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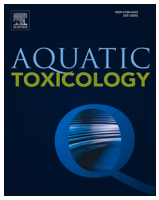

Using zooplankton metabarcoding to assess the efficacy of different techniques to clean-up an oil-spill in a boreal lake

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ABSTRACT

Regulators require adequate information to select best practices with less ecosystem impacts for remediation of freshwater ecosystems after oil spills. Zooplankton are valuable indicators of aquatic ecosystem health as they play pivotal roles in biochemical cycles while stabilizing food webs. Compared with morphological identification, metabarcoding holds promise for cost-effective, high-throughput, and benchmarkable biomonitoring of zooplankton communities. The objective of this study was to apply DNA and RNA metabarcoding of zooplankton for ecotoxicological assessment and compare it with traditional morphological identification in experimental shoreline enclosures in a boreal lake. These identification methods were also applied in context of assessing response of the zooplankton community exposed to simulated spills of diluted bitumen (dilbit), with experimental remediation practices (enhanced monitored natural recovery and shoreline cleaner application). Metabarcoding detected boreal zooplankton taxa up to the genus level, with a total of 24 shared genera, and while metabarcoding-based relative abundance served as an acceptable proxy for biomass inferred by morphological identification ($\rho \geq 0.52$). Morphological identification determined zooplankton community composition changes due to treatments at 11 days post-spill (PERMANOVA, $p = 0.0143$) while metabarcoding methods indicated changes in zooplankton richness and communities at 38 days post-spill (T-test, $p < 0.05$; PERMANOVA, $p \leq 0.0429$). Shoreline cleaner application overall seemed to have the largest impact on zooplankton communities relative to enhanced monitored natural recovery, regardless of zooplankton identification method. Both metabarcoding and morphological identification were able to discern the differences between the two experimental remediation practices. Metabarcoding of zooplankton could provide informative results for ecotoxicological assessment of the remediation practices of dilbit, advancing our knowledge of best practices for remediating oil-impacted aquatic ecosystems while serving to accelerate the assessment of at-risk freshwater ecosystems.

1. Introduction

Aquatic ecosystems are continuously threatened by global activities of extraction and transport of oil, especially in cases of accidental oil spills (Atlas and Hazen, 2011; Beyer et al., 2016). Diluted bitumen (dilbit) is a complex petroleum mixture produced by the dilution of bitumen, a viscous heavy oil, to form a mixture that is transportable through pipeline and rail but is toxic to aquatic organisms (Barron et al., 2018; Dew et al., 2015; Madison et al., 2015). Bitumen extracted from the Athabasca Oil Sands region in Alberta is diluted to form dilbit and transported across North America, leading to potential risks of spills occurring via pipeline or rail. North America has seen several large pipeline oil spills, including a 2010 spill of dilbit affecting the Kalamazoo River in Michigan (USA) and in 2016 where crude oil spilled into the North Saskatchewan River, Saskatchewan (Canada) (Dew et al., 2015; Yang et al., 2020). Several practices have been developed to help restore oil impacted marine aquatic ecosystems (Dave and Ghaly, 2011). These include active processes, such as shoreline cleaner application, and

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passive natural attenuation, such as nutrient enrichment application; however, the effects of these different oil remediation practices on boreal freshwater ecosystems of low energy need to be better understood.

Shoreline cleaner application can be an effective strategy for shoreline remediation of oil spills in marine ecosystems. Cleaners wash oil from surfaces to be collected by traditional physical methods, whereas dispersants promote dispersion of petroleum components (Pezeshki et al., 2000; Prince, 2015). Cleaners have been shown to be an effective strategy for cleaning oiled shorelines, including prevention of plant damage (Pezeshki et al., 1995; Teas et al., 1993). Shoreline cleaner toxicity to various aquatic organisms, however, requires more information (Barron et al., 2020; Chen et al., 2019). The potential use effects in freshwater ecosystems, let alone boreal ecosystems, is not well understood (Bhattacharyya et al., 2003; Hansen et al., 2014). A previous study has determined enhanced toxicity of oil exposure with the addition of shoreline cleaner, COREXIT® 9580, but it was to a lesser extent relative to the addition of a dispersant (Bhattacharyya et al., 2003). Shoreline cleaner application is currently not allowed to be used in freshwater ecosystems in Canada, as more investigation would be required prior to approval.

Nutrient enrichment is used to stimulate hydrocarbon-degrading microorganisms to break down oil-residue (Atlas and Hazen, 2011; Prince, 1993). Nutrient enrichment is an effective approach (Bragg et al., 1994), but it may cause eutrophication of the aquatic system leading to harmful algae bloom formation and expanding water hypoxia (Pretty et al., 2003; Pretty et al., 2003; Watson et al., 2016). Long-term nutrient enrichment in a Precambrian Shield lake has been shown to have negative consequences on the biomass of zooplankton (Malley et al., 1988; Paterson et al., 2011). The remediation practice that has the least impact on aquatic organisms has yet to be determined *in-situ* in a boreal freshwater ecosystem of low energy. Processes of wave energy to help break up oil into smaller droplets or remobilization of settled oil for optimal biodegradation is minimized in low energy environments, therefore efficacy and effects of these remediation practices needs to be better understood (Carls et al., 2001; Fitzpatrick et al., 2015).

Since zooplankton can respond quickly to altering environmental conditions and are sensitive to aquatic pollution, they are widely used as indicators of the status and trends of aquatic ecosystems (Parmar et al., 2016; Schindler, 1987). Zooplankton play pivotal roles in freshwater ecosystems by recycling nutrients (Steinberg et al., 2008) while also occupying central trophic positions, making them mediators of energy and material fluxes in ecosystems (Giering et al., 2019). The traditional visual identification of zooplankton based on morphology can be costly and time-consuming (Pan et al., 2008; Wheeler et al., 2004). Furthermore, it is difficult to standardize and requires individuals with taxonomical expertise, a collective skill that has declined in recent decades (Hopkins and Freckleton, 2002; Thomsen et al., 2012). Application of the emerging technology of metabarcoding has been suggested for describing communities of zooplankton (Yang et al., 2017d). DNA metabarcoding can provide robust reproducible identification of taxa during ecological assessments (Valentini et al., 2016), but DNA based metabarcoding cannot distinguish whether organisms are dead or alive (Pochon et al., 2017), which should be of importance when tracking rapid changes of communities exposed to environmental stressors. RNA metabarcoding may serve as a useful measure in this regard, as it can reflect the active community upon sampling (Baldrian et al., 2012). RNA is broken down within individual organism cells at a rate that balances energetic costs and adaptability to varying environmental conditions (Hui et al., 2014).

This study assessed the ability of zooplankton metabarcoding to provide data comparable to that produced by using morphology-based identification. We also compared the ecotoxicological effects on the zooplankton community of two different methods for oil-spill remediation, a shoreline cleaner and nutrient enhancement using shoreline mesocosms. The study was conducted in the summer of 2019 in a boreal lake and specific objectives were to: 1) compare the relative abundances or biomass of zooplankton taxa in communities as determined by the use of DNA or RNA metabarcoding and morphologically identified taxonomic (morph-taxa) techniques; 2) determine and compare ecotoxicological effects of remediation practices on zooplankton communities in mesocosms; 3) compare the performance of the three zooplankton identification methods (DNA metabarcoding, RNA metabarcoding, and morphological taxonomy) to elucidate the effects of oil-spill remediation practices.

2. Materials and methods

2.1. Experimental design

The experiment was conducted at the IISD Experimental Lakes Area (IISD-ELA), an area that contains 58 boreal lakes located in northwestern Ontario, Canada that have been set aside for whole-ecosystem experimentation (49°41′45.0″ N, 93°4603.4″ W) (Kidd et al., 2007; Schindler et al., 1996). In June 2019, seven mesocosms (enclosures of 15 × 5 m) were established along the shoreline of Lake 260 at the IISD-ELA in a wetland habitat type. On June 21st, 2019, after enclosure construction and baseline measurement were completed, six randomly selected enclosures were treated with model spills of dilbit (mean applications = 1300 g/enclosure) applied to the surface of the water approximately 50 cm from the shore. One enclosure remained untreated to serve as a reference.

The oil was allowed to interact with the shoreline soil, sediment and vegetation for 4 days to conservatively simulate spill response times, after which any oil remaining on the surface of the water was removed using pre-weighed oleophilic absorbent pads. Additionally, each enclosure, including the reference, was rinsed with 1200-L of water pumped from the interior of the enclosure over the oiled sections of the confined shoreline to mimic oil spill clean-up procedures typically used following a spill. Water was pumped under low pressure and returned to the interior of each enclosure. Any additional oil dislodged by flushing was also captured using absorptive pads.

Enclosures treated with dilbit were then randomly selected to receive one of two different remediation treatments to determine their effectiveness in promoting the longer-term recovery from residual oil contamination. The first method, enhanced monitored natural recovery (eMNR; $n = 3$), included addition of nutrients designed to promote the decomposition of remaining oil products. The second method consisted of active cleaning of the shoreline by use of the oil surface cleaning agent COREXIT® EC9580A (Nalco, Co., Illinois, USA) (SCA; $n = 3$) (Fig. S1; Appendix S1). One shoreline enclosure remained untreated serving as the reference (REF; $n = 1$).

2.2. Collection of zooplankton

Triplicate 20-L water samples for metabarcoding were collected consecutively from each experimental enclosure three days before the simulated spill of bitumen, and then 11 and 38 days after the spill (Fig. S1). Pre-installed tubing, with a funnel on the end inundated within the enclosure, was used to collect representative zooplankton samples without disturbing the water surface. Zooplankton were enriched by two-step filtering by use of a pump with an in-line 53 μm mesh filter and washed off with Nanopure™ water (Thermo Fisher Scientific, USA) for final enrichment with a 5 μm Durapore® PVDF membrane filter (Millipore, Germany). Samples were preserved in LifeGuard Solution (Qiagen, Germany) and stored at −80 °C before extraction of nucleic acid. To avoid cross-contamination, use of filter pumps specified for each treatment, single-use filter-units, changing of gloves at each enclosure, and strict protocols were enforced. Equipment was also decontaminated between each replicate using 15% bleach and 70% ethanol, while field blanks were collected frequently during sampling. Field blanks consisted of an opened, decontaminated 500 mL Nalgene™ bottle (Thermo Fisher

Scientific, USA) containing Nanopure™ water during the period of sampling, for each treatment.

