Regional Evaluation of Non-indigenous Marine Species in Prince William Sound

Smithsonian Environmental Research Center

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Acronyms and Abbreviations

ASVs	Amplicon Sequence Variants
CM	Centimeter
LCL	Lower Confidence Limit
KM	Kilometer
M	Meter
N	North
NEMESIS	National Estuarine and Marine Exotic Species Information System (SERC)
NIS	Non-Indigenous Species
PCR	Polymerase Chain Reaction
PWS	Prince William Sound
PWSRCAC	Prince William Sound Regional Citizens' Advisory Council
SC	Sample Coverage
SD	Standard Deviation
SE	Standard Error
SERC	Smithsonian Environmental Research Center
SI/HPC	Smithsonian Institution High Performance Computing
UCL	Upper Confidence Limit
W	West

Overview and Rationale

Prince William Sound (PWS) is at considerable risk for novel invasions due to the combined result of several key drivers. Specifically, a large number of non-indigenous species (NIS) are established already in coastal bays and estuaries along the Pacific coast of North America, with over 300 NIS known in California alone (Ruiz et al. 2015; Fofonoff et al. 2018). NIS are spreading northward as a result of coastwise transfers by human activities including especially vessels (ballast water and hull biofouling) and live trade (e.g., aquaculture, bait, fisheries).

Risk of new NIS invasions to PWS results from: (a) a relatively large number of vessel arrivals directly from California (and elsewhere), as well as other vectors that are known to transfer NIS; (b) the progressive northward spread of NIS, with several new species arriving to southeast Alaska in recent years; and (c) climate change that increases the environmental match (especially for temperature) for NIS to establish new populations from lower latitudes along the Pacific coast.

It has been many years since a broadscale survey of PWS to evaluate whether, and the extent to which, new NIS have colonized. While we have helped establish detection and monitoring programs for some selected species (e.g., PlateWatch, Green crab surveys), these are focused on an important but still narrow range of target species. Importantly, such efforts would not detect a very large spectrum of potential NIS in PWS that we know are present to the south, including some now present in southeast Alaska, including areas surrounding Ketchikan and Sitka.

A recent project by Pagenkopp Lohan et al. (2022) focused on analysis of zooplankton communities near Valdez to evaluate/detect the presence of NIS using genetic methods (meta-barcoding), which builds on previous work and methods by Geller and Ruiz. This was conceived as a first step to a broader analysis of PWS, especially including benthic communities, where most NIS along the Pacific coast occur (Ruiz and Hewitt 2009; Fofonoff et al. 2018).

Here, we report on an extensive broadscale survey and analysis of benthic marine communities in PWS, to detect new NIS and evaluate the current status of invasions in PWS. It has been over a decade since our last extensive analysis of invasions across PWS.

Our overall goal was to evaluate NIS present in PWS, using standardized measures, which allow direct comparison to previous surveys of PWS (2000, 2011). In addition, this approach allows quantitative comparisons with identical surveys at other sites along the Pacific coast (California to Alaska). This work aims both to advance invasion science and inform management and policy in this area.

Our specific objectives were to:

- Conduct a standardized survey of benthic marine invertebrate communities in PWS to detect NIS.

- Evaluate temporal change in marine NIS occurrence in PWS, based on our surveys and literature-based analyses, and update baseline regional measures.
- Characterize the northward progression of NIS and proximity to PWS, based on our ongoing literature and field-based measures.
- Estimate the environmental suitability of PWS for colonization by NIS, focusing on those which have been detected in Alaska waters, based on the current survey and synthesis of multiple other data sources.

Here, we report the results from the PWS surveys, which were conducted in 2023, along with occurrence records from our ongoing surveys and the literature to evaluate: (a) new NIS records to PWS; (b) proximity and progression of NIS toward PWS; and (c) model habitat suitability using statistical environmental niche models.

Overall, we detected 3 NIS of benthic marine invertebrates during the PWS surveys in this study. Two of these species appear to be new records to PWS. In a broader synthesis of NIS for PWS, we document 7 NIS of benthic marine invertebrates, including 3 species with the first detection for PWS in 2023. Of these 7 NIS detected in PWS to date, 2 are considered established, whereas it is not known whether the other 5 species are established. Vessels are a likely mechanism (vector) of introduction for all of these species, and local environmental conditions appear suitable for colonization of PWS by these species as well as many other NIS that are spreading northward along the Pacific coast of North America. We recommend sustained and targeted surveillance in PWS using several approaches to evaluate the performance of ongoing management actions to reduce invasion risk, including (1) expanded PlateWatch surveys with local communities, (2) focused surveys at key vessel hubs, and (3) a decadal detection survey at 5-10 year intervals.

Methods

We used surveyed PWS hard substrate communities, using standard protocols that we have developed over the past 25 years and which we have now applied for repeated measures as “NIS sentinel site surveys” in San Francisco Bay CA, Chesapeake Bay VA, Tampa Bay FL, and other coastal bays. In 2022, we also conducted an identical survey in Ketchikan, AK, funded by U.S. Coast Guard. Using the same approach here allows for direct comparisons, especially across latitude on the Pacific coast.

We sampled 11 sites throughout PWS using a stratified random sampling design to collect 10 replicate community samples per site, using settling panels (similar to those used in Platewatch; see also Marraffini et al. 2017; Chang et al. 2018). Specific locations and dates are indicated in Figure 1 and Table 1. These are 14x14cm gray PVC panels that serve as standard habitats and passive collectors for benthic invertebrate communities. Each panel is suspended 1m below the water surface (Mean Lower Low Water, or low tide level), left for 3 months to allow for invertebrate recruitment and growth, and retrieved for analysis.

The summer/warm season is targeted, as the warm temperatures, greater light, and higher food quality coincide with the timing of reproduction and greatest recruitment and growth for many invertebrates. Panels were deployed in June 2023 and retrieved for analysis in September 2023.

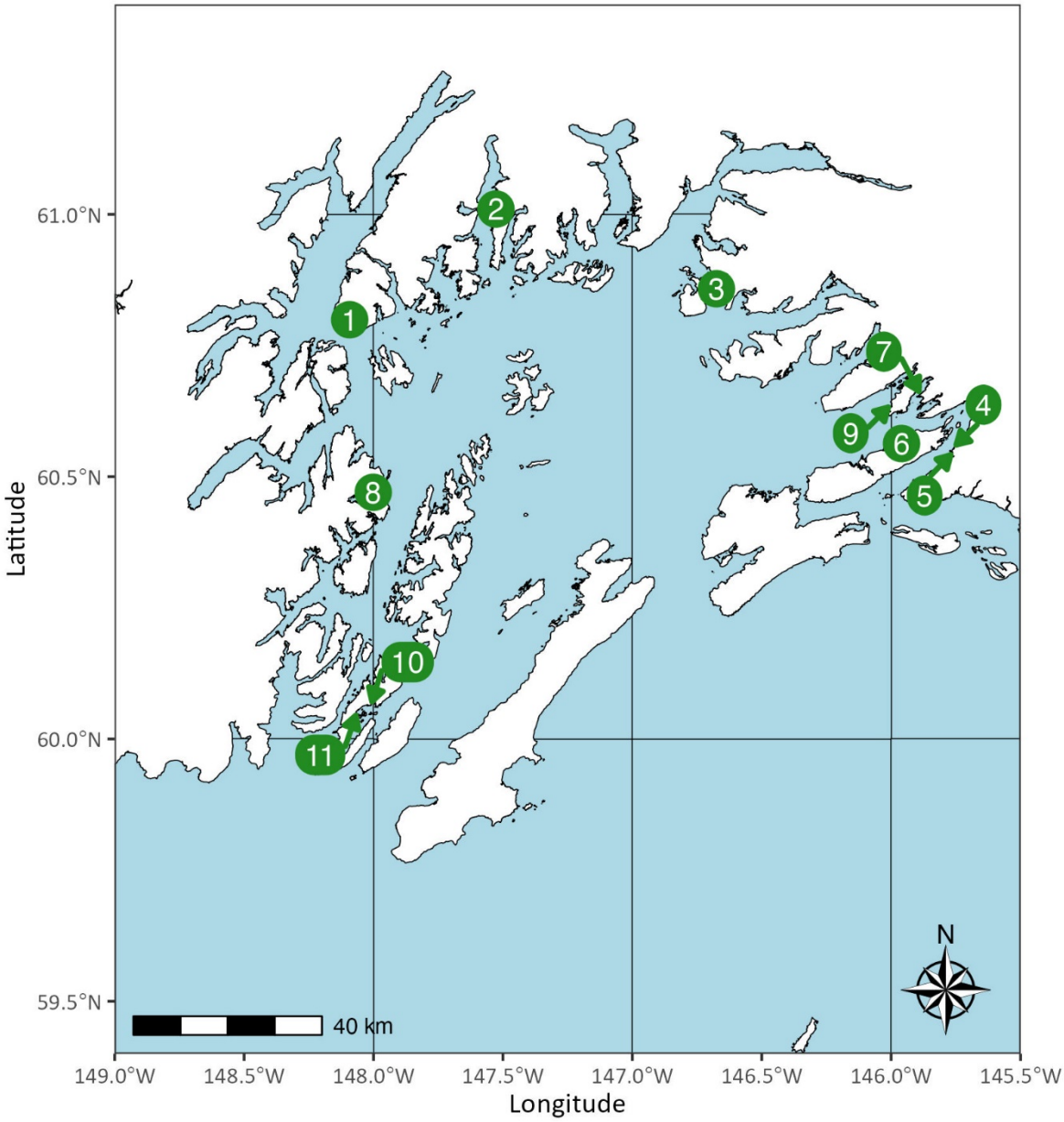

Figure 1: Map of hard substrate community sampling locations in PWS (2023).

Table 1: Hard substrate community sampling locations in PWS (2023).

Site No.	Site Name	Latitude (N)	Longitude (W)
1	Wally Noerenberg Hatchery	60.799	-148.092
2	Cannery Creek	61.010	-147.527
3	Tatitlek Ferry Dock	60.858	-146.675
4	Cordova Ferry Terminal	60.557	-145.756
5	Cordova Small Boat Harbor	60.545	-145.763
6	Windy Bay Kelp Farm	60.563	-145.960
7	Simpson Bay Oyster Farm	60.658	-145.888
8	Eshamy	60.470	-148.001
9	Sheep Bay	60.636	-146.004
10	Chenega Bay Marina	60.066	-148.009
11	AFK Hatchery	60.050	-148.065

For these 110 panels, we evaluated community composition using standardized morphological and genetic methods that we developed to detect NIS. Our approach also leverages a genetic barcode library that we have developed with collaborators Jonathan Geller (Moss Landing Marine Laboratories) and Katrina Pagenkopp Lohan (Smithsonian Environmental Research Center, or SERC) for NIS in California.

A. Morphological Analyses

For each panel, we identified all marine invertebrate species present using our standardized morphological and genetic methods. Upon retrieval of the panels, all sessile macroinvertebrates were processed live under a dissecting microscope to generate morphological vouchers for species-level identification on each plate. These "field vouchers" were later identified to species (or lowest taxonomic unit) based upon morphological characteristics using published taxonomic literature. A subset of these identifications was verified through additional consultation with outside taxonomic experts. In specific cases, results from morphological analyses were compared to results from genetic analyses using DNA barcoding to confirm taxonomic identification and test for the presence of cryptic species.

The morphological identifications of specimens produced a list of taxa identified to the lowest possible taxonomic level for each sample. For each taxon, we classified the invasion status in the bay where it was found as of the year of sampling, based upon previous analyses, the literature, and using a synthesis of information in the SERC National Estuarine and Marine Exotic Species Information System (NEMESIS) database (Fofonoff et al. 2018). Four categories were used for this classification: NIS, native, cryptogenic (of uncertain status, *sensu* Carlton (1996)), and unresolved (where species-level identification could not be made because specimens were juveniles or in poor condition). Putative records of new species were examined closely and compared to available databases and literature in consultation with taxonomic experts to evaluate their invasion status.

From these data, we compiled the number of NIS, native, cryptogenic, and unresolved taxa detected at each site and for the entire bay. We then constructed accumulation curves and calculated species richness estimators.

