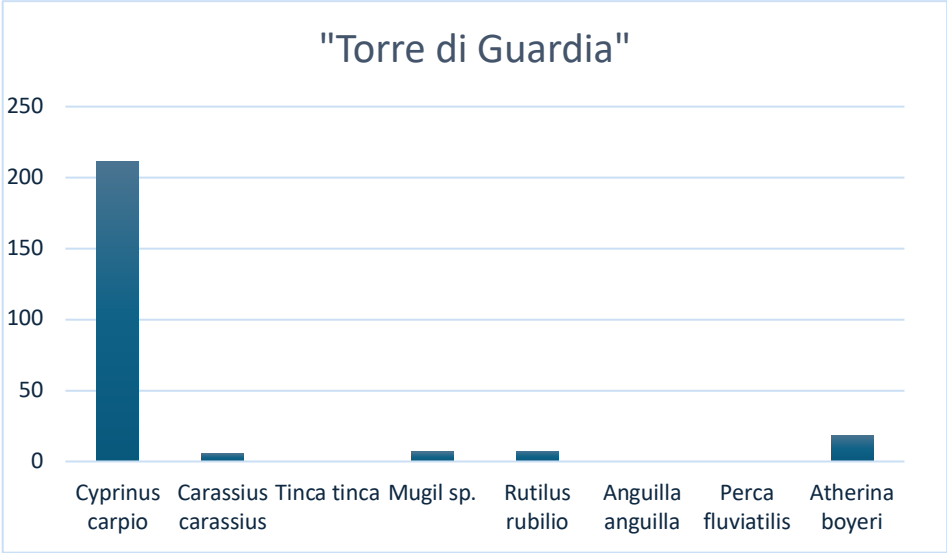
4.5.1 Analytical record of fish sampling

Fish caught in the sampling sites ST_TG (Torre guardia) and ST_TA (Torre sollevamento/ boa ARPA), were reported in the following tables (Tab. 4.5.1.1 and Tab 4.5.1.2) and figures 4.5.1.1, 4.5.1.2, 4.5.1.3.

Tab. 4.5.1.1 *Fish sampled in ST_TG*

"TORRE DI GUARDIA"							
Cyprinus carpio	*Carassius carassius*	*Tinca tinca*	*Mugil* sp.	*Rutilus rubilio*	*Anguilla anguilla*	*Perca fluviatilis*	*Atherina boyeri*
100m tremaglio net size 12							
97	3	0	0	0	0	0	0
100m invisible net size 10							
111	3	0	0	0	0	0	0
5 Bortovelli mezza luna size 06mm							
3	0	0	0	0	0	0	0
5 Sinking pot size 04mm							
0	0	0	3	2	0	0	8
Electrostunner							
0	0	0	4	6	0	0	11
1 Cage for Trachemis							
1 Male specimen							

Fig. 4.5.1.1 *Column charts of fish species sampled in ST_TG*



Tab. 4.5.1.2 *Fish sampled in ST_TA*

"TORRE SOLLEVAMENTO ACQUE" side BOA ARPA						
Carassius carassius	*Tinca tinca*	*Mugil* sp.	*Rutilus rubilio*	*Anguilla anguilla*	*Perca fluviatilis*	*Atherina boyeri*
100m tremaglio net size 12						
98	0	0	0	0	0	0
100m invisible net size 10						
67	0	0	0	0	0	0
5 "Bortovelli mezza luna" size 06mm						
0	0	1	3	0	0	2
5 Sinking pot size 04mm						
0	0	5	4	0	0	21

Electrostunner						
0	0	6	3	0	0	5
1 Cage for Trachemis						
1 Young specimen						

4.5.1.2 *Column charts of fish species sampled in ST_TA*

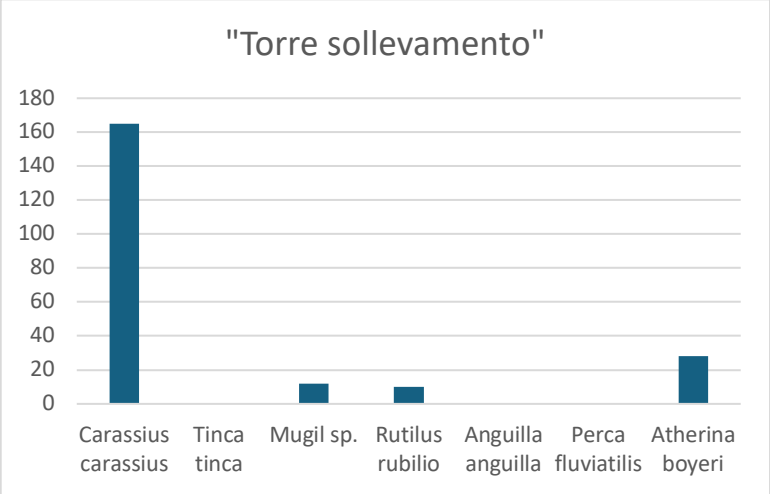
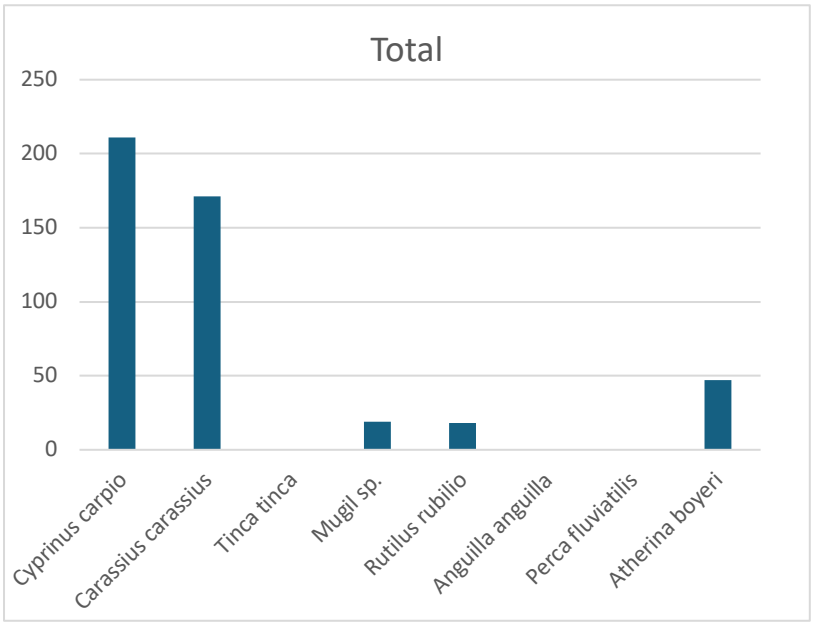


Fig. 4.5.1.3 *Column charts of both ST_TG and ST_TA Fish species sampled*



4.5.2 Other vertebrates recordings

From the sampling carried out, a good population of river crabs was found; Furthermore, the presence of juvenile fish on the surface was ascertained.

In addition to teleost fishes, the survey documented several other aquatic and semi-aquatic vertebrates. Individuals of the Sicilian pond turtle (*Emys trinacris,* Fritz et al 2005), an endemic species, were observed in both juvenile and adult stages, confirming active reproduction within the lake (fig.4.5.2). The non-native red-eared slider (*Trachemys scripta,* Thunberg in Schoepff, 1792) was detected only once, indicating a currently limited introduction.

Fig. 4.5.2.1 *Male and female of* Emys trinacris *during courtship in July 2023.*



Moreover, along the shoreline several specimens of lagomorphs have been observed with their known abundance in Mediterranean wetland margins. Terrestrial fauna also included the black rat snake (*Hyerophis viridiflavus carbonarius* Lacepede, 1789) was observed in November 2023 while resting in the vegetation near the shore of the lake (fig. 4.5.3).