Samples of zooplankton for morphological identification were collected simultaneously with one 60-L water sample being collected using the same protocol for metabarcoding collection, minus the final enrichment step. Taxonomic identification was conducted following procedures detailed previously (Paterson et al., 2010). Briefly, identification of zooplankton was completed using the taxonomic key of Balcer et al., 1984, as well as several other guides to North America's freshwater zooplankton (Balcer et al., 1984; Brandlova et al., 1972; Smith and Fernando, 1978; Witty, 2004). Biomass was determined using length-weight regression based on historic zooplankton weights (Schindler and Novén, 1971) and regression equations (Lawrence et al., 1987; Malley et al., 1989) obtained from IISD-ELA lakes.

2.3. Co-isolation of DNA and RNA, PCR amplification, and next-generation sequencing (NGS)

Zooplankton were thawed on ice and pelleted by centrifugation (8000 x g for 5 min.). LifeGuard Solution was removed with a sterile pipette. DNA and RNA were co-isolated by use of AllPrep DNA/RNA Mini Kit (Qiagen, Germany) following the manual. DNA contamination of extracted RNA was digested with RNase-Free DNase (Qiagen, Germany). The extracted DNA and RNA were measured and checked for quality using Qubit 4 Fluorometer (Thermo Fisher Scientific, USA) and purity by use of NanoDrop Spectrophotometer, respectively (Thermo Fisher Scientific, USA). One extraction blank was conducted with each batch for quality control (QC). Concentrations of DNA and RNA from extraction blanks were less than the limit of detection. Complementary DNA (cDNA) was synthesized using SuperScript IV Reverse Transcriptase (Invitrogen, CA, USA) along with ezDNase to remove residual DNA.

PCR amplification was performed on normalized cDNA and DNA samples (10 ng/μL) using unique dual tagged primers targeting a 313 bp region of the cytochrome oxidase subunit region 1 (COI) using the primers mICOIintF (5'-GGWACWGGWTGAACWGTWTAYCCYCC-3') and jgHCO2198R (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') with a "touchdown" cycle program (Leray et al., 2013; Yang et al., 2017a). To minimize potential bias during amplification, PCR was performed in triplicate using Platinum Taq Hot Start II High-Fidelity DNA Polymerase (Invitrogen, USA), with plate set-up containing multiple PCR blanks for QC. PCR products were checked with agarose gel electrophoresis and purified using the QIAquick PCR Purification kit (Qiagen, Germany). No bands of blanks for extraction and PCR were observed visually. Construction of the sequencing library and next-generation sequencing by use of Illumina chemistry were performed as described previously (DeBofsky et al., 2020). Sequencing data can be accessed at 10.20383/101.0313.

2.4. Bioinformatics

Raw reads were demultiplexed based on dual tags of both forward and reverse primers for each sample, with sequences of the forward and reverse primers being removed thereafter. Paired-end sequences were merged using VSEARCH (version 2.14.2), after filtering out lesser quality (ee > 1.0), chimeras, and shorter length (< 300 bp) sequences (Rognes et al., 2016). Zero-radius operational taxonomic units (ZOTUs) were generated using Unoise3, with a minimum frequency of 5 (Edgar, 2016) and their open reading frames (ORF) were searched *via* NCBI ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/). Pseudogenes and short open reading frames (< 300 bp) were discarded, with features occurring in only one sample subsequently removed.

To gain confidence in identifying species and genera referred to jointly as taxa, features were classified using several steps. BOLD was used to assign features using a percent similarity of greater than or equal to 98%, 95%, and 90% for species, genus, and family-level annotation, respectively (Ratnasingham and Hebert, 2007). Independently, taxonomical annotations were searched by use of an in-house curated database for zooplankton plus six barcoded taxa (e.g., *Diaphanosoma birgei, Epischura lacustris, Daphnia mendotae, Leptodiaptomus minutus, Holopedium glacialis*, and *Diacyclops thomasi*) with VSEARCH (percent identity = 0.98; query coverage = 0.8) being used to taxonomically assign ZOTUs (Bolyen et al., 2019). Taxonomic identification output from BOLD and VSEARCH were combined according to consensus, with lowest-level identification superseding. Unidentified sequences or annotations at a higher-level than family, underwent megablast searching, by use of the NCBI Nucleotide Blast Tool using the standard nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), returning up to 100 hits per query sequence (e-value = 1e-20, percent identity = 99%, and word size = 24). Taxonomy was assigned to the best attainable level by use of the lowest common ancestor (LCA) implemented using MEGANv6 (Default settings except for min score = 150, top percent = 2), with the highest assignable level allowed being genus.

ZOTUs that remained unassigned or that were nontarget taxa, with target taxa being Phylum Rotifera or select orders in Subphylum Crustacea (Orders Calanoida, Cyclopoida, and Cladocera), were removed (See Table S2 and Appendix S2 for sequence read counts). Replicate samples for each enclosure taken for metabarcoding at each time point were merged before downstream analyses. Singleton taxa and taxa found to occur in only one sample were subsequently removed. After collapsing features to the taxa-level for data analyses, unassigned sequences were removed and samples were rarefied to 9985 sequences per sample to avoid bias introduced by uneven sequencing depth (Weiss et al., 2017). Rarefied read count data and raw morphological abundance and biomass data can be found in the supporting information for family, genus, and species-level (Table S3–5). Further details on MiSeq sequencing output can be found in supporting information. Bioinformatics was conducted under QIIME2 (version 2020.2) and R environment (version 4.0.0) (Team, 2013).

2.5. Statistics

All statistics and graphics were performed in the R environment (version 4.0.0) by use of the Vegan package (version 2.5.6) (Oksanen et al., 2007) unless otherwise stated. Venn diagrams were applied to present the agreement and difference among identification methods. Spearman rank correlation was used to determine relationships between $\log_e$-transformed biomass and $\log_e$-transformed relative abundance for shared genera between morphological identification and DNA/RNA metabarcoding. Relative abundance refers to rarefied metabarcoding count data. Differences in genus richness between treatments at each time point were estimated by use of ANOVA, as sample size was not sufficient for interpretation, with Welch's *t*-test used to test between treatments SCA and eMNR. A random intercept model using packages lme4 and lmer Test was used to discern differences in richness between treatment groups while controlling for the effects of time (e.g., −3, 11 and 38 days), with differences for least squares means of respective treatments used for post-hoc testing (Bates et al., 2014; Kuznetsova et al., 2017). Differences in genus richness over time for each remediation practice (e.g., eMNR and SCA) was tested using ANOVA. Principal Coordinates Analysis (PCoA) was performed on genus-level count data to visualize β-diversities of zooplankton communities, with function envfit used to project genera with high correlation with sample ordination as vectors ($p < 0.01$; 9999 permutations). Treatment group differences of β-diversities within each time point were tested using adonis2 (PERMANOVA; 9999 permutations), with a pairwise test being conducted on complete distance matrix, including all samples for each identification method, testing differences between treatments while controlling for the effects of time (Martinez Arbizu, 2017).

3. Results

3.1. Validation of zooplankton metabarcoding with morphologically identified taxonomy (morph-taxa)

Metabarcoding inferred zooplankton taxonomy was consistent with morphological identification at the family and genus-level. The agreement between mb-taxa (taxa identified by both DNA and RNA metabarcoding) with morph-taxa decreased from 89.5% at family level (Fig. 1A), to 77.4% at genus level (Fig. 1C), to 30.0% at species level (Fig. 1E). Portion of shared taxa between mb-taxa with morph-taxa decreased from almost 100% at family level (Fig. 1B), to 98.9% at genus level (Fig. 1D), to 38.0% at species level (Fig. 1F). Within classified species, relative abundances (average ± standard deviation (SD)) of *Keratella cochlearis* and *Mesocyclops edax* determined by metabarcoding (*K. cochlearis*: 18.6 ± 22.0%, *M. edax*: 0.0323 ± 0.0720%) differed from those identified by morphology (*K. cochlearis*: 0.512 ± 0.820, *M. edax*: 25.0 ± 18.3%). Rare zooplankton genera inferred from morphological identification, for instance, *Lepadella* (Lepadellidae), *Macrocyclops, Microcyclops, Monostyla, Diacyclops*, and *Gastropus* were mis-detected by metabarcoding. Fourteen species were detected by morphological identification but not metabarcoding, specifically, *Bosmina longirostris, Chydorus sphaericus, Diacyclops thomasi, Diaphanosoma birgei, Keratella crassa, Keratella serrulata, Keratella taurocephala, Kellicottia longispina, Macrocyclops albidus, Microcyclops rubellus, Polyarthra vulgaris, Trichocerca cylindrica, Tropocyclops extensus*, and *Trichotria tetractis*. Within those, eight species, *Diacyclops thomasi, Kellicottia longispina, Keratella crassa, Keratella taurocephala, Microcyclops rubellus, Tropocyclops extensus Trichocera cylindrica*, and *Keratella serrulate* were unrepresented in the GenBank database (searched 2020–07–21). DNA and RNA