We conducted a standard series of statistical analyses to assess the completeness of our sampling efforts and estimate the number of NIS present. We used rarefaction to estimate the completeness of sampling for our level of sampling effort. This approach is combined with richness estimators calculated from our observations, to estimate the true (asymptotic) NIS richness detected using each method in each habitat, and to generate confidence intervals for detection. To estimate the number of NIS captured by our sampling methods, we used a relatively recently developed approach to species richness estimation that builds on traditional methods of rarefaction by combining rarefaction and extrapolation to make asymptotic estimates of richness along with quantifiable measures of sample completeness (Colwell et al. 2012; Chao et al. 2020).

Sample completeness, or sample coverage (SC), is a key determinant of how close the estimated number of species is to the true number of species present (observed + undetected) in a sampled assemblage. The more complete a set of samples is estimated to be, the more likely it is that all species actually present have been detected (Chao et al. 2014, 2020, 2021).

Statistical analyses were carried out using R 4.2.3 (R Core Team 2023) and the R packages vegan 2.6-4 (Oksanen et al. 2022), and iNEXT.3D 1.0.1 (Hu and Chao 2023).

B. Genetic Analyses

In addition to morphological analyses, we also sampled the entire community using genetic methods (DNA metabarcoding) to detect sequences present and identify NIS based on the COI gene. A brief summary of methods are outlined below with additional detail provided in Appendix A.

Sequencing Methods

Genomic DNA was extracted from a subsample (n=55) of blended biological material from fouling panels. Specific DNA tags were added to the beginning and end of the Polymerase Chain Reaction (PCR) products as indices to later identify the source sample for each DNA sequence. The sequences were then purified to remove small and spurious fragments. The concentration of DNA per sample was then quantified. Based on those calculations, DNA from each sample was then pooled based on equimolar concentrations into three libraries for sequencing, with the intent of having the same concentration of DNA lead to a similar number of sequences per sample. The final pooled libraries were sequenced using a MiSeq v3 600 Reagent Kit on an Illumina MiSeq platform at the Laboratories of Analytical Biology at the Smithsonian National Museum of Natural History. Additional details on DNA extraction, PCR amplification, and sequencing can be found in Appendix A.

Bioinformatic Pipeline and Taxonomic Assignment

Bioinformatic analyses were run on the Smithsonian Institution High Performance Computing Cluster (SI/HPC, 2024). Data curation, taxonomic assignment, and data analysis were done with the R (R Core Team, 2024) software. Before processing, we removed primer sequences using cutadapt (Martin, 2011; version 4.7). We used the dada2 package (Callahan et al., 2016) in R to trim, filter, assess, and correct sequencing errors, merge reads and remove chimeras (an artifact where partial PCR products from different species can be joined), and generate unique amplicon sequence variants (ASVs). ASVs summary tables were cropped to the desired target amplicon size (keeping sequences between 301 and 319 base pairs (bp).

To assign taxonomic ranks to ASVs, we ran the blastn algorithm from the BLAST software (Altschul et al., 1990; version 2.15) against two reference databases:

1) MIDORI2 (Leray et al., 2022; Machida et al., 2017). We used version MIDORI2_UNIQ_NUC_SP_GB259_CO1_BLAST downloaded from http://www.reference-midori.info/download.php#.

2) MLML reference database. This is a local database compiled by Jon Geller up to December 2023, that includes 310 sequences targeting marine invertebrates, many of them known NIS in California and the Pacific coast of North America.

BLAST results were filtered for quality, keeping only matches with percent coverage >95%, alignment length higher than 250 bp, and an e-value under 0.01. We used customized R functions to add higher taxonomic levels and select the best match from each database. When there were multiple "best" matches with identical similarity metrics, we assigned only the taxonomic level for which the reference sequences agreed on the classification (e.g., a match to *Balanus glandula* and *Balanus amphitrite* would be assigned only to *Balanus*). When there was only one best match, we kept the full taxonomy as provided. We then selected one final best match comparing both databases. To compare across databases, if only one match had a percent identity of 98% or more, we kept that one. If both matches were of 98% identity or more, we kept the one belonging to our local MLML database. If only one database returned a match, we kept that one. If both matches were lower than 98% percent identity, we kept the one with higher percent identity. We removed non-target taxa (e.g., terrestrial insects), and narrowed our analysis to marine and brackish metazoan species. The negative controls had only a low amount of reads and 7 ASVs. After close inspection of taxa names, we did not identify any true contaminant (e.g., human DNA) so only negative controls were removed before the analyses.

Data Analysis

ASVs were clustered based on unique taxa names using the aggregate taxa() function from the microbiome (Lahti & Shetty, 2012) R package. We then calculated species richness per location using the phyloseq (McMurdie & Holmes 2013) and vegan (Okasanen *et al.* 2014)

packages in R. We estimated rarefaction and extrapolation curves using the iNEXT approach described in the morphology chapter and the R package iNEXT.3D (Chao, A et al., 2021).

To evaluate NIS status for each unique taxa identified to species level, we compiled information from the following sources (in order of relevance):

- NEMESIS (Fofonoff et al. 2018), as downloaded on May 9, 2024.
- Surveys and reports produced by SERC on fouling plates, including both morphological and metagenetic analyses, of biota in California coastal waters.
- Simon et al (2022) detailed compilation of species in the Salish Sea. This publication list dozens of species most of them native to the area, but also highlights some introduced and cryptogenic species. We considered species native in the Salish Sea area would likely be native in PWS, if there was no other source of information available for the species.
- MarINVaders (Verones et al., 2023) list of alien species in the Cold Temperate North Pacific Province downloaded on Mar 14, 2024.
- Occurrence data from GBIF and OBIS, downloaded on Jun 13, 2024, using R packages rgbif (Chamberlain et al., 2024; Chamberlain & Boettiger, 2017) and robis (Provoost & Bosch, 2022). For taxa identified to species level and without occurrences reported in Alaska, we did an additional literature search and used phylogenetic trees to evaluate support for the name based on all available sequences for that genus.

Based on the information from all sources, we classified species as Introduced, Cryptogenic, Native, and Unknown. For taxa not identified to species level, we classified invasion status as Unknown, since we lacked sufficient resolution for further evaluation.

C. Range Expansion & Environmental Suitability of PWS

Over the past 25 years, SERC has conducted multiple surveys of marine communities along the Pacific coast, from Panama to Alaska, to detect NIS and evaluate invasion dynamics, focusing particular attention on detection of new NIS and geographic spread from California northward into Alaska. In addition to standard surveys and analyses by our team, we have implemented several collaborative participatory science programs to detect particular NIS, especially in Alaska (e.g., PlateWatch, Green Crab trapping, Bioblitz campaigns). The SERC team also has continued to synthesize new records of marine NIS in North America (as reported in publications, reports, and ongoing research) to track new detections and changes in distribution across time, creating the NEMESIS (Fofonoff et al. 2018), which is a web-based and searchable database available to the public.

In this report, we use NEMESIS to evaluate new detections of NIS over time in Alaska and PWS, noting the date of first record, reported occurrences, and what is known about the current population status of each species, evaluating specifically whether each is known to

have established (self-sustaining populations), or is only known from one or few records (creating uncertainty about establishment).

Modeling NIS Species Distributions in Alaska

In addition, in this report, we evaluate the potential of those NIS detected in Alaska to colonize and spread further along the coastline, including the potential for colonization of PWS. This is based on environmental modelling to consider both environmental suitability and habitat suitability.

In our previous work, led by Christina Simkanin (2019, unpublished report), we conducted species distribution modelling and range infilling analysis for 97 NIS on the U.S. Pacific coast, including marine invertebrates and algae across seven phyla, to examine the northern range distribution. Specifically, we aimed to predict: (a) northern range limit of each species based on environmental modelling of species distributions; (b) adjust or limit potential range to consider both environmental suitability and habitat suitability; (c) evaluate the percent of the range currently occupied, as a measure of range saturation (infilling) or potential for future spread; and (d) mapping current versus potential range along the Pacific coast of North America, which highlights potential for colonization of Alaska (including PWS).

Our earlier analyses found that 86 (or 89%) of the 97 species investigated had successfully spread and established populations beyond the bay or harbor of their first introduction. Critically, results from MaxEnt models showed that unoccupied environmentally suitable areas exist for nearly all 97 species – indicating that >95% of the species investigated have potential to continue expanding their non-native ranges northward along the Pacific coast.

A majority of the species investigated have filled only a limited proportion of their predicted range, indicating that they have high potential for future spread (Figure 2). Most of the 97 NIS have predicted distributions that extend throughout Alaska, indicating the potential for northward spread to this region. In this previous analysis, only 13 of the non-native species we investigated were known to occur as far north as Ketchikan (55°N) – but an additional 65 species had areas of predicted environmental suitability north of 55^{o}N latitude under current climate conditions.

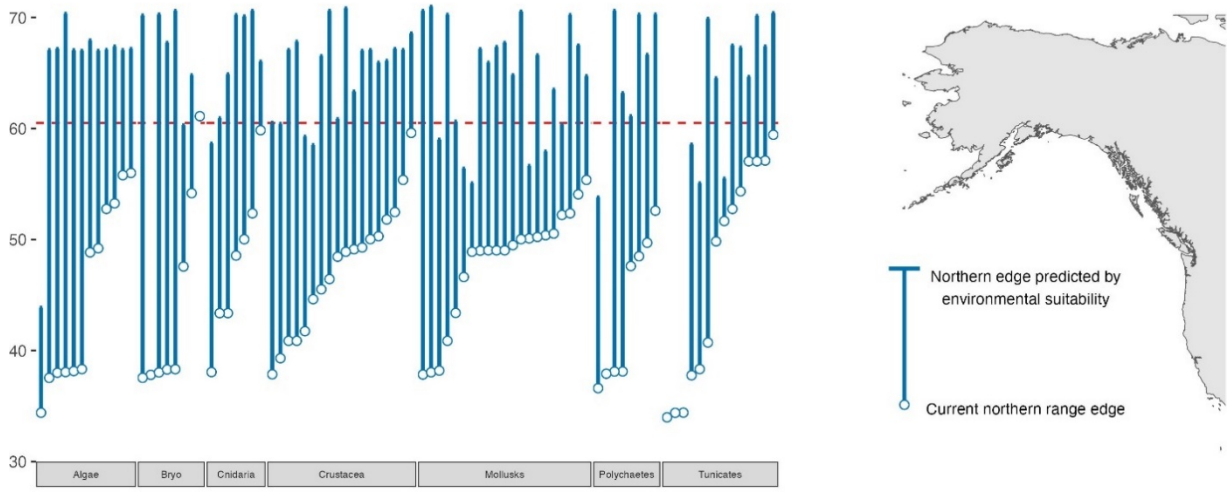

Figure 2: The potential for northward spread of non-native species based on species distribution modelling. Open circles represent the currently known northern range edge of a species distribution on the Pacific coast; blue bars represent the distance between the current most northern occurrence and the predicted most northern occurrence from MaxEnt models ('environmental suitability'). [Figure modified from Simkanin et al. 2019.]

In this report, we highlight the predicted range for the subset of NIS that have been reported to date in Alaska waters. We used output from our previous models along with updated distribution records, to provide a higher resolution snapshot of current and predicted NIS in Alaska as well as their proximity to PWS.

Results and Discussion

1. Environmental Setting of Survey

Average temperatures during the three-month (June to September 2023) period of the survey varied from 10.9°C to 14.0°C, with considerable variation among and within sites (Figure 3, Table 2). The warmest site was Tatitlek Ferry Dock and the coldest was Cannery Creek; interestingly, these two sites also had the least variation of all sites. Tatitlek also had the highest average salinity and Eshamy had the lowest (Table 2).

