ORIGINAL ARTICLE

Sampling and laboratory protocols to study the effects of water-level management on the littoral invertebrate fauna in deep and large temperate lakes

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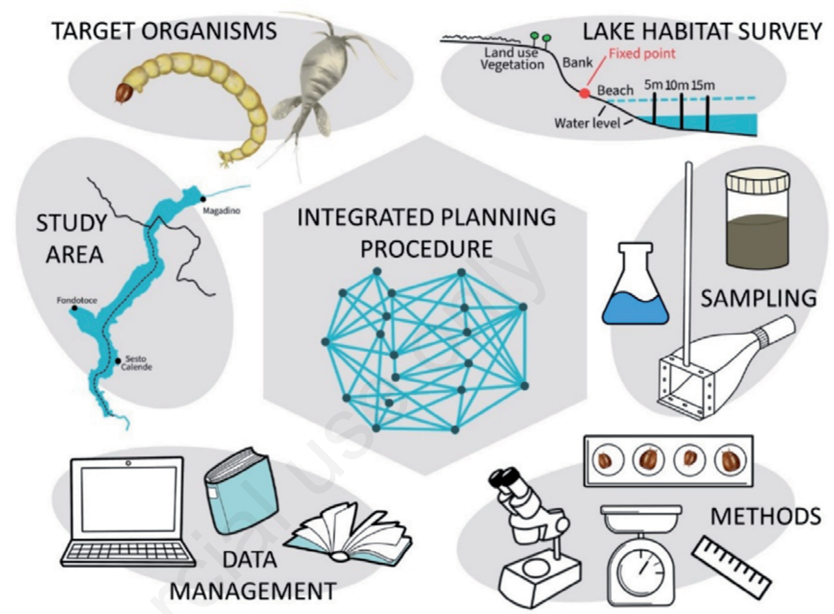
GRAPHICAL ABSTRACT



ABSTRACT

An integrated multidisciplinary protocol on monitoring, sampling, and laboratory procedures was developed and proposed as part of the Project "Parks Verbano Ticino" in the framework of the INTERREG V-A Italy-Switzerland 2014-2020 Cooperation Program. The project's overall goal is to evaluate the effects of water-level management (hydro-morphological stress) on both macro- and meio-fauna along the shores of Lake Maggiore, a large and deep temperate lake in northwestern Italy. Because of their importance in the aquatic food web, determining how this stress affects macro- and meio-faunal assemblages is pivotal. The protocol developed thus includes the evaluation of hydro-morphological impacts via the Lake Habitat Survey method, which entails monitoring human-induced impacts and related infrastructures, followed by an in-depth evaluation of the ecological health of lake habitats via chemical analyses. The protocol then describes the sampling methods for shallow lake waters (i.e., <1.5 m depth) of deep lakes, but it also provides guidance on the best time to sample, how to select sampling sites, and how to allocate sample replicates along transects. A detailed step-by-step laboratory procedure for sample treatment was provided in order to assess the structure of macro- and meio-fauna assemblages, as well as morpho-functional traits (e.g., body shape and size, biomass estimate) in response to water-level management. For the first time, a set of morphological and functional characteristics of macro- and meio-faunal taxa are proposed for comparison. The protocol for standardized trait measurement is intended to be widely used. We also proposed chironomid species-specific length-mass regression models for biomass estimation, which is important for determining the growth rate and secondary production of these taxa in temperate zone lakes. Length-mass equations could shed light on the role of specific species in the flow of energy through aquatic ecosystems. The proposed protocol was evaluated by team members to ensure common utility, accuracy, and repeatability of the procedures towards a feasible application by researchers and stakeholders involved in water management of lakes with similar physical characteristics to use it. The protocol, which has been adapted or simply developed to meet the needs of the Italian context, could be successfully applied to other Alpine and Mediterranean temperate, deep lakes, reservoirs, and other glacial, volcanic, and morainic lakes, as well as to a broader European context.

INTRODUCTION

In view of sustainability, the assessment of environmental and local resources is a critical step in the planning of water resource management. Usually, the complexity of the environment that characterizes an area makes it necessary to deepen the knowledge of the aquatic ecological processes and their monitoring to achieve a comprehensive assessment of the ecosystem health and human well-being. To acquire an adequate knowledge, it is necessary to identify suitable methodologies for detecting ecosystem health.

