

SHINING A SPOTLIGHT BELOW THE MEKONG'S SURFACE

How environmental DNA monitoring can support conservation efforts in the Lower Mekong Basin







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Who is the report for?

We aim for this report to be useful to anyone interested in biodiversity monitoring and the delivery of national and regional conservation targets. In particular, it is intended for those involved in decision-making related to biodiversity targets – across both the private and public sectors – to support meaningful and effective delivery.

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INTRODUCTION OUT OF SIGHT, OUT OF MIND

Healthy and connected rivers, lakes and wetlands are critical for people and nature. They are central to water and food security, tackling the climate crisis, reversing nature loss and driving sustainable development. Their importance has been recognized in key international agreements, including the Kunming-Montreal Global Biodiversity Framework, the Paris Agreement, and the Sustainable Development Goals.

However, many countries are struggling to achieve the targets set out in these global frameworks quickly enough. Indeed, rivers, lakes and other wetlands are the most threatened ecosystems globally. Two-thirds of long rivers no longer flow freely from source to sea¹. We have lost 35% of the world's wetlands since 1970, while freshwater species populations have collapsed by 85 per cent on average over the same period².

Despite these alarming statistics, the priceless biodiversity of our rivers, lakes, wetlands continue to be undervalued and overlooked, especially species that swim out-of-sight and out-of-mind, below the surface. This contributes to the rapid loss of freshwater species and ecosystems, undermining hard-won development gains and global efforts to tackle the climate and nature crises, and deliver sustainable development.

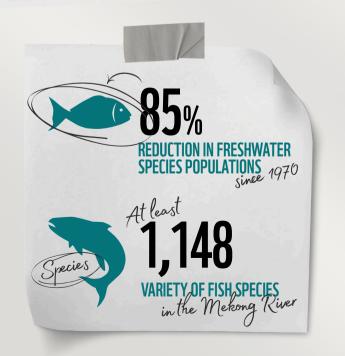
There is an urgent need for governments, businesses, non-governmental organizations and communities to accelerate efforts to protect, restore and sustainably utilise freshwater ecosystems, and to safeguard their biodiversity. Central to these efforts is accurate data, yet freshwater biodiversity remains understudied and poorly understood. The reasons for this are multifaceted, but include the difficulties of collecting data from remote, sediment-rich rivers and from ecosystems whose size and water flow can fluctuate significantly over the course of a year.



Patrol team in Paksanom village, Lakhonepheng District, Salavan Province. ©WWF-Laos



► 2 © Bristol Zoological Society





MEKONG RIVER: DAZZLING BIODIVERSITY UNDER THREAT

The Mekong River boasts extraordinary biodiversity, including an astonishing variety of fish species – at least 1,148 – making it the third most biodiverse river in the world, after the Amazon and Congo. These fishes are critical for the health, food security and livelihoods of tens of millions of people across the region, as well as the overall functioning of the river system. However, they are under ever-increasing pressure, with one in five already threatened with extinction.

The Mekong is home to the world's largest inland fishery. It accounts for over 15 per cent of the entire global inland catch, generates over US\$11 billion annually, and is central to the food security and livelihoods of over 40 million people in communities across the basin.

Yet the Mekong's fishes continue to be undervalued and overlooked by decision-makers. At least 19 per cent of assessed species are now estimated to be heading towards extinction, with 18 species already listed as Critically Endangered on the IUCN Red List of Threatened Species. Numerous species remain data deficient, while the knowledge of local fishers is not sufficiently recognized.

Many of the basin's other species are also increasingly at risk, from the Mekong's iconic river dolphins to the Critically Endangered Siamese crocodile.

An unprecedented combination of threats is driving the decline of the Mekong's fishes and other species, including habitat loss, hydropower dams, conversion of wetlands for agriculture and aquaculture, unsustainable sand mining, invasive species – and the worsening impacts of climate change.