Fig. 4.5.2.2 *The black rat snake*



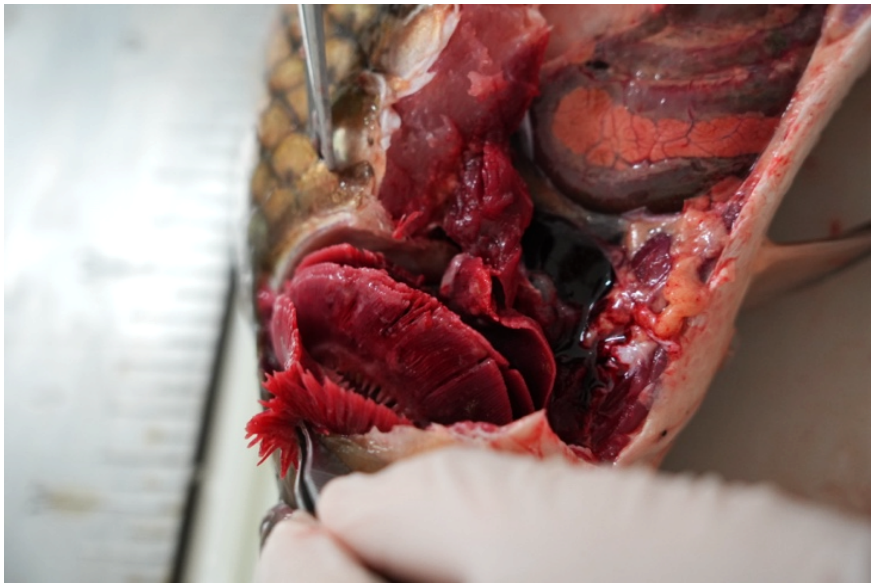
4.6 FISH HEALTH AND HISTOPATHOLOGICAL FINDINGS

The health assessment of the examined *Cyprinus carpio* specimens did not reveal gross pathological alterations suggestive of active infectious or parasitic disease. During external examination, the skin, fins, eyes, oral cavity, opercular region, and gills appeared macroscopically preserved. No ulcers, erosions, haemorrhages, abnormal pigmentation, excessive mucus production, fin necrosis, nodular lesions, deformities, or visible ectoparasites were observed. In particular, no external or branchial alterations compatible with major infectious diseases of cyprinids were detected (Figures 4.6.1 and 4.6.2).

Fig.4.6.1 *A specimen of* C. carpio *before the Histological examination*



Fig.4.6.2 *Histological examination on a specimen of* C. carpio



The gills did not show gross lesions such as pallor, congestion, necrosis, excessive mucus accumulation, lamellar adhesions, or visible parasitic infestation. The absence of marked skin and gill lesions was particularly relevant because these tissues are commonly involved in several infectious and environmental conditions affecting cyprinids. No macroscopic findings suggestive of *Cyprinid herpesvirus* 3, CyHV-3, infection were observed, including skin alterations, gill necrosis, or severe branchial damage. Similarly, no lesions compatible with other major bacterial, viral, or parasitic diseases were detected during the external examination.

Internal necropsy confirmed the absence of relevant gross lesions. The coelomic cavity appeared free from abnormal fluid accumulation, fibrin, adhesions,

haemorrhages, or visible parasitic forms. The liver, spleen, kidney, gastrointestinal tract, gonads, and other examined organs did not show evident enlargement, discoloration, nodular lesions, necrotic areas, abnormal consistency, or signs of systemic infection. No macroscopic alterations were observed that could indicate acute septicemic disease, chronic inflammatory processes, or severe organ impairment.

Histopathological examination confirmed the macroscopic findings. Across the examined organs, tissue architecture was generally preserved and no microscopic lesions indicative of active infectious disease were detected. The gills showed preserved branchial organization, without relevant epithelial hyperplasia, lamellar fusion, necrosis, inflammatory infiltrates, or parasite-associated changes. The absence of significant branchial lesions supported the macroscopic observation of a lack of active gill disease.

The liver showed preserved parenchymal architecture, with no evidence of severe hepatocellular degeneration, necrosis, inflammatory foci, granulomatous lesions, or pathogen-associated alterations. Hepatocyte arrangement and vascular organization were consistent with the absence of relevant hepatic pathology in the examined specimens. No microscopic findings suggestive of systemic infectious involvement or severe metabolic disturbance were observed.

Renal tissue did not show lesions compatible with active bacterial or viral disease. The haematopoietic and renal components appeared preserved, without relevant tubular necrosis, interstitial inflammation, haemorrhage, granulomatous reaction, or bacterial colonies. Similarly, splenic tissue did not reveal pathological

changes suggestive of systemic infection, and no severe inflammatory or necrotic lesions were observed.

The gastrointestinal tract also appeared histologically preserved. No relevant enteritis, mucosal necrosis, marked inflammatory infiltration, parasite-associated tissue reaction, or ulcerative lesions were detected in the examined sections. When parasitological findings were recorded in selected specimens, they were considered separately in the dedicated parasitological results section. Overall, histological evaluation did not reveal microscopic evidence of acute or chronic infectious processes in the examined fish.

The combined anatomopathological and histopathological findings therefore indicated that the sampled *C. carpio* specimens did not show evidence of active disease at the time of sampling. The absence of gross and microscopic lesions affecting the skin, gills, kidney, liver, spleen, and gastrointestinal tract suggests that no overt bacterial, viral, or parasitic disease outbreak was occurring in the examined fish population during the monitoring period.

Microbiological investigations were performed on fish tissues, water, and sediment samples in order to complement the pathological assessment and to characterize the cultivable bacterial component associated with the lake environment and fish health. This integrated approach allowed the evaluation of microorganisms present both in environmental matrices and in fish tissues, including bacterial taxa potentially relevant for freshwater teleost health.

Bacteriological examination of kidney samples was included to assess the possible occurrence of systemic bacterial colonization or infection. Although no gross or histological lesions suggestive of bacterial disease were observed, the microbiological screening allowed the detection of cultivable bacteria in internal tissues of selected specimens. These results are reported separately and are interpreted in relation to fish health and environmental microbial circulation in the Discussion.

Overall, the examined *C. carpio* specimens were not affected by clinically or histologically evident disease during the sampling period. The integration of gross pathology, histology, bacteriology, and parasitology provided a comprehensive evaluation of fish health within the broader ecosystem assessment of Lake Biviere di Lentini.

4.7 AVIFAUNAL RICHNESS AND SEASONAL OCCURRENCE

Avifauna monitoring carried out between 2022 and 2024 documented a highly diversified bird community associated with Lake Biviere di Lentini. Overall, approximately 25,000 bird records were obtained during the monitoring period, corresponding to approximately 150 species. Among these, 15 species were confirmed as breeding species, indicating that the lake is used not only as a temporary resting or

feeding site, but also as a reproductive habitat by several taxa. The complete list of recorded species is provided in the Supplementary Material.

The structure of the avifaunal community reflected the environmental heterogeneity of the reservoir. The large open-water surface, extensive reedbeds, marginal wetland vegetation, shallow areas, and availability of fish and aquatic invertebrates provided suitable conditions for different ecological guilds. Visual censuses, high-resolution photographic documentation, drone-assisted surveys, and fixed-camera observations confirmed the regular presence of waterfowl, herons and egrets, waders, raptors, reedbed-associated species, and piscivorous birds. This diversity of ecological groups highlights the role of Lake Biviere di Lentini as a multifunctional wetland system within the Sicilian and Mediterranean context.

Seasonal variation was evident throughout the monitoring period. During migration and wintering periods, the lake hosted large concentrations of aquatic birds, particularly ducks and other water-dependent species. Among the most frequently recorded waterfowl were mallards or wild ducks (*Anas platyrhyncus*) (fig.4.7.1), Eurasian teal (*Anas crecca*), common pochard (*Aythya ferina*), northern shoveler (*Spatula clypeata*), and ferruginous duck (*Aythya nyroca*). The presence of *A. nyroca* is particularly relevant because this species is of conservation concern and is closely associated with well-structured wetland habitats .

Fig. 4.7.1 *A Wild Ducks flock*



The repeated occurrence of these species indicates that the lake provides suitable feeding and resting conditions during key phases of the annual cycle.

Large wintering assemblages of ducks were commonly observed. During the study period, wintering waterfowl aggregations reached up to approximately 2,000 individuals per year, including different duck species. These concentrations confirm the importance of the lake as a wintering site for aquatic birds and suggest that the reservoir provides adequate open-water habitat, food resources, and refuge conditions during the colder months. In addition to ducks, other wetland-associated species were also recorded, including swans, geese, coots, moorhens, and water rails. Several of these taxa were observed in association with reedbed habitats, where evidence of breeding was also documented.

The great crested grebe (*Podiceps cristatus*) was regularly recorded (fig. 4.7.2), with wintering groups frequently exceeding 200 individuals. This represents one of the most relevant concentrations documented in Sicily during the monitoring period. The high number of individuals observed suggests that Lake Biviere di Lentini provides favourable conditions for this species, particularly in relation to open-water availability and fish resources. The recurring presence of large groups of *P. cristatus* further supports the relevance of the reservoir as a wintering and feeding area for piscivorous and fish-associated waterbirds.

Fig. 4.7.2 *Two great crested grebes inside a heronry*



Herons and egrets were regularly observed throughout the monitoring period. The recorded species included grey heron (*Ardea cinerea*), cattle egret (*Bubulcus ibis*), little egret (*Egretta garzetta*) fig. 4.7.3, and great egret (*Ardea alba*), fig. 4.7.4.

Fig. 4.7.3 *Two grey herons and a lot of little egrets on the shoreline of the lake*



Fig. 4.7.4 *A specimen of great egret*



These species, that usually live in large heronry (fig.4.7.5), use the lake and its margins for foraging, resting, and movement across wetland habitats. Their regular occurrence reflects the availability of shallow-water feeding areas, fish, amphibians, aquatic invertebrates, and marginal habitats suitable for wading birds.

