



OneAquaHealth 

PROTECTING URBAN AQUATIC ECOSYSTEMS TO PROMOTE ONE HEALTH

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OneAquaHealth Key Indicators of Ecosystem and Biological Health – Factsheets collection

Dirk Schmeller, Ana R. Calapez, Janine P. Silva, Ana Cláudia Norte, Sónia R. Q. Serra, Marcos Dias, Gabriel T. Silva, Rayan Bouchali, Adeline Loyau, Salomé F. P. Almeida, Rafael Schmitt, António Chen, Jaime A. Ramos, Maria João Feio



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Introduction

The OneAquaHealth project aims to identify and refine indicators at both ecosystem and biological levels that can reflect the potential risks to human and animal health originating from degraded freshwater ecosystems in urban areas. These indicators are essential for understanding how urbanization impacts ecosystem functions and the dissemination of health hazards, aligning with the broader concepts of One Health, EcoHealth and Planetary Health. Their monitoring can provide early warning signals of ecosystem degradation and potential disease emergence that may threaten human health in urban environments.

For each selected indicator, a factsheet has been developed outlining its rationale, sampling methods, metrics, and potential data sources. This collection supports harmonized monitoring approaches and interdisciplinary research linking environmental quality, biodiversity, and public health in urban aquatic systems. These factsheets are intended to inform decision-makers and other stakeholders working in public health, sustainable cities, nature conservation and environmental education.

Key indicators included in this collection:

- I. Diatoms and Diatom Teratology
- II. Benthic Macroinvertebrates
- III. Fish
- IV. Amphibians
- V. Birds
- VI. Microbial Diversity
- VII. Fecal Coliforms
- VIII. Pathogens
- IX. Antibiotic Resistance Genes
- X. Diptera Adults
- XI. Invasive Alien Plants of the Riparian Corridor



I. Diatoms and diatom teratology

Rational

Diatoms are ubiquitous in aquatic environments and a key component of river ecosystems, contributing to primary productivity, nutrient cycling and oxygenation.

Pollutants such as heavy metals, pharmaceuticals and others may cause deformities in the frustules (valves – external silicate walls) – teratologies, which reflect diatom health.

Certain diatom species can form harmful algal blooms (HABs) in freshwater environments. These blooms can produce toxins and/or deplete oxygen levels in the water, harm aquatic organisms, leading to fish kills and posing risks to human health through contaminated water or food consumption. These algal blooms can proliferate with water pollution through human activities, such as nutrient runoff from agriculture, urban development, and industrial activities.

How is the indicator measured?

Periphytic diatoms are scraped from the surface of submerged stones/substrate. They can also be collected from the sediment or from the surface of aquatic plants.

Samples are cleaned in the laboratory using nitric acid and potassium dichromate at room temperature for 24h (European Committee for Standardisation, 2003) to remove organic content. Permanent slides are prepared using Naphrax®. About 400 diatom valves are identified and counted per sample, following the European standard (European Committee for Standardisation, 2004).

The identification is performed under a microscope to species level based in their morphology. Deformities in the morphology of the valves should also be registered and counted. Samples can also be identified through metagenomics.

Common biotic indices used to reach the biological quality status are the Biological Diatom Index (IBD) (Coste et al. 2009), and the Indice de Polluosensibilité (IPS) (Coste in Cemagref, 1982). In the European Union each member-state adopted their own index, and reference values for the different types of rivers must be considered in the evaluation.

