

**Spatial and temporal analysis of marine invasions in California, Part II:
Humboldt Bay, Marina del Rey, Port Hueneme, and San Francisco Bay**

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Chapter 1: Introduction

Overview

The Smithsonian Environmental Research Center (SERC) and Moss Landing Marine Laboratories (MLML) have advanced an extensive program with California Department of Fish and Wildlife (CDFW) to analyze spatial and temporal patterns of nonindigenous species (NIS) invasions in marine and estuarine waters of California, as required by law.

As specified by Section 71211 of the California Public Resources Code, CDFW is responsible for meeting the following requirements in the analysis of NIS in California's coastal waters:

1. Add to its inventory of NIS in open waters, bays, and estuaries, and monitor for new introductions or the spread of existing NIS.
2. Make such data and analysis available to the public using the Internet.
3. Assess the effectiveness of ballast water management in reducing the introduction and spread of NIS.

SERC and MLML have designed and implemented analyses to explicitly (a) assist CDFW in meeting statutory requirements by reporting status, trends, mechanisms, and rates of biological invasions in California waters and (b) test key questions about NIS in California, in order to understand invasion processes and assess strategies for NIS management and prevention.

Our approach combines a statistically robust sampling design, traditional taxonomic and biogeographic analyses, and broad-scale application of genetic tools to understand invasion dynamics in California. First, sampling is designed explicitly to make formal quantitative estimates for both NIS richness and abundance, allowing statistical comparisons across locations and over time for each measure. Second, DNA-based tools are utilized to assure consistent taxonomic assignment and detect cryptic species. The latter approach provides a critical tool and means of taxonomic quality control, providing the necessary groundwork (and baseline) for high-throughput, high-sensitivity, and cost-effective future analyses.

Our sampling design aims specifically to measure and test for spatial, temporal, and taxonomic patterns in NIS diversity (including species richness and abundance). We use a question-driven approach to inform and refine the sampling effort. We seek to evaluate the (a) differences in NIS diversity across habitats, geographic regions, and taxonomic groups and (b) changes in invasion dynamics (NIS detection rate and spread) over time associated with different transfer mechanisms (vectors) and changing management practices, including especially those for ships' ballast water and hull biofouling. We also seek to increase the capacity and sensitivity of detection for NIS, by coupling morphological and molecular analyses.

More specifically, our approach is designed to establish a robust, quantitative baseline and implement a time-series of repeated measures, which serve to assess current status and also to evaluate temporal

changes in invasion rate / dynamics associated with management of ballast water and other vectors (Ruiz & Hewitt 2002; Ruiz & Carlton 2003).

In 2012, we launched a multi-year campaign of field-based surveys and associated analyses to characterize NIS in California's coastal waters, as part of a long-term program (hereafter the Program):

- For the initial phase, we designed a 3-part study to focus primarily on bays and estuaries, because (a) these are the primary foci for introduction of NIS and (b) past studies have detected very few NIS along exposed outer coasts, outside of bays, estuaries, and harbors (Wasson *et al.* 2001; Ruiz *et al.* 2009; Zabin *et al.* 2018). Of those NIS present on the outer coast of California (including those reported from recent CDFW-funded surveys), all occur in bays and estuaries and were found at transition zones in close proximity to the mouths of bays and estuaries, suggesting some "spill-over" from estuaries that may not be self-sustaining.
- This initial phase lays the groundwork and establishes the baseline measures to evaluate spatial patterns of invasion in bays and estuaries throughout the state.
- In subsequent phases, continued sampling (repeated measures) in some bays will evaluate temporal changes in invasion dynamics in response to vector management. Additional measures (surveys) may also assess the extent to which NIS are spreading to outer coastal regions that are adjacent to bays.

Approach

In this initial phase, we are intensively sampling the invertebrate communities in 10 different estuaries in California. Within each estuary, we are sampling hard-substrate invertebrate communities, soft-sediment communities, and plankton assemblages. The estuaries include those with commercial ports (n=5) and those without commercial ports (n=5), which are distributed throughout the state, allowing us to directly compare (a) differences between the two types of estuary, (b) biogeography of NIS as well as native and cryptogenic species along the axis of the state, and (c) differences among habitat types, including hard-substrate, soft-sediment, and plankton.

For all estuaries, we sample habitats in high salinity (> 20 ppt) waters, which are present in all focal estuaries. In addition, for San Francisco Bay, we include survey sites in low salinity waters, allowing a test of differences across the salinity gradient for each habitat type. Finally, we include survey sites for at least one outer coastal region, which serves as a pilot project for future surveys across a broader number of outer coast sites in out-years, beyond the current project (*i.e.*, in subsequent phases of the program).

All surveys occur in summer through mid-fall, to control for possible seasonal differences. This time of year is selected to encompass the season of maximum plankton abundance and larval recruitment, in order to maximize species detection. Each of ten estuaries is surveyed once during a 5-year period, and one estuary (San Francisco Bay) is surveyed in each year.

For each habitat surveyed, we use a stratified sampling design, with replicate samples collected and analyzed to identify the taxonomic composition for each habitat and bay. Although not required as part of our current contract, we also characterize abundances of taxa in samples based on morphological examination. In addition, the following metadata are collected for each of the sites surveyed per estuary: GPS location (latitude and longitude), salinity, temperature, dissolved oxygen, sample date, and weather conditions.

The taxonomic composition of samples is characterized using both morphological and genetic methods for identification of biota. Using established protocols that we have developed over the past decade, we sort and collect voucher specimens for each morpho-species per habitat and bay during field analyses, placing these into individually labeled vials. These “morphological vouchers” are identified subsequently to species (or lowest taxonomic unit) based upon morphological characteristics. A subset of the identifications is verified through additional consultation with taxonomic experts.

Results from morphological analyses are compared to results from genetic analyses, to confirm taxonomic identification and test for cryptic species, using DNA barcoding. Where available, a minimum of five specimens from every newly identified species are collected as “molecular vouchers” from all habitats to be sequenced for mitochondrial cytochrome c oxidase subunit I (COI), which has been highly successful in detecting species-level differences. We augment the standard COI sequence with a second, nuclear locus, a fragment of the large subunit (LSU) ribosomal RNA gene.

Goals

The overall goals of the long-term Program are to:

- Measure status and trends of biological invasions in coastal marine ecosystems of California, using statistically robust sampling and DNA-assisted taxonomic analysis;
- Understand geographic distribution, habitat distribution, and patterns of spread for non-native marine and estuarine species in the state;
- Assess the mechanism(s) of introduction and spread of non-native species in California;
- Detect changes in the patterns (rate, spread, prevalence) of non-native marine and estuarine species in response to management strategies, shifts in vector dynamics, and other forcing functions.

Objectives

Through intensive field-based surveys, morphological and molecular analyses, and statistical data analyses, the specific objectives of this initial phase of the Program are to:

1. Efficiently characterize native and non-native components of coastal and estuarine waters of California so that analyses can be parsed at regional, biome, landscape, and habitat levels.
2. Test for differences in NIS diversity across different geographic and habitat scales (zones).
3. Estimate total NIS and native species diversity across estuaries.
4. Estimate the relative strength of different vectors to the invasion and spread of NIS in California.
5. Test efficacy (performance) of ballast water control methods by establishing robust baseline data for holoplankton and testing for new invasions.
6. Develop a DNA barcode library for NIS that further advances rapid, sensitive, and cost-effective detection methods for NIS.
7. Maintain and grow a publicly-accessible database incorporating past and concurrent CDFW data, other related data, on NIS in California that uses SERC's National Exotic Marine and Estuarine Species Information System (NEMESIS) framework (Fofonoff *et al.* 2017).

Structure of this Report

This is the second of three expected reports for the initial phase, focusing on sampling and analyses from four bays and estuaries in California: Humboldt Bay, Marina del Rey, Port Hueneme, and San Francisco Bay. This expands the analyses presented in our previous report (Ruiz and Geller 2015) -- or Part I of the three-part series that included San Diego Bay, Mission Bay, Morro Bay, San Francisco Bay, and Bodega/Tomales Bay. The next report will include Los Angeles/Long Beach Harbors and Newport Bay under contracts P1675034 (MLML) and P1675035 (SERC).

The results are presented in multiple chapters, which are organized in sections, as follows:

- Section I describes the details for each of the surveys and provides results from the morphological analyses conducted by SERC. These are organized by habitat components, including hard substrate (Chapter 2), soft-sediment (Chapter 3), and zooplankton (Chapter 4). For Chapters 2-4, we include data from each of the four bays, allowing comparisons among bays within community type.
- Section II provides the genetic analyses and results for benthic invertebrates and zooplankton conducted by MLML. Chapter 5 provides results of DNA barcoding of benthic invertebrates collected during the surveys described in Section 1. Chapter 6 reports on analysis of plankton communities across the four bays, using a metagenetic approach. Together, these two chapters demonstrate the application of genetic approaches to evaluate species detection and community composition in coastal waters. Section III provides a brief synthesis, summarizing

results to date and next steps for the integrated program of morphological and molecular analyses of NIS in California.

In addition to being reported here, the occurrence records for each NIS will be made accessible through CalNEMO, which was launched as a California-specific portal of NEMESIS (<https://invasions.si.edu/nemesis/calnemo/intro.html>). This website provides specific georeferenced location and date, both in tabular and mapped format, for each record. A separate electronic archive of the occurrence records by site for this specific study (report) will also be made available through CalNEMO.

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Section I: Morphological Detection and Analysis of NIS by Habitat

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Chapter 2: Hard Substrate Communities

Introduction

To detect the presence of non-native invertebrate taxa in hard substrate communities, we sampled four estuaries: Humboldt Bay, Marina del Rey, Port Hueneme, and San Francisco Bay. We sampled 8-10 sites per estuary, and the specific locations and dates are indicated in Appendix 2.1.

For each site, we deployed 10 PVC plates (14 x 14 cm), facing downward and 1 m below MLLW, for a minimum of 3 months. Plates were deployed using a randomized design within site. These plates served as passive collectors for recruitment of marine invertebrates. Upon retrieval, we randomly selected at least 5 plates per site for analysis of biota. In general, our goal was to analyze 50 plates per bay (10 sites x 5 plates). In Port Hueneme, we selected 6-7 plates for analysis at some sites to reach a total of 50 plates for that bay, because there were fewer suitable sites that were available there.

Upon retrieval, all sessile and mobile macroinvertebrates were collected and processed live to generate morphological vouchers for species-level identification on each plate. Molecular vouchers were also collected for each species (at least n=5 per bay, when available), and the molecular vouchers were sent to MLML for DNA barcoding.

For the four estuaries, we surveyed the hard substrate invertebrate community from high salinity waters on a total of approximately 200 plates (4 estuaries x 10 sites x 5 replicates). In addition, we sampled the high salinity portion of San Francisco Bay in identical fashion in 2014 and 2016 (10 sites x 5 replicates x 2 years = 100 additional plates), providing repeated measures for three consecutive years. Thus, our analyses include data from 300 plates across these estuaries from 2014-2016.

Results

A. Spatial Variation in High Salinity Hard Substrate Communities of Four Estuaries.

The analyses indicate that our sampling program performed well at detecting and characterizing the NIS in the target hard substrate communities of these estuaries, for each the sessile and the mobile marine macroinvertebrates. This performance is shown in a series of figures below that examine the detection of species in high salinity regions among and within the five estuaries using species accumulation curves and richness estimators, calculated using R package *vegan* 2.3-0 (R Core Team 2015; Oksanen *et al.* 2015). The species accumulation curves show rarefaction of species richness and the associated standard error with sampling effort. Species richness estimators provide statistical estimates of total richness present, based on accumulation curves, and are reported as Chao, Jack1, Jack 2, and Boot values along with standard errors (Canning-Clode *et al.*, 2008).

Total NIS Richness among Estuaries.

When we combine samples across all four estuaries, species accumulation for NIS approaches an asymptote (Figure 2.1). We observed (detected) 26 NIS for mobile invertebrates and 46 NIS for sessile invertebrates. The total estimated richness for these two groups was approximately 28 and 48 NIS, respectively (Table 2.1), indicating that our surveys detected between 90-96% of the estimated total pool of NIS present, depending upon which estimator is considered. In contrast, the species accumulation curves for native species are further from their asymptotes, suggesting we detected 56.85% of the estimated total native species pool (Table 2.1).

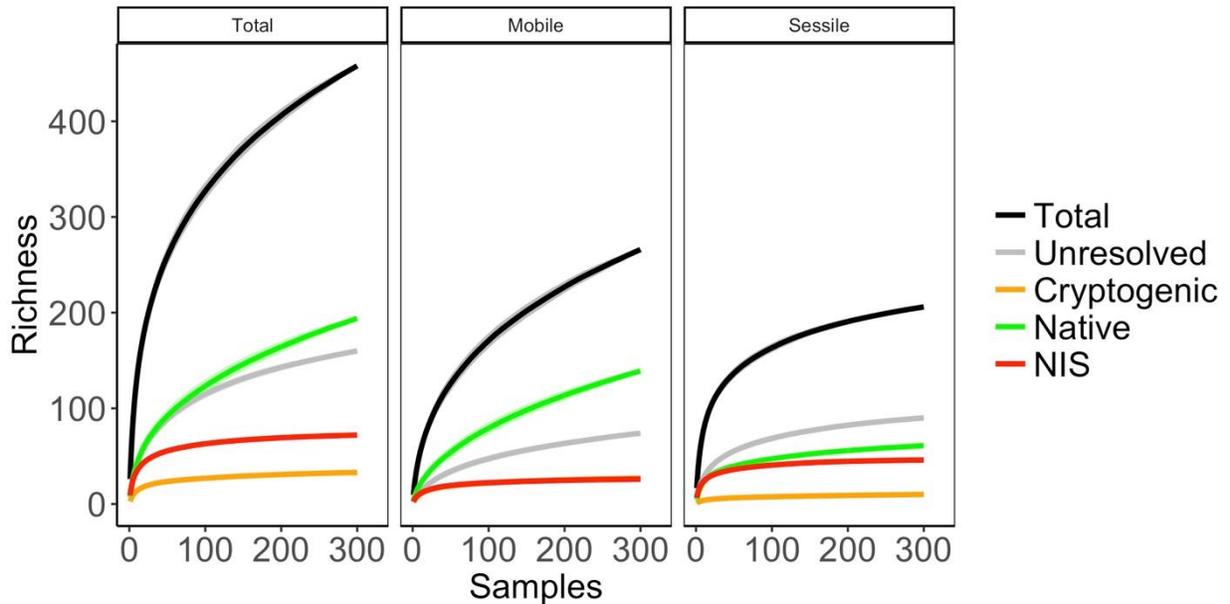


Figure 2.1. Species accumulation curves by invasion status for high salinity habitats across all four estuaries combined. Status is designated based on literature and SERC NEMESIS database. Samples (x-axis) represent the number of settlement plates examined across all four bays, including 100 plates in San Francisco and Humboldt Bay and 50 plates in Marina Del Rey and Port Hueneme (n=300 plates total across bays). All bays were sampled in 2015. NIS asymptotes agree strongly with species richness estimators (Table 2.1). Shading around each line represents 1 standard deviation (SD), which is often not visible at this scale due to low variation in estimates.

