MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Ibrahim *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 21 (2025) 2662–2674

RESEARCH ARTICLE

From Water to DNA: The Evaluation of Environmental DNA (eDNA) Sampling Volume in a Tropical Blackwater Peat Swamp in Dungun, Terengganu

Nur Ain Aqilah Ibrahım[a], Izzati Adilah Azmır[a,b*], Mohamad Aqmal-Naser[c], Yuzine Esa[d], Amirrudin Ahmad[c,e]

[a]Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia; [b]EmiBio Special Interest Group, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Negeri Sembilan, Kampus Kuala Pilah, Pekan Parit Tinggi, 72000 Kuala Pilah, Negeri Sembilan, Malaysia; [c]Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia; [d]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; [e]School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia

Abstract Blackwater peat swamps in Peninsular Malaysia support diverse aquatic species including cryptic and elusive taxa. However, extreme environmental conditions such as high acidity, turbidity and organic content hinder biodiversity monitoring, leaving these ecosystems largely understudied. Environmental DNA (eDNA) offers a non-invasive approach for species detection, thus providing a promising tool for biodiversity assessment. This study investigates the influence of water filtration volume and hydrological factors on DNA concentration in a blackwater peat swamp in Dungun, Terengganu. Water sampling was conducted between November and December 2023 at three substations—designated as upper (A), middle (B) and lower (C) which spaced at 50-meter intervals with five replicate samples filtered from each substation. A total of 2L blackwater per station was filtered using a 0.45 µm mixed cellulose ester (MCE) membrane and an oil-free vacuum pump. eDNA was extracted using a modified DNeasy® Blood & Tissue Kit, quantified with a NanoDrop spectrophotometer and assessed through gel electrophoresis. Results showed that eDNA concentration varied significantly across stations and was influenced by the filtered water volume. The highest eDNA concentrations were recorded at the middle stretch (B1: 480 mL, 32.00 ng/µL; B2: 480 mL, 38.22 ng/µL), followed by the lower stretch (C1: 460 mL, 14.00 ng/µL; C3: 400 mL, 11.00 ng/µL). Sample replicates with filtration volumes below 400 mL (A5, B4, B5, C4, C5) failed to yield detectable DNA, likely due to insufficient water filtration. Despite optimal filtration volumes (>400 mL), A1 (420 mL, 7.66 ng/µL) and C2 (450 mL, 9.10 ng/µL) exhibited low DNA concentrations, suggesting hydrological influences that resulted in localized accumulation or dilution of genetic material. Replicate B3 from Station B (400 mL, 19.50 ng/µL) recorded higher DNA compared to replicate C1 and C3 as this area has a moderate and consistent water flow which likely enhanced the dispersal of eDNA throughout the water column. Gel electrophoresis confirmed high molecular weight DNA in samples exceeding 400 mL, particularly in B1 and B2 which displayed the brightest bands. Statistical analysis (Spearman's ρ = 0.809, p = 0.005) revealed a strong positive correlation between filtration volume and eDNA yield. In conclusion, optimizing filtration volume and accounting for hydrological conditions are crucial for effective eDNA sampling, emphasizing the need for standardized protocols to improve biodiversity monitoring in blackwater peat swamp ecosystems.

Keywords: Environmental DNA, Blackwater Peat Swamps, Water Filtration, DNA Extraction, Biodiversity Monitoring.

***For correspondence:**
izzati_adilah@uitm.edu.my

Received: 4 March 2025
Accepted: 13 August 2025

Introduction

Environmental DNA (eDNA) is the genetic material which are released by the aquatic organisms through processes such as cell shedding, excretion or other external means [1, 2]. More specifically, exhalation or blow released by cetaceans at the water surface contains a mixture of respiratory tract cells mucus and fluids that are deposited onto the surrounding water [3]. This eDNA approach has gained a tremendous momentum in recent years of detecting the presence for targeted invertebrate and vertebrate species in freshwater environments [4], lakes [5], peat swamps [6] and marine ecosystems [7]. It serves as a non-invasive tool for biodiversity assessment as it relies solely on the detection of short extracellular DNA fragments released into the environment [8]. Additionally, rare aquatic species in oceanic and lacustrine systems particularly benefit from this method especially in settings where conventional survey techniques are limited [9]. Findings from studies in aquaria and small pond environments indicate that eDNA also used to estimate population abundance [10].

Among the water bodies in the world, research on the detection of aquatic life using eDNA in blackwater ecosystems remains limited primarily due to the high turbidity of these environments [11]. Blackwater peat swamps are distinguished by their dark-coloured water which results from high concentrations of dissolved organic matter primarily humic acids derived from decaying plant material [12]. The blackwater peat swamp forests of Sundaland which encompasses Peninsular Malaysia, Borneo and Sumatra support a diverse and ecologically significant assemblage of fish species, many of which are highly valued in the international ornamental fish trade [9 , 10]. In addition, species such as *Betta* spp., *Parosphromenus* spp., *Sphaerichthys* spp., *Rasbora* spp., and *Boraras* spp. play crucial roles in regulating algae populations, maintaining ecosystem balance and serving as bioindicators of aquatic health [9,10]. Many of these species exhibit life cycle adaptations to fluctuating water levels and seasonal rainfall, synchronizing their reproduction and survival strategies with hydrological changes [11, 12].

Despite the high species diversity in blackwater ecosystems, most blackwater peat swamps are currently facing significant threats including urbanisation, deforestation and pollution which collectively jeopardise their ecological integrity [17]. These anthropogenic disturbances alter key water parameters such as pH, turbidity, and dissolved oxygen [18]. Thereby, complicating the effectiveness of traditional physical sampling methods that rely on the capture or handling organisms such as gill netting, electrofishing and visual surveys [18, 19]. Moreover, blackwater peat swamp habitats are highly sensitive to rainfall which affect water level, flow and sedimentation [21]. These dynamic conditions influence species presence and detectability which further limiting conventional sampling approaches. In contrast, the application of eDNA presents an adaptive alternative, capable of detecting species even in turbid or seasonally fluctuating waters [22]. This method has shown superior performance in terms of detection rates and accuracy, enabling more effective conservation strategies tailored to protect vulnerable aquatic communities [23].

This technique facilitate rapid and efficient data collection which typically requiring less than 15 minutes from arrival to departure at a given site [15, 18]. A crucial step in the eDNA workflow is the capture phase, where filtration remains the most commonly employed method that involve the passage of water samples through a membrane to retain DNA fragments [25]. The efficiency of each step from filtration to extraction directly affects DNA yield and species detectability. The collected water samples in the field are transported in ice chests to the laboratory, filtered within 24 hours and subsequently frozen before processing [26]. The membranes used in the filtration of eDNA samples, such as polycarbonate (PC), nylon, glass fibre (GF), and mixed cellulose ester (MCE), vary not only in material composition but also in pore size, which plays a critical role in sample processing [27]. Since filter papers are composed of various materials, DNA is expected to exhibit differing binding affinities depending on the filter type [28]. Study by Liang [25] demonstrated that DNA binding varies across different filter papers. Furthermore, findings from previous studies suggest a potential interaction between filter paper type and extraction method, influencing overall DNA yield. Filters with pore sizes between 0.45 and 0.8 µm are commonly used for general sampling, effectively retaining most DNA fragments without clogging quickly [22 , 23]. Larger pores (e.g., 1.0 µm) allow for faster filtration but may reduce DNA capture efficiency while smaller pores (e.g., 0.22 µm) slow filtration but enhance retention of degraded eDNA, which are often more prevalent in aquatic environments [31].

