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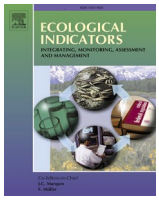

qPCR-based eDNA workflow for humic-rich lake sediments: Combined use of sedimentary DNA (sedDNA) and Indigenous Knowledge in reconstructing historical fish records

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ABSTRACT

Lake sediment serves as a natural archive of historical biological information. The use of sedimentary DNA (sedDNA), a form of environmental DNA (eDNA) shed by aquatic organisms and preserved in sediment, has been instrumental in reconstructing past faunal composition in aquatic communities. However, the low abundance of fish sedDNA and the often humic-rich nature of lake sediments create methodological challenges for the accurate detection of target sedDNA using quantitative polymerase chain reaction (qPCR)-based approaches. Herein, we present a consolidated qPCR-based eDNA workflow to reconstruct past and current fish fauna in Cowpar Lake located in the Oil Sands region in Alberta (Canada), which were then validated using Indigenous Knowledge from Chipewyan Prairie First Nation community members. The present study highlights the importance of combining column- and precipitation-based PCR inhibitor clean-up, nucleic acid concentration, incorporating endogenous chloroplast DNA as a sample integrity control. Robust qPCR-based eDNA assays were also useful in preventing the false-negative detection of low copies of target fish DNA. The presence of Northern pike (1905 to 2019) and Cisco (1919 to 1942) in Cowpar Lake was confirmed based on detected sedDNA from sediment core. The reconstructed fish records from sedDNA-inferred data aligned with the Indigenous accounts of natural and human-mediated changes in land use around the lake. Overall, the present study addresses common methodological concerns in processing lake sediment samples for fish eDNA detection and demonstrates the great potential of combined eDNA-inferred data and Indigenous Knowledge in reconstructing historical fish records in aquatic communities.

1. Introduction

The use of environmental DNA (eDNA) in fish biomonitoring offers an efficient method for detecting the presence of target species in aquatic ecosystems (Boivin-Delisle et al., 2021). In comparison to traditional ecological survey techniques, this method allows for the more sensitive, less intrusive, and inexpensive detection of cryptic low-density species (Boivin-Delisle et al., 2021; Doi et al, 2015; Evans et al., 2017). Studies have recently investigated the potential of using sedimentary DNA (sedDNA) to detect certain fish species in aquatic systems, and to infer the response of the fish faunal composition over time to anthropogenic influences and climate change using lake sediment core samples (Nelson-Chorney et al., 2019; Sakata et al., 2020; Sakata et al., 2022). Comparison between water and sediment samples shows more abundant fish eDNA in sediments (Turner et al., 2015). Extracellular DNA adsorbs to suspended particles and precipitates on the benthic

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floor, preventing DNA from deteriorating and allowing for long-term preservation (Levy-Booth et al., 2007; Pietramellara et al., 2009). Although there has been a handful of studies that have applied sedDNA approaches to examine past fish dynamics (Stager et al., 2015; Baldigo et al., 2017; Buxton et al., 2018; Nelson-Chorney et al., 2019; Kuwae et al., 2020; Olajos et al., 2018; Sakata et al., 2022), only a few of have success in using qPCR approach (Nelson-Chorney et al., 2019; Sakata et al., 2022).

The quantity of DNA shed from the target species, the movement of DNA from the water column to the sediment, and DNA degradation can all impact the detection of fish eDNA in sediment samples (Goldberg et al., 2015; Capo et al., 2021). Mobile meiofauna, such as fish, display significant spatial variability and low biomass, in contrast to bacteria and plankton, which affects the likelihood that DNA from the target organisms would be acquired in an environmental sample (Huston et al., 2023). To increase detection rates in lake sediments, extremely sensitive and robust qPCR-based eDNA assays (such as digital droplet PCR) are needed due to low abundance of fish DNA in aquatic sediments (Huston et al., 2023). Moreover, the presence of high organic matter in lake sediments can lead to low DNA yield and quality due to the presence of humic substances (Thomson-Liang et al., 2022). Co-precipitation of humic substances during DNA extraction may cause inhibition of downstream PCR applications (Sidstedt et al., 2015). As a result, it is essential to overcome frequent methodological difficulties in the PCR-based eDNA procedure to successfully detect fish sedDNA.

Herein, we demonstrate the potential of combined sedDNA-inferred data and Indigenous Knowledge in reconstructing historical fish records in Cowpar Lake located in the Oil Sands region in northeastern Alberta, Canada (Fig. 1). Fish sedDNA is used to assess the effects of long-term natural and human-mediated events on fish fauna in the lake. To do this, we consolidated best practices to provide a more comprehensive qPCR-based workflow for the accurate detection of fish sedDNA. Specifically, robust qPCR-based eDNA assays were designed to detect four freshwater fishes: (1) Lake whitefish [*Coregonus clupeaformis*, Dene name: łú]; (2) Northern pike [*Esox lucius,* Dene name: uldai]; (3) Walleye [*Sander vitreus,* Dene name: ëch'úi]; and (4) Cisco [*Coregonus artedi,* Dene name: dádúë] from a sediment core collected at Cowpar Lake. The sedDNA-inferred data was then validated using Indigenous Knowledge from Chipewyan Prairie First Nation (CPFN) community members, whose long-term relationship with Cowpar Lake continues to this day.

2. Materials and methods

2.1. Sediment core collection and dating

A sediment core (COW21-A) was collected on 27 July 2021 from the deposition basin, location 55.90680 N, 110.45936 W, water depth 3.8 m, with a Pylonex HTH gravity corer and extruded and sectioned lakeside with a procedure developed to prevent cross contamination of samples. Before coring, the corer, 7 cm polycarbonate core tube, and plastic bung and cap were cleaned with 3 % sodium hypochlorite (NaClO) solution and rinsed thoroughly with deionized water (DI) before use. The core recovered was 38 cm in length, with the surface intact and consisting of organic sediment (gyttja) and kept vertical with minimal disturbance until processing. Because extruding (pushing up the sediment) can cause smearing of the core on the core tube, we used a