As is generally known, freshwater is a crucial resource, which provides ecosystem services of pivotal utility to humans (Falkenmark, 1990). Fundamental benefits freely provided by the ecosystems include water supply (agriculture, livestock and industry, hydroelectric power generation), food resources like angling and aquaculture, navigation and water transports. Freshwaters also contribute to the local economy through tourism and recreation activities, exploiting aesthetic and landscape values of the area (European Environment Agency, 2018). Unfortunately, water quality degradation in its various forms (pollution, water abstraction, alien species introduction, *etc.*) is an increasingly common problem (Peters and Meybeck, 2000). In addition, human-induced water level fluctuations (WLFs) have become one of the hydro-morphological pressures that severely threaten the ecological integrity of the ecosystems (Bragg *et al.,* 2003; European Commission *et al.,* 2006; Wantzen *et al.,* 2008; Cardinale *et al.,* 2011) and greatly affect the food web and size structure of the littoral communities in shallow waterbodies (Špoljar *et al*., 2021). Poikane *et al.* (2020) reviewed all biological assessment methods currently applied in Europe that address the impact of hydro-morphological pressures (e.g., lakeshore alteration, and WLFs). Regarding the effect of WLFs, many of them impact on macrophytes and benthic invertebrates (Poikane *et al.,* 2020). Indeed, by implementing natural-based solutions to water-level management (gradual fluctuations more related to natural than anthropic events), we would combine the benefits of flood control, ecological restoration and water supply demands, to the reduction and the mitigation of the adverse effects of more rapid increment or decrement of WLFs as a consequence of coupled late spring-summer agricultural and hydropower water demands (Coops and Hosper, 2002).

An example of a temperate lake under water-level regulation regime is Lake Maggiore (North-Western Italy). Its basin is marked by high annual precipitations (1700 mm yr^{-1} *vs* the national average of about 900 mm yr^{-1}) (Saidi *et al*., 2013). Its very large catchment (compared to the lake area) involves such extreme events bringing significant amounts of water to the lake through the tributaries frequently causing remarkable floods both as quantity and as frequency distribution. At Lake Maggiore outlet (River Ticino), the Miorina dam was built between 1938 and 1942, to regulate the lake outflow and to optimize water supply for industrial, agricultural and hydro-power production purposes.

As a result of the Technical Table established in 2015 to respond to increased water demand by stakeholders to the water resource managers of the regulated Lake Maggiore, the INTERREG Italy-Switzerland Cooperation Program Parks Verbano Ticino Project (from here on PVT) was launched in 2019. The project's specific goal is to develop common implementation strategies for shared and sustainable water management, with a focus on protected natural areas (sites belonging to Natura 2000 network in Italy and sites of the Emerald Network in Switzerland). The PVT project's goal is to improve human well-being by promoting the conservation of these natural protected areas while also encouraging innovative approaches to the area's economic and environmentally sustainable development.

We used macroinvertebrates as bioindicators due to their common use to detect anthropic impacts, diffuse distribution, taxa diversity and abundance, almost sedentary nature, and relatively long-life cycles (Rosenberg and Resh, 1993; O'Connor *et al.*, 2000). Conversely, the use of meiofauna as a biomonitoring tool remained largely unexplored (Höss *et al.*, 2017) notwithstanding it serves, together with macro-invertebrates, as a link between primary and higher trophic levels (Schmid and Schmid-Araya, 2002; Majdi and Traunspurger, 2015; Weber and Traunspurger, 2015), and contribute substantially to benthic energy flow (Bergtold and Traunspurger, 2005; Reiss and Schmid-Araya, 2010).

Specific aim of the paper is to propose an integrated multidisciplinary standard protocol to highlight the significance of increased WLFs stress on different ecological aspects of both macro- and meio-benthic fauna (diversity, abundance, morpho-functional traits). To fulfil this purpose, the original version of the Lake Habitat Survey (LHS) for the hydro-morphological health assessment (Rowan *et al.*, 2006) of the whole lake area was implemented and tailored for the Italian context where many more lake types, anthropogenic impacts and vegetation cover are present (Mor *et al.,* 2022). Indeed, trait-based approaches that consider biological (e.g., maximum body size, number of reproduction cycles per year, types of aquatic stages, feeding habits) and ecological traits (e.g., current velocity and temperature preferences) seem to have particularly strong potential for detecting the impacts of amplified WLFs on lakes and streams (García-Roger *et al.,* 2013; Evtimova and Donohue, 2014). The concept of response/effect traits that determine which traits respond to environmental gradients ("response traits") and which traits affect ecosystem processes ("effect traits") (Suding *et al*., 2008; De Bello *et al.*, 2021) seems to be also very attractive in exploring the WLFs impacts. In that respect, there is a crucial need to develop biological assessment methods responding specifically to WLFs that should be included in routine monitoring programs of lakes (Poikane *et al*., 2020). Then, standard sampling and laboratory methods for the assessment of WLFs stress on the benthic invertebrate fauna of the littoral zone are here proposed. However, little is known about traits of macro- and meio-fauna in Lake Maggiore, and more in general in temperate lakes, and until now the trait-based approach has been applied just to benthic copepods (Cifoni *et al.,* 2021, 2022), which represent one of the most abundant meio-faunal taxa of the lake shorelines. That is why we decided to consider not only taxonomic diversity, but also biological traits such as body size, biomass and types of aquatic stages of macro-fauna as a proxy for stress conditions at the water/sediment interface, and of meio-fauna as a proxy for the stress at the superficial/sub-superficial sediment interface.

