Metagenetic Analysis of 2018 and 2019 Plankton Samples from Prince William Sound, Alaska.

Report to Prince William Sound Regional Citizens' Advisory Council (PWSRCAC)

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ABSTRACT

This report describes the methods and findings of the metagenetic analysis of plankton samples from the waters of Prince William Sound (PWS), Alaska, taken in May of 2018 and 2019. The study was done to identify zooplankton, in particular the larvae of benthic non-indigenous species (NIS). Plankton samples, collected by the Prince William Sound Science Center (PWSSC), were analyzed by the Molecular Ecology Laboratory at the Moss Landing Marine Laboratories. The samples were taken from five stations in Port Valdez and nearby in PWS. DNA was extracted from bulk plankton and a portion of the mitochondrial Cytochrome c oxidase subunit 1 gene (the most commonly used DNA barcode for animals) was amplified by polymerase chain reaction (PCR). Products of PCR were sequenced using Illumina reagents and MiSeq instrument. In 2018, 257 operational taxonomic units (OTU; an approximation of biological species) were found and 60 were identified to species. In 2019, 523 OTU were found and 126 were identified to species. Most OTU had no reference sequence and therefore could not be identified. Most identified species were crustaceans and mollusks, and none were non-native. Certain species typical of fouling communities, such as Porifera (sponges) and Bryozoa (moss animals) were scarce. Larvae of many species in these phyla are poorly dispersing, such that they will be found in abundance only in close proximity to adult populations. Because fouling communities are important reservoirs of NIS, the absence of NIS in the OTU list may not reflect the prevalence of NIS in Port Valdez. As in previous years, there was overlap but strong differences between years. This variation could be a sampling effect of low replication compounded by natural temporal variation.

INTRODUCTION

Marine invasive species are found in most harbors worldwide. Their ecological and economic impacts are widely variable, poorly predictable, and often place-specific. Implementation of eradication or mitigation measures are most likely to be successful when new invaders are detected when populations are small and localized. Thus, frequent monitoring of species near likely points of entry (e.g., docks, aquaculture facilities, disturbed habitat) can provide important information to managers and policy-makers.

Context for the present study in Port Valdez and Prince William Sound was given in a previous report to PWSRCAC (Geller et al. 2019): "Monitoring marine habitat for species of concern, including invasive species, can be costly and time-consuming, which limits the information available to resource managers, scientists, and the public. Two of the reasons for the high cost of monitoring are labor-

intensive sampling methods and the need for expert taxonomists to identify specimens. A genetic approach to species identification can reduce the reliance on taxonomic experts, as DNA sequences from all species are analyzed similarly (unlike morphological analysis). High throughput sequencing, particularly metagenetics or metabarcoding, is an increasingly popular tool for assessing aquatic biodiversity (Valentini et al. 2016, Borrell et al. 2017, Ransome et al. 2017). Metabarcoding, which allows for community level assessments of multispecies samples through the amplification of a single locus (Taberlet et al. 2012), is used to address questions in aquatic habitats such as community richness and composition (Ransome et al. 2017) and invasive species detection (Xiong et al. 2016, Borrell et al. 2017). Given the high sensitivity of the method for detecting low abundance or rare taxa (Zhan et al. 2013), this method has great appeal for early detection of aquatic invasive species (Xiong et al. 2016), an essential step to prevent the establishment of nuisance species."

We have conducted metabarcoding studies of marine and estuarine habitat in California for the detection of invasive species, sponsored by the California Department of Fish and Wildlife (https://wildlife.ca.gov/OSPR/Science/Marine-Invasive-Species-Program/Monitoring). Ten bays from San Diego to Humboldt Bay were sampled with settlement plates and plankton collections. Approximately 3000 species were detected in plankton samples, while settlement plates yielded more than 4000 species. Of these, we were able to identify 108 and 111 invasive species from plankton and plates, respectively. These results validate the utility of the metabarcoding method.

The studies in Port Valdez and Prince William Sound described here have focused on plankton as an easily accessible source of larvae of benthic invertebrates. Larvae can change in composition and abundance at diel, daily, monthly, or seasonal time scales. To date, these studies have not accounted for such changes in larvae composition and abundance because samples are only taken once per year. An important conclusion of this and our prior studies is that scales of variation for larvae in plankton need to be better understood if species lists are to be comprehensively created and invasive species identified in Port Valdez and Prince William Sound.

METHODS

Field sampling

Five and 11 plankton samples were collected in 2018 and 2019, respectively, by PWSSC from Prince William Sound (PWS), Valdez Arm (VA), Valdez Marine Terminal Station E (VTE), N (VTN), and W (VTW) (Figure 1). Casts of a 30 centimeter (cm) diameter, 80 micrometer (µm) mesh net were towed from a 5 meter (m) depth to the surface, except as noted below. Plankton was concentrated and preserved in DNE solution (20% DMSO, 500 mM EDTA, and NaCl at saturation). Two samples in 2019, from VA and VTE, were deep vertical tows from 50 meters to the surface. Plankton tows were not replicated except for five replicate samples that were collected at PWS in 2019. Further information and metadata are available from PWSSC.

Laboratory and bioinformatic analysis

Samples were homogenized and subsamples of the homogenates were used for genomic DNA (gDNA) extractions in October 2019. Metagenetic libraries of all samples were prepared and sequenced following protocols previously published (Lohan et al., 2019). This resulted in DNA reads (raw sequences from single molecules) for the Cytochrome c oxidase subunit 1 gene (COI) from species contained in each plankton sample.

Analysis of sequences was performed with the software program USEARCH 10 (Edgar, 2010). Reads were paired (combining the complementary sequences of double-stranded DNA) and filtered for quality. Replicates of identical sequences and sequences occurring once (singletons) were temporarily set aside to facilitate other computational steps. Sequences were then clustered at a 95% similarity threshold. These 95% clusters are considered to be operation taxonomic units (OTUs) which approximate biological species. For analysis of read abundance, all reads were mapped to an in-house database of plankton OTU sequences (including many from California as well as all Valdez OTUs found herein).

Statistical analysis was performed with the software package Plymouth Routines in Multivariate Ecological Research (PRIMER 7) software (Clark and Gorley 2015). Reads were rarefied to normalize sequence yield from each sample. First, permutational multivariate analysis of variance (PERMANOVA), a non-parametric analysis of variation, was used to test for significant differences between years. Next, non-metric multidimensional scaling (nMDS) plots were generated to visually compare similarity of samples. In the nMDS, distance between samples, represented by dots plotted in two dimensions, indicates community similarity as estimated by a Bray-Curtis similarity index. The Bray-Curtis similarity index considers both taxonomic composition and abundance of taxa.

Because of variation in the rate of molecular evolution among taxa, a 95% cluster of COI sequences is not a perfect approximation of biological species. It is possible that some OTUs contain more than one species that have little genetic separation. It is also possible that sequences from one biological species may separate into more than one OTU if that species is unusually variable. This is the same problem faced by morphological taxonomists with highly polymorphic species on the one hand or morphologically similar cryptic species on the other.

To assign taxonomy to OTUs, a representative sequence from each OTU was compared using the software program BLAST (www.ncbi.nih.gov) to a proprietary in-house COI reference database of invasive species and to a curated database extracted from Genbank (Heller et al., 2018). OTUs matching database records at 95% or higher similarity were considered as provisionally identified, after correcting taxonomic errors in Genbank known to us.

BLAST results were filtered by removing results that did not have full binomial names. Thus, genus-only or environmental samples, for example, are not listed here, as we cannot assign a species name. Without a species name, we cannot assess native or introduced status.

