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Flood-Pulse Effects on Environmental DNA Detection of Fish Biodiversity in the Kavango River

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Abstract

Seasonal flooding changes fish habitat availability and the transport, retention and recovery of genetic material, complicating environmental DNA assessments in connected river–floodplain systems. This review examines how these processes may influence fish biodiversity detection in the Kavango River and develops a framework for interpreting seasonal surveys. Published regional research is evaluated alongside studies of DNA transport, molecular bias and imperfect detection. A deterministic model separates source production, discharge dilution, recovery efficiency and repeated sampling; its numerical outputs are illustrative and do not represent Kavango field observations. Under a constant source, a fourfold discharge increase reduces relative concentration to one quarter and lowers modelled single-sample detection from 0.777 to 0.313. Three

independent samples increase detection to 0.989 under baseline conditions and 0.675 under the diluted scenario. These calculations show why changes in detections cannot be attributed to biodiversity change without accounting for the observation process. We propose sampling the rising, high-water, falling and low-water phases across channel, channel-margin and connected floodplain habitats, supported by replicated water samples, contamination controls, inhibition testing and regional reference sequences. Flooding may increase the spatial coverage of DNA while weakening local attribution and reducing recoverable concentration. Hydrologically explicit monitoring can improve interpretation, but the magnitude and direction of these effects require empirical validation in the Kavango River.

Keywords: Environmental DNA, Fish Biodiversity, Flood Pulse, Kavango River, Hydrological Connectivity, Imperfect Detection

Introduction

Flooding as an Ecological and Measurement Process

The same flood pulse that creates fish habitat also changes the information contained in a water sample. Environmental DNA (eDNA) consists of genetic material recovered from environmental media rather than directly from an isolated target organism. For fish, a water sample may contain material released locally, transported from another habitat, retained on particles or returned from previously deposited sediment. Detection therefore depends on ecological production and movement as well as the procedures used to collect, extract, amplify and identify DNA. In a seasonally connected river, the interpretation of a positive or negative detection must account for this combination of processes ^[1, 2, 5-7].

The flood-pulse concept emphasizes the ecological importance of repeated exchange between a river channel and its floodplain ^[1]. Applied to fish monitoring, it suggests that a fixed point in the channel may receive biological material from different source areas at different water levels. Flooding can connect habitats that are isolated during low water, while recession can concentrate fish and drain floodplain water back into channels. These are plausible mechanisms for seasonal variation in eDNA occurrence; they do not establish a uniform seasonal pattern for every taxon or river reach. Habitat preferences, movement timing, flood duration and local channel form may generate different responses within the same flood cycle.

The Kavango River provides an appropriate setting for examining this problem because seasonal water movement and fish biodiversity are closely related to the wider Okavango system. Existing basin research demonstrates the feasibility of fish eDNA surveys, while a Kavango transect report documents collection of eDNA samples along the Namibian river corridor ^[3].

⁴⁾. Nevertheless, downstream Delta findings cannot simply be applied as numerical expectations for the river near

Nkurenkuru. The two settings differ in channel structure, connectivity and the scale over which water and biological material mix. The present review treats regional research as contextual evidence and uses studies from other rivers to identify mechanisms requiring local testing.

There are two distinct management questions. An inventory asks which fish taxa contribute DNA to the sampled water and its connected source area. A local habitat assessment asks which fish occupy a particular channel or floodplain patch at the time of collection. A transported signal can contribute valid information to the first question while offering limited support for the second. Conversely, a species may occupy a site and remain undetected because its DNA was scarce, the filter captured little material, or amplification failed. Reporting these different questions under a single term such as presence obscures the evidence needed for each inference.

A seasonal monitoring programme should therefore define its unit of inference before it defines its sampling calendar. A reach, a connected habitat complex and a bottle of water are different units. Assigning a positive sequence to the entire reach may be reasonable for broad surveillance, whereas assigning it to a specific nursery habitat requires corroboration. Hydrological measurements make those choices more defensible by describing when source areas were connected and how long material could have travelled before sampling.

Purpose and Contribution

This article develops a hydrologically explicit interpretation of fish eDNA detection for the Kavango River. It integrates ecological hypotheses with a transparent observation model and a proposed monitoring protocol. Its contribution is the connection between flood phase, spatial attribution and detection probability, rather than a claim of newly measured Kavango fish diversity. The practical aim is to help future surveys distinguish a change in fish distribution from a change in the recoverability of fish DNA, and to express uncertainty in terms useful for biodiversity management.

Regional evidence and the transferability problem

A 2023 Okavango Delta study used fish metabarcoding and developed a regional mitochondrial reference resource [3]. It reported 211 reference barcodes covering 74 species and recovered 40 species through metabarcoding. Some closely related taxa could not be separated reliably with the marker. These findings establish regional feasibility and illustrate why a molecular inventory need not recover every known species. They do not provide a seasonal effect size for the Kavango River. Their value here is to identify two requirements for river surveys: regional taxonomic validation and sampling that represents environmental variation rather than a single convenient access point.

The Kavango River Transect 2021 report records eDNA sampling at nine river sites [4]. The existence of this effort prevents the present review from treating river eDNA work as wholly absent. A transect, however, answers a different question from repeated observations through a flood cycle. Seasonal inference requires comparable sites revisited under changing hydrological conditions, with replication sufficient to estimate observation error. Any future use of the transect material should begin with access to its original sample metadata, assay information and quality controls. The review does not reanalyse those samples or assume that their

results represent a complete fish community.

Recent studies outside the basin strengthen the case for a more explicit temporal design. Perry and colleagues showed that river eDNA can retain ecological spatial and seasonal information across intensively sampled systems [9]. Astorg and colleagues evaluated fish metabarcoding alongside electrofishing in a large floodplain and found that the approaches represented communities differently [12]. These studies support repeated surveys and independent validation. They also caution against treating the species list generated by one method as an interchangeable replacement for another. A change in the sampling method can change the apparent community without any ecological change.