Urgent action is needed to reverse this alarming trend. Alongside protecting and restoring freshwater ecosystems, Mekong countries should let rivers flow more naturally, improve water quality, and end unsustainable exploitation of resources. Crucially, fishers and communities possess knowledge, expertise and solutions - such as community Fish Conservation Zones - that have demonstrated local success.

But central to efforts to safeguard the Mekong's priceless biodiversity is the urgent need to overcome one major challenge: the lack of data.



THE MEKONG IS POOR IN FRESHWATER BIODIVERSITY MONITORING AND DATA

Like most regions, the Mekong basin lacks sufficient data on its freshwater biodiversity. This is a long-term problem rooted in a range of factors, including a lack of resources, capacity, and investment, and various challenges of collecting data.

There is a critical need for tools that harness Traditional Ecological Knowledge and community engagement. The Mekong's significant freshwater biodiversity and its large, complex ecosystems make data collection and analysis particularly challenging.

Fisheries-dependent surveys typically focus on commercially valuable species. However, like many rivers, the Mekong contains a rich assemblage of fishes with little or no commercial value – such as loaches, pipefishes and pufferfish. The question is: how can they be monitored? Even in the Mekong – the world's largest river fishery – commercially important species such as the long-fin glass catfish (*Ompok pinnatus*), the giant sharkminnow (*Osteochilus schlegelii*), the rat-faced pangasiid (*Helicophagus leptorhynchus*), and elongate catfish (*Pangasius elongatus*) are classified as 'data deficient'. Indeed, 38 per cent of the fish species in the Mekong that have been assessed by IUCN are considered data deficient. This means they are so poorly known that their threat status cannot be assessed. This figure is significantly higher than the global average of 14 per cent for data deficient species.

In addition to these, many Mekong species have yet to have their conservation status assessed. Some have not even been described by science, and new species continue to be discovered each year – such as the catfish *Glyptothorax irroratus* and the stone loach *Nemacheilus pullus*, both identified in 2023.

And it is not just fishes. There is also a lack of data on freshwater mammals, amphibians and reptiles – leaving communities, non-governmental organizations and governments with a major challenge: how can we protect and restore species populations without key data about them? This challenge also undermines efforts to achieve national and global biodiversity targets, as it makes it harder for authorities to demonstrate progress towards goals and targets.

This is where the rapidly evolving technology of environmental DNA (eDNA) monitoring can make a significant difference.

MONITORING BIODIVERSITY REQUIRES EXPERTISE IN DATA COLLECTION AND INTERPRETATION

Put simply, eDNA monitoring refers to the collection and analysis of genetic material shed by organisms into their surrounding environment. While typical biodiversity survey methods require experts on the ground and can be time-consuming, costly and intrusive – usually involving catching fishes and other aquatic animals – eDNA technology offers an innovative solution that is often far more efficient and can detect species that are difficult to observe with traditional surveys. It is also relatively easy to do – just one water sample can reveal what is living, often unseen, in our waters.

Monitoring can be undertaken by local communities and citizen scientists, working alongside expert ecologists to plan surveys and interpret data. This can generate significant volumes of data that would otherwise be very difficult – if not impossible – to gather. eDNA monitoring can be used to rapidly gather data in river basins with limited data, potentially expediting decisions and policy development.

In addition, eDNA can be easily integrated into other biodiversity surveys.

eDNA monitoring can tell us about species diversity and the presence of target species, such as those at risk of extinction, invasive non-native species, or even 'lost' species. It can also be used to monitor



the presence of species across large areas, and with careful interpretation, provide distribution information.

While not a silver bullet for eliminating biodiversity data challenges in the Mekong or elsewhere, eDNA technology complements other methods by providing essential evidence about the health of freshwater biodiversity. This evidence can be used to support communities' understanding of their resources and encourage stronger government commitments and policies for freshwater and fisheries conservation. It can also provide private companies and financial institutions developing their businesses in key freshwater habitats with the data needed to make more informed decisions and implement sustainable practices.

