

## Briefing for PWSRCAC Board of Directors – September 2020

**ACTION ITEM**

**Sponsor:** Austin Love and the Scientific Advisory Committee

**Project number and name or topic:** 9520 – Marine Invasive Species Metagenetics Analysis Report Acceptance

1. **Description of agenda item:** This agenda item seeks Board acceptance of the report titled “Metagenetic Analysis of 2018 and 2019 Plankton Samples from Prince William Sound, Alaska” by Dr. Jonathan Geller, Melinda Wheelock, and Martin Guo of Moss Landing Marine Laboratory. In 2018 and 2019, plankton samples were gathered in Port Valdez and Prince William Sound by the Prince William Sound Science Center and sent to the Moss Landing Marine Laboratory in California for genetic analysis. The overall goal of that genetic analysis was to identify any marine invasive species in the 2018 and 2019 plankton samples. The report by Moss Landing Marine Laboratory describes the methods used to collect and genetically analyze the 2018 and 2019 plankton samples provided by the Prince William Sound Science Center, and provides the results of the genetic analysis. Austin Love will provide a summary presentation of the results and Dr. Jonathan Gellar will be available to answer questions from the Board.

2. **Why is this item important to PWSRCAC:** The Marine Invasive Species Project helps PWSRCAC fulfill one of its duties detailed in the Oil Pollution Act of 1990. The Act instructs PWSRCAC to “devise and manage a comprehensive program of monitoring the environmental impacts of the operations of terminal facilities and of crude oil tankers while operating in Prince William Sound.” Crude oil tankers arriving at the Valdez Marine Terminal are a potential source of marine invasive species. Trans Alaska Pipeline System tankers dock in ports known to harbor marine invasive species. Those invasive species could be introduced into Prince William Sound from a ship’s ballast water or from organisms growing on its hull. Some invasive species could cause significant economic and environmental harm if reproducing populations became established locally. The work done under the Marine Invasive Species project is meant to understand and minimize the impacts of harmful invasive species arriving in the PWSRCAC region due to the operation of oil tankers in Prince William Sound.

3. **Previous actions taken by the Board on this item:** The following includes all Board actions taken related to Project Number 9520 – Marine Invasive Species.

<u>Meeting</u>	<u>Date</u>	<u>Action</u>
XCOM	6/3/2003	The Executive Committee approved the Non-Indigenous Species database and final report for distribution.
XCOM	7/31/2003	The Executive Committee approved comments on the Draft National Management Plan for the European Green Crab for immediate distribution.
XCOM	8/29/2003	The Executive Committee approved a letter to Senator Lisa Murkowski on the National Aquatic Invasive Species Act (NAISA) of 2003.
Board	3/11/2004	The Board approved Resolution 04-02 to the owner companies, shipping companies, and Alyeska to engage in a coordinated effort to evaluate viable shipboard and shoreside ballast water treatment technologies for implementation.

## Report Acceptance: Metagenetic Analysis of PWS Plankton Samples 4-3

XCOM	3/25/2004	The Executive Committee approved the report titled "Projecting Range Expansion of Invasive European Green Crabs ( <i>Carcinus Maenas</i> ) to Alaska: Temperature and Salinity Tolerance of Larvae" for distribution.
Board	5/12/2005	The Board approved a letter regarding S. 363 to Senator Stevens regarding the Ballast Water Management Act of 2005.
XCOM	12/6/2005	The Executive Committee approved the reported titled "Ballast Water Exchange: Efficacy of treating ship's ballast water to reduce marine species transfers and invasive success" for distribution and posting on PWSRCAC website.
XCOM	12/11/2006	The Executive Committee approved the report titled "Biological Invasions in Alaska's Coastal Marine Ecosystems: Establishing a Baseline" for distribution and posting on the PWSRCAC website.
XCOM	11/20/2007	The Executive Committee approved the report titled "Northward Spread of Marine Non-indigenous Species along Western North America: Forecasting Risk of Colonization in Alaskan Waters Using Environmental Niche Modeling" for distribution and posting on PWSRCAC's website.
XCOM	2/10/2009	The Executive Committee approved the report titled "Tunicate: Northward spread, diversity, source and impact of non-native tunicates in Alaska: Establishing a monitoring and education network" by Sarah Cohen and Greg Ruiz for distribution and posting on the PWSRCAC website.
XCOM	1/14/2010	The Executive Committee approved the report titled "Characterizing Risk Associated with Vessel Fouling and Non-Indigenous Species in Prince William Sound" for distribution.
Board	7/18/2011	The Board approved sending the proposed letter to Governor Sean Parnell regarding the designation of a non-indigenous species coordinator in each cabinet department of the State of Alaska.
XCOM	11/20/2012	The Executive Committee approved the report from the Smithsonian Environmental Research Center titled "Quantitative Survey of Non-indigenous Species (NIS) in Prince William Sound" for distribution.
Board	1/23/2014	The Board accepted the report titled "Non-Indigenous Species (NIS) in PWS: Plankton" by Greg Ruiz of the Smithsonian Environmental Research Center.
Board	9/25/2014	The Board accepted the report titled "Analysis of Crude Oil Tanker Ballast Water Data for Valdez and Prince William Sound, Alaska," by Danielle Verna.
Board	5/5/2016	The Board approved the report titled "Updated: Analysis of Crude Oil Tanker Ballast Water Data for Valdez and Prince William Sound, Alaska" by Danielle Verna for distribution and posting on the PWSRCAC web site.
Board	5/4/2017	The Board accepted the final reports titled "Analysis of Federal and state ballast water management policy as it concerns crude oil tankers engaged in coastwise trade to Alaska" and "Status of international and domestic regulations on installation and use of ballast waste management systems" by Danielle Verna, including a footnote added to page 7 of the second report (get note from minutes).
Board	9/14/2017	The Board accepted the report titled "BioBlitz 2016: Re-assessing marine invasions in Valdez, Prince William Sound, Alaska" by Greg Ruiz of the Smithsonian Environmental Research Center for distribution and posting on the PWSRCAC website.
Board	9/19/2019	The Board accepted the report titled "Metagenetic Analysis of 2017 Plankton Samples from Prince William Sound, Alaska" by Dr. Jonathan Geller, Melinda Wheelock, and Martin Guo, dated August 15, 2019 as meeting the terms and conditions, and for public distribution.