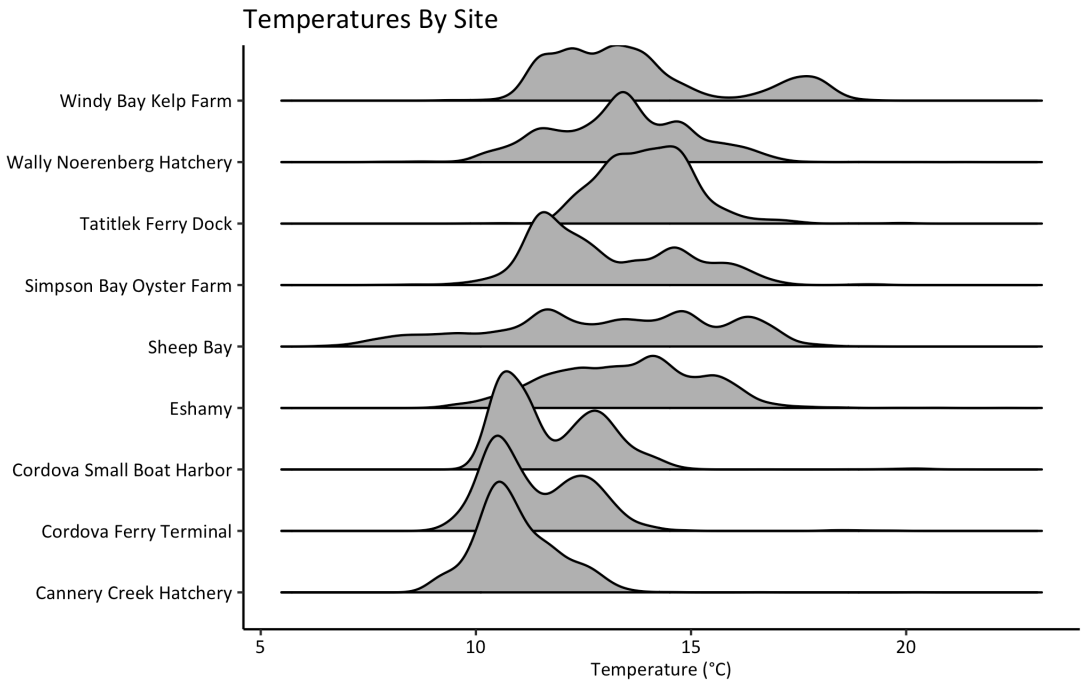

Figure 3: Distribution of temperatures at each site at 1m depth during the survey period in PWS in 2023. Temperature information was not available for Chenega Bay Marina.

Table 2: Environmental conditions at 1m depth at survey sites at PWS in 2023. Temperatures (mean and standard deviation (SD)) are summarized from loggers recording at 1-hour intervals during the settlement panel deployment period, while salinity values are averaged from spot samples at 1m depth taken at panel deployment and again upon retrieval.

Site	Mean Salinity (PSU)	Mean Temperature (°C)	SD Temperature
Wally Noerenberg Hatchery	21.9	13.4	1.6
Cannery Creek Hatchery	24.1	10.9	1.0
Tatitlek Ferry Dock	25.1	14.0	1.1
Cordova Ferry Terminal	26.3	11.4	1.2
Cordova Small Boat Harbor	25.5	11.8	1.3
Windy Bay Kelp Farm	25.9	13.7	2.1
Simpson Bay Oyster Farm	23.0	13.1	1.7
Eshamy	19.4	13.5	1.7
Sheep Bay	26.2	13.0	2.6

2. NIS Detected in PWS Survey: Morphological Analyses

Sampling Performance and Detection of Invasions

Our analyses indicate that our sampling program performed well in detecting and characterizing identifiable NIS in the PWS hard substrate community. This is shown below in a series of figures depicting the detection of NIS using species accumulation curves and richness estimators.

Species accumulation curves show the rate at which new species are found in a given area with additional sampling, and these are used to assess the completeness of sampling. An asymptote is reached nearly immediately for NIS, indicating complete sampling of the NIS community (Figure 4). Species richness estimators are reported here along with their respective standard errors (SE) for each type of organism (sessile, mobile, or total) and invasion status. The estimators generally agreed with the asymptote in NIS richness in Figure 5, further indicating that this result is robust (Table 6).

We detected a total of three NIS (one sessile taxon and two mobile taxa) in our hard substrate surveys in 2023.

SC estimators indicate that NIS were completely sampled for both sessile and mobile taxa (estimators table), with 100% SC. Overall survey performance was excellent, with 95% SC, indicating both that (1) there were likely few hard substrate species present that were not detected by the survey, and that (2) those species that did escape detection were most likely native or unresolved. In addition, most unresolved taxa are juveniles or specimens in poor condition that lack the features necessary for identification.

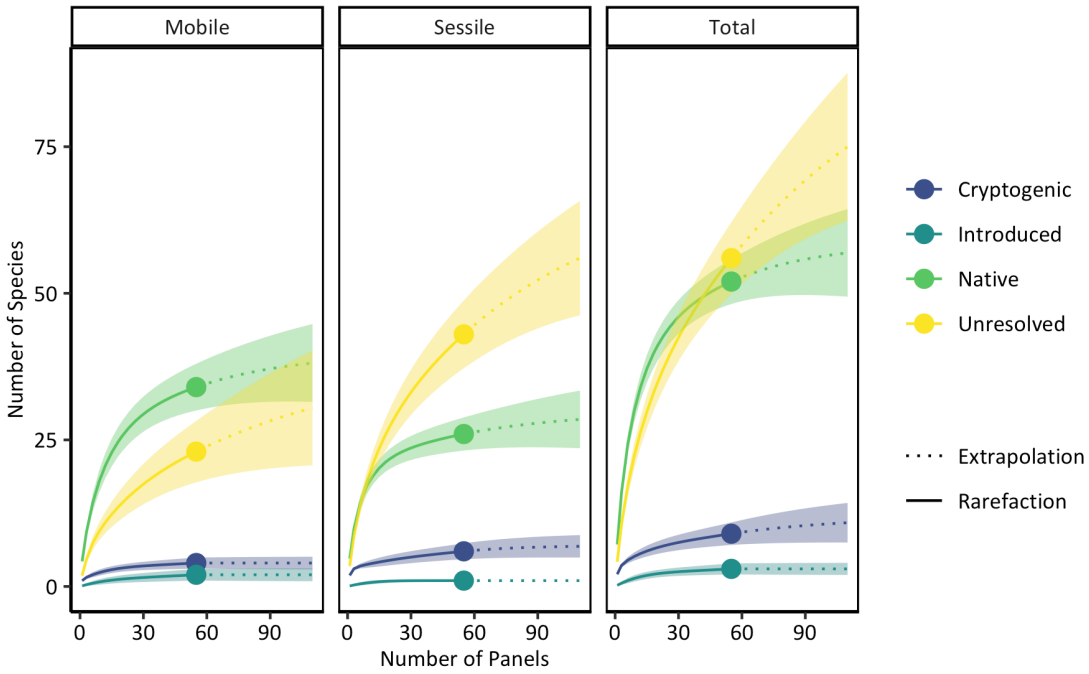

Figure 4: Species accumulation curves by invasion status for marine macroinvertebrates in PWS hard substrate communities in 2023. Number of species detected as a function of panels for each invasion status. Invasion status is assigned based on literature and the SERC NEMESIS database (Fofonoff et al., 2018). Here, a sample represents one settlement plate; up to 5 plates were analyzed from each of 11 sites in PWS in 2023. Shading around each line represents ± 1 SE. Rarefied estimates (solid line) up to the number of observed samples (dot), beyond which estimates are extrapolated (dashed line) up to twice the size of the reference (observed) samples.

Table 3: Species richness and SC estimators by invasion status for richness of marine macroinvertebrates in PWS hard substrate communities (2023). Invasion status is designated based on literature and SERC NEMESIS database (Fofonoff et al. 2018). The SC estimator, observed number of species (Observed), richness estimator (Estimator), standard error of the estimator (SE), 95% lower confidence limit (LCL), and 95% upper confidence limit (UCL) are given.

Type	Status	Observed	Estimator	SE	LCL	UCL	SC
Mobile	Cryptogenic	4	4.00	0.62	4.00	5.21	100.00
Mobile	Introduced	2	2.00	0.45	2.00	2.89	100.00
Mobile	Native	34	40.01	9.42	34.00	58.47	97.10
Mobile	Unresolved	23	39.36	15.67	23.00	70.09	90.30
Mobile	Total	63	88.32	13.80	63.00	115.36	95.29
Sessile	Cryptogenic	6	6.98	0.93	6.00	8.80	98.15
Sessile	Introduced	1	1.00	0.00	1.00	1.00	100.00
Sessile	Native	26	29.93	7.63	26.00	44.89	98.50
Sessile	Unresolved	43	69.51	18.69	43.00	106.14	90.79
Sessile	Total	76	111.35	11.05	89.69	133.00	95.79
Total	Cryptogenic	9	11.95	2.56	9.00	16.95	97.44
Total	Introduced	3	3.00	0.61	3.00	4.20	100.00
Total	Native	52	58.63	7.80	52.00	73.91	97.77
Total	Unresolved	56	99.83	23.69	56.00	146.26	89.33
Total	Total	120	174.53	20.78	133.80	215.25	95.01

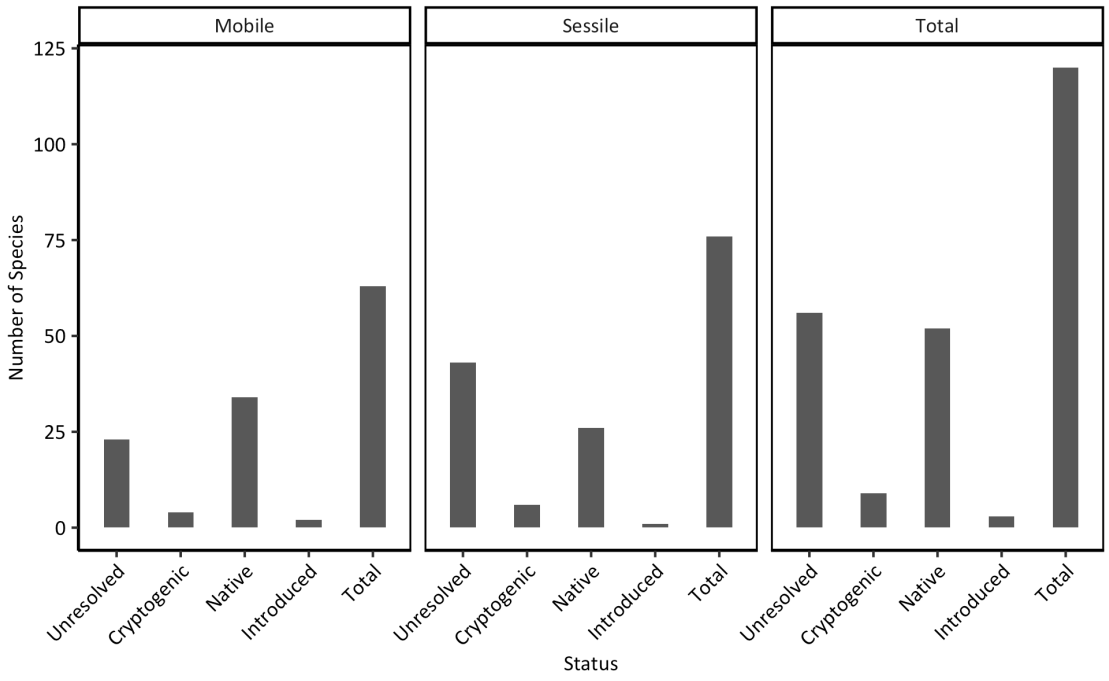

Figure 5: Number of unique species detected in PWS hard substrate communities in 2023 by invasion status. Status was assigned based on literature and SERC NEMESIS database.

NIS make up a very small percentage of the hard substrate community in PWS (Figure 5). This is even more evident in the low observed mean NIS richness per plate of 0.2 ± 0.65 (mean ± 1 SD), with three NIS recorded, and only at one location (Tatitlek Ferry Dock).