This common integrated procedure (hydro-morphological and chemical aspects characterization combined with macro- and meio-fauna taxonomic and functional approaches) goes beyond taxonomy and might provide a more complete assessment of ecological health of any freshwater ecosystem at the surficial/interstitial boundary conditions in relation to WLFs.

METHODS

Study area

Lake Maggiore is one of the largest and deepest subalpine regulated lakes in the southern side of the Alps, located at the confluence of the Alpine Ossola Valley with the Po Plain with particular physical characteristics (Tab. 1). The lake was used as a case study to implement the original version of the Lake Habitat Survey (LHS) method and to develop standard sampling and laboratory methods for assessment of lake-water level stress on the littoral benthic invertebrate fauna.

Tab. 1. Lake Maggiore: main morphometric and hydrological features.

Parameter	Units	
Altitude	m asl	194
Drainage basin area	km^2	6599
Perimeter	km	170
Area	km^2	212.2
Max depth	m	370
Mean depth	m	177.5
Volume	$m^3 x10^6$	37500
Theoretical renewal time	yrs	~ 4

Since 1942, the Miorina dam, built on the River Ticino outlet (Fig. 1), is regulating water levels between -0.50 and +1.00 m threshold, and leaving up to 200 $m^3 s^{-1}$ in the river to agriculture and industry. In the 1960s, the regulation thresholds were changed for the winter period (from November to March) to + 1.50 m, to have greater amounts of available water at the start of agricultural activities and in case of scarce rainfall. The increased frequency of prolonged drought led the decision to rise in 2015, when necessary, the threshold level to +1.35 m, and in the worst cases to +1.50, also during late spring and summer periods.

Monitoring of hydro-morphological and chemical aspects

Since the adoption of the Water Framework Directive 2000/60/EC (WFD; European Commission, 2000) a multidisciplinary approach for the assessment of freshwater ecological status was launched including hydro-morphological and chemical aspects. Morphological aspects such as lake depth variations along with lake level fluctuations, and ground waters/surface waters interactions became pivotal factors in the assessment of ecological quality under eutrophic conditions (Ciampittiello *et al.,* 2017).

The proposed method, derived from the Lake Habitat Survey (Acreman *et al*., 2006a, 2006b; Rowan *et al*., 2006; SNIFFER, 2008), is based on the consideration that a physical environment, such as the lake one, is the result of the natural evolution of the lake over time, and of a series

Fig. 1. Lake Maggiore: sampling sites and habitats (d, dry; w, wet; detailed description within the text); dashed line: border between Italy and Switzerland.

of anthropogenic pressures from human activities along the lake-shores. The method includes equally spaced Habitat Plot Observation Stations (or hab-plots) taking into consideration modified and natural characteristics of the riparian, shore and littoral zones (Fig. 2a). Thus, the method requires to collect, archive and elaborate information on habitat characteristics, human manufactures, and alien vegetal species occurrence, to provide synthetic indices (Lake Habitat Quality Assessment - LHQA, and the Lake Habitat Modification Score - LHMS) for the assessment of hydro-morphological quality of lakes in relation to their ecological quality (MacGoff and Irvine, 2009) . Since its first tuning, the method was adopted in Italy in 2010 and, after a first run, it was tailored to the Italian context introducing wider records of lake origins, anthropogenic pressures, and habitat features. In particular, the origin of the lake such as Fluvial Glacial (FG), Volcanic or pseudo-volcanic Lakes (VL), Landslide Lakes (LL) were included, together with the identification of some regional protection categories (e.g., National protected areas of local interest, law decree 49/95 of the Tuscany Region), anthropogenic pressures (e.g., docks, ports or marinas, piling, dredging, equipped beaches, intensive poplar grove), and habitat feature such as submerged trees, and nuisance vegetal species of the riparian zone or included within the aquatic vegetation. The results of LHQA and LHMS indices (taking into account all natural and artificial features, and also the anthropogenic infrastructures present in the Italian lakes), were integrated and the original Access database was converted into a more-easy-to-use-and-handle excel one. To the scope of our study, we evaluated LHMS and LHQA by scoring all the features mentioned in Tab. 2, considering different hab-plots and water-level fluctuations of different amplitudes.

The field form to compile include information that is easy to obtain and, at the same time, easy to insert in the excel database. Finally, the present database includes information and allowed not only a whole lake approach, but also the assessment of several specific hab-plots distributed along the lake shores.

Chemical analyses were performed on water samples taken with 0.5 L polyethylene bottles at the lake surface and preserved at dark at 4°C until laboratory analyses. At the same time and in the same places, water temperature was measured by a digital thermometer. Conductivity, pH, alkalinity, chloride, sulphate, and major nutrients were measured according to standard methods for freshwater samples (https://www.idrolab.irsa.cnr.it/en/analytical-methods; APAT IRSA-CNR, 2003). Both internal and external quality controls were performed.