4. **Summary of policy, issues, support or opposition:** None.

5. **Committee Recommendation:** The Scientific Advisory Committee recommends that the Board of Directors accept this report.

6. **Relationship to LRP and Budget:** Project 952 – Marine Invasive Species is in the approved FY2021 budget and annual work plan.

**9520-Marine Invasive Species**  
**As of August 17, 2020**

<b>FY-2021 Budget</b>	\$53,350.00
<b>Actual and Commitments</b>	
Actual Year-to-Date	
Commitments (Professional Services)	\$1,000.00
Actual + Commitments	<u>\$1,000.00</u>
Amount Remaining	<u>\$52,350.00</u>

7. **Action Requested of the Board of Directors:** Accept the report titled “Metagenetic Analysis of 2018 and 2019 Plankton Samples from Prince William Sound, Alaska” by Dr. Jonathan Geller, Melinda Wheelock, and Martin Guo dated April 13, 2020 as meeting the terms and conditions of Purchase Order 17255, and for distribution to the public.
8. **Alternatives:** Do not accept the report, or accept the report with recommended revisions.
9. **Attachments:** Draft report titled “Metagenetic Analysis of 2018 and 2019 Plankton Samples from Prince William Sound, Alaska” by Dr. Jonathan Geller, Melinda Wheelock, and Martin Guo.

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

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

From Molecular Ecology Laboratory  
Moss Landing Marine Laboratory

Dr. Jonathan Geller  
Melinda Wheelock  
Martin Guo

Any opinions expressed in this PWSRCAC-commissioned report  
are not necessarily those of PWSRCAC.

April 13, 2020

## 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](http://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 ( $\log e$ ) $\pm$ SE	Mean Simpson's Index ( $1-\lambda$ ) $\pm$ SE	Mean Shannon's Index ( $\log e$ ) $\pm$ SE	Mean Simpson's Index ( $1-\lambda$ ) $\pm$ SE
PWS	2.32 $\pm$ 0.001	0.777 $\pm$ 0.0003	2.01 $\pm$ 0	0.649 $\pm$ 0.0001
VA	2.90 $\pm$ 0.002	0.881 $\pm$ 0.0001	2.72 $\pm$ 0.001	0.867 $\pm$ 0.0002
VTE	2.91 $\pm$ 0.002	0.887 $\pm$ 0.0001	2.91 $\pm$ 0.001	0.915 $\pm$ 0.0001
VTN	1.23 $\pm$ 0	0.374 $\pm$ 0	3.32 $\pm$ 0.001	0.932 $\pm$ 0.0001
VTW	2.01 $\pm$ 0.001	0.656 $\pm$ 0.0002	2.78 $\pm$ 0.001	0.902 $\pm$ 0.0001