Together with greater investment in other forms of assessment, eDNA can help address the lack of data on the status of species and the rate of freshwater biodiversity loss – and, where efforts are successful, freshwater biodiversity gains. This will help accelerate the implementation of locallevel projects and transformational river basin actions needed to safeguard and restore the Mekong's freshwater biodiversity and the ecosystem services it provides.

> Bueng Khong Long, Th © Kelsey Hartman / WWF-Greater Mekong

WWF's eDNA PROJECT In the mekong

Given the urgent need for more data on freshwater biodiversity in the Mekong, WWF partnered up with experts from FISHBIO to conduct and analyse eDNA studies in a range of countries and ecosystems across the region in 2024. eDNA technology was used to improve our understanding of threatened and poorly understood freshwater biodiversity in the Lower Mekong Basin – a biodiversity hotspot and the world's largest inland fishery, but also a hotspot of risk.

METHODOLOGY

Building on eDNA research from around the world and a few pilot surveys supported by WWF in the Mekong in 2021, the teams selected 24 sites in Laos, Thailand and Cambodia for this project.

In Cambodia, 50 samples were collected: 30 from sites in Stung Treng Province and 20 from sites in Kratie Province.

In Laos and Thailand, a total of 70 samples were collected: 30 from six villages in Thailand and 40 from eight villages in Laos.

Samples Cambodia Thailand Laos 50 30 40

At each site, four in-river samples were collected across the river's width, along with a single negative control collected (filtered pure water) to ensure accuracy.

In addition to generating broader data on freshwater biodiversity, these surveys aimed to improve understanding of the presence and distribution of:

- Critically Endangered freshwater species, particularly fishes prioritised under the Asian Species Action Partnership (ASAP) – a network of over 200 organizations dedicated to catalysing and accelerating conservation action for Southeast Asia's most threatened species, including in the Mekong;
- All threatened species on the IUCN Red List of Threatened Species;
- Data deficient species on the IUCN Red List of Threatened Species;
- Species of economic or cultural importance; and
- Invasive non-native species.

	Laos/Thailand	Cambodia	Overall
2024			
Survey period	April	June	
Number of sites	14	10	24
Number of samples	70	50	120



2024

Number of potential species Number of confirmed species Number of threatened species Number of ASAP species Other target species Invasive non-native species



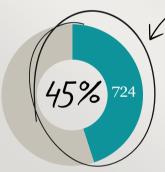
Laos/Thailand	Cambodia
106	117
66	80
3	3
3	1
1	-
3	3



GENERAL FINDINGS

Cambodia

- Among the samples with detectable DNA, the number of fish sequences per sample ranged from 16 to 89, with a mean of 40.
- The cleaned dataset of 1,604 sequences contained 724 unique sequence variants, assigned to 117 distinct fish taxa from 12 orders, 30 families, 72 genera and 80 species. Of the 21 sequences identified to order level, 17 were Siluriformes and four were Osteoglossiformes.
- Of the species identified, three were threatened, one prioritised under ASAP, and three were invasive non-native species. Notably, the total diversity detected was significantly higher than in a previous study in the same region (117 taxa in this study versus 63 taxa in a 2024 survey³)



Unique sequence variants

Cambodia 1,604



- Among samples with detectable DNA, the number of fish sequences per sample ranged from two to 50, with a mean of 21.71.
- The cleaned dataset of 1,194 sequences contained 486 unique sequence variants, assigned to 106 distinct fish taxa from 29 families, 64 genera and 66 species. Of the 12 sequences identified to order level, 11 were Siluriformes and one belonged to Osteoglossiformes.
- · Of the species identified, three were threatened, three prioritised under ASAP, and three were invasive non-native species. The total diversity detected in these samples was slightly higher than that reported in 2022^4 (106 versus 93 taxa) and the level of taxonomic resolution was considerably higher (66 species-level identifications in 2024 versus 50 in 2022).