Extraction is the process of isolating and purifying DNA from environmental samples such as water, soil, and sediment which contain genetic material shed by organisms in the environment [32]. Cells or biological materials in the sample are disrupted to release DNA into the solution, which is accomplished using chemical agents or physical methods such as bead-beating or vortexing to break down cell walls and membranes. Certain samples like water require more intensive mechanical disruption to release

DNA [26 , 27]. Next, the bound DNA is eluted using a buffer solution that releases the DNA from the purification medium (e.g., silica or beads) into a clean, ready-to-use solution [31]. Generally, a larger volume of water sampled enables the detection of a greater number of species [35]. However, processing large amounts of water can be challenging because of long filtration times, difficult sample transportation or both [36]. Therefore, it is imperative to determine the appropriate sample volume.

Therefore, the aim of this study was to evaluate the environmental DNA (eDNA) sampling techniques by examining the effect of water filtration volume on DNA detection in samples collected from a blackwater peat swamp in Dungun, Terengganu with consideration of hydrological factors. The results of this study are anticipated to offer insights into optimal filtration volumes for eDNA recovery in acidic and humic-rich blackwater environments. The outcomes reveal variations in DNA yield and detection success across different sample volumes, thereby providing guidance for future biomonitoring initiatives. These findings will contribute to the development of an enhanced, volume-sensitive protocol for eDNA sampling in blackwater systems and may support more precise biodiversity assessments and conservation strategies in vulnerable peat swamp habitats.

Materials and Methods

Collection of Water Samples from Blackwater Peat Swamp

Water samples were collected from a blackwater peat swamp in Dungun, Terengganu, Peninsular Malaysia, between November and December of 2023 at (4°48′33″N; 103°20′59″E) (Figure 1) between 9:00 a.m. and 2:00 p.m. to minimise fluctuations in water flow conditions and reducing potential variability in eDNA concentration [37]. Sampling was performed during non-precipitation hours to avoid sedimentation and debris accumulation on the filter membrane which maintain consistent filtration efficiency [38]. The sampling site was characterized by shallow and turbid water due to organic matter decomposition while the dense canopy covers limited sunlight penetration.

Three substations were selected along the swamp, each located 50 meters apart: Station A (upper stretch), Station B (middle stretch), and Station C (lower stretch). At each substation, a sterile 2L high-density polyethylene (HDPE) container was submerged at the surface layer of the water column at each site to collect a representative sample [39]. Triplicates 2-liter water samples were collected from all stations within 4 hours. All water samples were labeled and immediately placed in an icebox at 0–4 °C to prevent eDNA degradation [7, 35].

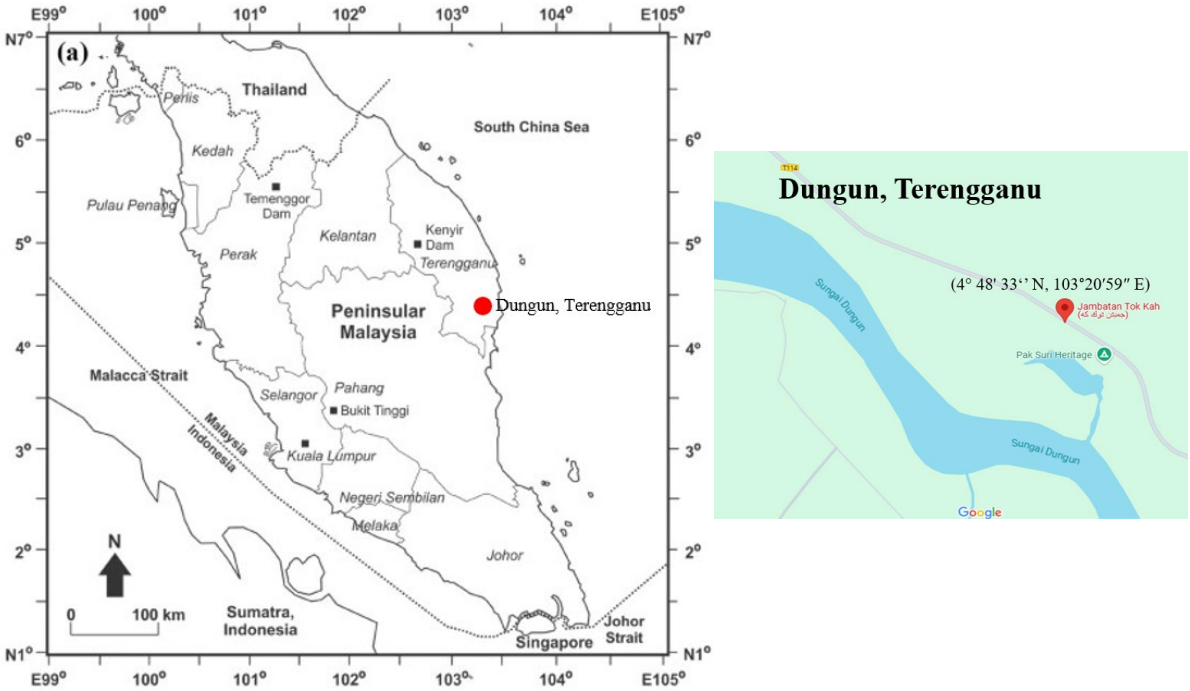


Figure 1. Location of the eDNA sampling station at Jambatan Tok Kah, Dungun, Terengganu, Malaysia. The site is situated along Sungai Dungun with coordinates (4°48'33"N, 103°20'39"E)

Filtration and Preservation of Blackwater Peat Swamp Samples

A field control consisting of 2L of sterile pure water was used at each site to monitor contamination from field equipment [41]. Filtration was performed using an oil-free vacuum pump (Rocker, Taiwan) with a pressure range of 200–400 mbar to balance flow rate and prevent filter clogging or loss of sample integrity. (Figure 2). A mixed cellulose ester (MCE) filter membrane (47 mm diameter, 0.45 µm pore size; Evergreen Engineering, Malaysia) was chosen for its efficiency in retaining eDNA while minimizing clogging [28].

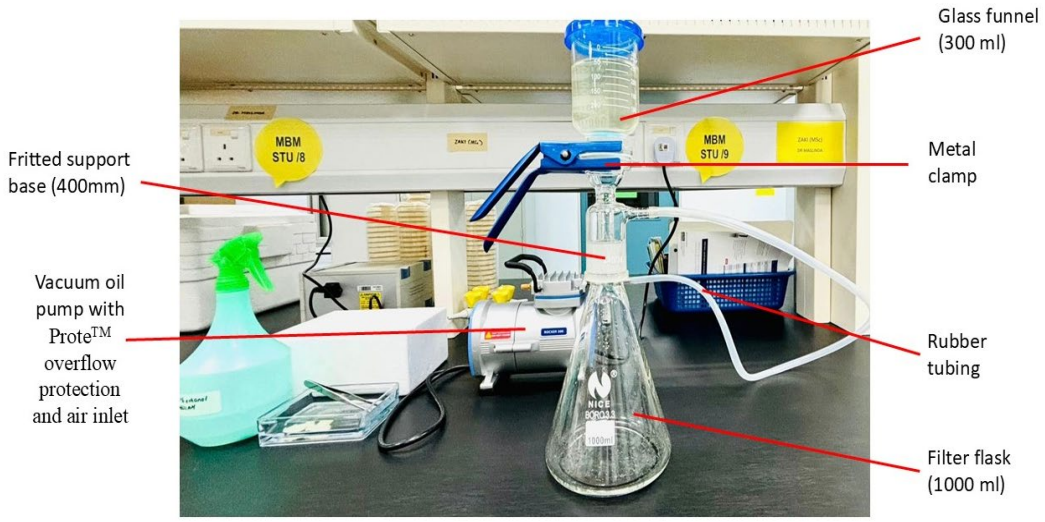


Figure 2. Components of filtration system for blackwater peat swamp samples using oil-free vacuum pump