The possibility of non-native species among identifiable OTUs was evaluated by examining geographic distributions as reported in the World Register of Marine Species (WoRMS) or in published literature. The geographic source of reference sequences in Genbank was also examined, when such information was provided by depositors. Lastly, external experts (Drs. James Carlton, Williams College, and Paul Fofonoff, Smithsonian Institution) examined the list to flag any species that were probably NIS.

RESULTS

Samples collected in 2018 yielded 3,752,656 reads and 1,774,972 sequences (after merging forward and reverse reads). After rejecting low quality sequences, 1,680,320 sequences remained, which formed 257 OTU when clustered at the 95% threshold. Samples collected in 2019 returned 8,919,216 reads and 7,051,176 sequences after merging. After rejecting low quality sequences, 6,587,376 sequences remained, which were clustered at 95% similarity to yield 523 OTUs (Table 1).

Data Rarefaction

The total of reads per sample mapped to the 95% OTU table for the five samples collected in 2018 ranged from 89,664 to 229,113. After rarefying to progressively higher numbers of reads, we found that the number of recovered 95% OTUs became relatively constant after approximately 10% of the total reads were subsampled for all five samples (Figure 2). The data were therefore rarefied in 10 trials to the level of the lowest read yield (89,664), which exceeded the 10% level, to compare samples and to compute diversity indices.

The total reads per sample mapped to a 95% OTU table for the 11 samples collected in 2019 ranged from 149,692 to 444,289. The number of recovered 95% OTUs became relatively constant as approximately between 10% and 30% of the total reads were examined for all the samples (Figure 3). The data were rarefied in 10 trials to 149,692 reads per sample to retain all samples for the 95% OTUs and diversity indices comparisons. Additionally, sequencing data of the 2018 and 2019 samples were combined and rarefied to 94,405 reads per sample to compare results across years.

OTUs, Diversity Indices, and Composition Analysis

Comparisons of OTUs and diversity indices were based on the rounded average of 10 repeated rarefactions since there was no biological replication at the sample collection sites in 2018 and 2019, except for the PWS site in 2019. Biological replicates of the PWS samples collected in 2019 were averaged and the two deep vertical tow samples collected at VA and VTE were excluded, as ecological outliers, from the OTU and diversity comparisons but retained on the nMDS plots.

The numbers of total 95% OTUs recovered from the samples collected at PWS, VA, VTE, VTN, and VTW in 2018 were 146, 170, 170, 116, and 121, respectively; the numbers of unique 95% OTUs recovered at these sites were 27, 23, 33, 0, and 5, respectively (Figure 4). Seventy 95% OTUs were found at all sites (Figure 4). Furthermore, the plankton composition was relatively more diverse, as measured by Shannon and Simpson indices, at VTE and VA followed by PWS and the rest of the Valdez Marine Terminal stations (Table 2). The Shannon and Simpson indices incorporates species abundance such that rare species contribute less to diversity, whereas species richness is simply the number of species present regardless of their abundance. Neither measure is “better” – they provide a different perspective on the same sample.

For the shallow towed samples collected in 2019, the numbers of 95% OTUs recovered from the samples at PWS, VA, VTE, VTN, and VTW were 276, 238, 223, 238, and 213, respectively; the numbers of unique 95% OTUs were 116, 17,17, 25, and 10, respectively, and 86 OTUs were recovered from all sites (Figure 5). Although more 95% OTUs were recovered from the PWS samples, plankton composition was relatively more diverse at the rest of the sites (Table 2).

Multiyear (2018 and 2019) analysis, using samples retained after rarefying in 10 trials to 94,405 reads per sample in 10 times, showed distinct plankton communities in each year, but not among within-bay sites. This was found using either the Jaccard (Figure 6) or Bray-Curtis (Figure 7) similarity index. The Bray-Curtis index incorporates abundance and is often preferred for ecological studies. However, for metabarcoding, abundance of DNA reads per OTU is a proxy for organism counts, but read abundance is indirectly related to organism abundance. For this reason, we also use the Jaccard index which is based only on species presence or absence. The sample from PWS collected in 2019 was distinct from those within Valdez Bay (Figure 6 and 7).

Taxonomically Assignable 95% OTUs

The representative 95% OTU sequences recovered from the plankton samples collected in 2018 and 2019 were compared to the MLML COI references and a modified GenBank COI references (CoArbitrator version 1) databases for searching potential biological species. Queries returned with high-quality results (query coverage and pairwise identity thresholds were set to be 90% or above and 95% or above, respectively) were retained and summarized in Table 3.

Non-Indigenous Species

None of the species that could be identified in the 2018 or 2019 samples were flagged as potential NIS.

DISCUSSION

The aim of this study was to describe the species composition in the zooplankton of the Port of Valdez, with focus on the detection of NIS. After sequencing the DNA barcode typically used for animals (a portion of the mitochondrial COI gene) from whole-plankton DNA extractions, and clustering similar sequences into groups (OTUs) that represent presumed biological species, we found 257 and 523 species in 2018 and 2019, respectively. Of these, 60 and 126 OTU in each year could be taxonomically identified by comparison to existing genetic databases (Table 3).

Takeaway 1*: Improving genetic databases by barcoding expertly identified voucher specimens will improve the ability of metabarcoding to assess communities.*

The majority of species that could be identified were crustaceans and dominated by copepods, which is expected for zooplankton. The second most prevalent phylum was Mollusca, dominated by the larvae of benthic gastropods and bivalves. Together, these results illustrate the potential for plankton metabarcoding to describe both holoplanktonic and benthic communities. However, with little doubt we found far fewer benthic invertebrate species than must actually exist in Port Valdez. This is true for each phylum in Table 3. Particularly worrisome is the paucity or absence of bryozoans, ascidians, and sponges, all important and abundant members of NIS assemblages worldwide. These groups are typified by larvae that are short-lived in the plankton and do not disperse great distances from adults. We hypothesize that our sampling was too distant from hard substrata to effectively capture larvae of these phyla.

Takeaway 2*: Plankton sampling from dockside or closely adjacent to docks, floats, boats, piers, and rip-rap will improve detection of certain phyla such as bryozoans, sponges, and ascidians.*

A final observation of the make-up of the species list in Table 3 is the relative scarcity of taxa likely to be common in the local fauna. In particular, peracarid crustaceans (e.g., isopods and amphipods) are barely represented, but are often among the most abundant benthic invertebrates everywhere. Peracarids do not have planktonic larvae, but are instead hatched as benthic juveniles.

Takeaway 3*: While plankton sampling is logistically simpler, occasional benthic sampling may be advised to fill in taxa expected on biological grounds to be rare in the plankton. Occasional sampling for adults also will help assess the efficacy of plankton sequencing in detection of known NIS.*

The overarching aim of this study was to detect the presence of NIS in the zooplankton of the Port of Valdez. None were found, which contrasts with our experience in 10 California bays where essentially all plankton tows reveal introduced species (MLML, unpublished data).

However, the present result should be interpreted with caution and in context of the nature of the sampling. Sample sizes were small (5 and 11 plankton tows, in 2018 and 2019) and were made in open water channels some distance from fouling communities where NIS are expected to be most abundant (Figure 1). We found relatively small variation in community composition between sites (with the exception of the outlier site in Prince William Sound, distant from the actual port), suggesting that the zooplankton were reasonably well mixed horizontally. On the other hand, higher variation exists between years (Figure 6 and 7, Table 3).

Few species (6% of the total identified) were found in all four years and most (94%) were found in one year only (Figure 7). Some species were found in consecutive years, while others were observed after a year or two of absence (Table 3). While not impossible (given only these data), we have no reason to believe in a real pattern of local extinction and re-emergence. Therefore, the data seems best explained by variation in detection over time. However, without structured sampling over several time scales (diel, daily, weekly, seasonally, or yearly), we do not know which scale of temporal variation is most responsible for the differences among the yearly samples, or the role of sample size itself.