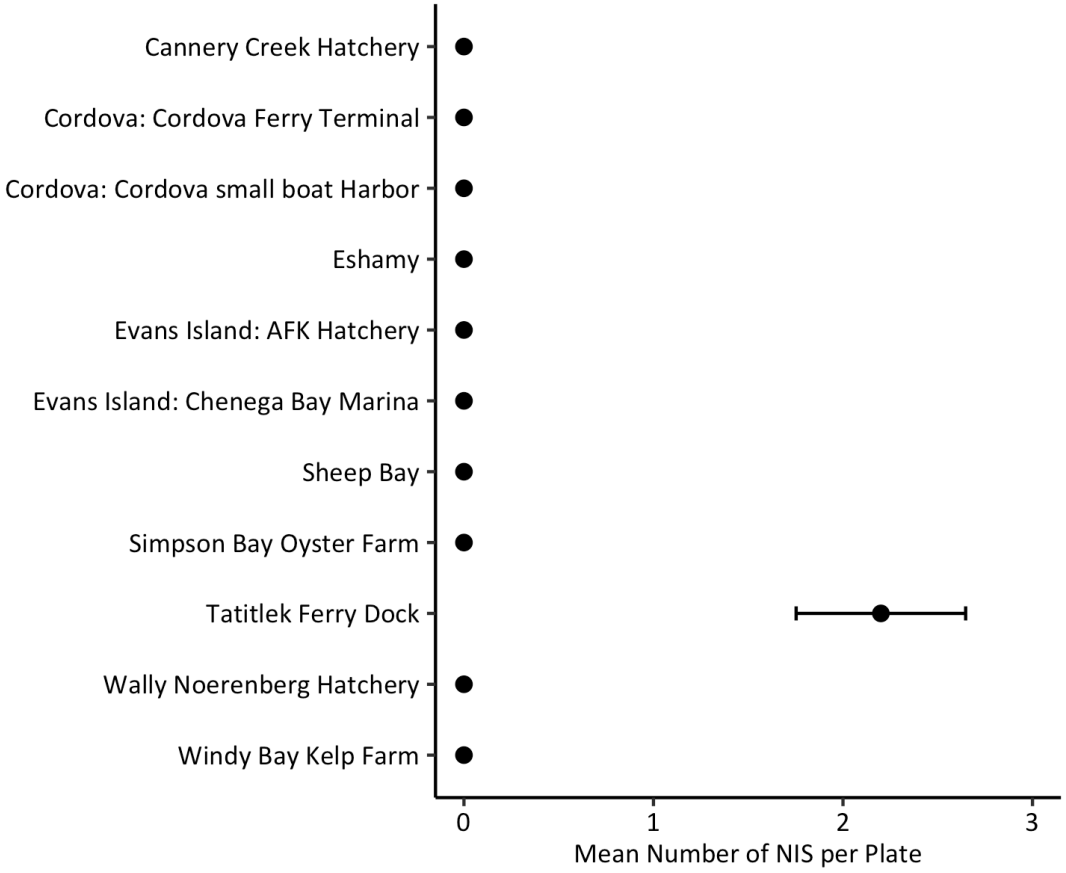

Figure 6: Mean number of NIS detected per plate averaged within sites in PWS in 2023. Error bars equal ± 1 SD.

Introduced Species Detected by Morphological Analyses

Three introduced taxa were identified in our survey of PWS, two of which had previously been detected from the region (Table 4; Table 6; Figure 4). All three NIS were detected only at the Tatitlek Ferry Dock (Figure 6), which also was the warmest site on average as well as one of the higher salinity locations (Table 2). Two species were previously known from the region, the broadly distributed temperate bryozoan *Schizoporella japonica* and the caprellid amphipod *Caprella mutica*. Although *C. mutica* has been detected previously in many regions of Alaska, including nearby Kachemak Bay (Ashton et al. 2008; Fofonoff et al. 2018), its detection in this study may be the first confirmed report within PWS.

Table 4: Introduced species detected in the 2023 PWS hard substrate surveys. The number of panels per site with each species is presented.

Group	Taxon	Number of Panels
Amphipoda	*Monocorophium acherusicum*	1
Bryozoa	*Schizoporella japonica*	5
Caprellidae	*Caprella mutica*	5

The gammarid amphipod *Monocorophium acherusicum*, which was detected on one plate at Tatitlek Ferry Dock, is a new record for both PWS and the broader region. The previous northernmost record of this species on the North American Pacific coast - and the only other record of this species in Alaska - is from our 2022 survey of Ketchikan, over 1000 km to the south. It was not found in our 2003 survey of PWS.

A species that dwells in tubes constructed on hard surfaces and firmer sediment, *M. acherusicum* is likely a native of the northern Atlantic Ocean and was originally described from Europe (Costa 1851). Broadly dispersed by shipping and oyster transplants, *M. acherusicum* has a nearly global distribution in tropical and temperate waters today, and it is considered to be introduced the northeastern Pacific, including Alaska (Fofonoff et al. 2018). Likely vectors include both vessel hull fouling as well as ballast water (Fofonoff et al. 2018).

The broad geographic range and environmental tolerances of *Monocorophium acherusicum*, including tolerance for ice-covered winter conditions and temperatures as high as 30^{o}C (Lee et al. 2005) and salinities as low as 6 (Takashi 1966), indicated significant potential for further spread. Corophiid amphipods like *M. acherusicum* are generally thought to graze on detritus and benthic microalgae, and are in turn eaten by fishes (Fofonoff et al. 2018).

3. NIS Detected in PWS Survey: Genetic Analyses

Synthesis of Sampling Performance and Taxonomic Detection

We found a total of 7,169 ASVs from 55 samples in the filtered reads. After removing non-target taxa, we ended up with 3,453 metazoan ASVs. Of these, we reviewed 550 ASVs that had a scientific name assigned and at least 95% identity; these ASVs included 73 unique taxa (Table A2). Taxa were identified to the lowest taxonomic level possible, usually to species. To be more conservative, we focused our analysis on ASVs identified to at least 98% identity (515 ASVs corresponding to 68 unique taxa, with 51 of those identified to species level).

Species accumulation curves (Figure 7) show that for unique marine invertebrates identified to a binomial species name, both morphological and genetic analysis reached a similar estimated species richness. On the other hand, for analysis of unique taxa identified at any taxonomic level, the morphological analysis of plates yielded a higher number of unique taxa, and of estimated total species richness than the genetic approach, likely due to the conservative threshold used for species level identification based on genetics.

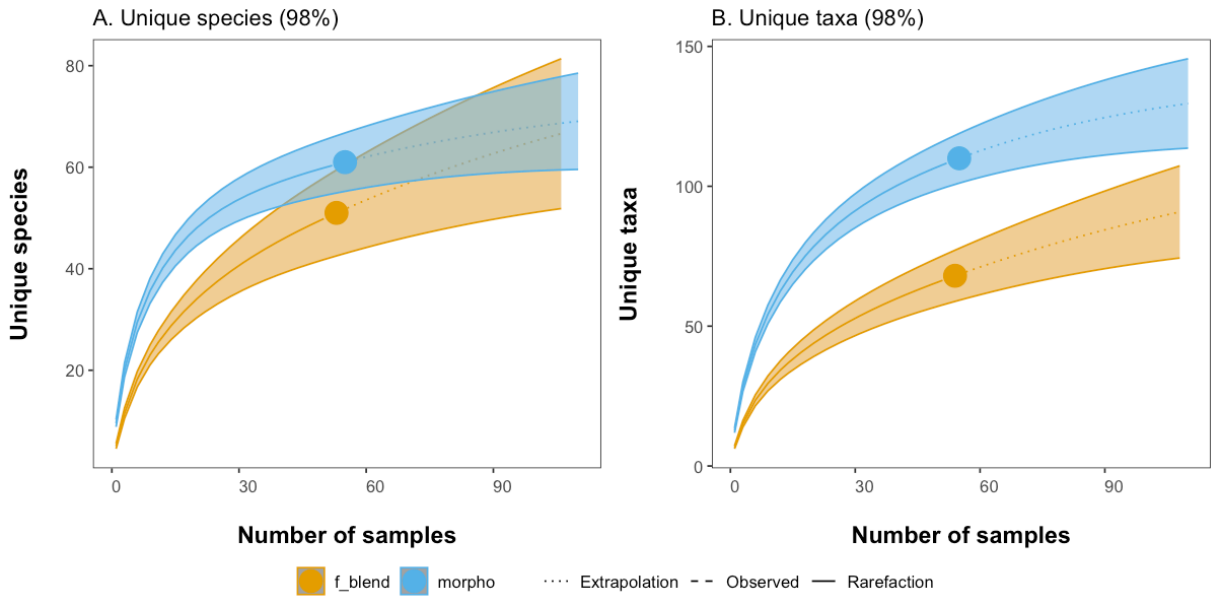

Figure 7: Species accumulation by sampling type comparing marine macroinvertebrates in PWS from hard substrate community surveys (in blue) and metagenetic analysis of the settlement panels (in orange) in 2023. A) Number of species and B) Number of unique taxa detected as a function of panels for each type of method, using a 98% identity criteria for the genetic results. Here, a sample represents one settlement plate. Shading around each line represents ± 1 SE. Rarefied estimates (solid line) up to the number of observed samples (dot), beyond which estimates are extrapolated (dashed line) up to twice the size of the reference (observed) samples.

We present the observed species richness with each method and sample estimators in Table 5. As expected by both methods, estimated species richness is higher than observed, indicating that additional taxa remain undetected in the survey. This is not surprising, especially given the spatial scale and environmental variation among sample sites. The SC for the genetic methods was fairly high (93%) but slightly lower than the morphology approach (95%).

Table 5: Species richness and SC estimators by sampling method for richness of marine macroinvertebrates in PWS (2023) metagenetic samples from settlement panels (blend) and community samples from hard substrate. We present data for unique taxa and unique species identified with 98% identity. The SC estimator, observed number of species (Observed), richness estimator (Estimator), standard error of the estimator (SE), 95% lower confidence limit (LCL), and 95% upper confidence limit (UCL) are given.

	Type	Observed	Estimator	SE	LCL	UCL	SC
Unique taxa	Blend	66	117.11	50.35	18.42	215.79	92.55
	Morphology	110	141.45	19.61	103.01	179.90	95.64
	Total	156	226.51	22.02	183.35	269.66	95.12
Unique species	Blend	51	90.25	25.98	39.32	141.17	92.61
	Morphology	61	75.14	9.01	57.47	92.80	97.75
	Total	94	135.86	13.31	109.77	161.95	96.74

Overall, there were fewer sequences in genetic analyses than expected and the relatively low reads per sample (see Appendix A) could directly impact richness assessments. Thus, it is possible that greater sequencing depth could improve the performance of the genetic approach. We note that there were samples containing only a small amount of tissue and primarily sediment, and samples for which DNA quantification was low and had low amplification success. This outcome may reflect relatively lower biomass in 2023, compared to our previous surveys (personal observation), possibly due to interannual variation in temperature and other environmental conditions. In addition, further modifying field protocols in the future to reduce sediment load (from glacial silt) and additional optimization of genetic methods may also yield higher reads per sample, and therefore species richness.

When looking at the identity of the species found with each method (Figure 8), using 98% identity for genetics, only 18 species were shared by both methods, and different species were found by the genetic and morphological approach. This complementarity is expected and consistent with results of our surveys (using these same methods) in other locations, when comparing morphological and genetic analyses. Each method has limitations. While

genetic methods have the potential to detect many taxa, including immature stages and damaged specimens which simply cannot be identified morphologically (due to lack of key characters), we currently can only assign a species name for those sequences which have been paired or linked to a valid morphological identification, using available bar code libraries. Unfortunately, many if not most sequences detected lack a known species identity, because bar code libraries remain very incomplete for marine invertebrates; although, we point out that these sequences (from past samples) can be identified in the future, as the bar code libraries mature. In contrast, for many of the larger marine invertebrates, especially in the biofouling community, taxonomy is relatively well developed, allowing us to detect many species for which genetic sequences are not yet available or adequately resolved.

Figure 8: Venn diagram showing the number of shared or unique species (with 98% identity) found using genetic methods (blend) and the morphological surveys (morphology).

Introduced Species Detected by Genetic Analyses

Most species identified by genetic analyses at the 98% identity threshold were native (Figure 9), followed by unresolved and cryptogenic species. We identified 2 introduced species, *Caprella mutica* and *Schizoporella japonica,* using genetic analyses. These species were found in low abundances and only in the Tatitlek Ferry Dock location. *Caprella mutica* was represented by two ASVs from 37 reads; *Schizoporella japonica* was represented by three ASVs from 281 reads. Both of these taxa were also detected by morphological analyses.

We also detected the polychaete *Polydora onagwaensis* at the AFK Hatchery on a single panel in western PWS and this was only detected with genetic methods (Appendix C). We currently have classified this species as cryptogenic, pending further evaluation. We consider this to possibly be introduced and a species of potential concern. *Polydora onagwaensis* was recently described from Onagawa Bay, Miyagi Province, Japan, from cultured oysters (*Magallana gigas*) and scallops (*Mizuhopecten yessoensis*), and has also been reported from the Bohai Sea and Yellow Sea in China (Sato-Okoshi et al. 2023). This

appears to be a species native to the western Pacific, and it also has been reported in European waters, including Normandy and the Contentin Peninsula of France (Sato-Okoshi et al. 2023). It also has been reported by morphological and genetic analyses in the northeastern U.S., where it associated with mud-blisters on shells of cultured oysters (Silverbrand 2019; Silverbrand et al. 2021; Rodewald et al. 2021).