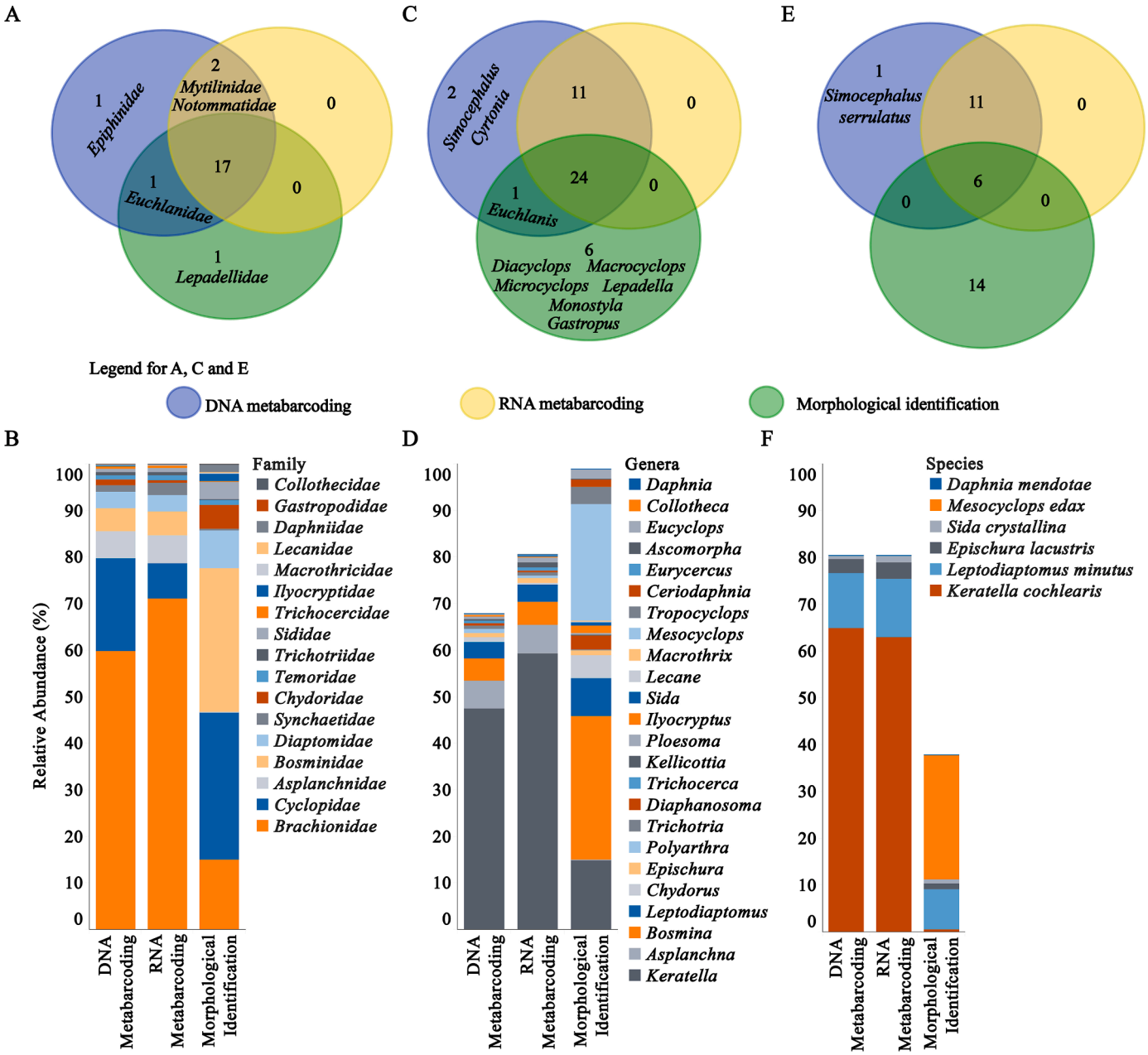

Fig. 1.. Comparison of zooplankton metabarcoding with morph-taxa. (A) Shared families among identification methods; (B) Relative abundance of shared families; (C) Shared genera among identification methods; (D) Relative abundance of shared genera; (E) Shared species among identification methods; (F) Relative abundances of shared species. Unclassified species were filtered out, with relative abundance being adjusted accordingly.

metabarcoding revealed similar profiles of zooplankton communities (Fig. 1 and S3). Mismatched taxa between DNA and RNA metabarcoding were rare (relative abundances < 0.01%).

Metabarcoding based relative abundance of shared genera of Rotifera and Arthropoda phyla revealed the distribution of morphology-based densities. Relative abundances (log_e-transformed) of shared genera for both phyla Rotifera and Arthropoda had similar trends with that of morphology-based densities (log_e-transformed). For Arthropoda genera, both DNA and RNA metabarcoding based relative abundances (log_e-transformed) of zooplankton were significantly correlated with log_e-transformed biomass (Spearman rank correlation, Fig. 2B, $r = 0.66$, $p = 0.013$; Fig. 2C, $r = 0.60$, $p = 0.028$). For Rotifera, both DNA (Fig. 2B, $r = 0.52$, $p = 0.11$) and RNA (Fig. 2C, $r = 0.52$, $p = 0.13$) metabarcoding based relative abundances were moderately correlated with Rotifera biomass, although the level of significance was marginal. Significant correlations between DNA and RNA metabarcoding of shared Arthropoda and Rotifera genera showed that the two methods gave similar estimates for relative abundances of target taxa (Fig. 2A, $r \geq 0.93$, $p \leq$ 3.1e-06).

3.2. Zooplankton metabarcoding revealed effects of remediation practices on communities of zooplankton

SCA and eMNR caused two different outcomes on zooplankton genus richness in the enclosures over time. No significant differences were observed among enclosures prior to the application of oil spill and cleaning practices (Fig. 3, T-test, $p \geq 0.425$) and day 11 post-spill (Fig. 3, T-test, $p \geq 0.570$). At day 38 post-spill, DNA metabarcoding showed an increase in zooplankton genus richness for eMNR relative to SCA (Fig. 3A, T-test, $p = 0.0357$) while for RNA metabarcoding, observed genus richness in SCA declined significantly relative to eMNR (Fig. 3B, T-test, $p = 0.0478$). For remediation practices, for instance, eMNR and SCA, genus richness for eMNR increased over time for DNA metabarcoding (Fig. 3A, ANOVA, $p = 0.026$), while genus richness of SCA practice decreased for RNA metabarcoding over time (Fig. 3B, ANOVA, $p = 0.00418$). Shannon diversity of zooplankton genera had moderately similar trends as genus richness for DNA and RNA metabarcoding (Fig. S4; Appendix S3). The random intercept model and corresponding computed least square means determined differences for REF vs SCA (Table S6, $p = 0.00968$) and eMNR vs SCA (Table S6, $p = 0.0129$) for DNA metabarcoding, while difference was determined for REF vs. SCA (Table S6, $p = 0.00203$) and REF vs eMNR (Table S6, $p = 0.0473$) for RNA metabarcoding.

Differences in structures of zooplankton communities between SCA and eMNR at 38 days post-spill were greater than that of day 11 post-spill. Results for PERMANOVA based on DNA and RNA metabarcoding showed that treatments did not differ significantly at 11 days post-spill (Fig. 4B, C, $p \geq 0.171$). Reference was more closely related to SCA relative to eMNR for both DNA and RNA metabarcoding according to PCoA plots at day 11 post-spill, with *Keratella* being significantly correlated with eMNR sample ordination (Fig. 4A, B). At 38 days post-spill, PERMANOVA for DNA and RNA metabarcoding were significant (Fig. 4C, $p \leq 0.0429$). PCoA plot for DNA and RNA metabarcoding indicated strong clustering for SCA whereas eMNR was more variable in its community composition (Fig. 4C, D). From sample position on the PCoA plots at 38 days post-spil1, reference seemed more closely related to eMNR relative to SCA, with *Kertella* being significantly correlated with SCA sample location (Fig. 4C, D). Pairwise comparison between all samples, blocking the effect of time, determined differences for REF vs eMNR (Table S7, $p = 0.173$) and REF vs SCA (Table S7, $p = 0.0174$) for DNA metabarcoding, while difference was determined for treatment combination REF vs SCA (Table S7, $p = 0.0142$) for RNA metabarcoding.

3.3. Performance to distinguish ecological effects of remediation practices

Morphological identification differed from metabarcoding in the ability to determine ecotoxicological effects of remediation practices. No statistical differences were observed among remediation practices for genus richness based on morphology. A trend towards lesser richness was observed for SCA on day 11 relative to eMNR (Fig. 5A, T-test, $p = 0.183$). Morphological identification indicated that zooplankton richness increased for SCA on day 38 (Fig. 5A, T-test, $p = 0.519$). The random intercept model and corresponding computed least squares means determined no differences between treatment groups (Table S6, $p \geq 0.114$). Based on morphometry, treatment groups differed at 11-days post-spill for community composition (Fig. 5B, PERMANOVA, $p = 0.0143$). Treatment groups did not differ in centroid position at day 38 (Fig. 5C, PERMANOVA, $p = 0.486$), with eMNR being closer in distance to reference relative to SCA, indicating a stronger relationship between the two communities at day 38 (Fig. 5C). Pairwise comparison of the distance matrix based on all samples, blocking the effect of time, determined differences for REF vs eMNR (Table S6, $p = 0.0478$).

4. Discussion

4.1. Overall agreement between metabarcoding and morphological identification

Zooplankton taxonomy as determined by metabarcoding was analogous to morph-taxa at the family- and genus-levels. Overall, relatively similar trends in the responses of zooplankton communities to differing remediation practices were observed, even with differences existing

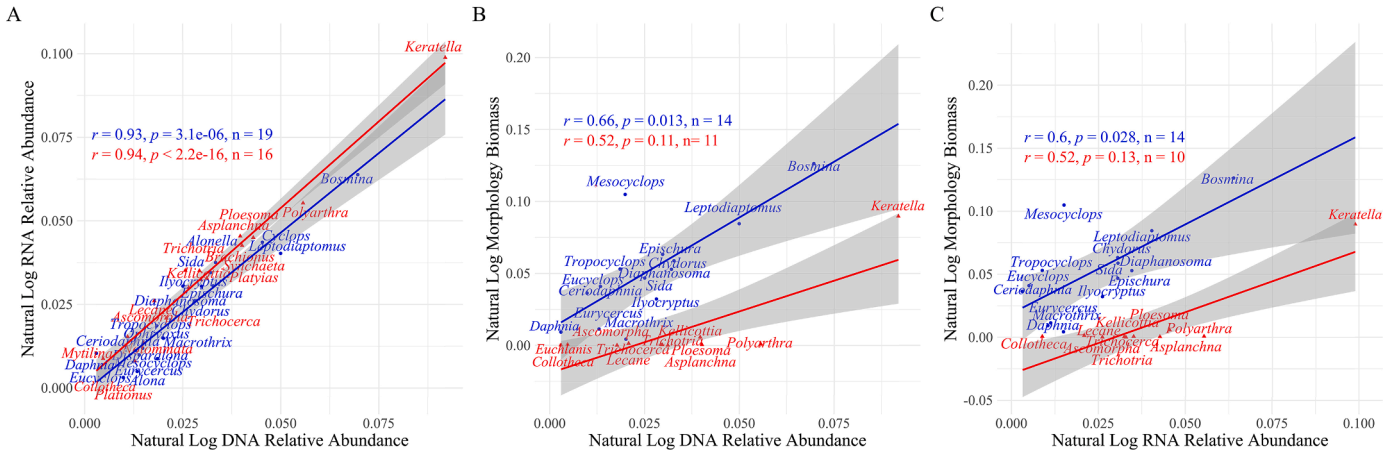

Fig. 2.. Correlations of shared genera of the log_e-transformed metabarcoding relative abundance data and log_e-transformed morphology biomass using Spearman rank correlation. Blue text indicates phylum Arthropoda and red text indicates phylum Rotifera.