Takeaway 4: *An increased and structured program of sampling is necessary to understand the variability in the species found each year. Expanding collections to the night, in addition to daytime, and throughout the summer and spring seasons, will both increase the total species found.*

In summary, the absence of NIS in the species list derived from metabarcoding plankton samples is an indication of low penetration of NIS into the local fauna of the Port of Valdez. However, variation in the number of species identified and the low overlap in species composition from year to year suggests that a higher intensity of sampling spread longer over the spring and summer will be necessary to build a more comprehensive annual species list. We also strongly suspect that sampling in closer proximity to fouling communities will better target NIS.

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Table 1. Summary of bioinformatics of the internal COI metagenetic data on Valdez plankton samples collected in 2018 and 2019.

	2018	2019
Number of Samples	5	11
Number of Total Reads	3,752,656	8,919,216
Number of Merged Reads	1,774,972	7,051,176
Number of Quality Filtered Reads	1,680,320	6,587,376
Number of 99% OTUs	925	1,284
Number of 95% OTUs	257	523

Table 2. Average Shannon's diversity and Simpson's indices of 2018 and 2019 Valdez plankton samples based on the 95% OTU compositions. Data were rarefied to 89,664 and 149,692 reads per sample, in 10 times, respectively. Site names are denoted as follow: PWS= Prince William Sound, VA= Valdez Arm, VTE= Valdez Marine Terminal Station E, VTN= Valdez Marine Terminal Station N, and VTW= Valdez Marine Terminal Station W.

	2018		2019	
	Mean Shannon's Index (log e) ± SE	Mean Simpson's Index (1-λ) ± SE	Mean Shannon's Index (log e) ± SE	Mean Simpson's Index (1-λ) ± SE
PWS	2.32±0.001	0.777±0.0003	2.01±0	0.649±0.0001
VA	2.90±0.002	0.881±0.0001	2.72±0.001	0.867±0.0002
VTE	2.91±0.002	0.887±0.0001	2.91±0.001	0.915±0.0001
VTN	1.23±0	0.374±0	3.32±0.001	0.932±0.0001
VTW	2.01±0.001	0.656±0.0002	2.78±0.001	0.902±0.0001

Table 3. Assignable taxonomy for 95% OTUs from 2016-2019 Valdez and Prince William Sound plankton. "X" indicates a species was detected. 2016 and 2017 were previously reported (Geller et al. 2019).

Species	Phylum	Class	2016	2017	2018	2019
Arctonoe vittata	Annelida	Polychaeta				X
Glycera nana	Annelida	Polychaeta				X
Harmathoe rarispina	Annelida	Polychaeta		X		
Harmothoe fragilis	Annelida	Polychaeta				X
Harmothoe imbricata	Annelida	Polychaeta				X
Laonice sp.	Annelida	Polychaeta	X		X	X
Magelona sp.	Annelida	Polychaeta				X
Mesochaetopterus taylori	Annelida	Polychaeta				X
Micronereis nanaimoensis	Annelida	Polychaeta				
Neanthes acuminata	Annelida	Polychaeta			X	
Nereis vexillosa	Annelida	Polychaeta	X	X		
Pectinaria granulata	Annelida	Polychaeta				X
Pholoides asperus	Annelida	Polychaeta	X			X
Phyllodoce groenlandica	Annelida	Polychaeta				X
Prionospio steenstrupi	Annelida	Polychaeta		X		X
Rhynchospio glutaea	Annelida	Polychaeta			X	
Sabellariidae sp.	Annelida	Polychaeta				X
Scolelepis squamata	Annelida	Polychaeta			X	X
Spionidae sp.	Annelida	Polychaeta				X
Spiophanes norrisi	Annelida	Polychaeta				X
Spiophanes uschakowi	Annelida	Polychaeta				X
Tomopteris sp.	Annelida	Polychaeta				X
Evadne nordmanni	Arthropoda	Branchiopoda				X
Pleopsis polyphemoides	Arthropoda	Branchiopoda	X			
Podon leuckartii	Arthropoda	Branchiopoda				X
Acartia (Acanthacartia)	Arthropoda	Copepoda	X		X	X
Acartia (Acanthacartia) tonsa	Arthropoda	Copepoda			X	X
Acartia (Acartiura) hudsonica	Arthropoda	Copepoda			X	X
Bomolochus cuneatus	Arthropoda	Copepoda	X			X
Calanus glacialis	Arthropoda	Copepoda			X	X
Calanus marshallae	Arthropoda	Copepoda	X	X	X	X
Calanus pacificus	Arthropoda	Copepoda	X		X	X
Centropages abdominalis	Arthropoda	Copepoda	X	X	X	X
Clausocalanus pergens	Arthropoda	Copepoda				X
Ctenocalanus vanus	Arthropoda	Copepoda		X		X
Ectinosoma melaniceps	Arthropoda	Copepoda	X			X
Epilabidocera amphitrites	Arthropoda	Copepoda	X	X		
Eucalanus bungii	Arthropoda	Copepoda		X	X	X
Eurytemora pacifica	Arthropoda	Copepoda			X	
Ismaila belciki	Arthropoda	Copepoda		X		
Lepeophtheirus salmonis	Arthropoda	Copepoda	X			
Mesochra sp.	Arthropoda	Copepoda				X
Metridia lucens	Arthropoda	Copepoda	X			
Metridia pacifica	Arthropoda	Copepoda			X	X
Neocalanus cristatus	Arthropoda	Copepoda			X	
Neocalanus flemingeri	Arthropoda	Copepoda	X	X	X	X
Neocalanus plumchrus	Arthropoda	Copepoda		X	X	X
Oithona similis	Arthropoda	Copepoda	X	X	X	X