Fig. 4.7.5 *A heronry along the lakeshore*



The lake also supported black-winged stilt (*Himantopus himantopus*), a species historically associated with nesting colonies in the area, confirming the importance of the reservoir and surrounding wetland habitats for breeding and migratory waders (fig. 4.7.6).

Fig. 4.7.6 *A specimen of black winged stilt*



The great cormorant (*Phalacrocorax carbo*) was among the most abundant piscivorous species recorded during the monitoring period. The species occurred in high numbers and formed large communal roosts within the lake area. Peak counts exceeded 2,000 individuals, representing one of the largest concentrations documented in Sicily during the study period .

The abundance of *P. carbo* is particularly relevant within the integrated ecological framework of this thesis, because the species is strictly piscivorous and may exert predation pressure on the fish community. Moreover, as a fish-eating bird, it may

contribute to the maintenance of parasite life cycles involving fish as intermediate or paratenic hosts and birds as definitive hosts (fig. 4.7.7).

Fig. 4.7.7 *Cormorants resting on the dry branches of a tree submerged in the waters of the lake*



Several raptor species were regularly detected in the study area, confirming the suitability of the lake and surrounding habitats for birds of prey. Among the most relevant species were peregrine falcon (*Falco peregrinus*), western marsh harrier (*Circus aeruginosus*), and osprey (*Pandion haliaetus*). The regular occurrence of *C. aeruginosus* is consistent with the presence of extensive reedbeds and wetland vegetation, while the detection of *P. haliaetus* reflects the availability of fish resources in the reservoir.

Additional species of conservation interest, including Bonelli's eagle (*Aquila fasciata*) and spotted eagles belonging to the genus *Clanga*, were also recorded during the monitoring period.

Among large birds, the white stork (*Ciconia ciconia*) represented one of the key breeding species at the site. Its confirmed breeding highlights the importance of Lake Biviere di Lentini not only as a feeding and resting area but also as a reproductive site for large wetland-associated birds.

The lake also hosted wintering common cranes (*Grus grus*), with increasing numbers observed during the monitoring period. This trend suggests that the reservoir may be gaining importance as a wintering or stopover site for this species within the regional wetland network (fig. 4.7.8), this is also the only place in Sicily where this species nests.

Fig. 4.7.8 *Sixteen specimens of common cranes observed in January 2024*



The Eurasian spoonbill (*Platalea leucorodia*) was frequently observed during migration. Flocks exceeding 200 individuals were recorded, indicating that the lake provides suitable stopover and feeding conditions for this large wading bird. The recurrent presence of *P. leucorodia* further confirms the importance of the reservoir for migratory species moving through the central Mediterranean region.

Overall, the avifauna recorded at Lake Biviere di Lentini included a wide range of species belonging to different ecological guilds, including resident, migratory, wintering, breeding, piscivorous, herbivorous, omnivorous, and predatory birds. The high number of records, the large species richness, the presence of confirmed breeding species, and the occurrence of several taxa of conservation interest confirm the high biodiversity value of the site. The lake therefore represents an important Mediterranean wetland ecosystem and a major ornithological hotspot in Sicily.

Within the broader framework of this study, avifaunal data also provided relevant ecological information for interpreting cross-trophic processes in the reservoir.

The abundance of piscivorous species, especially great cormorants, herons, grebes, and ospreys, indicates strong ecological links between bird communities and fish populations. These interactions may influence fish community structure, nutrient redistribution, and parasite transmission dynamics. Therefore, avifauna monitoring contributed not only to biodiversity assessment, but also to the integrated interpretation of Lake Biviere di Lentini as a complex freshwater ecosystem connecting water quality, fish assemblages, parasitological findings, and higher trophic levels.

4.8 MACROZOOBENTHIC FAUNA

The results, currently representing a non-exhaustive snapshot of the animal component present in the sediment, highlighted a prevalence in terms of abundance relative to the sampled portion of sediment of larval forms belonging to the Phylum Arthropoda and more specifically to the Chironomidae family (Newman, 1836), Philopotamidae Stephens, 1829 and Culicidae (Meigen, 1818).

Chironomid dipterans are small-medium sized aquatic insects, which spend at least part of their life cycle in contact with the substrate (benthic organisms in the larval stage). They belong to the order Diptera and the suborder Nematocera (fig.4.8.1),

Fig.4.8.1 *A Chironomidae specimen recorded*



They are characterized by long and thread-like antennae, slender and elongated body, long legs and narrow and elongated wings. They are holometabolous insects, that

is, they complete complete metamorphosis, passing from a larval stage to a structurally and visually different adult stage and occupy different ecological niches.

The postembryonic development phases of these insects, as in all holometabolans, are: egg, larva, pupa and finally adult or mago insect.

Philopotamidae is a family of insects in the order Trichoptera, the caddisflies. They are commonly known as net caddis. The aquatic larvae of these caddis fly silk-mesh nets in flowing water to capture food. A larva can weave more than a kilometer of paper-thin silk to create its intricate web.

Members of the Culicidae family are found throughout the world and attack a wide variety of different species. They are holometabolous and have four stages in their life cycle: egg, larva, pupa and adult. Mosquitoes are most active at night and feed largely on nectar, with only females consuming a blood meal to produce eggs. Aside from the nuisance of bites, Culicidae mosquitoes carry a number of other pathogens.

The life cycle of Culicidae is holometabolous, meaning they have a number of distinct morphological states. The early stages require areas of standing water in which to develop. Their life cycle can last from two weeks to several months depending on environmental conditions, mainly temperature.

At the moment, the data obtained have not been processed statistically as, for processing relating to the zoobenthic biodiversity associated with the lakebed, the following are necessary: further sampling aimed at the seasonal evaluation of the variability of the populations; the expansion of the sampled areas and the processing of

genomic data deriving from the analyzes currently being carried out on the area in question.

The following table 4.8.1 are representative of the families of some identified specimens recorded in S1 to S5 samples.

Table 4.8.1 *Families recorded macrozoobenthic Arthropoda*

	S 1		**S 2**		**S 3**		**S 4**		**S 5**	
Family	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2
Chironomidae	1	3	2	3	3	6	1	2	7	4
Culicidae	1	3	4	2	1	3	2	0	1	3
Philopotamidae	1	1	4	7	1	4	0	3	5	2

5 DISCUSSIONS

5.1 INTEGRATED INTERPRETATION OF LAKE BIVIERE DI LENTINI AS A MEDITERRANEAN RESERVOIR ECOSYSTEM

The present study provides an integrated assessment of the ecological status of Lake Biviere di Lentini by combining physicochemical, chemical, microbiological, macrozoobenthic, ichthyological, pathological, parasitological, and avifaunal data. This multidisciplinary approach allowed the lake to be interpreted not only as an artificial water-storage infrastructure, but also as a complex Mediterranean freshwater ecosystem characterized by seasonal variability, biological richness, and cross-trophic interactions.

Artificial reservoirs in Mediterranean regions are strongly influenced by the interaction among hydrological management, climatic seasonality, catchment inputs, nutrient dynamics, sediment–water exchanges, and biological communities (Naselli-Flores and Barone, 2005; Cantonati et al., 2020; Palma et al., 2021). In these systems, ecosystem functioning is often highly dynamic, and ecological conditions may change rapidly between seasons as a consequence of precipitation–evaporation cycles, water-level fluctuations, temperature changes, mixing processes, and biological productivity. Therefore, single-compartment or single-season assessments may fail to capture the actual ecological complexity of these environments.

The results obtained in this study indicate that Lake Biviere di Lentini is a biologically rich but environmentally sensitive reservoir. Chemical contamination was generally low or below detection limits for most investigated contaminants, suggesting the absence of marked pollution by the analyzed trace metals and organic compounds during the sampling period. However, the lake showed clear evidence of seasonal trophic variability, microbial circulation, benthic-community simplification, asymptomatic occurrence of potentially pathogenic bacteria in fish, detection of anisakid nematode larvae, and high avifaunal richness. These findings collectively suggest that the ecological status of the lake cannot be interpreted solely through chemical water quality, but requires an ecosystem-level perspective integrating environmental, biological, and health-related indicators.

This interpretation is consistent with current approaches to freshwater ecosystem health, which emphasize the need to assess ecological functioning through multiple lines of evidence, including water quality, biological communities, trophic structure, microbial processes, habitat conditions, and anthropogenic pressures (Rapport et al., 1998; Dudgeon et al., 2006; Reid et al., 2019; Tickner et al., 2020). In this framework, Lake Biviere di Lentini represents a relevant case study because it combines hydrological importance, high biodiversity value, and sensitivity to Mediterranean climatic and seasonal drivers.