Laos and Thailand

SPECIFIC TARGET SPECIES

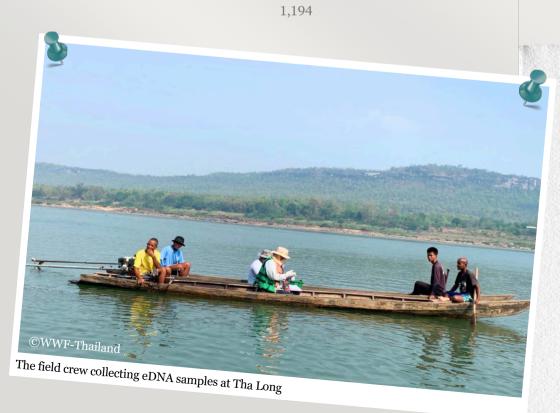
In Cambodia, three priority and threatened freshwater fish species were found, including:

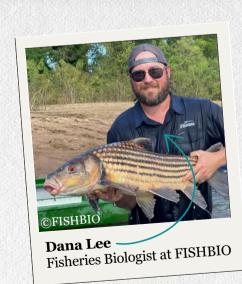
- Critically Endangered Jullien's golden carp (Probarbus jullieni) – detected in nine of the 10 sampled locations (all except Kong Konsat);
- Vulnerable goonch (Bagarius yarrelli) detected in Anlung Cheuteal, Koh Hip, Kang Konsat, Tbaung Khlar, Khsach Makak, and Kampi;
- Vulnerable Laotian shad (Tenualosa thibaudeaui) - detected in Anlung Cheuteal, Koh Hip, Stung Treng, Koh Santuk, Kang Konsat, and Koh Khnhae.

In Laos and Thailand, four priority species were found, including three threatened fishes:

- Critically Endangered Jullien's golden carp (Probarbus jullieni) - detected in Kudjub, Thapea, and Heunhin villages;
- Vulnerable goonch (Bagarius yarrelli) detected in Poung-Nua;
- Lesser tapah (Wallago attu) detected in Kudjub village.







3. Eschenroeder, J.C., Pilger, T.J., Chea, S., and Hogan, Z. 2024. What's in the water: using environmental DNA metabarcoding to detect fish biodiversity in the Cambodian Mekong. *Cambodian Journal of Natural History*. 4. WWF, 2022, Lao-Thai Fisheries (LTF) Project report

Another important species was detected on the transboundary Mekong of Laos and Thailand:

• Silver barb (*Barbonymous gonionotus*) – a key food fish, detected in 11 of the 14 sampled villages - all except Boungkuang, Tha Long, and Kan Tha Kaewn.

The surveys are just a start, but they have already had a significant impact.

By investing in these eDNA surveys in the Lower Mekong Basin, WWF has helped to:

- Create extensive new and transparent freshwater biodiversity datasets;
- Improve understanding of some Critically Endangered freshwater ASAP species, as well as some threatened, data deficient, economically/culturally important, and invasive non-native species;
- Provide evidence for updating IUCN Red List assessments, Key Biodiversity Assessments and other conservation planning tools;
- Strengthen understanding of freshwater biodiversity among communities, governments, non-governmental organizations and the private sector.

"We weren't sure what the eDNA results would show. Of course, we had hoped to find evidence of all the threatened target species, but we knew that was a long shot. What we got wasn't surprising, but it was a rich addition to our knowledge of what's in these stretches of the Mekong and other rivers in the region. It was especially encouraging to see the amount of diversity detected with a minimal number of samples as well as the detection of several rare species that aren't commonly captured. This is vital information and highlights the urgent need to invest more in eDNA and other data gathering."

CONCLUSION

The Lower Mekong Basin is astonishingly rich in freshwater biodiversity but remains critically poor in data.