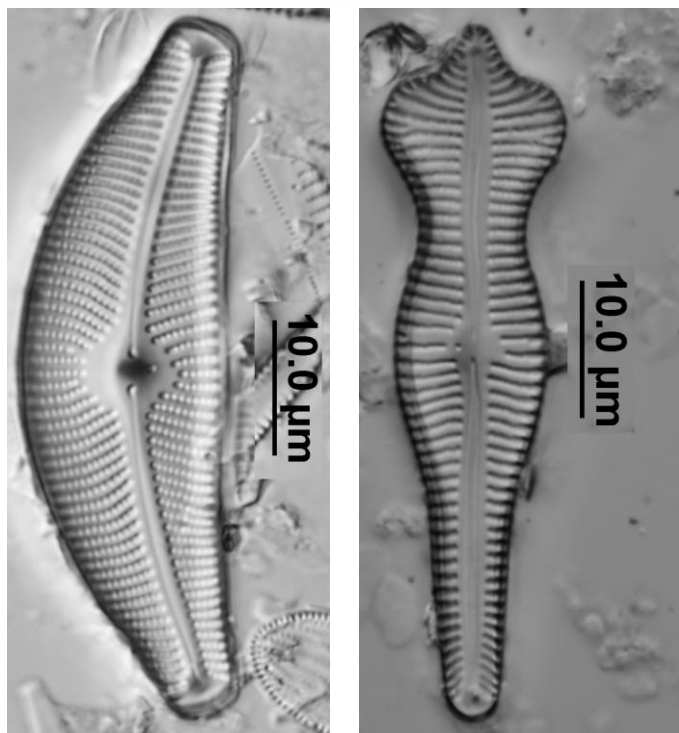
Importance of indicator

They are widely used as bioindicators of water quality, presenting high sensitivity to environmental changes, organic pollution, and eutrophication. Induce selection pressure, change in abundances, diversity loss, and increase mortality rate.

They are among the biological quality elements used in the assessment of the ecological quality status of rivers, according to the European Water Framework Directive (2000).

The alteration in the structure of the aquatic communities indicates alteration in ecosystem functioning and ecosystem services.

Representation of a diatom



Photos: S. F. P. Almeida



II. Benthic Macroinvertebrates

Rational

Macroinvertebrates are ubiquitous in rivers and include small aquatic organisms such as insect larvae, crustaceans, worms and molluscs. They are key biological quality elements under the Water Framework Directive, widely used as sensitive indicators of freshwater ecosystem health. Because they have limited mobility, relatively long life cycles, and variable tolerance to pollution, changes in their communities reflect cumulative environmental conditions. They also play a key role as links between primary producers (e.g. algae) and higher trophic levels (e.g. fish, birds), directly influencing the functioning and stability of ecosystem food chains.

Healthy macroinvertebrate communities indicate clean water and functioning ecological processes, which are directly linked to human and animal health by supporting safe drinking water sources, fisheries and disease-limiting environments.

Importance of indicator

Macroinvertebrates community structure reveals patterns of water quality, habitat integrity and ecosystem functioning, including long-term exposure to pollutants, physical disturbances, and changes in biodiversity.

Regular monitoring help identify trends in ecosystem degradation or recovery, assess restoration efforts, monitoring climate changes effects and detect early signals of emerging environmental stressors and health risks.

Their use is highly relevant for environmental policy, supporting regulatory frameworks (e.g. Water Framework Directive), providing solid biological evidence for environmental protection decisions, guiding water management for human consumption, recreation, and fisheries, and enhancing public health by identifying ecosystems at risk of contamination or conditions favourable to disease.

How is the indicator measured?

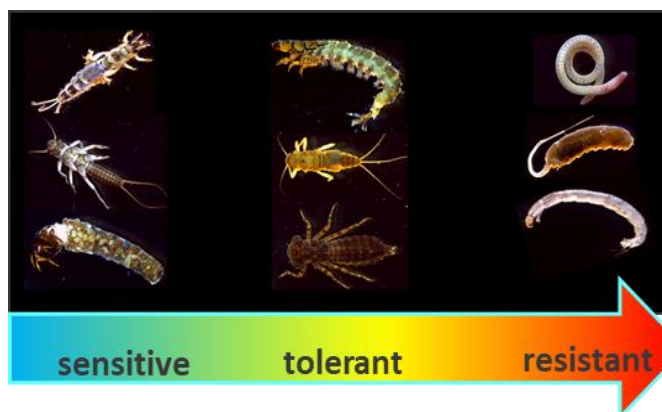
Macroinvertebrates assemblages are collected in streams with a hand-net (500 µm mesh size, 0.25 × 0.25 m opening) by kick sampling covering six sub-samples (× 1 m length) allocated across habitat types (organic and inorganic) according to their relative availability within the reach. Collected organisms are preserved in ethanol and later processed in the laboratory, where they are sorted, identified under a stereomicroscope and counted. From these data, metrics such as taxa richness, diversity and biotic scores (e.g. BMWP) are calculated to reflect the community's sensitivity or tolerance to pollution.