The spatial extent of a waterborne signal should be established locally. Stream experiments have identified retention and resuspension as relevant processes, while work on a large European river has demonstrated that DNA can integrate information over substantial distances [6, 8]. These studies concern different flow regimes and substrates. They justify a transport hypothesis, but they do not justify assigning a fixed transport distance to the Kavango. A channel sample taken during high water may represent a larger connected source area than a sample collected from an isolated margin. The extent of that difference remains an empirical question.

Regional reference coverage also limits species-level interpretation. A taxon with an informative, validated reference sequence can be reported more specifically than a closely related taxon lacking an identifiable barcode. Expanding a database may increase named species detections even if the underlying biological community remains unchanged. Consequently, comparisons across years should reprocess all archived sequences against the same reference release. Otherwise, improved annotation can be mistaken for ecological recovery or invasion.

Why contemporary evidence matters

Recent reviews discuss the promise and continuing methodological limitations of freshwater fish eDNA monitoring [11, 15]. Their relevance to the Kavango lies in the need to validate assays and interpretation within the regional fauna. General protocols are useful starting points, but confidence depends on performance with the taxa and sample matrices actually encountered. In addition, regional research capacity affects continuity: a programme must preserve sample histories and analytical expertise if it is to compare several flood cycles [16].

The evidence gap is therefore narrowly defined. Published feasibility studies and general process experiments do not yet establish how flood phase changes detection probability and local attribution for fish in the proposed Kavango monitoring area. Addressing that gap requires ecological observations, hydrological context and technical controls collected together. A large number of sequences cannot compensate for the absence of those links.

Research questions and testable expectations

The first question is whether changes in discharge and lateral connectivity alter the probability of detecting a taxon after habitat and sampling effort are accounted for. The central expectation is conditional: greater discharge can reduce DNA concentration when source production increases more slowly than water flux, whereas additional sources from connected habitats can partly offset that

reduction. This hypothesis allows a null result and a reversal of direction. It avoids assuming that high water necessarily produces either the richest molecular inventory or the weakest signal.

The second question concerns the difference between a broad inventory and local habitat attribution. Floodplain exchange may add taxa to a channel sample while weakening the interpretation that all detected taxa occupy that channel segment. A future study should test whether high-water channel detections include taxa independently observed in connected off-channel habitats. Samples collected before connection, during connection and after separation would be more informative than an undifferentiated wet-season comparison. Where both fish observations and DNA signals change together, local ecological redistribution becomes more plausible; where only the molecular signal changes, transport and observation effects require closer examination.

The third question asks whether the rising and falling limbs produce different detections at similar discharge. Such a difference would indicate hydrological hysteresis, meaning that current water level alone does not determine the system state. Recently inundated vegetation, suspended sediment, fish movement and drainage from storage habitats can differ between those limbs. A comparison matched on discharge but separated by phase would test this expectation. It should include temperature, suspended solids and inhibition measurements so that a change in DNA recovery is not attributed automatically to fish behaviour.

A fourth question is how much replication is required for uncommon taxa. The appropriate effort depends on the probability of capturing and identifying their DNA, which may change seasonally. The expected benefit of additional samples can be calculated under specified assumptions, but the assumptions must be evaluated with pilot data. Repeated bottles from the same mixed water mass may add less information than samples from distinct microhabitats. Laboratory replication addresses another component of uncertainty and cannot substitute for spatially independent water samples.

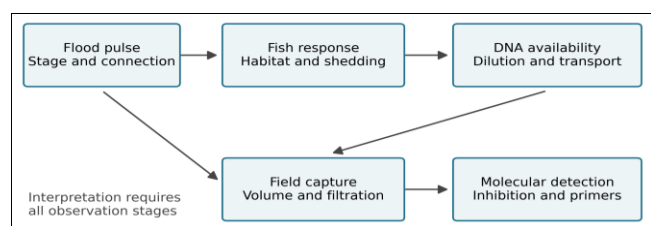


Fig 1: Conceptual pathways linking the flood pulse to fish DNA detection. Arrows identify plausible dependencies, not estimated causal effects. The diagram is original and synthesizes mechanisms discussed in references [1, 5-7, 10]

The objectives are to synthesize transferable evidence, derive reproducible illustrative calculations and propose an implementable river–floodplain design. These objectives are linked: the review identifies mechanisms, the calculations clarify their implications and the protocol states what would need to be measured to test them. No hypothesis is claimed to have been confirmed in the Kavango by this manuscript.

Materials and Methods

Study type and evidence selection

We prepared a focused narrative review with an analytical

illustration and a proposed field protocol. The source searches were conducted on 8 October 2026 using combinations of Kavango, Okavango, fish, environmental DNA, flood pulse, discharge, transport, inhibition, metabarcoding and occupancy. Publisher records, primary research articles and institutional publication records were used to verify bibliographic details. Searches emphasized literature published in 2023–2025 and retained older papers when they established a mechanism or statistical method. This was not a registered systematic review or meta-analysis; no exhaustive coverage, formal screening flow or pooled effect estimate is claimed.

Eligible evidence comprised regional fish eDNA research, river transport experiments, floodplain fish monitoring, molecular performance studies and methods for imperfect detection or diversity comparison. Laboratory and non-Kavango studies were retained for mechanism development when their setting was explicit. Studies of marine or lake systems were used only for relevant methodological comparisons, without transferring their quantitative outcomes to the Kavango. The Kavango transect report was treated as regional grey literature and described separately from peer-reviewed experimental evidence [4].

For each retained source, interpretation focused on location, sampling medium, target community, hydrological setting, analytical method and the scope of inference. We distinguished measurements reported by another study from our own proposed applications. Because study designs and response variables differed, we did not pool species richness, detection probabilities or transport distances. The narrative synthesis instead examined where different findings were compatible, where apparent differences could follow from measurement scale and which mechanisms require local validation.

Proposed spatial and temporal design

A future pilot could use six fixed reaches distributed across accessible sections of the Namibian Kavango corridor, including the Nkurenkuru area where feasible. Within each reach, the design would distinguish the flowing channel, the channel margin and a floodplain habitat connected during part of the cycle. Reach coordinates and boundaries would be set after reconnaissance and retained across visits. The six-reach configuration is a planning example, not a report of established sampling stations or a claim that six reaches adequately represent the entire river.