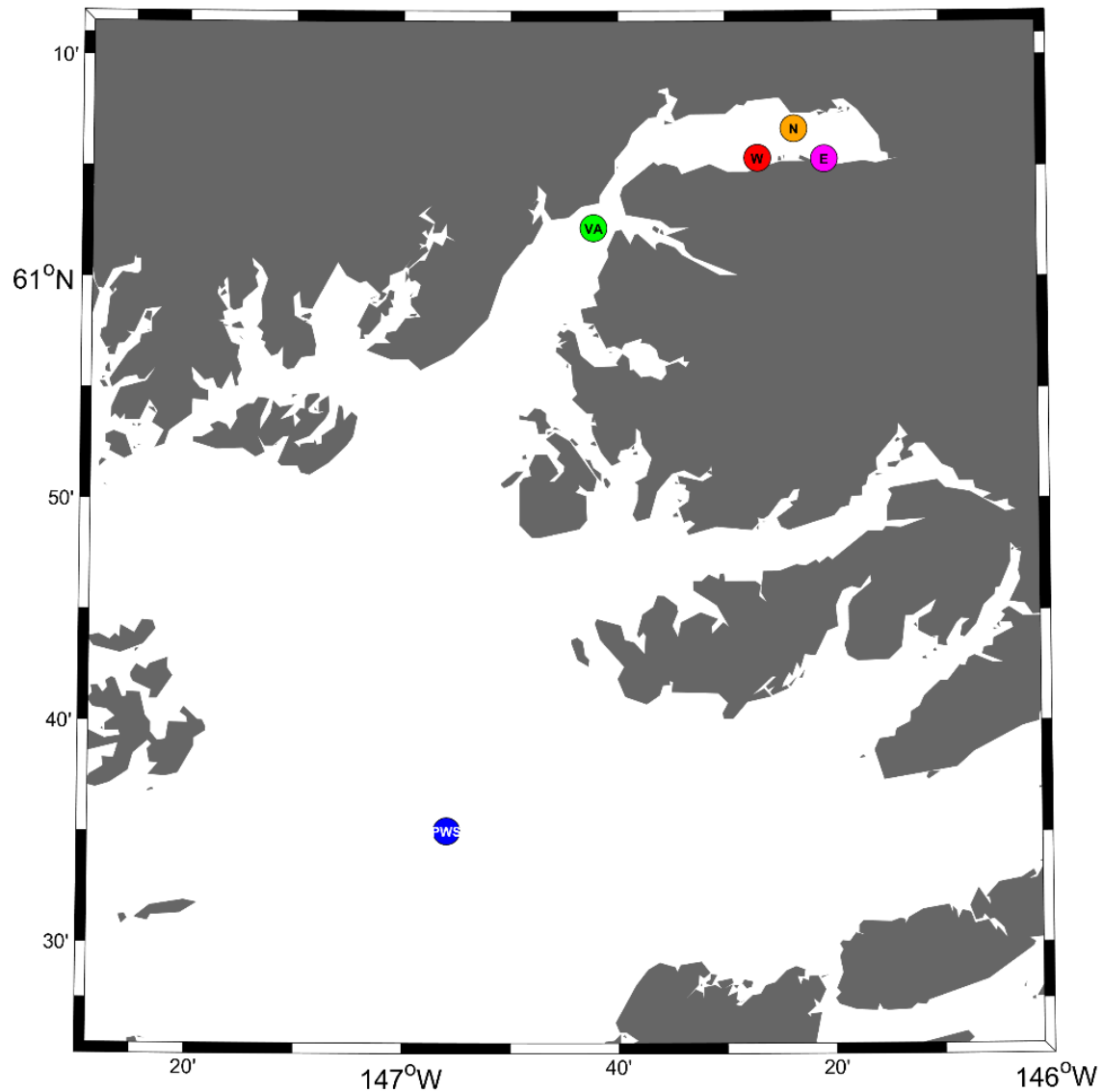
Table 3. Assignable taxonomy for 95% OTUs from 2016-2019 Valdez and Prince William Sound plankton. “X” indicates a species was detected. 2016 and 2017 were previously reported (Geller et al. 2019).