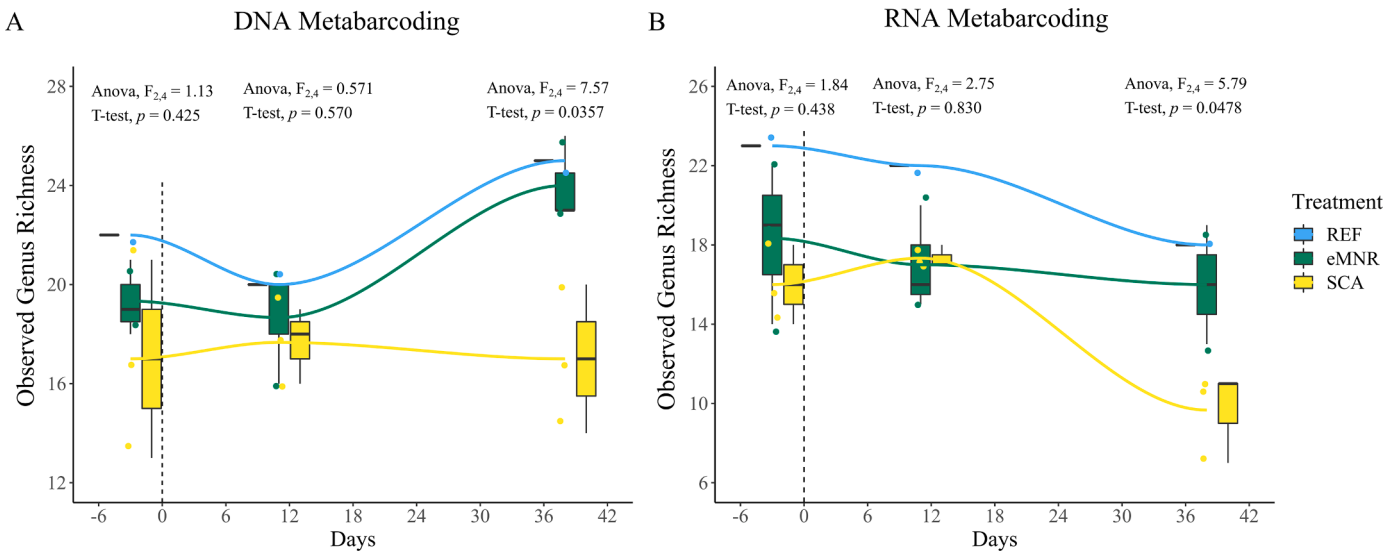

Fig. 3.. Observed zooplankton genus richness over time for (A) DNA and (B) RNA metabarcoding. Treatment groups consisted of enhanced monitored natural recovery (eMNR; $n = 3$), shoreline cleaner application (SCA; $n = 3$), and reference (REF; $n = 1$). ANOVA was computed between remediation practices at each time point of interest with Welch's *t*-test used to test observed genus richness between treatments eMNR and SCA. Dashed line represents beginning of simulated dilbit spill and treatment trend is represented by local polynomial regression.

between the profiles of relative abundances of zooplankton among methods of identification. Metabarcoding of zooplankton has previously been shown to effectively capture spatial and temporal trends determined by morphological identification, even when differences existed in community profiles of zooplankton between the two methods (Abad et al., 2016). Furthermore, monitoring of macroinvertebrates in boreal stream ecosystems by use of DNA metabarcoding was consistent with results based on morphological metrics at family and genus level (Emilson et al., 2017). Abundant zooplankton genera, *Keratella, Bosmina, Leptodiaptomus, Mesocyclops*, and *Polyarthra*, detected with metabarcoding were consistent with results of previous papers examining other local boreal lakes as well as the lake used in this study (Drouin et al., 2009; Kidd et al., 2014). Abundant zooplankton genera, *Keratella, Bosmina, Polyarthra, Asplanchna*, and *Diaphanosoma* additionally had global comparability with a boreal lake in Finland (Arvola et al., 1996). Metabarcoding provides a high throughput method for analyzing various ecological communities but has yet to be optimized for zooplankton communities in boreal ecosystems.

Use of DNA and RNA metabarcoding, or present and active taxa, respectively, resulted in similarly measured zooplankton communities; however, they differed from morph-taxa in species composition, with only six shared species. Different taxonomic levels can be used to assess the status of aquatic ecosystems and classifying individuals to the level of family, with 17 shared families, or genus level, with 24 shared genera, was shown to be relatively sufficient for comparing the two identification methods: metabarcoding and morphological identification. Relative abundances as determined by use of metabarcoding could be an acceptable proxy for biomass of zooplankton inferred from morphological identification at genus level. Results of previous studies have shown that eDNA/DNA copies can be correlated with organism biomass (Elbrecht and Leese, 2015; Takahara et al., 2012; Yang et al., 2017c). The genus richness is a useful metric to measure temporal dynamics of zooplankton communities. Zooplankton identification methods had comparatively similar temporal changes in genus richness, although differences did exist (Fig. S5; Appendix S4). Mitochondrial COI has been shown previously to be a valuable metabarcoding marker for zooplankton biodiversity assessment (Clarke et al., 2017).

4.2. *Potential reasons for discrepancy between zooplankton identification methods*

Several underlying factors could have affected results inferred from metabarcoding when comparing to morphological identification. Some boreal freshwater zooplankton species are not yet barcoded and represented in public databases, explaining the number of species not detected by metabarcoding. To better determine taxonomic composition through next-generation sequencing, more representative taxa of typical watersheds should be barcoded (Yang et al., 2017d). Some of the species missed by metabarcoding were included in the in-house curated database (e.g., *Diacyclops thomasi*). This seems to indicate that even in local watersheds, zooplankton can have sequence divergence and adequate number of individuals need to be barcoded in order to accurately detect at the species-level. Naming conventions of zooplankton, which can be highly variable, can also lead to differences in the species detected due to database limitations of the corresponding metadata (Visco et al., 2015), including *Keratella cochlearis.* a species complex that morphological taxonomy has not been fully worked out. More examples of variable naming conventions or validation of morphological identification consensus include *Trichotria tetractis* (e.g. *Dinocharis tetractis*), *Chydorus sphaericus, Diacyclops thomasi* (e.g. *Diacyclops/Cyclops bicuspidatus thomasi*), *Polyarthra vulgaris* (e.g. *Polyarthra trigla*), *Kellicottia longispina* (e.g. *Anuraea longispina* or *Notholca longispina*), *Bosmina longirostris, Trichocerca* (e.g. *Acanthodactylus, Coelopus, Diurella, Mastigocerca, Monocerca, Rattulus, or Vaginaria*), *Tropocyclops extensus* (e.g. *Tropocyclops prasinus mexicanus*), *Keratella crassa* (e.g. *Keratella cochlearis*), *Microcyclops rubellus* (e.g. *Microcyclops varicans rubellus*), and *Diaphanosoma birgei* (e.g. *Diaphanosoma leuchtenbergianum* or *D. brachyurum*). Metabarcoding can potentially detect taxa not from target habitat (e.g. pelagic) due to residual DNA, and possibly RNA, adhered to the zooplankton tissue and within the gut or adhered to algae likely collected by the 53 μm mesh filter used, leading to differences in species composition relative to morphological identification (Barnes et al., 2020.; Siegenthaler et al., 2019). This could explain the greater detection of zooplankton not primarily planktonic but associated with surfaces or sediments via metabarcoding methods (e.g., *Eurycercus, Macrothrix, Eucyclops, Ilyocryptus*, and *Chydorus*).

Relative abundance of detected taxa also varied between identification methods. One reason for the discrepancy of differential portions

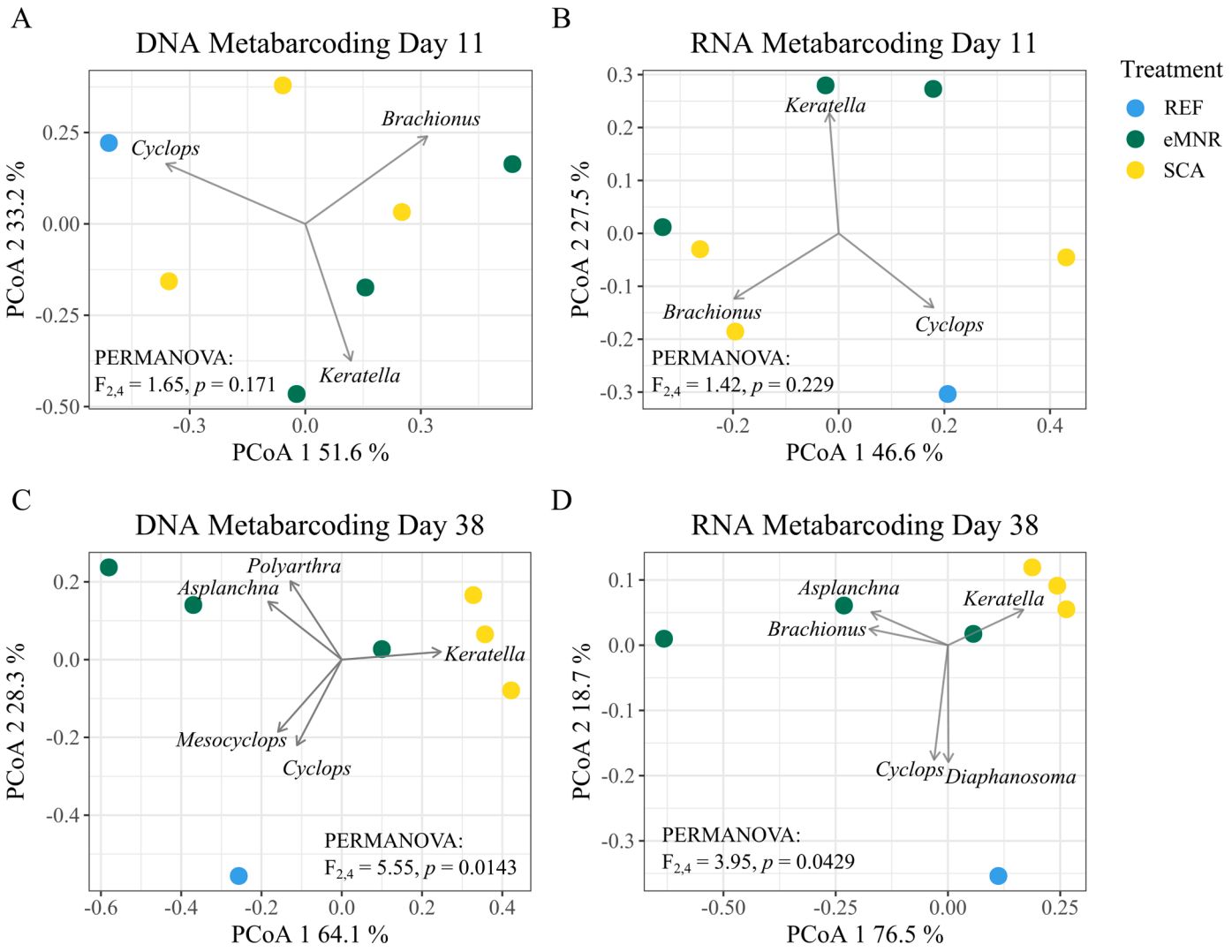

Fig. 4.. PCoA plots between treatment groups for genus-based matrix. (A) DNA metabarcoding at 11-days post-exposure; (B) RNA metabarcoding at 11-days post-exposure; (C) DNA metabarcoding at 38-day post-exposure; (D) RNA metabarcoding at 38-days post-exposure. Treatment groups consisted of enhanced monitored natural recovery (eMNR; $n = 3$), shoreline cleaner application (SCA; $n = 3$), and reference (REF; $n = 1$). Associated PERMANOVA statistic is shown on the respective plots. Genera plotted inferred to be highly correlated with sample ordination ($p < 0.01$).