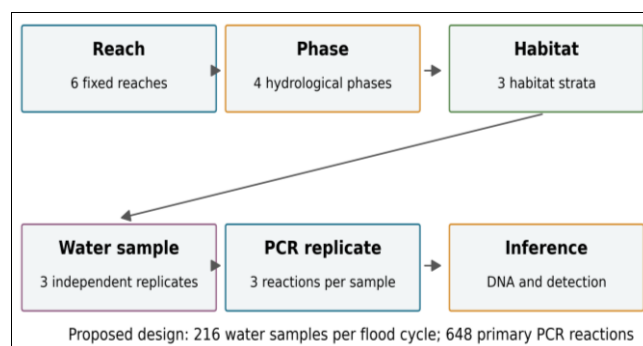


Fig 2: Proposed nested monitoring design. Numbers are planning choices, not completed sampling effort. Six reaches × four phases × three habitat strata × three water samples gives 216 water samples per flood cycle; three primary polymerase chain reactions per sample gives 648 reactions, excluding controls and additional assays

The four phases are rising water, sustained high water, falling water and low water. They would be assigned using the local stage record and observed connection state rather than fixed calendar months. If a floodplain stratum is dry, it should be recorded as structurally unavailable rather than replaced silently with channel water. This distinction preserves the meaning of habitat comparisons and prevents seasonal sampling opportunities from being confused with species absence.

Field collection and hydrological observations

Within every available reach–phase–habitat combination, the proposed protocol collects three independent water samples. Independence should be encouraged through separate collection points and recorded timing, avoiding three aliquots from one container. At channel sites, the chosen cross-section should include a documented margin location and a safely accessible flowing-water point when conditions allow. Water collection should precede any fish capture or sediment disturbance. The location, depth and method of collection must be consistent enough that a seasonal comparison does not mainly reflect a change from bank sampling to mid-channel sampling.

The initial filtration target is one litre per sample through a sterile 0.45 µm membrane. This is a proposed pilot configuration requiring validation, not a demonstrated optimum for the Kavango. Turbid water may prevent the target volume from passing through a filter. The operator should record actual volume and filtration duration, rather than force a clogged membrane or discard the sample without documentation. When several filters are needed for one bottle, they should be recorded as parts of one sample and either co-extracted under a validated procedure or analysed with their nesting retained. They are not additional independent field replicates.

Samples would be filtered on site where possible, with gloves changed between collections and sterile single-use collection equipment preferred. If filtration is delayed, bottles should be cooled immediately, protected from light and filtered within a predefined, pilot-validated holding period. A pragmatic initial target is within six hours, with the actual interval logged. This target is an operational proposal and should be tightened or revised if preservation trials show loss of sensitivity. Preservation method, solution lot, freezer history and transport interval should be recorded for every filter.

At the time of collection, field staff should measure water temperature, dissolved oxygen, conductivity, pH and turbidity with calibrated instruments. Suspended solids should be measured from a paired water sample where resources allow. Stage, recent rainfall, connection state, visible flow direction in connecting channels and evidence of recent inundation should be documented. Discharge should come from a suitable gauging record or a validated local measurement; stage should not be relabelled as discharge without a defensible rating relationship. The record must identify whether the hydrological value is local, interpolated or derived from another station.

Flood phases should be defined before outcome analysis. Rising water requires a sustained positive change in stage and a documented expansion of connected water area; falling water requires a sustained negative change after high water. A stable low-water period is assessed against the local hydrograph. Exact thresholds and averaging windows

should be chosen from reconnaissance and historical records, then frozen in the analysis plan. Multiple classifications can be examined in sensitivity analyses, but selecting phase definitions to maximize a species difference would weaken the inference.

Each sampling day should include at least one field blank, processed with DNA-free water using the same filtration handling. Equipment blanks are required whenever reusable apparatus contacts water. Additional blanks are appropriate after a contamination incident or unusually high-template handling. The field team should keep fish tissues, preserved specimens and amplified DNA away from water-sampling materials. Such separation addresses a known source of avoidable false detections [21].

All filters should receive unique identifiers linked to reach, phase, habitat and replicate. A chain-of-custody record should preserve handling dates and operators. Samples that exceed holding limits, lose their identifiers or undergo documented contamination should be flagged before taxonomic results are inspected. Their exclusion, reanalysis or retention in a sensitivity analysis must follow a stated rule. Ordinary non-detections should remain in the data, because deleting them would bias the estimate of detection probability.

Laboratory processing and taxonomic assignment

The proposed laboratory workflow separates pre-amplification extraction from post-amplification work. Filters should be extracted in randomized batches containing samples from several phases and reaches so that season is not identical to extraction date or plate. Each batch should include an extraction blank and a suitable positive control. A pilot comparison should test extraction performance in clear and turbid river water before selecting the final kit and elution volume. The chosen protocol must then be reported with reagent amounts, incubation times and filter handling details to support reproducibility.

Fish community screening would use a validated 12S mitochondrial metabarcoding assay such as MiFish as a starting point [2]. Before routine use, the laboratory should evaluate primer compatibility with regional references and test representatives of poorly resolved groups. A second marker or species-specific assay may be needed for management-relevant taxa that share the primary barcode. Assay choice should reflect the taxonomic question: a family-level inventory and confirmation of a rare species require different evidence. The existence of a universal primer pair does not guarantee equal amplification efficiency for all fishes.

A candidate primary polymerase chain reaction (PCR) protocol follows the published MiFish framework: 12 µL reactions containing 6.0 µL of 2× high-fidelity mix, 0.7 µL of each 5 µM primer, 2.6 µL of DNA-free water and 2.0 µL of template. The published cycling conditions use initial denaturation at 95 °C for 3 min and 35 cycles of 98 °C for 20 s, 65 °C for 15 s and 72 °C for 15 s, followed by 72 °C for 5 min [2]. These are starting conditions; local validation, inhibitors and laboratory chemistry may justify changes that must be disclosed. Three PCR replicates per extract should initially remain identifiable rather than being pooled before their detection histories can be recovered.