Table 2.1. Species richness estimators by invasion status for high salinity sites for all four estuaries (combined). Column 3 shows observed species richness by category followed by multiple estimators for total species richness, and associated standard errors (except for Jack 2), to show concordance across estimates.

Data	Status	Species	Chao	Chao SE	Jack1	Jack1 SE	Jack2	Boot	Boot SE	n
All	Cryptogenic	33	36.11	3.65	37.98	2.23	38.99	35.48	1.30	300
	Introduced	72	76.49	4.79	77.98	2.44	79.98	75.17	1.60	300
	Native	194	345.87	49.03	273.73	12.05	332.41	227.17	6.30	300
	Unresolved	160	213.60	21.78	203.85	7.33	229.74	179.48	3.98	300
	Total	458	651.24	45.76	592.55	15.80	680.12	516.30	8.42	300
Mobile	Cryptogenic	27	29.66	3.48	30.99	1.99	31.99	28.98	1.17	300
	Introduced	26	28.24	3.39	28.99	1.73	29.99	27.48	1.01	300
	Native	139	254.21	39.49	206.77	10.83	254.52	167.34	5.62	300
	Unresolved	74	126.39	27.82	102.90	5.90	123.79	86.13	3.09	300
	Total	266	429.33	44.70	369.65	13.53	440.29	309.93	7.02	300
Sessile	Cryptogenic	10	12.99	4.48	12.99	1.73	15.97	11.16	0.90	300
	Introduced	46	48.24	3.39	48.99	1.73	49.99	47.69	1.15	300
	Native	61	75.04	10.37	73.96	3.59	80.93	66.86	1.98	300
	Unresolved	90	103.46	8.29	107.94	4.23	113.94	98.68	2.46	300
	Total	206	240.11	14.77	242.88	6.22	259.83	223.39	3.61	300

Total NIS Richness within Individual Estuaries.

A similar pattern exists within the four individual estuaries: NIS detection approaches an asymptote rapidly compared to that for native and other taxa (Figure 2.2, Table 2.2). For mobile biota, we detected 7-21 NIS per estuary, and we detected 15.36 NIS per estuary for sessile invertebrates (Table 2.2). It is also noteworthy that for sessile taxa in San Francisco Bay, our observed NIS richness was 95.100% of total estimated NIS richness values.

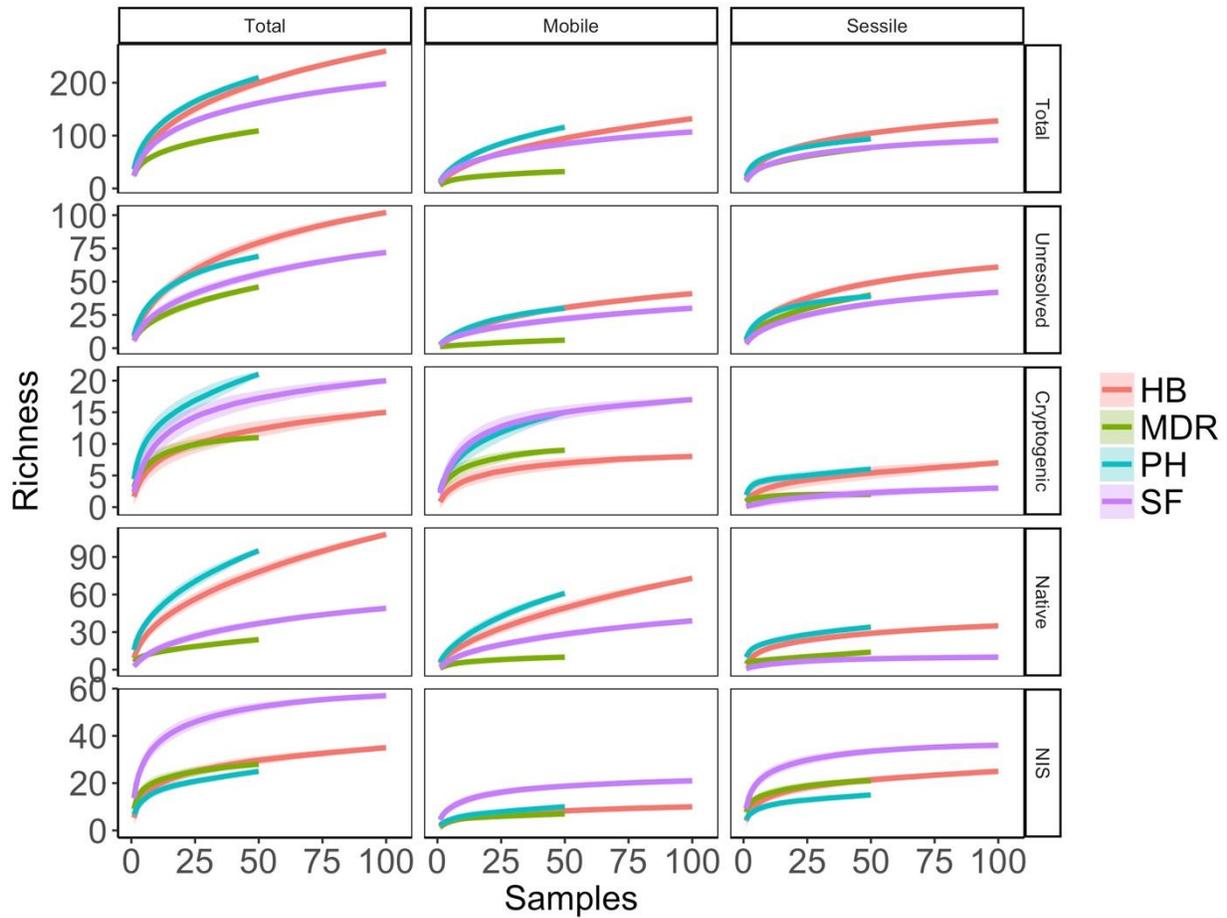


Figure 2.2. Species Accumulation curves separated by invasion status for high salinity portions of each bay sampled. Status is designated based on literature and SERC NEMESIS database. Samples (x-axis) represent the number of settlement plates examined among sites for each bay (≤ 10 plates/site), including 100 plates in San Francisco and Humboldt Bay and 50 plates in Marina Del Rey and Port Hueneme ($n=300$ plates total). NIS asymptotes agree strongly with species richness estimators (Table 2.2). Shading around each line represents 1 standard deviation (SD), which is often not visible at this scale due to low variation in estimates.

Table 2.2. Species richness estimators by invasion status for high salinity sites within four estuaries (separately). Column 3 shows observed species richness by category followed by multiple estimators for total species richness, and associated standard errors (except for Jack 2), to show concordance across estimates.

	Bay	Status	Species	Chao		Jack1		Jack2	Boot	Boot SE	n
				Chao	SE	Jack1	SE				
All	Humboldt Bay	Cryptogenic	15.00	18.96	5.24	18.96	1.98	20.94	16.79	1.14	100
		Native	108.00	177.91	27.50	156.51	9.15	188.04	128.58	4.68	100
		NIS	35.00	55.05	19.99	43.91	2.97	50.79	38.68	1.65	100
		Unresolved	102.00	140.15	17.50	135.66	7.56	154.43	117.04	3.94	100
	Marina del Rey	Cryptogenic	11.00	11.25	0.72	11.98	0.98	11.06	11.67	0.70	50
		Native	24.00	73.00	58.40	33.80	4.18	42.46	27.91	2.07	50
		NIS	28.00	29.96	2.60	31.92	2.41	32.00	30.03	1.63	50
		Unresolved	46.00	85.20	25.57	65.60	5.56	80.10	54.18	2.93	50
	Port Hueneme	Cryptogenic	21.00	45.01	30.49	27.86	2.59	33.64	23.82	1.37	50
		Native	95.00	155.31	26.53	134.20	9.96	160.37	111.66	5.17	50
		NIS	25.00	52.44	21.00	32.84	2.77	40.52	27.98	1.38	50
		Unresolved	69.00	91.11	13.64	87.62	5.48	98.34	77.49	2.94	50
San Francisco Bay	Cryptogenic	20.00	23.96	5.24	23.96	1.98	25.94	21.81	1.10	100	
	Native	49.00	69.05	12.63	66.82	4.65	76.70	57.00	2.57	100	
	NIS	57.00	59.06	2.48	61.95	2.21	61.03	59.89	1.58	100	
	Unresolved	72.00	91.01	10.16	95.76	5.24	104.73	83.05	3.05	100	
Mobile	Humboldt Bay	Cryptogenic	8.00	8.25	0.72	8.99	0.99	8.03	8.65	0.73	100
		Native	73.00	148.65	33.97	113.59	8.48	143.10	89.68	4.22	100
		NIS	10.00	12.97	4.45	12.97	1.71	15.91	11.18	0.87	100
		Unresolved	41.00	69.61	19.62	57.83	4.54	69.64	48.14	2.33	100
	Marina del Rey	Cryptogenic	9.00	9.25	0.72	9.98	0.98	9.06	9.67	0.70	50
		Native	10.00	14.41	7.06	12.94	1.70	14.88	11.31	0.95	50
		NIS	7.00	7.98	2.20	8.96	1.39	10.88	7.73	0.69	50
		Unresolved	6.00	10.41	7.06	8.94	2.20	10.88	7.27	1.16	50
	Port Hueneme	Cryptogenic	15.00	27.25	16.80	19.90	2.19	23.76	17.09	1.22	50
		Native	61.00	105.10	22.31	90.40	7.76	109.80	73.51	4.03	50
		NIS	10.00	15.88	7.00	13.92	1.96	17.76	11.47	0.96	50
		Unresolved	30.00	41.86	9.55	40.78	3.81	46.64	34.93	2.09	50
San Francisco Bay	Cryptogenic	17.00	21.46	7.12	19.97	1.71	21.94	18.31	0.94	100	
	Native	39.00	54.84	10.48	54.84	4.43	62.76	46.13	2.42	100	
	NIS	21.00	23.23	3.37	23.97	1.71	24.97	22.45	1.01	100	
	Unresolved	30.00	41.88	9.08	41.88	3.96	47.82	35.30	2.29	100	
Sessile	Humboldt Bay	Cryptogenic	7.00	9.97	4.40	9.97	1.71	12.91	8.15	0.89	100
		Native	35.00	40.28	4.89	42.92	3.13	44.94	38.90	1.77	100
		NIS	25.00	33.91	10.08	30.94	2.42	34.88	27.50	1.35	100
		Unresolved	61.00	75.31	9.11	77.83	4.32	84.79	68.90	2.46	100
	Marina del Rey	Cryptogenic	2.00	2.00	0.00	2.00	0.00	2.00	2.01	0.07	50
		Native	14.00	34.58	16.97	20.86	3.55	27.58	16.59	1.71	50
		NIS	21.00	21.49	1.02	22.96	1.97	21.12	22.30	1.31	50
		Unresolved	40.00	75.40	25.37	56.66	4.50	69.22	46.92	2.36	50
	Port Hueneme	Cryptogenic	6.00	6.98	2.18	7.96	1.39	9.88	6.73	0.68	50
		Native	34.00	50.33	14.56	43.80	3.40	50.58	38.15	1.71	50
		NIS	15.00	20.88	7.07	18.92	1.96	22.76	16.51	0.97	50
		Unresolved	39.00	49.45	10.08	46.84	3.11	51.70	42.56	1.79	50
San Francisco Bay	Cryptogenic	3.00	3.50	1.31	3.99	0.99	4.00	3.50	0.59	100	
	Native	10.00	10.99	2.25	11.98	1.40	13.94	10.87	0.78	100	
	NIS	36.00	36.50	1.02	37.98	1.40	36.06	37.45	1.11	100	
	Unresolved	42.00	49.92	5.98	53.88	3.43	56.91	47.75	2.03	100	

Percent Contribution of NIS to Total Species Richness per Estuary

NIS contributed 16.40% of total observed species richness per estuary for sessile invertebrates, and the percent contribution was highest in San Francisco Bay and Marina del Rey (Figure 2.3). For mobile invertebrates, NIS species richness accounted for 7-22% among estuaries, being greatest in the same two estuaries. When considering mobile and sessile invertebrates combined, NIS represented 12-29% of the total species richness, again with the highest levels in San Francisco Bay and Marina del Rey. The relative dominance of NIS in San Francisco Bay and Marina del Rey was driven by both high NIS richness and low native species richness in these estuaries compared to Humboldt Bay and Port Hueneme (see red and green respectively in Figure 2.3).

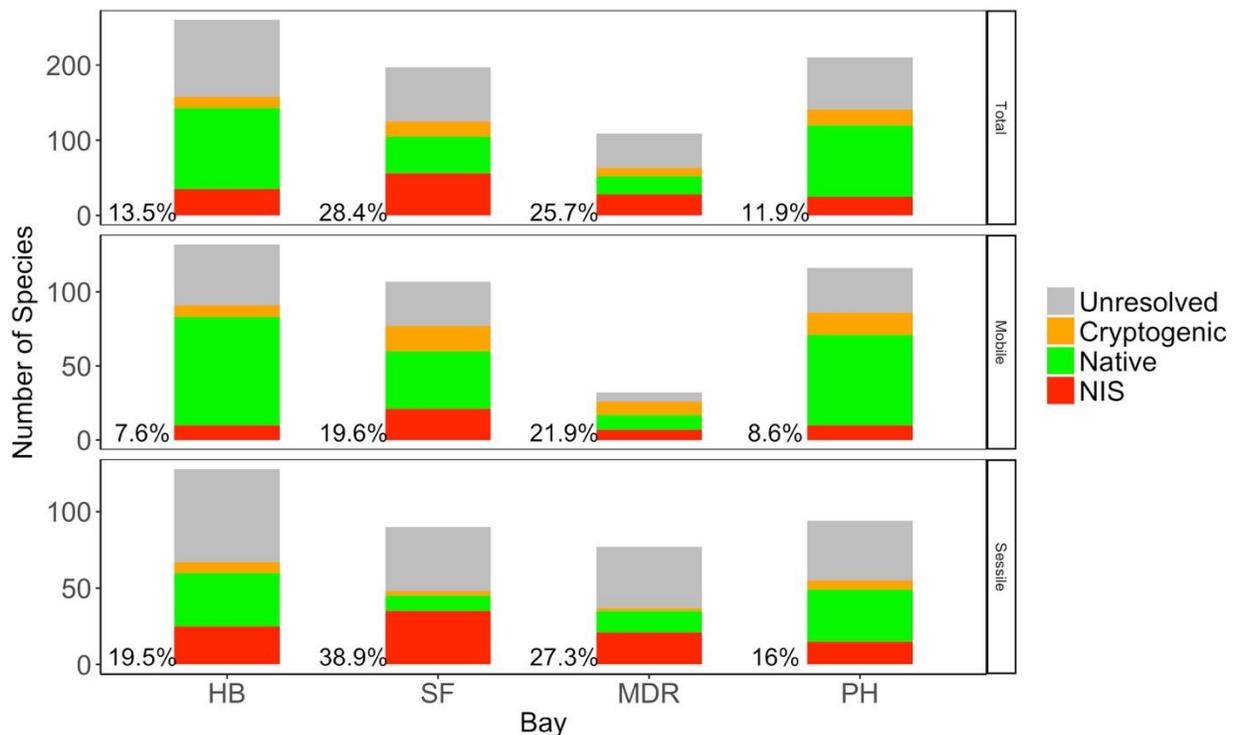


Figure 2.3. Number of unique species for each bay, functional type, and status. Status is designated based on literature and SERC NEMESIS database. Percentages to the left of each bar indicate the percentage contribution of NIS to total species richness per bay for each sessile invertebrate, mobile invertebrates, and all invertebrates combined.