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

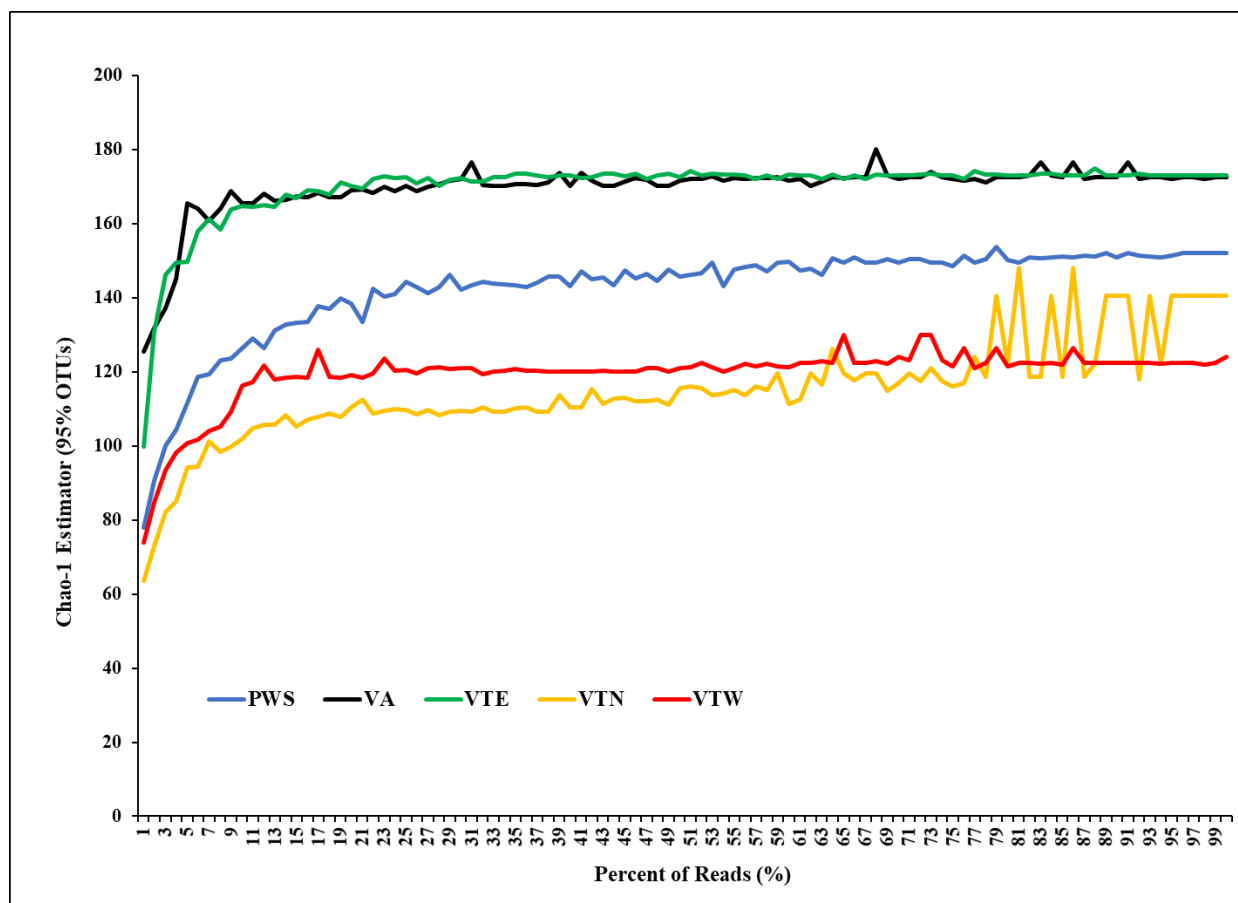
Species	Phylum	Class	2016	2017	2018	2019
<i>Paracalanus sp.</i>	Arthropoda	Copepoda				X
<i>Paradactylopodia sp.</i>	Arthropoda	Copepoda			X	
<i>Pareucalanus attenuatus</i>	Arthropoda	Copepoda		X	X	X
<i>Pseudocalanus acuspes</i>	Arthropoda	Copepoda	X	X	X	X
<i>Pseudocalanus mimus</i>	Arthropoda	Copepoda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

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

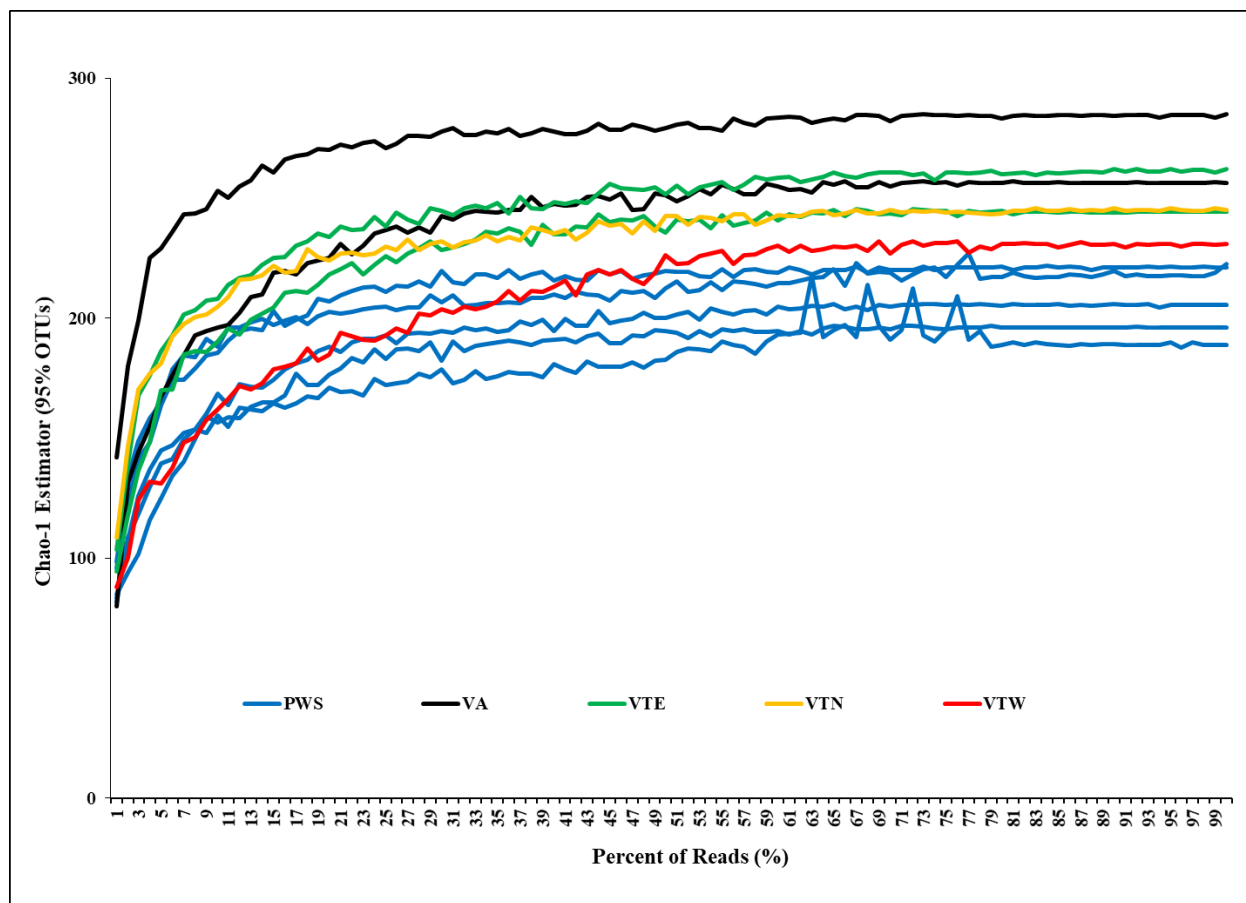
Species	Phylum	Class	2016	2017	2018	2019
<i>Haminoea virescens</i>	Mollusca	Gastropoda	X			
<i>Hermisenda crassicornis</i>	Mollusca	Gastropoda	X	X		
<i>Knoutsodonta jannae</i>	Mollusca	Gastropoda	X	X		
<i>Lacuna vincta</i>	Mollusca	Gastropoda	X	X	X	X
<i>Limacina helicina</i>	Mollusca	Gastropoda	X	X	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