Species	Phylum	Class	2016	2017	2018	2019
Paracalanus sp.	Arthropoda	Copepoda				X
Paradactylopodia sp.	Arthropoda	Copepoda			X	
Pareucalanus attenuatus	Arthropoda	Copepoda		X	X	X
Pseudocalanus acuspes	Arthropoda	Copepoda	X	X	X	X
Pseudocalanus mimus	Arthropoda	Copepoda	X	X	X	X
Pseudocalanus minutus	Arthropoda	Copepoda	X	X	X	X
Pseudocalanus newmani	Arthropoda	Copepoda	X	X	X	X
Pseudocalanus sp.	Arthropoda	Copepoda			X	X
Tisbe sp.	Arthropoda	Copepoda				X
Bopyroides hippolytes	Arthropoda	Malacostraca			X	
Cancer oregonensis	Arthropoda	Malacostraca				X
Chorilia longipes	Arthropoda	Malacostraca				X
Eualus avinus	Arthropoda	Malacostraca				X
Euphausia pacifica	Arthropoda	Malacostraca		X	X	X
Hippolytidae sp.	Arthropoda	Malacostraca				X
Hyas coarctatus	Arthropoda	Malacostraca				X
Hyperiidae sp.	Arthropoda	Malacostraca			X	X
Isopoda sp.	Arthropoda	Malacostraca				X
Metacarcinus gracilis	Arthropoda	Malacostraca	X			
Oregonia gracilis	Arthropoda	Malacostraca	X	X		
Pagurus hirsutiusculus	Arthropoda	Malacostraca		X		
Pandalopsis dispar	Arthropoda	Malacostraca			X	
Pandalus borealis	Arthropoda	Malacostraca			X	
Pandalus dispar	Arthropoda	Malacostraca			X	
Pugettia gracilis	Arthropoda	Malacostraca			X	X
Themisto pacifica	Arthropoda	Malacostraca	X	X		
Thysanoessa inermis	Arthropoda	Malacostraca			X	X
Thysanoessa longipes	Arthropoda	Malacostraca			X	X
Thysanoessa raschii	Arthropoda	Malacostraca				X
Thysanoessa spinifera	Arthropoda	Malacostraca		X	X	X
Discoconchoecia elegans	Arthropoda	Ostracoda				X
Balanus balanus	Arthropoda	Thecostraca			X	X
Balanus crenatus	Arthropoda	Thecostraca	X		X	X
Balanus glandula	Arthropoda	Thecostraca	X		X	X
Chthamalus dalli	Arthropoda	Thecostraca		X		X
Semibalanus cariosus	Arthropoda	Thecostraca		X	X	
Penicillium digitatum	Ascomycota	Eurotiomycetes	X			
Alcyonidium polyoum	Bryozoa	Gymnolaemata				X
Membranipora membranacea	Bryozoa	Gymnolaemata	X	X	X	X
Clupea pallasii	Chordata	Actinopterygii				X
Leuroglossus schmidti	Chordata	Actinopterygii		X		X
Limanda aspera	Chordata	Actinopterygii	X			X
Microstomus pacificus	Chordata	Actinopterygii		X		
Oncorhynchus kisutch	Chordata	Actinopterygii	X			
Aequorea sp.	Cnidaria	Hydrozoa				X
Agalma elegans	Cnidaria	Hydrozoa			X	
Aglantha digitale	Cnidaria	Hydrozoa			X	X
Bougainvillia superciliaris	Cnidaria	Hydrozoa				X
Catablema vesicarium	Cnidaria	Hydrozoa			X	
Clytia gregaria	Cnidaria	Hydrozoa		X	X	X
Corynidae sp.	Cnidaria	Hydrozoa			X	X

Species	Phylum	Class	2016	2017	2018	2019
Mitrocomella polydiademata	Cnidaria	Hydrozoa				X
Nanomia bijuga	Cnidaria	Hydrozoa				X
Obelia longissima	Cnidaria	Hydrozoa				X
Proboscidactyla flavicirratta	Cnidaria	Hydrozoa	X	X		
Rathkea octopunctata	Cnidaria	Hydrozoa				X
Aurelia labiata	Cnidaria	Scyphozoa	X			X
Chrysaora melanaster	Cnidaria	Scyphozoa				X
Pleurobrachia bachei	Ctenophora	Tentaculata				X
Strongylocentrotus	Echinoderm	Echinoidea		X		
Strongylocentrotus pallidus	Echinoderm	Echinoidea				X
Ophiopholis kennerlyi	Echinoderm	Ophiuroidea	X			
Ophiura sarsii	Echinoderm	Ophiuroidea				X
Azadinium dalianense	Miozoa	Dinoflagellata	X			
Angulus nuculoides	Mollusca	Bivalvia	X			
Clinocardium nuttallii	Mollusca	Bivalvia				X
Compsomyax sudiaphana	Mollusca	Bivalvia	X			
Hiatella sp.	Mollusca	Bivalvia			X	X
Humilaria kennerleyi	Mollusca	Bivalvia	X			
Keenocardium californiense	Mollusca	Bivalvia		X		X
Kellia suborbicularis	Mollusca	Bivalvia	X			
Leukoma staminea	Mollusca	Bivalvia				X
Limecola balthica	Mollusca	Bivalvia				X
Macoma bathica	Mollusca	Bivalvia	X	X		
Macoma calcarea	Mollusca	Bivalvia			X	X
Modiolus modiolus	Mollusca	Bivalvia	X			
Mytilus trossulus	Mollusca	Bivalvia	X	X	X	X
Pandora bilirata	Mollusca	Bivalvia	X			
Saxidomus gigantea	Mollusca	Bivalvia		X		X
Acanthodoris atrogriseata	Mollusca	Gastropoda	X			
Acanthodoris nanaimoensis	Mollusca	Gastropoda	X			
Aglaja ocelligera	Mollusca	Gastropoda	X			
Alderia modesta	Mollusca	Gastropoda				X
Alia gausapata	Mollusca	Gastropoda	X			
Amphissa columbiana	Mollusca	Gastropoda				X
Aplysiopsis enteromorphae	Mollusca	Gastropoda	X		X	X
Clione limacina	Mollusca	Gastropoda		X	X	X
Corambe steinbergae	Mollusca	Gastropoda	X	X		X
Coryphella verrucosa	Mollusca	Gastropoda			X	X
Crepipatella lingulata	Mollusca	Gastropoda	X			
Cryptonatica aleutica	Mollusca	Gastropoda				X
Dendronotus albus	Mollusca	Gastropoda	X	X		X
Dendronotus frondosus	Mollusca	Gastropoda				X
Dendronotus rufus	Mollusca	Gastropoda				X
Dendronotus venusta	Mollusca	Gastropoda	X			
Elysia hedpethi	Mollusca	Gastropoda		X		
Eubranchus rupium	Mollusca	Gastropoda			X	X
Flabellina sp.	Mollusca	Gastropoda			X	X
Flabellina trilineata	Mollusca	Gastropoda				X
Flabellina verrucosa	Mollusca	Gastropoda				X
Fusitriton oregonensis	Mollusca	Gastropoda	X			
Gastropteron pacificum	Mollusca	Gastropoda				X

Species	Phylum	Class	2016	2017	2018	2019
Haminoea virescens	Mollusca	Gastropoda	X			
Hermissenda crassicornis	Mollusca	Gastropoda	X	X		
Knoutsodonta jannae	Mollusca	Gastropoda	X	X		
Lacuna vincta	Mollusca	Gastropoda	X	X	X	X
Limacina helicina	Mollusca	Gastropoda	X	X		
Limneria prolongata	Mollusca	Gastropoda				X
Margarites pupillus	Mollusca	Gastropoda		X		X
Melanochlamys diomedea	Mollusca	Gastropoda	X	X		X
Microchlamylla gracilis	Mollusca	Gastropoda			X	
Nassarius mendicus	Mollusca	Gastropoda	X	X		X
Odostomia tenuisculpta	Mollusca	Gastropoda	X			
Olea hansineensis	Mollusca	Gastropoda	X	X		X
Onchidoris bilamellata	Mollusca	Gastropoda	X	X	X	X
Onchidoris muricata	Mollusca	Gastropoda		X		
Placida dendritica	Mollusca	Gastropoda			X	
Stiliger fuscovittatus	Mollusca	Gastropoda	X			X
Trichotropis cancellata	Mollusca	Gastropoda				X
Amphiporus formidabilis	Nemertea	Hoplonemertea				X
Carcinonemertes errans	Nemertea	Hoplonemertea	X			X
Emplectonema sp.	Nemertea	Hoplonemertea				X
Gurjanovella littoralis	Nemertea	Hoplonemertea		X	X	X
Paranemertes californica	Nemertea	Hoplonemertea	X			X
Poseidonemertes collaris	Nemertea	Hoplonemertea		X		X
Cerebratulus californiensis	Nemertea	Pilidiophora				X
Cerebratulus herculeus	Nemertea	Pilidiophora			X	X
Cerebratulus sp.	Nemertea	Pilidiophora				X
Lineus flavescens	Nemertea	Pilidiophora				X
Maculaura aquilonia	Nemertea	Pilidiophora			X	X
Ditylum brightwelli	Ochrophyta	Diatoms	X			
Melosira nummuloides	Ochrophyta	Diatoms	X			
Thalassionema nitzschioides	Ochrophyta	Diatoms	X			
Ectocarpus siliculosus	Ochrophyta	Phaeophyceae		X		
Leathesia difformis	Ochrophyta	Phaeophyceae		X		
Pylaiella littoralis	Ochrophyta	Phaeophyceae	X	X		
Phoronopsis harmeri	Phoronida					X
Polycladida sp.	Platyhelmint	Polycladida				X
Phascolosoma agassizii	Sipuncula	Phascosomatid	X			