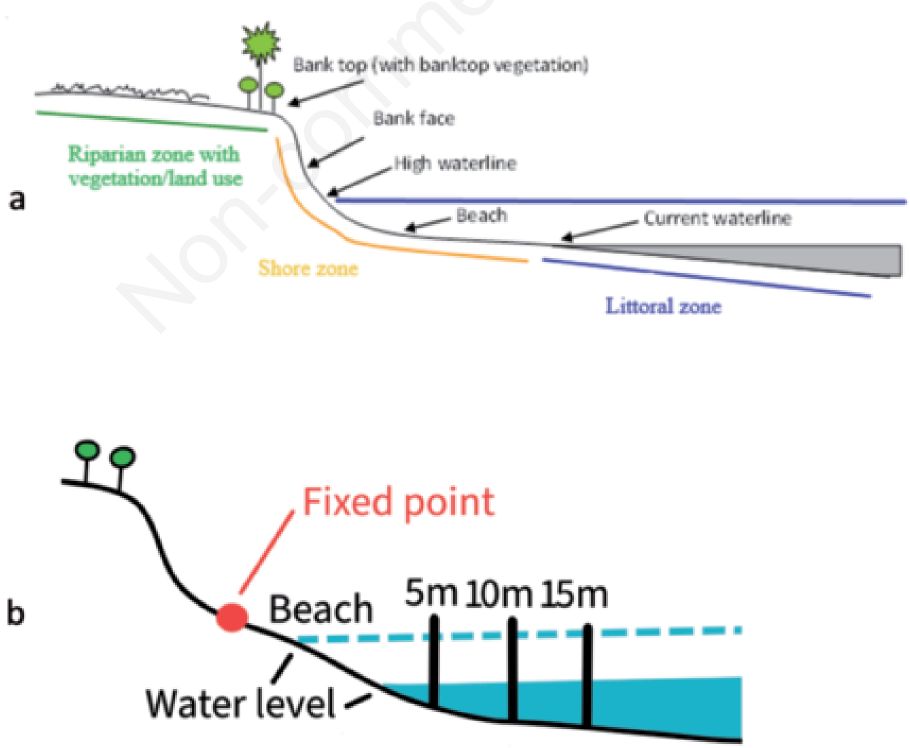


Fig. 2. a) Schematic section of Hab-plot zonation (redrawn by Rowan *et al.*, 2004). b) Schema of replicates distribution at fixed distances from the shore in two different conditions (dry – only at low water level, and wet – permanently under water). See more details within the text.

Tab. 2. Features scored in Lake Habitat Modification Score (LHMS) and in Lake Habitat Quality Assessment (LHQA) for Lake Maggiore.

LHMS
Shore modification
Max value among the following shore modifications: • Poaching • Impoundments • Hard bank engineering closed • Hard bank engineering open • Soft engineering • Flow and sediment control • Piled structures • Floating and tethered structures • High density of moorings • Outfalls and intakes • Sediment extraction • Recreational beaches
Shore uses
Sum of the following shore uses: • Flood embankment • Land claim • Commercial • Residential • Roads or railways • Unsealed tracks and footpaths • Parks, gardens • Camping and caravans • Walls, dykes, revetments • Docks, marinas, jetties • Flow sediment control structures • Commercial activities • Educational recreation • Quarrying or mining • Coniferous plantations • Tillend land • Orchard • Pasture • Litter, dump, landfill • Recreational beaches
In-lake pressure
Total recreational pressures
Hydrology
Main water use plus annual flux
Max value among the following characteristics: • Raise/decrease of lake level • Dam presence (no fish pass) • Number of infrastructures • Any upstream impoundment
Sediment
Sedimentation
Number of plots with sedimentation
% Shore erosion
% Delta deposition
Vegetal nuisance species
Total nuisance species
Riparian nuisance species
Littoral nuisance species

LHQA
Riparian score
Final riparian score of the sum of the following scores: • Final score of vegetation complexity • Final score of vegetation longevity • Final score of natural land cover types • Final score of the number of natural cover types • Final score of the number of bank top features
Shore score
Sum of the following final shore score: • Final score of bank material and bank height • Sum of final score of habitat diversity trash • Final score of bank naturalness
Max value of final score of number of natural bank materials: • Max value of the final score of beach naturalness • Final number of natural beach material types
Littoral final score
Sum of the following final scores: • Final score of the coefficient of variation of water depth at the observation point and of its distance from the shore • Final score of extent of natural substrate • Final number of natural littoral substrate types • Final score of average macrophytes cover • Final score of extent of macrophytes lakewards • Final number of macrophytes cover types • Final score of average of littoral habitat features • Final score of number of features
Final score whole lake
Final score of specific hab features
Final score of number of islands
Final score of deltas deposit features

Biological sampling design

Sampling design (location, frequency, sampling equipment, number of samples) has been elaborated mainly to highlight possible assemblage modifications associated with low/high water level fluctuations due to water-level management at the Miorina dam.