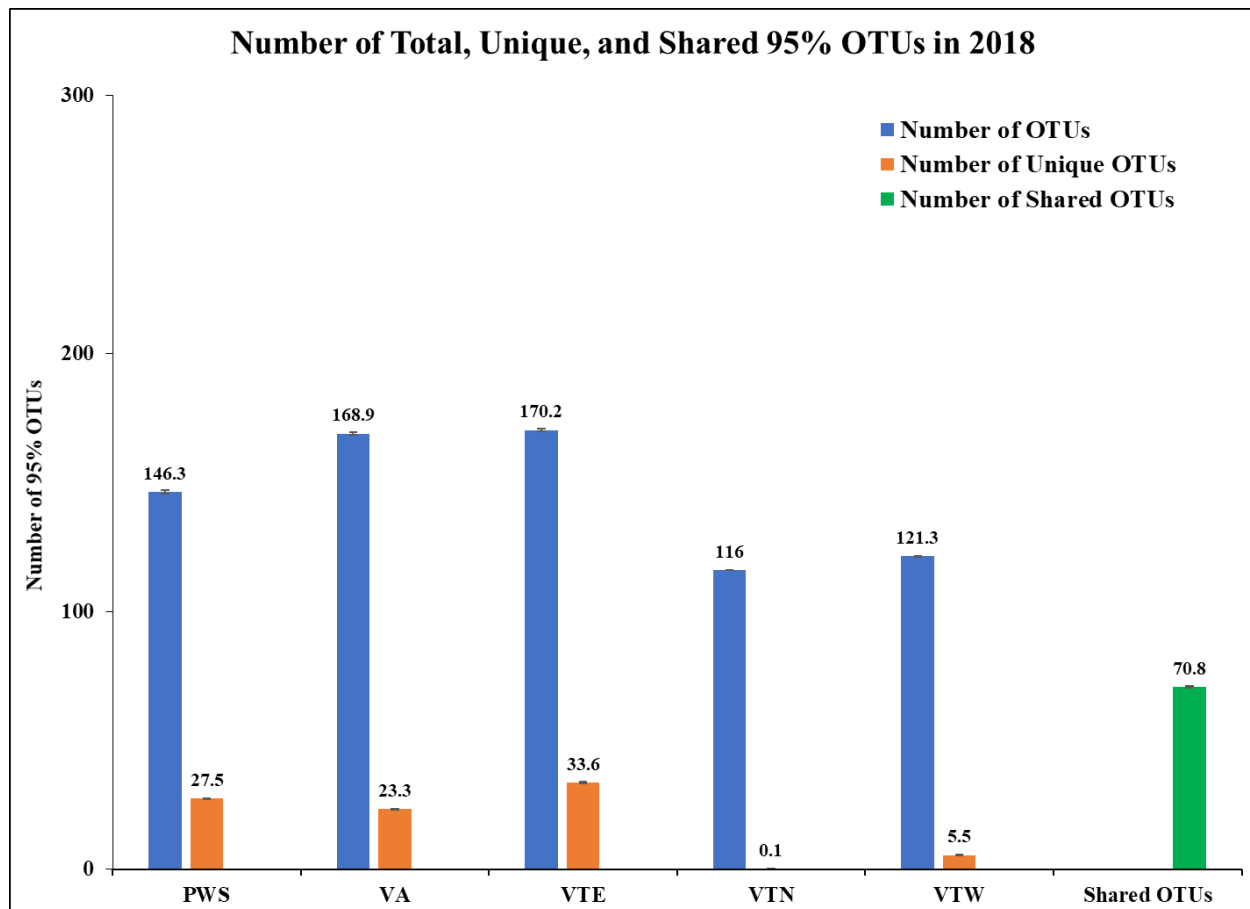
**Figure 1:** Valdez plankton sample collection sites. Map was from the sample collection report edited by Rob Campbell. Site names are denoted as follow: PWS= Prince William Sound, VA= Valdez Arm, E= Valdez Marine Terminal Station E, N= Valdez Marine Terminal Station N, W= Valdez Marine Terminal Station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