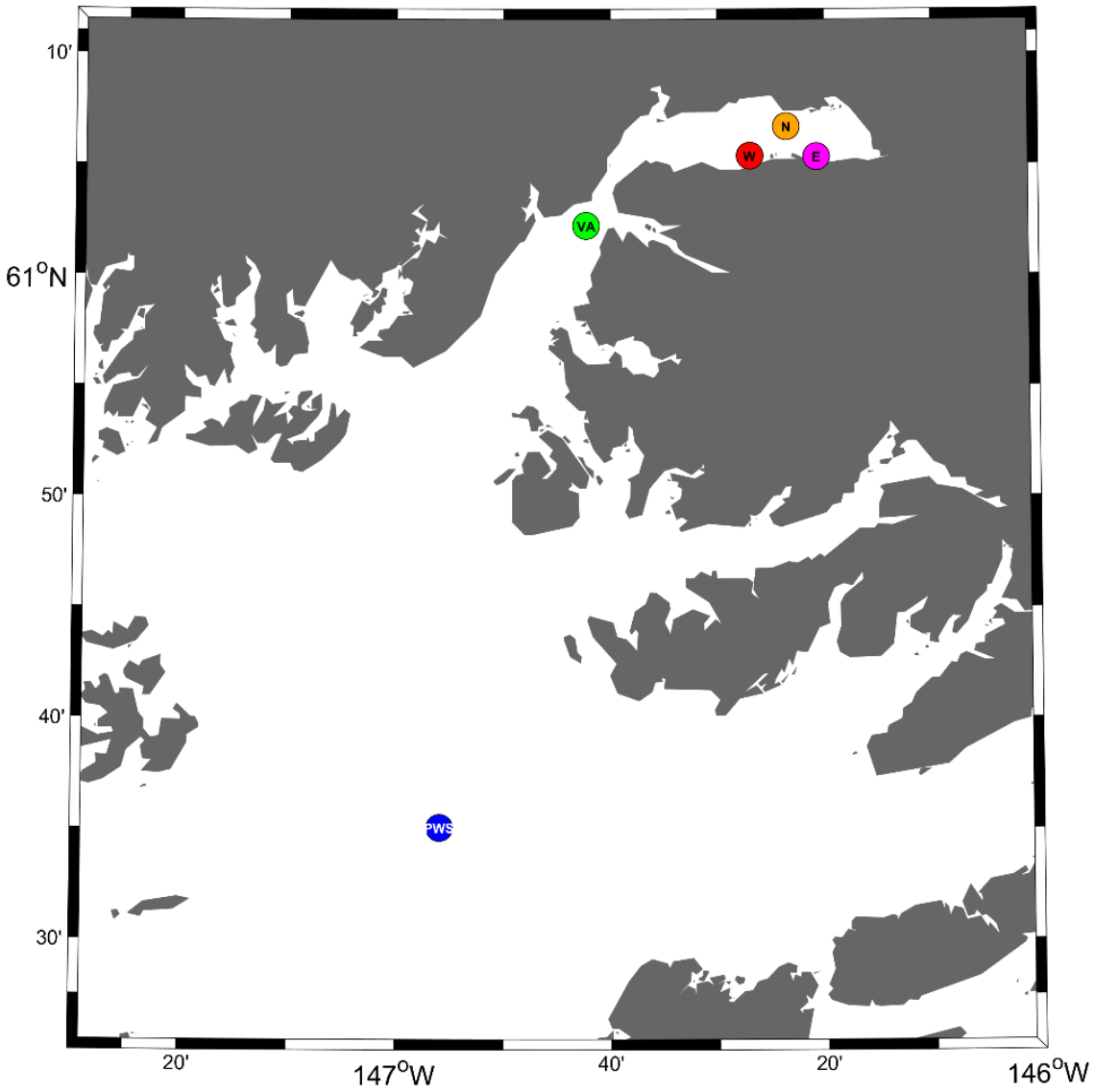

Figure 1: Valdez plankton sample collection sites. Map was from the sample collection report edited by Rob Campbell. Site names are denoted as follow: PWS= Prince William Sound, VA= Valdez Arm, E= Valdez Marine Terminal Station E, N= Valdez Marine Terminal Station N, W= Valdez Marine Terminal Station W.