Although macro- and meio-fauna can colonize any type of substrate, including artificial ones, in this study we focused on sandy substrates, common and frequent littoral substrate in the lake where both macro- and meio-fauna are easily found (Atilla and Fleeger, 2000; McLachlan and Defeo, 2018). In particular, meiofauna are mostly found in and on soft sediments (Giere, 2009). Therefore, the sampling sites and stations were selected considering i) sand sediment composition, ii) position along the lake shores, iii) slope of the beaches, and iv) water-level fluctuations of different amplitude representing the northern, central and the southern parts of the lake. All of them undergo different water level amplitude during water-level regulation period (from March to September – usually corresponding to very high and low level conditions, respectively). At each site (Fig. 1), two sampling stations featuring two different habitat types, were designated, one partially or totally dry during the period of minimum water level (dry), and the second one permanently immersed and entirely under water all year round (wet) (Fig. 2a). The sampling periods were chosen by following the lake level trends at the hydrometer of Verbania local station. Daily lake levels were also recorded and archived for further statistical analysis. Sampling was performed during the period of water-level management (from March to September), following a standardized protocol that allows comparing the three littoral sampling sites and their habitats (dry and wet).

The effects of water-level management were then studied by analysing eight main bio-ecological parameters:

i) taxonomic: richness, density and abundance of each taxon (both for macro- and meio-fauna);

ii) functional: biomass and body length (evaluated only for Diptera chironomids - macro-fauna, and for Copepoda - meio-fauna, taken as representative taxa of the littoral lake area) body shape (for meio-fauna), age-size classes and sexual gender (for Crustacea Copepoda, only).

Macro-fauna was semi-quantitatively sampled through a handled net (24 x 24 cm - 576 cm^2) equipped with a 250 µm opening mesh net (Boggero *et al.,* 2011), while meio-fauna through a handled net equipped with a 60 µm opening mesh net (Malard *et al*., 2002). Both handled nets were equipped with a bottle to trap the collected organisms (Fig. 3). Macro-fauna samples were taken kicking the first 5 cm of sediments for 1 min (Frost *et al*., 1971): to ensure the capture of even the most mobile individuals, the net was passed several times through the water column above the sampled area. Concurrently, in a very close area, meiofauna semi-quantitative samples were taken following the methods in Cifoni *et al.* (2022) that involves disturbing an area of about 625 cm^2 by foot, up to 7 cm in-depth, for 30 sec (Ausden, 1997), following the methods in Pozojević *et al.* (2019). To avoid filtering the water column, the net was quickly dragged over the disturbed area and closed at the opening before being withdrawn to the surface.

Once collected, the biological samples were fixed with 80% ethanol and bottled for further laboratory analysis (sorting, identification to the minimum taxonomic resolution level - genus or species, photography under a microscope or stereoscope, measurements of length, width and body weight, counting of individuals, biomass estimation) (Fig. 4).

Fig. 3. Handled net, field thermometer, bottle and funnel useful for collecting and treating macro-fauna samples in the field (left). Surber net for collecting meio-fauna samples and filtering procedure (right).

At each sampling site and in each habitat (dry and wet), three spatial replicates were taken along a cross-shore transect to a maximum depth of 1.2 m. The replicates were taken at fixed distances from the shore (both in wet and dry habitats) subjected to different water-levels depending on the local weather-climatic conditions, on the thawing period, on the beach slope, and on the water regulation management towards the Po Valley. The distances of the three spatial replicates from the shore varied among 3-5, 10, and 20-25 m, taking as a fixed point a plant, a trunk, or other stable objects on the shore (Fig. 2b). According to the local conditions, the definition of the fixed points and the protocol can be adapted either to small and shallow lakes (including a variation in the distance from the fixed points).

Protocol for the analysis of macroinvertebrates

Collected macroinvertebrate samples were transferred to the laboratory for subsequent analyses.

i) *Macroinvertebrates sorting*:

The samples were completely analysed under a stereomicroscope (Zeiss Stemi 2000, magnification: 80×) to ensure the collection of all organisms.

ii) *Measurement of linear dimensions*:

Guidelines for linear dimensions measurement (expressed in mm) of different macroinvertebrate ranks are presented in Tab. 3 (modified by Sangiorgio *et al.,* 2009). We focused mainly on Diptera chironomids because the

Tab. 3. Linear dimensions (L) for large macroinvertebrates taxon ranks (modified from Sangiorgio *et al.*, 2009; with permission). Only Insecta Diptera dimensions were considered in the present paper. The remaining information is provided for completeness.