5.2 PHYSICOCHEMICAL CONDITIONS AND SEASONAL TROPHIC VARIABILITY

The physicochemical results showed generally stable water-quality conditions across depths, with slightly alkaline pH values, high dissolved oxygen saturation, and moderate conductivity. These findings suggest that, during the two sampling campaigns, the central area of the lake did not experience severe oxygen depletion or acute physicochemical deterioration. However, several parameters showed relevant seasonal variation, indicating that the reservoir is subject to marked temporal dynamics.

The most evident seasonal pattern was observed for chlorophyll-*a*. The extremely high chlorophyll-*a* concentration recorded in March, especially in the surface layer, indicates intense phytoplankton development during the spring sampling. Conversely, the negligible chlorophyll-*a* values observed in November across all depths indicate a phase of very low phytoplankton biomass. This contrast suggests a seasonally pulsed trophic regime, rather than a continuously eutrophic condition.

In shallow lakes and reservoirs, chlorophyll-*a* is widely used as an indicator of phytoplankton biomass and trophic status (Carlson, 1977; OECD, 1982). However, its interpretation requires caution, particularly in Mediterranean reservoirs where seasonal hydrology and climatic variability can generate short-lived but intense productivity peaks. A single high chlorophyll-*a* value may indicate a bloom event or transient eutrophic phase, but it does not necessarily imply stable or chronic eutrophication. In

Lake Biviere di Lentini, the spring chlorophyll-*a* peak, followed by negligible values in autumn, supports the occurrence of a highly seasonal phytoplankton dynamic.

This pattern is consistent with the known behaviour of Mediterranean reservoirs, where water-level fluctuations, nutrient accumulation, thermal conditions, and residence time may influence phytoplankton biomass and community structure (Naselli-Flores and Barone, 2005; Naselli-Flores, 2011; Almeida et al., 2021). In such environments, periods of increased light availability, nutrient availability, and favourable temperature may promote rapid phytoplankton growth. Conversely, changes in hydrology, grazing pressure, nutrient limitation, mixing, or sedimentation may lead to a sharp decline in phytoplankton biomass.

The spring chlorophyll-*a* peak is ecologically relevant because high phytoplankton biomass may influence oxygen dynamics, microbial activity, food-web structure, and organic matter deposition. When algal biomass declines, sedimentation and decomposition of organic matter may increase microbial oxygen demand and affect nutrient cycling at the sediment–water interface (Wetzel, 2001; Scheffer, 2004). Although severe hypoxia was not observed during the sampling campaigns, the presence of high spring phytoplankton biomass suggests that the lake may be vulnerable to episodic eutrophic conditions, especially under warmer temperatures, prolonged stratification, or reduced water renewal.

The COD pattern further supports the interpretation of seasonal organic matter redistribution. In March, COD was higher at the surface and decreased with depth, which

may be consistent with surface biological productivity and suspended organic material associated with phytoplankton development. In November, higher COD values were observed at mid-depth and bottom layers, suggesting a shift in the vertical distribution of oxidizable material. This may reflect settling of organic particles, sediment–water exchange, resuspension processes, or seasonal mixing. Although COD values alone do not identify the source of oxidizable matter, their vertical and seasonal variation indicates that organic load dynamics are not constant throughout the year.

TDS and conductivity also showed seasonal variation. Conductivity increased markedly in November across all depths, while TDS showed differences between March and November. These changes may be influenced by evaporation, inflowing waters, mineralization processes, and reservoir management. In Mediterranean reservoirs, seasonal concentration of dissolved ions may occur during dry periods or when water renewal decreases. However, the ionic composition remained relatively stable overall, and major anions and cations did not indicate major salinization processes during the study period.

Alkalinity was higher in March than in November, while acidity showed the opposite trend. This suggests seasonal modification of carbonate equilibrium and buffering capacity. In productive lakes, photosynthesis, respiration, CO_2 dynamics, and carbonate precipitation or dissolution may influence pH and alkalinity. The slightly alkaline pH values recorded throughout the study are consistent with a buffered aquatic

system, but the seasonal variation in alkalinity indicates that the lake's carbonate system may respond to biological activity and hydrological conditions.

The low concentrations of trace metals and the absence of detectable VOCs, SVOCs, and organochlorine pesticides indicate that the central sampling site was not affected by measurable contamination from the investigated chemical classes during the monitoring period. This is an important finding because it suggests that the main ecological pressures observed in the lake are not primarily related to acute chemical pollution by the analyzed contaminants. Nevertheless, the absence of detectable chemical pollutants does not exclude ecological imbalance, because freshwater ecosystem status may be affected by nutrient dynamics, organic enrichment, hydrological instability, biological invasions, sediment stress, and trophic alterations even when regulated contaminants are low or absent (Dudgeon et al., 2006; Reid et al., 2019).

5.3 SEDIMENT–WATER INTERACTIONS AND INTERNAL NUTRIENT CYCLING

The marked seasonal variability in chlorophyll-*a*, COD, ammonium, and microbial indicators suggests that the water column of Lake Biviere di Lentini is dynamically connected to the sediment compartment. In shallow and lowland reservoirs, sediments are not passive depositional environments, but active biogeochemical compartments

that influence nutrient cycling, microbial persistence, organic matter degradation, and redox-dependent processes (Søndergaard et al., 2003; Smolders et al., 2006; Wetzel, 2001).

Internal nutrient loading, particularly phosphorus release from sediments, may sustain phytoplankton production even when external nutrient inputs are limited or intermittent. Under low-oxygen or anoxic conditions, phosphorus bound to iron compounds may be released into the water column, increasing bioavailable phosphorus and promoting algal growth (Søndergaard et al., 2003). High pH values may also affect phosphorus dynamics by influencing adsorption and precipitation processes. Although sediment phosphorus fractions and fluxes were not measured in the present study, the high chlorophyll-*a* concentrations observed in March despite undetectable phosphate in water samples may be consistent with rapid biological uptake of available phosphorus or with transient nutrient pulses not captured by the sampling frequency.

The absence of detectable phosphate in the water column should therefore be interpreted carefully. In productive aquatic systems, dissolved phosphate may remain low because it is rapidly assimilated by phytoplankton or microorganisms. Thus, low phosphate concentrations do not necessarily indicate low trophic potential. Instead, the combination of high chlorophyll-*a* and undetectable phosphate may indicate that phosphorus was biologically consumed during or shortly before the sampling event.

The ammonium peak observed in November at mid-depth may also reflect localized nitrogen transformation processes. Ammonium can originate from organic

matter mineralization, excretion by aquatic organisms, sediment release, or reduced nitrification under oxygen-limited microconditions. Although dissolved oxygen saturation was generally high at the measured scale, microzones within sediments or particulate organic aggregates may support different redox conditions. In this sense, ammonium variability may represent an early signal of internal nutrient processing rather than widespread nitrogen pollution.

Future monitoring should therefore include sediment nutrient fractions, sediment oxygen demand, high-frequency oxygen and temperature profiling, and direct measurements of nutrient fluxes at the sediment–water interface. Such data would clarify whether episodic phytoplankton blooms are primarily driven by external inputs, internal loading, hydrological operations, or a combination of these mechanisms.

5.4 MICROBIOLOGICAL INDICATORS AND SANITARY INTERPRETATION

The microbiological results showed the presence of total coliforms, *E. coli*, enterococci, sulphite-reducing Clostridia, and presumptive *Vibrio* spp. in water and sediment samples, while *Salmonella* spp. were consistently absent. This pattern suggests that Lake Biviere di Lentini was characterized by detectable microbial loading, but without evidence of contamination by the investigated enteric pathogen during the two sampling campaigns.

Total coliforms were the most abundant microbial group in the water column. Their widespread occurrence across depths and seasons indicates the presence of a broad environmental coliform component. Total coliforms are not exclusively fecal in origin, as they may include environmental bacteria associated with soils, vegetation, sediments, and decaying organic matter. Therefore, high total coliform values should be interpreted as indicators of general microbial load rather than direct evidence of recent fecal pollution.

By contrast, *E. coli* values were much lower than total coliform counts. This suggests that recent fecal contamination was present at low levels or occurred intermittently. The highest *E. coli* values were observed in bottom water, which may indicate particle association, sediment resuspension, or localized accumulation near the sediment–water interface. The absence of *E. coli* in sediments, despite the presence of total coliforms, enterococci, and sulphite-reducing Clostridia, indicates that the sediment microbial community was not dominated by recent fecal indicators at the time of sampling.

Enterococci were consistently detected, although with variable values across depths and seasons. Their presence may reflect fecal inputs from wildlife, birds, livestock, or runoff, but also their greater persistence in the environment compared with *E. coli*. In a reservoir supporting abundant avifauna, bird-derived microbial input may represent an important natural source of fecal indicators. However, without microbial

source tracking, it is not possible to distinguish among avian, livestock, human, or environmental sources.