Water samples were collected from three substations with 2L of surface water obtained at each location. Each 2L sample was filtered to produce five replicate filters, resulting in a total of 15 replicates and a cumulative filtered volume of 6L across all sampling sites. Each replicate represented a variable volume of filtered water, ranging from 300 mL to 480 mL, depending on the degree of particulate matter and filter clogging. The filter membrane was replaced when clogging was evident. Then, filter membrane was carefully removed using sterile forceps and rolled with the filtrate side inward as illustrated in Figure 3. Forceps were disinfected in a 10% bleach solution followed by rinsing in sterile distilled water after each use to prevent cross-contamination [42]. All filter membranes were placed in sterile 1.5mL tubes (Eppendorf, Germany) and stored at −20 °C until DNA extraction [43].

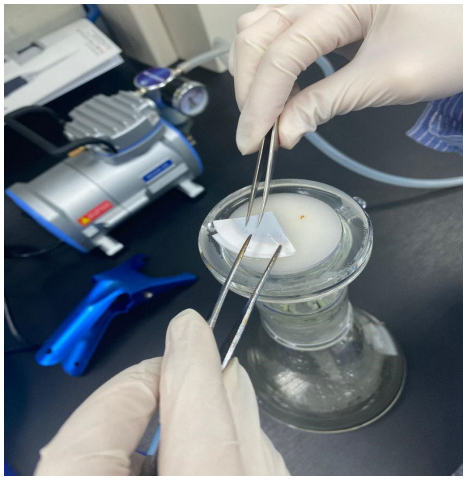


Figure 3. Folding the MCE filter membrane with the filtrate side (usually brown) in using a sterile forceps

Extraction of Environmental DNA (eDNA)

The DNeasy® Blood & Tissue Kit (Qiagen, Germany) was used for DNA extraction, following a modified protocol by Kawato [44]. The filter membrane was sectioned into small fragments using a sterile disposable scalpel (no 22, Swann Morton, UK) shown in Figure 4. These fragments were transferred into a 2.0mL microtube containing 220 µL PBS, 200 µL Buffer AL, and 20 µL proteinase K. The lid of microtube was securely closed and the contents thoroughly mixed by manual inversion. Subsequently, the microtubes were incubated at 56 °C for 2 hours in a microtube block (Eppendorf ThermoMixer®, Germany). An extended incubation time facilitates the breakdown of cellular components, thereby enhancing DNA release into the extraction buffer [45]. The microtubes were gently agitated at 30-minute intervals during the 2-hour incubation period. Following incubation, the tubes were centrifuged at 15,000 × *g* for 1 min to separate the lysate. Subsequently, 400 µL ethanol (96%–100%) was added, and 700 µL of the mixture was transferred to a DNeasy mini spin column. The column was washed with 500 µL Buffer AW1 and AW2 and centrifuged at 6000 × *g* and 20,000 × *g*, respectively. DNA was eluted with 80 µL Buffer AE after a final 1 min incubation at room temperature. The eluate was stored at −20°C until further analyses. One litre of sterile water as negative control in all processing steps to ensure no contamination of eDNA from external sources [46].

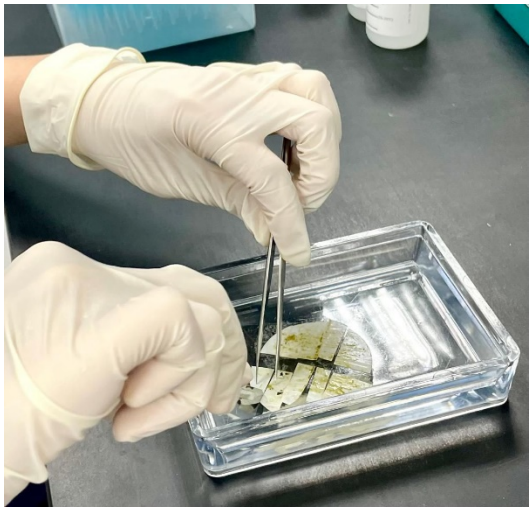


Figure 4. Fragmentation of a mixed cellulose ester (MCE) filter membrane using a sterilized scalpel and surgical blade for environmental DNA (eDNA) extraction

Gel Electrophoresis and Environmental DNA (eDNA) Quantification

The DNA concentration and purity were measured using a Nanodrop Lite Plus spectrophotometer (Thermo-Scientific, USA) at 260 and 280 nm absorbance (A260/A280) with values ranging between 1.8 and 2.0 considered optimal for DNA samples. Deviations from this range were attributed to RNA contamination or degraded DNA [47]. Gel electrophoresis on 1% agarose gel at 80 V for 60 min was used to assess DNA integrity. Successful DNA isolation was confirmed by bright, intact bands above the 1 kb marker, indicating high molecular weight DNA [48].

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26 [49]. To assess the correlation between filtration volume and DNA concentration, the normality of the data was initially examined using the Shapiro-Wilk test, as the sample size, $n < 50$. The Shapiro-Wilk test evaluates whether the data significantly deviate from a normal distribution. If the test indicated a non-normal distribution ($p < 0.05$), a non-parametric approach was employed. Specifically, Spearman's rank correlation coefficient (ρ) was utilized to determine the strength and direction of the monotonic relationship between filtration volume and DNA concentration.

The Spearman's rank correlation coefficient is calculated using the formula:

$$\rho = 1 - \frac{6\ \sum d_i^2}{n(n^2-1)}$$

Spearman's correlation coefficient (ρ) ranges from -1 to 1, where values closer to +1 or -1 indicate stronger positive or negative correlations, respectively. A two-tailed significance test was conducted to assess the statistical significance of the correlation [50].

Results and Discussion

Water Sample Collection and Normality Test

Table 1 presents a summary of the sampling stations, membrane designation, volume of water collected per station and volume of water filtered per replicate from the three blackwater peat swamp stations in Dungun, Terengganu. Notably, there was a variation in the volume of water successfully filtered per replicate, ranging from 300 mL to 480 mL across all stations (A, B and C). This variability may be attributed to the highly organic and humic nature of blackwater that causes membrane clogging [25]. Replicates from Station B which was located the middle stretch of the swamp recorded relatively high filtration volume, with two replicates (B1 and B2) reaching up to 480 mL indicating less clogging and possibly lower suspended solids [36]. Reduced membrane clogging enhances filtration efficiency by decreasing filtration time and permitting greater volumes of water to be processed for eDNA extraction [28, 51]. In contrast, replicates from Station C which were located at the lower stretch consistently yielded lowest volume at only 330 mL. This could be attributed to higher particulate matter which accelerate membrane saturation. The variation reflects the influence of microhabitat condition on eDNA sampling efficiency. In order to improve filtration success, Capo [52] recommended the utilization of 0.8 µm filters as the optimal choice for turbid, eutrophic and high fish density ponds as they achieve an effective balance between filtration efficiency and the likelihood of species detection. Hence, the need to balance between achieving adequate water volume and preventing filter saturation is critical for sampling consistency.