between methods is zooplankton species could have shed DNA, in the form of exoskeleton or sloughed tissue, at variable rates into the water column or differ in target gene copies within individuals, resulting in potential biases of relative abundance (Harvey et al., 2017; Sassoubre et al., 2016). Total DNA amounts of zooplankton could have also influenced the inferred relative abundance. Taxa within zooplankton communities can have various life-histories, with rotifers and cladocerans being opportunistic and other plankters, such as copepods, exhibiting longer life cycles and fewer generations (Allan, 1976). This variability could have direct impacts on the inferred activity of select taxa (Blazewicz et al., 2013), while differences in zooplankton habitat preference could impact the suggested presence upon collection (Leduc et al., 2019). RNA "production" could also vary according to the life-history and biology of the target zooplankton genera, influencing the relative detection and abundance. Zooplankton DNA and RNA could also have been extracted with varying levels of recovery for different taxa, potentially affecting relative abundances inferred (Liu et al., 2019). Biases in PCR and body-size have been shown to impact inference of species presence and relative abundances in target ecosystems, affecting total species detected and their relative portions in zooplankton communities (Elbrecht and Leese, 2015; Gibson et al., 2014; Harvey et al., 2017; Polz and Cavanaugh, 1998).

Comparing to biomasses of genera determined by morph-taxa, results of metabarcoding indicated that genera in the phylum Rotifera had greater relative abundances compared to genera in the phylum Arthropoda. Traditional morphological estimates for biomasses of genera within the phylum Rotifera may not be representative due to too large of filter mesh utilized when collecting in the field (Chick et al., 2010), or due to small sizes. Differences in life history of the two phyla could also have impacts on inferred biomass. However, additional reasons could be due to sampling, extraction, and PCR steps within the metabarcoding pipeline.

4.3. DNA and RNA metabarcoding: advantages and disadvantages for ecotoxicological assessment

Metabarcoding-based genus richness could be a sensitive method to measure the ecotoxicological response of communities to environmental disturbances. SCA had the greatest negative impact on richness of the zooplankton community based on both DNA and RNA metabarcoding. COREXIT® EC9580A has been shown to be acutely toxic to the pelagic copepod species *Acartia tonsa*, and a mysid, *Americamysis bahia*, with a 48 h-LC50 of 50.4 ± 4.47 mg/L and 32 mg/L, respectively (Bi et al., 2020; Fingas, 2013; Hansen et al., 2014). Nutrient enrichment has been

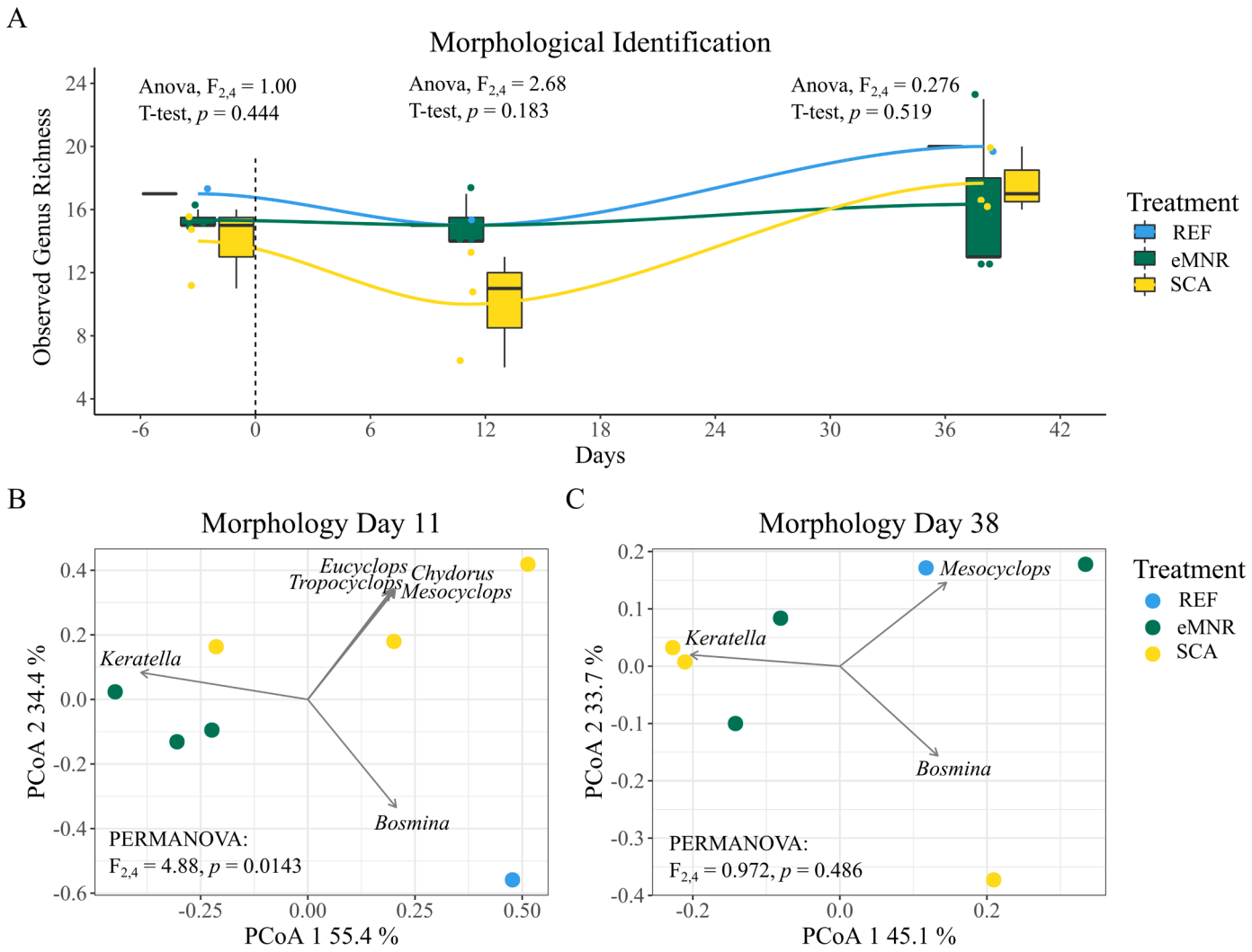

Fig. 5.. Observed zooplankton genus richness for treatments over time and β-diversity analyses of selected time points, (B) 11-days post-exposure and (C) 38-days post-exposure, for morphological identification. Treatments consisted of enhanced monitored natural recovery (eMNR; $n = 3$), shoreline cleaner application (SCA; $n = 3$), and reference (REF; $n = 1$). ANOVA was computed between treatment groups, with Welch's t-test used to test observed genus richness between treatments eMNR and SCA. Dashed line represents beginning of simulated dilbit spill and treatment trend is represented by local polynomial regression. Associated PERMANOVA statistics are shown on the respective plots PCoA plots. Genera plotted inferred to be highly correlated with sample ordination ($p < 0.01$).

found to increase richness of zooplankton, due primarily to increases in rotifer species (Azevêdo et al., 2015), which can be seen with increases in total dissolved phosphorus at 38 days post-spill for eMNR (Table S8). Too large of an increase in primary productivity, however, can lead to an overall decrease in zooplankton species richness (Dodson et al., 2000). Since RNA metabarcoding can measure response of communities at the time of sampling, without the common issue of persistence of DNA in the environment (Cristescu, 2019) or identification of nonviable zooplankton (Zetsche and Meysman, 2012), it could be advantageous for measuring changes in community structure due to exposures to stressors. Results of previous studies have shown that RNA can decipher more significant changes in taxa richness due to treatment relative to DNA metabarcoding (Laroche et al., 2017). RNA metabarcoding could be influenced by the variability in production due to life-history traits of different zooplankton genera. The variability of the enclosures at day −3 could have also impacted inferred differences between treatments for RNA metabarcoding.

Monitoring changes in community composition is a powerful method to measure effects of environmental stressors. Over time, SCA seemed to have an overall negative effect on the composition of the zooplankton community at the genus level as measured with metabarcoding techniques (Parsons et al., 1984), which could explain the greater distance between SCA and REF at day 38, relative to eMNR, and the closer clustering of the SCA samples for RNA metabarcoding. Due to the variable tolerance of zooplankton taxa to nutrient enrichment, eMNR could have had contrasting magnitudes of effects on zooplankton communities over time (Yang et al., 2017b). With variable zooplankton communities in enclosures at day −3, greater dispersion between eMNR treated enclosures over time could occur (Strecker and Arnott, 2005). Overall, DNA metabarcoding, relative to RNA metabarcoding, may be more reliable for assessing treatment effects on community composition (Laroche et al., 2017), which was observed in the current study with a larger magnitude of differences in zooplankton community composition between remediation practices for DNA metabarcoding.

DNA metabarcoding can commonly be the result of legacy contamination in the ecosystem, as DNA is typically more stable and persistent than RNA in the environment (Cristescu, 2019). RNA metabarcoding can act as an effective method to depict responses of active communities (Baldrian et al., 2012); however, variation in activities of organisms and life history can generate taxonomic biases as well as PCR artifacts formed from cDNA synthesis (Blazewicz et al., 2013; Brandt et al., 2020; Houseley and Tollervey, 2010). Resulting shared genera between

metabarcoding methods reflected the potential of life-history differences and contrasting persistence of the two nucleic acids, with greater percentage of shared genera to overall genera at 11-days post-spill (90.9%) versus day 38 (76.3%) (Appendix S5; Figure S6). It has been suggested to use both DNA and RNA metabarcoding when assessing ecosystems for these reasons, among others (Laroche et al., 2017; Pochon et al., 2017). Coupled DNA and RNA metabarcoding could serve as a stand-alone assessment of ecosystem status or can be used as a complementary method to morphology-based monitoring (Laroche et al., 2018).