NIS Richness per Plate within Bays.

The observed mean NIS richness varies >3-fold among sites across the four different estuaries when combining mobile and sessile taxa (Figure 2.4). This was driven largely by variation in sessile species, with sites in San Francisco Bay being at the highest end of the range. While this figure emphasizes

differences among sites, the elevated NIS richness per plate in San Francisco Bay was also observed at the bay level when comparing mean NIS richness for all plates within each of the four bays (Figure 2.5). Thus, overall, San Francisco Bay had the highest per-plate NIS richness within individual sites as well as for the entire bay. [An additional analysis of total NIS richness per site (instead of plate) is also presented in Appendix 2.3.]

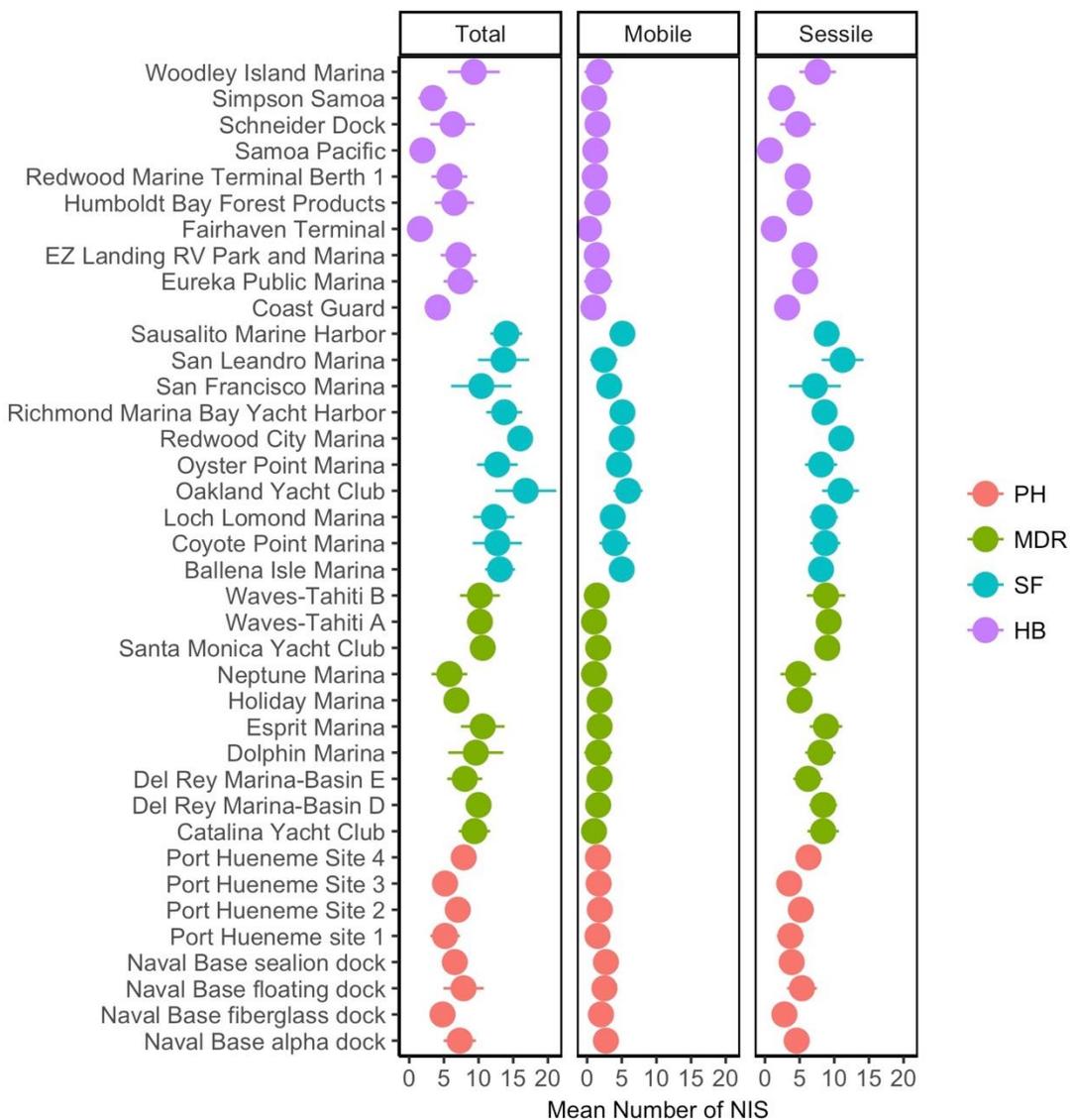


Figure 2.4. Mean number of NIS detected per plate within each site. Error bars equal $\pm 1SD$.

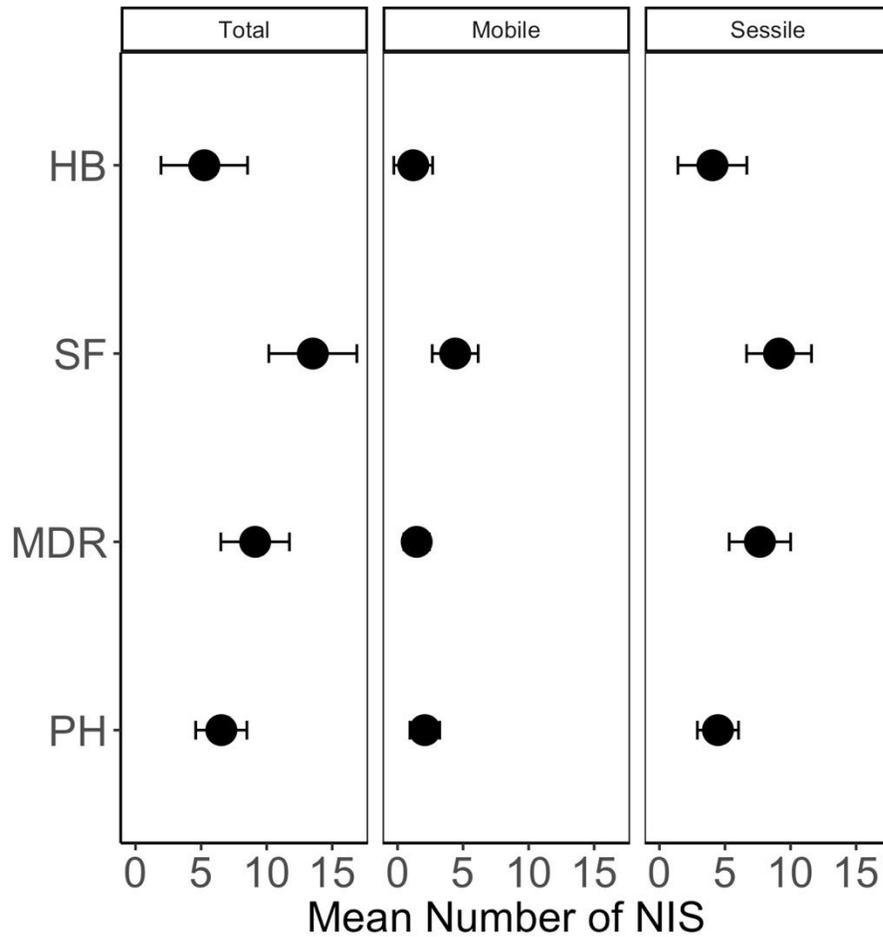


Figure 2.5. Mean number of NIS detected per plate within each bay. Error bars equal $\pm 1SD$.

Relative Abundance of Sessile NIS per Plate within Bays.

For sessile invertebrates alone, excluding mobile taxa, we estimated relative abundance using percent cover based on point count measurements. NIS occupied the highest percent cover in San Francisco Bay, followed by Marina del Rey (Figure 2.6), with median NIS cover exceeding 50% per plate in each bay. Moreover, San Francisco Bay had the lowest percent cover for native species. Thus, sessile invertebrate communities in San Francisco Bay differed from the other three bays in relative abundance of both native species and NIS. In comparison to NIS and native species, cryptogenic and unresolved taxa occupied a relatively small percentage of space in each bay.

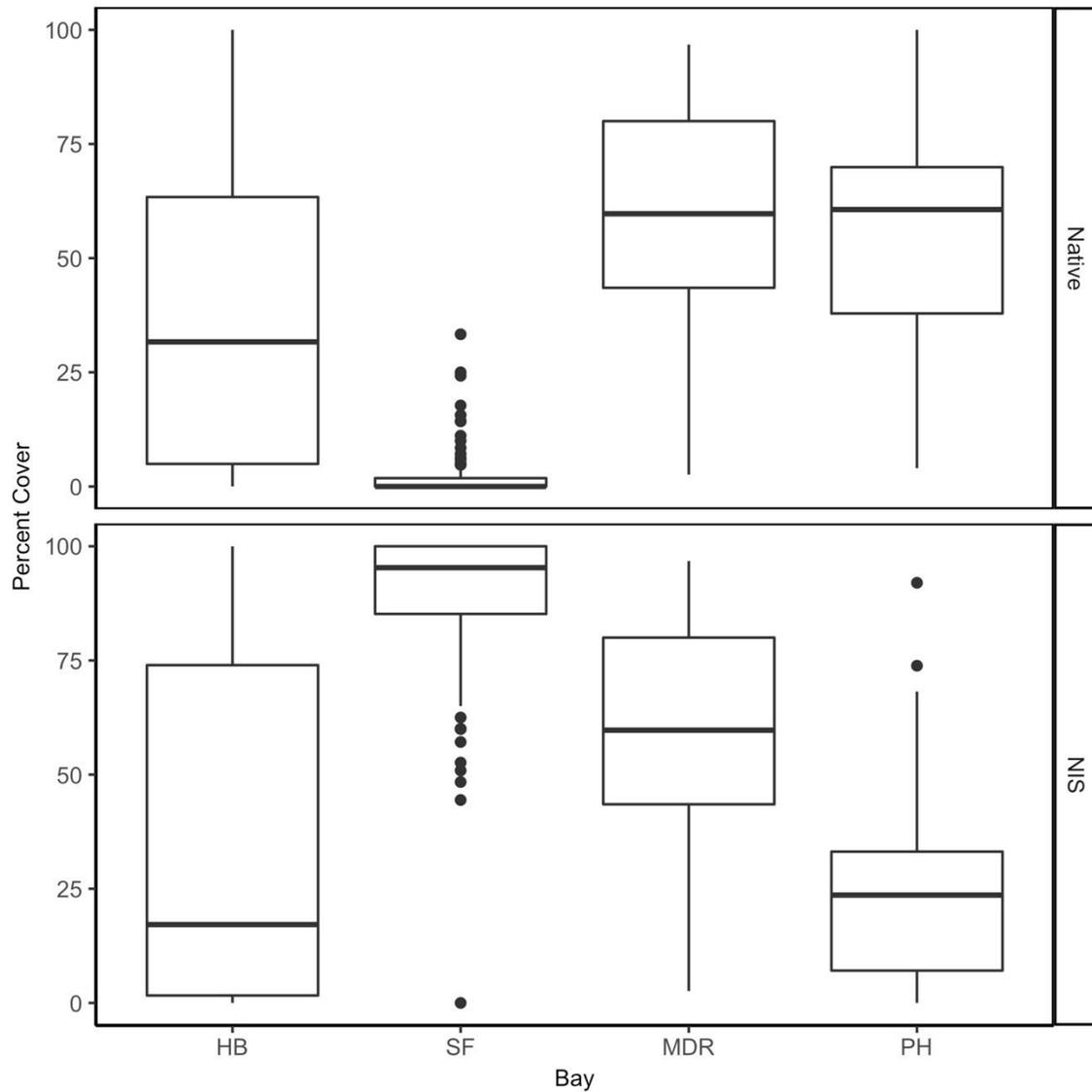


Figure 2.6. Measures of percent cover of native species (upper) and NIS (lower) on plates in each bay for sessile taxa only. Percent cover was calculated using point count measurements (50 points/plate and ≥ 50 plates per estuary), identifying primary (base) space occupation by sessile invertebrate species. Status as native or NIS is designated based on literature and SERC NEMESIS database. The lower and upper hinges of the box plots correspond to the first and third quartiles (the 25th and 75th percentiles); center line with boxes indicates medians, lines indicate 95% confidence limits, and points indicate samples that extended beyond these limits.

B. Temporal and Salinity Variation in San Francisco Bay: Sessile Invertebrates.

Total NIS Richness among Years at High Salinity Sites.

The results of repeated measures (surveys) among three years shows a high degree of consistency both in the number of NIS detected each year and also in reaching an asymptote, suggesting that these surveys are sampling a high percentage of the total species pool for this focal habitat in San Francisco Bay. Figure 2.7 compares the species accumulation curves for NIS in 2015 (also shown in previous section in Figure 2.2) to that in 2014 and 2016 in San Francisco Bay, using only high salinity sites surveyed with identical methods. Figure 2.8 shows the total number of NIS observed in each year and the percent contribution of NIS to total species richness.