Unlike *C. mutica* and *S. japonica*, which have been reported in many other locations for Alaska in recent years and are considered established, this appears to be the first record of *P. onagwaensis* in PWS. We also detected the same sequence in Ketchikan in 2022, during our recent surveys (Ruiz et al., unpublished data). In both cases, detected only by genetic methods to date, the sequence matches a reported bar code for the species, and we are now examining this in greater depth to evaluate its known biogeography. It is unknown whether a population of this organism is established in Alaska.

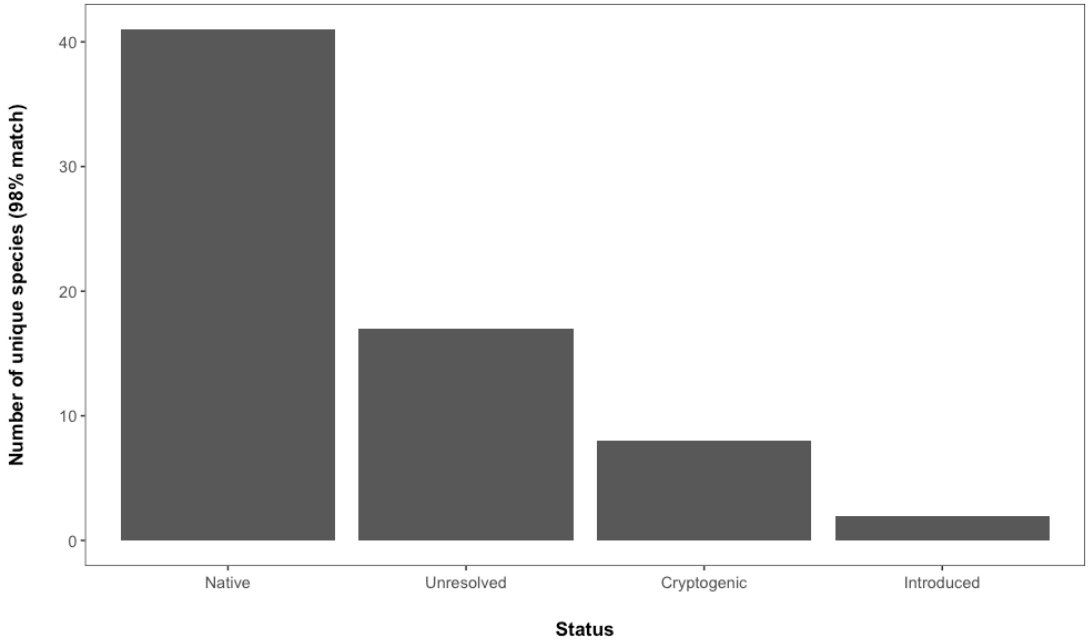

Figure 9: Number of unique species detected in PWS using metagenetic analysis of settlement panels with a 98% percent identity threshold. Status was assigned based on the literature, SERC NEMESIS database, and SERC previous status assignments in other projects. Source details were provided in Methods.

4. NIS Detected in Alaska and PWS

Combining results from the current survey with those of our past surveys and literature synthesis for benthic marine invertebrates, we can identify 21 NIS that have been reported in Alaska based on confirmation of morphological specimens. This excludes plants, vertebrates, and algae. Of these 21 NIS, 12 species are considered to have an established population in at least one location in Alaska (Table 6; Fofonoff et al. 2018). One additional species, the Asian oyster *Magallana gigas,* is cultured in Alaska waters but is not currently

known to have an established, self-sustaining population in the wild. The population status of the remaining 8 NIS is unknown, as to whether each is established or not.

Table 6: Benthic marine invertebrate NIS detected morphologically in Alaska by region and population status. Population status is shown as either established or unknown (Estab and Unk, respectively). Unknown status is highlighted in grey; taxa highlighted in grey are not known to be established in any region of Alaska. PWS is shown separately from Central Alaska in this table.

Taxa Group	Taxon	Aleutians	Kodiak	Central Alaska (Cook Inlet)	Prince William Sound	Southeast Alaska
Brozoan	*Bugula neritina*					Unk
Bryozoans	*Cryptosula pallasiana*					Unk
Bryozoans	*Schizoporella japonica*				Estab	Estab
Bryozoans	*Watersipora subatra*					Unk
Cnidarians-Anthozoans	*Diadumene lineata*					Unk
Cnidarians-Hydrozoans	*Ectopleura crocea*					Estab
Crustaceans-Amphipods	*Ampithoe valida*					Estab
Crustaceans-Amphipods	*Caprella mutica*	Estab		Estab	Unk	Estab
Crustaceans-Amphipods	*Monocorophium acherusicum*				Unk	
Crustaceans-Amphipods	*Monocorophium insidiosum*					Unk
Crustaceans-Barnacles	*Amphibalanus improvisus*				Unk	
Crustaceans-Crabs	*Carcinus maenas*					Estab
Crustaceans-Isopods	*Orthione griffenis*					Estab
Mollusks-Bivalves	*Magallana gigas*					Stock
Mollusks-Bivalves	*Mya arenaria*				Estab	
Tunicates	*Botrylloides violaceus*				Unk	Estab
Tunicates	*Botryllus schlosseri*					Estab
Tunicates	*Ciona savignyi*				Unk	Unk
Tunicates	*Didemnum vexillum*					Estab
Tunicates	*Molgula citrina*			Estab		

Of the 21 introduced taxa known from morphological specimens collected in Alaska, 17 are likely to be detected with the survey methods used in the current study, as they are sessile or small mobile invertebrates associated with hard surfaces; we have detected all 17 of these species using this methodology in California and elsewhere.

This total number of NIS reported in Alaska to date contrasts sharply with approximately 300 NIS known to be established on the Pacific coast of North America, of which most occur in California, and the total number decline with latitude (Ruiz et al. 2015; Fofonoff et al. 2018). It is also noteworthy that (1) most NIS in Alaska were detected in the past 25 years, occurring first in the continental U.S. and spreading northward and (2) the number of NIS detected within Alaska also declines from southeast Alaska northward (Figure 10).

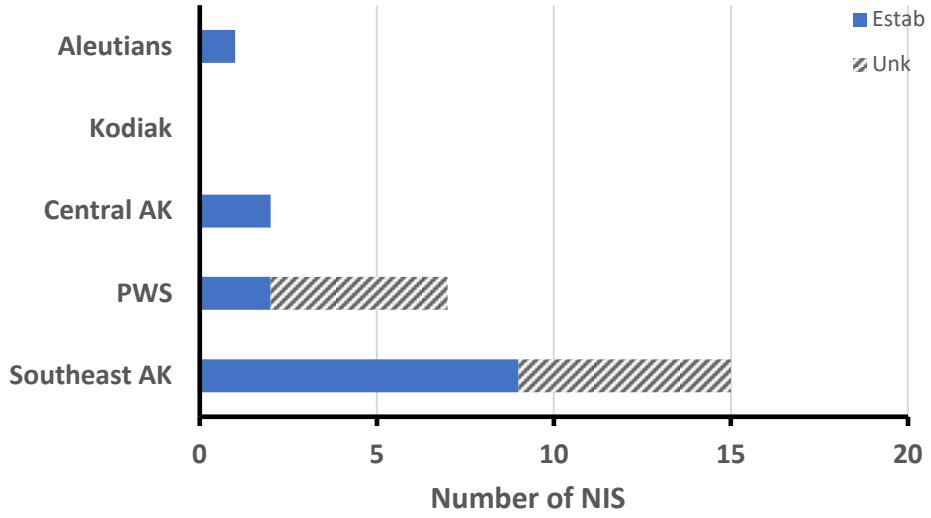

Figure 10: Number of benthic marine invertebrate NIS reported with confirmed morphological specimens in regional coastal waters of Alaska, shown as distance from the southern border. Shown are the number of with established populations (solid) and those with uncertain population status (hatch). PWS is shown separately from Central Alaska in this figure.

To date, in PWS, we have documented records of 7 NIS for benthic marine invertebrates, of which 2 are now considered established, whereas the population status of the other 5 remains unknown. Two of the latter were new records in 2023, including the tunicate *Ciona savignyi* and the amphipod *Monocorophium acherusicum.* The amphipod was detected in the current study. The tunicate was detected as part of our PlateWatch participatory science program (https://platewatch.nisbase.org) at the same time our staff were conducting this study and we confirmed the identification based on our subsequent morphological analysis.

As indicated in Table 6, the solitary tunicate *Ciona savignyi* has been reported in both southeast Alaska and PWS. Two specimens were found in Ketchikan in 2016 (Jurgens et al. 2018), and one specimen was found in PWS during our 2023 work. Prior to these records, there was a single specimen detected in 1903 in Ketchikan, as noted in Jurgens et al. 2018. Given the paucity of historical records and the conspicuous nature of this species, along with the well-known invasion history and spread along the Pacific U.S. coast (Fofonoff et al. 2018), we consider this to be an introduced species to Alaska waters and it is unknown whether a population is established in Alaska.

We exclude from these totals the polychaete *Polydora onagwaensis*, which is currently classified as cryptogenic, pending further analyses (as noted in section 3, above).

5. Environmental Suitability for NIS Colonization of PWS

Of the 21 NIS for benthic marine invertebrates reported in Alaska, including populations with both established and unknown status shown in Table 6, we have sufficient data (from their distributions and associated environmental conditions) to model the potential distribution in Alaska by latitude for 14 of these invertebrate species. We also have done this for two of five marine macroalgal NIS that have been reported in Alaska water to date.

In Figure 11, we show the current northern (established) range edge as well as the potential range for these, as predicated by MaxEnt models based on current environmental conditions. Among these taxa, only the bryozoan *Schizoporella japonica* is known to be established in PWS or further north, although several other species are considered established to the west of PWS (Table 6). Our models predict suitable environment exists in PWS and further north for all 16 of these taxa, including the five with unknown population status in Table 6. We also surmise that suitable habitat exists for all of these taxa, most of which colonize hard substrate and artificial structure (such as docks and marinas).

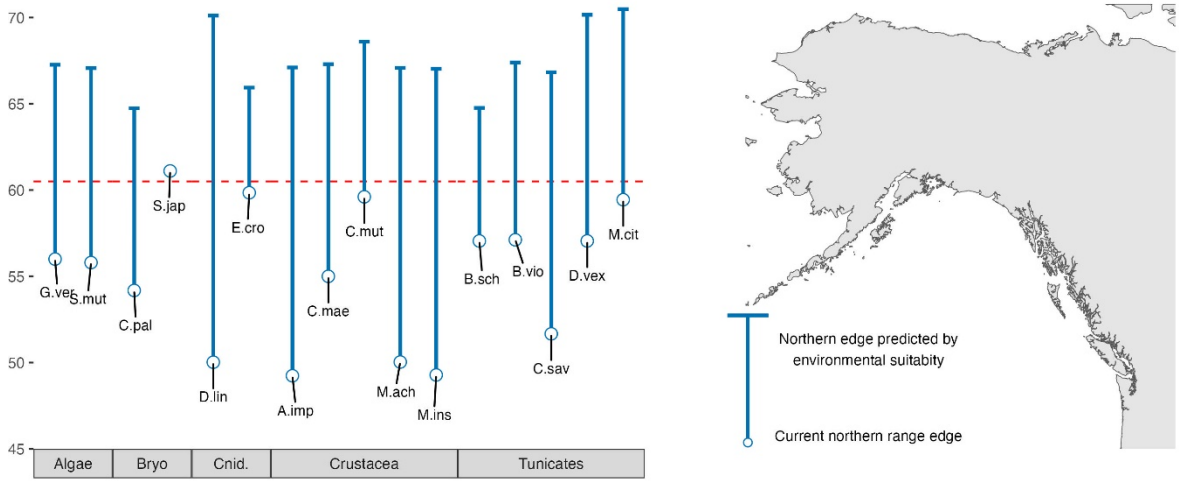

Figure 11: Potential for northward spread to PWS of 16 non-native species reported in Alaska, including some not known to be established. Open circles represent the currently known northern range edge of a species distribution on the Pacific Coast; blue bars represent the distance between the current most northern occurrence and the predicted most northern occurrence from MaxEnt models ('environmental suitability'). Abbreviated species names are indicated with each line within taxonomic group (see Table 6 for full names of invertebrates). Dashed horizontal line in red indicates latitude for PWS. [Figure modified from Simkanin et al. 2019.]