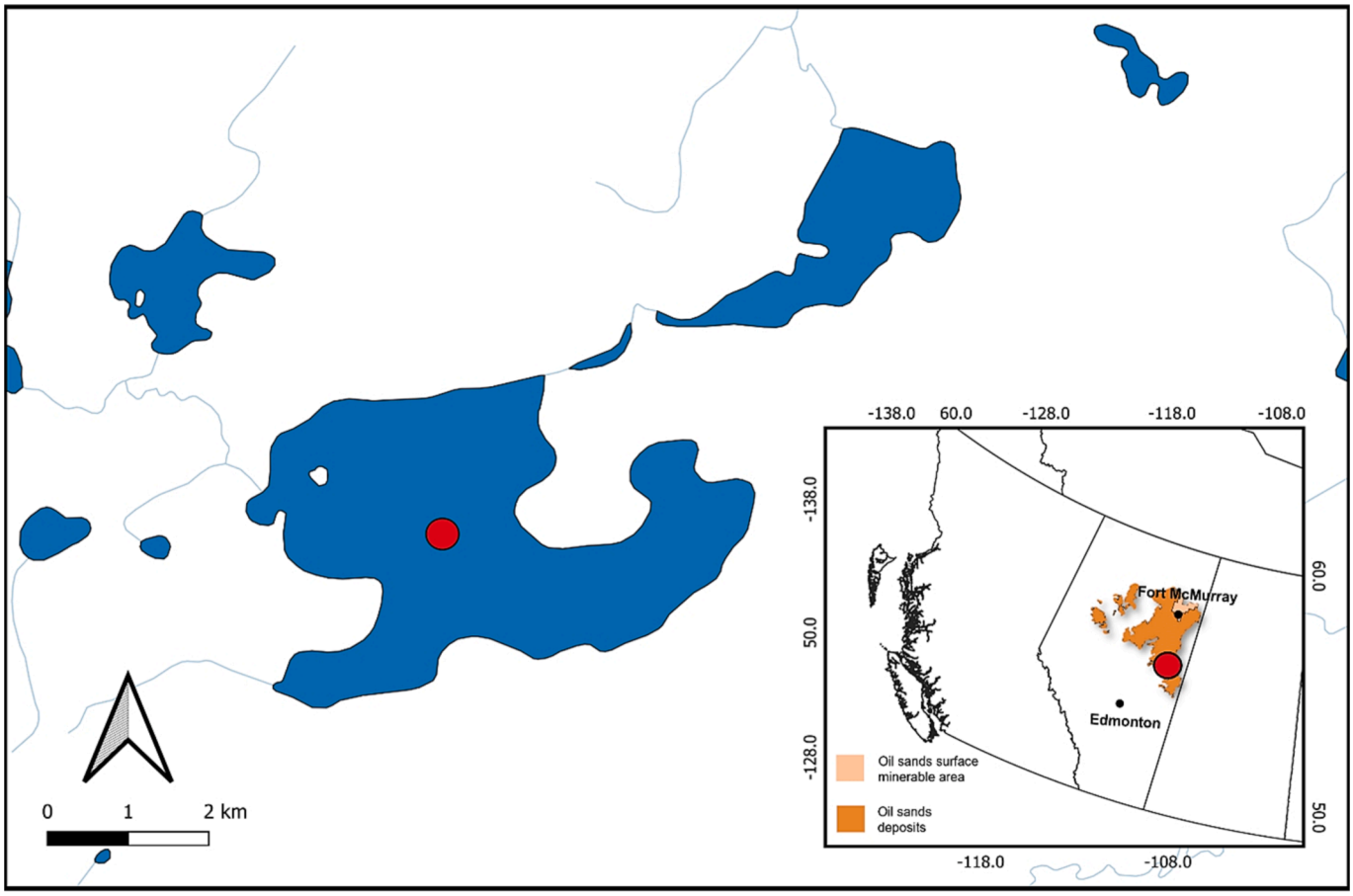

Fig. 1. Location of the sampling site. The sediment core was collected at the center of Cowpar Lake (water depth of 3.8 m; 55.90, −110.45). The circle in the right panel indicates the location of Cowpar Lake within the sand and oil region (orange region) in Alberta, Canada. The dot in the left panel indicates the sampling site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

procedure to prevent cross-contamination of samples, as used by Nelson-Chorney et al. (2019) and briefly described here. First, the Pylonex extruding device, and all implements used were cleaned with 3 % bleach solution and rinsed thoroughly with DI water. Then, an interval of sediment (1 cm intervals for 0–30 cm; 2 cm intervals below 30 cm) was extruded out of the top of the core tube and a plastic scraper was pushed under the bottom of the interval. A metal spatula was used to subsample sediment (on the plastic scraper) that had not touched the core tube and to place the subsample into a falcon tube designated for the analysis of sedDNA. The rest of the sample from the interval was put into a separate container or bag for sediment dating *via* radiochemical analysis. Between intervals, the plastic scraper and metal spatula were again cleaned with 3 % bleach solution and rinsed thoroughly with DI water. All subsamples were frozen (−20 °C) and later shipped to either the University of Victoria (Uvic, British Columbia, Canada) for eDNA analysis or Institut National de la Recherche Scientifique – Eau Terre Environnement Research Centre (INRS-ETE, Quebec, Canada) for radiochemical analysis.

For estimating ages and sedimentation rates for the core, subsamples were freeze-dried, homogenized, and analyzed for Pb-210, Ra-226, and Cs-137 with a high-purity germanium coaxial well detector at INRS-ETE. Data for Pb-210 and Ra-226 were used to model age and sedimentation rates, according to the constant rate of supply (CRS) model (Appleby and Oldfield 1978). Data for Cs-137, an artificial radionuclide introduced to the environment with nuclear weapons testing that began in 1952 and peaked in 1963, was used as a chronostratigraphic marker to validate dates from the CRS model.

2.2. SedDNA extraction and viability testing

Each sediment core section was assigned to a randomized DNA processing number (DPN). Sediment samples were centrifuged at 3,220 × g at 4 °C for 30 min before DNA extraction to remove excess water. Using the soil DNA Isolation Maxi Kit (Cat. 62000; Norgen Biotek, Ontario, Canada), sedDNA was extracted from 2 g of wet weight sediment. A final elution volume of 3 mL was collected. The eluted sedDNA was then concentrated and purified using the modified ethanol precipitation protocol. The DNA pellets were resuspended in 300 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The IntegritE-DNA^{TM} assay targeting endogenous chloroplast DNA was used to assess the presence of any residual PCR inhibitory compounds and ascertain the viability of the extracted sedDNA samples (Veldhoen et al., 2016). Successful amplification of endogenous plant chloroplast DNA contained within the sedDNA samples confirms that the recovered DNA is viable and sufficient inhibitory compounds have been removed. Two μL of sedDNA samples in four technical replicates were analyzed, with eight technical replicates receiving ultrapure distilled water acting as non-template controls, and two technical replicates receiving synthetic plant DNA (250 copies/μL; gBlocks^{TM}, Integrated DNA Technologies [IDT], Iowa, USA) to act as the positive control. The TaqMan thermocycler profile was as follows: initial denaturation of 9 min at 95 °C followed by 50 cycles of 15 s at 95 °C, 30 s at 64 °C, and 30 s at 72 °C. The samples were considered to pass the IntegritE-DNA^{TM} assay if the recorded C_t value is significantly lower (~30 cycles) than the negative control (~33 cycles). The OneStep PCR Inhibitor Removal Kit (D6030; Zymo Research, California, USA) was used for another round of PCR inhibitor removal for samples that failed DNA viability testing. The cleaned-up DNA was stored at − 20 °C until needed for qPCR analysis.