Sulphite-reducing Clostridia were detected at low levels in both water and sediment. Their presence is relevant because spore-forming bacteria may persist in aquatic environments for longer periods than vegetative cells and may indicate historical or persistent contamination. The detection of Clostridia in sediments supports the interpretation of the sediment compartment as a reservoir for persistent microbial forms. This is consistent with the broader role of sediments as sinks and potential secondary sources of microorganisms, organic matter, and nutrients.

Presumptive *Vibrio* spp. were detected in all water layers and in sediments. Although Vibrio species are often associated with marine or brackish environments, several taxa can occur in freshwater or low-salinity aquatic habitats, especially under favourable temperature and organic matter conditions. The detection of presumptive Vibrio spp. should therefore be interpreted as part of the aquatic microbial profile of the reservoir rather than as evidence of a specific disease process. Nevertheless, their occurrence supports the need for continued microbiological surveillance, particularly in the context of climate warming, because temperature is an important driver of aquatic bacterial dynamics.

The absence of *Salmonella* spp. in all water and sediment samples is a positive sanitary finding. It indicates that this pathogen was not detected at the central sampling site during the monitored periods. However, this result should not be generalized to the

entire lake or to all seasons, because *Salmonella* occurrence in environmental waters may be intermittent and influenced by rainfall, runoff, wildlife activity, livestock presence, and sampling location. Therefore, repeated monitoring across multiple sites and seasons would be required to confirm the persistence of this pattern.

Overall, the microbiological data support the interpretation of a reservoir characterized by background microbial loading and environmental bacterial circulation rather than acute pathogen contamination. The combined presence of fecal indicators, persistent spore-forming bacteria, and aquatic opportunistic taxa highlights the need to interpret microbiological quality in relation to hydrology, sediment dynamics, avifaunal abundance, organic matter, and land use.

5.5 MICROBIAL PROCESSES AND FUTURE MOLECULAR MONITORING

Although the present study relied mainly on culture-based microbiological indicators, the results suggest that microbial processes may play an important role in the functioning of Lake Biviere di Lentini. In freshwater ecosystems, microorganisms regulate organic matter degradation, nutrient regeneration, nitrogen cycling, methane production and oxidation, and the transfer of dissolved organic matter into higher trophic levels through the microbial loop (Azam et al., 1983; Cotner and Biddanda, 2002; Pomeroy et al., 2007).

The vertical distribution of microbial indicators, together with seasonal changes in COD and ammonium, suggests that microbial activity may be linked to organic matter redistribution and sediment–water interactions. In reservoirs, microbial communities are strongly influenced by temperature, oxygen availability, suspended particles, organic matter, nutrient concentrations, and hydrological mixing. These factors may vary substantially across seasons and depths, especially in Mediterranean environments.

Future studies should therefore integrate culture-based microbiology with molecular tools. High-throughput 16S rRNA gene sequencing could provide information on bacterial community composition in water and sediments, while shotgun metagenomics or targeted qPCR could identify functional genes involved in nitrogen cycling, phosphorus metabolism, organic matter degradation, cyanotoxin production, antimicrobial resistance, and methane cycling. Functional markers such as amoA, nirS, nirK, nosZ, nrfA, and hzo could help characterize nitrification, denitrification, dissimilatory nitrate reduction, and anammox pathways. Similarly, pmoA could be used to investigate methane-oxidizing bacteria.

Although methane dynamics were not directly assessed in this study, they may represent a relevant future research target. Shallow reservoirs can contribute to greenhouse gas emissions through sedimentary organic matter decomposition, methanogenesis, ebullition, and diffusive fluxes. At the same time, aerobic and microaerophilic methanotrophs may oxidize part of the methane before it reaches the

atmosphere. The inclusion of methane-related measurements would therefore broaden the ecosystem-level understanding of carbon cycling in Lake Biviere di Lentini.

5.6 FISH COMMUNITY, FISH HEALTH, AND ABSENCE OF ACTIVE DISEASE

The fish community of Lake Biviere di Lentini was dominated by cyprinids, consistent with the ecological characteristics of lowland lentic freshwater systems.

According to local historical sources and sports fishermen, the presence of tench (*Tinca tinca*) and perch (*Perca fluviatilis),* once considered highly important for the local economy and feeding, can be hypothesized but there were not records by fish sampling.

Common carp, *Cyprinus carpio*, was selected for detailed health assessment because of its abundance, benthivorous behaviour, interaction with sediments, and potential role as a host for parasites and opportunistic bacteria.

Macroscopic and histopathological analyses did not reveal lesions compatible with active infectious disease in the examined specimens. The absence of gross lesions affecting the skin, gills, internal organs, and coelomic cavity suggests that no overt disease outbreak was occurring in the sampled fish at the time of investigation. Histology confirmed these findings, showing preserved tissue architecture and no microscopic evidence of acute or chronic infectious processes.

The absence of branchial lesions is particularly relevant because gills are highly sensitive to infectious, parasitic, and environmental insults. In cyprinids, severe gill necrosis, epithelial hyperplasia, lamellar fusion, and inflammatory lesions may be associated with viral, bacterial, parasitic, or water-quality-related conditions. No histological evidence suggestive of *Cyprinid herpesvirus* 3 infection was observed. However, histology alone cannot formally exclude pathogen circulation. Targeted molecular testing would be required to determine whether specific pathogens, including CyHV-3, are present at subclinical levels.

The lack of histopathological lesions does not imply the absence of environmental stress, but it suggests that the sampled fish were not undergoing active tissue-level disease during the sampling period. In wild or reservoir fish populations, health status may vary seasonally and may be influenced by temperature, oxygen availability, reproductive cycle, nutritional condition, parasite exposure, microbial pressure, and environmental quality. Therefore, the present findings provide a useful snapshot of fish health but should be integrated with repeated seasonal surveys.

5.7 OCCURRENCE OF *AEROMONAS VERONII* IN CLINICALLY HEALTHY FISH

The isolation of *Aeromonas veronii* from kidney samples of *C. carpio* represents an important finding within the integrated fish health assessment. Members of the

genus Aeromonas are widespread in aquatic environments and include species commonly isolated from freshwater, sediments, fish, amphibians, and aquatic invertebrates (Janda and Abbott, 2010; Austin and Austin, 2016). *A. veronii* is increasingly recognized as an opportunistic pathogen of freshwater fish and has been associated with septicemia, ulcerative lesions, hemorrhagic disease, and mortality events in aquaculture and wild fish contexts (Li et al., 2020; Sun et al., 2016; Chen et al., 2019).

In the present study, *A. veronii* was detected in fish tissues without associated gross or histopathological lesions. This is a crucial point for interpretation. The bacteriological finding should not be considered evidence of an active outbreak, because no clinical signs, ulcerative lesions, septicemic changes, or microscopic inflammatory processes were observed. Instead, the result suggests asymptomatic carriage, subclinical colonization, or internal presence of cultivable bacteria in apparently healthy fish.

This condition is ecologically plausible in freshwater environments. Opportunistic bacteria may persist in water, sediments, and host-associated microbiota without causing disease under stable conditions. Disease expression may occur only when environmental or host-related stressors compromise immune competence or favour bacterial proliferation. In fish, stressors such as high temperature, hypoxia, organic enrichment, handling, crowding, poor water quality, sudden environmental change, and

coinfections may increase susceptibility to opportunistic bacterial disease (Noga, 2010; Austin and Austin, 2016).

The detection of *A. veronii* is therefore relevant as an early-warning element rather than as evidence of current pathology. In Mediterranean reservoirs, seasonal warming, eutrophic episodes, oxygen fluctuations, and increased organic matter may create conditions favourable to opportunistic bacteria. The high chlorophyll-*a* values observed in March and the seasonal redistribution of organic matter indicated by COD may be important contextual factors, although no direct causal link can be established from the present data.

The potential virulence of *A. veronii* depends on strain-specific characteristics. Several virulence-related factors have been described in *Aeromonas* spp., including aerolysin, cytotoxic enterotoxins, hemolysins, proteases, lipases, DNases, secretion systems, motility-related factors, biofilm formation, iron acquisition systems, and quorum sensing mechanisms (Janda and Abbott, 2010; Li et al., 2020; Talagrand-Reboul et al., 2017; Zhu et al., 2022; Behera et al., 2023). Genes such as aer, act, ser, lip, exu, and luxS are frequently discussed in relation to pathogenicity, tissue damage, host colonization, and coordinated bacterial behaviour. However, the present study did not investigate the virulence gene profile of the isolated strain; therefore, no conclusions can be drawn regarding its pathogenic potential.

Future studies should characterize *A. veronii* isolates using molecular virulence screening, antimicrobial susceptibility testing, and, if possible, whole-genome

sequencing. This would allow discrimination between low-virulence environmental strains and potentially pathogenic strains circulating asymptomatically in fish. Moreover, antimicrobial resistance profiling would be useful because *Aeromonas* spp. may carry resistance determinants of relevance for aquatic animal health and public-health surveillance.