Taxonomic identification

Sampling by kick and sweep upstream sediment by foot with a hand-net

Biotic indices scores are assigned to taxa based on their tolerance to pollution



III. Fish

Rational

Fish are widespread and an essential component of aquatic ecosystems. As biological quality elements under the Water Framework Directive, they are effective indicators of ecosystem and biological health.

Changes in fish presence, diversity, and health reveal water quality, habitat integrity, and general ecological functioning, providing reliable information for assessing the status of water bodies. Their monitoring is also relevant for human and animal health: fish are important food sources for humans and wildlife, can accumulate pollutants such as heavy metals and pesticides, and can be vectors and reservoirs of pathogens transmissible across species including to humans. For these reasons, fish monitoring supports both ecological assessment and public health vigilance, ensuring cleaner, safer and more resilient freshwater systems.

How is the indicator measured?

Fish communities in wadeable streams are assessed using standardized field sampling electrofishing (single-pass using backpack battery-powered electrofishing gear). A sampling reach is selected (minimum of 100 m), ideally containing a representative mix of habitats such as riffles, runs, and pools. The operator walks upstream through the entire reach, ensuring all habitats are sampled evenly. Captured fish are identified to the species level, counted and measured, with data recorded on species richness and abundance (number of individuals per species captured within the 100m reach or per time effort – CPUE, catch per unit effort). Native species are returned alive to the stream at the end of sampling. Exotic or invasive species are removed according to local regulations.

Common indices used to assess biological quality include the Fish-based Index of Biotic Integrity, or the European Fish Index, alongside diversity metrics and species richness.



Electrofishing sampling to catch stream ichthyofauna

Importance of indicator

Freshwater fish respond consistently to cumulative and long-term environmental conditions, reflecting changes in water chemistry, pollution, hydrology, landscape features, habitat quality, climate impacts and overall ecosystem health. Regular monitoring reveals spatial and temporal trends that help identify degradation or recovery, assess restoration efforts and detect early signals of ecological stress and health risks.

The results support environmental policy, including the Water Framework Directive and guiding management actions, contributing to the protection of human and animal health by monitoring pollutant accumulation, pathogen risks and overall freshwater ecosystem resilience.

$$H = - \sum_{i=1}^s p_i \ln p_i$$

where H is the Shannon-Weaver diversity index, s is the total number of species, p_i is the proportion of all individuals in the sample that belongs to species i , and $\ln$ is the natural logarithm.

$H' < 1$: Very low diversity; often associated with highly disturbed or degraded streams.

H' between 1 and 2: Moderate diversity; indicates some ecological stress.

H' between 2 and 3: Good diversity; relatively healthy ecosystem.

$H' > 3$: High diversity; near-pristine or minimally disturbed conditions.



European eel (*Anguilla anguilla*), a critically endangered species of high ecological, cultural and economic value, traditionally consumed by humans.



Some fish species found in urban streams sampled by electrofishing



Achondrostoma oligolepis



Luciobarbus bocagei



Gobio lozanoi



Gasterosteus aculeatus

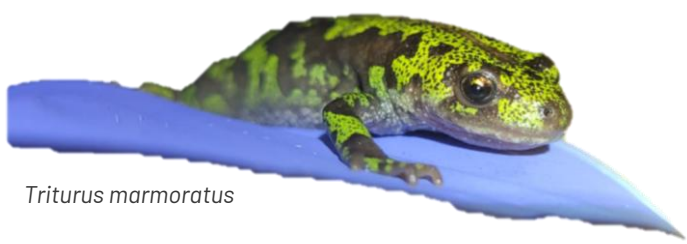


IV. Amphibians

Rational

Amphibians are vital vertebrates whose dependence on aquatic ecosystems for reproduction makes them vulnerable to environmental changes. Their physiological sensitivity, especially their permeable skin, allows them to function as bioindicators of ecosystem health, providing early warnings of environmental contamination.

Amphibians also deliver important ecosystem services by controlling pest populations, such as mosquitos and thereby reducing the transmission of mosquito-borne diseases, serving as a food source for other wildlife, and facilitating nutrient cycling between terrestrial and aquatic ecosystems.



Triturus marmoratus

Importance of indicator

Urbanization seems to influence the diversity, population, and disease dynamics of amphibians.

Habitat loss and water contamination are extensively documented causes of amphibian population declines.

The number of endemic and vulnerable species is associated to a better ecological condition of streams while more degraded streams may be a source of several pathogens affecting amphibians.