While our models predict that suitable environmental conditions already exist for colonization of PWS by many NIS, the probability of establishment is likely to increase with warming temperature for NIS that arrive here from further south along the Pacific coast. Historically, the seasonal window for reproduction and recruitment of marine invertebrates in PWS was more limited for many taxa, compared to bays at lower latitudes such as in California, and we expect this would have served to reduce the likelihood of colonization if

and when introductions occurred. Recent and ongoing warming in Alaska is likely widening the temporal window for such species to successfully reproduce, establish, and spread. Thus, while some biosecurity steps, such as ballast water treatment in recent years, have reduced the number of organisms delivered to PWS and Alaska, there may also be a per capita increase in the chance of colonization for those organisms that do arrive. The quantitative relationship between propagule release and colonization success remains poorly resolved (National Research Council 2011), making it difficult to assess the isolated or compensatory effects of temperature (climate change) on invasion outcomes.

Conclusions and Recommendations

Our survey and analyses confirmed the presence of 3 NIS for PWS in 2023. We also confirmed the identity of a fourth NIS, which was collected by our PlateWatch Program in 2023 for PWS. Two of these species appear to be new morphological records for PWS and the broader region, including the amphipod *Monocorphium acherusicum* and the tunicate *Ciona savignyi*. Two of these four species were also detected by genetic methods, along with the polychaete *Polydora onagwaensis*, which also appears to be a new record to the region; we are now conducting a more formal analysis of this polychaete to evaluate whether it may also be introduced.

In a broader synthesis of NIS records for marine invertebrate NIS detected in PWS, we identified 7 species with confirmed morphological records, of which 2 are considered to have established populations and 5 are not known to be established. It appears that 3 (43%) of these 7 first morphological records occurred in 2023.

Vessels are a possible mechanism (vectors) of introduction for all of these species, based on known life-histories and habitat distributions, although several vectors are possible for most (Fofonoff et al. 2018). Specifically, all of these species can be transferred by hull biofouling associated with commercial and other vessel types. Ship's ballast water is considered a possible mechanism for at least two of these species, including the amphipod *Monocorphium acherusicum* and the barnacle *Amphibalanus improvisus*. Most of these species are also associated with oysters and mussels as well, although this seems a less likely mechanism than vessels due to current management practices.

Overall, our analyses indicate that new NIS are being detected in PWS. We surmise that several of these are likely very recent arrivals, although we cannot determine the actual date of introduction or whether most have established populations. We hypothesize that these new records result from continuing transfers by vessels, including especially via hull biofouling. Further, our modelling indicates PWS currently has environmental conditions suitable for all of these species to establish self-sustaining populations. It is also likely that the opportunity for local establishment is improving, due to climate change.

To date, NIS arriving to Alaska and PWS have resulted primarily by human-mediated transfers from lower latitudes along the Pacific coast of North America (Ruiz et al. 2015).

Our current results suggest that new invasions to Alaska have occurred in recent years (especially in southeast Alaska) and that this pattern is likely to continue, since vessel biofouling contributes strongly to this northward spread, biofouling management is not required for vessels arriving to Alaska, and environmental conditions in PWS are suitable for colonization for most species. In addition, warming climates, sea ice declines, and the projected long-term opening of the Arctic Northwest Passage, along with planned construction of a deep draft port at Nome, may also enhance vessel traffic to multiple regions of Alaska, which could increase the likelihood of NIS transport and novel introductions (Miller and Ruiz 2014) from other global regions to Alaska, unless adequate biosecurity is in place.

Importantly, such northward spread and invasions to Alaska are not inevitable. Many of the NIS detected are not likely to arrive in Alaska without human transfers, due to their limited ability for natural dispersal. Thus, understanding invasion dynamics in Alaska provide critical information on whether management actions are working and sufficient to reduce invasion risks, or whether pathways for invasion remain open (National Resource Council 2011; Ruiz & Carlton 2003).

From this perspective, to evaluate the ongoing performance of biosecurity to reduce invasion risk in PWS, including especially those associated with vessel operations, we recommend several steps for sustained surveillance that also consider efficiency in cost and effort. We outline these briefly below:

Expanded PlateWatch Surveys. PlateWatch provides an efficient approach to detection of NIS and also engages local communities. To date, several NIS have been detected by PlateWatch, which has focused primarily on morphological detection of large, conspicuous organisms (such as tunicates). While we recommend sustaining this program, it is also feasible to include a genetic component and this could enable detection of many additional species, including those that are challenging to identify morphological. We recommend a training workshop in PWS for PlateWatch participants, to incorporate genetic sampling into the detection program, following new protocols SERC has developed for detecting DNA by soaking panels. This methodology is now being used in our broader research program and can be applied readily in PlateWatch.

Valdez Marine Terminal and Tatitlek Ferry Dock Repeated Measures. We recommend repeated measures at both Valdez Marine Terminal and Tatitlek (near the ferry dock), using panels, for both morphological and genetic analyses. We have detected all known marine invertebrate NIS for PWS at these two locations, including the four species not known to be established. In essence these two sites appear to be hotspots for detection, likely due to marine transportation and possibly environmental conditions. Moreover, the Valdez terminal could allow sampling across salinities, since there is a salinity gradient with depth. Frequent sampling at these two sites could serve as sentries for new NIS as well as evaluate whether the four recent

NIS (of unknown population status) are established. Moreover, including short (3-month) and long (>6-month) duration panels may increase the total number of species detected at these sites.

Decadal Survey of PWS. We recommend repeating the current survey and analyses every 5-10 years for PWS, to evaluate long-term changes. The panel surveys aim to detect NIS and also provide an assessment of community composition, including native, non-native and cryptogenic species. Thus, these data serve to evaluate invasion dynamics, while also assessing broader community-level changes, which may be expected in response to climate change or other pulse disturbance events. SERC has established sentinel sites for repeated measures, using panels and zooplankton sampling (analyzed by both morphology and genetics) in San Diego, Long Beach, San Francisco Bay, Humboldt Bay, and Ketchikan. These sentinel site surveys are repeated at least every 3-5 years and serve as part of a decadal survey along the Pacific coast, including 12 locations from San Diego to Homer, to evaluate northward spread of NIS. Including PWS every 5-10 years would leverage the extensive data being collected across latitude to evaluate northward spread and changing risk of invasions in Alaska waters.

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Appendix A: Sequencing and Bioinformatics Details

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from 0.25g of blended biological material from fouling panels. Negative extraction controls were included to identify potential contaminants in the library preparation. A portion of the mitochondrial COI gene was amplified using primers fbLCOF1 (J. Geller, unpublished) and jgHCO2198 (Geller et al. 2013). This COI gene fragment is a genetic marker, or "DNA barcode," commonly used to identify animals and so is well represented in public databases to aid taxonomic assignment of DNA sequences. All PCR reactions were generated in triplicate to mitigate potential variation across replicates in PCR. To increase sequence diversity, additional base pairs (0, 1, 2 and 3 bp) were added to each forward and reverse primer in an equal-volume mix. All PCRs were generated in triplicate. PCR reagents consisted of 1 x GeneAmp 10 x PCR Gold Buffer (150 mM Tris-HCL, pH 8.0; 500 mM KCl; Applied Biosystems, Carlsbad, CA), 2.0 mM MgCl2, 0.2 mM each nucleotide, 0.4 µM each primer, 0.2 mg mL-1 bovine serum albumin (BSA; New England Biolabs), and 0.025 units µL-1 of AmpliTaq Gold with water to a final volume of 20 µL. Thermal cycling was carried out using a S1000 Thermal Cycler (Bio-Rad, Hercules, CA) with an internal denaturation of 95ºC for 10 min, 35 cycles of 95ºC for 60 s, 50ºC for 90 s, 72ºC for 60 s and a final elongation of 72ºC for 5 min. For screening the assays, an aliquot of PCR product (5 µL) was electrophoresed on agarose gel (2% w/v) stained with GelRed (Phenix Research) and visualized under UV light. Triplicate PCR amplicons were pooled for each sample based on gel band intensity.

We used dual-indexing with Nextera adapters with a unique combination to each sample. PCR reagents consisted of 12.5 µL KAPA Ready Mix, 1 µL each index (i7 or i5), 1 µL amplicon (pooled product), and 9.5 µL water for a final reaction volume of 25 µL. Thermal cycling was carried out with an initial denaturation of 95ºC for 5 min, followed by 12 cycles of 98ºC for 20 s, 60ºC for 45 s, and 72ºC for 45 s, and a final extension of 72ºC for 5 min. To verify that indexing was successful, an aliquot of indexed product and unindexed product were both electrophoresed on agarose gel (2% w/v) stained with GelRed and visualized under UV light. The indexed product was purified with AMPure XP Beads (Beckman-Coulter, USA) by following the manufacturer's instructions for 10 µL sample reaction volume and 1.5X ratio.

The bead-cleaned samples were quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) with a Qubit 2.0 Fluorometer using manufacturer instructions. Samples were equally divided and pooled based on equimolar concentrations into three separate libraries, which were independently sequenced on three runs. The final pooled libraries were sequenced using a MiSeq v3 600 Reagent Kit (Illumina) on an Illumina MiSeq platform at the Laboratories of Analytical Biology at the Smithsonian National Museum of Natural History.

Numbers of Reads at Each Stage of the Dada2 Pipeline

sample_names	input	filtered	denoisedF	denoisedR	merged	nochim
control-EC-20240401-PWS-2023-fb	136	78	48	52	48	48
control-EC-20240402-PWS-2023-fb	41	27	27	27	27	27
control-EC-20240404-PWS-2023-fb	45	30	27	27	27	27
control-EC-20240405-PWS-2023-fb	16	1	1	1	0	0
control-EC-20240408-PWS-2023-fb	2	2	1	1	0	0
f-blend-22324-1-PWS-2023-fb	100424	83144	80914	81141	77817	70368
f-blend-22328B-1-PWS-2023-fb	76391	65092	64527	64815	63302	61588
f-blend-22334-1-PWS-2023-fb	63474	54721	54433	54364	53918	53903
f-blend-22334B-1-PWS-2023-fb	62138	52160	50303	50582	48674	44533
f-blend-22335-1-PWS-2023-fb	20353	16662	16077	16075	15715	15300
f-blend-22336-1-PWS-2023-fb	195871	159977	158008	158169	152933	141068
f-blend-22339-1-PWS-2023-fb	2975	2397	2324	2329	2306	2306
f-blend-22341-1-PWS-2023-fb	28309	22610	21644	21631	20914	19942
f-blend-22342-1-PWS-2023-fb	145938	120319	116328	116910	111633	106861
f-blend-22346-1-PWS-2023-fb	59399	49537	49232	49237	48670	47390
f-blend-22349-1-PWS-2023-fb	15968	8642	8583	8568	8545	8545
f-blend-22350-1-PWS-2023-fb	33474	25333	25101	25101	24579	24579
f-blend-22351-1-PWS-2023-fb	5663	2924	2702	2765	2583	2583
f-blend-22352-1-PWS-2023-fb	197	126	90	105	20	20
f-blend-22358-1-PWS-2023-fb	56087	46778	45570	45551	44106	42290
f-blend-22360-1-PWS-2023-fb	69501	60624	59738	59783	58179	50999
f-blend-22367-1-PWS-2023-fb	102902	84855	82464	82964	79355	71604
f-blend-22369-1-PWS-2023-fb	74996	64206	63898	63811	63514	62958
f-blend-22370-1-PWS-2023-fb	261606	218278	214082	214722	206966	170945
f-blend-22371-1-PWS-2023-fb	40138	32209	30940	30964	29639	29366
f-blend-22377-1-PWS-2023-fb	35698	30931	30315	30386	29572	27294
f-blend-22383-1-PWS-2023-fb	42238	36325	35292	35280	34473	31391
f-blend-22387-1-PWS-2023-fb	109749	82777	81097	81243	78266	74309
f-blend-22388-1-PWS-2023-fb	64390	56241	55901	55952	55319	53872
f-blend-22392-1-PWS-2023-fb	45576	39006	38219	38141	37568	37096