.

Table 1. Summary of sampling stations, membrane designations, volume of water collected (L) per station and volume of water filtered per replicates (mL) from blackwater peat swamp samples

Sampling Station	Membrane Designation (replicates)	Volume of Water collected per station (L)	Volume of water filtered per replicates (mL)
A (Upper stretch)	A1	2.0	420
	A2		420
	A3		420
	A4		370
	A5		370
B (Middle stretch)	B1	2.0	480
	B2		480
	B3		400
	B4		340
	B5		300
C (Lower stretch)	C1	2.0	460
	C2		450
	C3		400
	C4		360
	C5		330

Table 2 presents the results of Spearman's rank correlation coefficient between volume of water filtered (mL) and DNA concentration (ng/µL). The Spearman's correlation coefficient (ρ) between volume of water filtered and DNA concentration is 0.809. Given that Spearman's ρ ranges from -1 to +1, this value indicates a strong positive correlation between the two variables. An increase in the volume of filtered water is associated with a higher DNA concentration. The p-value is 0.005, below the conventional significance threshold of 0.01. This result indicates that the correlation is statistically significant at the 0.01 level, suggesting

that the observed relationship is unlikely to be attributable to random chance, based on the sample size (n=10). The high correlation coefficient (ρ=0.809) by Spearman's rank analysis support that filtering larger volumes of water may enhance DNA capture efficiency, likely due to the increased probability of collecting target DNA molecules. However, other factors environmental or technical factors such as DNA degradation rates, filtration efficiency and potential inhibitors in the water samples should be considered when interpreting these results [43].

Table 2. Correlation analysis of water volume filtered and DNA yield using Spearman's Rank test

			Volume of water filtered (mL)	Concentration of DNA (ng/μL)
Spearman's rho	Volume of water filtered (mL)	Correlation coefficient	1.000	0.809
		Sig. (2-tailed)	0	0.005
		N	10	10
	Concentration of DNA (ng/μL)	Correlation coefficient	0.809	1.000
		Sig, (2-tailed)	0.005	0
		N	10	10

Correlation is significant at the 0.01 level (2-tailed)

Nanodrop Spectrophotometer-based Quantification of Genomic DNA

DNA was successfully extracted from 10 out of 15 filter membranes (A = 4 membranes; B = 3 membranes; C = 3 membranes) shown in Table 3. Most of the highest DNA concentrations were documented in Station B (B1: 480 mL, 32.00 ng/μL; B2: 480mL, 38.22 ng/μL), followed by Station C (C1: 460 mL, 14.00 ng/μL; C3: 400 mL, 11.00 ng/μL). The relationship between sampled water volume and eDNA concentration has been shown to exhibit variability in species detection. According to Mächler [53], increased sample volumes led to higher detection rates for macroinvertebrate species such as *Anguilla fluviatilis* and *Baetis buceratus* which benefits from larger volume that suggest a minimum of 1 L water required to be collected for optimal results. Similarly, Margaret [54] has discovered that increasing the filtered water volume by four times resulted in a 4.4X increase in target DNA yield. Higher eDNA yields were likely resulted from filtering larger water volumes containing organism-shed particles in the blackwater peat swamp which increase the chances of detecting DNA even in low-concentration environments [48 , 49].

Table 3. Summary of sampling stations, membrane designations, volume of water filtered water per replicate (mL), DNA purity ratio (A260/A280) and DNA concentration from blackwater peat swamp samples

Sampling station	Membrane designation (replicates)	Volume of water filtered per replicate (mL)	DNA purity $A_{260/A280}$ ratio	Concentration of DNA (ng/μL)
A	A1	420	1.667	7.66
	A2	420	1.680	7.44
	A3	420	1.981	12.0
	A4	370	2.571	3.63
	A5	370	N/A	N/A
B	B1	480	2.143	32.00
	B2	480	1.834	38.22
	B3	400	1.887	19.50
	B4	340	N/A	N/A
	B5	300	N/A	N/A
C	C1	460	2.0	14.00
	C2	450	1.942	9.10
	C3	400	1.849	11.00
	C4	360	N/A	N/A
	C5	330	N/A	N/A

N/A: Not applicable

The higher DNA concentrations observed in replicates B1, B2 and B3 which were obtained from the middle stretch of the blackwater peat swamp may also be attributed to the moderate and consistent water flow within this area. This likely enhanced the transport and dispersal of eDNA throughout the water column. The finding of Wood [57] on the eDNA dynamics of *Salmon salar* juveniles further supports this statement with the concept of predictable plume behaviour, with eDNA initially concentrating in the midstream before being dispersed towards stream margins. A similar finding by Jeunen [7], reported that eDNA tends to concentrate in areas with minimal water movement such as the middle stretch of the swamp where conditions support higher concentrations. This aligns with our findings in the middle stretch of the blackwater swamp (Station B), where consistent water flow helps in maintaining a higher concentration of eDNA. In contrast with replicates C1, C2 and C3 from Station C which exhibited low DNA concentration which was likely due to dilution effects and stagnant water conditions at lower stretch of blackwater peat swamp. The accumulation of organic matter might interfere with eDNA preservation and detection, potentially contributing to the observed variations in DNA concentrations across replicates [58].

However, Station A documented most of the lowest DNA concentrations (A3: 420 mL, 12.0 ng/µL; A1: 420 mL, 7.66 ng/µL) despite filtration volumes exceeding 400 mL per replicate. This was presumably influenced by site-specific hydrological and ecological conditions. Station A, located in the upper stretch of the blackwater peat swamp features by slow and intermittent water flow that lead to spatially uneven distribution of eDNA and localized accumulation zones [51 , 52]. Additionally, the upper stretch of blackwater peat swamps is characterized by low dissolved oxygen levels, which limit the presence of oxygen-sensitive taxa such as certain cyprinids and labyrinthine gouramies (e.g., *Boraras spp., Trichogaster spp.*) that typically inhabit more oxygen. In this study, replicate A5 has the same filtered volume as replicate A4 (370 mL) but no DNA detected possibly due to the inherently stochastic distribution of extracellular DNA at low concentrations [2]. This phenomenon also occurs in five membranes (A5, B4, B5, C4, C5) where no DNA were successfully detected using Nanodrop spectrophotometer. Bottle neck effect occurs when actual number of target DNA molecules is very low, even slight differences in sampling or pipetting can lead to detection in one replicate and not the other [54]. Additional factors including variability in filtration such as particulate matter interfere with how consistently DNA is retained across filters and sample-specific degradation may also contribute to detection discrepancies as similarly reported by [51, 52]. Hence, filtering larger water volumes may be beneficial for increased eDNA yield depending on the experimental system, as it accounts for the stochastic properties of source water containing only minute amounts of eDNA and improves the precision of detection estimates [54].