When habitats are degraded or fragmented, amphibian populations become isolated and stressed. This stress can weaken their immune systems, making them more susceptible to pathogens such as *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal), which are responsible for chytridiomycosis, a devastating skin disease. Ranaviruses might also more easily infect stressed individuals, including fish and amphibians.

How is the indicator measured?

Amphibians are sampled with a hand net (mesh size 3-5mm), by wading a total length of 100 meters in each stream, covering various habitat types (i.e., lentic and lotic zones). Along each transect, amphibians (adults, larvae and eggs) are captured, identified and counted.

The body condition of the animals is assessed through their weight and size (using a calliper). In addition, buccal and skin swabbing may be conducted. The swabs are placed in a sterile, labelled tube and stored on ice for later analysis for Ranaviruses, the chytrid fungi *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, and the protective skin microbiome.

All animals are immediately released. Standardised biosecurity and sanitation procedures (<https://shorturl.at/ftJuP>) should be implemented between each individual sampled and across various sampling sites. Nitrile gloves should be used to handle the animals, and callipers and weigh pans must be thoroughly wiped down with 70% ethanol and rinsed with distilled water after each use.



Mind that all amphibians are protected and for handling and capturing them in the field, you need special permits from your national authorities.

Photos of amphibian surveys



Photos: C. Vigileos and G.T. Silva



V. Birds

Rational

A healthy ecosystem harbours a diverse and balanced community of birds, including more sensitive bird species like insectivorous birds.

These birds are benefitted by the presence of orchards, small woody features, scrubs and shrubs, hedgerows and other boundaries that provide resources and constitute connectivity network in the landscape.

How is the indicator measured?

This indicator is based on bird censuses (point counts) through the detection of their sounds (songs and calls).

Bird censuses should be performed at the early hours of the day (up to three hours after sunrise), during 10 minutes, at each location under evaluation, using the mobile app Merlin (Cornell Lab Lab of Ornithology, Cornell University) or similar, for those with no experience in ornithology.

The bird species detected are classified according to its feeding guild following Wilman et al. (2014), and the number of insectivorous bird species are counted.

Wilman, H., Belmaker, J., Simpson, J., de la Rosa, C., Rivadeneira, M. M., & Jetz, W. (2014). *Ecology*, 95, 2027. doi: <https://doi.org/10.1890/13-1917.1>

Importance of indicator

Provides data about the presence and number of insectivorous bird species.

The number of insectivorous bird species between areas differering in degree of urbanisation, and accross habitat changes evaluated in long-term monitoring, will elucidate about the resilience of the ecosystem regarding **pest control** (disease vector mosquitoes, plant pests, etc), and the **quality of cultural services** that contribute to human well-being.

For example, an increase in population density per km² from 500 to 3000, or an increase of impervious area by 35%, may lead to a **loss of one insectivorous bird species** in that area.

A breeding pair of one such species, like the blue tit *Cyanistes caeruleus*, can feed their nestlings with about 700 aphids during the breeding season (Cowie and Hinsley 1988). Estimating a population of 7800 breeding pairs of blue tits in a 150km² city, this means **less 5 460k aphids consumed** each spring.

Cowie, R. J., & Hinsley, S. A. (1988). *Journal of Animal Ecology*, 57, 611-626. doi: 10.2307/4928



Blue tit *Cyanistes caeruleus*, an insectivorous bird species, feeding one of its newly fledged chicks (photo from pixabay.com)



VI. Microbial diversity

Rational

Microbial diversity refers to the variety of microorganisms—bacteria, archaea, fungi, and micro-eukaryotes—present in a given environment, such as freshwater, soil, or sediment.

High microbial diversity generally reflects a healthy and resilient ecosystem, where different species perform complementary functions beneficial for the ecosystem, such as nutrient cycling, organic matter decomposition, and pathogen biocontrol.

Low diversity can indicate environmental stress, pollution, or the dominance of opportunistic or pathogenic microbes, making microbial diversity a useful indicator of ecosystem health and water quality.

How is the indicator measured?

Microbial diversity is typically assessed using molecular methods targeting environmental DNA that capture the identity and abundance of microbial taxa.

Common approaches include sequencing of universal marker genes (16S rRNA for bacteria, 18S rRNA for micro-eukaryotes) or shotgun metagenomics (i.e. the sequencing of the totality of DNA), which provides functional information in addition to taxonomic profiles.