f-blend-22398-1-PWS-2023-fb	5537	4287	3749	3775	3443	3396
f-blend-22399-1-PWS-2023-fb	120056	97775	96626	96687	94406	81449
f-blend-22527-1-PWS-2023-fb	53684	46032	45595	45652	44500	39859
f-blend-22528-1-PWS-2023-fb	79513	68403	67753	67883	66140	55169
f-blend-22535-1-PWS-2023-fb	4610	3838	3766	3761	3728	3728
f-blend-22538-1-PWS-2023-fb	49055	43640	43267	43243	43024	43000
f-blend-22539-1-PWS-2023-fb	5461	682	644	646	639	639
f-blend-22540-1-PWS-2023-fb	29804	23940	23197	23242	22645	22595
f-blend-22541-1-PWS-2023-fb	13524	11150	10494	10492	10003	10003
f-blend-22545-1-PWS-2023-fb	1707	1323	1081	1063	1029	1029
f-blend-22551-1-PWS-2023-fb	54577	47649	46506	46450	45723	42466
f-blend-22557-1-PWS-2023-fb	12112	9542	9040	9007	8514	8507
f-blend-22558-1-PWS-2023-fb	89	56	45	43	43	43
f-blend-22559-1-PWS-2023-fb	106545	88834	87595	87993	85244	76718
f-blend-22561-1-PWS-2023-fb	49316	39537	38722	38629	37535	37424
f-blend-22562-1-PWS-2023-fb	737	457	443	446	440	440
f-blend-22566-1-PWS-2023-fb	104336	83369	81539	81619	78162	74946
f-blend-22568-1-PWS-2023-fb	2697	2060	1974	1966	1841	1779
f-blend-22571-1-PWS-2023-fb	85765	72342	71202	71401	69632	62541
f-blend-22573-1-PWS-2023-fb	58127	51366	50921	50937	50702	49997
f-blend-22575-1-PWS-2023-fb	169065	139435	135206	135977	127348	118649
f-blend-22577-1-PWS-2023-fb	141003	118299	116302	116256	112436	98123
f-blend-22753-1-PWS-2023-fb	78460	62001	61567	61476	60807	59280
f-blend-22762-1-PWS-2023-fb	26623	21983	20890	21018	20105	19753
f-blend-22783-1-PWS-2023-fb	3035	2391	2091	2079	1853	1847
f-blend-22784-1-PWS-2023-fb	84175	72640	72243	72242	72143	72131
f-blend-22785-1-PWS-2023-fb	61890	51327	50748	50896	49454	45200
f-blend-22787-1-PWS-2023-fb	39976	32037	30794	30799	29653	29264
f-blend-22788-1-PWS-2023-fb	156899	129428	125031	126235	119821	111807
f-blend-22789-1-PWS-2023-fb	86224	62460	59588	60007	56819	56320

Appendix B: Taxa Identified in Survey by Morphological Analyses

Table B1: Species detected by morphological analyses in the 2023 PWS hard substrate survey. The number of plates per site with each species is shown, along with the total across all sites. Invasion status is shown for each species as Introduced (I), Native (N), Crytopgenic (C), or Unresolved (U).

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Harbor	Windy Bay Kelp Farm	Total
Phylum: Annelida													
Group: Serpulidae													
Pseudochitinopoma occidentalis	N	1	0	0	2	1	0	4	0	5	0	5	18
Serpulidae	U	0	0	0	0	1	0	1	0	0	0	0	2
Group: Spionidae													
Polydora websteri	C	2	0	0	0	0	0	0	1	0	0	0	3
Prionospio cirrifera	C	0	0	0	0	0	0	0	1	0	0	0	1
Spio sp.	U	1	0	0	0	0	0	0	0	0	0	0	1
Group: Spirorbidae													
Bushiella sp.	U	0	0	3	1	0	0	0	0	0	0	0	4
Circeis armoricana	N	0	0	1	2	1	1	0	0	0	0	0	5
Circeis sp.	U	0	0	0	1	0	0	0	0	0	0	0	1
Circeis spirillum	C	4	2	3	2	5	1	0	0	4	0	4	25
Paradexiospira vitrea	N	0	0	4	0	0	0	0	0	0	0	0	4
Spirorbidae	U	1	0	0	0	0	1	0	0	0	0	1	3
Phylum: Arthropoda													
Group: Amphipoda													
Allorchestes sp.	U	0	0	0	1	0	0	0	0	0	0	0	1
Americorophium brevis	N	0	1	0	0	0	0	0	0	0	0	0	1
Ampithoe dalli	N	0	2	0	0	0	3	0	0	0	0	0	5
Ampithoe sp.	U	1	0	0	0	0	0	0	0	0	0	0	1
Anisogammaridae	U	0	1	0	0	0	0	0	0	0	0	0	1

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Anisogammarus pugettensis	N	0	0	0	1	0	0	0	0	0	2	0	3
Aoroides columbiae	N	0	0	0	2	0	0	0	0	0	0	0	2
Aoroides sp.	U	0	0	1	0	0	0	0	0	0	0	0	1
Corophiidae	U	0	0	1	0	0	0	0	0	0	0	0	1
Eogammarus confervicolus	N	0	4	0	0	0	0	0	1	0	5	0	10
Gnathopleustes pachychaetus	N	5	5	3	1	0	4	0	0	0	1	0	19
Ischyrocerus anguipes	C	0	0	1	1	1	1	0	0	0	0	0	4
Ischyrocerus sp.	U	1	0	0	1	0	0	0	0	0	0	1	3
Jassa staudei	N	1	0	1	0	0	0	0	4	0	0	0	6
Monocorophium acherusicum	I	0	0	0	0	0	0	0	0	1	0	0	1
Pontogeneia inermis	N	0	0	0	0	0	0	0	0	0	1	0	1
Pontogeneia rostrata	N	0	0	0	0	0	1	0	0	0	0	0	1
Group: Caprellidae													
Caprella alaskana	N	0	1	0	0	0	0	0	0	1	0	0	2
Caprella gracilior	N	0	0	0	0	0	0	1	0	0	0	2	3
Caprella irregularis	N	0	0	0	2	1	0	0	0	0	0	0	3
Caprella laeviuscula	N	0	1	1	0	0	1	0	0	0	0	0	3
Caprella mutica	I	0	0	0	0	0	0	0	0	5	0	0	5
Caprella sp.	U	1	0	0	1	1	2	1	0	0	0	3	9
Deutella californica	N	0	0	0	5	2	0	0	0	0	0	0	7
Metacaprella kennerlyi	N	0	1	0	0	0	0	0	4	2	0	0	7
Group: Cirripedia													
Balanidae	U	0	0	0	0	0	0	0	0	0	0	1	1
Balanus crenatus	N	4	0	0	5	5	4	0	1	2	2	3	26
Balanus glandula	N	0	1	0	2	1	3	2	0	0	2	2	13

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Balanus sp.	N	0	0	1	0	0	0	2	0	0	1	0	4
Cirripedia	U	1	0	0	0	0	0	0	0	0	0	0	1
Group: Decapoda													
Caridea	U	0	0	0	0	0	0	0	0	0	0	1	1
Glebocarcinus oregonensis	N	0	0	1	0	0	0	0	0	0	0	0	1
Heptacarpus brevirostris	N	0	0	0	3	0	1	0	0	0	0	0	4
Group: Isopoda													
Gnorimosphaeroma oregonense	N	0	0	0	0	0	0	0	0	0	2	0	2
Munna stephenseni	N	0	0	1	2	0	0	0	0	0	0	1	4
Pentidotea schmitti	N	0	1	0	0	0	1	0	0	0	0	0	2
Phylum: Bryozoa													
Alcyonidium sp.	U	0	0	0	1	1	1	0	1	4	0	1	9
Amathia sp. *Bowerbankia*	U	0	0	0	0	3	0	0	0	1	0	0	4
Callopora craticula	N	3	0	2	1	0	0	0	0	1	0	1	8
Celleporella hyalina	C	5	1	5	5	4	5	0	4	2	0	0	31
Crisia sp.	U	0	0	1	0	0	0	0	0	0	0	0	1
Crisiidae	U	0	0	1	0	0	0	0	0	0	0	0	1
Crisularia pacifica	N	1	0	3	2	5	0	0	0	0	0	0	11
Dendrobeania lichenoides	N	0	0	0	0	0	1	0	0	0	0	0	1
Fenestrulina delicia	C	0	0	1	0	0	0	0	0	0	0	0	1
Fenestrulina sp.	U	0	0	3	0	0	0	0	0	1	0	0	4
Filicrisia franciscana	N	2	0	0	0	0	0	0	0	0	0	0	2
Filicrisia sp.	U	3	0	2	0	0	0	0	0	0	0	0	5
Juxtacribrilina corbicula	N	3	0	3	1	4	3	0	0	5	0	0	19
Juxtacribrilina sp.	N	0	0	2	1	1	2	1	0	0	0	1	8

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Lichenoporidae	U	0	0	1	0	0	0	0	0	0	0	0	1
Membranipora villosa	N	0	0	2	0	0	0	3	0	3	0	3	11
Patinella verrucaria	N	5	0	0	0	0	0	0	0	0	0	0	5
Schizoporella japonica	I	0	0	0	0	0	0	0	0	5	0	0	5
Tegella aquilirostris	N	2	0	0	4	0	0	0	0	0	0	0	6
Tubulipora sp.	U	0	0	1	0	0	0	0	0	0	0	1	2
Phylum: Chlorophyta													
Chlorophyta	U	0	1	0	1	3	1	0	5	0	5	1	17
Phylum: Chordata													
Group: Tunicata													
Aplousobranchia	U	0	0	0	3	2	0	0	0	0	0	0	5
Corella inflata	N	2	0	5	2	5	0	0	0	0	0	0	14
Distaplia occidentalis	N	0	0	0	0	1	0	0	0	0	0	0	1
Distaplia sp.	N	0	0	0	1	1	0	0	0	0	0	0	2
Stolidobranchia	U	0	0	0	0	3	0	0	0	1	0	0	4
Styela sp.	U	0	0	0	2	0	0	0	0	0	0	0	2
Phylum: Cnidaria													
Group: Anthozoa													
Actiniaria	U	0	0	1	0	0	1	0	0	0	0	0	2
Unidentified Anthozoa	U	0	0	0	0	1	0	0	0	0	0	0	1
Metridium sp.	U	0	0	0	1	0	0	0	0	0	0	0	1
Group: Hydrozoa													
Athecata	U	0	1	0	0	0	0	0	0	0	0	0	1
Campanulariidae	U	0	5	0	5	2	5	1	1	1	5	2	27
Campanulinidae	U	0	1	0	0	0	0	0	0	0	0	0	1

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Hydrozoa	U	0	0	1	0	0	0	0	0	0	0	0	1
Thecata	U	0	0	0	0	1	0	2	0	0	0	0	3
Phylum: Echinodermata													
Group: Asteroidea													
Asteroidea	U	0	0	0	0	0	1	4	3	2	1	1	12
Group: Echinoidea													
Echinoidea	U	0	0	0	0	0	0	0	0	0	0	1	1
Phylum: Mollusca													
Group: Bivalvia													
Hiatella arctica	N	5	5	5	5	5	5	5	5	5	5	4	54
Modiolus modiolus	N	1	1	2	4	4	0	1	0	5	0	5	23
Mya truncata	N	0	0	0	0	0	1	0	0	0	0	0	1
Mytilus galloprovincialis/trossulus complex	C	5	4	4	3	2	5	5	5	2	5	3	43
Pododesmus macrochisma	N	0	0	0	0	0	0	1	0	4	0	4	9
Vilasina vernicosa	N	0	1	0	0	0	0	0	0	0	0	0	1
Group: Gastropoda													
Alvania compacta	N	1	0	1	0	0	1	0	0	0	0	2	5
Calyptraeidae	U	0	0	0	0	0	0	0	0	1	0	1	2
Columbellidae	U	0	0	0	0	0	2	0	0	0	0	1	3
Crepipatella lingulata	N	0	0	0	1	0	0	0	0	1	0	3	5
Crepipatella sp.	U	0	0	1	0	0	0	0	0	0	0	0	1
Unidentified Gastropoda	U	0	0	1	0	0	0	0	0	0	0	0	1
Lacuna sp.	U	0	0	0	0	0	0	0	0	1	0	1	2
Lacuna vincta	U	1	2	1	4	0	4	2	0	0	5	2	21

