Technical Workshop of the Advancing Sustainable Hydropower series:

Good International Industry Practices for Aquatic Biodiversity Baseline Determination and Monitoring Protocols

eDNA-Metabarcoding: Novel approaches for aquatic surveys

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Monitoring aquatic biodiversity:

Conventional methods

- Fish pots
- Angling
- Push net
- Fyke nets
- Multi-mesh gill net
- Day-snorkeling*
- Beach seine*
- Bottom trawl*
- Night-snorkeling*
Conventional survey methods

• Species monitoring relies on physical identification by visual **distinction of morphological characters**
  – heavily dependent on taxonomic expertise

• **Invasive** on species or ecosystem
• Expensive
• Time consuming
eDNA-based non-invasive meta-barcoding: 
A Novel tool for monitoring aquatic biodiversity
Environmental DNA (eDNA) is genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material.
eDNA-based metabarcoding

- For higher organisms, this DNA may come from excreted cells or tissue such as urine, hairs and skin and dead individuals leaking genetic material.

Free-floating DNA (from sloughed skin cells, faeces/urine, gametes, decaying matter) and microscopic taxa
Studies show that eDNA-based meta-barcoding outperforms other survey methods.
Application of eDNA

- reduce field survey time and
- have little or no impact on ecosystems
- species identification from DNA sequences is often easier and accurate than identification by observation of external morphology
- variety of aquatic species may be detected from a single water sample
Application of eDNA

• eDNA collection can successfully monitor
• (qualitative)presence/absence of rare, endangered, indicator and invasive species, assess biodiversity, and
Application of eDNA

• Relationships between eDNA concentration and species (quantitative) abundance/biomass have previously been reported.

• eDNA concentration in water samples may provide similar fish abundance index of invasive capture methods used in fisheries management.
Environmental DNA (eDNA) technique

1. For aquatic species, eDNA is usually captured by filtration of large volumes of water
Environmental DNA (eDNA) technique
Environmental DNA (eDNA) technique

2. Followed by PCR Amplification of a targeted region of DNA \(\text{(metabarcode analysis)}\) making it possible to detect and quantify species-specific DNA sequence \(\text{(taxonomic assignment)}\)
Environmental DNA (eDNA) technique

- PCR analysis involves amplification of mitochondrial gene using:
  a) Fish *species-specific* PCR primer sets
  b) Generic/ universal PCR primer sets
eDNA: Metabarcoding using universal primers

- Ideal universal primers for fish eDNA:
- amplify a short fragment (<200 bp)
- contain sufficient sequence variation to correctly assign fish to taxonomic family, genus and species level
- Versatile enough across a taxonomically and ecologically diverse range of fishes
PROPOSED MOLECULAR STUDY FOR LONG TERM MONITORING OF AQUATIC BIODIVERSITY
Sampling design

Fig 5: Map showing major existing or planned hydroelectricity projects
Study parameters

• **Analysis of physical parameters:** water temperature, pH, transparency, color, velocity, turbidity, dissolved oxygen, total alkalinity, hardness and free carbon dioxide

• **Analysis of biological parameters:** phytoplankton and zooplanktons
eDNA study:

a. REFERENCE FISH DNA Library

Fish DNA barcoding

DNA Extraction → Amplification using universal fish barcode primer → Sanger sequencing → S. marmoratus
eDNA study:
b. eDNA Metabarcoding of fish

**eDNA metabarcoding of Fish**

Water sample → DNA Extraction → Amplification using universal fish barcode primer → Next Gen sequencing

Comparison with Reference database

Fish species 1

Fish species 2

Fish species 3
Spotted Bass

IDENTIFICATION: The spotted bass looks very much like the largemouth bass. However, the spotted bass has a dark spot on the gill cover and spots or streaks on the lower side of the body. The mouth, when closed, does not extend beyond the eye. Also, the spotted bass has a rough tongue patch located in the middle of the tongue while largemouth bass lack the tongue patch.

Species Facts

Science Name: Micropterus punctulatus
Other Names: Kentucky bass, Kentucky spotted bass, northern spotted bass, Alabama spotted bass
Ideal Temp: 70 to 78
World Record: 9 lbs. 9 ounces, Ca.
Environment: stream, river

Sequence data

CTTTATATTTAATAATTGGAGCTTGGAGCAATAAGTGACTGCACT
AAAGTATTAAAATCAGCAGCAGAATTAGGACAACAGCGAGTGCACACTCTGGAGAG
TGACCAAAATTATAAGGTGACTGCCAGCATTGTATTAATTTTCT
TTATAGTAATACCAATAATAATTGGAGCTTGGAGAAACTGACTTTGACCA
CTAATAATTGGAGGCCAGATATGCGATCCCCAGAATAATAATATAA
TTTTGACTCTTACCCCATCAATTTCTCTCCTCTCTCTAGCATACAAATAG
AAAGGAGAAAGAGGAGGCACAGGATGACAGTCTATCCGGCCTAGCGCTGAAT
TTAGCACCATGCAAGGAGCATCAAGTTGACCTAAACAAATTTTTTTCTTACACTTAG
CTGGAGTCTTTCTCTATTGTGGTTGTGCAATTATTATTTATTACTACATTATCAA
TATAAAAAACCCACGGCAGCTGAATATCAATACACACCCCATATTGTGATCTC
CGTCTAAATTACGGCTGACTGCTCTCTACATTCCGCCCAGTACTAGCTCA
GGTATTACTATACTATAACCCAGCAGAAAATCTAAAAATCAACCTTTCTTGAC
CTGCTCGAGGAGGAGGACCCAATCTCTATACACATCTTTCTGATTTTTG
GCACCAGGAAATTTAACTTAACCTTGTGTTACCAACATCCTTTCTGATTTTTG
GCACCAGGAAATTTAACTTAACCTTGTGTTACCAACATCCTTTCTGATTTTTG
THANK YOU