Analyses generate metrics such as species richness (number of microbial taxa), Shannon (H) or Simpson (S) diversity indices (accounting for abundance and evenness J), and beta diversity (differences between communities), with:

$$H = - \sum_{i=1}^s p_i \ln p_i$$

$$S = - \sum_{i=1}^s p_i^2$$

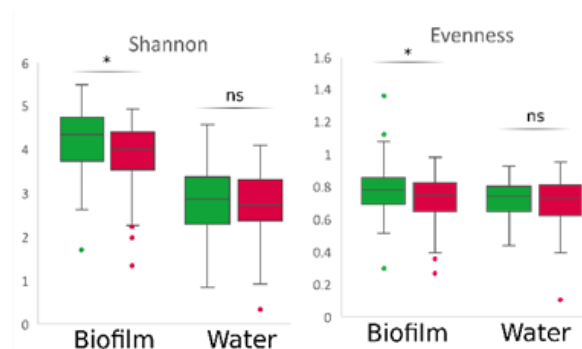
$$J = \frac{H}{\ln(\text{richness})}$$

These measures can be expressed as diversity indices, relative abundance of taxa, or the presence/absence of key functional groups.

Importance of indicator

Monitoring microbial diversity provides insight into ecosystem stability and function. High diversity supports ecosystem resilience, nutrient cycling, and suppression of pathogens, whereas low diversity can signal pollution, habitat degradation, or increased disease risk.

Tracking microbial diversity over time helps evaluate the impact of environmental changes, restoration efforts, or pollution mitigation strategies, and informs water management decisions aimed at maintaining healthy and balanced microbial communities.



Boxplot showing two diversity metrics (Shannon and Evenness) decreasing in biofilms from disturbed ecosystems.



VII. Fecal Coliforms

Rational

Fecal coliforms living naturally in the intestinal tract of warmed-animal, including humans. They are used as indicator of recent animal and human fecal contamination in water. Their presence suggests inputs from human or animal waste and therefore a potential exposure to enteric pathogens including pathogenic bacteria, and parasites.

Monitoring of fecal coliforms provide a simple way to assess the sanitary quality of surface water, recreational water, and drinking-water sources, by estimating potential infectious health risks.

Importance of indicator

Fecal coliforms provide essential information about the sanitary quality of water. Because they come from the digestive systems of humans and animals, their presence indicates that fecal material has entered the environment. This makes them a reliable early warning signal for the potential presence of harmful pathogens such as *E. coli* O157, *Salmonella*, or viruses (e.g. norovirus causing gastroenteritis) and parasites associated with gastrointestinal diseases.

Fecal coliforms are widely used in water-quality regulations for drinking water, recreational waters, shellfish harvesting areas, and environmental monitoring programs. Because they are simple and inexpensive to measure, they remain a cornerstone indicator for public health protection, ecosystem management, and policy decision-making.

How is the indicator measured?

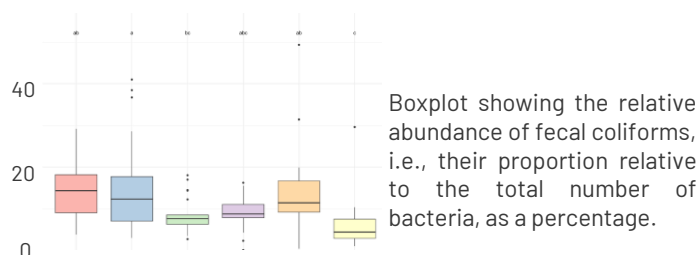
Water samples are collected and analyzed using several complementary methods. The most common approach is culture-based testing, where a known volume of water is filtered and the filter is placed on a selective growth medium (i.e. allowing the growth of fecal coliforms only). When incubated at warm temperatures fecal coliforms will grow, forming visible colonies. These colonies are then counted and the result is expressed as colony-forming units per 100 mL (CFU/100 mL). Enzyme-substrate tests work in a similar way but use color changes or fluorescence to detect these bacteria more quickly.

In addition to culture methods, molecular tools such as qPCR can be used. qPCR detects and counts specific DNA sequences from fecal coliforms, offering rapid and sensitive results, even when bacteria are present at low levels or are no longer alive.

