

Detection of invasive freshwater fish in British Columbia lakes, using eDNA metabarcoding

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Introduction

Early detection of invasive fish species is critical to enable effective management intervention and limit harmful ecological impacts that can occur if invasive populations become established. Despite the increasing use of eDNA metabarcoding applications in conservation science, standard operating procedures are still under development. We compared different sample collection methodologies for their ability to detect invasive fish presence in a lake with well-known fish diversity. We used a Canadian freshwater fish custom 12S reference library to assign species identities to our sequencing results.

Methods

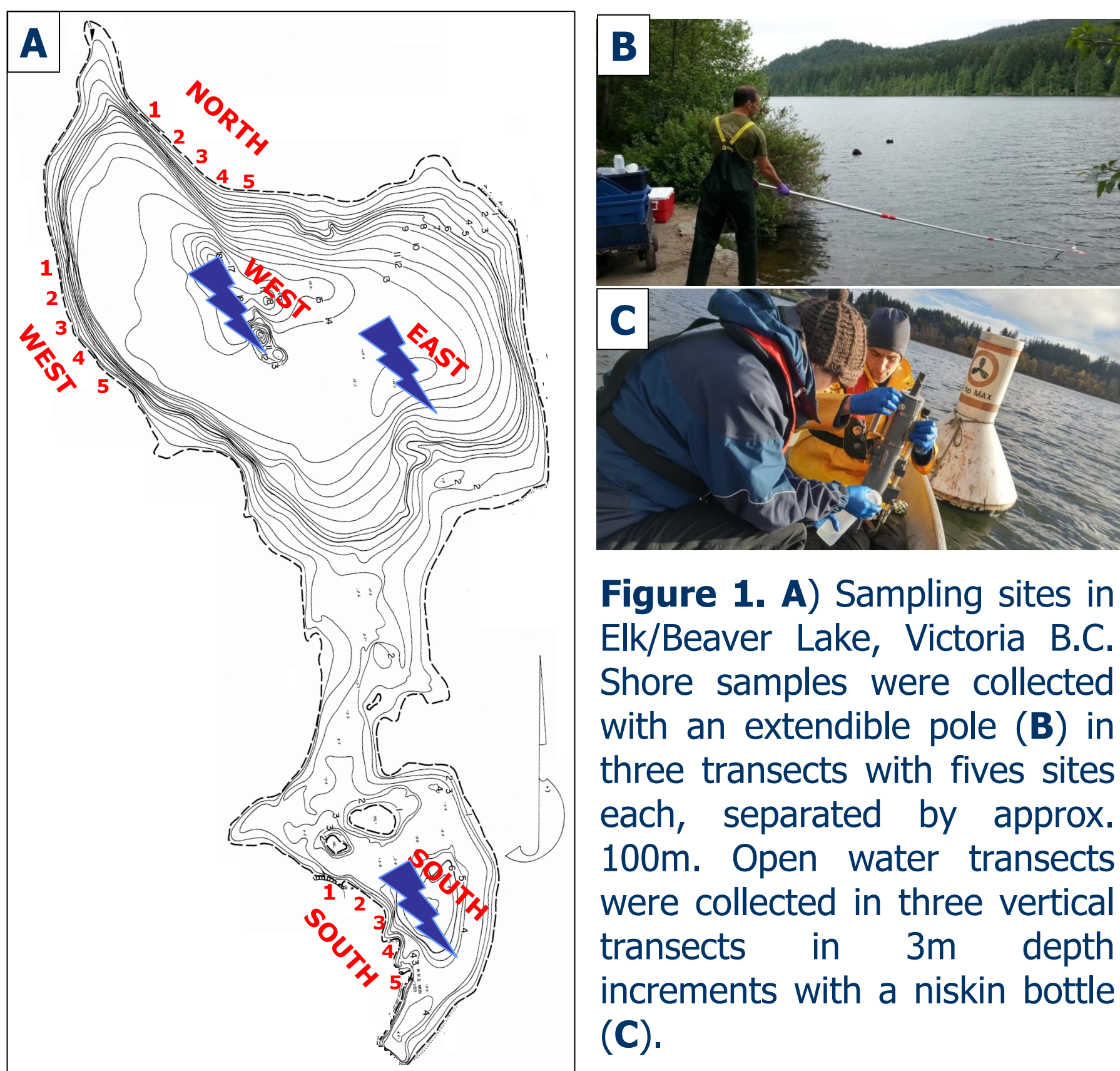


Figure 1. A) Sampling sites in Elk/Beaver Lake, Victoria B.C. Shore samples were collected with an extendible pole (B) in three transects with five sites each, separated by approx. 100m. Open water transects were collected in three vertical transects in 3m depth increments with a niskin bottle (C).