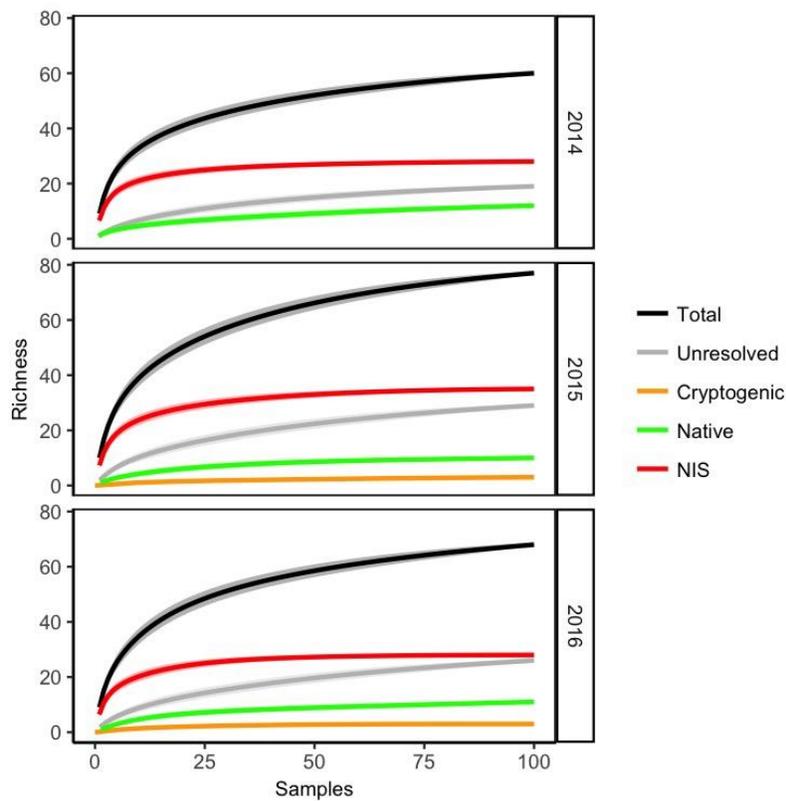


Figure 2.7. Species accumulation curves (sessile taxa only) by invasion status for high salinity sites in San Francisco Bay in three successive years. Status is designated based on literature and SERC NEMESIS database. Shading around each line represents 1SD.

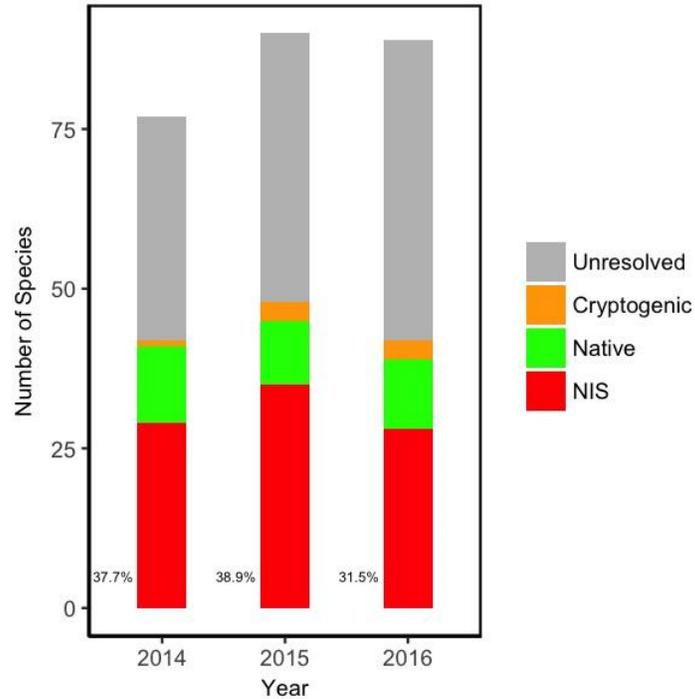


Figure 2.8. Number of unique species (sessile taxa only) detected in each year by invasion status for high salinity sites in San Francisco Bay in three different years. Status is designated based on literature and SERC NEMESIS data base. Percentages to the left of each bar indicate the percentage contribution of NIS to the total .

NIS Richness per Plate among Years.

While the surveys exhibited high consistency in performance and overall (cumulative) detection of NIS among years, there was also considerable variation among years and sites in the mean NIS richness observed per plate (Figure 2.9). We hypothesize that much of this variation was caused by environmental conditions, and especially differences in rainfall (and salinity in the winter and spring, prior to the summer surveys), following previous work (Chang *et al.* 2017). This result suggests that the probability of NIS detection may vary among years when controlling for sampling effort, including the number of sites and number of plates.

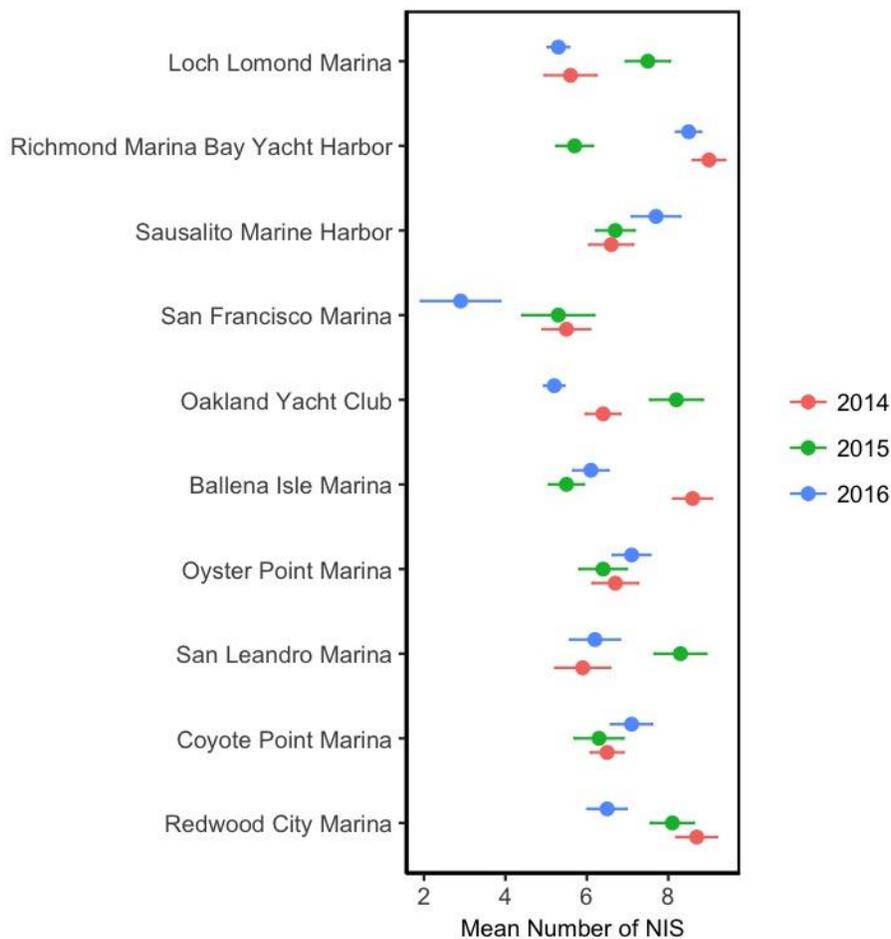


Figure 2.9. Mean number of NIS (sessile taxa only) detected per plate averaged within site per year in San Francisco Bay. This shows high salinity sites sampled over time. Error bars equal $\pm 1SD$.

Relative Abundance of NIS per Plate among Years and Sites.

The mean percent cover of NIS at high salinity sites exceeded 75% for all sites and years (Figure 2.10). Six of the sites showed considerable variation across years, including Richmond Marina Bay Yacht Harbor, Oakland Yacht Club, Ballena Isle Marina, and San Francisco Marina.

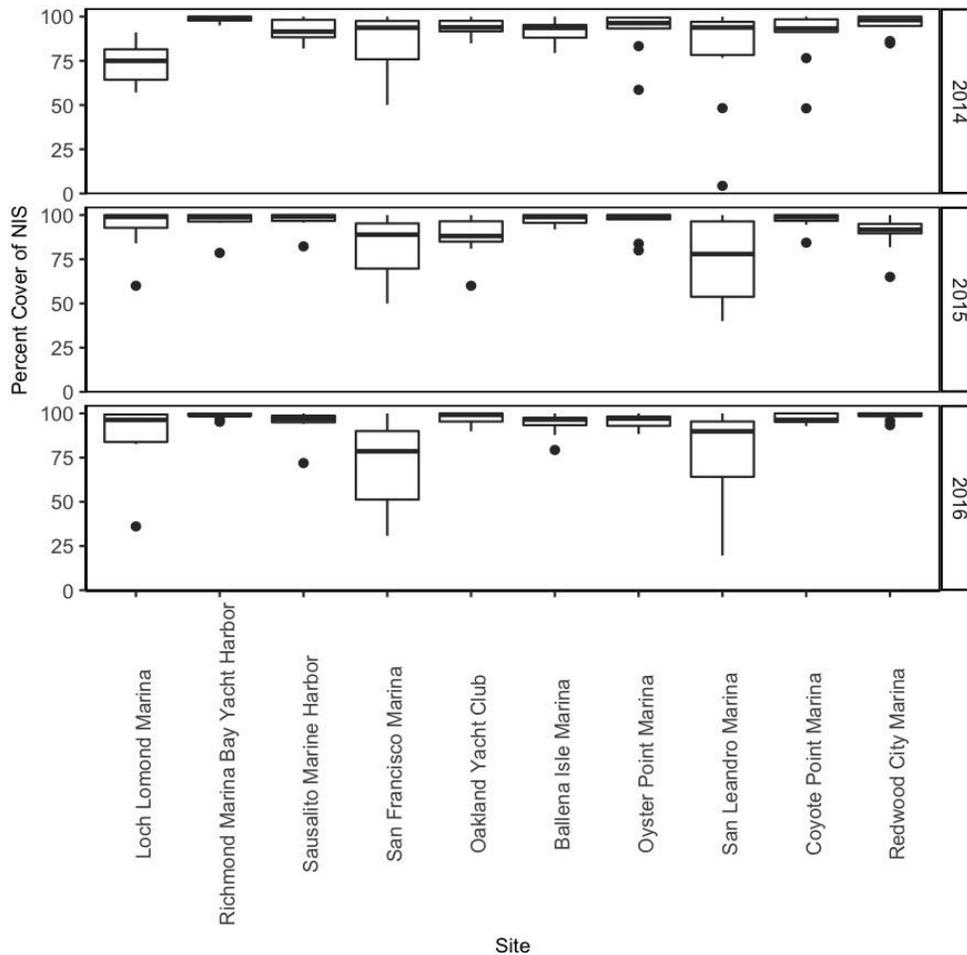


Figure 2.10. Measures of percent cover of NIS (sessile taxa only) on plates within sites in each year in San Francisco Bay, based on point count measurements. Approximately 50-point count measurements were made per plate, identifying sessile invertebrates to species. Status is designated based on literature and SERC NEMESIS database. The lower and upper hinges of the box plots correspond to the first and third quartiles (the 25th and 75th percentiles); center line with boxes indicates medians, lines indicate 95% confidence limits, and points indicate samples that extended beyond these limits.

C. Newly Detected NIS across Years (2014-2016) in the San Francisco Bay Hard Substrate Community.

For the hard substrate surveys, we detected ten NIS on panels for the first time in San Francisco Bay, using this repeated measure across years (Table 2.3). Four of these species were tunicates (*Microcosmus squamiger*, *Molgula ficus*, *Perophora japonica*, and *Styela canopus*), and the remaining –six were polychaetes. Two of the ten species have been previously reported in San Francisco Bay (but not on panel surveys), and others appear to be new records for San Francisco Bay. However, all these species were reported previously elsewhere in California (Fofonoff *et al.* 2017).

Table 2.3. Year of first detection in plate surveys for San Francisco Bay. Asterisks indicate species previously reported for San Francisco Bay, and all species were reported previously elsewhere in California.

<u>Species</u>	Year of First Detection for Surveys		
	<u>2014</u>	<u>2015</u>	<u>2016</u>
<i>Amblyosyllis speciosa</i> D	x		
<i>Branchiomma</i> sp. 2	x		
<i>Myrianida pentadentata</i>	x		
<i>Neoamphitrite</i> sp. A	x		
<i>Perophora japonica</i>	x		
<i>Microcosmus squamiger</i>		x	
<i>Molgula ficus</i> *		x	
<i>Myrianida pachycera</i>		x	
<i>Styela canopus</i>		x	
<i>Pseudopolydora paucibranchiata</i> *			x

Several of the new tunicate records for San Francisco Bay appear to be northward range expansions from southern California that coincide with unusually warm water, suggesting a change of environmental conditions may have contributed to colonization (Tracy *et al.*, 2017). Whether these populations will persist in San Francisco Bay, especially during normal (colder) years, is not yet clear.

While our repeated measures across years suggest new invasions may have occurred recently for San Francisco Bay, some of the newly detected polychaete species may simply reflect an increased search effort for this group, which has received relatively little scrutiny (for plate surveys) until the last four years.

D. Newly Detected NIS across Other Sampled Bays in the Hard Substrate Community.

Aside from San Francisco Bay, our surveys of Port Hueneme, Marina del Rey, and Humboldt Bay detected one new NIS: the bryozoan *Cradoscrupocellaria bertholletii*, which was found at two sites in Humboldt Bay in 2015 (Appendix 2.2), representing a first record for the Pacific coast of North America. *C. bertholletii* is widely reported from the Mediterranean. This paucity of new records, despite the extensive survey effort outlined in this report, is noteworthy and discussed further in Section III.

References

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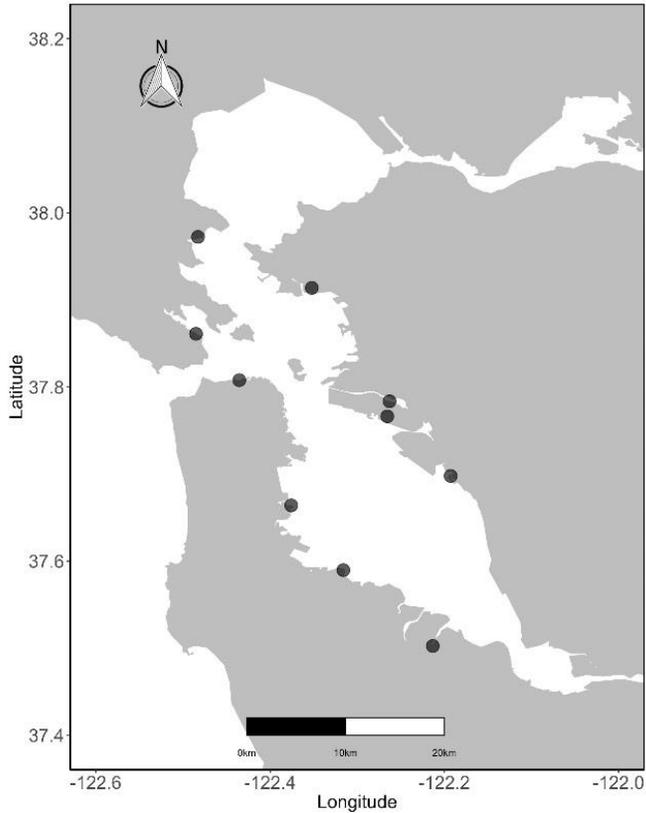
R Core Team. 2015. *R: A Language and Environment for Statistical Computing*. <https://www.R-project.org/>

Tracy B, Larson K, Ashton G, Lambert G, Chang AL, & Ruiz GM. (2017) Northward range expansion of three non-native ascidians on the west coast of North America. *BioInvasions Records*. DOI: 10.3391/bir.2017.6.3.04

Appendix 2.1: Survey Locations by Estuary and Year

The maps and tables below indicate locations and dates for hard substrate surveys for each estuary and year.