Environmental DNA metabarcoding can provide a broader picture of microbial communities in water. Although not used to *quantify* fecal coliforms directly, metabarcoding can confirm the presence of related indicator bacteria and identify other microorganisms associated with fecal contamination.



Agar plate showing the colonies of fecal coliforms and enabling their quantification.



VIII. Pathogens

Rational

Pathogen indicator refers to the detection of microorganisms (bacteria, viruses, fungi, or protozoa) that can cause disease in humans, domestic animals, or wildlife.

These organisms are naturally present in the environment at low levels, but their abundance can increase due to pollution, wastewater discharge, or environmental disturbances.

Monitoring pathogens in water is essential to assess health risks and prevent outbreaks of infectious diseases.

How is the indicator measured?

Pathogens can be detected using culture-based methods, molecular techniques such as qPCR targeting specific pathogen genes, or metagenomics and metabarcoding, which identifies a broad range of microbial pathogens from environmental DNA.

Measurements may include the presence/absence of specific pathogens, their abundance, or the relative frequency of pathogenic taxa within the microbial community.

$$\text{Pathogen abundance} = \frac{\text{Pathogen number}}{\text{Total microbe number}} \times 100$$

Pathogens metrics can also be converted into infectious health risk indexes (between 0 and 1), mixing pathogen richness, abundance and their severity (i.e., virulence, number of infectious cases).

$$\text{Pathogen risk} = \frac{\text{Patho. richness} + (\text{Patho. abundance} * \text{severity})}{2}$$

Advanced methods also allow detection of toxin-producing strains or antibiotic-resistant genes associated with pathogens, providing more detailed health risk information.

Importance of indicator

Monitoring pathogen presence is critical for protecting public health, livestock, and wildlife. High levels of pathogens in water can indicate contamination from sewage, agricultural runoff, or wildlife, posing risks for drinking water, recreation, and aquaculture.

Tracking pathogens over time supports early warning systems, informs treatment and sanitation strategies, and helps reduce the spread of infectious diseases in both humans and animals.



Pseudomonas aeruginosa (human pathogen) growing on selective media.



IX. Antibiotic Resistance Genes

Rational

Antibiotic Resistance Genes (ARGs) indicate the presence of bacteria that can resist to antibiotic treatments.

They originate from human, animal, or environmental microbes and can be spread through wastewater, agriculture, or urban runoff.

Their detection in water reveals potential risks for the emergence and circulation of antibiotic-resistant bacteria relevant to human and animal health.

How is the indicator measured?

ARGs are detected using molecular methods (i.e., detection of DNA) because they cannot be reliably measured through culture alone. However, culture on selective medium, amended with antibiotic, allow the detection of antibiotic-resistant bacteria. Targeted qPCR quantifies specific resistance genes, while metagenomics (i.e., sequencing of the totality of DNA) allows the detection of a broad range of ARGs and links them to microbial communities.

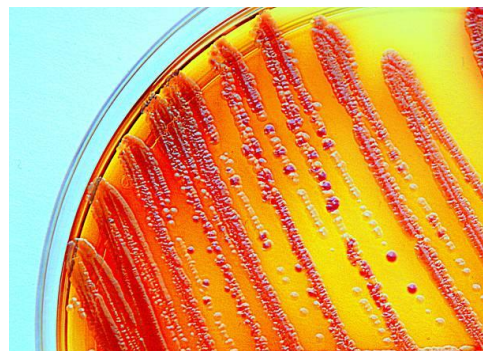
Results are generally expressed as gene copies per volume of water or as relative abundance.

Importance of indicator

Monitoring of ARGs help identify areas where antibiotic resistance is accumulating and spreading in the environment.

They provide insights into contamination sources, such as wastewater treatment plants or livestock operations.

Because antibiotic resistance affects both human and animal health, monitoring ARGs supports One Health strategies and helps anticipate future public-health risks linked to resistant infections.



Agar plate amended with antibiotic showing the growth of antibiotic-resistant bacteria, allowing their detection and quantification



X. Diptera Adults

Rational

By tracking how different **Diptera adults** respond to urban pressures and restoration, this indicator highlights when ecological imbalances may translate into increased vector presence and health risks for humans and other animals.

Diptera include some of the most medically relevant taxa worldwide, such as mosquitoes (Culicidae), biting midges (Ceratopogonidae), sand flies (Phlebotominae), and blackflies (Simuliidae).