2.3. eDNA assay design and validation

A total of five fish eDNA qPCR-based assays were used in the present study: eFISH1 (general fish DNA, Klymus et al., 2019), eESLU1 (*E. lucius*), eSAVI2 (*S. vitreus*), eCOCL1 (*C. clupeaformis*), and eCOAR7 (*C. artedi*). All assays were designed and validated according to the suggested workflow by Langlois et al. (2021). The mitochondrial genome for target species as well as any closely related and co-occurring fish species were obtained from Genbank. The mitogenomes were then aligned with MAFFT (v7.490, Katoh et al., 2002) and the aligned sequences were used for constructing a phylogenetic tree using RAXml (Stamatakis, 2014). Mitogenomes of target species were run through the Unikseq pipeline (Allison et al., 2023) to identify regions of the mitogenome unique to the target species exclusively. The identified unique region was then used for primer and probe design with Beacon Designer^{TM} 8.21 (PREMIER Biosoft, California, USA). Primer and probe sequences for each eDNA assay used in the present study are listed in Table 1. For *in vitro* specificity validation, SYBR green qPCR (QIAcuity EG PCR Kit [250111, Qiagen, Hilden, Germany]) validation was run using several primer pairs together with target and sympatric species' genomic DNA (gDNA) as a template. The used thermocycler profile is as follows: initial denaturation of 2 min at 95 °C followed by 50 cycles of 15 s at 95 °C, 30 s at 64 °C, and 45 s at 72 °C, followed by a melt curve in 0.5 °C increments from 65 to 95 °C. The resulting amplicon was then run using gel electrophoresis for amplicon size validation. Afterward, high-end specificity validation with primer and probe was done through TaqMan qPCR (QIAcuity Probe PCR Kit [250101, Qiagen, Hilden, Germany]) using species of interest gDNA as a template having 25 technical replicates per sample. To characterize assay sensitivity, serial dilutions of synthetic DNA amplicon (gBlocks®, IDT) were prepared to construct a standard curve.

Based on the constructed standard curve, eLowQuant was used to calculate the limit of detection (LOD) and limit of quantification (LOQ) based on a modified Binomial-Poisson distribution model (Lesperance et al., 2021). These measurements generally describe the smallest concentration of DNA that can be reliably measured by eDNA assays with reasonable statistical certainty. The LOD from continuous data ($LOD_{continuous}$) was also determined as the lowest copy number where there is a ≥ 95 % detection (Klymus et al., 2019). This $LOD_{continuous}$ indicates the breakpoint for continuous and discontinuous data defining the computational approaches for determining sample copy number. Lastly, the PCR assay efficiency, measuring the ability of the designed primers and probe to amplify the target DNA region for every PCR cycle, was computed for each designed eDNA assay using the equation shown in Supplemental Table S1.

2.4. SedDNA analyses

Following the IntegritE-DNA^{TM} assay, the eDNA samples were run through the general fish detection assay (eFISH1) to establish a background fish presence for each sediment core section. Following the confirmation of the presence of fish eDNA in the sediment layers, several species were identified based on CPFN Indigenous Knowledge for further eDNA analysis. Two species of interest, Cisco (eCOAR7) and Lake whitefish (eCOCL1) were tested as targets, while Northern pike (eESLU1) and Walleye (eSAVI2) were selected as field positive and negative controls, respectively. Each sediment layer sample was analyzed using 16 technical replicates per assay to improve the detection probability of target eDNA (Matthias et al., 2021). Eight replicates received UltraPure-dH_2O (Invitrogen, Massachusetts, USA) acting as a non-template control (NTC), and two replicates received 20 copies/reaction of synthetic target DNA fragment of the appropriate DNA sequence (gBlocks^{TM}, IDT Supplemental Table S1) to act as a positive control for each assay on every 96-well qPCR plate. Each qPCR reaction consisted of two μL of purified sediment eDNA, 700 nM forward and reverse primers, 100 nM TaqMan probe, and 1X of QIAcuity Probe Master Mix (QIAcuity Probe PCR Kit, QIAGEN) for a final reaction volume of 15 μL. The following TaqMan thermocycler profile was used for all assays: initial denaturation of 9 min at 95 °C followed by 50 cycles of 15 s at 95 °C, 30 s at 64 °C, and 30 s at 72 °C. The eDNA concentration (copies/g) of amplified samples was extrapolated from C_t values using the previously generated standard curves. Any calculated eDNA concentrations higher than the LOD and LOQ values of the respective eDNA

Table 1
Summary of primer and probe sequences for qPCR-based environmental DNA assay developed in this study.

Assay	Target species	Common name/ Indigenous name	Sequence type	Sequence 5′ → 3′	Target gene	Amplicon size	Source
IntegritE-DNA™	Plant DNA	General plant	Forward	TCTAGGGATAACAGGCTGAT	*cl-23S*	130	Veldhoen et al., 2016
			Reverse	TGAACCCAGCTCACGTAC			
			Probe	FAM-TTTGGCACCTCGATGTCGG-ZEN/IB			
eFISH1	Fish DNA	General fish	Forward	CACCTAGAGGAGCCTGTTCTA	*mt-rnr1*	153	Klymus et. al., 2019
			Reverse	CTACACCTCGACCTGACGTT			
			Probe	FAM-TATATACCRCCGTCGTCAGCTTACCC-ZEN/IB			
eCOCL1	*Coregonus clupeaformis*	Lake whitefish/łú	Forward	CATCATTCCTCTCATAGCA	*mt-nd2*	162	The present study
			Reverse	ATTGGGTGGGTTAATTGT			
			Probe	FAM-CCATTCTCCAACCAGTCAAGCATTAGT-ZEN/IB			
eESLU1	*Esox Lucius*	Northern pike/uldai	Forward	TCTCCACAGCCTTCTCATC	*mt-cytb*	325	The present study
			Reverse	CCGCCTCAGATTCATTGG			
			Probe	FAM-CTCCTCCTAACAATAATAACCGCCTTCGT-ZEN/IB			
eSAVI2	*Sander vitreus*	Walleye/ëch'úi	Forward	CTCGGGATCTTGTTTCTA	*mt-nd1*	331	The present study
			Reverse	CTGATACTAATTCGGATTCG			
			Probe	FAM-CCTATCAAGCCTAGCAGTCTACTCTATTCT-ZEN/IB			
eCOAR7	*Coregonus artedi*	Cisco/dádúë	Forward	CACCACAAATAGCGTTAG	*mt-nd5*	78	The present study
			Reverse	GTAGCCCTAATATACTCTTCA			
			Probe	FAM-CACACACCACCAACAGTCCC-ZEN/IB			

assay (Klymus et al., 2019; Lesperance et al., 2021) were selected for Sanger sequencing to verify amplicon sequence was that of the desired target species.

2.5. Gathering Indigenous Knowledge from CPFN

Gathering of Indigenous Knowledge from elders and knowledge holders was independently conducted by Dr. Ave Dersch as CPFN's principal archaeologist. Historical accounts of fish species present in Cowpar Lake along with observations of environmental events affecting the lake were collected through personal communications and small group discussions. The list of noted fish species from the past was consolidated with the generational ecosystem changes noted by CPFN community. All discussions and information were gathered before the genetic analysis of sediment core samples. All Indigenous narratives and sedDNA-inferred diversity data were consolidated to reconstruct past and current fish fauna in Cowpar Lake.