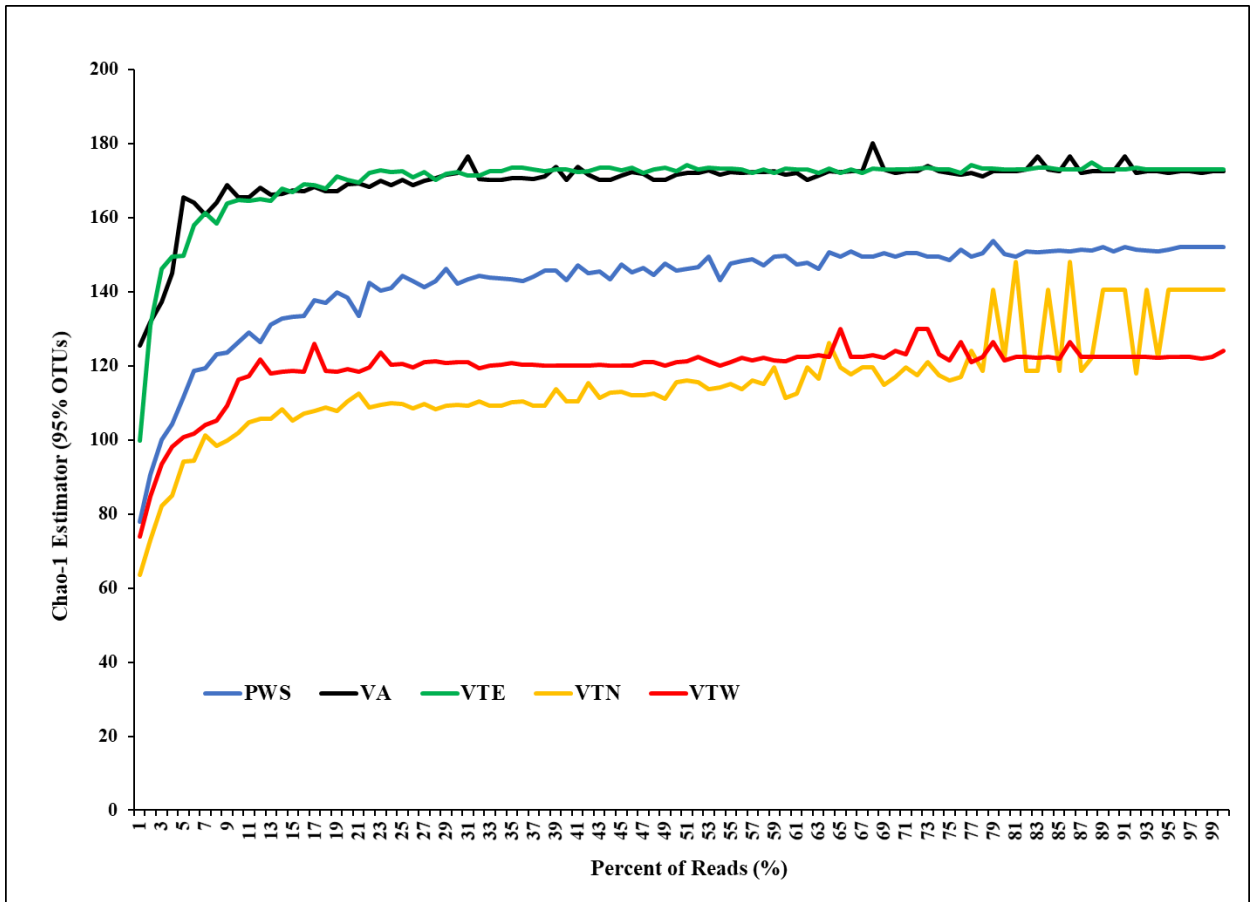

Figure 2. Rarefaction curves on alpha diversity (Chao-1 estimator) of 2018 Valdez plankton samples. This relates diversity discovered to the number of reads obtained. Site names are denoted as follow: PWS= Prince William Sound, VA= Valdez Arm, VTE= Valdez Marine Terminal Station E, VTN= Valdez Marine Terminal Station N, VTW= Valdez Marine Terminal Station W.

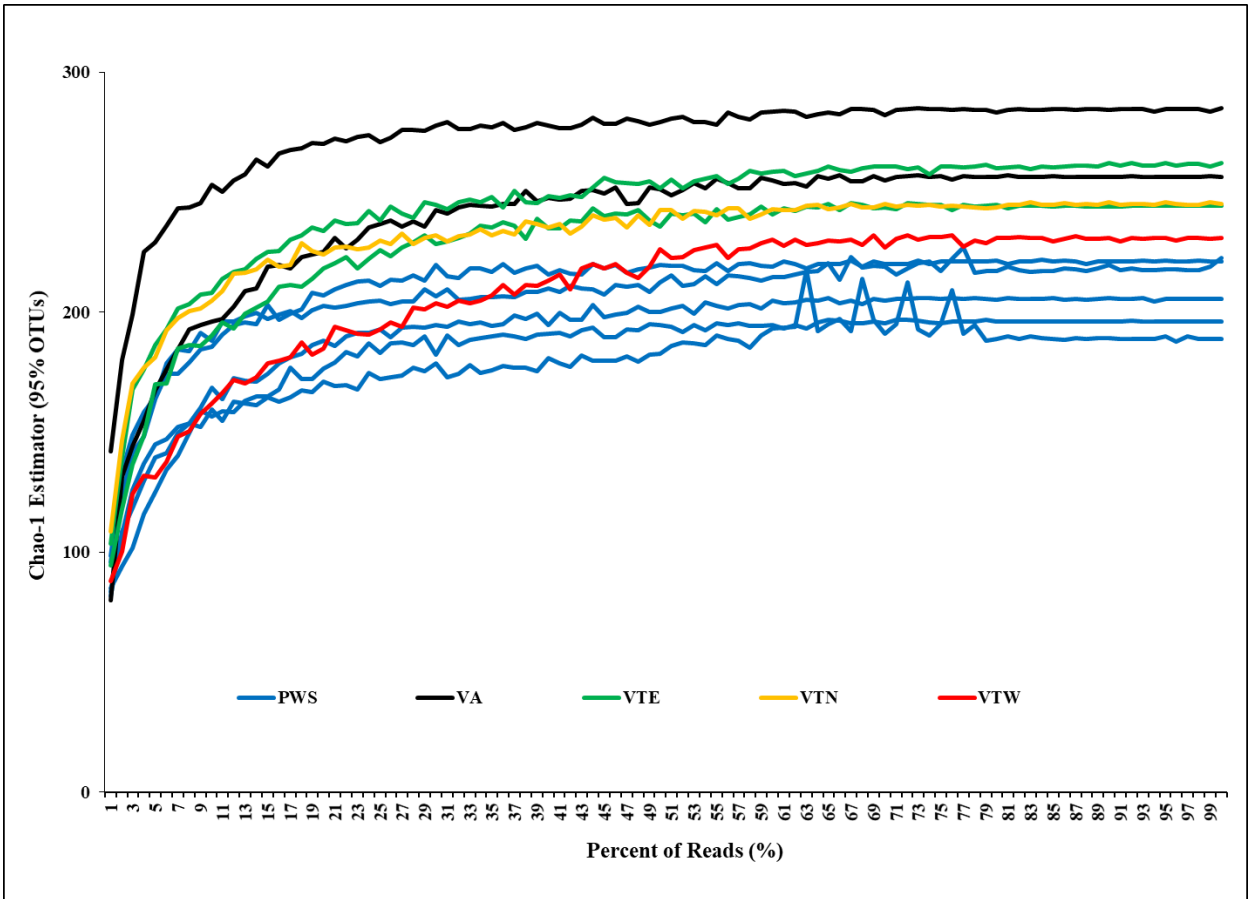

Figure 3. Rarefaction curves on alpha diversity (Chao-1 estimator) of 2019. This relates diversity discovered to the number of reads obtained. Site names are as in Figure 2.