4.4. Comparison of metabarcoding and morphological identification for ecotoxicological assessment of remediation practices of oil spills

Metabarcoding methods overall were more sensitive relative to morphology in measuring changes in genus richness caused by various remediation practices. There are however limitations in the statistical power of statistical tests conducted, due to small sample sizes. Alpha diversity as determined by metabarcoding has been shown to be consistent with that calculated from taxa defined by morphology, although typically more sensitive to spatial or environmental differences (Frontalini et al., 2020; Mauffrey et al., 2020). Regardless of identification method, SCA seemed to have the largest negative impact on richness of the zooplankton community over time. Morph-genera community composition shifted significantly on day 11; however, on day 38 no difference was seen between treatment groups, which disagreed with metabarcoding methods. A previous study determined that zooplankton metabarcoding can be a more sensitive method for analyzing community composition differences relative to morphology, which was seen at 38 days post-spill (Yang et al., 2017c).

5. Conclusions

This study revealed that identification of zooplankton based on ZOTUs from metabarcoding and morphological identification were relatively consistent in their ability to identify the presence of each zooplankter to the genus-level and detect changes in zooplankton communities over time due to remediation practices, although differences in the timing of the measured response was apparent. Metabarcoding could be more sensitive relative to morphological identification for detecting changes in zooplankton genus richness over time due to remediation practices. There were limitations in inferences of results when comparing between methods due to small sample sizes, including having only one reference enclosure and variability of enclosures at day −3. For the metabarcoding methods, DNA metabarcoding was the most sensitive in detecting changes in zooplankton community composition due to remediation practices. For all identification methods, SCA had the greatest impact on zooplankton genus richness relative to eMNR and REF. β-diversity analyses showed that both shoreline cleaner application and nutrient enrichment can cause changes in zooplankton community composition. Overall, as shown by both α- and β-diversity analyses, while surfactants can release stranded petroleum constituents of dilbit from shoreline substrates to be mechanically removed, shoreline cleaner application has the greatest, acute effect on the zooplankton community.

CRediT authorship contribution statement

Phillip J. Ankley: Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Yuwei Xie:** Conceptualization, Methodology, Validation, Writing – review & editing. **Tyler A. Black:** Methodology, Validation, Resources, Writing – review & editing. **Abigail DeBofsky:** Methodology, Writing – review & editing. **McKenzie Perry:** Resources, Writing – review & editing. **Michael J. Paterson:** Validation, Resources, Writing – review & editing. **Mark Hanson:** Writing – review & editing. **Scott Higgins:** Writing – review & editing. **John P. Giesy:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Vince Palace:** Funding acquisition, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.105847.

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Supporting Information for

Using zooplankton metabarcoding to assess the efficacy of different techniques to clean-up an oil-spill in a boreal lake

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Appendix S1: Experimental shoreline enclosure descriptions

Beginning in April 2019, when water temperatures had warmed enough to allow work to begin (>8°C), enclosures (15 X 5m) (Curry Industries, Winnipeg) were deployed in Lake 260 shorelines of organic wetland type sediment. The enclosures' consisted of a polystyrene foam floatation collar encased in a polyvinyl shell. The floatation collar suspended an impermeable polypropylene curtain that extended to the bottom of the lake, where it was sealed to the aquatic and terrestrial sediment/soil using a double row of sandbags. A total of six enclosures were treated with oil in shoreline areas of organic/wetland sediments. One enclosure, not treated with oil, was included to serve as a reference (a total of seven enclosures). Water depth measurements were obtained at 1m intervals from the shoreline to determine slope of the lake bottom and estimate enclosure volumes (28,500 ± 1650L). Enclosures were assigned to a given treatment, or to reference designations, randomly. Table S1 indicates the specific locations for each enclosure.

Appendix S2: MiSeq sequencing output

Metabarcoding consisted of a total of 1,637,206 sequences after demultiplexing from the two MiSeq runs, with run 1 having 698,592 and run 2 having 938,614 sequences. Technical replicates had sequence counts of 8798 ± 6914 (mean ± standard deviation (SD)), while blanks had sequence counts of 322.4 ± 455 (mean ± SD) prior to merging of the two libraries, with some samples being re-sequenced on run 2. After

denoising and merging technical replicates, a total of 1,301,361 sequences were assigned to target metazoan (Phylum Rotifer and Orders Calanoida, Cyclopoida, and Cladocera) to at least the family level, with merged technical replicates having sequence counts of 30984.8 ± 18821.9 (mean ± SD) (Table S2). After collapsing features to the taxa-level and removing unassigned taxa or taxa occurring in only one sample, samples were rarefied to equal read depths of 9985 (Figure S2).

Appendix S3: Shannon diversity for identification methods between remediation practices

ANOVA was used to estimate differences between Shannon diversity of treatments at each time point, as sample size was not sufficient for interpretation, with a student's t-test used to test for differences between eMNR and SCA. No differences in Shannon diversity were seen between treatment groups, eMNR and SCA, at day -3 (Fig. S4, T-test $p \geq 0.662$). Welch's t-test was significant at day 11 for morphological identification (Fig S4, T-test, $p = 0.0149$). SCA and eMNR differed significantly for DNA metabarcoding at day 38 (Fig. S4, T-test, $p = 0.00571$).

Appendix S4: Temporal zooplankton genus richness between identification methods

Differences in genus richness between identification methods at each time point were tested by use of Kruskal-Wallis (KW) with Dunn's Kruskal-Wallis multiple

comparison with p-values adjusted with the Holm method for post-hoc testing. No difference was observed between identification methods at days -3 (Fig. S5, Kruskal-Wallis, $p = 0.0740$), however, significant differences were observed at day 11 and 38 post-spill (Fig. S5, Kruskal-Wallis, $p \leq 0.0191$). At day 11 post-spill, DNA and RNA metabarcoding were found to have a greater richness relative to morphological identification (Fig. S5, Dunn's test, $p \leq 0.0185$), while at day 38 post-spill, DNA metabarcoding was found to have a greater zooplankton genus richness than RNA metabarcoding (Fig. S5, Dunn's test, $p = 0.0147$).

Appendix S5: Proportion of shared genera for 11- and 38-days post-spill between metabarcoding methods

A total of 33 genera were detected overall by DNA ($n = 31$) and RNA ($n = 32$) metabarcoding for 11 days post spill with metabarcoding methods sharing 90.9% or 30 genera. At 38 days post-spill, DNA ($n = 37$) and RNA ($n = 30$) metabarcoding detect an overall 38 genera with the metabarcoding methods sharing a total of 29 genera or 76.3%.

Figure S1: Aerial photo of experimental design used in comparing metabarcoding and traditional morphological identification of zooplankton in assessing the ecotoxicological effects of two remediation practices – enhanced monitored natural recovery (eMNR) using the addition of nitrogen and phosphorous, and a shoreline cleaner, COREXIT EC9580A (SCA) – relative to a reference enclosure (REF). Diluted bitumen was applied to enclosures on June 21st, with the selected remediation practices being applied on June 25th. Unlabeled enclosures are not part of this select experiment.

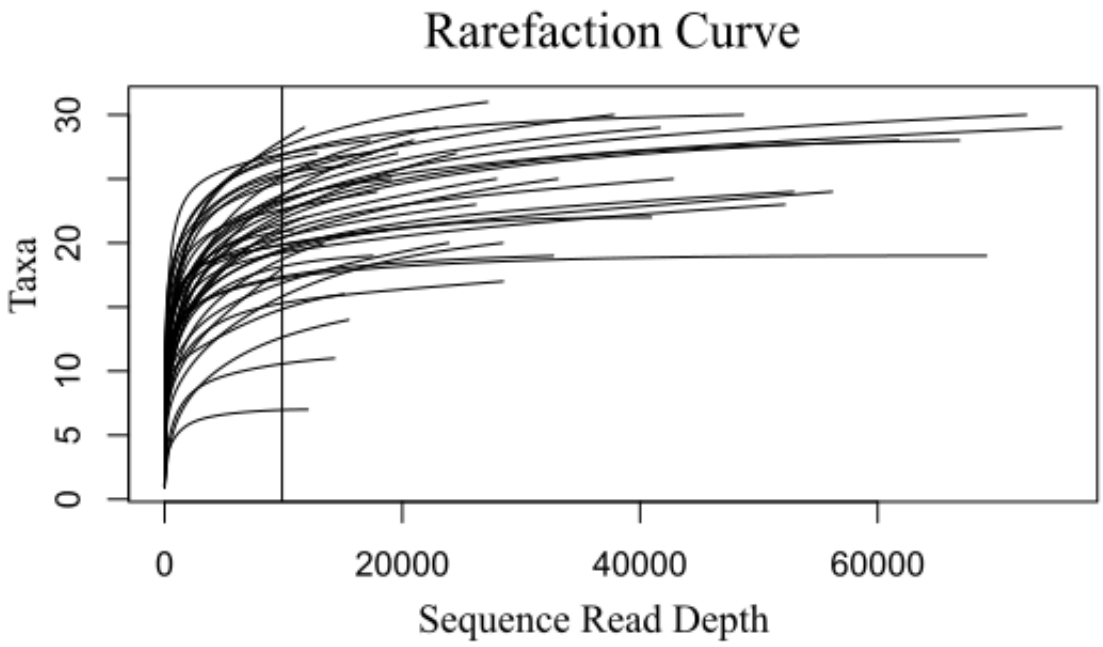
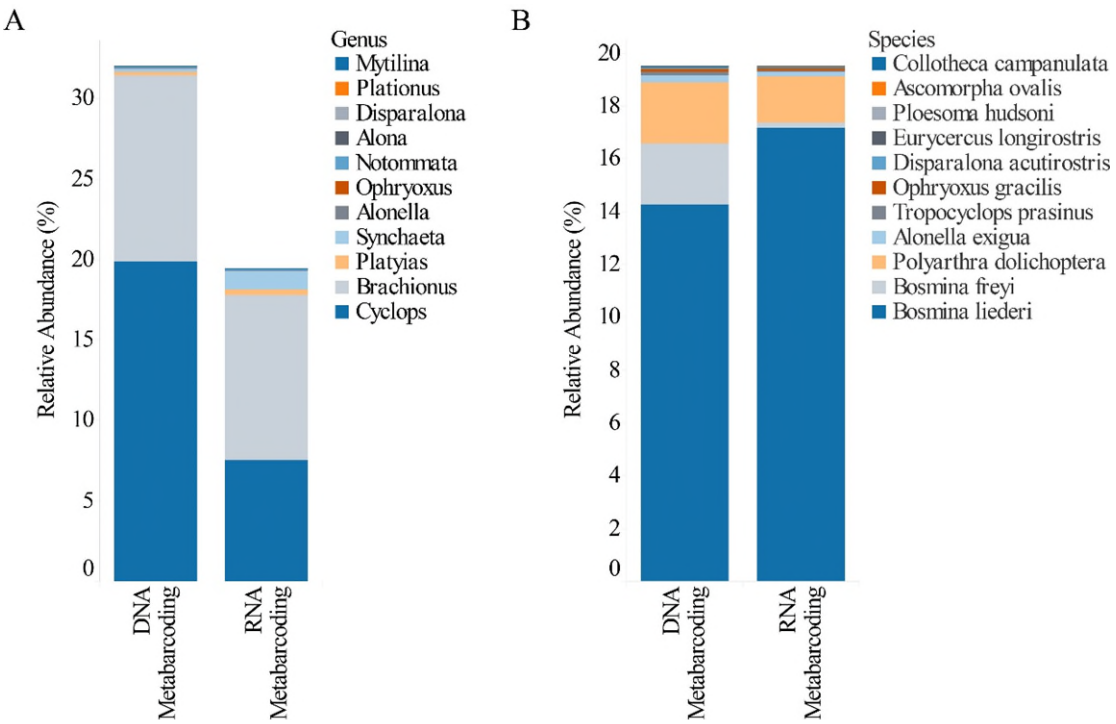


Figure S2: Rarefaction curve of number of detected taxa for each sample. The vertical line indicates the rarefied read depth of 9885.