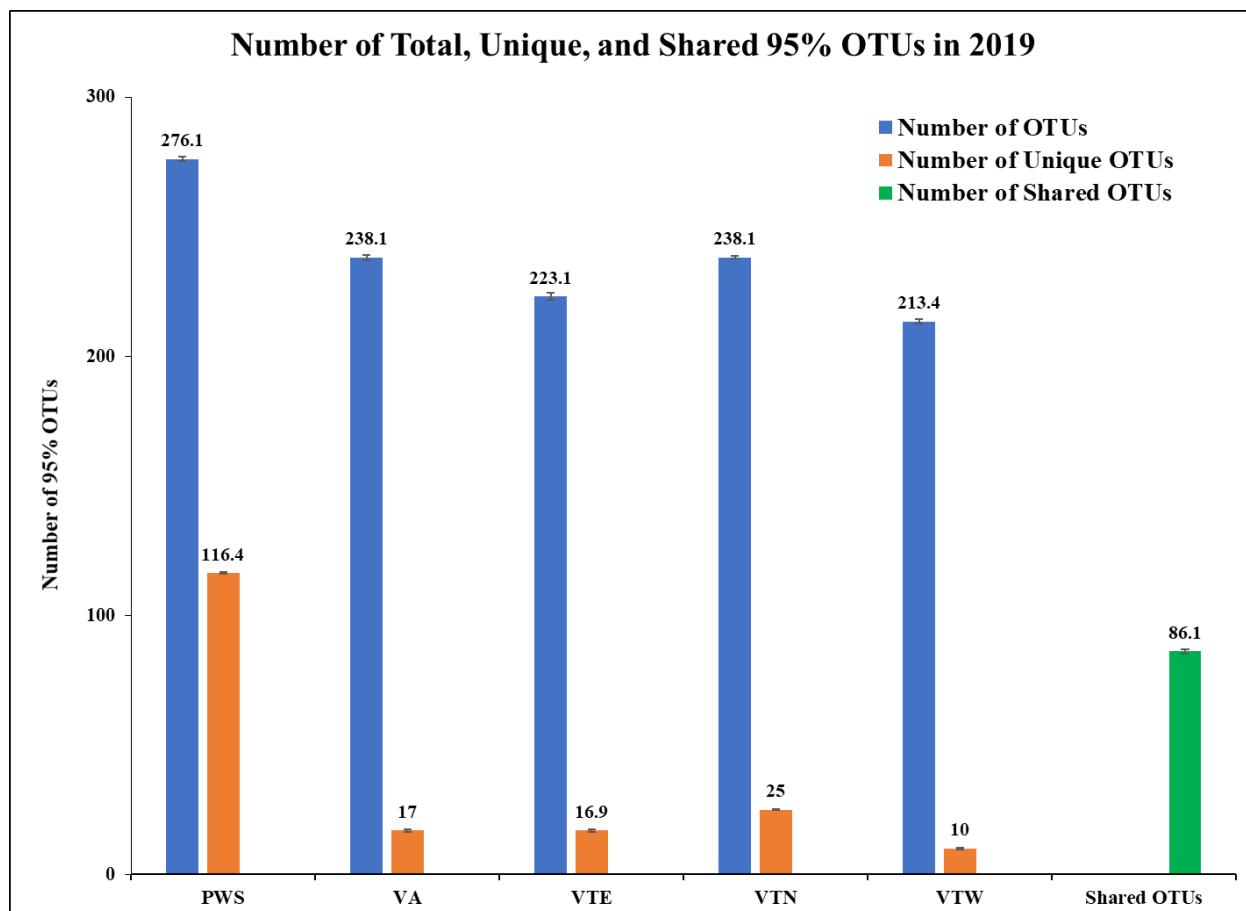
**Figure 2.** Rarefaction curves on alpha diversity (Chao-1 estimator) of 2018 Valdez plankton samples. This relates diversity discovered to the number of reads obtained. Site names are denoted as follow: PWS= Prince William Sound, VA= Valdez Arm, VTE= Valdez Marine Terminal Station E, VTN= Valdez Marine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