Inhibition should be assessed with an exogenous internal amplification control or a validated spike assay. A positive control in clean water does not demonstrate that a river

extract is uninhibited. The pilot should compare neat template with a specified dilution and, if necessary, a clean-up treatment. Dilution can improve amplification while reducing available template, so its net benefit must be measured. Quantitative PCR used for inhibition assessment or focal species confirmation requires reporting its assay performance, limit of detection, limit of quantification and calibration range.

Indexed libraries should use a documented indexing strategy and include no-template PCR controls, a mock community and blank-derived libraries. A compatible paired-end sequencing configuration should be selected for the amplicon length and verified in a pilot run. Samples from different phases should be balanced across libraries or sequencing runs. Read depth, sequencing failure, control behaviour and contamination screening must be reported before ecological interpretation. Additional sequencing of a failed sample should be recorded rather than replacing its original outcome without explanation.

Sequence processing should remove primers, reject low-quality reads, infer amplicon sequence variants, merge valid read pairs and remove chimeras using documented software and settings. DADA2 provides an established approach to variant inference [18]. This manuscript does not specify an installed software release or a completed pipeline. A future analysis should archive the exact versions, parameters and database release used, together with intermediate quality summaries, so that a change in processing can be distinguished from a biological change.

Taxonomic assignment should prioritize validated regional sequences and retain ambiguous assignments at the lowest defensible rank. A species threshold based only on percentage identity is insufficient when several regional taxa share the marker. Candidate rare or unexpected detections should be checked against controls, replicate consistency and alternative assignments. Reference gaps should be reported as gaps, not converted into species absence. Where annotations are updated, all seasons should be reprocessed under the same taxonomic rules [3, 10].

Analytical model and statistical plan

The illustrative calculations describe a simplified, well-mixed channel receiving a target DNA source. Let S denote the flux of target DNA entering the water per unit time and Q the water discharge. Ignoring transient storage and degradation within the mixing reach, concentration is $C = S/Q$. Define relative discharge $q = Q/Q_0$ and a source response $S/S_0 = q^a$, where a is an assumed exponent. Relative concentration is then $c = C/C_0 = q^{a-1}$. Values $a = 0$, 0.5 and 1 represent a constant source, a sublinear source increase and a proportional source increase, respectively. None is estimated from Kavango observations.

Let λ_0 denote the expected recoverable target count at baseline discharge for one standardized sample, after all capture and analytical losses. Assuming a Poisson target count and detection whenever at least one effective target is recovered, $p = 1 - \exp(-\lambda_0 r c)$, where r is relative recovery efficiency. This idealization collapses extraction, amplification and identification into an effective recovery stage. It assumes specificity and does not model contamination. We set $\lambda_0 = 1.5$ and $r = 1$ for the dilution examples. For n independent samples, the probability of at least one detection is $P_n = 1 - (1 - p)^n$. These inputs are selected to demonstrate the equations and are not calibrated

assay parameters.

The transport illustration uses $C(x)/C(0) = \exp(-x/L)$, where x is downstream distance and L is an effective attenuation length. In a steady one-dimensional advection model with constant velocity u and first-order loss k , $L = u/k$. We plot $L = 1, 5$ and 10 km and an illustrative relative threshold of 0.10 . The purpose is to show sensitivity to attenuation, not to predict a transport distance in the Kavango. Dispersion, tributary input, lateral exchange and episodic resuspension are omitted. Captions and discussion retain those limitations. A future field analysis should keep separate latent variables for DNA occurrence in the sampled reach, DNA capture in a water sample and laboratory detection in a PCR replicate. A possible hierarchy is $z_{its} \sim \text{Bernoulli}(\psi_{its})$, $w_{itjs} | z \sim \text{Bernoulli}(z\theta_{itjs})$ and $y_{itjks} | w \sim \text{Bernoulli}(w p_{itjks})$. Indices denote reach i , occasion t , water sample j , PCR replicate k and taxon s . Here ψ is DNA occurrence, not automatically organism occupancy; θ is sample capture and p is analytical detection. Organism occupancy would require a transport-aware model and independent validation [17, 20].

The DNA occurrence component could include habitat, connectivity, phase and reach effects. Capture could include volume and suspended solids, while laboratory detection could include inhibition, marker, run and assay-specific performance. Identifiability should be examined through simulated pilot designs before fitting many correlated covariates. Uncertainty intervals, convergence diagnostics and predictive checks should accompany estimates. False-positive extensions require information from controls or independent confirmation; stringent filtering alone does not prove the absence of false positives.

Community comparisons should use a consistent taxonomic resolution and sample completeness. Coverage-based rarefaction provides a defensible option for unequal sampling [22]. Presence-absence dissimilarity is useful when abundance calibration is unavailable. Permutational multivariate analysis may assess community differences, with permutations restricted to the repeated design and dispersion examined separately [23]. No such significance tests are performed on the illustrative curves. The numerical results are deterministic outputs, so confidence intervals and empirical P values would be inappropriate.

Results and Discussion

Results of the evidence synthesis

The review supports a process-based interpretation rather than a universal ranking of flood phases. Regional work establishes that fish eDNA can detect a substantial part of an assemblage and that reference resolution can constrain species identification [3]. River experiments establish that material can be transported and retained, while contemporary monitoring studies show that ecological spatial structure can remain detectable despite movement [6, 8, 9]. These findings are compatible: transport broadens a signal, but broadening does not necessarily erase every habitat or reach difference. The amount of integration depends on conditions and on the scale at which the biodiversity question is asked.

Evidence from different media further shows why sampling context matters. Water and sediment samples represented fish assemblages differently in a Chinese river-lake system [13], while a South African estuarine comparison favoured aquatic eDNA for its particular setting [14]. The present review therefore does not prescribe sediment as universally

more informative or water as universally sufficient. For a flood-pulse study concerned with contemporary channel detections, water provides the primary sampling medium; sediment could be included in a separate investigation of retention and resuspension, with its distinct temporal interpretation preserved.