Phylum/Classe	Order	Linear dimension (L) expressed in mm	Dimension code
Insecta	Diptera	Total length (from the anterior edge of the head to the last abdominal segment)	TL (BL+HL)
		Body length (from the rear edge of the head to the last abdominal segment)	BL
		Head length	HL
Insecta	Coleoptera	Total length (from the anterior edge of the head to the last abdominal segment)	TL
Insecta	Ephemeroptera Plecoptera Odonata	Total length (from the anterior edge of the head to the last abdominal segment excluding antennae and appendages)	TL
Insecta	Hemiptera	Total length (from the anterior edge of the head to the last abdominal segment excluding antennae)	TL
Bivalvia		Major axis of the valves	SL
		Minor axis of the valves	SH
Crustacea	Amphipoda/Isopoda	Body without antennae and uropods	TL
Crustacea	Decapoda	Body without antennae	TL

TL, total body length; SL, length or major axis of the shells; SH, height or minor axis of the shells; BL, body length; HL, head length.

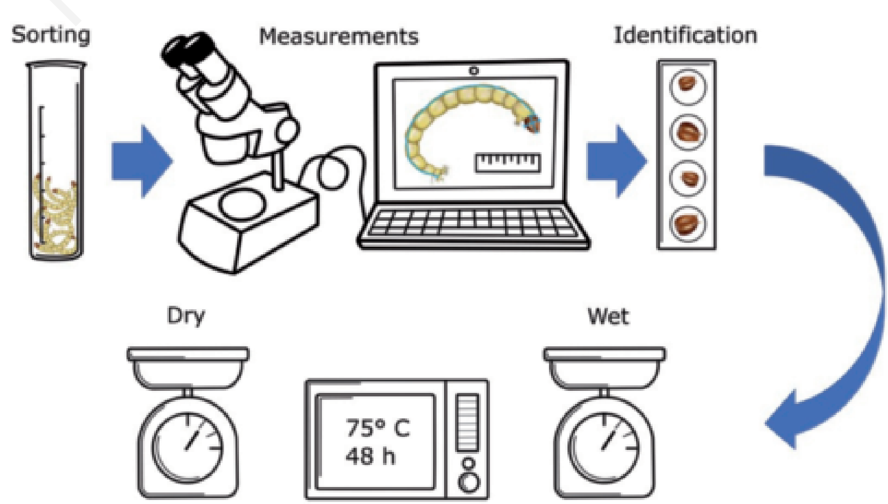


Fig. 4. Scheme of laboratory procedures for the determination of the size structure and biomass of Diptera Chironomidae.

other taxa were very poorly represented in lakes and therefore it was not possible to obtain robust data for subsequent statistical analyses.

- The Diptera chironomids individuals were placed in a Petri dish and each of them photographed with OPTIKA integrated camera on stereomicroscope.
- The image was subsequently processed with the ImageJ Software (imagej.nih.gov) to measure some biometric parameters (e.g., body length, head width and length - Schütz and Füreder, 2018). Only undamaged individuals were measured.

iii) *Taxonomic identification*:

Specimens of oligochaetes and chironomids were identified to species level under a microscope (Zeiss Axiolab - maximum magnification 1000×) on slides using Faure as mounting medium for temporary mounts. Ephemeroptera, Plecoptera, Trichoptera, Odonata, Coleoptera, Mollusca (Gastropoda and Bivalvia) and Hirudinea were identified to species level under a stereomicroscope (Zeiss Stemi 2000, magnification 50×). Taxa specific keys were used to identify species (AA.VV., 1977-1985; Wiederholm, 1983; Timm, 2009).

Chironomid larvae were dissected to separate the head capsule, which contains the main structures used in taxonomic identification, from the abdomen. The head capsule was mounted ventrally on a slide using water as mounting medium. Once the organism had been identified, the head capsule was removed from the slide and used for biomass determination (Fig. 4).

iv) *Density estimation*:

- After counting specimens belonging to different taxa, the total number of individuals at each sampling station of each site was obtained. The values obtained from each replicate were then used to calculate the average density, expressed as individuals per square meter (ind m^{-2}). The area of each replicate is 576 cm^2 (24 x 24 cm).
- The total number of individuals for each replicate was compared to 1 m^2 (10,000 cm^2) according to the following proportion:

$$\frac{N}{576} = \frac{D}{10000} \qquad \text{(eq. 1)}$$

from which: $D = \frac{N \times 10000}{576}$
hence: $D = N \times 17.36$
where: D = density (ind m^{-2})
N = total number of individuals per replicate of each site.

v) Biomass estimate of chironomids:

- Chironomids were chosen as reference organisms for biomass estimate by direct weighing, dry mass and length-mass regression.
- Determination of wet weight (WW in mg): individuals were blotted on filter paper, and subsequently weighed using a Mettler Toledo XP105DR balance (x ± 0.01 mg).
- Determination of dry weight (DW in mg) as a proxy of biomass: after taxonomic identification, each individual was placed in aluminium crucible of known weight. Then, crucibles were placed in a Heraeus thermostat-controlled oven at 75°C for 48 h. This temperature allows complete drying without alterations in the organic matter content of invertebrates (Stoffels *et al*., 2003). The dry weight of each specimen was obtained by subtracting the weight of the empty crucible from the total weight obtained with a Sartorius micrometric balance (x ± 0.001 mg).
- The biomass was calculated also indirectly through a length-weight relationship: dry weight of chironomids as a power function of a total body length (TL). This allometric weight-length relationship is presented as a power function of the form:

$$DW = a\,TL^b \qquad \text{(eq. 2)}$$

where DW is the dry weight (the response variable), expressed in mg
TL is the total body length (the predictor variable), expressed in mm
a is the DW-intercept of the regression equation
b is the slope of the line (or the increase of the weight)

By using a logarithmic transformation (3), equation 2 was converted to a linear form (4)

$$lnDW = lna + b[lnTL] \qquad \text{(eq. 3)}$$
$$DW = a + bTL \qquad \text{(eq. 4)}$$

Protocol for the analysis of benthic meiofauna

Meiofaunal samples collected in the field were then transferred to the laboratory and stored for further analyses.

i) *Sorting of microinvertebrates:*

The semi-quantitative samples were sorted under a stereomicroscope (Leica M80, magnification 16), without stain addition, and standardized by picking up 150 meiobenthic animals using a glass pipette, based on a preliminary sampling survey that aimed at maximizing taxa richness at the class/order level (Metzeling and Miller, 2001). The standardization was such to capture the whole expected benthic copepod biodiversity along the lake shorelines (Cifoni *et al.,* 2022).

ii) *Taxonomic approach:*

The individuals were then identified to the lowest possible taxonomic resolution based on updated literature. Copepoda were the dominant taxon in terms of abundances and were chosen as reference group, identified to species level after dissection under a stereomicroscope (Leica M80, magnification 40×) and mounted on slides using glycerin as medium. Copepoda specific keys were used to identify species: e.g. Kiefer (1960), Dussart and Defaye (2001, 2006). The sex and ontogenetic stage (adults, copepodites and nauplii) of each specimen of Copepoda were also recorded on a dedicated form for subsequent analyses on assemblage structure. The remaining taxa were identified to class/order levels (e.g., nematodes, rotifers, tardigrades, oligochaetes, bivalves, mites, ostracods, and early larval stages of chironomids and ephemeropterans - these latter being considered "temporary meiofauna", according to Giere (2009)).

iii) *Functional approach:*

Afterward, five functional traits (size, body form, locomotion and substrate relation, diet and feeding habits), described by the modalities reported in Cifoni *et al.* (2022), were measured for each meiofaunal specimen (Tab. 4). The trait profiles were described at the species levels for the dominant taxon (Copepoda) and using the mean trait profiles of the class/order in the corresponding biogeographic region for the remaining taxa.

Tab. 4. Functional traits (in bold) and modalities (normal font) of the meiofaunal taxa.

Maximal potential size (for copepods only)
• ≤0.25 mm
• 0.26–0.50 mm
• 0.51–1.00 mm
• 1.01–2.00 mm
• >2.00 mm
Body form
• Streamlined
• Flattened
• Cylindrical (+ geometric)
• Spherical
Locomotion and substrate relation
• Swimmer
• Crawler
• Burrower (epibenthic)
• Interstitial (endobenthic)
• Attached (temporarily or permanently)
Diet
• Fine sediment + microorganisms
• Fine detritus (<1 mm)
• Dead plants (>1 mm)
• Living microphytes
• Living macrophytes
• Dead animal (>1 mm)
• Living microinvertebrates
• Living macroinvertebrates (+ vertebrates)
Feeding habits
• Deposit-feeder (+ absorber)
• ShredderScraper
• Filter-feeder
• Piercer (plants or animals)
• Predator (carver/engulfer/swallower)
• Parasite

iv) *Body size measurements:*

a) At the stereomicroscope, copepods were photographed under a stereomicroscope with an integrated camera and the images were captured using the LAS software (Leica Application Suite, version 4.7.1 of Leica Microsystems, Wetzlar, Germany).

b) The images were subsequently processed with the ImageJ software, to measure body length and body width considering the maximum body extensions excluding appendages.

v) *Biomass estimates:*

The dry carbon biomass of the dominant taxon (Copepoda) was estimated as follows: body dimensions (see above) were then converted to biovolume using the equation in Reiss and Schmidt-Araya (2008) (Tab. 5). First, meiofaunal body volume was converted into individual fresh weight assuming a specific gravity of 1.1. The dry/wet weight ratio was assumed to be 0.25. The individual dry carbon content was estimated as 40% of the dry weight.

POTENTIAL RE-USE OF THE INTEGRATED MULTIDISCIPLINARY PROTOCOL

Here, an integrated multidisciplinary standard method (LHS application, chemical conditions and macro- and meio-faunal analyses) to assess the stress of water-level management on the littoral areas of temperate large lakes, is proposed to highlight mainly the WLFs effects on the littoral assemblage structure, biodiversity, and morpho-functional traits (body shape and volume, biomass, body size) (for results of application see this Special Issue Boggero *et al.*, 2022).