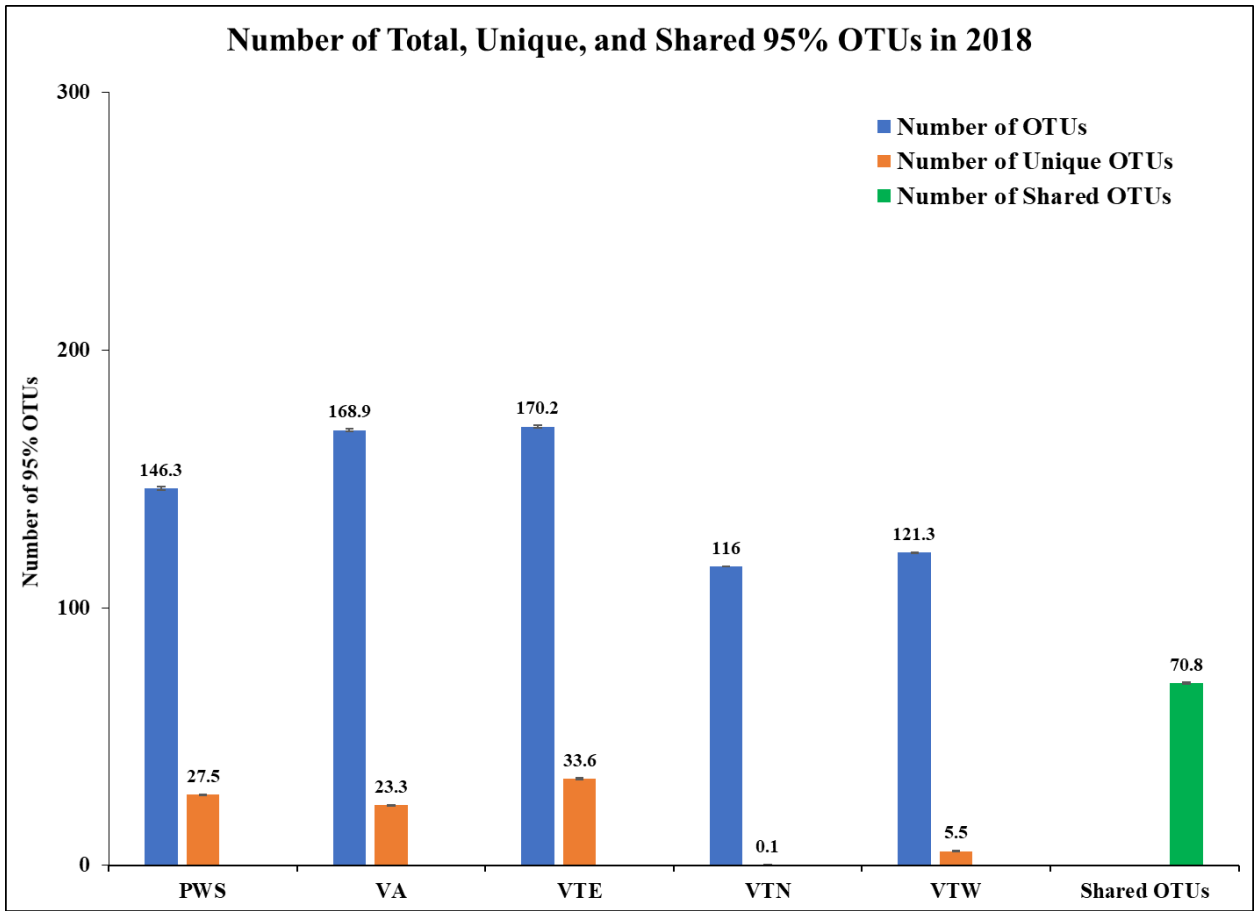

Figure 4. Number of total (blue), unique (orange), and shared (green) 95% OTUs comparison across 2018 Valdez plankton samples. Data were rarefied to 89,664 reads per sample in 10 times. Site names are as in Figure 2. Error bars indicate standard errors.

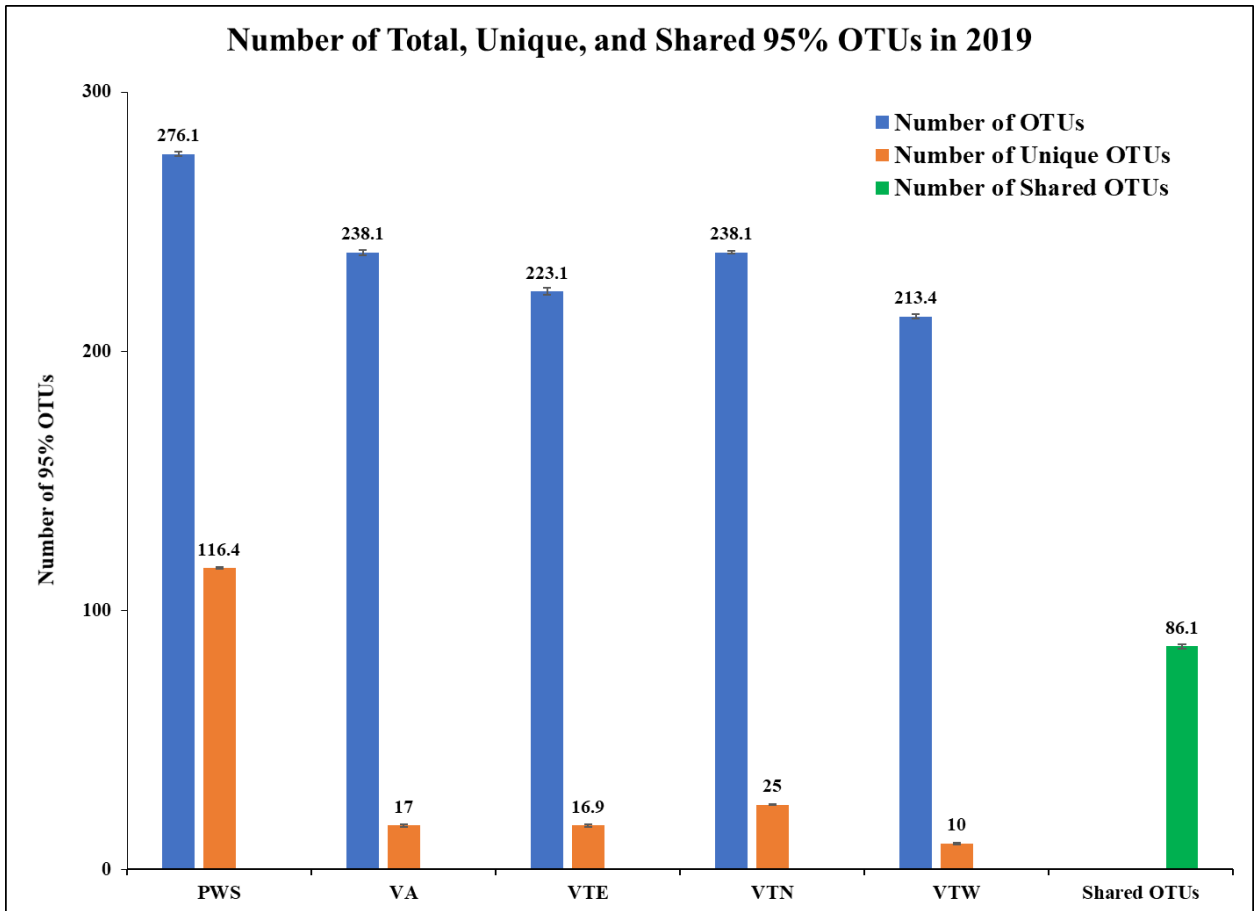

Figure 5. Number of total (blue), unique (orange), and shared (green) 95% OTUs comparison across 2019 Valdez plankton samples. Data were rarefied to 149,692 reads per sample in 10 times. Site names are as in Figure 2. Deep tows collected at VA and VTE were excluded. Error bars indicate standard errors.