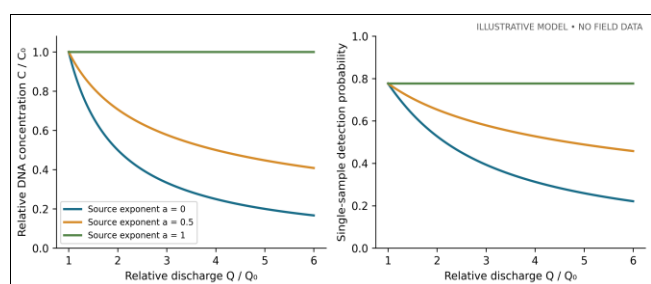


Fig 3: Deterministic dilution and detection curves. Left: $c = q^{a-1}$ for a constant, sublinear or proportional source response. Right: $p = 1 - \exp(-1.5c)$, with relative recovery fixed at one. The source exponent is assumed; no curve is fitted to Kavango data and no uncertainty interval is implied

The analytical illustration identifies a simple condition for declining concentration: source production must increase more slowly than discharge. At four times baseline discharge, the constant-source case yields $c = 0.25$, the sublinear case yields $c = 0.50$ and the proportional-source case yields $c = 1.00$. Flooding can therefore produce the same hydrological change alongside very different concentration responses. The observation cannot determine the source response without additional information about fish distribution, shedding or material entering from connected habitats.

Fig 3 should be read as a set of conditional expectations. It does not demonstrate a seasonal decline in Kavango DNA. It clarifies why an observed decline could occur even without a loss of fish and why additional connected sources could maintain a signal despite dilution. This distinction is essential when a monitoring report describes fewer detections during a large flood.

Dilution and the interpretation of negative detections

With $\lambda_0 = 1.5$ and constant recovery, baseline single-sample detection is $1 - \exp(-1.5) = 0.7769$. Under fourfold discharge and a constant source, the expected recoverable count falls to 0.375 and single-sample detection becomes 0.3127. Three independent samples produce at least one detection with probability 0.9889 at baseline and 0.6753 under dilution. All values follow directly from the specified equations. They quantify a model consequence, not a measured flood effect or an estimate for a particular fish species.

Table 1: Illustrative outputs under fourfold discharge with three independent water samples

Assumed source response	Relative concentration	One sample p	Three samples P_3
Baseline discharge	1.00	0.777	0.989
Constant source $a = 0$	0.25	0.313	0.675
Sublinear source $a = 0.5$	0.50	0.528	0.895
Proportional source $a = 1$	1.00	0.777	0.989

In the constant-source example, a non-detection in one bottle has probability 0.687 during the higher-discharge condition. Treating that bottle as proof of absence would therefore be a poor decision even within the simplified model. A field estimate should be species-specific and should include uncertainties in capture and amplification. The main implication is that the evidential strength of a negative result depends on conditions under which it was obtained. A dry-season non-detection and a flood-season non-detection need not carry the same information.

A useful reporting convention is to accompany negative records with the available detection effort and quality information. For each target taxon, the report should distinguish a valid analysed sample with no target sequence from a failed extraction, an inhibited reaction and a habitat that could not be sampled. These outcomes have different likelihoods. Combining them as zero detections makes a seasonal contrast difficult to interpret and can create the appearance of a decline whenever laboratory or field conditions deteriorate.

An apparent richness decline can arise from the accumulation of species-specific non-detections. If rare taxa begin with low template availability, a modest concentration reduction can remove them from the detected list before common taxa disappear. This produces a shorter inventory without requiring an equal decline in underlying richness. The sensitivity of uncommon taxa should be evaluated through mock communities, targeted assays and replicate histories. A common-taxon signal remaining strong does not demonstrate that sampling was adequate for all fishes.

Dilution is also incomplete as a sole explanation. A flood may recruit new source habitats, stimulate reproductive shedding or redistribute fish into the sampled channel. Under such conditions, source production could rise enough to offset reduced concentration. The constant-source calculation deliberately holds those processes fixed. Empirical monitoring must measure plausible source changes or use independent observations to decide whether this limiting case is informative for a particular reach and phase.

Discharge normalization should therefore be used with care. Multiplying measured concentration by discharge estimates DNA flux under suitable mixing and measurement assumptions, but it does not transform flux into fish abundance. Changes in shedding, degradation or source area still affect the result. DNA concentration and DNA flux should be reported as distinct variables with their assumptions, rather than combined into a biomass interpretation unsupported by calibration.

Transport and the spatial meaning of a positive detection

A positive sequence establishes that target DNA was recovered and assigned under the stated analytical rules. It does not alone specify the distance to the organism producing it. Flood-related velocity changes can alter the distance travelled before loss, and newly connected habitats can add lateral sources. In Fig 4, the attenuation model makes this dependence explicit. The effective length L combines velocity and loss in a simplified representation, while the actual river may contain storage zones and several simultaneous sources.

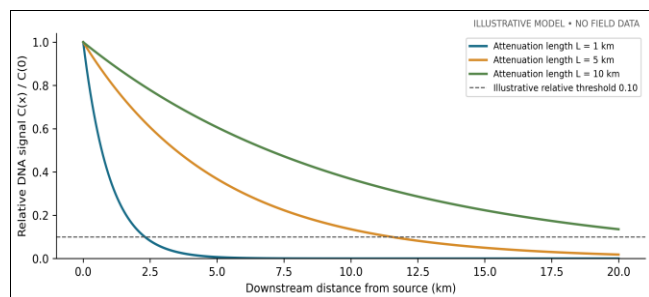


Fig 4: Illustrative downstream attenuation, $C(x)/C(0) = \exp(-x/L)$. Effective attenuation lengths of 1, 5 and 10 km are assumptions. The horizontal line marks an assumed relative threshold of 0.10. The curves demonstrate sensitivity to transport and loss; they are not Kavango transport estimates

The distance at which the illustrative signal falls to 0.10 is $x = L \ln(10)$. It is approximately 2.30 km for $L = 1$ km, 11.51 km for $L = 5$ km and 23.03 km for $L = 10$ km. These calculations show that the same relative detection threshold can correspond to very different spatial footprints. A threshold in actual molecular analysis also depends on source strength, filtered volume, recovery and assay performance. The model threshold therefore cannot be interpreted as an absolute limit of detection or a defensible station-spacing rule.