San Francisco Bay (2014-2016)



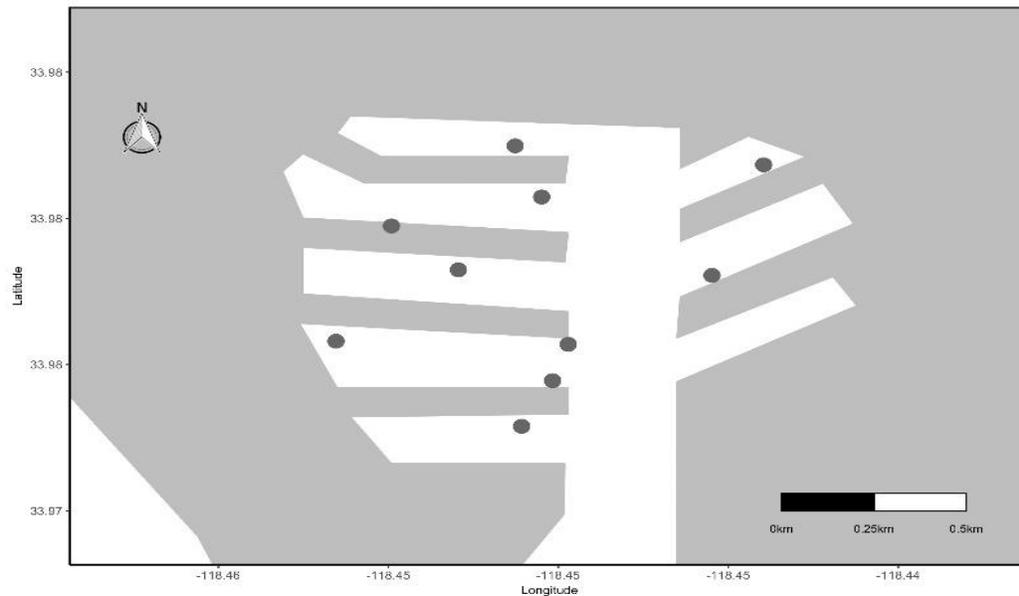
Site Name	Latitude	Longitude	2014 Deploy Date	2014 Retrieval Date	2015 Deploy Date	2015 Retrieval Date	2016 Deploy Date	2016 Retrieval Date
San Francisco Marina	37.80777	-122.43544	6/10/2014	9/8/2014	6/18/2015	9/15/2015	6/21/2016	9/7/2016
San Leandro Marina	37.69776	-122.19279	6/11/2014	9/9/2014	6/11/2015	9/16/2015	6/13/2016	9/14/2016
Sausalito Marine Harbor	37.86108	-122.48511	6/10/2014	9/19/2014	6/16/2015	9/25/2015	6/15/2016	9/23/2016
Coyote Point Marina	37.58952	-122.31601	6/12/2014	9/16/2014	6/18/2015	9/22/2015	6/20/2016	9/22/2016
Oyster Point Marina	37.66393	-122.37583	6/12/2014	9/17/2014	6/18/2015	9/23/2015	6/20/2016	9/21/2016
Redwood City Marina	37.50243	-122.2134	6/12/2014	9/11/2014	6/18/2015	9/21/2015	6/17/2016	9/26/2016
Ballena Isle Marina	37.7662	-122.26561	6/11/2014	9/12/2014	6/11/2015	9/17/2015	6/13/2016	9/13/2016
Richmond Marina Bay Yacht Harbor	37.91377	-122.3522	6/9/2014	9/10/2014	6/17/2015	9/14/2015	6/17/2016	9/12/2016
Loch Lomond Marina	37.97231	-122.48293	6/10/2014	9/15/2014	6/16/2015	9/18/2015	6/15/2016	9/8/2016
Oakland Yacht Club	37.78352	-122.263	6/19/2014	9/24/2014	6/19/2015	9/24/2015	6/17/2016	9/27/2016

Humboldt Bay 2015



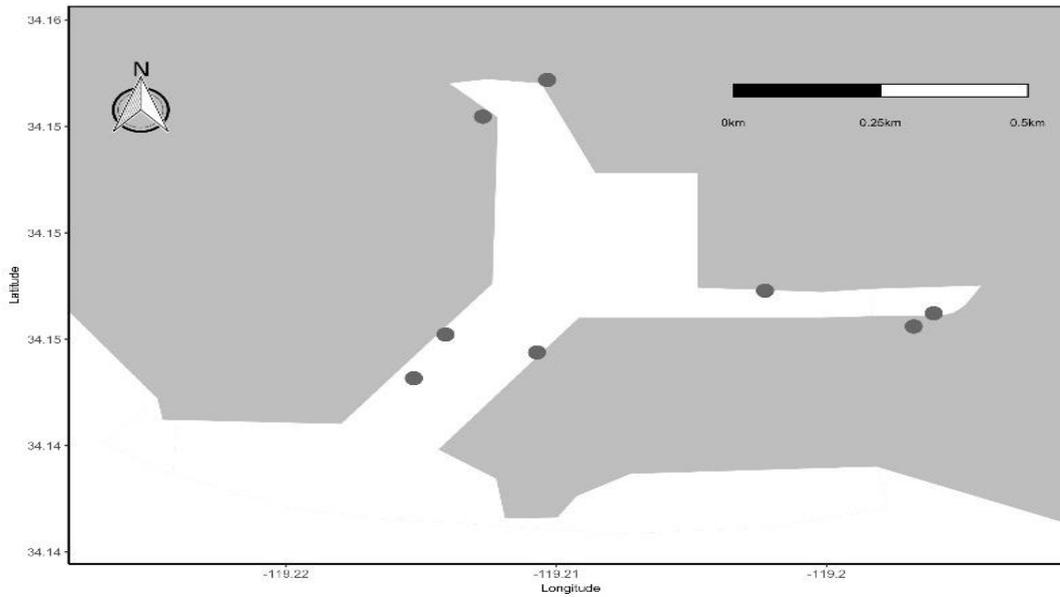
Site Name	Latitude	Longitude	Deploy Date	Retrieval Date
EZ Landing RV Park and Marina	40.73703	-124.21757	5/18/2015	8/17/2015
Coast Guard	40.76691	-124.2174	5/19/2015	8/11/2015
Fairhaven Terminal	40.78842	-124.19461	5/19/2015	8/18/2015
Samoa Pacific	40.79869	-124.19067	5/19/2015	8/19/2015
Redwood Marine Terminal Berth 1	40.81733	-124.18038	5/19/2015	8/12/2015
Simpson Samoa	40.80359	-124.18906	5/20/2015	8/13/2015
Woodley Island Marina	40.80784	-124.16101	5/20/2015	8/10/2015
Schneider Dock	40.77746	-124.1865	5/20/2015	8/14/2015
Humboldt Bay Forest Products	40.73262	-124.21914	5/21/2015	8/20/2015
Eureka Public Marina	40.8026	-124.17892	5/21/2015	8/15/2015

Marina del Rey 2015



Site Name	Latitude	Longitude	Deploy Date	Retrieval Date
Waves-Tahiti A	33.97289	-118.45108	4/29/2015	7/29/2015
Waves-Tahiti B	33.97445	-118.45017	4/29/2015	7/29/2015
Esprit Marina	33.97569	-118.44971	4/29/2015	7/27/2015
Neptune Marina	33.9758	-118.45654	4/28/2015	7/27/2015
Catalina Yacht Club	33.98183	-118.44396	4/28/2015	7/28/2015
Del Rey Marina-Basin D	33.98073	-118.45049	4/28/2015	7/31/2015
Del Rey Marina-Basin E	33.98248	-118.45127	4/28/2015	7/31/2015
Dolphin Marina	33.97824	-118.45294	4/26/2015	7/30/2015
Holiday Marina	33.97974	-118.45491	4/26/2015	8/1/2015
Santa Monica Yacht Club	33.97805	-118.44548	4/26/2015	7/28/2015

Port Hueneme 2015



Site Name	Latitude	Longitude	Deploy Date	Retrieval Date
Naval Base floating dock	34.15369	-119.20897	5/4/2015	7/25/2015
Naval Base fiberglass dock	34.14771	-119.21085	5/4//2015	7/23/2015
Naval Base alpha dock	34.15283	-119.21016	5/4//2015	7/23/2015
Naval Base sealion dock	34.146583	-119.212639	5/4//2015	7/24/2015
Port Hueneme site 1	34.14729	-119.20916	4/27/2015	7/22/2015
Port Hueneme Site 2	34.1479	-119.20219	4/27/2015	7/20/2015
Port Hueneme Site 3	34.14821	-119.20182	4/27/2015	7/22/2015
Port Hueneme Site 4	34.14874	-119.20494	4/29/2015	7/21/2015

Appendix 2.2 Taxa Identified Morphologically by Estuary and Year

Humbolt Bay

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS	Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
ANNELIDA											
Capitellidae											
Capitellidae	U			1							
Chrysopetalidae											
Chrysopetalidae	U	6	1			2	1	2	1	2	2
<i>Paleanotus bellis</i>	N	5	2	6	11	9	5	8	4	4	1
Fabriciidae											
Fabriciidae	U		1								
Nereididae											
<i>Alitta succinea</i>	I				2	1					1
Nereididae	U		1		2				1		
<i>Nereis latescens</i>	N										1
<i>Platynereis bicanaliculata</i>	N	10	9	10	9	9	8	10	7	10	10
Opheliidae											
<i>Armandia brevis</i>	N		1								
Orbiniidae											
<i>Naineris dendritica</i>	N										1
Phoronida											
<i>Phoronis</i> sp.	N		1	1							
Phyllodoceidae											
Eteoninae	U							1		1	
<i>Eulalia quadrioculata</i>	N	7	1	1	8	9	6	9	3	5	3
<i>Eulalia</i> sp.	U				2	1		1		1	
<i>Hypereteone lighti</i>	N					1					
<i>Mystides</i> sp.	N							1			
<i>Phyllodoce</i> sp.	N									1	
Phyllodoceidae	U	1						1	1	1	1
Polynoidae											
<i>Halosydna brevisetosa</i>	N	4	3		2	1		2	1	2	3
<i>Halosydna johnsoni</i>	N		1		2	1		2	1		
<i>Halosydna</i> sp.	N		1	1							
<i>Harmothoe imbricata</i>	C		1								3
Polynoidae	U	1				1		2			
Sabellariidae											
<i>Neosabellaria cementarium</i>	N								1		
<i>Sabellaria gracilis</i>	N		1						1		
Sabellariidae	U		1								

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS	Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
Sabellidae											
<i>Chone</i> sp.	N	1				1					
<i>Myxicola</i> sp. A	C		1								
Sabellidae	U	1			1	3					
<i>Schizobranchia insignis</i>	U	2							1		
Serpulidae											
<i>Crucigera zygophora</i>	N	1		3	1	3		1			
<i>Pseudochitinopoma occidentalis</i>	N	8	5	8	3	10			5	1	
Serpulidae	U		1	1		1		1	1		1
Spionidae											
<i>Dipolydora socialis</i>	C		2						1		
<i>Polydora narica</i>	N		1	1							
<i>Polydora</i> sp.	U			1	1						
Spionidae	U				1			1		1	
Spirorbidae											
<i>Neodexiospira pseudocorrugata</i>	C	2	8	4						1	
Spirorbidae	U	1									
Syllidae											
Eusyllinae	U				1				2		
<i>Eusyllis</i> sp.	U									1	
Exogoninae	U									1	
<i>Myrianida</i> sp.	U	1									
<i>Odontosyllis phosphorea</i>	N				2						
<i>Sphaerosyllis californiensis</i>	N		2	1				1			
Syllidae	U							2			
<i>Syllis elongata</i> complex	N							1			
<i>Typosyllis</i> sp.	U				1			1			
Terebellidae											
<i>Eupolytmia heterobranchia</i>	N			1							1
<i>Polycirrus</i> sp.	U		6	1	1		1		1	2	2
Terebellidae	U	1								1	
ARTHROPODA											
Amphipoda											
<i>Allorchestes rickeri</i>	N					1					
<i>Ampithoe lacertosa</i>	C							1			
<i>Ampithoe valida</i>	I							1		2	
<i>Anisogammarus pugettensis</i>	N			1							
Aoridae	U					1					
<i>Aoroides columbiae</i>	N	4			2						
<i>Aoroides inermis</i>	N	4	2	5	3	5	5	5	3	5	1

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS										
		Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
<i>Aoroides secunda</i>	I		1								
<i>Erichthonius brasiliensis</i>	C			1				1			
<i>Gnathopleustes serratus</i>	N									1	1
<i>Grandidierella japonica</i>	I		1					2			1
Hyalidae	U									1	
<i>Incisocalliope derzhavini</i>	I	1									
<i>Jassa slatteryi</i>	C	4	3		4	5	5	5	5	5	5
<i>Jassa</i> sp.	U	1			1						3
<i>Jassa staudei</i>	N	4	1		2					1	
<i>Laticorophium baconi</i>	N					1					
<i>Metopa cystella</i>	N								1		
<i>Microjassa</i> sp.	N					1					
<i>Monocorophium</i> <i>acherusicum</i>	I		4	5	1	3	2	3	5	4	5
<i>Monocorophium</i> <i>californianum</i>	N	1									
<i>Monocorophium insidiosum</i>	I	2	1	1		4	1	2			1
<i>Monocorophium</i> sp.	U				1						
<i>Paracorophium</i> sp.	I		1								
<i>Photis brevipes</i>	N	1	3	2	1	4		1	4		
<i>Photis</i> sp.	N							1			
<i>Podocerus brasiliensis</i>	C				3				4		
<i>Podocerus cristatus</i>	C	5	1		3	4			1		
<i>Pontogeneia rostrata</i>	N			2							
<i>Protohyale frequens</i>	N	2									
Caprellidae											
<i>Caprella alaskana</i>	N	1									
<i>Caprella californica</i>	N	3	4	5		5	4	2	5	3	4
<i>Caprella drepanochir</i>	I	2	4	3		3	4	4	4	2	4
<i>Caprella equilibra</i>	C					1	2	1	5	3	4
<i>Caprella ferrea</i>	N				1						
<i>Caprella laeviuscula</i>	I	1						1			
<i>Caprella mutica</i>	I	4	4	5		4	2	3	3	2	5
<i>Caprella</i> sp.	U	1				1		1			
<i>Caprella</i> sp. 11	U							1			
Caprellidae	U								1		
<i>Deutella californica</i>	N	1			3	5					
<i>Tritella pilimana</i>	N	1									
Cirripedia											
<i>Amphibalanus amphitrite</i>	I	1									
<i>Amphibalanus improvisus</i>	I								1		