While fisheries surveys are relatively common, biodiversity surveys are scarce – making data validation a persistent challenge. There is an urgent need for greater investment in a range of complementary approaches, including eDNA monitoring and assessments, to provide more accurate data to enhance conservation efforts.

To fully realise the potential of eDNA, stakeholders must also invest in expanding reference databases. Currently, only around 50 per cent of fish species in the Lower Mekong Basin are represented in <u>GenBank</u> – an open access genetic sequence database. Adding more species to this dataset will improve future surveys and enable reanalysis of existing data, significantly increasing the evidence base. Capacity building within local communities is also essential. Utilising Traditional Ecological Knowledge and building local expertise will ensure biodiversity monitoring is more inclusive, resilient and sustainable.

Two key priorities for improving the effectiveness of eDNA are:

1. Addressing gaps in existing reference libraries, with a focus on obtaining genetic sequences for priority species; and

2. Conducting targeted studies to evaluate eDNA field sampling and analysis methods, in order to develop protocols that maximize the detection of fish diversity.

These priorities are particularly urgent in light of the accelerating impact of human-driven change, and can inform more responsible operations of existing infrastructure and the planning of future developments.



Tapah (Wallago attu)



WWF's eDNA PROJECT IN MYANMAR

Although Myanmar's Ayeyarwady (Irrawaddy) and Thanlwin (Salween) rivers lie outside the Mekong basin, the findings are presented separately here because they offer valuable, complementary insights. These results not only reinforce key themes from the wider report - such as the potential of eDNA to uncover freshwater biodiversity in under-surveyed systems - but also contribute important baseline data in their own right for two of Southeast Asia's most ecologically significant river basins.

Overview

WWF's eDNA project in Myanmar focused on evaluating fish diversity in the Ayeyarwady and Thanlwin river basins to build a freshwater biodiversity baseline for the basin by 2025. This survey was conducted to address knowledge gaps such as species composition in largely unsurveyed sub-catchments of the Ayeyarwady and Thanlwin systems, identify habitation ranges of endemic and / or threatened species, and to compare and contrast detected biodiversity composition between different habitats and past survey records in response to anthropogenic changes.

Summary of findings

A total of 82 eDNA samples were collected during the first half of 2024 across 14 sites, yielding a total of 2,027 sequences. After review and cleaning of the sequence data, 135 distinct fish taxa were detected, representing 12 orders, 36 families, 85 genera and 93 species. The order Cypriniformes (Carps and minnows) forms the largest proportion of detected fish taxa at 55 per cent, followed by Siluriformes (Catfishes) at 17 per cent and Anabantiformes (Labyrinth fish) at 11 per cent.

Seven species listed on the IUCN Red List of Threatened Species were definitively identified through species-level sequence assignments. These included:

- Tiger botia (Syncrossus berdmorei),
- Sawbwa barb (Sawbwa resplendens)
- Red dwarf rasbora (*Microrasbora rubescens*)
- Inle loach (*Petruichthys brevis*)
- Pengba / Manipur osteobrama (Osteobrama belangeri)
- Sareng catfish (*Wallago attu*)
- Goonch catfish (Bagarius yarrelli)
- Bombay duck lizardfish (Harpadon nehereus)

In addition, several priority species of particular interest to WWF-Myanmar were detected. These include Barilius, Devario, Neolissochilus, Schistura, Sicyopterus, and Tor - genera known to contain many stream-

dwelling species often considered indicators of healthy water quality. The detection of the endangered red dwarf rasbora, a species considered endemic only to the Inle Lake watershed, in the Naga region could indicate the presence of an unknown additional population of this threatened fish species.

Diadromous migratory species detected in this sampling include Burmese mullet (Sicamuqil hamiltonii), Indian mottled eel (Anguilla bengalensis), pangas catfish (Pangasius pangasius), and pama croaker (Otolithoides pama).