2.6. Sediment chemistry

To aid in interpretation of factors affecting fish presence/absence, subsamples of sediment were subject to geochemical analyses. One subsample from each interval was digested and analyzed for elements at Institut national de la recherche scientifique (INRS, Quebec, Canada). Samples were subject to a total digestion of ultra-trace metal grade nitric, perchloric, and hydrofluoric acids (Optima™, Fisher Chemical™, Washington, USA). Major and minor elements were analyzed by ICP-OES with a Varian Agilent Dual View (Agilent Scientific Instruments, USA), per US EPA Method 200.7 (US EPA 1994a). Minor and trace elements were analyzed by ICP-MS with a Thermo iCAP (Thermo Scientific, USA), per US EPA Method 200.8 (US EPA 1994b). Certified reference materials were also analyzed for major, minor, and trace elements, in triplicate, with percent recovery averaging 94 % and 99 % for LKSD-2 (NRCAN) and Buffalo River sediment 8704 (NIST), respectively, among elements. Another subsample from each interval was analyzed for total Hg and organic matter (OM) content using loss on ignition (LOI) at the University of Alberta. Both total Hg and LOI were analyzed with a Milestone DMA-80 (Milestone Srl, Italy) per US EPA Method 7473 and Chen et al. (2015), respectively. MESS-4 (NRCAN) was analyzed in triplicate, with all results within the certified range for total Hg. LOI is a direct measure of organic matter and an indirect measure of inorganic matter, with the equation: inorganic matter (%) = 100 % – LOI (%).

3. Results

3.1. Assay sensitivity

Details on the sensitivity characterization of all eDNA assays designed in the present study are presented in Table 2. The R^2 values of the standard curve calibration for all assays were > 0.98. The calculated PCR assay efficiency values for all assays are above 85 % (86 – 99 %). For limit of detection (LOD) and limit of quantitation (LOQ; n = 16, Supplemental Table S1), the calculated values for all assays range from 0.3 to 3.0 and 0.7 – 3.0 DNA copies/reaction, respectively. The LOD and LOQ measurements describe the smallest concentration of DNA that can be reliably detected and measured, respectively, by each eDNA assay with reasonable statistical certainty, thus increasing the confidence in the reported results from downstream eDNA analyses. Moreover, the designed assays have highly comparable sensitivity with other published fish eDNA assays with reported LOD and LOQ based on discontinuous data (Fig. 2).

3.2. DNA viability testing and fish sedDNA detection

To address the requirement for a false negative control in the eDNA workflow, we tested the presence of amplifiable internal chloroplast DNA in all extracted sedDNA samples using the IntegritE-DNA™ assay. Earlier detection of the target gene (C_t < 30) was noted in all samples other than the no template control containing UltraPure water (C_t > 33). Moreover, the calculated values for DNA copies per sample for all replicates are above the set C_t threshold for IntegritE-DNA™ assay (Fig. 3; LOD and LOQ not shown). These observations indicate that the recovered total DNA is of sufficient quality to evaluate further in the eDNA workflow and can then be run in species-specific qPCR reactions.

Table 2
Summary of sensitivity parameters of the fish eDNA assays used in the present study. The values presented for the limits of detection (LOD) and quantification (LOQ) were computed based on n = 16 technical replicates.

Assay	Target species	Binomial data						Continuous data					Source
		LOD (c/rxn)	LOD 95 % CI Lower	LOD 95 % CI Upper	LOQ (c/rxn)	LOQ 95 % CI Lower	LOQ 95 % CI Upper	$LOQ_{continuous}$ (c/rxn)	Slope	% Efficiency	Y-Intercept	R^2 value	
eFISH1	General fish	3.0	1.8	6.1	4.7	3	9.5	20	−3.52	92	37.39	0.98	Klymus et al., 2019
eCOCL1	*Coregonus clupeaformis*	0.8	0.6	1.3	3.0	2.2	4.8	20	−3.35	98	35.99	0.99	The present study
eCOAR7	*Coregonus artedi*	0.6	0.5	1.1	2.4	1.7	4.0	20	−3.45	95	36.24	0.99	The present study
eESLU1	*Esox lucius*	0.4	0.2	0.7	1.3	0.9	2.6	20	−3.35	99	−3.35	0.99	The present study
eSAVI2	*Sander vitreus*	0.3	0.2	0.5	1.0	0.7	1.8	4	−3.70	86	38.53	0.99	The present study

c/rxn, copies/reaction; CI, Confidence interval; LOD, Limit of detection; LOQ, Limit of quantification.

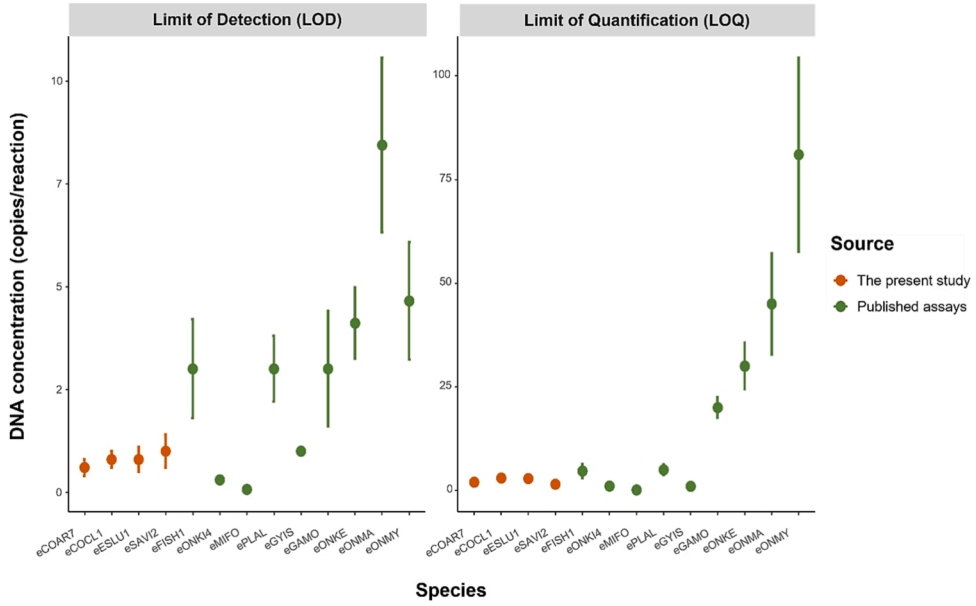

Fig. 2. Calculated limit of detection (LOD) and limit of quantification (LOQ) for the developed assays in the present study (Coregonus artedi, eCOAR7; Sander vitreus, eSAVI2; C. clupeaformis, eCOCL1; Esox lucius, eESLU1) based on a modified Binomial-Poisson model (Lesperance et al., 2021) for n = 16 technical replicates. Published fish eDNA assays: Klymus et al., 2019 (general freshwater fish, eFISH1; Oncorhynchus kisutch, eONKI4); Brys et al., 2021 (Misgurnus fossilis, eMIFO); Sakata et al., 2022 (Plecoglossus altivelis, ePLAL, and Gymnogobius isaza, eGYIS); Salter et al., 2019 (Gadus morhua, eGAMO); and Fu'adil Amin et al., 2021 (Oncorhynchus keta, eONKE; Oncorhynchus masou, eONMA; Oncorhynchus mykiss, eONMY). Note: Values for eGAMO, eONKE, eONMA, and eONMY were based on the continuous linear model.