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS										
		Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
Balanidae	U	2			1	1					
<i>Balanus crenatus</i>	N	9	10	7	11	9	8	11	7	10	6
<i>Balanus glandula</i>	N	9			1		1	3	2	8	4
<i>Balanus nubilis</i>	N	9			6	3	3	7	2	1	2
<i>Balanus</i> sp.	U	1		2	1		2	3	1		4
Cirripedia	U										1
<i>Megabalanus californicus</i>	N	2									
Decapoda											
<i>Hemigrapsus oregonensis</i>	N	1									
<i>Heptacarpus</i> sp.	N			1							
<i>Pachycheles pubescens</i>	N	1									
<i>Pachycheles rudis</i>	N				2	1				1	
<i>Pachycheles</i> sp.	N							1			
<i>Pugettia gracilis</i>	N		1								
<i>Pugettia richii</i>	N					1					
<i>Romaleon branneri</i>	N	2	1		1	1	2		1	1	
Isopoda											
<i>Ianiropsis</i> sp.	U					1			2		
<i>Idotea rufescens</i>	N							1			
<i>Idotea</i> sp.	N					1					
<i>Limnoria</i> sp.	U							1			
<i>Munna</i> sp.	U					1					
<i>Munna stephensi</i>	N	2									
Oniscidea	U								1		
<i>Paracerceis sculpta</i>	N			2			1		1		2
Pycnogonida											
<i>Achelia</i> sp.	N								1		
<i>Tanystylum occidentale</i>	N									1	
Tanaidae											
<i>Leptochelia</i> sp.	C			1						1	
<i>Zeuxo</i> sp.	U						1		1	5	4
BRYOZOA											
Bryozoa											
<i>Aetea pseudoanguina</i>	N					4					
<i>Alcyonidium</i> sp.	U	5	4	2	11	7	4	11	4	2	
<i>Amathia</i> sp. <i>bowerbankia</i>	U	8	10	10	7	9	1	4	4	6	
<i>Anguinella palmata</i>	I		1								
<i>Bugula neritina</i>	I	3	9	10	3	10	6	1	4	2	10
Bugulidae	U								1		

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS	Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
<i>Bugulina californica</i>	N		1								
<i>Bugulina longirostrata</i>	N		10		2	6			3	1	
<i>Bugulina stolonifera</i>	I		5	5	5	4	3		3		9
Candidae	U	1	2				1				
<i>Celleporaria brunnea</i>	N								1		
<i>Celleporaria</i> sp.	U								1		
<i>Celleporella hyalina</i>	C	1	10	5	4	7	7	8	4	8	5
<i>Conopeum</i> sp.	U					1	1		2	1	1
<i>Conopeum tenuissimum</i>	I		1		1		1	1	3	1	1
<i>Copidozoum adamantum</i>	N	1									
<i>Cradoscrupocellaria bertholletii</i>	U		2		1						
<i>Crisia occidentalis</i>	N	6	3		5	8	1	2	4		1
<i>Crisularia pacifica</i>	N	1	3	9		9					
<i>Cryptosula pallasiana</i>	I		5	4		2					7
Electridae	U								1		
<i>Fenestrulina delicia</i>	C		6	2		1					1
<i>Fenestrulina</i> sp.	U		3	1					2	1	1
<i>Filicrisia franciscana</i>	N	2	5			6			2		
<i>Filicrisia</i> sp.	U					1					
Hippothoidae	U						1				
<i>Licornia diegensis</i>	N	1	5	1	2	2	3	1	4	1	
<i>Microporella californica</i>	N		2	1	1	7	7		3	1	1
<i>Parasmittina collifera</i>	N		5		1	5	3		3	1	
<i>Parasmittina</i> sp.	U								1		
<i>Pomocellaria varians</i>	N		6	1							
<i>Rhynchozoon spicatum</i>	N								1		
<i>Schizoporella errata</i>	I		2								
<i>Schizoporella japonica</i>	I	1	9	10		4	1		2	1	2
<i>Schizoporella</i> sp.	U		1				2				1
<i>Scruparia ambigua</i>	C				1						
<i>Smittoidea prolifica</i>	N		2						1		2
<i>Tubulipora</i> sp.	U	2			1						
<i>Watersipora</i> sp.	I	3					3			3	
<i>Watersipora subtorquata</i> complex	I	3	10	10	1	10	8		8	3	10

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS	Coast Guard		Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
CNIDARIA												
Anthozoa												
Actinaria	U	2						2	1			
Anthozoa	U	1									1	
<i>Diadumene</i> sp.	U				1	1	2			1		
<i>Metridium</i> sp.	N	2				5	1		1			1
Hydrozoa												
Athecata	U		1			3		2	1		4	
Corynidae	U	2										
Hydrozoa	U									2		
Hydrozoa sp. A	U	11	3		8	10	10	11	11	4	5	9
Hydrozoa sp. C	U	1					1			2	1	
Hydrozoa sp. D	U											1
Plumularioidea	U	4	1				1	2		3	3	
Thecata	U		2				1				1	1
Tubulariidae	U								1			
ECHINODERMATA												
Asteroidea												
Asteroidea	U	1						1	1			
Echinodermata												
Echinodermata	U				1							
Echinoidea	U	1										
<i>Ophiopholis kennerlyi</i>	N	1										
<i>Ophiothrix spiculata</i>	N							2		2		
ENTOPROCTA												
Kamptozoa												
Kamptozoa	U		2							2		
MOLLUSCA												
Bivalvia												
Anomiidae	N						1					
Bivalvia	U	1						1		1		
Cardiidae	U	1										
Heterodonta	U	1				1			1	3		
<i>Hiatella arctica</i>	C	1										
<i>Modiolus modiolus</i>	N							1				
<i>Modiolus</i> sp.	N	3										
Mytilidae	U	7	5		1	9	7	6	9	3	5	6
<i>Mytilus californianus</i>	N					1					1	1
<i>Mytilus galloprovincialis/trossulus</i> complex	U		1			1					1	

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS											
		Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock		Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
<i>Mytilus</i> sp.	U	3	1	2	1		1		1		3	1
<i>Ostrea lurida</i>	N						1					
Ostreidae	U		1									
Pectinidae	U	2		1	2							
<i>Pododesmus</i> sp.	N				1	3			1			
Pteriomorpha	U	1	2		3				1			
Gastropoda												
<i>Alia carinata</i>	N										1	
<i>Alia gouldi</i>	N											
<i>Alia</i> sp.	N		1						1			
<i>Alia tuberosa</i>	N											1
<i>Alvania</i> sp.	N		1									
Columbellidae	U					1	1		2		1	
Gastropoda	U		1		1	3			1		1	
Nudibranchia												
<i>Acanthodoris rhodoceras</i>	N				3							
<i>Dendronotus</i> sp.	N								1			
<i>Dendronotus venustus</i>	N							3	1			
<i>Diaphorodoris lirulatocauda</i>	N		1			1						
Doridacea	U		1		1							
<i>Doto</i> form A of Goddard	N											1
<i>Doto</i> sp.	N											1
<i>Eubrancheus doriae</i>	N								1			
<i>Hermisenda</i> sp.	N	2	6		7	6	1	4	5		3	3
Nudibranchia	U	2				1	2	2			2	
<i>Onchidoris bilamellata</i>	N	1			1							
<i>Onchidoris muricata</i>	N				1							
<i>Polycera</i> sp.	N		1									
Opisthobranchia												
<i>Stiliger fuscovittatus</i>	N	1										
Polyplacophora												
Chitonida	N					1			1			
Ischnochitonidae	U								1			
<i>Mopalia hindsii</i>	N								1			
Polyplacophora	U					1			1			
NEMERTEA												
Nemertea												
Nemertea	U	2	1		1	1	1		1		1	2
<i>Tubulanus</i> sp.	N											

HUMBOLDT BAY Fouling Panel Species List	BAY SPECIES STATUS	Coast Guard	Eureka Public Marina	EZ Landing RV Park and Marina	Fairhaven Terminal	Humboldt Bay Forest Products	Redwood Marine Terminal Berth 1	Samoa Pacific	Schneider Dock	Simpson Samoa	Woodley Island Marina
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
PLATYHELMINTHES											
Platyhelminthes											
<i>Acanthozoon lepidum</i>	N							2	1		
<i>Acerotisa</i> sp.	U					1					
Leptoplanoidea	U							1			
Platyhelminthes	U	2	1	2	6	7	7	3	2	6	8
<i>Pleioplana inquieta</i>	N					1					
<i>Pseudoceros</i> sp.	N						1		1		1
PORIFERA											
Porifera											
Porifera	U			1							
Porifera sp. A	U	3	7	2	1	1		4	3		3
Porifera sp. B	U	1			1						
Porifera sp. C	U	2	7		5	10	1		1		
UROCHORDATA											
Tunicata											
<i>Aplidium</i> sp.	U	1				1		1			
Aplousobranchia	U	3	3	3	2		2	1	4	4	
Botryllinae	I	6	3	2	1	8	5	1	3	4	3
<i>Botrylloides</i> sp.	I		2	2					1		1
<i>Botrylloides violaceus</i>	I	2	5	8		2	8		5	2	10
<i>Botryllus schlosseri</i>	I			5		2			2		6
<i>Botryllus</i> sp.	I			1							
<i>Botryllus</i> sp. A	I								1		3
<i>Ciona savignyi</i>	I										4
<i>Ciona</i> sp.	I		4		2	2	5	3	2	4	2
<i>Corella inflata</i>	I	7									
<i>Corella</i> sp.	U		2								
Didemnidae	U	1	5	2	4	8	5	6	3	4	4
<i>Didemnum</i> cf. <i>vexillum</i>	I						1	2	1	2	2
<i>Diplosoma listerianum</i>	I	6	1		1	6	6		2	3	3
<i>Diplosoma</i> sp.	U		1		5	3		3	1		
<i>Distaplia occidentalis</i>	N	2	1	5		1	1	4	2		7
<i>Distaplia</i> sp.	U		4	3	7	8		5	4		1
<i>Molgula manhattensis</i>	I		1								1
<i>Perophora</i> sp.	U			1		1					
Phlebobranchia	U	1			2	1					
Stolidobranchia	U	5	4	3		1	2	1	2	5	2
<i>Styela clava</i>	I										1
<i>Styela</i> sp.	U		1	4	4	4		1	1		
Tunicata	U		2								

Marina del Rey

MARINA DEL REY Fouling Panel Species List	BAY SPECIES STATUS	Catalina Yacht Club	Del Rey Marina Basin D	Del Rey Marina Basin E	Dolphin Marina	Esprit Marina	Holiday Marina	Neptune Marina	Santa Monica Yacht Club	Waves Tahiti A	Waves Tahiti B
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
ANNELIDA											
Capitellidae											
<i>Capitella capitata</i> complex	C		1		1	2				1	
Cirratulidae											
<i>Cirriformia</i> sp.	N										1
Dorvilleidae											
<i>Schistomeringos longicornis</i>	N								3		
Eunicidae											
<i>Marphysa</i> sp.	C		1	1							
Nereididae											
<i>Neanthes acuminata</i> complex	C						1				1
Sabellidae											
<i>Parasabella</i> sp.	N	5	5	5	4	5	5	3	5	4	5
Serpulidae											
<i>Hydroides elegans</i>	I	4	5	5	2	1	3	1	4	4	4
<i>Hydroides gracilis</i>	N										1
<i>Hydroides</i> sp.	U	1	1		1		1				2
<i>Salmacina tribranchiata</i>	N	3	3	4	4		5	5	5	5	4
Spionidae											
<i>Boccardiella</i> sp.	U	1									
Spirorbidae											
Januini	U		1								
<i>Neodexiospira brasiliensis</i>	I				2						
<i>Neodexiospira pseudocorrugata</i>	C	3	3	4	3	2	4	4	5	3	4
<i>Pileolaria</i> sp.	N					1					
<i>Simplaria pseudomilitaris</i>	N	4	5	5	4	3	4	5	5	5	5
Syllidae											
<i>Megasyllis nipponica</i>	I				1						
<i>Myrianida pachycera</i>	I			1	1	1	1		1		
<i>Salvatoria californiensis</i>	N									1	
Syllidae											
<i>Syllis gracilis</i> complex	C					1			1	1	
ARTHROPODA											
Amphipoda											
<i>Aoroides secunda</i>	I	4	4	3	3	2	2	1	3	1	3

MARINA DEL REY Fouling Panel Species List	BAY SPECIES STATUS	Catalina Yacht Club	Del Rey Marina Basin D	Del Rey Marina Basin E	Dolphin Marina	Esprit Marina	Holiday Marina	Neptune Marina	Santa Monica Yacht Club	Waves Tahiti A	Waves Tahiti B
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
<i>Elasmopus bampo</i>	C	4	5	5	5	5	5	5	5	5	4
<i>Erichthonius brasiliensis</i>	C	3	5	2	4	5		4	5	5	4
<i>Laticorophium baconi</i>	N		2	1	1	3	1		2	3	4
<i>Leucothoe alata</i>	C				1	2			2	2	1
<i>Monocorophium acherusicum</i>	I				1	3	1	2		2	1
<i>Paradexamine</i> sp. 1	I				1						
<i>Podocerus brasiliensis</i>	C					4		1		4	2
<i>Podocerus cristatus</i>	C		1	1	1	1	2			2	2
<i>Stenothoe valida</i>	I		1	4		3	3	1		2	1
Caprellidae											
<i>Caprella equilibra</i>	C					1					
Cirripedia											
<i>Amphibalanus</i> sp.	U						1				
Isopoda											
<i>Paracerceis sculpta</i>	N				1						
<i>Paranthurus japonicus</i>	I	1	3		2		2	1	4		2
Tanaidae											
<i>Zeuxo</i> sp.	U	5	3	4	4	4	4	4	5	5	4
BRYOZOA											
Bryozoa											
<i>Bugula neritina</i>	I	2	3		3	3	2	1	2	5	5
Bugulidae	U					1					
<i>Bugulina stolonifera</i>	I	5	4	1	1	1	1	3	5	3	4
<i>Celleporaria brunnea</i>	N	5	5	4	3	5	4	5	5	4	5
<i>Crisia</i> sp. A	U					1			1		
<i>Crisulipora occidentalis</i>	N	5	3	2	4	5		3	5	4	3
<i>Cryptosula pallasiana</i>	I	4	3	5		4	3	1	4	4	4
Cyclostomatida	U						1			1	1
<i>Diaperoforma californica</i>	N							1			
<i>Disporella</i> sp.	N									1	
<i>Thalamoporella californica</i>	N	1	1		1	5	3	2	1	5	3
<i>Tubulipora</i> sp.	U									1	
<i>Watersipora</i> sp.	I				1	1					
<i>Watersipora subtorquata</i> complex	I	5	5	3	4	5	3	4	3	5	4