Laboratory Methods:

- Extract DNA in DNA-free space
- SYBR qPCR testing of DNA quality
- Amplification with unique tags with 12S fish-specific primers¹
- NGS library build, TruSeq PCR-Free library prep
- Bioinformatics: ObiTools²
- Taxonomic assignment in Blast and Megan³ using custom reference library (Fig. 2)

Summary of custom Canadian Freshwater Fish Reference Library used here

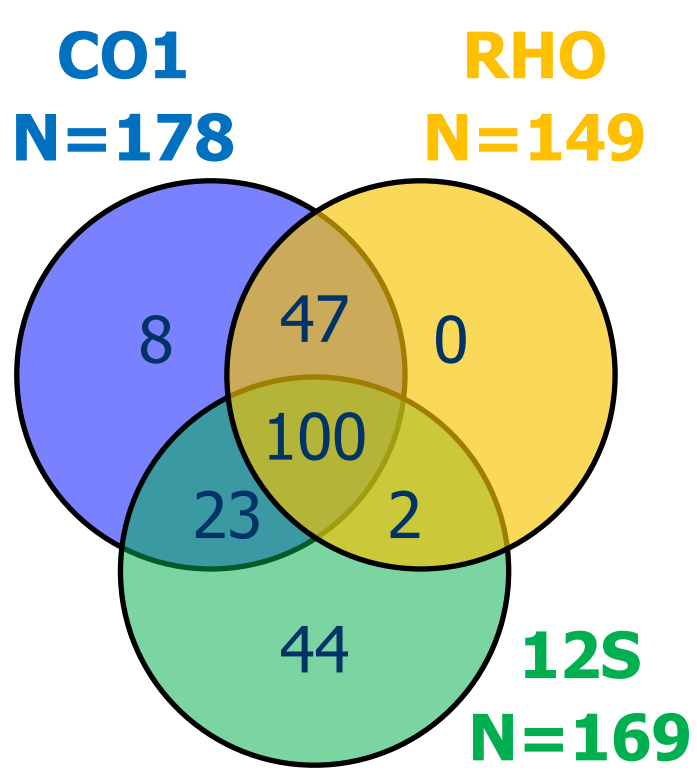


Figure 2. The number of species sequenced with each marker is depicted in the Venn diagram. 835 individual fish samples were collected, vouchered, and sequenced using up to three markers. This sequencing effort covers 178 species, which comprises 88% of Canadian freshwater fish species⁴.

Main Findings

- Species detections were **similar between vacuum and syringe**, only syringe data shown here.
- Species detections in **summer and fall were similar** (Fig. 3).
- **More species were detected from shore sampling** and fewer species were detected with increasing depth (Figs. 3 and 4).
- Two species were detected with **very low read abundance** (*O. clarkii* and *G. aculeatus*) and **detected only in summer samples** (Fig. 4A,C).
- Relative **read abundances were highly variable** among samples (Fig. 4) in both seasons and locations (shore and open water).
- **All known fish species that occupy lake (n=10) were detected.**