Some taxa may transmit **viruses, parasites and bacteria** through blood-feeding. Changes in their abundance or distribution can therefore signal shifts in the risk of **vector-borne diseases** within **urban environments**.



Culicidae, *Aedes* sp.



Simuliidae

Importance of indicator

Diptera adult assemblages integrate signals from **aquatic, semi-aquatic, and terrestrial** taxa, capturing ecological responses across stream channels, riparian zones, and wet urban habitats. These groups include key **ecological functional roles** (decomposers, pollinators, herbivores, predators, parasites) as well as **medically relevant vectors** capable of transmitting pathogens, including **invasive** species expanding under favorable conditions.

Repeated measurements across space and time provide insight into ecosystem condition and **vector-borne health risks** for humans, livestock, pets, and wildlife.

Changes in Diptera communities can reveal environmental **changes**, habitat **degradation**, shifts expected under **climate, land-use and restoration scenarios**. While some taxa (e.g., Culicidae) are already monitored for public-health purposes, other complex but informative groups strengthen ecological assessment.

This indicator supports **One Health policy** by linking biodiversity, environmental quality, and human health and well-being, helping define city-specific baselines for informed urban planning.

How is the indicator measured?

Riverine Diptera adult assemblages are assessed using **standardized CO₂-baited traps** (e.g., Biogents BG-Pro, with CO₂ generated by dry-ice sublimation powered by a powerbank).

At least two traps are placed per site along the urban stream, ideally 25 m apart (minimum 5 m), avoiding direct exposure to sunlight.

Traps operate **overnight**, from a few hours before sunset to a few hours after sunrise. After collection, samples are transported in a cooler and stored at -80 °C upon arrival in the laboratory.

Specimens are then **morphologically identified** under a stereomicroscope, and community metrics (richness, abundance, taxonomic composition) are quantified. These metrics are then related to environmental and climatic variables to assess ecological patterns and responses.

Additionally, **pathogen screening** can be performed by extracting DNA and RNA from pools of previously identified frozen specimens (grouped by species, site and date), or by preserving individuals in RNAlater. This enables extraction, amplification and sequencing for the detection of viral, bacterial or parasitic pathogens.

Importance of indicator



→ CO₂ trap at site

Morphological ID ←

Dipteran adults offer a sensitive **early-warning indicators** of urban stream health



XI. Invasive Alien Plants of the riparian corridor

Rational

Invasive Alien Plants (IAP) are plant species introduced – intentionally or accidentally – outside their natural geographic range that spread rapidly and cause ecological, economic, and/or social harm.

IAPs are a typical feature of biological communities in urban, peri-urban and human-disturbed ecosystems.

In terms of health impacts, AIPs, particularly angiosperms, are frequently associated with the production of allergenic pollen and toxic compounds, which can cause wounds, skin irritation, allergic reactions and poisoning. Additionally, IAPs may act as mediators of disease vectors (e.g., dipteran vectors, ticks) and allow the occurrence and proliferation of vector-borne diseases.

How is the indicator measured?

The non-native community in the riparian corridor is visually surveyed along a 100-meter stretch of the stream on both banks. Ten checkpoints are established at 10-meter intervals. At each checkpoint, the assessment is conducted from the water's edge toward the riverside, covering a linear distance of 5 meters and a width of 2 meters.



Whenever possible, species are identified *in situ*. The relative coverage (%) of each species is visually estimated, and each species is classified as cosmopolitan, alien, naturalized, potentially invasive, or invasive, in accordance with national legislation.

Importance of indicator

IAPs, due to their negative impacts on ecosystems and human health, can serve as indicators of ecological degradation and disturbance.

These species tend to thrive in disturbed, nutrient-enriched, or poorly managed environments and their presence can reflect habitat disturbance (e.g., soil movement, bank erosion, vegetation clearing), hydrological alteration (e.g., modified flow regimes, channelization), nutrient enrichment or pollution (which favours fast-growing opportunistic species), loss of native biodiversity (as invasives displace or suppress indigenous plants), and reduced ecosystem stability or resilience (where natural communities recover incompletely or cannot recover).

AIPs are a cost-effective, sensitive, and easily observable indicator of disturbance, ecosystem stress, and biodiversity decline, making them a good tool in freshwater health monitoring.

Invasive Alien Plants examples



Examples of IAPs that can be found in the riparian corridors of European streams and rivers.