3.3. Detection of fish sedDNA from sediment core

The results of all fish eDNA analyses performed on the sedDNA extracted from Cowpar Lake sediment core are summarized in Fig. 3. The DNA copy estimates presented in the results are all based on dewatered wet weight (weight after centrifuging) sediment sample. Fish sedDNA was detected in 16 sections of the sediment core that were dated from 1905 ± 8.1 to 2019 ± 1.0, where detection frequency varies for each section (n = 16). The DNA concentrations from sediment layers with detected non-specific fish, ranging from 6.67 to 29.73 copies/g of wet weight sediment sample, are all within eFISH1 LOD (±95 % CI) range. For the field positive control, Northern pike, positive detections were noted in 10 sections of the sediment core spanning from oldest to most recent samples. The estimated DNA concentrations for the wet weight sediment sample range from 4.98 to 28.95 copies/g and do not significantly differ from the eESLU1 LOD (±95 % CI) value. Walleye which serves as the negative field control was not detected in any section of the sediment core with eSAVI2 assay. In terms of the Whitefish species, only Cisco was detected in six sections of the sediment dated from 1919 to 1943. The DNA concentrations range from 4.12 to 967.60 copies/g of sediment sample, where one section (from 1919 sediment section) had value lower than the eCOAR7 LOD and LOQ (±95 % CI). Positive controls containing 20 copies gBlocks®/reaction appropriate for each eDNA assay showed expected amplification of the target amplicon, whereas NTCs resulted in no amplification on every plate.

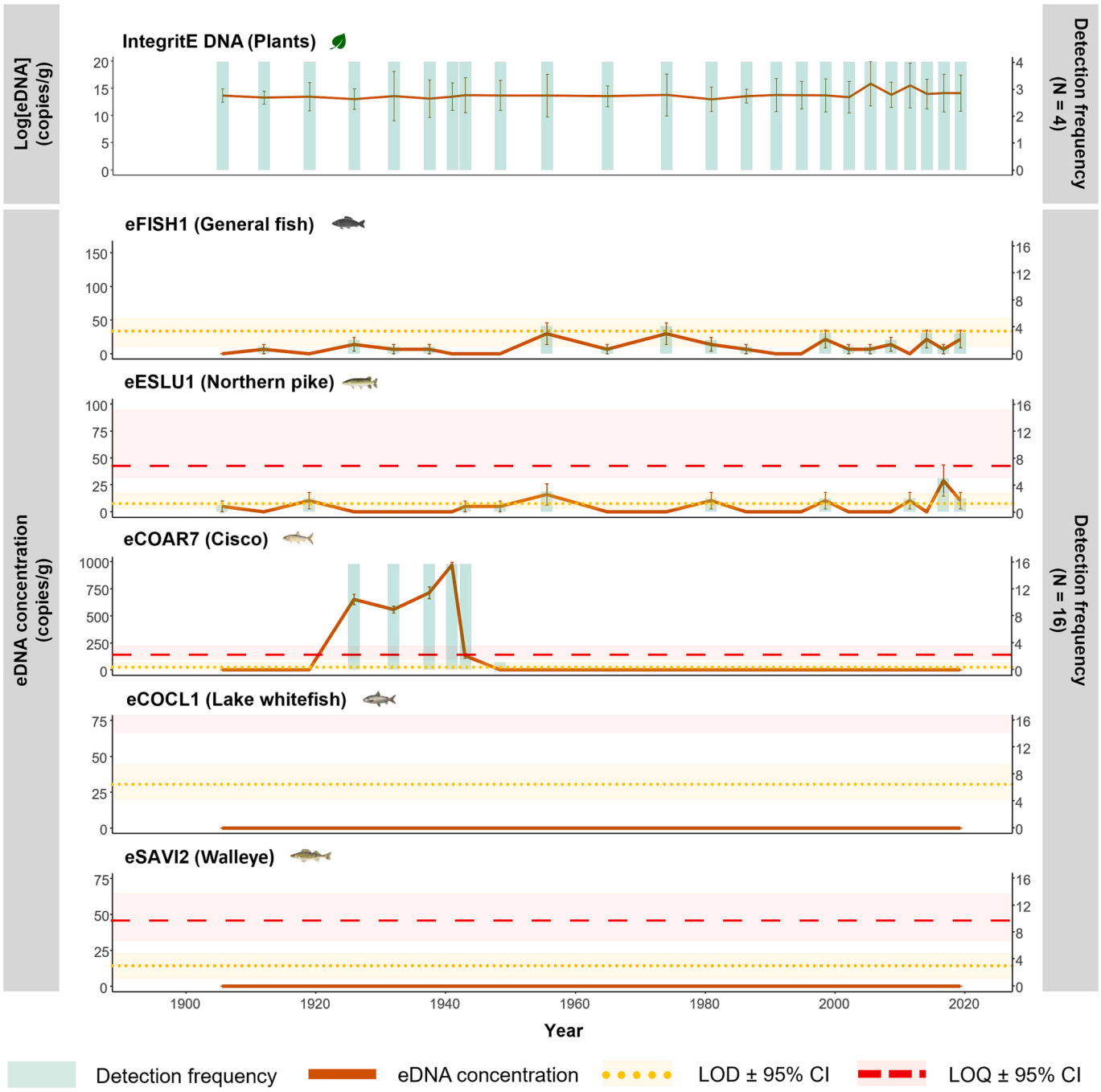

Fig. 3. eDNA concentration (copies/g [wet weight]) and detection frequency (n = 16) for each gram of sediment sample. Dashed lines show the limits of detection (LOD: gray line) and quantification (LOQ: red line) for each assay. Shading around the respective dashed and dotted lines indicate the 95 % CI. Not shown in the figure due to axis scale: eFish1 LOQ: 176.25 (95 % CI Lower limit 112.5, Upper limit 356.25); and eCOCL1 LOQ: 112.50 (95 % CI Lower limit 82.5, Upper limit 180.0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Chronology of Cowpar lake sediment core

The observed stratigraphic profiles of Pb-210 and Ra-226 met the assumptions of the CRS model, yielding reliable estimates for dates and sedimentation rates that are validated by Indigenous Knowledge and Cs-137 data (Supplementary Fig. S7). Total Pb-210 declines with depth, although not simply exponential, indicating there are changes in sedimentation rate in the historical record. Activities of total Pb-210 and Ra-226 are equivalent below 24 cm depth, and the CRS model assigned the dating horizon (24 cm) a date of 1905.6 CE (Fig. 4). Linear sedimentation rates and mass accumulation rates are relatively constant for approximately three decades until a large sedimentation event occurred in the early 1940 s, recorded in the core at depths 17–19 cm. Following this event, sedimentation stabilized to previous (baseline) rates. Post c.1990 CE, linear sedimentation rates and mass accumulation rates are again above baseline and increasing, though not as a short-term event but more as a multi-decadal change to a different (steady or unsteady) state. For further validation of the model estimates, the Cs-137 profile shows a marked increase in activity at 13 – 14 cm depth, dated to 1964.9 CE. Diffusion of Cs-137 (i.e., upward and downward movement) in the sediment column is apparent, however, that is common in lake sediments (e.g., Wang et al. 2017).