From an ecosystem-health perspective, the isolation of *A. veronii* from clinically healthy fish reinforces the importance of integrating bacteriology with pathology. If bacteriology had been interpreted alone, the detection of this species might have suggested disease risk. Conversely, histopathology alone would not have revealed bacterial carriage. The combination of both approaches allowed a more balanced interpretation: *A. veronii* was present in the fish population, but without evidence of active disease at the time of sampling.

5.8 PARASITOLOGICAL FINDINGS AND FISH–BIRD TROPHIC LINKS

The parasitological examination did not reveal widespread ectoparasitic or endoparasitic infections in the examined fish. External assessment, gill biopsies, and coelomic cavity inspection were negative for visible ectoparasites, branchial parasites, and generalized parasitic disease. This finding suggests that, during the sampling period, the examined fish were not affected by major external parasitic burdens.

The absence of ectoparasites may reflect seasonal variation in parasite transmission, environmental conditions not favourable to parasite proliferation, low host density in the sampled areas, or effective host responses. Parasite abundance in fish populations is strongly influenced by temperature, host density, intermediate host availability, water quality, and seasonal cycles (Poulin, 2007; Marcogliese, 2016). Therefore, negative findings should be interpreted as representative of the sampling period rather than as definitive evidence of the absence of ectoparasites throughout the year.

The detection of anisakid nematode larvae morphologically identified as *Contracaecum rudolphii* L3 larvae in two adult *C. carpio* specimens is ecologically relevant. *Contracaecum* spp. have complex life cycles involving aquatic intermediate or paratenic hosts and piscivorous birds as definitive hosts (Anderson, 2000; Moravec, 2013). Fish may acquire larvae through ingestion of infected invertebrates or smaller fish and may subsequently transmit the parasites to fish-eating birds. Thus, the occurrence of *Contracaecum* larvae in common carp provides evidence of trophic links between fish and avifauna.

The localization of larvae within the intestinal lumen and encysted in the intestinal wall suggests exposure to larval nematodes and possible tissue interaction. Encystment is compatible with chronic host–parasite relationships and larval persistence in fish tissues. The low number of positive fish may reflect localized exposure, differences in feeding behaviour, heterogeneity in intermediate host distribution, or spatial variation in contamination by definitive hosts. In reservoirs with abundant piscivorous birds,

parasite transmission may be highly patchy, depending on roosting sites, feeding areas, defecation patterns, and fish movement.

The presence of large numbers of great cormorants and other piscivorous birds in Lake Biviere di Lentini provides an ecological context for the detection of *Contracaecum* larvae. Cormorants are known definitive hosts for members of the *C. rudolphii* complex, and their feeding ecology can support parasite transmission in freshwater systems (Mattiucci and Nascetti, 2008; Moravec, 2013; Vuić et al., 2022). Therefore, the parasitological finding should be interpreted not only as a fish health observation, but also as an indicator of food-web connectivity.

From a sanitary perspective, anisakid larvae are relevant because some species have zoonotic potential. Human infection by *Contracaecum* is considered less frequent than infection by *Anisakis*, but the presence of anisakid larvae in fish remains relevant where raw, marinated, smoked, or undercooked fish products are consumed (Audicana and Kennedy, 2008; EFSA BIOHAZ Panel et al., 2010; EFSA BIOHAZ Panel et al., 2024). In the present study, larvae were detected in the intestinal lumen and wall rather than reported from edible musculature; nevertheless, their presence supports the value of parasitological surveillance in fish from natural and artificial freshwater systems.

Definitive species-level identification within the *Contracaecum rudolphii* complex may require molecular confirmation, because larval morphology alone can be insufficient to discriminate closely related taxa (Mattiucci and Nascetti, 2008; Abdullah et al., 2021). Therefore, future studies should combine morphological identification with

molecular tools, such as ITS or mitochondrial markers, to confirm species identity and clarify parasite diversity in the lake.

5.9 MACROZOOBENTHIC COMMUNITIES AS INDICATORS OF SEDIMENTARY CONDITIONS

The macrozoobenthic assemblage, characterized by dominance of Chironomidae with contributions from other taxa such as Culicidae and Philopotamidae, provides additional information on the ecological status of the benthic compartment. Macrozoobenthic communities are widely used as indicators because they integrate environmental conditions over time and respond to oxygen availability, organic matter, sediment type, habitat complexity, hydrological disturbance, and trophic status (Rosenberg and Resh, 1993; Bonada et al., 2006; Birk et al., 2012).

Chironomidae are common inhabitants of freshwater sediments and include taxa with different ecological preferences and tolerance levels. Many chironomid larvae are able to colonize fine sediments and tolerate organic enrichment, low oxygen availability, and fluctuating conditions (Armitage et al., 1995; Pinder, 1986; Rossaro et al., 2022). Their dominance in Lake Biviere di Lentini is therefore compatible with a soft-sediment lentic habitat characterized by seasonal organic matter deposition and variable oxygen regimes.

The dominance of tolerant taxa does not necessarily indicate severe degradation, particularly in artificial lowland reservoirs where fine sediments, high productivity, water-level fluctuations, and organic deposition are common. However, when associated with limited taxonomic diversity, Chironomidae dominance may suggest moderate ecological stress or unstable benthic conditions. In the present study, this pattern is consistent with the physicochemical evidence of seasonal trophic variability and with microbiological findings indicating sediment-associated microbial persistence.

The benthic community also has functional relevance for the broader food web. Chironomid larvae and other benthic invertebrates represent important prey for fish and waterbirds. Therefore, the macrozoobenthic compartment may mediate the transfer of organic matter and microbial-derived energy to higher trophic levels. In this sense, benthic data support the interpretation of Lake Biviere di Lentini as a system in which sedimentary processes, fish feeding ecology, and avifaunal use are closely interconnected.

Future macrozoobenthic monitoring should include replicated seasonal sampling across multiple lake sectors, taxonomic identification to the lowest possible level, functional trait analysis, and the use of biotic indices adapted to lentic Mediterranean systems. Such an approach would allow a more robust assessment of benthic ecological quality and would clarify whether Chironomidae dominance reflects natural reservoir conditions, seasonal instability, organic enrichment, or habitat simplification.

5.10 AVIFAUNAL DIVERSITY AND CROSS-TROPHIC ECOLOGICAL PROCESSES

Avifauna monitoring confirmed the high biodiversity value of Lake Biviere di Lentini. Approximately 25,000 bird records corresponding to about 150 species were obtained between 2022 and 2024, with 15 confirmed breeding species. These data demonstrate that the lake functions as an important wetland habitat for resident, breeding, wintering, and migratory birds.

The large water surface, extensive reedbeds, shallow margins, fish resources, and aquatic invertebrate availability create suitable conditions for multiple ecological guilds. Ducks, grebes, herons, egrets, waders, rails, raptors, storks, spoonbills, cranes, and piscivorous birds were recorded during the monitoring period. This confirms that the reservoir is not only a water-storage basin, but also a major ornithological hotspot within the Sicilian and Mediterranean wetland network.

The high number of wintering waterfowl, including Eurasian teal, common pochard, northern shoveler, and ferruginous duck, indicates the importance of the lake as a winter refuge and feeding area. The presence of ferruginous duck is particularly relevant because this species is of conservation concern and depends on well-structured wetland habitats. The recurrent presence of large groups of great crested grebe further indicates the availability of fish resources and open-water habitat suitable for piscivorous and fish-associated birds.

The abundance of great cormorants is particularly important for ecosystem interpretation. Peak counts exceeding 2,000 individuals suggest that the species may exert substantial predation pressure on fish communities. Bioenergetic studies indicate that cormorant food intake can reach several hundred grams of fish per individual per day, depending on species, season, prey energy density, temperature, and activity (Dirksen et al., 1995; Ridgway, 2010). Therefore, large roosting aggregations may represent a meaningful top-down force in the lake ecosystem, especially if prey fish are spatially concentrated or recruitment is limited.

At the same time, piscivorous birds may contribute to parasite circulation. The detection of *Contracaecum* larvae in common carp is ecologically coherent with the high abundance of fish-eating birds, because these birds can act as definitive hosts for anisakid nematodes. Thus, avifaunal data help interpret parasitological findings and highlight the importance of considering bird–fish interactions in ecosystem-health assessments.

Birds may also influence nutrient and microbial dynamics. Guano deposition can contribute organic matter, nitrogen, phosphorus, and fecal microorganisms to water and sediments, particularly near roosting or resting areas (Green and Elmberg, 2014; Gwiazda, 2014). In large bird aggregations, these inputs may be ecologically relevant, although their magnitude was not quantified in the present study. Future studies should therefore evaluate whether bird abundance, roosting locations, and microbial indicators are spatially associated.