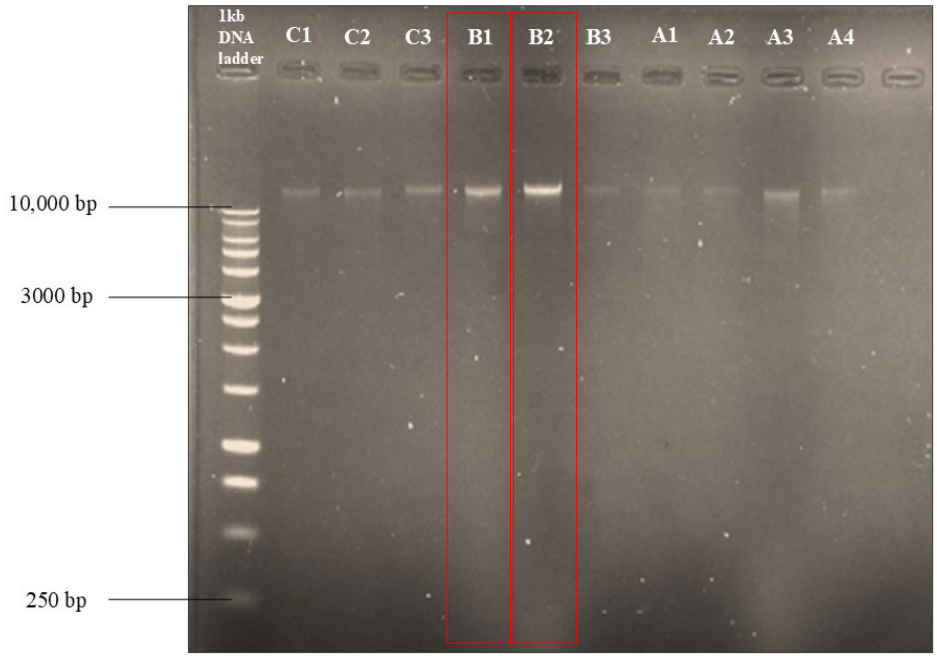
Meanwhile, the A260/A280 ratio that used in nucleic acid quantification to evaluate the presence of contaminants has observed six of the membranes had ratios ranging from 1.8 to 2.0 (A3: 1.981, B2: 1.834, B3: 1.887, C2: 1.942, C3: 1.849, and C2: 2.0) indicating high purity of genomic DNA [63]. This demonstrated that cutting the MCE filter membrane into smaller fragments was crucial in extraction protocol. This is due to the filter matrix possesses a three-dimensional fibre network capable of capturing significant particulate matter, including eDNA-containing biological cells, within its porous structure, entrapping eDNA within the matrix rather than solely on its surface [37 , 40]. This fragmentation also effectively increases the surface area available for reagent interaction, thus facilitating reagent penetration into the filter [58 , 59]. Additionally, extending the incubation period of MCE fragments in the lysis buffer ensures the complete breakdown of cellular structures, thereby maximising eDNA release [66]. Two samples exhibited elevated A260/A280 ratios of 2.143 (B1) and 2.571 (A4), which could be due to RNA contamination, DNA degradation, or the presence of contaminants like polyphenols [47]. RNA contamination is common in eDNA extractions. As RNA absorbs more strongly at 260 nm than DNA, this may have caused the elevated ratio. In addition, DNA degradation can also cause an elevated ratio, as the breakdown of DNA may alter its absorption characteristics [67]. In addition, contaminants may also be introduced through direct contact with non-sterile surfaces, cross-contamination between samples, or environmental factors such as airborne particles and microorganisms, thereby compromising the integrity of eDNA extraction [5 , 62].

Visualization of Genomic DNA by Agarose Gel Electrophoresis

Gel electrophoresis was performed to assess the quality and integrity of the extracted genomic DNA. This technique allows for the separation of DNA fragments based on size which aids in evaluating the purity and overall integrity of the DNA sample [69]. Figure 5 shows 10 bands that appeared above the top band of the 1 kb ladder which corresponds to DNA fragments larger than 10,000 base pairs (bp). This result indicates that the extraction yielded intact and high molecular weight DNA from the samples. Particularly, lanes B1 and B2 which corresponded to DNA concentrations of 32.00 ng/µL and 38.22 ng/µL respectively, displayed the brightest and most well-defined bands. Thus, suggesting that these samples were of high quality and had undergone effective DNA extraction [28]. These findings highlight

the importance of an efficient DNA extraction protocol to ensure effective cell lysis and purification while enabling the recovery of high-quality DNA from the captured material. [70]. Removing inhibitors such as humic acids and tannins from environmental samples are crucial to avoid interference with DNA migration and to preserve intact genomic DNA, resulting in bright bands on gel electrophoresis [71]. Additionally, high eDNA yields were likely result from filtering larger water volumes containing organism-shed particles in the blackwater peat swamp, increasing the chances of detecting DNA even in low-concentration or dispersed environments [1, 49]. However, filtering larger volumes may pose technical challenges including an increased risk of contamination and potential eDNA degradation due to prolonged filtration time. [72]. Hence, the appropriate volume of filtration samples should be determined considering those difficulties and risks: the 2-L filtration of blackwater peat swamp from Dungun, Terengganu have been demonstrated to be suitable in the present study.

The faint bands observed in lanes B3, A1, A2 and A4 suggest low DNA yields, which may be due to inhibitors or challenges inherent in extracting eDNA from complex environmental matrices like blackwater peat swamp [73]. The complex matrices such as water, soil or sediment can occasionally provide low levels of DNA, especially if the target organisms are few or there is severe degradation owing to environmental conditions such as UV exposure and microbial activity [66 , 67]. Notably, lane B3 exhibited a fainter band than lane C3 despite having a higher DNA concentration of 19.5 ng/µL. This observation suggests that the DNA in B3 may have been fragmented or partially degraded, while the brighter band in C3 indicates better genomic DNA integrity even with a lower measured concentration. Similar findings were reported by Hunter [54], where high spectrophotometric DNA readings did not always correspond to intact DNA, as degradation often results in faint or smeared bands in gel electrophoresis. Meanwhile, the absence of a band in the negative control lane indicates the absence of contamination and non-specific amplification. This is essential to validate the reliability and accuracy of the electrophoresis analysis and ensures that the observed bands in the experimental lanes are specific to the eDNA extracted from the water samples [76].



Note: C1; 460 mL, C2; 450 mL, C3; 400 mL, B1; 480 mL, B2; 480 mL, B3; 400 mL, A1;420 mL, A2; 420 mL, A3; 420 mL, A4; 370 mL

Figure 5. Successful genomic eDNA visualisation of water samples from 3 stations (A, B, and C) following volume of water filtered.

The findings from this study are comparable to the study by Nur [48] which utilized freshwater sample (Table 4). In the present study, Sample 1 (32.00 ng/µL) and Sample 2 (38.22 ng/µL) from the blackwater peat swamp exhibited higher DNA concentrations compared to Sample 1 (27.80 ng/µL) and Sample 2

(15.90 ng/μL) from the freshwater ecosystem. This difference is primarily attributed to difference in the volume of water filtered between these ecosystems. The larger water volume processed from blackwater swamp samples (480 mL for Sample 1 and Sample 2) compared to freshwater samples (200 mL) likely enhanced DNA recovery, leading to higher measured concentrations. Increased water volume improves the probability of capturing environmental DNA (eDNA), particularly in ecosystems where DNA may be heterogeneously distributed or susceptible to rapid degradation [77]. Additionally, the DNA purity values (A260/A280 ratios) for most freshwater samples exceeded 2.0, indicating potential contamination or impurities compared to the blackwater peat swamp samples. This discrepancy may be attributed to insufficient incubation time during the lysis buffer step, which could have resulted in incomplete cell disruption and inefficient removal of contaminants [78]. As a result, incomplete lysis may lead to higher A260/A280 and A260/A230 ratios due to the presence of residual proteins, phenolic compounds, or polysaccharides, ultimately affecting DNA purity [70].