MARINA DEL REY Fouling Panel Species List	BAY SPECIES STATUS	Catalina Yacht Club	Del Rey Marina Basin D	Del Rey Marina Basin E	Dolphin Marina	Esprit Marina	Holiday Marina	Neptune Marina	Santa Monica Yacht Club	Waves Tahiti A	Waves Tahiti B
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
<i>Watersipora subtorquata</i> sensu Vieira et al. 2014	I	1		1			2		1		
CNIDARIA											
Anthozoa											
Actinaria	U		2	1	1			2	2		1
Anthozoa	U				1						
<i>Diadumene franciscana</i>	I					1					
<i>Diadumene leucolena</i>	I					1					
<i>Diadumene lineata</i>	I			1	1		3	1			
<i>Diadumene</i> sp.	U			2	2	1	3	2	1		2
Hydrozoa											
Athecata	U	1				1	2	1			
Hydrozoa	U								1	1	
Hydrozoa sp. A	U					1					
Hydrozoa sp. D	U	2	4	4	1	1	6	2	2	3	5
Plumularioidea	U					1					
Tubulariidae	U		3	4		4	1	2		4	3
ENTOPROCTA											
Kamptozoa											
Kamptozoa	U	1									
MOLLUSCA											
Bivalvia											
Mytilidae	U					1					
<i>Mytilus californianus</i>	N				1				1	1	
<i>Mytilus galloprovincialis/trossulus</i> complex	U					3					1
<i>Mytilus</i> sp.	U	1	2							1	
<i>Ostrea lurida</i>	N										1
Nudibranchia											
<i>Polycera atra</i>	N	1								2	
<i>Tenellia adspersa</i>	I			1							
NEMERTEA											
Nemertea											
Nemertea	U					1				1	
Palaeonemertea	U					1					

MARINA DEL REY Fouling Panel Species List	BAY SPECIES STATUS	Catalina Yacht Club	Del Rey Marina Basin D	Del Rey Marina Basin E	Dolphin Marina	Esprit Marina	Holiday Marina	Neptune Marina	Santa Monica Yacht Club	Waves Tahiti A	Waves Tahiti B
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
PLATYHELMINTHES											
Platyhelminthes											
<i>Acerotisa californica</i>	N				2	1	1	3	1	2	2
<i>Eurylepta aurantiaca</i>	N		2		1	2	2			4	1
<i>Hoploplana californica</i>	N	2									
<i>Notocomplana acticola</i>	N		1			1	2	1		3	2
Platyhelminthes	U				1	1					1
<i>Pseudoceros</i> sp.	N				1						
PORIFERA											
Porifera											
Porifera sp. A	U	3	3	4	1	3	5	2	3	1	3
Porifera sp. B	U						1				
Porifera sp. C	U							1	1		
Sycon sp.	U								1		
UROCHORDATA											
Tunicata											
<i>Aplidium</i> sp.	U	1	2		3			2	3	4	3
<i>Aplidium</i> sp. A	U									2	
<i>Aplousobranchia</i>	U	1	1		1	1		2	2		1
<i>Ascidia</i> sp.	U				1						
<i>Ascidia zara</i>	I	1			1						
Asciidiidae	U									1	
Botryllinae	I	4	1	1	5	4	1	2	4	2	2
<i>Botrylloides diegensis</i>	I					1					1
<i>Botrylloides giganteum</i>	I					2				1	
<i>Botrylloides</i> sp.	I		1	1	1				2	1	2
<i>Botrylloides violaceus</i>	I	1			2	1			1	1	
<i>Botryllus schlosseri</i>	I	5	5	3	5	5	2	2	4	5	5
<i>Ciona robusta</i>	I	3	4	3	4	4	1	3	5	5	4
<i>Ciona</i> sp.	U	4	1	3	2	2	1	3	3	1	1
<i>Diplosoma listerianum</i>	I	3	4	4	5	5		4	5	5	5
<i>Diplosoma</i> sp.	U	2			2						
<i>Distaplia occidentalis</i>	N									1	
<i>Distaplia</i> sp.	U									1	

MARINA DEL REY Fouling Panel Species List	BAY SPECIES STATUS	Catalina Yacht Club		Del Rey Marina Basin D		Del Rey Marina Basin E		Dolphin Marina		Esprit Marina		Holiday Marina		Neptune Marina		Santa Monica Yacht Club		Waves Tahiti A		Waves Tahiti B	
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	
<i>Microcosmus</i> sp.	U																		1		
<i>Molgula</i> sp.	U																1				
<i>Perophora</i> sp.	U	2	1														3				
<i>Polyandrocarpa zorritensis</i>	I	4	5	4	2	1	5	1	5	1	5	1	5	1	5	1	5	1	1	1	1
Stolidobranchia	U	2			1				1										1		
<i>Styela plicata</i>	I	1	2		1	4		1	4	1		1		1		4		4	3	3	3
<i>Styela</i> sp.	U		2	2	2	2		2	2	3		3		3		3		3	3	3	3
Styelidae	U			1	1			1						1		1		1	2	2	2
Tunicata	U	1			2				2												

Port Hueneme

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpha Dock		Naval Base Fiberglass Dock		Naval Base Floating Dock		Naval Base Sealion Dock		Port Hueneme Site 1		Port Hueneme Site 2		Port Hueneme Site 3		Port Hueneme Site 4	
		2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015
ANNELIDA																	
Chrysopetalidae																	
Chrysopetalidae	U			1													1
<i>Paleanotus bellis</i>	N	5	4	5	2	3	2	3	2	3	5	3	5	3	5	3	5
Cirratulidae																	
Cirratulidae	U							1									
Nereididae																	
<i>Platynereis bicanaliculata</i>	N	1		3	1	3	1	3	1	2	1	2	1	2	1	2	1
Opheliidae																	
<i>Polyophthalmus</i> sp.	N				1												
Phyllococidae																	
<i>Eulalia quadrioculata</i>	N							1				1					
Phyllococidae	U							1									
<i>Pterocirrus burtoni</i>	N		1														

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Hueneme Site 1	Port Hueneme Site 2	Port Hueneme Site 3	Port Hueneme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
Polynoidae									
<i>Halosydna brevisetosa</i>	N		1	1					1
<i>Halosydna johnsoni</i>	N			1					
Sabellariidae									
<i>Neosabellaria cementarium</i>	N		1						
Sabellidae									
<i>Paradialychone ecaudata</i>	N	2	2	1	3	2	3	3	1
<i>Parasabella</i> sp.	N							1	
Serpulidae									
<i>Hydroides gracilis</i>	N	6		6	5	5	5	6	6
<i>Hydroides</i> sp.	U		2						
<i>Pseudochitinopoma occidentalis</i>	N	5			1		5	6	6
<i>Salmacina tribranchiata</i>	N						2		
Spionidae									
<i>Boccardiella</i> sp.	U				1				
<i>Polydora narica</i>	N	1							
Spirorbidae									
<i>Neodexiospira pseudocorrugata</i>	C	4		4	7		6	6	5
<i>Pileolaria berkeleyana</i>	N	2			2				
<i>Pileolaria</i> sp.	N			1					
<i>Protolaespira eximia</i>	N	7	3	3	1	4	5	4	3
<i>Simplaria pseudomilitaris</i>	N	4	3	4	7	5	5	6	5
Spirorbidae	U				2				
Syllidae									
<i>Amblyosyllis speciosa</i> D	I				1				
Eusyllinae	U		2			1	1	1	1
<i>Eusyllis</i> sp.	U	1		1		1			1
<i>Eusyllis</i> sp. 1	U	1			2			1	
<i>Exogone lourei</i>	C		1		1				1
Exogoninae	U	2				1			
<i>Megasyllis nipponica</i>	I	2		3	2				
<i>Myrianida</i> sp.	U	1							
<i>Odontosyllis parva</i>	N					1			
<i>Odontosyllis phosphorea</i>	N		1					1	

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Hueneme Site 1	Port Hueneme Site 2	Port Hueneme Site 3	Port Hueneme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
<i>Salvatoria californiensis</i>	N		1			1			
Syllidae	U							2	
<i>Syllis gracilis</i> complex	C						1		2
<i>Typosyllis</i> sp.	U		1	1			1	1	1
Terebellidae									
<i>Eupolymnia</i> sp.	N			1		2			
<i>Nicolea</i> cf. <i>amnis</i>	U								1
ARTHROPODA									
Amphipoda									
<i>Ampithoe lacertosa</i>	C			1					
<i>Ampithoe</i> sp.	U					1			
<i>Aoroides secunda</i>	I	4	2	5	6	3	3	3	2
<i>Coboldus hedgpethi</i>	N		1						
<i>Elasmopus bampo</i>	C						1		
<i>Elasmopus</i> sp.	U						2	1	
<i>Erichthonius brasiliensis</i>	C	2	5	4	2	5	3	5	6
<i>Gammaropsis shoemakeri</i>	N			5					
<i>Gammaropsis</i> sp.	N								2
<i>Grandidierella japonica</i>	I								1
<i>Houstonius vilordes</i>	N		1			1		2	
<i>Incisocalliope derzhavini</i>	I		1						
Ischyroceridae	U		1						
<i>Jassa</i> cf. <i>pusilla</i>	N				1				
<i>Jassa slatteryi</i>	C	7	5	6	5	6	6	6	3
<i>Jassa</i> sp.	U		1		2	3	1		4
<i>Jassa</i> sp. 3	C				1	1			1
<i>Jassa staudei</i>	N						1		
<i>Laticorophium baconi</i>	N	7	4	4	7	4	6	6	7
<i>Metopa cystella</i>	N	1		2		1	1	1	
<i>Microjassa</i> sp.	N		1			1			
<i>Monocorophium acherusicum</i>	I	3	2		2		1	1	
<i>Paradexamine</i> sp.	I				3				
<i>Paradexamine</i> sp. 1	I		1		1		1		
<i>Peramphithoe tea</i>	N						1		

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Huenueme Site 1	Port Huenueme Site 2	Port Huenueme Site 3	Port Huenueme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
<i>Photis brevipes</i>	N		4			2	1	1	2
<i>Podocerus brasiliensis</i>	C		4			6		1	
<i>Podocerus cristatus</i>	C		4					1	
<i>Podocerus</i> sp.	C								1
<i>Protohyale frequens</i>	N					1			
<i>Stenothoe valida</i>	I		2	1			1		
Caprellidae									
<i>Caprella californica</i>	N	7	5	6	3	5	5	5	7
<i>Caprella equilibra</i>	C			6	1		1		
<i>Caprella mutica</i>	I	3		1	2	1			1
<i>Caprella natalensis</i>	C			1			1		
<i>Caprella penantis</i>	C	1	2		1	3	2	2	3
<i>Caprella simia</i>	I	6	3	5	6	5	6	6	7
<i>Caprella</i> sp.	U				1		1		
<i>Caprella verrucosa</i>	N					1			
<i>Deutella californica</i>	N					2			
Cirripedia									
Balanidae	U	1	2				2		
<i>Balanus crenatus</i>	N		1	1			1		
<i>Balanus glandula</i>	N		1	2				1	
<i>Balanus</i> sp.	U				1	1		1	
<i>Balanus trigonus</i>	N	6	2	5	6	2	6	6	5
<i>Megabalanus californicus</i>	N	7	3	5	1	5	4	3	4
<i>Megabalanus</i> sp.	N							1	
Decapoda									
Decapoda	U						1		
<i>Hemigrapsus oregonensis</i>	N				1				
<i>Hemigrapsus</i> sp.	U				2				
<i>Heptacarpus</i> sp.	N				1				
<i>Palinurus</i> sp.	N			1					

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Huene Site 1	Port Huene Site 2	Port Huene Site 3	Port Huene Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
Isopoda									
<i>Ianiropsis</i> sp.	U	3	1	3			4	4	5
<i>Paracerceis</i> sp.	N	1		1		1			
<i>Paranthura japonica</i>	I	1							
Pycnogonida									
<i>Achelia chelata</i>	N								1
<i>Ammothella biunguiculata</i>	N								2
<i>Anoplodactylus pacificus</i>	N		1						
Tanaidae									
<i>Leptochelia</i> sp.	C								1
<i>Zeuxo</i> sp.	U	6	2	5	7	3	5	5	5
BRYOZOA									
Bryozoa									
<i>Aetea pseudoanguina</i>	N					1			
<i>Amathia</i> sp. <i>bowerbankia</i>	U	6	2	5		6	2	6	7
<i>Bugula neritina</i>	I	6	5	4	6	4	6	5	7
<i>Bugulina longirostrata</i>	N		2	1	1			1	6
<i>Bugulina stolonifera</i>	I			4	1	3	1	1	1
<i>Celleporaria brunnea</i>	N	7	5	6	7	6	6	6	7
<i>Celleporella hyalina</i>	C					4		4	4
<i>Crisia occidentalis</i>	N	7	2	3	7	5	4	6	4
<i>Crisia</i> sp.	U		1	1					1
Crisiidae	U	2			1	1	3	1	3
<i>Crisulipora occidentalis</i>	N	5		3	4		3	4	4
<i>Cryptosula pallasiana</i>	I	7	2	3	2	4	4		6
<i>Fenestrulina delicia</i>	C							1	
<i>Fenestruloides eopacifica</i>	N	6	2	3	4	2	2		2
<i>Filicrisia franciscana</i>	N	3	2		3	5		2	5
<i>Filicrisia</i> sp.	U					1			
<i>Licornia diegensis</i>	N	5	2			3			4
<i>Membranipora villosa</i>	N					1			
<i>Schizoporella japonica</i>	I	6		2	3	3	5	5	2
<i>Scruparia ambigua</i>	C	2	1	2		4		2	4
<i>Tegella circumclathrata</i>	N	3	3		2				

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Hueneme Site 1	Port Hueneme Site 2	Port Hueneme Site 3	Port Hueneme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
		<i>Thalamoporella californica</i>	N		1			1	
<i>Tubulipora aliciae</i>	N							1	
<i>Tubulipora pacifica</i>	N	7	2	5	7	4	6	6	4
<i>Tubulipora</i> sp.	U	3	1	2	2	2	1		2
<i>Tubulipora</i> sp. 2	U		1						
Tubuliporidae	U					1			
<i>Watersipora arcuata</i>	I	1		1	1				
<i>Watersipora</i> sp.	I					1			
<i>Watersipora subtorquata</i> complex	I	7	5	6	7	5	6	6	7
CNIDARIA									
Anthozoa									
Actiniaria	U			1	1		1		
Hydrozoa									
Hydrozoa	U								1
Hydrozoa sp. A	U		2			1	1	1	2
Hydrozoa sp. D	U		1						4
Plumularioidea	U		3	5		5			
Tubulariidae	U	4	2	4	1				
ECHINODERMATA									
Echinodermata									
<i>Amphipholis squamata</i>	C							1	
Amphiuridae	U	1						1	
Ophiuroidea	U		1						
<i>Strongylocentrotus purpuratus</i>	N	1	1	1	1	1	1		
ENTOPROCTA									
Kamptozoa									
Kamptozoa	U		3			3			
MOLLUSCA									
Bivalvia									
Anomiidae	N						2		
Bivalvia	U		2		2	1	1		
<i>Crassadoma gigantea</i>	N							1	
<i>Hiatella arctica</i>	C	5	4	4	4	4	5	4	1
<i>Hiatella</i> sp.	C				1				
<i>Leptopecten latiauratus</i>	N			3					1