Local attribution should be assessed by collecting samples across suspected source pathways. For a newly connected floodplain, upstream channel water, the floodplain water body, the connecting outlet and downstream channel water can be sampled within the same visit. A taxon concentrated in the outlet and appearing downstream after connection would support a transport explanation. A taxon found repeatedly in the habitat by an independent method would strengthen organism-level attribution. Neither pattern should be interpreted without considering differences in sample volume and inhibition.

Fish markets, landing sites, processing areas and wastewater inputs are additional candidate sources of exogenous DNA. These should be documented during reconnaissance, especially if a surprising species is detected near a human-use site. Repeat sampling upstream and downstream can help evaluate the source. A control-clean positive remains a real DNA detection but may have a source unrelated to a live local fish. That distinction protects the usefulness of broad surveillance while avoiding an unsupported local habitat claim.

Transport uncertainty need not invalidate eDNA monitoring. It determines which question can be answered at which scale. Reach-level or connected-system inventories can remain useful when local patch attribution is uncertain. Monitoring designs should make that scale explicit rather than presenting all detections as if their spatial precision were identical.

Replication and detection of uncommon taxa

The gain from independent water samples is especially large when per-sample detection is low. Fig 5 evaluates $P_n = 1 - (1 - p)^n$ for $p = 0.15, 0.35$ and 0.65 . These values are arbitrary examples spanning weaker and stronger detection conditions. They are not seasonal estimates or species-specific measurements. The curves help identify what a pilot study must estimate before a final sampling budget is set.

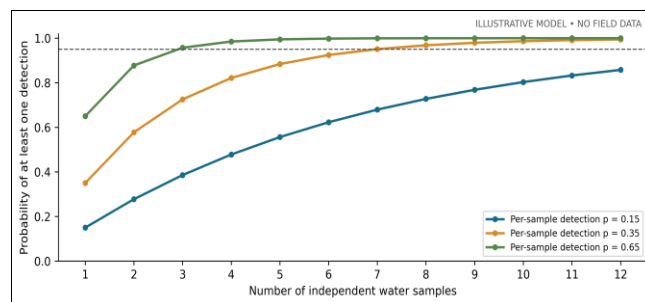


Fig 5: Calculated probability of at least one detection under independent samples. Per-sample probabilities of 0.15, 0.35 and 0.65 are illustrative assumptions. The dashed line indicates 0.95. The curves do not show observed detection rates and do not include field or laboratory correlation

For a target probability of 0.95, the smallest required integer n is the ceiling of $\ln(0.05)/\ln(1 - p)$. The examples require 19 samples when $p = 0.15$, seven when $p = 0.35$ and three when $p = 0.65$. The first requirement lies beyond the displayed range. This contrast explains why a protocol that is adequate for a common taxon may be poorly suited to a rare one. It also shows why simply using three replicates everywhere should be understood as a pilot starting point rather than a guarantee of complete detection.

The independence assumption is consequential. Three bottles taken side by side may share the same patch of DNA-rich or DNA-poor water, and repeated PCR reactions share the same extract. Such correlation reduces the information gained relative to the idealized curves. A future model should retain sample and reaction nesting and examine whether additional microhabitats provide more value than additional aliquots. Lake sampling research offers methods for evaluating effort, although its recommended spatial arrangement cannot be imported directly into a flowing floodplain river [19].

Effort allocation should follow the decision. A broad community baseline can prioritize representation across reaches and habitats. Confirmation of a rare management-relevant taxon may require targeted sampling at likely habitats and additional validated assays. Once a taxon is detected, the inference should still distinguish DNA occurrence from living-organism occupancy. Increasing the number of samples improves sensitivity but can also increase the opportunity to encounter contamination, making controls and confirmation rules more important rather than less.

The proposed six-reach pilot is intended to estimate feasibility and variability. It is not powered in this review to detect a specified ecological effect. A defensible power assessment would use pilot detection histories, realistic correlation and a minimum effect relevant to management. Simulation can then compare extra visits, extra reaches and extra within-visit samples under the same budget. This approach avoids calculating nominal power from an assumed sample size while ignoring the actual observation process.

Recovery efficiency and analytical confounding

Hydrological effects can enter the measurement process through turbidity and inhibitor content. A sample may contain target DNA but produce fewer detectable molecules after filtration, extraction or amplification. Fig 6 uses r to

represent this combined effective recovery. It does not measure chemical inhibition and should not be read as a calibrated efficiency curve. Its purpose is to show that effort and analytical recovery jointly determine detection under the same initial template expectation.

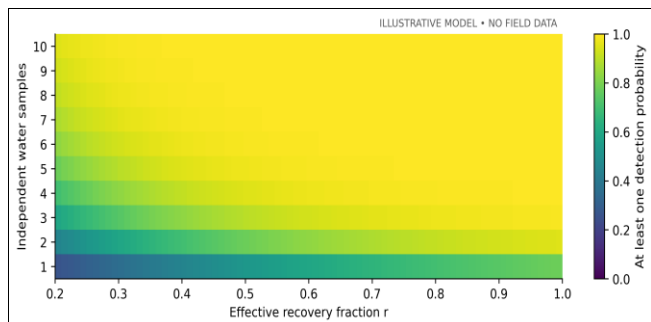


Fig 6: Sensitivity of detection to recovery and effort. The calculation is $P_n = 1 - \exp(-1.5nr)$, where r ranges from 0.20 to 1.00 and n is the number of independent standardized water samples. Discharge is held at baseline. Values are mathematical illustrations; the recovery fraction has not been measured in Kavango samples

At $r = 0.20$, one standardized sample has detection probability 0.259 and three samples have probability 0.593. At $r = 1.00$, the corresponding values are 0.777 and 0.989. Even if DNA production and concentration were unchanged, this difference in recovery would create a strong contrast in observed detections. In practice, r may vary among taxa as well as among samples. An inhibition control can inform one component of the problem, but it does not directly reveal losses on a clogged filter or differences in primer amplification.

Matrix effects require paired technical observations. Actual filtered volume, suspended solids and filtration duration document capture conditions. Internal controls document amplification performance, while mock communities and reference tissues inform taxonomic bias. The combination allows analysts to ask whether a seasonal signal persists after poor-performing samples are accounted for. If high-water samples are disproportionately inhibited, a simple wet-versus-dry richness comparison is partly a comparison of assay performance.