131

132 **Figure S3:** Shared taxa between DNA metabarcoding and RNA metabarcoding at (A)

133 genus level and (B) species level. Unclassified species were filtered out.

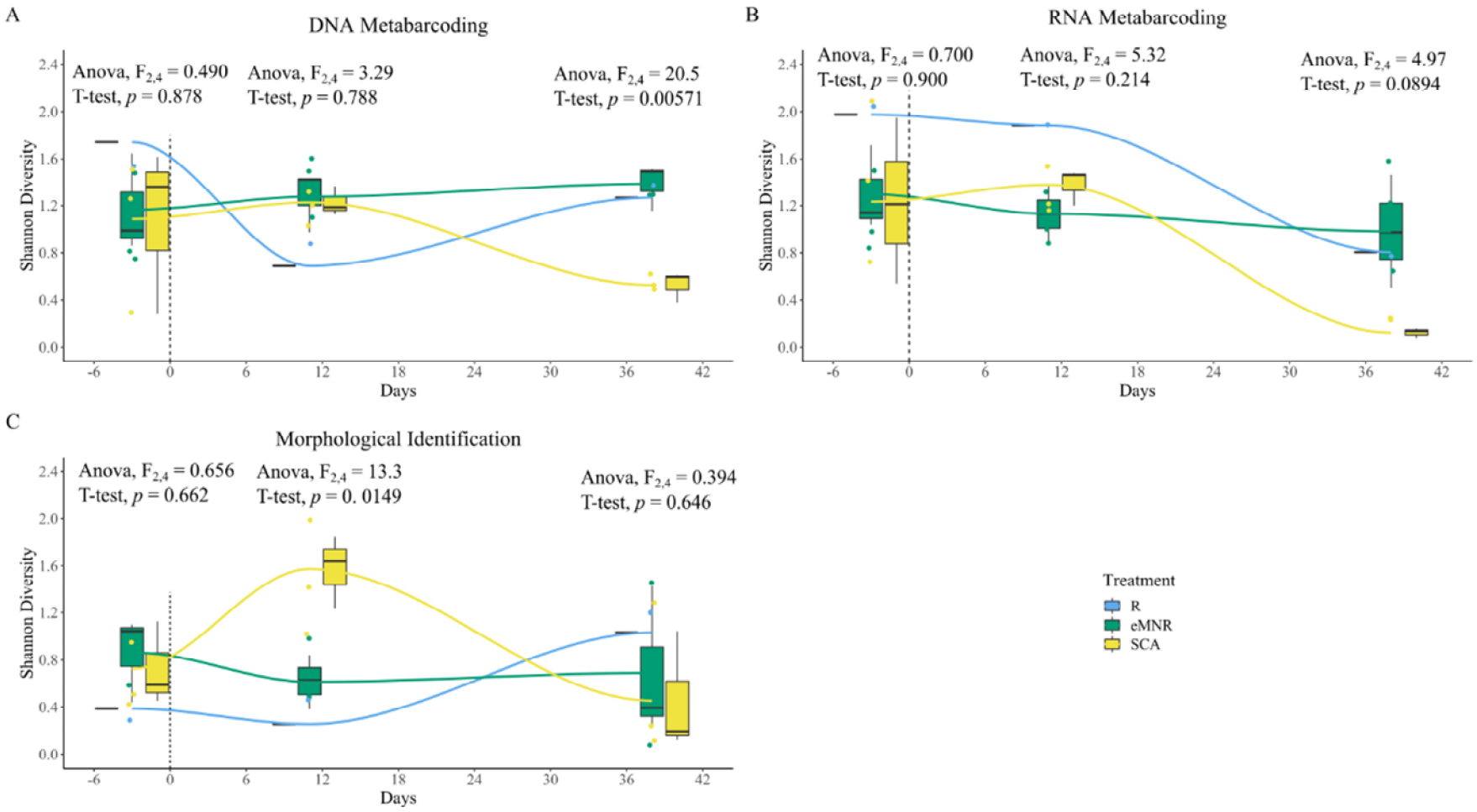


Figure S4: Zooplankton Shannon diversity for treatments over time for each zooplankton identification methods applied. Treatment groups consisted of enhanced monitored natural recovery (eMNR; n =3), shoreline cleaner application (SCA; n = 3), and reference (REF; n =1). ANOVA was used to estimate differences between treatment diversity at each time point, with a Welch's t-test being used to test between eMNR and SCA. Dashed line represents beginning of simulated dilbit spill and treatment trend is represented by local polynomial regression.

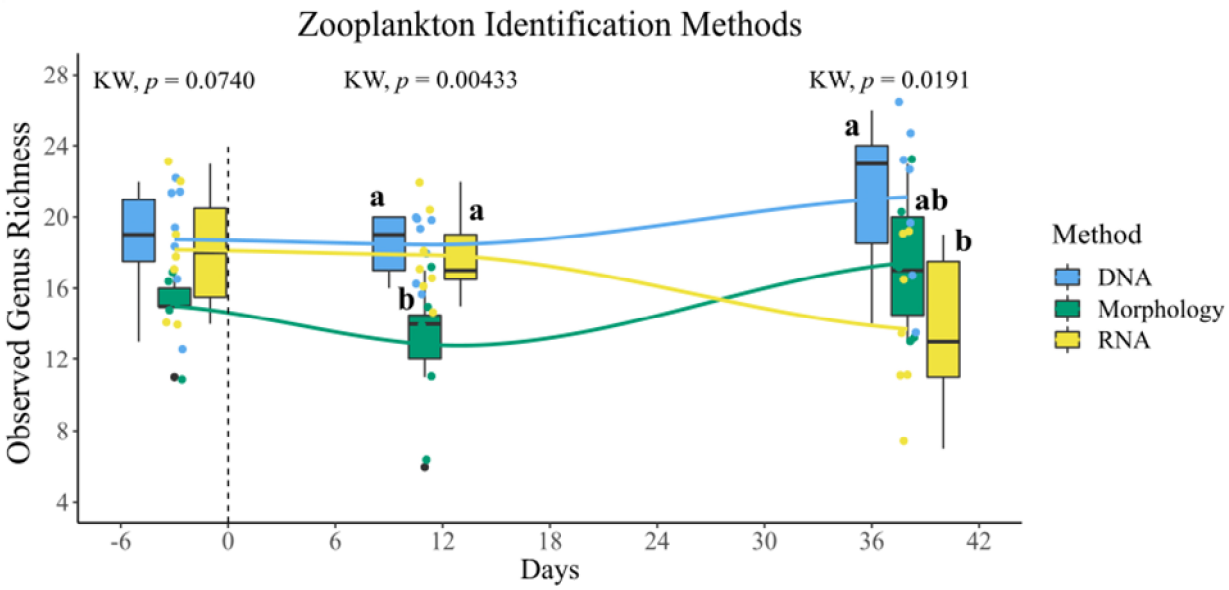
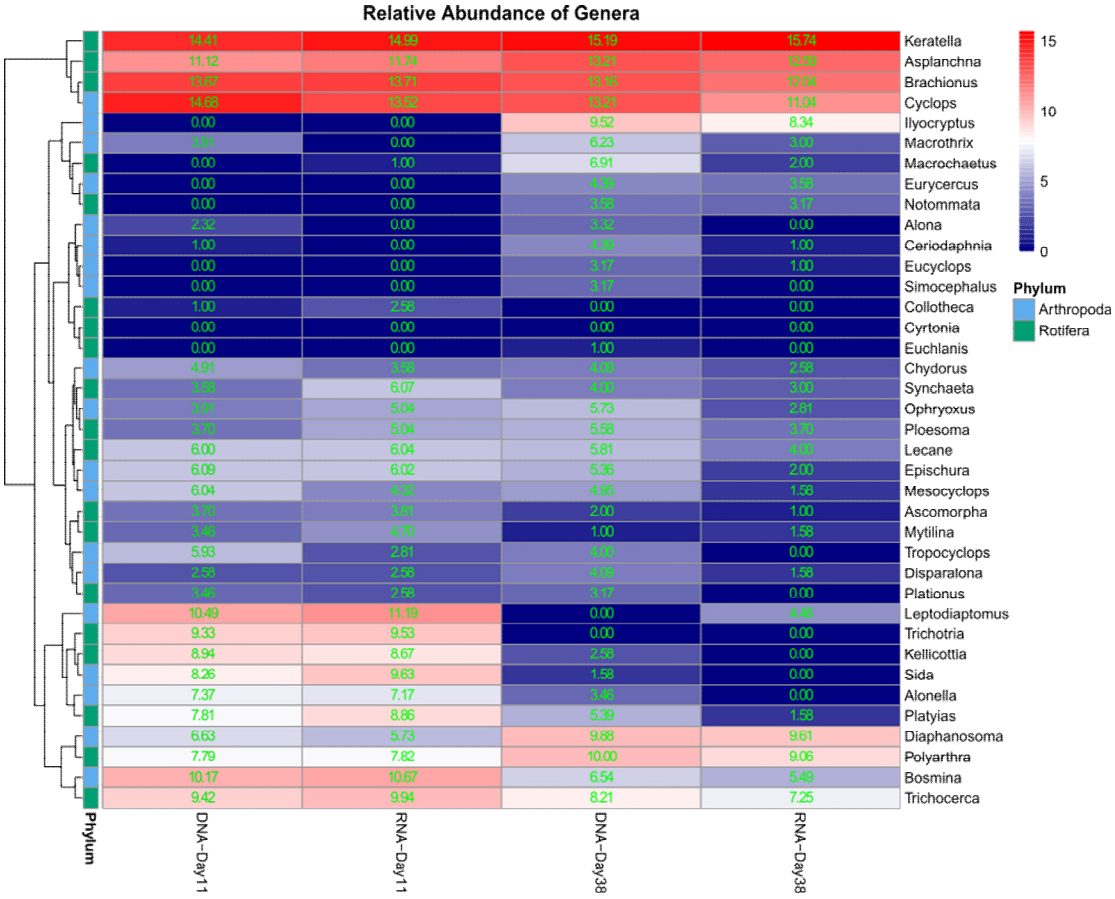


Figure S5: Overall genus richness over time for zooplankton identification methods. Kruskal-Wallis was computed between identification methods at each sampling time point. Identification methods consisted of morphological identification (Morphology), DNA metabarcoding (DNA), and RNA metabarcoding (RNA) with sample size $n = 7$ for each method. Lowercase letters indicated significance level of $\alpha < 0.05$ inferred from Dunn's Kruskal-Wallis multiple comparison with p-values adjusted with the Holm method. Dashed line represents beginning of simulated dilbit spill and treatment trend is represented by local polynomial regression.