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Unidentified Limpet	U	0	0	0	0	0	0	0	0	1	0	0	1
Margarites pupillus	N	1	1	0	0	1	0	2	0	0	0	0	5
Odostomia sp.	U	0	0	0	0	0	1	5	3	1	0	2	12
Group: Nudibranchia													
Aeolidioidea	U	0	0	0	1	1	0	0	0	0	0	0	2
Coryphella verrucosa	N	0	2	0	2	1	0	0	0	0	0	0	5
Eubranchus olivaceus	C	0	4	0	0	0	2	0	0	0	0	0	6
Eubranchus rupium	C	0	0	0	0	0	0	0	0	0	1	0	1
Eubranchus rustyus	N	0	0	0	0	0	0	0	0	0	1	0	1
Eubranchus sp.	U	0	0	0	0	0	2	0	0	0	1	0	3
Hermissenda crassicornis	N	1	2	2	1	0	0	4	4	3	0	4	21
Onchidoris bilamellata	N	2	0	0	0	0	1	0	0	0	1	1	5
Onchidoris muricata	N	3	0	0	1	0	1	2	1	4	0	0	12
Onchidoris sp.	U	1	0	0	0	0	1	0	0	0	0	1	3
Trinchesia albocrusta	N	0	0	0	0	0	0	1	0	1	0	1	3
Phylum: Phaeophyceae													
Phaeophyceae	U	0	0	0	0	0	0	0	1	0	0	0	1
Phylum: Porifera													
Porifera sp. A	U	0	0	0	1	5	0	0	0	0	0	0	6
Porifera sp. B	U	0	0	0	0	1	0	0	0	0	0	0	1
Porifera sp. C	U	0	0	0	3	4	0	0	0	0	0	0	7
Phylum: Protozoa													
Protista sp. C	U	0	0	0	0	0	0	0	0	4	0	0	4

Taxon	Invasion Status	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat Harbor	Eshamy	Sheep Bay	Simpson Bay Oyster Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Protista sp. D	U	1	0	2	2	4	1	1	0	5	0	0	16
Phylum: Rhodophyta													
Rhodophyta	U	0	1	0	1	4	1	0	1	0	0	1	9

Appendix C: Taxa Identified in Survey by Site

Table C1: Species detected by genetic analyses in the 2023 PWS hard substrate survey by site. The number of plates per site with each species is shown, along with the total across all sites; genetic match indicates ≥ 98% to indicated taxon (yes) or 95-98% (no). Invasion status is shown for each species as Introduced (I), Native (N), Crytopgenic (C), or Unresolved (U).

Class	Taxon	Invasion Status	98% sequence match	AFK Hatchery	Cannery Creek Hatchery	Chenega Bay Marina	Cordova Ferry Terminal	Cordova Small Boat.Harbor	Eshamy	Sheep.Bay	Simpson Bay Oyster.Farm	Tatitlek Ferry Dock	Wally Noerenberg Hatchery	Windy Bay Kelp Farm	Total
Phylum Annelida															
Clitellata	Enchytraeidae	U	yes	1	0	0	0	0	0	0	0	0	0	0	1
Polychaeta	*Paleanotus bellis*	N	yes	0	0	0	0	0	0	0	2	0	0	0	2
Polychaeta	*Nereis vexillosa*	N	yes	3	2	4	3	0	1	0	3	0	0	0	16
Polychaeta	*Platynereis bicanaliculata*	N	yes	0	0	0	1	0	2	0	0	0	0	0	3
Polychaeta	*Halosydna brevisetosa*	N	yes	1	0	1	0	0	1	0	0	0	0	0	3
Polychaeta	*Harmothoe*	U	yes	0	0	0	0	0	0	0	0	0	0	1	1
Polychaeta	Syllidae	U	yes	5	0	0	2	2	0	0	0	2	3	1	15
Polychaeta	*Polydora onagawaensis*	C	yes	1	0	0	0	0	0	0	0	0	0	0	1
Polychaeta	*Terebellides stroemii*	N	yes	0	0	0	0	0	0	0	0	0	0	1	1
Polychaeta	*Capitella capitata*	C	no	0	1	0	0	2	0	0	0	0	0	0	3
Phylum Arthropoda															
Hexanauplia	*Paracalanus*	U	yes	0	0	0	0	0	0	1	0	0	0	0	1
Hexanauplia	*Euryte*	U	no	1	0	1	0	0	0	0	0	0	0	0	2
Hexanauplia	*Oithona similis*	N	no	0	1	0	2	1	0	0	0	1	0	0	5
Hexanauplia	*Ameira longipes*	C	yes	0	0	0	1	0	0	0	1	0	0	0	2
Hexanauplia	*Paradactylopodia*	U	yes	4	0	0	2	2	0	0	0	0	0	0	8
Hexanauplia	*Ectinosoma melaniceps*	C	yes	0	0	0	0	0	0	0	0	0	0	1	1
Hexanauplia	*Harpacticus*	U	yes	0	0	0	0	0	0	0	0	0	0	1	1
Hexanauplia	Laophontidae	U	yes	0	0	1	0	0	1	0	0	0	1	0	3
Hexanauplia	*Amonardia normani*	C	yes	4	0	0	5	1	0	0	0	0	0	0	10

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Hexanauplia	*Tisbe*	U	yes	0	0	0	0	0	0	0	0	1	0	0	1
Malacostraca	*Eogammarus confervicolus*	N	yes	0	0	0	0	0	0	0	0	0	1	0	1
Malacostraca	*Aoroides columbiae*	N	yes	0	0	0	1	0	0	0	0	0	0	0	1
Malacostraca	*Caprella laeviuscula*	N	no	0	1	0	0	0	0	0	0	0	0	0	1
Malacostraca	*Caprella mutica*	I	yes	0	0	0	0	0	0	0	0	2	0	0	2
Malacostraca	*Metacaprella kennerlyi*	N	yes	1	1	0	0	0	0	0	2	0	0	1	5
Malacostraca	*Jassa staudei*	N	yes	0	0	0	0	0	0	0	3	0	0	0	3
Malacostraca	*Microjassa*	U	yes	0	0	0	2	0	0	0	0	0	0	0	2
Malacostraca	*Gnathopleustes pachychaetus*	N	yes	3	5	0	0	0	4	0	0	0	0	0	12
Ostracoda	Podocopida	U	no	0	0	0	1	0	0	0	0	0	0	0	1
Ostracoda	Podocopida	U	yes	0	0	0	0	1	0	0	1	2	0	0	4
Thecostraca	*Balanus crenatus*	N	yes	1	0	0	4	5	4	0	0	0	0	1	15
Thecostraca	*Balanus glandula*	N	yes	0	2	0	1	2	2	0	0	0	2	0	9
Phylum Bryozoa															
Gymnolaemata	*Crisularia pacifica*	N	yes	1	0	4	1	5	0	0	0	0	0	0	11
Gymnolaemata	*Celleporella hyalina*	C	no	4	0	2	4	4	5	0	2	0	0	0	21
Gymnolaemata	*Celleporella hyalina*	C	yes	3	0	1	2	2	5	1	2	0	0	0	16
Gymnolaemata	*Membranipora serrilamella*	N	yes	0	0	0	0	0	0	0	0	1	0	0	1
Gymnolaemata	*Membranipora villosa*	N	yes	0	0	0	0	0	0	1	0	0	0	0	1
Gymnolaemata	*Schizoporella japonica*	I	yes	0	0	0	0	0	0	0	0	3	0	0	3
Gymnolaemata	*Alcyonidium*	U	yes	2	0	0	3	0	1	0	2	3	0	2	13
Phylum Cnidaria															
Anthozoa	*Metridium senile*	N	yes	0	0	0	1	0	0	0	0	0	0	0	1
Hydrozoa	*Bougainvillia superciliaris*	N	no	0	0	0	0	1	0	0	0	0	0	0	1
Hydrozoa	*Bougainvillia superciliaris*	N	yes	0	0	0	0	0	0	1	0	0	0	0	1

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Hydrozoa	*Gonothyraea loveni*	C	no	0	0	0	0	0	2	0	0	0	0	0	2
Hydrozoa	*Clytia gregaria*	N	yes	3	5	0	3	2	0	0	1	1	0	0	15
Hydrozoa	*Melicertum octocostatum*	N	yes	0	0	0	0	1	0	0	0	0	0	0	1
Hydrozoa	*Obelia dichotoma*	C	yes	0	4	1	3	1	5	0	0	0	1	1	16
Hydrozoa	*Tiaropsis multicirrata*	N	no	0	0	0	0	1	2	0	0	0	0	0	3
Scyphozoa	*Aurelia labiata*	N	yes	0	0	0	0	1	0	0	1	0	0	1	3
Phylum Mollusca															
Asteroidea	*Evasterias troschelii*	N	yes	0	0	0	0	0	1	2	3	3	0	0	9
Echinoidea	*Strongylocentrotus droebachiensis*	N	yes	0	0	0	0	0	0	0	0	0	0	1	1
Bivalvia	*Hiatella*	U	yes	4	5	4	3	5	5	3	2	2	3	1	37
Bivalvia	*Mytilus edulis*	N	yes	0	0	0	0	0	0	0	1	0	0	0	1
Bivalvia	*Mytilus trossulus*	N	yes	5	5	4	4	5	5	5	5	2	4	4	48
Bivalvia	*Ostrea lurida*	N	yes	0	0	0	1	0	0	0	0	0	0	0	1
Bivalvia	*Pododesmus macrochisma*	N	yes	0	0	0	0	0	0	0	0	0	0	1	1
Bivalvia	*Saxidomus gigantea*	N	yes	0	0	0	1	0	0	0	0	0	0	0	1
Gastropoda	*Crepipatella lingulata*	N	yes	1	0	0	2	0	0	0	0	0	0	4	7
Gastropoda	*Fusitriton*	U	yes	0	0	1	0	0	0	0	0	0	0	0	1
Gastropoda	*Coryphella trophina*	N	yes	0	2	0	2	0	0	0	0	0	0	0	4
Gastropoda	*Dendronotus*	U	yes	0	0	1	0	0	0	0	0	0	0	0	1
Gastropoda	*Eubranchus*	U	yes	0	2	0	0	0	0	0	0	0	0	0	2
Gastropoda	*Hermissenda crassicornis*	N	yes	1	0	0	0	0	0	2	1	1	0	0	5
Gastropoda	*Knoutsodonta jannae*	N	no	0	0	0	0	0	0	0	0	2	0	0	2
Gastropoda	*Onchidoris bilamellata*	N	yes	3	0	0	1	0	4	0	0	1	0	0	9
Gastropoda	*Onchidoris muricata*	N	yes	0	0	0	0	0	0	0	0	1	0	0	1
Gastropoda	*Trinchesia albocrusta*	N	yes	1	0	0	0	0	0	0	0	0	0	0	1

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Gastropoda	*Zelentia ninel*	C	yes	2	0	0	0	1	0	0	0	0	0	0	3
Gastropoda	*Calliostoma ligatum*	N	yes	0	0	1	0	0	0	0	0	0	0	0	1
Gastropoda	*Odostomia tenuisculpta*	N	yes	1	0	0	0	0	0	0	1	0	0	0	2
Gastropoda	*Odostomia tenuisculpta*	N	no	0	0	0	0	0	0	0	1	0	0	0	1
Polyplacophora	*Mopalia hindsii*	N	yes	1	0	0	0	0	0	0	0	0	0	0	1
Phylum Platyhelminthes															
Enopla	*Emplectonema viride*	N	yes	1	0	0	0	0	1	0	0	0	1	0	3
Enopla	*Paranemertes californica*	N	yes	0	0	0	0	0	0	0	0	0	3	0	3
Pilidiophora	*Maculaura cerebrosa*	N	yes	0	0	0	0	0	0	0	2	0	0	0	2
Rhabditophora	*Kaburakia excelsa*	N	yes	0	0	0	0	0	0	1	0	0	0	0	1
Rhabditophora	*Astrotorhynchus hakaiensis*	C	yes	1	1	0	2	0	0	0	0	0	0	0	4
Phylum Porifera															
Demospongiae	Halichondria panicea	N	yes	0	0	0	0	3	0	0	0	0	0	0	3