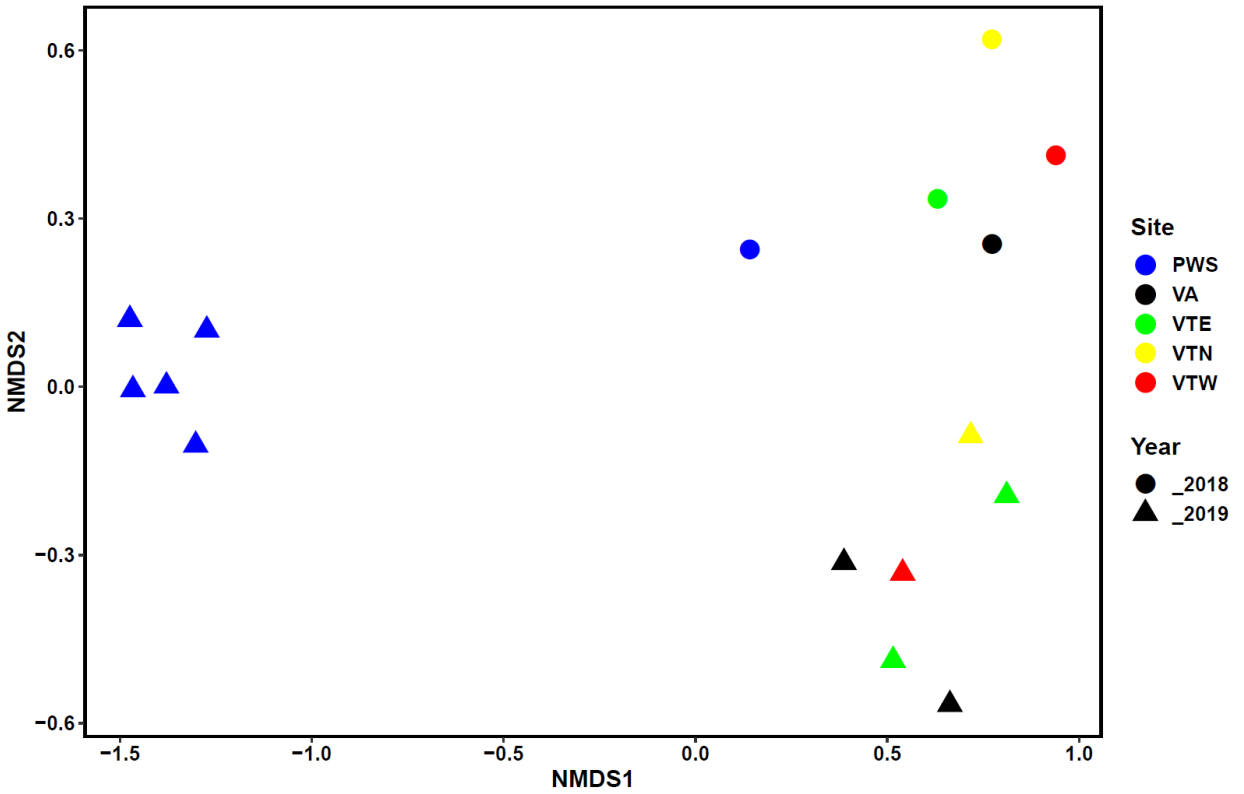

Figure 6. nMDS plot showing plankton composition based on Jaccard similarities among the Valdez plankton samples collected in 2018 (circles) and 2019 (triangles). Samples were collected at Prince William Sound (PWS, blue), Valdez Arm (VA, black), Valdez Marine Terminal Station E (VTE, green), N (VTN, yellow), and W (VTW, red). Data were rarefied to 94,405 reads per sample in 10 times.

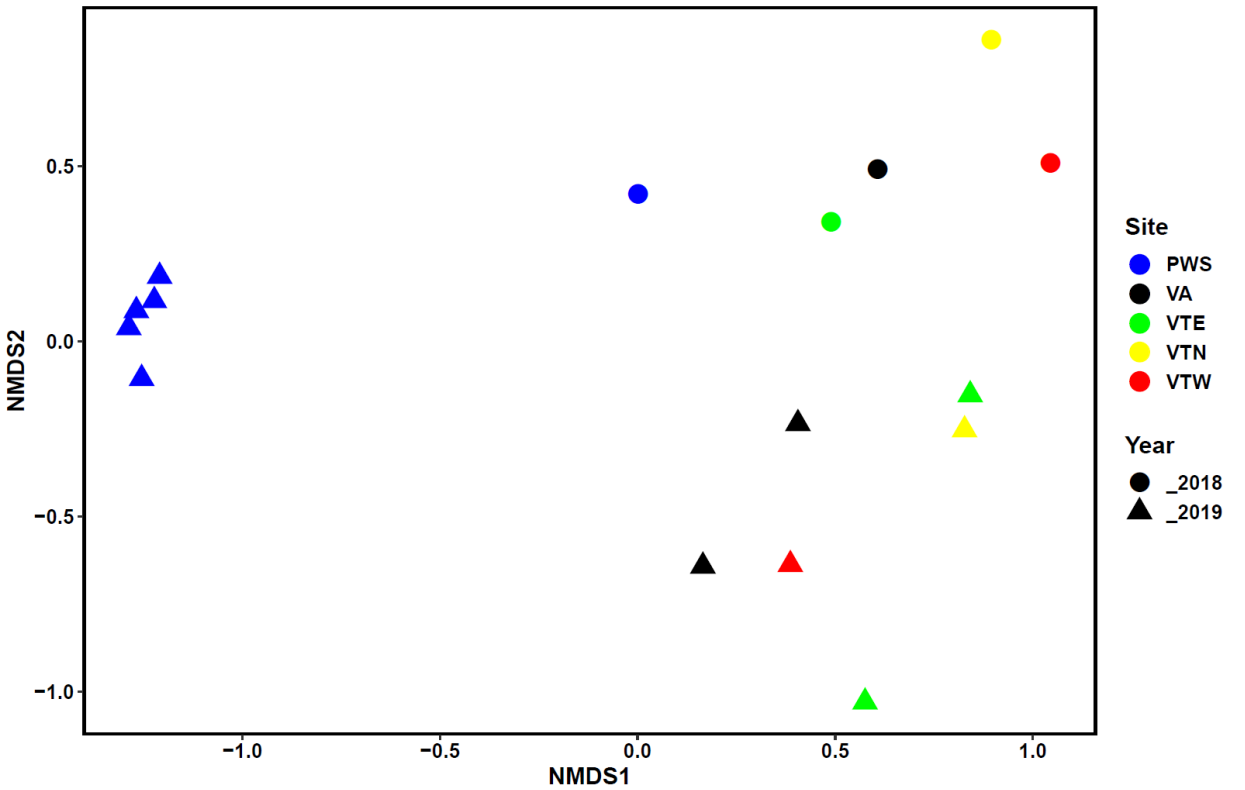

Figure 7. nMDS plot showing plankton composition based on Bray-Curtis similarities among the Valdez plankton samples collected in 2018 (circles) and 2019 (triangles). Samples were collected at Prince William Sound (PWS, blue), Valdez Arm (VA, black), Valdez Marine Terminal Station E (VTE, green), N (VTN, yellow), and W (VTW, red). Data were rarefied to 94,405 reads per sample in 10 times.

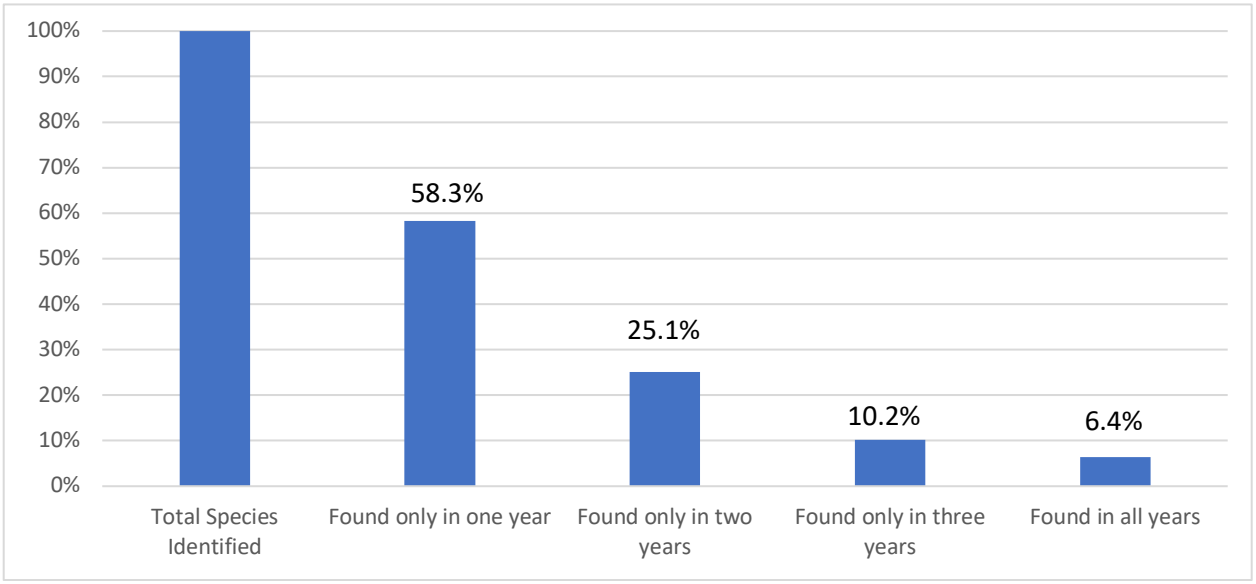

Figure 8. Distribution of repeat occurrences among 187 species that were identified in samples from 2016, 2017, 2018, and 2019 (Table 3). A majority of species were found in one year only, highlighting the high variability between years. Variability between sites within years was much less (Figures 4 and 5).