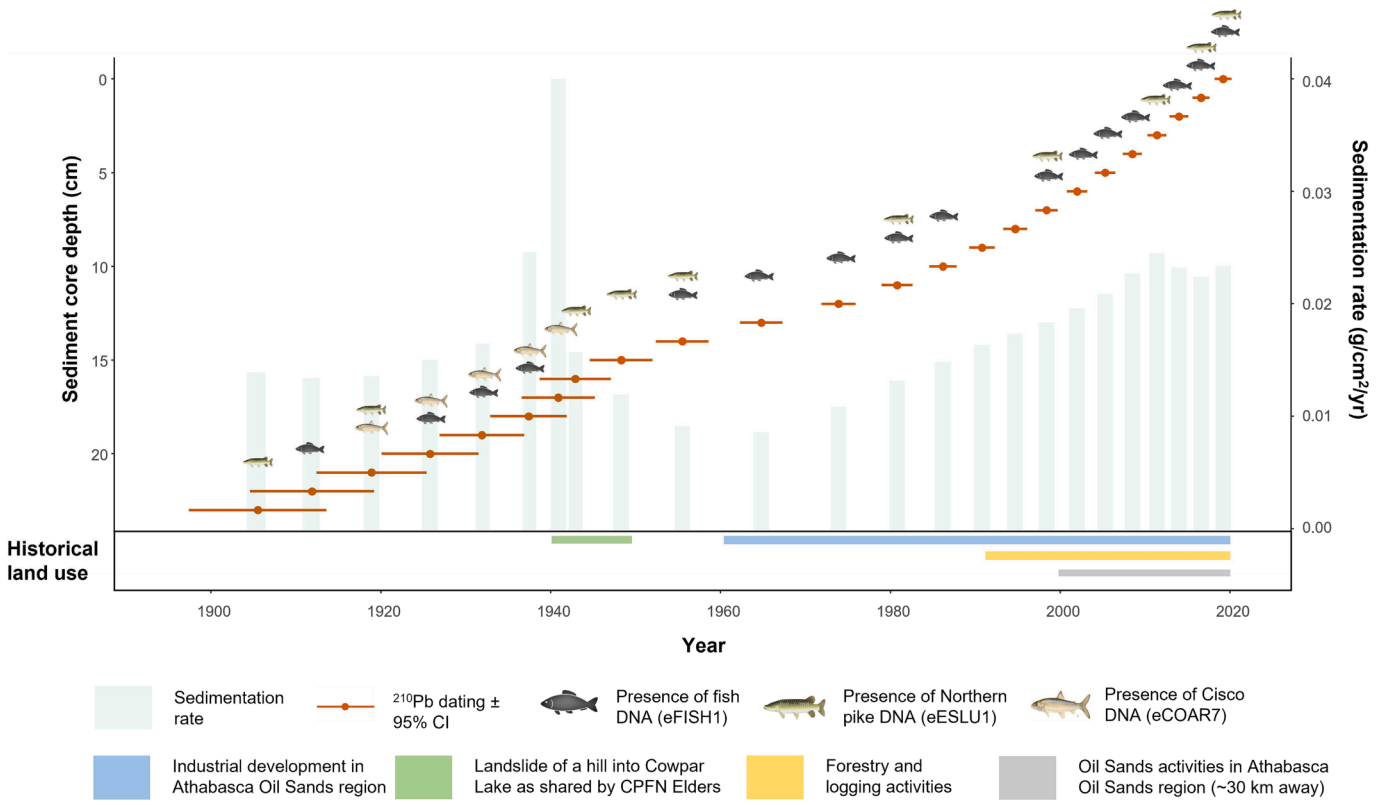

Fig. 4. Detect/no detect data for detected fish DNA across sections of Lake Cowpar sediment core and documented historical land use in Athabasca Oil Sands region.

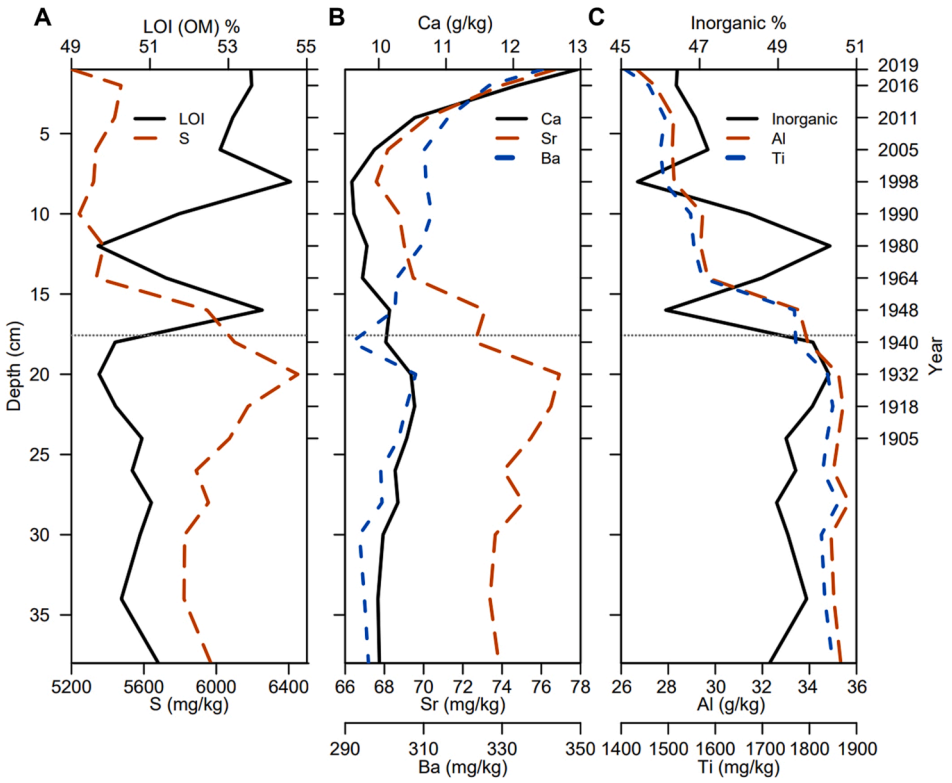

Fig. 5. Sediment chemistry. (a) LOI peak records organic matter pulse from landslide; sulfur (S) peak records metabolism of organic matter pulse. (b) Increasing sediment concentrations (and fluxes) of organic matter and the alkaline earth metals Ca, Sr, Ba suggest recent increase in algal production, as documented by Indigenous Knowledge. (c) Dilution of inorganic matter and lithogenic elements (Al and Ti shown) are a result of increases in within-lake production.

3.5. Braiding sedDNA-inferred data and Indigenous Knowledge in reconstructing historical fish faunal composition

Personal communications with CPFN elders and Indigenous Knowledge holders described a hill completely collapsing into the east side of the lake in the 1940 s after which time whitefish disappeared. This account of the landslide, which would have resulted in a flux of soil and parent material into Cowpar Lake, corresponds with a spike in the sedimentation rate observed c. 1941 (Fig. 4). This event is also recorded in the sediment profile via peaks in organic matter (as LOI) and sulfur, a redox sensitive element (Fig. 5a). Microbial respiration of organic matter – and thus the consumption of oxygen – may have made Cowpar Lake an unfavorable habitat for whitefish, a species intolerant of dissolved oxygen concentrations less than 6.5 mg/L (Taylor and Barton 1992).