These integrated multidisciplinary aspects allowed to better understand the functioning of lakes and interpret their ecological conditions, and the relationships among hydro-morphological parameters, habitat naturalness, human impacts and biological elements (in our case, macro- and

Tab. 5. Taxon-specific equations with factors to convert body dimensions (in mm) into bioVolumes (V in mL or nL) following Reiss and Schmid-Araya (2008).

Taxon	BioVolume (V)
Copepoda Harpacticoida	$V (nL) = L \times W^2 \times 490$
Copepoda Cyclopoida	$V (nL) = L \times W^2 \times 560$
Copepoda nauplii	$V (mL) = (L \times W^2 \times \Pi)/6$

L, length; W, width.

meio-fauna) useful to put into action the best water-management practices or to modify the existing ones. The originality of this approach stands precisely in collating different lake health assessments (approaches never applied before to stress the impact of WLFs on the littoral benthic biota) to achieve a more robust single end. This method, developed to relate the hydro-morphological and chemical condition of a lake with its littoral benthic invertebrate fauna, could also be useful to set a base for future studies allowing the implementation of the WFD when considering hydro-morphology as pressure.

The hydro-morphological aspects related to lake water bodies, habitats and human manufactures along the banks/shores, are strongly related to the ecological quality of lake ecosystems (Strayer and Findlay, 2010; Zaupa *et al.,* 2017). Therefore, the ecological quality assessment is a crucial step for the implementation of sustainable environmental management. Having an official method tailored to the Italian context, but comparable with other European methods is thus extremely useful and effective for the management of lake ecosystems (Poikane *et al*., 2020). The method has been adapted to the Alpine and Mediterranean temperate areas where a wide range of human-induced changes in morphological and hydrological quality occurred, and habitats and biocoenosis are more diverse. Then, the conversion of the original access to an easy-to-manage excel database of LHS provides an unchallenging way to easily understand and improve the assessment of ecological health of the system. Subsequently, a higher control on the calculation of the LHQA and LHMS indices makes the implemented method even more attractive and effective. Furthermore, the possibility of managing directly the data structure increases the understanding of the method itself, and its wider use allows to gain further knowledge of its potential benefits and limitations.

Then, macro-fauna assemblage structure analysis, such as biomass and diversity, are included within the WFD. However, weight-length equations for the most common species of chironomids and algorithms to estimate their body mass are here developed and provided for the first time for a temperate and regulated large lake like Lake Maggiore. Although several studies on this issue were carried out in various lakes in the Northern hemisphere and in the tropics (Towers *et al*., 1994; Stoffels *et al*., 2003; Schütz and Füreder, 2018), their results were not applicable for Lake Maggiore as a temperate one, with highly human-induced water stress and with pronounced increase of water temperature (Rogora *et al*., 2021).

Furthermore, for the first time, meiofauna, macrofauna and the abiotic conditions of lakes are integrated using a mixed approach involving the taxonomy and functionality of the meiofaunal assemblages. The trait protocols are designed for standardized measurement of traits to ease their widespread use. Carbon biomass for the dominant meiofaunal taxon (Copepoda) was also quantified (Cifoni *et al*., 2022).

These methods, adapted or just developed in line with the needs of the Italian context, may be successfully applied to other Alpine and Mediterranean, temperate, deep lakes, reservoirs and other glacial, volcanic and morainic lakes, but also to a wider European context.

CONCLUSIONS

The present standardised protocol aims to propose an integrated and multidisciplinary approach, never thought and exploited before to detect the effects of WLFs (one specific pressure of LHS application) on the littoral benthic invertebrate biota. The synchronous collection of biological information and hydro-morphological data, together with chemical monitoring, has become increasingly important to establish quantitative relationships between physical-chemical and hydro-morphological pressures and biological indicators (Boggero *et al*., 2022 in press).

Therefore, an integrated and multidisciplinary approach including an accurately planned sampling of biological elements and application of hydro-morphological methods and monitoring of physical-chemical conditions may be the best approach for detecting the effects of regulated water-level fluctuations. Moreover, an in-depth analysis of biological elements considering not only their spatial distribution, or their assemblage structure but also their functional traits may also allow to find specific relationships with the environmental parameters to better understand to what extent hydro-morphology in concomitance with chemical quality could trigger changes in the biota. Assemblage structure and functional traits analyses are important in defining causal relationships between pressures, impacts and biological elements. Highlighting the contextuality of natural or unnatural relationships is the basis of every future action of requalification, management and protection of lake ecosystems, highly valued for their recreational, aesthetic, and water-supply qualities.

All the aspects here proposed would require more in-depth analysis for a sound establishment of methodology to quantify the ecological health of water bodies with respect to WLFs, especially in the today's global water crisis, to fully harmonize the methodologies adopted.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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