Recommendations

As the Avevarwady and Thanlwin watersheds face increasing threats from population growth, land use change, mining of sand, gold and other metals, hydropower development, and climate change, obtaining reliable data on fish species distributions and habitat occupancy is critical for informing adaptive management of these important fisheries.

The findings of this project demonstrate the value of eDNA monitoring as a cost-effective, non-intrusive and highly-accessible alternative to traditional survey methods – especially in hard-to-reach areas. In parallel, eDNA approaches also foster collaboration between communities and experts, combining high-tech and lowtech tools to strengthen community understanding and engagement with conservation efforts.

While eDNA monitoring cannot generate data on abundance, size or age structure, or other key population demographics, it can still provide essential data on species presence in priority ecosystems. With sampling done across spatial and temporal distributions, we can build a better understanding of key ecological processes such as the migratory behaviour of economically significant food fish species and trends in biodiversity abundance and evenness in response to environmental changes.

Ongoing data collection and archiving of both extracted DNA and sequence data will be valuable for monitoring changes in fish communities in response to local management interventions and broader shifts in climate and hydrology. Developing and expanding the use of eDNA monitoring in Myanmar will be critical to understanding and protecting the country's freshwater biodiversity.



Throw a bucket out to take a water sampling

Push the water in the syringe through the filter disk (water will drip out the other side)



WIDER SIGNIFICANCE OF THIS WORK

BENEFITS OF eDNA FOR THE MEKONG

This project has highlighted several key benefits of eDNA monitoring in the Mekong:

- It can provide an inventory of species present across large areas and a broad range of taxa.
- It can target species of conservation importance, including elusive, low density, or even presumed 'lost' species.
- It is relatively easy to execute and a powerful tool for engaging donors and businesses.
- It is an effective citizen science tool and method that enables direct collaboration with communities (though results are not instant, as expert laboratory analysis takes 1-2 months and requires ongoing funding).
- It is non-invasive, causing minimal ecology disturbance compared to conventional monitoring tools.
- It complements existing biodiversity monitoring programmes and can drive important activities, such as completing species descriptions and inclusion in DNA reference databases.

CHALLENGES FOR eDNA IN THE MEKONG

As this report makes clear, eDNA is not a silver bullet but an important complementary tool. Current challenges include:

- The lack of standardised methodologies;
- The risk of false negatives;
- Gaps in the existing DNA reference databases;
- The need for careful interpretation of eDNA results regarding relative abundance and distribution;
- The requirement for expertise in local biology, ecology or zoology to accurately interpret findings.



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Mekong tiger perch subspecies

MONITORING BIODIVERSITY: A FOUNDATION FOR CONSERVATION

Biodiversity monitoring is the essential foundation for effective conservation and restoration strategies - and for demonstrating progress towards global and local targets.

This project has demonstrated how useful eDNA is as a tool for enhancing data and knowledge in the Mekong River – a system notoriously difficult to monitor using traditional methods due to its size, low visibility due to high sediment concentrations, and dynamic flow that fluctuates significantly both seasonally and year to year.

The project has generated a number of important insights:

- Community participation is critical;
- eDNA sample collection is simple to implement, though analysis requires time and trained laboratory experts;
- Working with expert ecologists is essential to design effective surveys and interpret results;
- There is great value in investing in the inclusion of additional species in GenBank;
- A mosaic of monitoring types is important for verification and completeness.

CONSERVATION STARTS WITH DATA: SO, LET'S GET STARTED

All stakeholders and decision-makers have an opportunity to chart a new course - to restore, protect and sustainably manage the Mekong for the benefit of people and nature today and into the future. A future in which the river's extraordinary freshwater biodiversity and ecosystems can thrive. But before this can happen, we need to know what species are present, where they are, and how healthy their populations are.

Environmental DNA cannot tell us everything - but it can tell us so much more. It offers priceless data to protect species, and does so in a way that is accessible, cost-effective and non-intrusive compared to traditional survey methods.

Mekong giant catfish





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