149

150 **Figure S6:** Relative abundance for genera identified by metabarcoding for 11- and 38-

151 days post-spill. DNA-Day11 refers to DNA metabarcoding at 11 days post-spill, with

152 the additional acronyms following this pattern. Total sequence read counts were log

153 base 2 transformed, and rows were clustered according to complete linkage for data

154 presentation.

155 **Table S1:** Experimental shoreline enclosure locations within Lake 260.

Encosure	Treatment	Latitude	GPS Latitude	Longitude	GPS Longitude
wR1	Reference	N	49.69983	W	93.76760
wEMNR1	EMNR	N	49.69994	W	93.76750
wSC1	SC	N	49.69997	W	93.76740
wSC2	SC	N	49.70000	W	93.76730
wEMNR2	EMNR	N	49.70000	W	93.76721
wEMNR3	EMNR	N	49.70041	W	93.76715
wSC3	SC	N	49.70048	W	93.76711

156

Table S2: Resulting annotated sequence read output post-denoising and ORF correction. Non-target indicates non-metazoan sequence reads, metazoan indicates all sequence reads assigned to metazoan, and target indicates zooplankton annotated sequence reads.

Total Reads	Non-target Reads	Metazoan Reads	Zooplankton Reads
1,552,241	57458	1494783	1301361

162 **Table S3:** Family-level rarefied (9885) metabarcoding count data and raw

163 morphological abundance and biomass data summed for each identification method.

Family	DNA Metabarcoding	RNA Metabarcoding	Morphological Abundance	Morphological Biomass
Asplanchnidae	12274	12578	1.944	0.02527
Bosminidae	10165	10408	1597.7	264.8
Brachionidae	123956	147648	3772.8	51.89
Chydoridae	2416	1044	20.77	12.46
Collothecidae	3	7	0.5556	0.03056
Cyclopidae	41467	15635	125.89	121.91
Cyprididae	2	0	0	0
Daphniidae	34	13	5.277	5.635
Diaptomidae	7248	7500	30.51	41.014
Euchlanidae	2	0	0.3162	0.009486
Eurycercidae	21	12	0.3299	0.6597
Gastropodidae	20	53	1.994	0.03408
Lecanidae	131	102	4.541	0.14212
Lepadellidae	0	0	0.1325	0.0006630
Macrothricidae	917	413	3.475	3.377
Notommatidae	34	20	0	0
Sididae	1429	1708	14.44	22.33
Synchaetidae	3079	5595	8.497	0.3292
Temoridae	1850	2098	4.335	9.367
Trichocercidae	1051	1298	0.2792	0.01954
Trichotriidae	1486	1450	0.2406	0.007219

164

Table S4: Genera-level rarefied (9885) metabarcoding count data and raw morphological abundance and biomass data summed for each identification method.

Genus	DNA Metabarcoding	RNA Metabarcoding	Morphological Abundance	Morphological Biomass
Alona	32	14	0	0
Alonella	182	152	0	0
Ascomorpha	20	53	0.4529	0.006341
Asplanchna	12274	12578	1.944	0.02527
Bosmina	10165	10408	1597.7	264.8
Brachionus	23934	21300	0	0
Ceriodaphnia	23	3	5.127	5.127
Chydorus	2176	869	20.77	12.46
Collotheca	3	7	0.5556	0.03056
Cyclops	41274	15598	0	0
Cyrtonia	2	0	0	0
Daphnia	2	10	0.1500	0.5087
Diacyclops	0	0	7.062	4.772
Diaphanosoma	1075	852	13.98	15.41
Disparalona	26	9	0	0
Epischura	1850	2098	4.335	9.367
Euchlanis	2	0	0.3162	0.009486
Eucyclops	10	2	3.064	3.999
Eurycercus	21	12	0.3299	0.6597
Gastropus	0	0	1.541	0.02774
Ilyocryptus	732	324	1.582	3.1630
Kellicottia	956	2362	2.096	0.03144
Keratella	98530	123168	3770.7	51.86
Lecane	131	102	1.961	0.06472
Lepadella	0	0	0.1325	0.0006630
Leptodiaptomus	7248	7500	30.51	41.014
Macrocyclops	0	0	0.2480	1.171
Macrochaetus	0	6	0	0
Macrothrix	111	27	1.894	0.2143
Mesocyclops	106	28	83.79	102.1
Microcyclops	0	0	0.2817	0.3934
Monostyla	0	0	2.580	0.07739
Mytilina	13	30	0	0
Notommata	34	20	0	0

Ophryoxus	74	62	0	0
Plationus	20	6	0	0
Platyias	503	782	0	0
Ploesoma	928	1961	7.307	0.2923
Polyarthra	1742	1230	1.191	0.03691
Sida	354	856	0.4615	6.923
Simocephalus	9	0	0	0
Synchaeta	409	2404	0	0
Trichocerca	1051	1298	0.2792	0.01954
Trichotria	1486	1444	0.2406	0.007219
Tropocyclops	77	7	31.45	9.421

167

Table S5: Species-level rarefied metabarcoding count data and raw morphological abundance and biomass data summed for each identification method. Note, due to taxa not assigned to species-level being filtered out, the read counts do not equal to the rarefied depth of 9885.

Species	DNA Metabarcoding	RNA Metabarcoding	Morphological Abundance	Morphological Biomass
Alonella exigua	167	117	0	0
Ascomorpha ovalis	4	7	0	0
Bosmina freyi	1429	134	0	0
Bosmina liederi	8736	10274	0	0
Bosmina longirostris	0	0	1597.7	264.8
Chydorus sphaericus	0	0	20.77	12.46
Collotheca campanulata	3	7	0	0
Daphnia mendotae	2	10	0.1500	0.5087
Diacyclops thomasi	0	0	7.0618	4.772
Diaphanosoma birgei	0	0	13.98	15.41
Disparalona acutirostris	26	9	0	0
Epischura lacustris	1850	2098	4.335	9.367
Eurycercus longirostris	21	12	0	0
Kellicottia longispina	0	0	2.0962	0.0314
Keratella cochlearis	39751	37656	201.06	2.011
Keratella crassa	0	0	28.920	0.2892
Keratella serrulata	0	0	1.5176	0.01518

Keratella taurocephala	0	0	3539.16	49.55
Leptodiaptomus minutus	7231	7500	30.508	41.01
Macrocyclops albidus	0	0	0.2480	1.171
Mesocyclops edax	106	28	83.79	102.1
Microcyclops rubellus	0	0	0.2651	0.3686
Ophryoxus gracilis	74	62	0	0
Ploesoma hudsoni	12	12	0	0
Polyarthra dolichoptera	1407	1026	0	0
Polyarthra vulgaris	0	0	1.1905	0.03691
Sida crystallina	354	856	0.4615	6.923
Simocephalus serrulatus	9	0	0	0
Trichocerca cylindrica	0	0	0.2792	0.01954
Trichotria tetractis	0	0	0.2406	0.007219
Tropocyclops extensus	0	0	31.449	9.4214
Tropocyclops prasinus	77	7	0	0

172

Table S6: Resulting t-test statistics from pairwise comparison of computed least square means for treatments based on random intercept model. Treatment groups consisted of enhanced monitored natural recovery (eMNR), shoreline cleaner application (SCA), and reference (REF).

	Comparison	Estimate	Std. Error	df	t value	Pr(>|t|)
DNA Metabarcoding	REF - eMNR	1.67	1.74	16	0.958	0.353
	REF - SCA	5.11	1.74	16	2.94	0.00968
	eMNR - SCA	3.44	1.59	16	2.80	0.0129
RNA Metabarcoding	REF - eMNR	3.89	1.81	16	2.15	0.0473
	REF - SCA	6.67	1.81	16	3.69	0.00203
	eMNR - SCA	2.78	1.28	16	2.17	0.0613
Morphological identification	REF - eMNR	1.78	2.06	16	0.864	0.401
	REF - SCA	3.44	2.06	16	1.67	0.114
	eMNR - SCA	2.67	1.46	16	1.15	0.269

178 **Table S7:** Pairwise comparison between treatment groups based on total distance
179 matrix for all samples, while blocking the effects of time. Treatment groups consisted
180 of enhanced monitored natural recovery (eMNR), shoreline cleaner application (SCA),
181 and reference (REF).

Comparison	DNA Metabarcoding				RNA Metabarcoding			Morphological Identification		
	DF	F-Statistic	R2	Pr(>F)	F-Statistic	R2	Pr(>F)	F-Statistic	R2	Pr(>F)
REF -eMNR	1,10	2.91	0.226	0.0173	1.58	0.136	0.0974	2.01	0.168	0.0478
REF - SCA	1,10	4.13	0.292	0.0174	2.16	0.178	0.0142	1.96	0.164	0.0796
eMNR - SCA	1,16	1.10	0.0642	0.203	0.979	0.0576	0.267	0.568	0.0343	0.498

182

183 **Table S8:** Total dissolved phosphorus and chlorophyll *a* measurement for treatments

184 (average ± SD) over time points sampled. Treatment groups consisted of enhanced

185 monitored natural recovery (eMNR), shoreline cleaner application (SCA), and

186 reference (REF).

Date	Total Dissolved Phosphorus			Chlorophyll *a*		
	REF	eMNR (avg. ± SD)	SCA (avg. ± SD)	REF	eMNR (avg. ± SD)	SCA (avg. ± SD)
6/18/2019 (Day -3)	6.00	6.03 ± 1.10	5.80 ± 0.889	4.10	3.83 ± 0.953	4.05 ± 1.27
7/02/2019 (Day 11)	6.80	6.87 ± 1.66	6.60 ± 0.458	2.60	3.60 ± 1.16	4.57 ± 1.38
7/29/2019 (Day 38)	4.90	6.27 ± 1.10	4.60 ± 0.794	1.43	1.92 ± 0.0874	3.68 ± 1.65

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