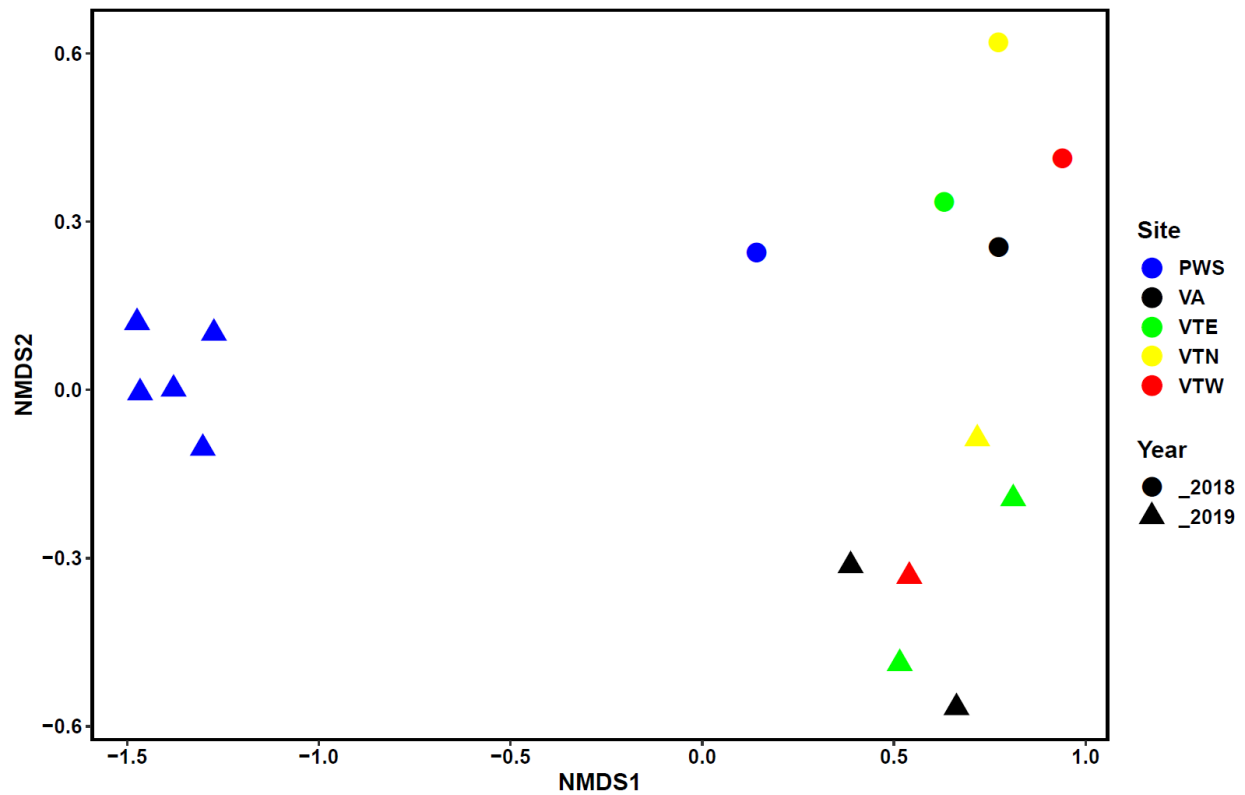
**Figure 3.** Rarefaction curves on alpha diversity (Chao-1 estimator) of 2019. This relates diversity discovered to the number of reads obtained. Site names are as in Figure 2.