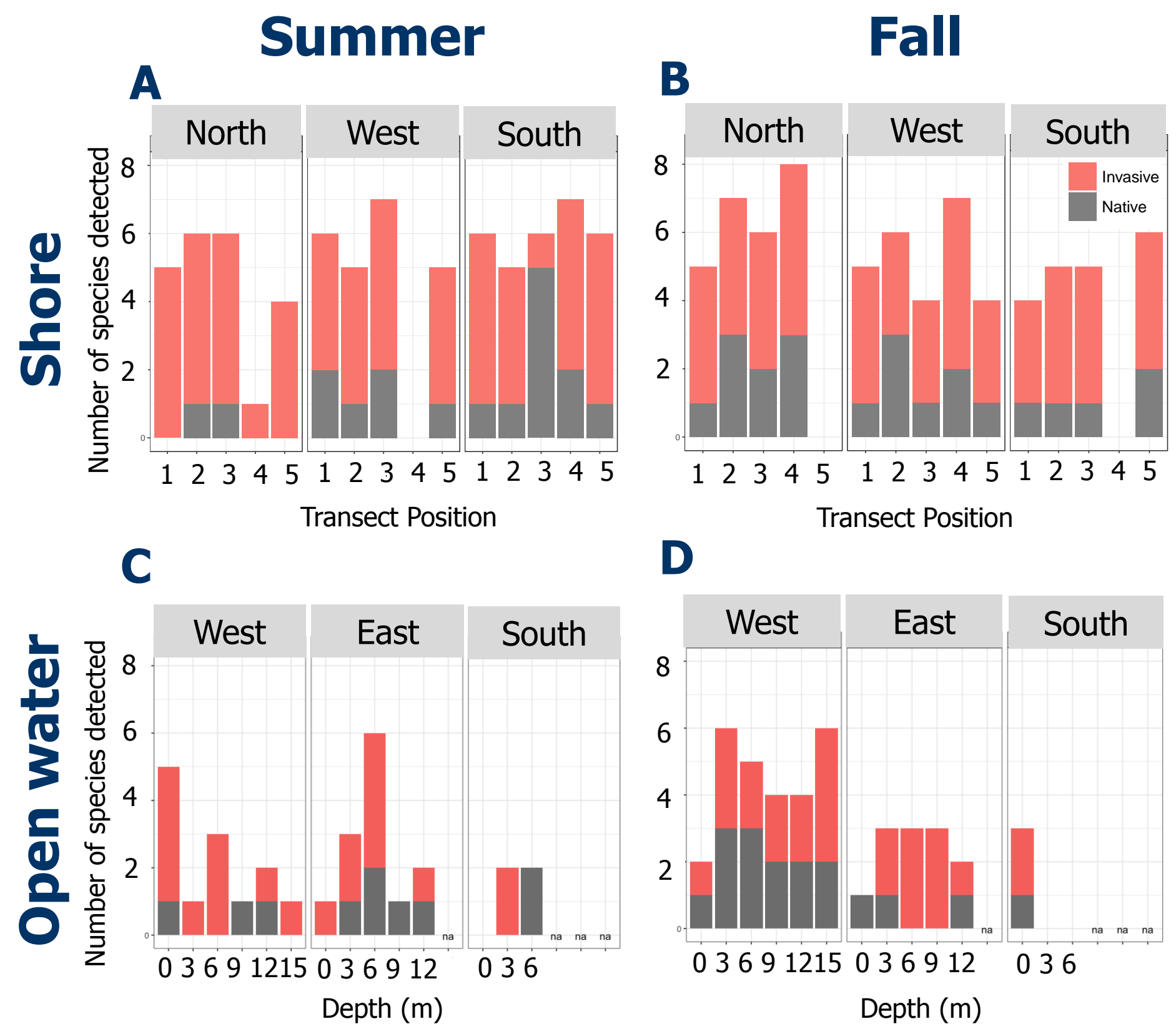


Figure 3. Number of invasive and native fish species detected with 250ml syringe samples in summer and fall from shore or open water samples. Open water transects were not all the same depth.

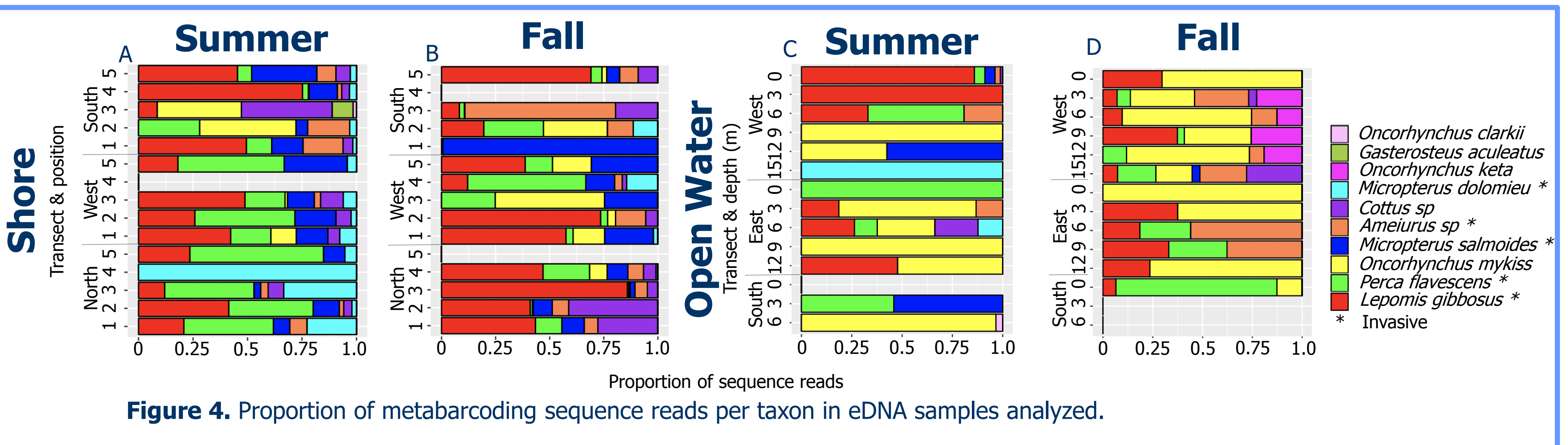


Figure 4. Proportion of metabarcoding sequence reads per taxon in eDNA samples analyzed.

References: ¹Valentini et al., 2016, Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding, Mol Ecol, 24(4):929-42. ²Boyer et al., 2016, obitools: a unix-inspired software package for DNA metabarcoding, Mol Ecol Res 16(1):176-82. ³Huson et al., 2007, MEGAN analysis of metagenomic data, Genome Res, 17(3):377-86. ⁴Canadian endangered species conservation council (CESCC) (2001) Wild Species 2000: The general status of species in Canada. Ottawa, Ontario: Minister of Public Works and Government Services Canada.

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