Table 4. Summary of sampling stations, site descriptions, membrane designations, filtered water volume, purity ratio (A260/A280), and DNA concentration from blackwater peat swamp samples

	Volume of water filtered (mL)		DNA concentration (ng/μL)		DNA Purity (A260/280)	
Sample source	Blackwater peat swamp	Freshwater	Blackwater peat swamp	Freshwater	Blackwater peat swamp	Freshwater
Sample 1	480	200	32.00	27.80	2.143	2.17
Sample 2	480	200	38.22	15.90	1.843	2.30
Sample 3	300	200	N/A	6.91	N/A	1.77
Sample 4	370	200	3.63	26.78	2.571	2.74

Note: N/A = Not applicable

Conclusions

This study confirms that eDNA concentration is significantly influenced by the volume of water filtered, with a strong positive Spearman's correlation coefficient indicating that larger filtered volumes lead to higher DNA concentrations. High DNA purity was observed in most samples, though some deviations suggest potential RNA contamination or degradation. Gel electrophoresis supported these findings by showing brighter DNA bands in samples with higher filtration volumes. Thus, reinforcing the positive relationship between sample volume and DNA yield. However, certain sample replicates with higher water volumes showed lower eDNA concentrations likely due to hydrological factors such as water flow and discharge rates which can influence eDNA distribution. These results highlight the importance of optimised sampling strategies and standardised protocols to improve eDNA-based ecological monitoring and species detection, particularly for rare or elusive organisms. Future studies should explore potential thresholds beyond which increasing the filtered volume no longer enhances DNA yield, as well as the effects of environmental factors like turbidity and microbial activity on eDNA concentration. Implementing best practices in eDNA sampling will enhance data reliability and contribute to more effective biodiversity conservation efforts.

Conflicts of Interest

The authors declared that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work was fully supported by the Malaysian Ministry of Higher Education through the Fundamental Research Grant Scheme (Ref. No: FRGS/1/2023/WAB11/UITM/02/3), administered by Universiti Teknologi Mara (UiTM) in Shah Alam, Selangor.

References

[1] Wu, P., *et al*. (2024). *Water filter: A rapid water environmental DNA collector in the field. Frontiers in Environmental Science*, 1–11. https://doi.org/10.3389/fenvs.2024.1415338.

[2] Goldberg, C. S., *et al.* (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution, 7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595.

[3] Suarez-Bregua, P., *et al*. (2022). Environmental DNA (eDNA) for monitoring marine mammals: Challenges and opportunities. *Frontiers in Marine Science*, 1–9. https://doi.org/10.3389/fmars.2022.987774.

[4] Schenekar, T. (2023). The current state of eDNA research in freshwater ecosystems: Are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiologia, 850*(6), 1263–1282. https://doi.org/10.1007/s10750-022-04891-z.

[5] Di Muri, C., *et al.* (2020). Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. *Metabarcoding and Metagenomics, 4*, 97–112. https://doi.org/10.3897/mbmg.4.56959.

[6] Nordstrom, B., *et al.* (2024). Environmental DNA reflects spatial distribution of a rare turtle in a lentic wetland assisted colonization site. *Environmental DNA, 6*(1), 1–16. https://doi.org/10.1002/edn3.507.

[7] Jeunen, G. J., *et al.* (2019). Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Molecular Ecology Resources, 19*(2). https://doi.org/10.1111/1755-0998.12982.

[8] Handley, L. L., Read, D. S., Hahn, C., & Winfield, I. A. N. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, 25, 3101–3119. https://doi.org/10.1111/mec.13660.

[9] Hallam, J., Clare, E. L., Iwan, J., & Julia, J. (2021). Biodiversity assessment across a dynamic riverine system: A comparison of eDNA metabarcoding versus traditional fish surveying methods. *Environmental DNA*, 1–20. https://doi.org/10.1002/edn3.241.

[10] Lacoursière-Roussel, A., Côté, G., Leclerc, V., & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: A promising tool for fisheries management. *Journal of Applied Ecology, 53*(4), 1148–1157. https://doi.org/10.1111/1365-2664.12598.

[11] Jaqueline, C., *et al.* (2023). Important role of endogenous microbial symbionts of fish gills in the challenging but highly biodiverse Amazonian blackwaters. *Nature Communications*, 14, 1–15. https://doi.org/10.1038/s41467-023-39461-x.

[12] Yule, C. M., Lim, Y. Y., & Lim, T. Y. (2018). Recycling of phenolic compounds in Borneo's tropical peat swamp forests. *Journal of Tropical Forest Science*, 30(2), 256–266.

[13] Hubert, N., et al. (2016). DNA barcoding Indonesian freshwater fishes: Challenges and prospects. *DNA Barcodes, 3*(1). https://doi.org/10.1515/dna-2015-0018.

[14] Sule, H. A., Ismail, A., Noor, M., & Azmai, A. (2016). A review of the ichthyofauna of Malaysian peat swamp forest. *Malaysian Applied Biology, 45*(2), 1–10.

[15] Wittmann, F., *et al.* (2022). A review of the ecological and biogeographic differences of Amazonian floodplain forests. *Water (Switzerland), 14*(21), 1–28. https://doi.org/10.3390/w14213360.

[16] Cheshire, D. L. M. (2015). *A conceptual review of aquatic ecosystem function and fish dynamics in the Lake Eyre Basin (Technical Report Series No. 15/36).* https://doi.org/10.13140/RG.2.2.11685.50406.

[17] Norhisyam, M. S., *et al.* (2012). *Water quality, diversity and distribution of blackwater fishes in selected locations of Raja Musa Peat Swamp Forest Reserve*. Water Quality, Diversity and Distribution of Blackwater Fishes in Selected Locations of Raja Musa Peat Swamp Forest Reserve, June 2014, 1–10.

[18] Azalina, N., *et al.* (2023). Anthropogenic disturbance of aquatic biodiversity and water quality of an urban river in Penang, Malaysia. *Water Science and Engineering.* https://doi.org/10.1016/j.wse.2023.01.003.

[19] Pont, D., Meulenbroek, P., Valentini, A., & Erős, T. (2021). Congruency between two traditional and eDNA-based sampling methods in characterising taxonomic and trait-based structure of fish communities and community–environment relationships in lentic environments. *Ecological Indicators, 129,* 107952. https://doi.org/10.1016/j.ecolind.2021.107952.

[20] Sciencedirect, S., et al. (2012). Fish sampling with active methods. *Fisheries Research, 124,* 1–3. https://doi.org/10.1016/j.fishres.2011.11.013.

[21] Syazwan, M., *et al.* (2022). Peatlands in Southeast Asia: A comprehensive geological review. *Earth-Science Reviews, 232,* 104149. https://doi.org/10.1016/j.earscirev.2022.104149.

[22] Sahu, A., Singh, M., Amin, A., & Mehboob, M. (2025). A systematic review on environmental DNA (eDNA) science: An eco-friendly survey method for conservation and restoration of fragile ecosystems. *Ecological Indicators, 173,* 113441. https://doi.org/10.1016/j.ecolind.2025.113441.

