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Discussion

# Citizen science meets eDNA: A new boom in research exploring urban wetland biodiversity

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## Summary

The biodiversity of urban wetlands provides crucial ecosystem services and contributes to cities' livability. However, such ecosystems are threatened by multiple stressors, such as climate change, environmental pollution, and species invasion. Citizen participation in biodiversity and ecological conservation is becoming an increasingly well-developed and valued approach worldwide. However, few citizen science programs have focused on aquatic organisms. In Nanjing, China, a group of junior students and scientists have been collaborating on a novel environmental DNA toolkit to enable the monitoring of multitrophic biodiversity in urban wetlands. The toolkit encompasses the full life cycle of citizen science practice, from design to implementation, evaluation, and data management. It enables citizens to collect samples with reduced contamination, which is a critical factor for the success of citizen biodiversity monitoring. Numerous native and non-native species across multitrophic levels that inhabit the water and sediment in urban wetlands were identified, increasing public awareness of the need to protect native diversity in these, and other, urban wetlands.

According to the United Nations, 55% of the world's population currently lives in urban areas. This number is projected to grow to 68% by 2050. The continuous expansion of urbanization has severely affected the ecological functions of the urban environment, leading to vegetation degradation and biodiversity loss. As an island of biodiversity, urban wetlands are often the last refuge for wildlife in urban settings, and a treasure trove of urban biodiversity [1] but threatened by species invasion, environmental pollution, and habitat degradation, among other factors [2]. Understanding the biodiversity of urban wetlands is vital for urban biodiversity conservation. However, surveying large urban wetlands is challenging and often requires specialized equipment and extensive fieldwork. Additionally, traditional biodiversity surveys are generally based on capturing organisms and are invasive for the biological community, which is contrary to the original intent of biodiversity conservation. Fortunately, environmental DNA (eDNA) citizen science is transforming this quandary.

## The charm of citizen science

Citizen science, also known as public participation in scientific research or community-based monitoring, involves members of the general public using scientific methods to collect and analyze authentic data as part of original research projects [3]. Citizen science is growing rapidly and is driven by the availability of handy equipment and infrastructure, such as mobile phones, low-cost sensors, and mobile internet [4]. Citizen science initiatives involve the public in the research process to generate genuine scientific outcomes [5], such as discoveries, evidence-based policymaking, and environmental governance. Citizen science has a long history in developed countries and plays an important role in knowledge dissemination and science popularization. However, citizen science still faces some significant challenges in environmental and ecological science, including how to: (i) obtain large-scale and high-quality biological samples and (ii) accurately identify species.

## A new boom in research

To monitor biodiversity, eDNA-based technologies analyze DNA that is shed from organisms into their surrounding environment in the form of skin, urine, feces, and mucus, which bypasses the need for labor-intensive and destructive capture processes and morpho-

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taxonomic expertise [6]. The combination of eDNA and citizen science provides new possibilities (e.g., high-quality, large-scale, and accurate identification of species) for biodiversity surveys. For example, in 2018, New York City citizen scientists (high school students and their teachers) were trained during a two-week study to identify species using DNA barcoding [7]. In 2019, James Julian et al. used quantitative polymerase chain reaction (PCR) to detect amphibian pathogens by training volunteers to collect eDNA samples from 21 ponds in the United States [8]. Additionally, in 2020, Julie Sheard et al. launched the 'Ant Hunt' citizen science project and established the presence of the first exotic ant species in Denmark [9].

eDNA citizen science still faces challenges, such as method standardization, especially for collecting eDNA samples. This is mainly because eDNA is easily degraded and contaminated; thus, it requires professional sampling equipment. Therefore, improving eDNA sampling technologies and standardizing participation processes are crucial to enhance the contribution of eDNA citizen science toward ecological conservation.

## Developing a free and easy-to-use eDNA toolkit for junior students

A novel eDNA sampling toolkit is developed to collect aquatic eDNA efficiently without contamination and the need for professional equipment (Fig. 1). The toolkit includes an eDNA sampler, a syringe, and a bottle of eDNA Longmire's solution. It is designed to be easily used by the public (Fig. S1). To collect a sample, water is drawn into a syringe and then filtered into an eDNA sampler until the filter membrane is saturated. After filtering the water sample, as much air as possible is removed by repeatedly pumping the sample with a syringe. Finally, 1 mL of eDNA Longmire's solution is added, and the samples are sealed and mailed to a professional eDNA laboratory for analysis.

## Training to use the toolkit

A vending machine that provides an eDNA sampling toolkit was developed to encourage public engagement in scientific activities, enabling citizens to receive the toolkit for free via express delivery or by scanning a QR code at the machine. A short training course on the eDNA concept and protocol guidelines was provided to the volunteers at the start of the project, specifically on September 4th, 2021. A video demonstration of the sampling toolkit was made available to help the volunteers use the toolkit appropriately. A total of 44 high school students were recruited in this pilot project, of which 42 participated in eDNA sampling and 6 participated in some laboratory operations. The samples were sent by express delivery to the eDNA laboratory of the Yangtze River Ecological Civilization Innovation Center (Figs. S2–S4). Because of funding constraints, only 50 sampling toolkits were produced, and 42 have been successfully recycled. The biological composition of each sample was analyzed using standard eDNA metabarcoding processes, which were mainly based on DNA extraction, PCR amplification, high-throughput sequencing, and species annotation (for more details, please see the Methods section of the supplemental information).