After the landslide/sedimentation event, climate warming may contribute to conditions that continue to be unfavorable for whitefish. Moreover, CPFN elders and Indigenous Knowledge holders reported Cowpar Lake as having more algae in recent years than in the past, including nuisance blooms. Sediment chemistry has fundamentally shifted, with increases in organic matter (as LOI) and the alkaline earth metals Ca, Sr, Ba (Fig. 5b). Warmer temperatures are increasing primary production in lakes regionally (Summers et al. 2016), and photosynthesis can increase sediment fluxes of organic matter, and Ca, Sr, and Ba through association with organic matter (uptake or adsorption) and/or pH-dependent mineral precipitation (McGrath et al. 1989, Stabel 1989). In Cowpar Lake, summer water temperatures can exceed both the chronic (20 °C) and acute (23 °C) thermal criteria for whitefish (Taylor and Barton 1992), and during winter ice cover dissolved oxygen concentrations approach 6.5 mg/L. These conditions may preclude re-establishment of a whitefish population in the lake if it were possible through migration.

Land use changes, including forestry and *in situ* oil sands development beginning in the 1990s and 2000s, respectively, do not have a clear, direct impact on Cowpar Lake geochemistry. The recent increases in linear sedimentation rates and mass accumulation rates appear (see above) to be driven by greater primary production within the lake, and the increased organic flux to sediments is diluting inputs of inorganic matter and lithogenic elements from the watershed (Fig. 5c). The lake's shoreline is undeveloped, possibly buffering impacts from disturbance occurring more distant (~30 km away) from the lake. Metals subject to regional and global atmospheric transport and deposition, e.g., Pb and Hg, show recent increases in sediment as expected (Cooke et al. 2017), but concentrations of these metals in fish are not at levels that would cause overt toxicity (and affect presence/absence).

4. Discussion

SedDNA has proven useful in reconstructing native fish records and detecting non-native fish invasion in aquatic systems (Nelson-Chorney et al., 2019). Recently, sedDNA was also found to reflect fluctuations in fish abundance caused by changes in ecological conditions (Kuwae et al., 2020; Sakata et al., 2022). However, detection of fish sedDNA using qPCR-based methods remains highly variable due to ecological and methodological uncertainties. Herein, we aimed to reconstruct historical fish records of Cowpar Lake, located within the Alberta Oil Sands region, using a comprehensive qPCR-based eDNA workflow for the analysis of humic-rich lake sediments.

The proper preservation of fish DNA in the sediment is crucial for the successful detection of fish sedDNA. Fish eDNA is transported from the water column to sediments inside carcasses or by binding to particulate organic matter (Turner et al., 2015). Fish eDNA preservation following deposition into sediments is significantly influenced by the physical and geochemical composition of aquatic sediments as well as DNA form (intra- or extracellular) (Huston et al., 2023). Most fish biomass is made up of unprotected cells, making it more susceptible to degradation than other taxa with resistant structural components (such as resting stages (e.g., seeds or ephippia) or lignin for terrestrial plants). Accordingly, sedDNA is impacted by the mineralogic composition, pore-water pH, and the valence and concentrations of cations in the sediments (Torti et al., 2015; Kanbar et al., 2020). SedDNA preservation is further influenced by the adsorption and desorption of DNA to mineral particles. The relative ratio of fish DNA to all other macrobial taxa is largely unknown and is expected to differ between ecosystems. What is known is that the majority of the sedDNA pool in both surface and deep sediment layers is comprised of bacterial and archaeal DNA. This is because of their relatively high densities in the water column and sediments (Capo et al., 2022). To successfully detect fish sedDNA, a thorough workflow using highly sensitive detection tools is required.

Based on our experience in processing Cowpar Lake sediment samples, we constructed an optimized qPCR-based workflow summarized in Fig. 6 for detecting fish sedDNA in humic-rich sediment samples. First, randomized processing numbers should be designated to each sediment core section to eliminate inherent biases in the succeeding downstream analyses. In the present study, we used 2 g of dewatered wet weight sediment samples (weight after centrifugation) for DNA extraction (due to limited availability) that was eluted with 3 mL buffer. Depending on sample availability, this can be adjusted up to 10 g of input material as recommended by most commercial soil DNA extraction kits (Thomson-Liang et al., 2022). The extracted sedDNA can then be further concentrated and purified through ethanol precipitation to increase the total amount of sedDNA in the sample volume that will be used for each qPCR reaction. For complete removal of co-precipitated humic substances, two independent column-based clean-up steps are included in this workflow: (1) built-in Norgen soil DNA extraction kit humic acid removal columns treated with organic substance removal (OSR) solution; and (2) Zymo Research OneStep PCR inhibitor removal spin column. The complete removal of organic contents helps avoid reporting false negative detection due to failed amplification of the target gene caused by co-precipitated PCR inhibitors (Thomson-Liang et al., 2022). A step for DNA viability testing using IntegritE-DNA^{TM} assay is also added to detect PCR amplifiable endogenous plant chloroplast DNA in the sedDNA samples. This assay detects the chloroplast 23S ribosomal RNA that is ubiquitously present in almost all types of environmental samples (Veldhoen et al., 2016). DNA viability testing allows the identification of non-viable DNA that excludes the potential inclusion of false negative observations in an eDNA field survey. For fish sedDNA detection, eFISH1 assay that targets a conserved region in fish mitochondrial 12S ribosomal RNA (Table 1, mt-*rnr1*) can be used in screening the presence of non-species-specific fish DNA. Samples containing general fish DNA can then be processed for species-targeted assays to identify fish species present. Compared to more abundant taxa (microbes and plankton), captured fish DNA from sediment samples is expected to be in trace amounts (Capo et al., 2021). With this, an increased number of technical replicates (n = 16) for each qPCR analysis per sample is recommended to improve the detection of the target species (Veldhoen et al., 2016; Matthias et al., 2021; Lesperance et al., 2021). In reporting the presence of fish species DNA, only those sediment sections with DNA concentration (copies/g sediment) values within and above the eDNA assays' LOD and LOQ ((±95 % CI) range will be reported for positive detection (Lesperance et al., 2021). The use of standardized LOD and LOQ allows enhanced reproducibility of eDNA qPCR assay results (Klymus et al., 2019). This echoes the need for a well-established workflow to develop robust qPCR-based eDNA assays (Langlois et al., 2021). Last, the resulting amplicon from qPCR runs were sent for Sanger sequencing, if possible, to validate the amplification of the target gene region. Overall, this comprehensive workflow addresses common methodological uncertainties regarding the accurate detection of fish sedDNA from humic-rich sediment samples in aquatic systems, thus increasing the confidence in reported eDNA results.