[23] Ruppert, K. M., Kline, R. J., & Rahman, S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation, 17,* e00547. https://doi.org/10.1016/j.gecco.2019.e00547.

[24] Shu, L., Ludwig, A., & Peng, Z. (2020). Standards for methods utilizing environmental DNA for detection of fish species. *Genes, 11(3),* 296. https://doi.org/10.3390/genes11030296.

[25] Liang, Z., & Keeley, A. (2013). Filtration recovery of extracellular DNA from environmental water samples. *Environmental Science & Technology, 47(16),* 9324–9331. https://doi.org/10.1021/es401342b.

[26] Stoeckle, M. Y., et al. (2022). Current laboratory protocols for detecting fish species with environmental DNA optimize sensitivity and reproducibility, especially for more abundant populations. *Environmental DNA, 79,* 403–412.

[27] Kirsch, J. E., *et al.* (2024). Imperfect detection and misidentification affect inferences from data informing water operation decisions. *North American Journal of Fisheries Management, 44(2),* 335–358. https://doi.org/10.1002/nafm.10974.

[28] Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). *Methods to maximise recovery of environmental DNA from water samples* (pp. 1–22).
[29] Thalinger, B., et al. (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environmental DNA, 3(4),* 823–836. https://doi.org/10.1002/edn3.189.
[30] Jiang, P., *et al.* (2023). Comparison of environmental DNA metabarcoding and bottom trawling for detecting seasonal fish communities and habitat preference in a highly disturbed estuary. *Ecological Indicators, 146,* 109754. https://doi.org/10.1016/j.ecolind.2022.109754
[31] Takahashi, M., *et al.* (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of the Total Environment, 873,* 162322. https://doi.org/10.1016/j.scitotenv.2023.162322.
[32] Shu, L., Chen, S., Li, P., & Peng, Z. (2022). Environmental DNA metabarcoding reflects fish DNA dynamics in lentic ecosystems: A case study of freshwater ponds. *Fishes, 7(5),* 257. https://doi.org/10.3390/fishes7050257.
[33] Díaz, C., *et al.* (2020). Aquatic suspended particulate matter as source of eDNA for fish metabarcoding. *Scientific Reports, 10(1),* 1–8. https://doi.org/10.1038/s41598-020-71238-w.
[34] Vourka, A., Karaouzas, I., & Parmakelis, A. (2023). River benthic macroinvertebrates and environmental DNA metabarcoding: A scoping review of eDNA sampling, extraction, amplification and sequencing methods. *Biodiversity and Conservation, 32*(13), 4221–4238. https://doi.org/10.1007/s10531-023-02710-y.
[35] Simpfendorfer, C. A., Jaiteh, V. F., Wiley, T. R., Heupel, M. R., White, W. T., & Kyne, P. M. (2016). Environmental DNA detects critically endangered largetooth sawfish in the wild. *Endangered Species Research, 30,* 109–116. https://doi.org/10.3354/esr00731.
[36] Djurhuus, A., Pitz, K., Sawaya, N. A., Rojas-Márquez, J., Michaud, B., Montes, E., Muller-Karger, F., & Breitbart, M. (2017). Evaluation of filtration and DNA extraction methods for environmental DNA biodiversity assessments across multiple trophic levels. *Frontiers in Marine Science, 4,* 314. https://doi.org/10.3389/fmars.2017.00314.
[37] Su, G. Y., Jeffery, N. W., Nelson, R. J., Whelan, K., Smith, C., & Horne, J. B. (2024). Enumeration potential of environmental DNA for Pacific salmon stock assessments. *Environmental DNA, 6*(1), 1–20. https://doi.org/10.1002/edn3.508.
[38] Munian, K., Naim, D. M., Tan, M. P., Ibrahim, M., Ismail, M. H., & Esa, Y. (2024). Environmental DNA metabarcoding of freshwater fish in Malaysian tropical rivers using short-read nanopore sequencing as a potential biomonitoring tool. *Molecular Ecology Resources, 24*(4), 1–14. https://doi.org/10.1111/1755-0998.13936.
[39] Foote, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A., Salling, A. B., Galatius, A., Orlando, L., & Gilbert, M. T. P. (2012). Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS ONE, 7*(8), e41781. https://doi.org/10.1371/journal.pone.0041781.
[40] Mathon, L., Dufresne, F., Clusa, L., & Fernández, S. (2021). Benchmarking bioinformatic tools for fast and accurate eDNA metabarcoding species identification. *Molecular Ecology Resources, 21*(7), 2565–2578. https://doi.org/10.1111/1755-0998.13430.
[41] Mariani, S., Fernandez, C., Baillie, C., Magalon, H., & Jaquemet, S. (2021). Shark and ray diversity, abundance and temporal variation around an Indian Ocean island, inferred by eDNA metabarcoding. *Conservation Science and Practice, 3*(6), e407. https://doi.org/10.1111/csp2.407.
[42] Laramie, M. B., Pilliod, D. S., Goldberg, C. S., & Strickler, K. M. (2015). *Environmental DNA sampling protocol: Filtering water to capture DNA from aquatic organisms* (Techniques and Methods 2–A13). U.S. Geological Survey. https://doi.org/10.3133/tm2A13.
[43] Segherloo, I. H., Abdoli, A., Freyhof, J., Arman, H., Mousavi-Sabet, H., & Esmaeili, H. R. (2022). eDNA metabarcoding as a means to assess distribution of subterranean fish communities: Iranian blind cave fishes as a case study. *Environmental DNA, 4*(3), 402–416. https://doi.org/10.1002/edn3.264.
[44] Kawato, M., Kondo, A., Kubokawa, K., & Watanabe, H. (2021). Optimization of environmental DNA extraction and amplification methods for metabarcoding of deep-sea fish. *MethodsX, 8,* 101238. https://doi.org/10.1016/j.mex.2021.101238.
[45] Rieder, J., Jemmi, E., Hunter, M. E., & Adrian-Kalchhauser, I. (2024). A guide to environmental DNA extractions for non-molecular trained biologists, ecologists, and conservation scientists. *Environmental DNA, 6*(5). https://doi.org/10.1002/edn3.70002.
[46] Fujii, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H., & Yamanaka, H. (2019). Environmental DNA metabarcoding for fish community analysis in backwater lakes: A comparison of capture methods. *PLoS One, 14*(1), 1–17. https://doi.org/10.1371/journal.pone.0210357.
[47] Lucena-Aguilar, G., Marí, A., Barbera, C., Carrillo-Á, A., & Lo, A. (2016). DNA source selection for downstream applications based on DNA quality indicators analysis. *BioTechniques, 14*(4), 264–270. https://doi.org/10.1089/bio.2015.0064.
[48] Nur, M. N., Ulayya, N., Azis, M., Maryanto, A. E., & Andayani, N. (2020). Methods to maximize environmental DNA (eDNA) for detection of the presence of Alligator Gar (*Atractosteus spatula*). *IOP Conference Series: Earth and Environmental Science, 538*(1). https://doi.org/10.1088/1755-1315/538/1/012018.
[49] Fernández, S., Rodríguez-Martínez, S., Martínez, J. L., Garcia-Vazquez, E., & Ardura, A. (2019). How can eDNA contribute in riverine macroinvertebrate assessment? A metabarcoding approach in the Nalón River (Asturias, Northern Spain). *Environmental DNA, 1*(4), 385–401. https://doi.org/10.1002/edn3.40.
[50] Schober, P., & Schwarte, L. A. (2018). Correlation coefficients: Appropriate use and interpretation. *Anesthesia & Analgesia, 126*(5), 1763–1768. https://doi.org/10.1213/ANE.0000000000002864.
[51] Deiner, K., Walser, J. C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation, 183*, 53–63. https://doi.org/10.1016/j.biocon.2014.11.018.
[52] Capo, E., Spong, G., Königsson, H., & Byström, P. (2020). Effects of filtration methods and water volume on the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) eDNA concentrations via