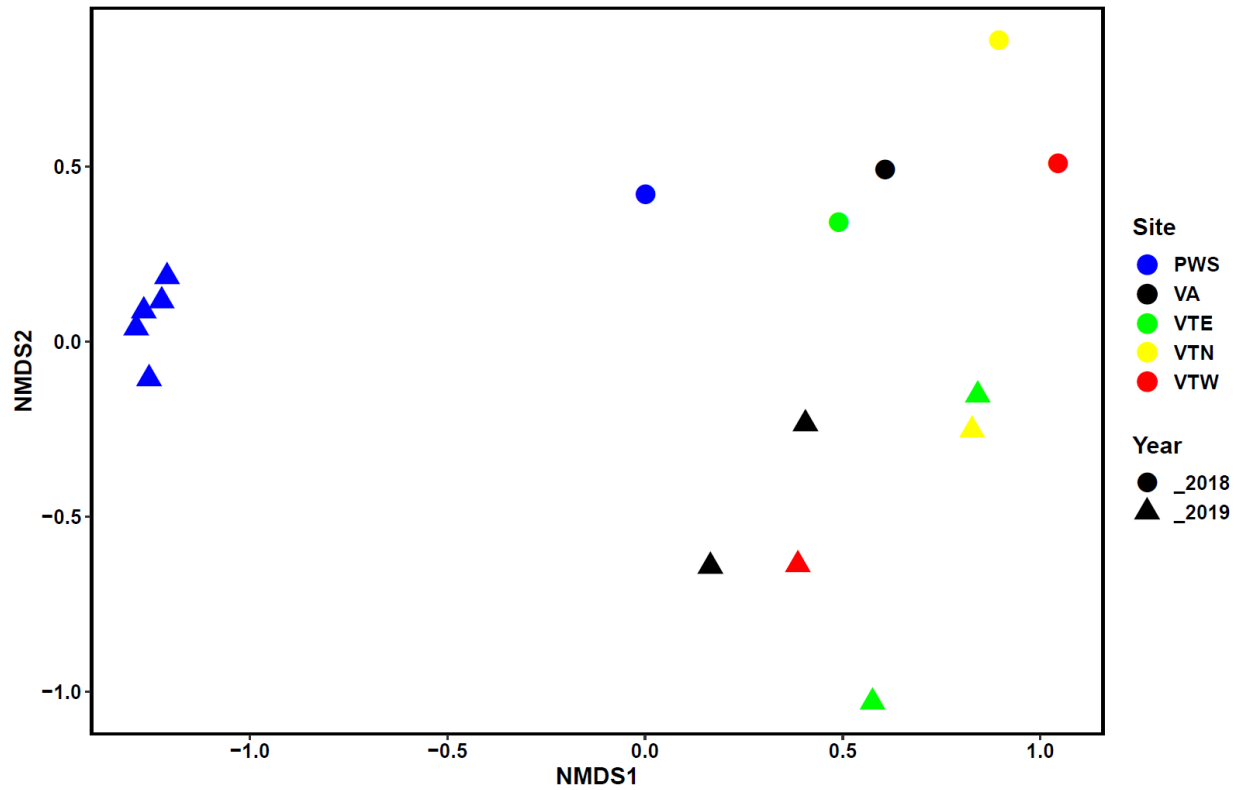
**Figure 4.** Number of total (blue), unique (orange), and shared (green) 95% OTUs comparison across 2018 Valdez plankton samples. Data were rarefied to 89,664 reads per sample in 10 times. Site names are as in Figure 2. Error bars indicate standard errors.



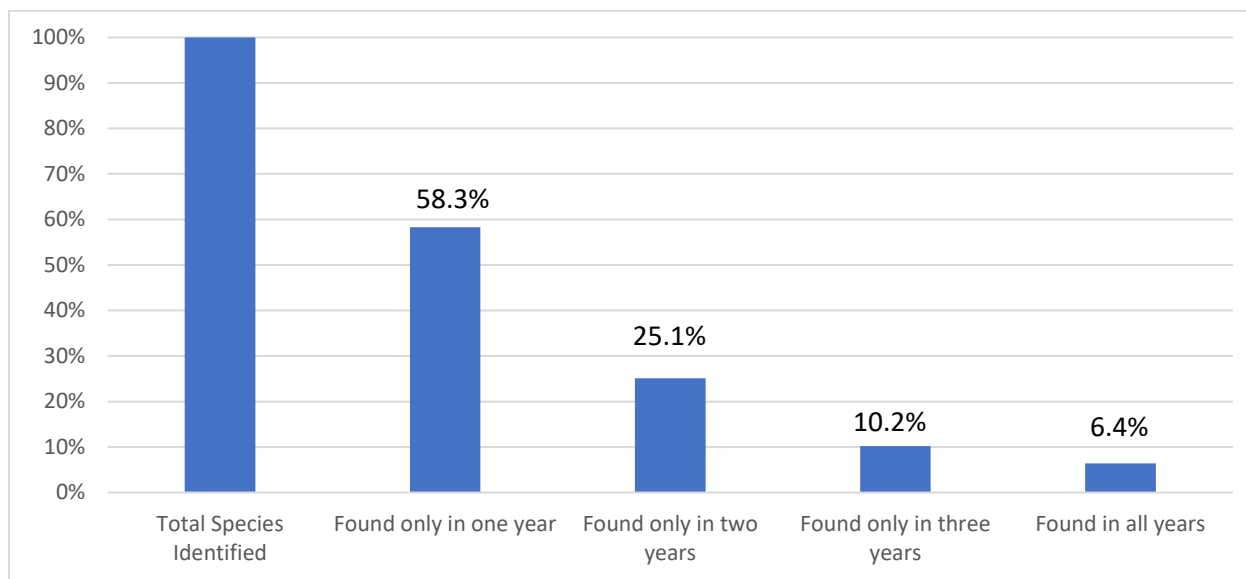
**Figure 5.** Number of total (blue), unique (orange), and shared (green) 95% OTUs comparison across 2019 Valdez plankton samples. Data were rarefied to 149,692 reads per sample in 10 times. Site names are as in Figure 2. Deep tows collected at VA and VTE were excluded. Error bars indicate standard errors.



**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).