Metabarcoding read counts add another source of uncertainty. Gold and colleagues demonstrated that amplification and stochastic sampling can affect the relation between initial template and final sequence data [10]. The appropriate interpretation of read abundance therefore depends on validation. For this review, presence-absence summaries and detection histories are preferred for the proposed field design. A relative read increase may reflect a competing taxon declining or a primer favouring one lineage, rather than a proportional increase in fish biomass. Standardization should focus on traceable performance rather than forcing identical output. Equal nominal bottle volume does not mean equal filtered volume, and equal sequence depth does not mean equal target recovery. Analysts should retain these measurements and compare alternative quality thresholds. If a result depends entirely on one threshold or one sequencing batch, that dependence belongs in the discussion. Replication and laboratory controls can identify the limitation; they cannot manufacture

an ecological interpretation when the samples contain insufficient usable information.

Flood phase and hydrological hysteresis

The rising limb, high-water period and falling limb should not be treated as interchangeable expressions of a wet season. Rising water opens new connections and can introduce material from recently inundated surfaces. At high water, sustained exchange may broaden the source area while increasing dilution. During recession, drainage from off-channel habitats can provide delayed signals. These are process hypotheses. Their occurrence and relative strength depend on the local flood and should be tested rather than inferred from the calendar.

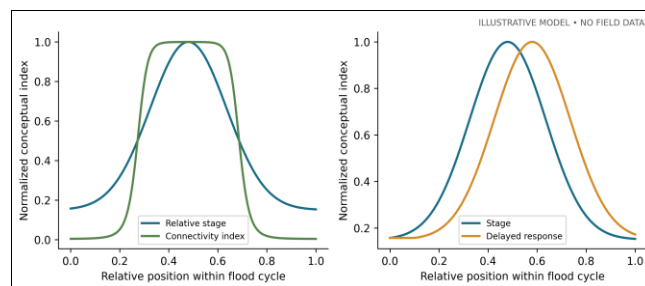


Fig 7: Conceptual flood-cycle indices and a delayed response.

Relative stage is $h(t) = 0.15 + 0.85 \exp[-((t - 0.48)/0.22)^2]$, for relative time t from zero to one. Connectivity is $1/\{1 + \exp[-16(h - 0.50)]\}$; the delayed response is $h(\max[0, t - 0.10])$. These arbitrary functions illustrate timing only and are not a Kavango hydrograph or fish-response prediction

Fig 7 illustrates why a lagged response can differ between rising and falling water even where current stage is similar. The selected lag is one tenth of the normalized cycle; it has no time unit and is not a biological parameter. Actual lag estimation would require visits sufficiently close together to resolve change. Four isolated visits may characterize phases but provide weak evidence for a short delay, so the proposed design should include an intensified subset of reaches around connection and disconnection if hysteresis becomes a principal objective.

Table 2: Mechanistic expectations to test in future observations

Phase	Candidate process	Measurement needed
Rising water	New habitat connection and entrainment	Connection dates, turbidity, paired source samples
High water	Expanded DNA source area and dilution	Discharge, volume, habitat-specific DNA
Falling water	Drainage, fish redistribution and storage release	Outlet direction, lagged detections, fish observations
Low water	Reduced exchange and concentration in residual habitats	Isolation status, temperature, local validation

The distinction between contemporary and stored signals deserves particular attention during recession. Repeated detections after habitat disconnection may reflect surviving local fish, residual DNA, continued exchange not captured by field observations, or resuspended material. A sequence alone cannot decide among these explanations. Combining repeated water samples, sediment measurements where justified and independent fish observations provides a route to discrimination. Absence of one supporting measurement

should limit the claim rather than being filled by a preferred narrative.

Biodiversity metrics and taxonomic uncertainty

Detected richness is the number of taxa recovered under the stated sampling and identification rules. It is an observable survey outcome, while true richness includes taxa that may escape detection. A flood-phase comparison should report both raw detected richness and an uncertainty-aware analysis when sufficient replicate information exists. The taxonomic rank must remain consistent. Counting one lineage as a species in one season and only as a genus in another introduces a methodological change into the richness comparison.

Sequence variants are not interchangeable with fish species. Several variants can occur within one species, and several species can share an assayed barcode. A variant-level change may therefore be informative about molecular composition while remaining inconclusive about species turnover. This is particularly relevant where regional references reveal limited resolution^[3]. Reports should retain the variant data for future reanalysis and present a taxon table stating assignment rank, reference support and any unresolved species complexes.

For repeated samples, incidence-based completeness and diversity estimates can account for uneven detection effort. The proposed use of coverage standardization follows an established principle: compare inventories at comparable completeness rather than assume that equal sample numbers provide equal coverage^[22]. In a floodplain design, however, an unsampled dry habitat is not an ordinary low-coverage water sample. Its structural unavailability must be retained in habitat-level analysis. Mixing those records with sampled non-detections would create a misleading appearance of community loss during low water.

Beta diversity describes differences among assemblages. A high-water decrease in between-reach dissimilarity might reflect greater biological movement, a broader integrated DNA signal or lower recovery of uncommon taxa. These mechanisms make similar predictions for a simple distance matrix. Analysis should therefore test whether patterns persist after excluding low-recovery samples, restricting assignments to well-resolved taxa and comparing habitat-specific subsets. Spatially repeated measurements can reveal a pattern without proving which mechanism produced it.

Ordination is useful for visualizing community differences but does not provide a causal test. A future ordination plot should display the relevant sample unit, confidence summaries where justified and the role of repeated observations. Permutational analysis should use a scheme respecting reach and time, and a separate dispersion assessment should determine whether apparent group differences reflect greater within-group variation^[23]. The present manuscript intentionally provides no invented ordination scores, taxon-by-site matrices or seasonal significance levels.

Interpreting agreement between methods

Agreement with fish capture or visual records can strengthen interpretation, but complete agreement is not expected. Traditional gears sample selected sizes and habitats, whereas eDNA reflects recoverable material in water. A negative capture record may arise from gear selectivity; a positive eDNA record may arise from

transport. The floodplain comparison by Astorg and colleagues illustrates the value of treating methods as complementary observations^[12]. Validation should align their spatial and temporal support as closely as possible before disagreement is interpreted as evidence that either method failed.