## An eco-friendly approach for monitoring biodiversity

A comprehensive understanding of biodiversity without disturbing the ecosystem is one of the highlights of eDNA citizen science. Although many members of the public have a strong desire to explore biodiversity, they lack the appropriate technologies and approaches, which makes it difficult for them to understand local ecosystems directly. The participants in the current case were most impressed by the huge abundance of species information that could be obtained from a single water sample. The eDNA sampling toolkit standardizes the sampling process, improves its efficiency, and reduces contamination, which is a critical factor for the success of citizen biodiversity monitoring. First, a new mold was redesigned to integrate the filter membrane directly into the filter to avoid contamination caused by direct touch by operators during the sampling process. Secondly, traditional membranes were replaced by a multilayer filter plate (one 3-μm filter membrane and two 200-μm filter plates) to increase the filtration area, which allowed citizen scientists to filter water quickly using a syringe without the need for specialized equipment. Finally, after sampling, eDNA Later was added to avoid DNA degradation during transport.

## Wetland biodiversity on a sub-microscopic level

Traditional citizen science activities focus on large animals or plants visible to the naked eye because they tend to be familiar to the public. Thus, eDNA technology extends the reach of citizen science to organisms that are sub-microscopic level (i.e., those with a body length <1 mm or hidden in the water and sediment). In this

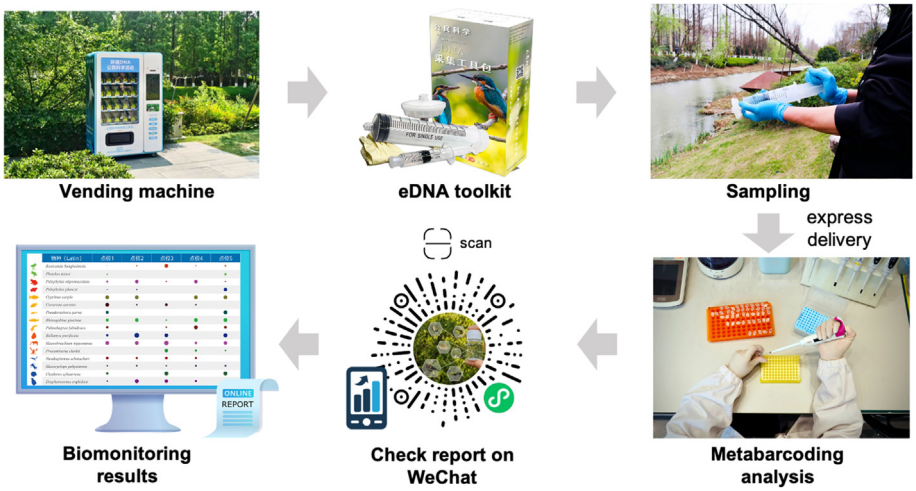


**Fig. 1.** Schematic of the environmental DNA (eDNA) citizen science process for urban biodiversity monitoring.

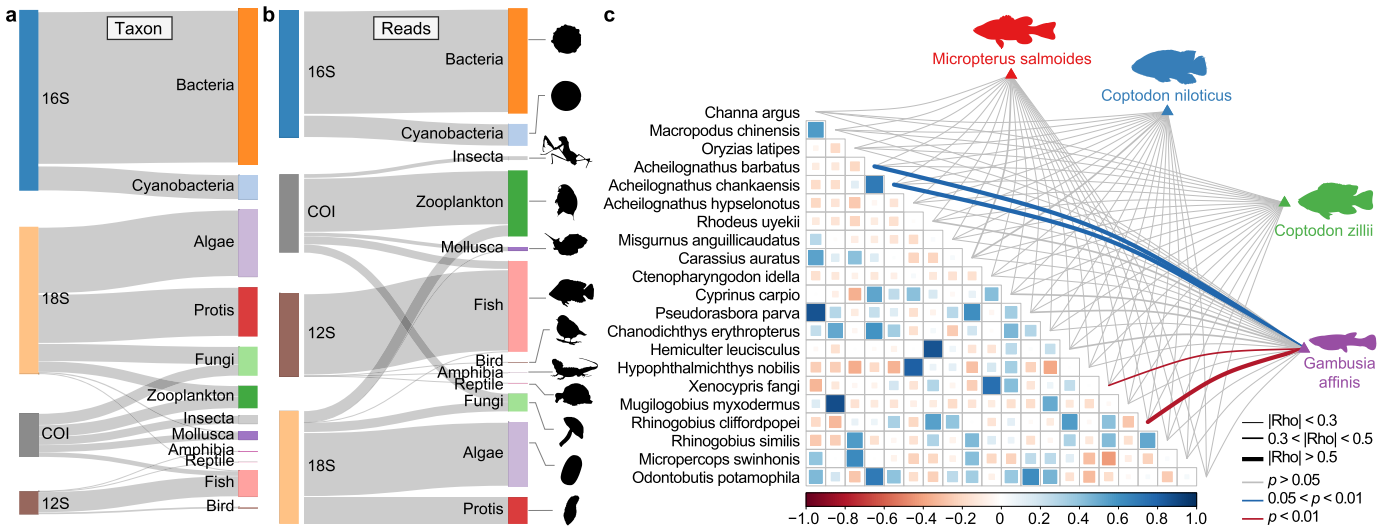


**Fig. 2.** Species composition of 14 major urban wetlands in Nanjing, China. **(a)** The biological taxa that were detected based on eDNA, **(b)** the proportion of DNA sequences that were detected based on eDNA, and **(c)** the effect of four non-native fish species on the native urban wetland fish.