droplet digital PCR. *Environmental DNA, 2*(2), 152–160. https://doi.org/10.1002/edn3.52.
[53] Mächler, E., Deiner, K., Spahn, F., & Altermatt, F. (2016). Fishing in the water: Effect of sampled water volume on environmental DNA-based detection of macroinvertebrates. *Environmental Science & Technology, 50*(1), 305–312. https://doi.org/10.1021/acs.est.5b04188.
[54] Hunter, M. E., Ferrante, J. A., Meigs-Friend, G., & Ulmer, A. (2019). Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. *Scientific Reports, 9*(1), 1–9. https://doi.org/10.1038/s41598-019-40977-w.
[55] Wu, J., *et al.* (2018). Spent substrate of *Ganoderma lucidum* as a new bio-adsorbent for adsorption of three typical dyes. *Bioresource Technology, 266*(June), 134–138. https://doi.org/10.1016/j.biortech.2018.06.078
[56] Weitemier, K., Penaluna, B. E., Hauck, L. L., Longway, L. J., Garcia, T., & Cronn, R. (2021). Estimating the genetic diversity of Pacific salmon and trout using multigene eDNA metabarcoding. *Molecular Ecology, 30*(20), 4970–4990. https://doi.org/10.1111/mec.15811.
[57] Wood, Z. T., Lacoursière-Roussel, A., Leblanc, F., & Trudel, M. (2021). Spatial heterogeneity of eDNA transport improves stream assessment of threatened salmon presence, abundance, and location. *Frontiers in Ecology and Evolution, 9*(April). https://doi.org/10.3389/fevo.2021.650717.
[58] Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences, 286*(1915). https://doi.org/10.1098/rspb.2019.1409.
[59] Bird, S., Dutton, P., Wilkinson, S., Smith, J., Duggan, I., & McGaughran, A. (2024). Developing an eDNA approach for wetland biomonitoring: Insights on technical and conventional approaches. *Environmental DNA, 6*(3), 1–19. https://doi.org/10.1002/edn3.574.
[60] Brockerhoff, E. G., et al. (2017). Forest biodiversity, ecosystem functioning and the provision of ecosystem services. *Biodiversity and Conservation, 26*(13), 3005–3035. https://doi.org/10.1007/s10531-017-1453-2.
[61] Holmes, A. E., Baerwald, M. R., Rodzen, J., Schreier, B. M., Mahardja, B., & Finger, A. J. (2024). Evaluating environmental DNA detection of a rare fish in turbid water using field and experimental approaches. *PeerJ, 12*, 1–26. https://doi.org/10.7717/peerj.16453.
[62] Holman, L. E., de Bruyn, M., Creer, S., Carvalho, G., Robidart, J., & Rius, M. (2019). Detection of introduced and resident marine species using environmental DNA metabarcoding of sediment and water. *Scientific Reports, 9*(1), 1–10. https://doi.org/10.1038/s41598-019-47899-7.
[63] Chong, G. O., Han, H. S., Lee, S. D., & Lee, Y. H. (2020). Improvement in RNA quantity and quality in cervico-vaginal cytology. *Virology Journal, 17*(1), 1–7. https://doi.org/10.1186/s12985-020-1282-x.
[64] Smith, T. B., & Skúlason, S. (1996). Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annual Review of Ecology and Systematics, 27*, 111–133. https://doi.org/10.1146/annurev.ecolsys.27.1.111.
[65] Jo, T. S. (2023). Utilizing the state of environmental DNA (eDNA) to incorporate time-scale information into eDNA analysis. *Proceedings of the Royal Society B: Biological Sciences, 290*(1999). https://doi.org/10.1098/rspb.2023.0979.
[66] Ruan, H., *et al.* (2022). Effects of sampling strategies and DNA extraction methods on eDNA metabarcoding: A case study of estuarine fish diversity monitoring. *Zoological Research, 43*(2), 192–204.
[67] Olson, N. D., & Morrow, J. B. (2012). DNA extract characterization process for microbial detection methods development and validation. *BMC Research Notes, 5*(1), 1. https://doi.org/10.1186/1756-0500-5-668.
[68] Macher, T. H., Schütz, R., Yildiz, A., Beermann, A. J., & Leese, F. (2023). Evaluating five primer pairs for environmental DNA metabarcoding of Central European fish species based on mock communities. *Metabarcoding and Metagenomics, 7*, 217–245. https://doi.org/10.3897/mbmg.7.103856.
[69] Lee, P. Y., Costumbrado, J., Hsu, C., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, (April), 1–5. https://doi.org/10.3791/3923.
[70] You, A., Be, M. A. Y., & In, I. (2021). Gel electrophoresis: The applications and its improvement with nuclear technology. *AIP Conference Proceedings, 050008*(April).
[71] Hamedi, J., Danaiefar, M., & Moghimi, H. (2016). Rapid and efficient method for environmental DNA extraction and purification from soil. *Journal of Microbiology, Biotechnology and Food Sciences, 5*(6), 530–533. https://doi.org/10.15414/jmbfs.2016.5.6.530-533.
[72] Yoshida, T., Kawato, M., & Fujiwara, Y. (2023). Optimization of environmental DNA analysis using pumped deep-sea water for the monitoring of fish biodiversity. *Frontiers in Marine Science*. https://doi.org/10.3389/fmars.2022.965800.
[73] Petit-Marty, N., Casas, L., & Saborido-Rey, F. (2023). State of the art of data analyses in environmental DNA approaches towards its applicability to sustainable fisheries management. *Frontiers in Marine Science*, 1–16. https://doi.org/10.3389/fmars.2023.1061530.
[74] Takahashi, M., et al. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of the Total Environment, 873*, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322.
[75] Mauvisseau, Q., Harper, L. R., Sander, M., Hanner, R. H., Kleyer, H., & Deiner, K. (2022). The multiple states of environmental DNA and what is known about their persistence in aquatic environments. *Environmental Science & Technology, 56*(9), 5322–5333. https://doi.org/10.1021/acs.est.1c07638.
[76] Deng, J., Zhang, H., Wang, Q., Kong, F., Zhao, H., & Zhang, L. (2023). An optimized environmental DNA method to improve detectability of the endangered Sichuan taimen (*Hucho bleekeri*). *Fishes*. *8*(7), 339. https://doi.org/10.3390/fishes8070339.
[77] Sint, D., Kolp, B., Rennstam Rubbmark, O., Füreder, L., & Traugott, M. (2022). The amount of environmental DNA increases with freshwater crayfish density and over time. *Environmental DNA, 4*(2), 417–424. https://doi.org/10.1002/edn3.249.
[78] Roopnarain, A., Mukhuba, M., Adeleke, R., & Moeletsi, M. (2017). Biases during DNA extraction affect bacterial and archaeal community profile of anaerobic digestion samples. *3 Biotech, 7*(6), 1–12. https://doi.org/10.1007/s13205-017-1009-x.