For a confirmed local occurrence, the strongest practical evidence may combine repeated control-clean DNA detections with independently identified fish from the same habitat and period. For a broad inventory, repeated DNA detection across a connected reach may be sufficient for a qualified record. An invasive or threatened species claim may justify additional confirmation because its consequences are greater. Reporting categories should reflect those evidential differences, with unresolved detections preserved for further assessment.

Limitations and implications for management

This article provides no direct estimate of the flood-pulse effect in the Kavango River. It uses a focused narrative synthesis rather than an exhaustive systematic review, and its calculations are deliberately simple. The review can identify plausible mechanisms and improve the design of an empirical study, but it cannot establish observed seasonal richness, species abundance, molecular concentration or the best sampling month. Published results from the Okavango Delta and other rivers support feasibility and hypotheses; they are not substitute field data for the proposed study reaches.

The analytical models omit transient hydrodynamics, particle-size variation, exchange with storage zones and explicit degradation during mixing. They assume that effective target counts follow a Poisson process and that samples are independent. Environmental aggregation or technical correlation may violate those assumptions. A modelled detection probability is conditional on the selected source and recovery values. Changing those values changes the outputs. Numerical precision in the calculations does not imply ecological precision in the river.

Several causal processes may vary together through a flood. Higher discharge can coincide with temperature change, fish movement and different filtration performance. A regression of detection on discharge can therefore describe an association while leaving the mechanism unresolved. Independent measurements of ecological source changes and analytical performance improve identifiability, but observational surveys may still leave residual confounding. A future article should state the causal interpretation warranted by its design and report competing explanations where they remain viable.

A single flood cycle would provide a seasonal comparison for that year, with limited generalization to other floods. The relation between hydrology and detection may differ during unusually weak, prolonged or abrupt inundation. Multi-year observations are needed to distinguish a recurring phase effect from one particular event. Long-term continuity also requires archived extracts, sequence data and a stable reference strategy. Otherwise, improved technology or changed personnel may become entangled with the environmental trend.

For management, the most defensible immediate output is a qualified biodiversity inventory accompanied by detection effort, assay quality and hydrological context. A report should identify whether each taxon is a validated local

record, a recurring DNA record for a connected reach or a provisional assignment requiring confirmation. It should avoid converting eDNA reads into fisheries yield or biomass without calibration. These distinctions allow authorities to use evidence appropriately while retaining uncertain records that may become informative later.

Table 3: Suggested reporting categories for future Kavango monitoring

Evidence category	Appropriate statement	Additional requirement
Repeated validated DNA	Taxon DNA detected in the sampled water	State controls, effort and spatial scope
DNA plus local fish record	Local occurrence supported for this period	Align habitat and timing of methods
Single unexpected detection	Provisional molecular record	Repeat sampling and independent assay
Valid non-detection	No target recovered under the protocol	Report detection performance and effort
Failed or unavailable sample	No interpretable molecular result	Retain missingness reason

The programme could contribute to evaluation of habitat connectivity or water-use changes once a reliable baseline exists. Such evaluations should compare like hydrological conditions and retain reference reaches where possible. A detected-richness decline alone would be insufficient evidence that abstraction or fishing caused biodiversity loss. The evidence becomes stronger when repeated biological changes remain after observation effects and natural flood variation are accounted for.

Conclusion

Flood-pulse effects on fish eDNA detection should be interpreted through two connected processes: ecological changes in the distribution and production of DNA, and changes in its transport and analytical recovery. Regional evidence supports the feasibility of fish metabarcoding in the wider Okavango system, but it does not establish a numerical seasonal effect for the Kavango River. The review therefore identifies mechanisms and a testable design rather than presenting an unmeasured fish inventory as an observed result.

The illustrative calculations explain a specific inference problem. When source production remains constant, fourfold discharge reduces relative DNA concentration to 0.25 and lowers single-sample detection from 0.777 to 0.313 under the stated Poisson model. Repeated sampling partly compensates: three independent samples yield probabilities of 0.989 and 0.675 in the baseline and diluted conditions. These values demonstrate how fewer detections can occur without fewer fish. They do not predict that this effect will dominate during an actual Kavango flood, where connectivity and source production may also increase.

Positive detections have a complementary limitation. They can provide valid information about a connected source area while offering uncertain evidence of occupancy at the collection point. Effective attenuation, lateral exchange and storage determine the spatial and temporal support of that information. Neither complete local attribution nor complete spatial homogenization should be assumed. A monitoring programme must establish the scale of its inference through paired habitats, repeated observations and independent validation.

The proposed protocol links hydrological phase to replicated sampling, quality controls and regional taxonomic references. Its practical value lies in preserving the information needed to separate a biological response from an observation effect. Further research should estimate species-specific capture and analytical detection, evaluate transport under contrasting phases and repeat observations across several flood cycles. Those measurements would allow the present framework to become an empirical assessment of the title's central question.

Recommendations

Begin with a pilot spanning the rising, high-water, falling and low-water phases and retain fixed reach boundaries. Record actual filtered volume, local stage, discharge source, connection state and inhibition performance for each sample. Intensify sampling around connection and drainage at a subset of reaches if estimating lagged responses is a priority. Dry habitats and failed assays should be retained as distinct forms of missing information.

Develop a regional reference and validation programme before relying on species-level lists for management. Resolve ambiguous barcodes with additional markers or targeted assays where feasible, and corroborate unexpected or consequential records with repeated sampling. Preserve raw sequences and annotations so that every season can be reprocessed consistently when reference coverage improves. Combine eDNA with appropriately matched traditional observations for questions requiring local fish occupancy or abundance.

Use pilot-informed simulations to allocate effort between reaches, visits and replicates. Report uncertainty and the intended spatial unit alongside richness and taxon lists. A long-term programme should retain shared analytical procedures, documented training and responsibilities for sample custody and reference maintenance. Interpretation should be reviewed jointly by fish ecologists, hydrologists and molecular analysts.

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