Moreover, the present study highlights the potential use of sedDNA and Indigenous Knowledge in reconstructing fish records in aquatic

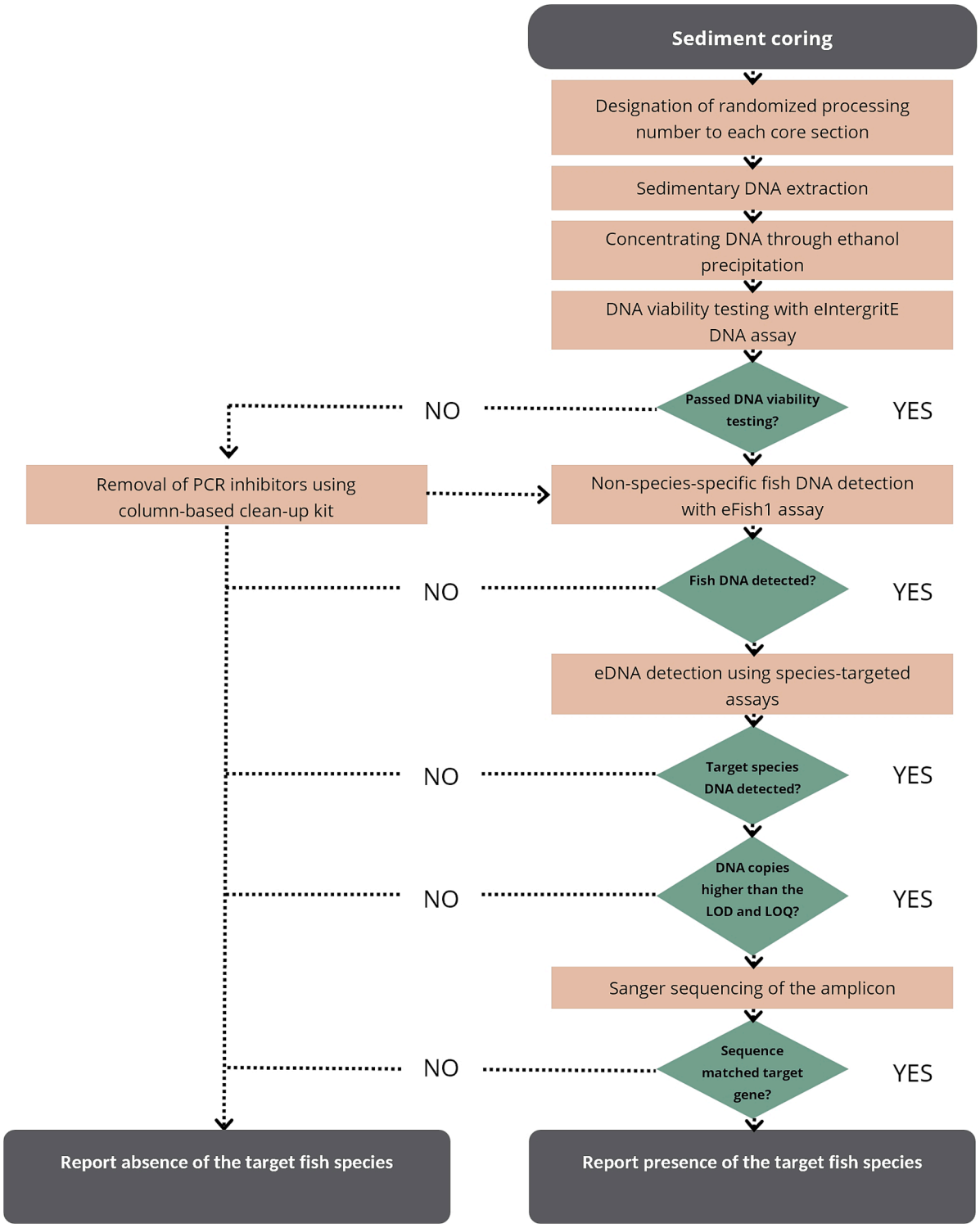

Fig. 6. Overall eDNA workflow for processing sediment samples from Cowpar Lake for the detection of fish sedDNA.

ecosystems. The noted changes in land use around the region according to CPFN Indigenous Knowledge holder are aligned with the modeled shifts in sedimentation rates (Fig. 4). Lake sedimentation processes are often linked to natural and/or human-mediated events that increase sediment input from terrestrial zones around the lake (Jenny et al., 2019). The highest sediment influx was noted in a sediment section dating back to 1941 ± 4.3 years, the same time (~1940 s) that CPFN Indigenous Knowledge holders described a hill collapsing into the east side of the lake. With the use of sedDNA from lake sediment cores, we demonstrated that previous fish fauna can be reconstructed and aligned to historical and/or human-mediated shifts in land use. Detection of one *Coregonus* species, Cisco (dádúë), followed by their disappearance around the 1940 s based on sedDNA data aligned with CPFN Indigenous knowledge of Whitefish in Cowpar lake. According to McKenna et al. (2020), both species have overlapping habitat niches and could inhabit the same lake. If there was a period when both Cisco and Lake whitefish were present in the lake, mitochondrial recombination could happen due to hybridization of this closely related species (Tsaousis et al.,

2005). This could have resulted in the cisco DNA detected in our core having a lake whitefish phenotype. Thus, examination of additional cores is needed to confirm the actual identity of the Whitefish species observed in the lake.

The present study showcases the benefit of integrating Indigenous Knowledge with western scientific approaches to improve system-understanding that can guide fisheries resource governance (Reid et al., 2020). Enhanced knowledge of changes to lake fish fauna improves understanding of how these fish populations respond to their dynamic natural habitat as well as human anthropogenic impacts. This provides critical information for lake managers in developing conservation policies that could directly benefit wildlife and the Indigenous Peoples governing the area.

5. Conclusion

The use of sedDNA can help determine past fish records, and aid in the understanding of how these fish populations respond to natural and human-mediated land use changes. Indigenous Knowledge is an invaluable historical record of natural events and anthropogenic activities around aquatic systems. Combined sedDNA data and Indigenous Knowledge can be a powerful tool in reconstructing historical fish records in aquatic communities. The current study presents a comprehensive qPCR-based eDNA workflow, which utilizes column- and precipitation-based PCR inhibitor clean-up, nucleic acid concentration, sample integrity control, and robust qPCR-based eDNA assays to address methodological and ecological uncertainties. This workflow increases confidence in reported fish eDNA detection from humic-rich aquatic sediment samples. As further advancements in sampling design, extraction and purification, detection method, and reporting of results emerge, the repeatability and confidence of fish sedDNA-inferred biological data will be strengthened to support historical biodiversity reconstructions.

CRediT authorship contribution statement

Mark Louie D. Lopez: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Matthew Bonderud:** Methodology, Writing – original draft. **Michael J. Allison:** Methodology, Writing – review & editing. **Findlay MacDermid:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Erin J. Ussery:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Mark E. McMaster:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Ave Dersch:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Kasia J. Staniszewska:** Investigation, Visualization, Writing – review & editing. **Colin A. Cooke:** Investigation, Formal analysis, Visualization, Writing – review & editing. **Paul Drevnick:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Caren C. Helbing:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Caren Helbing reports financial support was provided by Oil Sands Monitoring program. Mark Louie D. Lopez reports financial support was provided by Liber Ero. Caren Helbing reports financial support was provided by Genome Canada. Caren Helbing reports financial support was provided by Genome British Columbia. Caren Helbing reports financial support was provided by Génome Québec.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.111014.

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