study, more than 900 taxa of producers, consumers, and decomposers were found in the urban wetlands, including bacteria, fungi, algae, zooplankton, insects, mollusks, fish, amphibians, reptiles, and birds (Fig. 2a and b and Table S2). More than 90% of these species were organisms that could hardly be seen with eyes. This raised the public's awareness of the need to extend conservation to micro-organisms. This shift in public awareness is perhaps more important than discovering new species. We believe that, with increasing public participation, and, consequently, increased awareness, various environmental protection measures will be easier to implement.

## Non-native species as a particular concern

Urban wetlands are considered to be high-risk areas for invasive species because of frequent human activity. Even with a low number of sites, this pilot eDNA citizen survey found 57 local species and five non-native vertebrates in the urban wetlands. Many of the volunteers were shocked by the detection of non-native species and showed a keen interest in them, even though the five non-native species have previously been reported because of their widespread invasion in China and elsewhere. The volunteers were very interested in the ecological consequences of the non-native species, so we analyzed the effect of the non-native species on the indigenous biodiversity in our study sites and found that some non-native species significantly reduced the local biodiversity (Fig. 2c). By discovering non-native species through hands-on sampling, citizens can better understand the ecological consequences of invasive species. In particular, after learning that some non-native species, such as red-eared slider turtles (*Trachemys scripta* elegans), were established after humans' release, all the volunteers said that they would not release such species in the future to reduce the spread of non-native species.

## How eDNA citizen science could help biodiversity conservation in the future?

Notably, eDNA citizen science not only changes the public's perception of biodiversity but also provides new opportunities for scientists to solve scientific problems. For example, the public has become increasingly aware that tiny organisms deserve as much attention as big animals. In fact, the public's understanding of biodiversity is generally limited to a few large and protected animals that can be accessed in zoos, and the public knows little about the numerous smaller species that serve essential ecological functions. Since identifying smaller organisms usually requires specialized taxonomic knowledge, this limits public participation in relevant investigations and research [10]. Through eDNA citizen science activities, the public can fully participate in the investigation process and gain a comprehensive understanding of the biological characteristics of their local environment.

Moreover, citizen science activities can be used for scientists to obtain a broader range of data and samples, providing more opportunities for solving scientific problems. Specifically, the eDNA sampling toolkit allows citizens to collect samples without losing too much valuable information, which is crucial for reaching robust scientific conclusions. For example, this citizen science case study revealed a significant positive correlation between the total biodiversity and the water area in urban wetlands, which contributes directly to future urban wetland construction and biodiversity conservation. Therefore, eDNA citizen science could be a promising method for increasing awareness and expanding the use of citizen science to sub-microscopic aquatic organisms.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2023.100275.

## References

[1] N.B. Grimm, et al., Global change and the ecology of cities, Science 319 (5864) (2008) 756–760.
[2] T.M. Koel, et al., Predatory fish invasion induces within and across ecosystem effects in Yellowstone National Park, Sci. Adv. 5 (3) (2019) 1–11.
[3] R. Bonney, et al., Next steps for citizen science, Science 343 (6178) (2014) 1436–1437.
[4] D. Fraisl, et al., Citizen science in environmental and ecological sciences, Nature Reviews Methods Primers 2 (1) (2022) 1–20.
[5] J.R.B. Palmer, et al., Citizen science provides a reliable and scalable tool to track disease-carrying mosquitoes, Nat. Commun. 8 (2017) 1–13.
[6] R.P. Kelly, et al., Harnessing DNA to improve environmental management, Science 344 (6191) (2014) 1455–1456.
[7] C. Marizzi, et al., DNA barcoding Brooklyn (New York): a first assessment of biodiversity in Marine Park by citizen scientists, PLoS One 13 (7) (2018) 1–18.
[8] J.T. Julian, G.W. Glenney, C. Rees, Evaluating observer bias and seasonal detection rates in amphibian pathogen eDNA collections by citizen scientists, Dis. Aquat. Org. 134 (1) (2019) 15–24.
[9] J.K. Sheard, et al., Monitoring the influx of new species through citizen science: the first introduced ant in Denmark, PeerJ 8 (2020) 1–19.
[10] M. Seymour, et al., Environmental DNA provides higher resolution assessment of riverine biodiversity and ecosystem function via spatio-temporal nestedness and turnover partitioning, Commun Biol 4 (1) (2021) 512.