The presence of raptors, including peregrine falcon, western marsh harrier, osprey, Bonelli's eagle, and spotted eagles, further emphasizes the trophic complexity of the site. These species reflect the availability of prey resources and habitat heterogeneity in the lake and surrounding landscape. The breeding of white stork and the increasing occurrence of common cranes indicate that Lake Biviere di Lentini may be gaining increasing importance within regional migratory and breeding networks.

Overall, the avifaunal results reinforce the interpretation of the lake as a high-value biodiversity reservoir. From an ecosystem perspective, birds should not be interpreted only as conservation targets, but also as active ecological components influencing fish predation, parasite life cycles, microbial inputs, nutrient redistribution, and habitat use.

5.11 ECOSYSTEM RESILIENCE AND SENSITIVITY TO ENVIRONMENTAL CHANGE

The combined results indicate that Lake Biviere di Lentini is a functionally active and biodiversity-rich reservoir, but also a sensitivity-prone system. Its resilience depends on the balance among hydrological management, seasonal mixing, oxygen availability, sediment–water exchanges, nutrient dynamics, microbial processes, fish-community structure, and avifaunal use.

The absence of severe chemical contamination and active fish disease suggests that the lake was not undergoing acute environmental or pathological crisis during the study period. However, the high spring chlorophyll-*a* concentration, presence of fecal indicators, detection of opportunistic bacteria, Chironomidae-dominated benthic assemblage, and occurrence of anisakid larvae collectively indicate the presence of dynamic ecological processes that require continued monitoring.

Mediterranean reservoirs are particularly vulnerable to climate change because warming, drought, altered rainfall, increased evaporation, and water abstraction may intensify trophic instability and reduce ecosystem resilience (Cramer et al., 2018; Tramblay and Llasat, 2020; Almeida et al., 2021). Under future scenarios, higher temperatures may extend the growing season for phytoplankton, favour cyanobacterial blooms, reduce oxygen solubility, increase stratification stability, and promote internal nutrient loading. These processes may also influence microbial communities and opportunistic pathogens.

In this context, the spring bloom observed in Lake Biviere di Lentini should be considered an early management signal. Even if the lake does not appear chronically eutrophic, episodic high-productivity phases may become more frequent or intense under climate-driven warming and altered hydrology. Therefore, the lake should be monitored not only for chemical pollutants, but also for bloom risk, oxygen dynamics, cyanobacteria, cyanotoxins, microbial indicators, and fish health.

5.12 MANAGEMENT IMPLICATIONS

The results of this study have several implications for the management of Lake Biviere di Lentini and similar Mediterranean artificial reservoirs. First, monitoring should be seasonal and multi-compartmental. The strong contrast between March and November chlorophyll-*a* values demonstrates that single-season sampling could lead to misclassification of trophic status. High-frequency monitoring of temperature, dissolved oxygen, pH, conductivity, turbidity, chlorophyll-*a*, and redox potential would improve the ability to detect short-term changes and identify bloom-prone periods.

Second, sediment processes should be incorporated into management plans. Sediments may store nutrients, microorganisms, organic matter, and contaminants, and may influence the overlying water column through resuspension and internal loading. Sediment phosphorus fractionation, organic matter content, microbial profiling, and nutrient-flux measurements would help identify whether internal loading contributes to seasonal productivity.

Third, microbiological monitoring should continue. The presence of total coliforms, enterococci, sulphite-reducing Clostridia, presumptive *Vibrio* spp., and *A. veronii* indicates that microbial circulation is an important component of the lake

ecosystem. Future monitoring should include both culture-based indicators and molecular tools, including microbial source tracking, antimicrobial resistance genes, and virulence markers in opportunistic bacteria.

Fourth, fish health surveillance should be maintained and expanded. The absence of active disease is reassuring, but the detection of *A. veronii* in clinically healthy fish suggests that potentially opportunistic bacteria may circulate asymptomatically. Seasonal health assessments, bacteriology, histopathology, targeted PCR for notifiable pathogens, and antimicrobial susceptibility testing would improve early detection of emerging risks.

Fifth, parasitological monitoring should be linked to avifaunal data. The detection of *Contracaecum* larvae in common carp suggests that fish–bird parasite cycles occur within the reservoir. Monitoring should therefore include fish parasitology, bird abundance, roost location, fish diet, and molecular identification of nematode larvae. This would clarify transmission pathways and help assess both ecological and sanitary relevance.

Sixth, reedbed and littoral habitats should be protected. These habitats support breeding birds, waterfowl, fish nurseries, macroinvertebrates, and microbial processes. Their conservation is essential for maintaining the biodiversity value of the lake. At the same time, management should consider how water-level regulation affects reedbeds, benthic habitats, bird feeding areas, and fish reproduction.

Finally, adaptive hydraulic management should be considered. In Mediterranean reservoirs, water-level fluctuations can influence stratification, nutrient cycling, bloom risk, habitat availability, and littoral ecology. Management strategies should aim to balance water-storage needs with ecological functioning. A before–after control–impact design could be useful for evaluating how specific hydrological operations affect water quality, phytoplankton biomass, oxygen dynamics, fish health, and avifaunal use.

5.13 METHODOLOGICAL CONSIDERATIONS AND FUTURE PERSPECTIVES

The present study provides a first integrated and multidisciplinary assessment of Lake Biviere di Lentini, combining physicochemical, microbiological, macrozoobenthic, ichthyological, pathological, parasitological, and avifaunal data. This approach represents one of the main strengths of the work, as it allowed the lake to be interpreted as a complex ecosystem rather than through isolated environmental parameters. At the same time, some methodological aspects should be considered in order to guide future research and improve long-term monitoring strategies.

The sampling design was appropriate to establish a preliminary ecosystem-level baseline, but future investigations could benefit from a broader spatial and temporal resolution. In particular, the inclusion of additional sampling stations located near inflow areas, reedbeds, littoral zones, bird roosting sites, shallow margins, and outlet

structures would allow a more detailed characterization of spatial heterogeneity within the reservoir. These areas may differ from the central open-water station in terms of nutrient inputs, microbial load, sediment characteristics, fish activity, and avifaunal influence. Similarly, repeated sampling across additional seasonal phases would improve the understanding of short-term fluctuations in trophic status, oxygen dynamics, microbial indicators, and biological communities.

Future monitoring should also include more detailed analyses of sediment-related processes. The marked seasonal variability in chlorophyll-*a*, COD, ammonium, and microbial indicators suggests that benthic–pelagic coupling may play an important role in the ecological functioning of the lake. Therefore, sediment phosphorus fractionation, organic matter content, sediment oxygen demand, redox profiling, and nutrient-flux measurements would be useful to clarify the contribution of internal loading to seasonal productivity and bloom risk.

The microbiological component could be further strengthened by integrating culture-based methods with molecular approaches. While traditional indicators remain essential for sanitary assessment, molecular tools such as 16S rRNA gene sequencing, quantitative PCR, microbial source tracking, and screening for antimicrobial resistance or virulence markers would provide a more complete understanding of microbial diversity, ecological function, and potential health relevance. This would be particularly useful for interpreting the occurrence of opportunistic bacteria such as *Aeromonas veronii*, whose ecological significance depends not only on its presence, but also on

strain-specific virulence traits, antimicrobial resistance profile, and environmental conditions favouring pathogenicity.

Similarly, fish health surveillance could be expanded through repeated seasonal sampling, targeted molecular screening for relevant pathogens, and a larger number of specimens and species. In the present study, the absence of gross and histopathological lesions provided reassuring evidence of the lack of active disease in the examined fish. However, future studies could investigate whether subclinical pathogen circulation occurs in the reservoir, particularly under environmental conditions such as high temperature, reduced oxygen availability, or increased organic load.

Parasitological monitoring should also be continued and integrated with avifaunal data. The detection of *Contracaecum rudolphii* L3 larvae in common carp suggests the presence of fish–bird parasite cycles within the lake. Future investigations combining morphological and molecular identification of nematode larvae, fish diet analysis, seasonal parasite prevalence, and mapping of piscivorous bird abundance and roosting areas would help clarify transmission pathways and the ecological role of these parasites within the food web.

Finally, the exceptional avifaunal richness documented during the study confirms the importance of Lake Biviere di Lentini as a biodiversity hotspot. Future work should further explore the ecological relationships between bird communities, nutrient redistribution, microbial inputs, fish predation, and parasite transmission. In this perspective, long-term integrated monitoring would provide valuable information for

adaptive management, supporting both water-resource use and biodiversity conservation.

Overall, the present study should be considered as a baseline framework for future ecosystem-health monitoring of Lake Biviere di Lentini. The results highlight the importance of multidisciplinary approaches in Mediterranean artificial reservoirs and provide a foundation for more detailed investigations aimed at understanding seasonal dynamics, cross-trophic interactions, and the effects of environmental change on reservoir functioning.

6 CONCLUSIONS

This study provides a preliminary but comprehensive integrated baseline for the ecological and health assessment of Lake Biviere di Lentini, one of the most important artificial freshwater reservoirs in south-eastern Sicily and a key ecological node within the Mediterranean landscape. By combining physicochemical, chemical, microbiological, macrozoobenthic, ichthyological, histopathological, parasitological, and avifaunal data, this work offers a multidisciplinary interpretation of the lake as a biologically rich, environmentally dynamic, and ecologically sensitive system.

The results indicate that Lake Biviere di Lentini is characterized by generally stable physicochemical conditions and low detectable chemical contamination during the investigated period. Trace metals and organic contaminants were detected at low concentrations or remained below detection limits, suggesting the absence of relevant chemical contamination from the analysed compound classes. However, the marked seasonal variability observed in trophic indicators, particularly chlorophyll-*a*, highlights the dynamic nature of the reservoir.

The very high chlorophyll-*a* concentrations recorded in spring were consistent with transient bloom conditions and high phytoplankton biomass, whereas the negligible values observed in autumn indicated a strong seasonal reduction in primary productivity.

These findings suggest that the lake should not be interpreted as a chemically degraded system, but rather as a reservoir subject to pronounced seasonal trophic fluctuations.

Microbiological analyses revealed the presence of fecal indicator bacteria, sulphite-reducing Clostridia, and presumptive aquatic opportunistic bacterial groups in water and sediment samples. At the same time, *Salmonella* spp. were consistently absent from all analysed matrices. This microbiological profile indicates a background level of microbial circulation within the lake system, likely influenced by environmental conditions, sediment–water interactions, organic matter dynamics, wildlife presence, and hydrological processes. The detection of microbial indicators does not necessarily point to acute contamination, but it confirms the importance of including microbiological monitoring within long-term ecosystem surveillance programs.

The fish health assessment provided reassuring evidence regarding the absence of active disease in the examined common carp specimens. Macroscopic and histopathological analyses did not reveal lesions compatible with ongoing bacterial, viral, or parasitic disease. Tissue architecture was generally preserved across the examined organs, and no inflammatory, necrotic, degenerative, or pathogen-associated alterations were observed. These findings indicate that, during the sampling period, the examined fish did not show clinical or histopathological evidence of active infectious disease.

Nevertheless, bacteriological investigation documented the presence of *Aeromonas veronii* in fish samples. This result is relevant because *A. veronii* is an opportunistic bacterial species commonly associated with freshwater environments and may become pathogenic under unfavourable conditions, including high temperature, reduced oxygen availability, organic enrichment, or other stressors affecting fish immune competence. In the present study, its detection was not associated with gross or microscopic lesions, suggesting asymptomatic circulation rather than active disease. This finding reinforces the value of combining bacteriology with pathological evaluation, since the detection of potentially relevant bacteria requires interpretation in relation to fish health, environmental context, and tissue-level evidence.

Parasitological findings further supported the absence of severe parasitic disease in the examined specimens. External assessment, gill inspection, and coelomic cavity examination did not reveal widespread ectoparasitic or endoparasitic infections. However, the detection of morphologically identified *Contracaecum rudolphii* L3 larvae in two adult *Cyprinus carpio* specimens represents an ecologically meaningful finding. Although prevalence was low and no associated lesions were observed, the presence of anisakid larvae highlights the importance of parasitological surveillance in freshwater reservoirs. These parasites provide useful information on trophic links involving fish and piscivorous birds and may serve as biological indicators of cross-trophic ecological interactions within the lake.

The macrozoobenthic component contributed additional information on the benthic compartment. The dominance of tolerant taxa, particularly Chironomidae, was consistent with soft-sediment lentic habitats exposed to seasonal organic matter deposition and possible fluctuations in oxygen availability. Although this pattern does not necessarily indicate severe degradation, it suggests that the benthic environment may experience variable or moderately stressed conditions. Macrozoobenthic data therefore complemented the physicochemical and microbiological results, supporting the interpretation of the lake as a system in which sedimentary processes, trophic dynamics, and biological communities are closely connected.

Avifauna monitoring confirmed the exceptional biodiversity value of Lake Biviere di Lentini. The high number of bird records, the presence of approximately 150 species, and the confirmation of breeding species demonstrate that the reservoir functions as a major ornithological hotspot in Sicily. The lake provides feeding, resting, wintering, migratory, and breeding habitats for a wide range of ecological guilds, including waterfowl, herons, egrets, waders, raptors, grebes, storks, cranes, spoonbills, and piscivorous birds. The extensive water surface, reedbeds, riparian vegetation, and availability of fish and aquatic invertebrates contribute to habitat heterogeneity and support both aquatic and terrestrial biodiversity.

The presence of large numbers of piscivorous birds, particularly great cormorants, herons, grebes, and ospreys, is especially relevant within the integrated ecological framework of this thesis. These species may influence fish populations

through predation and may contribute to the maintenance of parasite life cycles involving fish as intermediate or paratenic hosts and birds as definitive hosts. In this sense, the avifaunal data provide an essential interpretative context for the parasitological findings and confirm that Lake Biviere di Lentini should be understood as a cross-trophic ecosystem rather than as an isolated aquatic basin.

Overall, the lake emerges as a biodiversity-rich but environmentally sensitive Mediterranean reservoir, where ecosystem functioning is shaped by the interaction among abiotic drivers, microbial processes, benthic dynamics, fish communities, parasite circulation, and avifaunal use. The present study does not aim to demonstrate direct causal relationships among these compartments. Rather, it documents complementary indicators of lake condition across multiple ecological levels, providing a first integrated framework for interpreting the environmental status and biological functioning of the reservoir.

The multidisciplinary approach adopted in this thesis represents one of the main strengths of the work. Chemical analyses alone would have described Lake Biviere di Lentini as a system with low detectable contamination. However, when chemical data are interpreted together with chlorophyll-*a* dynamics, microbiological indicators, fish health assessment, bacteriological findings, parasitological evidence, macrozoobenthic composition, and avifaunal richness, a more complete picture emerges. The lake appears neither severely degraded nor ecologically static; instead, it is a dynamic

reservoir characterized by high ecological value, strong seasonal variability, and sensitivity to environmental change.

From a management perspective, the results underline the need for long-term, integrated, and seasonal monitoring. Particular attention should be given to trophic dynamics, bloom risk, oxygen conditions, microbial indicators, fish health, opportunistic bacteria, parasite occurrence, benthic communities, and avifaunal trends. Future monitoring should also include additional lake sectors, such as inflow areas, reedbeds, littoral zones, bird roosting sites, and shallow margins, in order to better characterize spatial heterogeneity and identify areas of higher ecological or sanitary relevance.

The ecological importance of Lake Biviere di Lentini is further reinforced by its extensive riparian and reedbed vegetation, which contributes to habitat complexity and supports a wide range of biological communities. The conservation of these habitats should be considered a priority, as they provide shelter, feeding areas, nesting sites, nursery habitats, and ecological connectivity. At the same time, hydrological regulation and water-level management should be carefully balanced with biodiversity conservation, especially under the increasing pressure of Mediterranean climate variability, drought events, and rising temperatures.

In conclusion, this study establishes Lake Biviere di Lentini as a key site for integrated ecosystem-health monitoring in Mediterranean artificial reservoirs. The findings provide a valuable baseline for future research and management, supporting adaptive strategies aimed at safeguarding water quality, fish health, biodiversity, and

ecosystem resilience. Continued multidisciplinary surveillance will be essential to detect early signs of ecological imbalance, understand cross-compartment interactions, and preserve the ecological value of this reservoir in a context of increasing environmental and anthropogenic pressure.

7 FUNDING

This study was developed within the framework of the project “LAGHI VIVI 2.0 – Construction of an Ichthyogenic Centre for the enhancement, conservation and sustainable development of Sicilian lake heritage”, implemented under Measure 2.51 of the PO-FEAMP 2014–2020. The project was funded by the Sicilian Region, Department of Mediterranean Fisheries, and carried out in the area of Lake Biviere di Lentini, Municipality of Lentini, Syracuse, Italy.

The project supported the establishment of an Ichthyogenic Centre aimed at the production and conservation of endemic fish species for the restocking of Sicilian inland waters. In addition to its aquaculture and conservation objectives, the project included an integrated assessment of the environmental and health status of Lake Biviere di Lentini and of its aquatic fauna, with particular attention to the fish species inhabiting the lake.

Beyond its scientific and conservation value, the Ichthyogenic Centre is intended to serve as a research infrastructure for the study and management of Sicilian inland water bodies, as well as a potential driver of local development. The initiative involves the wider territorial district surrounding Lake Biviere di Lentini, including the municipalities of Lentini, Carlentini, Francofonte, Militello, and Scordia, and is expected to contribute to the ecological, productive, and socio-economic enhancement of the area between the provinces of Syracuse and Catania.

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