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Hueneme Site 1	Port Hueneme Site 2	Port Hueneme Site 3	Port Hueneme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
<i>Mytilus galloprovincialis/trossulus</i> complex	U	4	2	5	2	5	2	3	4
<i>Mytilus</i> sp.	U	4		2	3	1	1	2	3
Pectinidae	U	1		1	4		1		
<i>Pododesmus</i> sp.	N		2		6		3	1	
Gastropoda									
<i>Barleeia californica</i>	N				1				
<i>Barleeia</i> sp.	N		2						
Caenogastropoda	U	1							
Calyptraeidae	U				1				1
<i>Crepidula convexa</i>	I	5		6	4	1	4	4	5
<i>Crepidula</i> sp.	U	1		1			1	1	
<i>Lacuna</i> sp.	N						1		
<i>Lacuna unifasciata</i>	N	1		1				1	
<i>Lottia</i> sp.	N							1	
<i>Odostomia astricta</i>	N		1						
<i>Odostomia</i> sp.	U							1	
<i>Serpulorbis</i> sp.	N							1	
Nudibranchia									
<i>Aegires albopunctatus</i>	N				1				
Aeolidiidae	U				1				
<i>Corambe steinbergae</i>	N		1						
<i>Cuthona</i> sp.	U								1
<i>Dendronotus venustus</i>	N					1			1
<i>Dirona picta</i>	N				1				
<i>Doto amyra</i>	N					1			
<i>Doto</i> form A of Goddard	N		3			3			1
<i>Doto kya</i>	N		1			2			
<i>Eubranchus rupium</i>	N		1						
<i>Eubranchus rustyus</i>	N		1						
<i>Eubranchus</i> sp.	U		1						
<i>Hermisenda</i> sp.	N			2		1			
<i>Okenia angelensis</i>	N	3		3		6		5	3

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Hueneme Site 1	Port Hueneme Site 2	Port Hueneme Site 3	Port Hueneme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
<i>Polycera atra</i>	N	2		1		3	2	4	5
<i>Polycera hedgpethi</i>	N	2				1			1
NEMERTEA Nemertea									
<i>Lineus torquatus</i>	N				1				
Nemertea	U	1				1			1
PLATYHELMINTHES Platyhelminthes									
<i>Acerotisa californica</i>	N	2	1	3	1		1		4
<i>Hoploplana californica</i>	N	1	2	1			1		
<i>Notocomplana acticola</i>	N		1					1	
Platyhelminthes	U		1	1	1			1	1
<i>Polycystididae</i> sp. HYP1	N							1	
<i>Pseudoceros mexicanus</i>	N			1					
<i>Pseudoceros</i> sp.	N								1
PORIFERA Porifera									
Porifera	U							1	1
Porifera sp. A	U	2	2	3	7	1	4	5	
Porifera sp. B	U	5	4	5	7	4	7	4	5
Porifera sp. C	U	1	1	1	3	2	2	5	
<i>Sycon</i> sp.	U	1	1	3	1	1	2	2	1
UROCHORDATA Tunicata									
<i>Aplidium</i> sp.	U								1
Aplousobranchia	U					1	1		2
<i>Ascidia ceratodes</i>	N				1				
<i>Ascidia</i> sp.	U		1	3	2		2		3
Botryllinae	I		1	3	1	1	3		1
<i>Botrylloides diegensis</i>	I								1
<i>Botrylloides violaceus</i>	I			2					5
<i>Botryllus schlosseri</i>	I						1		4
<i>Ciona savignyi</i>	I				1				
<i>Ciona</i> sp.	U	1	1	4	5	1	2		5

PORT HUENEME Fouling Panel Species List	BAY SPECIES STATUS	Naval Base Alpa Dock	Naval Base Fiberglass Dock	Naval Base Floating Dock	Naval Base Sealion Dock	Port Hueneme Site 1	Port Hueneme Site 2	Port Hueneme Site 3	Port Hueneme Site 4
		2015	2015	2015	2015	2015	2015	2015	2015
Didemnidae	C						1		5
<i>Didemnum cf. vexillum</i>	I						1		
<i>Diplosoma listerianum</i>	I		1	1	1				5
Phlebobranchia	C	1					1	1	
Stolidobranchia	C	2				2			
<i>Styela</i> sp.	C	1	1	1	4	1	4	2	4
Tunicata	C		1		1	1			1

San Francisco Bay

SAN FRANCISCO BAY Fouling Panel Species List	BAY SPECIES STATUS	Ballena Isle Marina			Coyote Point Marina			Loch Lomond Marina			Oakland Yacht Club			Oyster Point Marina			Redwood City Marina			Richmond Marina Bay Yacht Harbor			San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor		
		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
ANNELIDA																															
Arenicolidae																															
<i>Branchiomaldane</i> sp.	N																								1						
Capitellidae																															
<i>Capitella capitata</i> complex	C	2	1		1	3	1		1	2		1		4	3		1		1	1		1		1		2	2				
Capitellidae	U									1																		1			
Chrysopetalidae																															
<i>Paleanotus bellis</i>	N																				2										
Cirratulidae																															
Cirratulidae	U	3			1						1		3		1	1		1		1				1							
<i>Cirratulus dillonensis</i>	N																							1							
<i>Cirratulus</i> <i>multioculatus</i>	N																							1							
<i>Cirriformia</i> sp.	N								1		2	2		2		2	1	1			2		2		1						
<i>Cirriformia</i> sp. A	U				2		4		8			2		2		1	3		1					1	3			1			
SERC <i>Cirriformia</i> <i>spirabrancha</i>	N	1	3	1	1	2	1		4		1	1	4	1	2	5	2		1	1			4	5	1						
<i>Ctenodrilus serratus</i>	C		1	1			2		1			1						2						2				1			
<i>Protocirrinieris</i> sp.	U	1																													

SAN FRANCISCO BAY Fouling Panel Species List	BAY SPECIES STATUS	Ballena Isle Marina			Coyote Point Marina			Loch Lomond Marina			Oakland Yacht Club			Oyster Point Marina			Redwood City Marina			Richmond Marina Bay Yacht Harbor			San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor		
		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		Dorvilleidae																													
Dorvilleidae	U																														
<i>Ophryotrocha</i> sp.	U	1	3	2		1	2	1		2	1	1	4	1	1	1	7	2	6	1											
<i>Schistomeringos</i> <i>annulata</i>	N		2						1					1						1						1					
<i>Schistomeringos</i> <i>longicornis</i>	N		2	1									1		1							1									
<i>Schistomeringos</i> sp.	N													1																	
Lumbrineridae																															
<i>Lumbrineris</i> sp.	N												1															1			
Nereididae																															
<i>Alitta succinea</i>	I													1	1		1														
<i>Neanthes acuminata</i> complex	C										6	5	6			3	2	9	8						1						
Nereididae	U											1							1												
<i>Nereis latescens</i>	N														1							1	4	3			1	1			
<i>Platynereis</i> <i>bicanaliculata</i>	N													2						1	4		5	5			8	4	3		
Oligochaeta																															
Oligochaeta	U																									1	1				
Orbiniidae																															
<i>Naineris dendritica</i>	N																			1								1			
Orbiniidae	U																								1						
Phyllococidae																															
<i>Eumida longicornuta</i>	N																								1						
<i>Eumida</i> sp.	U																						1								

SAN FRANCISCO BAY Fouling Panel Species List	BAY SPECIES STATUS	Ballena Isle Marina			Coyote Point Marina			Loch Lomond Marina			Oakland Yacht Club			Oyster Point Marina			Redwood City Marina			Richmond Marina Bay Yacht Harbor			San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor		
		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		Polynoidae																													
<i>Halosydna brevisetosa</i>	N																			1								3			
<i>Halosydna johnsoni</i>	N													1						1						1					
<i>Halosydna</i> sp.	N																						1								
<i>Harmothoe imbricata</i>	C	6		4	4	2	3	1	3		5	7	9	1	6	6			3	2		4	2	1	4	2	1	1	2	1	7
Polynoidae	U																											1			
Sabellidae																															
<i>Branchiomma</i> sp.	U		4	6			1					8	1			3												1			
<i>Branchiomma</i> sp. 2	U										2																				
<i>Parasabella</i> sp.	N		2	5		2	3				1	2	5			1		6	2			5				6		3	1		
Sabellidae	U																											1			
Serpulidae																															
<i>Ficopomatus enigmaticus</i>	I							6	5		4						1	8	3							4	2				
<i>Hydroides elegans</i>	I		1								4									1											
<i>Hydroides gracilis</i>	N																						1					1			
<i>Hydroides</i> sp.	U										1									1											
<i>Pseudochitinopoma occidentalis</i>	N																		2					1							
<i>Salmacina tribranchiata</i>	N		1								1																				
<i>Serpula</i> sp.	N										1																				
Serpulidae	U							1			1																	1			

SAN FRANCISCO BAY Fouling Panel Species List	BAY SPECIES STATUS	Ballena Isle Marina			Coyote Point Marina			Loch Lomond Marina			Oakland Yacht Club			Oyster Point Marina			Redwood City Marina			Richmond Marina Bay Yacht Harbor			San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor		
		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		Spionidae																													
<i>Dipolydora socialis</i>	C	1																					4	2	1				5	4	2
<i>Polydora cornuta</i>	I		1	1	1	2	1	2	1	3		1	2	2	4	1	6	2	3				2	2		1			3		5
<i>Polydora narica</i>	N																							1					1	1	
<i>Polydora nuchalis</i>	N									1					1																
<i>Polydora</i> sp.	U													1	1					1											1
<i>Pseudopolydora paucibranchiata</i>	I																										1				
Spirorbidae																															
<i>Neodexiospira alveolata</i>	C		2	8																											
<i>Neodexiospira pseudocorrugata</i>	C		6	3			1		2		1	2	5			1			1						2			1			1
Spirorbidae	U					1						2														3					
Syllidae																															
<i>Amblyosyllis speciosa</i> D	I	2				1						3	1					4				2				1					
Autolytinae	U												1				1						1						3	3	2
<i>Erinaceusyllis</i> sp.	N		2		1		2	1		1		1	1				1	1	2							1	3		1	1	
<i>Exogone lourei</i>	C	2	3	3	4	2	4						1	2	2	1	1	2				1	1		1	3	1	4	5	5	9
<i>Exogone</i> sp.	N																								1						
Exogoninae	U						1																								1
<i>Megasyllis nipponica</i>	I	7	8	9	7	6	6	9	9	1	7	8	8	1	1	9	2	2	6	9	1	5	6	8	8	3	2	4	1	5	7
<i>Myrianida pachycera</i>	I		2			1	1								1			3	3												
<i>Myrianida pentadentata</i>	I			1					1				2			2		1					1						3	8	5
<i>Myrianida</i> sp.	U		1							1	1						3			1									6		

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		Caprellidae																																
<i>Caprella californica</i>	N				1			1						1									5	8							7	9	3	
<i>Caprella cf. ferrea</i>	N																		1															
<i>Caprella equilibra</i>	C																						3	3										
<i>Caprella mutica</i>	I	5	3	9		3		3	1	1	4	5		1		7	6	1	3	1	1	1	0	0	0	5	6	6			2	9	1	9
<i>Caprella penantis</i>	C																						4	1							1			
<i>Caprella scaura</i>	I		2			3	3		3		4	4		3	5	7		2		1	3							1			1	2		
<i>Caprella simia</i>	I	7	9	6	7	8	6	1	3	2	1	1	6	7	9	9	1	7	8	5	9	1	0	7	9	1	7	9	4	4	2	1	1	1
<i>Caprella sp.</i>	U					1										1		1		1														
<i>Caprella sp. 11</i>	U																				2													
<i>Metacaprella anomala</i>	N																								1									
Cirripedia																																		
<i>Amphibalanus amphitrite</i>	I	2									7						1	4	6															
<i>Amphibalanus improvisus</i>	I	4									5	1	1	2			7		1	6	2	3	3	4	2	2	2				3			
<i>Amphibalanus sp.</i>	U									1					1	1			1															1
Balanidae	U														1										1									1
<i>Balanus crenatus</i>	N																	1		2			1	2	1				1	5				
<i>Balanus trigonus</i>	N																						1											
Cirripedia	U															2																		

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		Decapoda																													
<i>Hemigrapsus oregonensis</i>	N																									1					
<i>Pugettia producta</i>	N																											1			
<i>Romaleon branneri</i>	N							1																							
Isopoda																															
<i>Gnorimosphaeroma oregonensis</i>	N	1																										1			
<i>Ianiropsis</i> sp.	U	5	8	6	2	2	1	3			6	2	6	1	2	1	7	5		1	1	8	6	3		1	1	5			
<i>Munna</i> sp.	U																									1		8			
<i>Munna stephensi</i>	N	2												1														1			
Munnidae	U												2																		
<i>Paracerceis sculpta</i>	N		1									7																			
<i>Paranthura japonica</i>	I	8	1	9	9	9	7	1	8	1	8	9	9	8	6	1	1	1	1	1	7	3	1	1	2	7	8	5			
<i>Sphaeroma quoianum</i>	I										1																				
<i>Uromunna</i> sp.	U			4		1	2						1			3								1		3	1	1			
<i>Uromunna</i> sp. A	I																											4			
Pycnogonida																															
<i>Ammothea hilgendorfi</i>	N											1							1												
<i>Ammothella biunguiculata</i>	N				1	1																				1	1	1			
<i>Anoplodactylus erectus</i>	C								1																						
<i>Anoplodactylus</i> sp.	U							1	2																	1					
Pycnogonida	U					2																									

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
		<i>Crisularia pacifica</i>	N								3						1				1										
<i>Crisulipora occidentalis</i>	N											1																			
<i>Cryptosula pallasiana</i>	I	3	7	4			2		2			1		1			7	4	1 0	2	1 0	7	5	2	4	9	6	6	7	9	
Ctenostomatida	U																													2	
Cyclostomatida	U											2																			
<i>Electra</i> sp.	U																													1	
<i>Fenestrulina delicia</i>	C																						1							1	3
<i>Fenestrulina</i> sp.	U																		2			1								1	
<i>Licornia diegensis</i>	N														4																7
<i>Schizoporella errata</i>	I																										2				
<i>Schizoporella japonica</i>	I																					2	4	1	1	1					1
<i>Schizoporella</i> sp.	U																											1			
<i>Smittoidea prolifica</i>	N	5	1	1	2		3			1				2	1				1							3	1			1	
<i>Thalamoporella californica</i>	N												1																		
<i>Tricellaria occidentalis</i>	N								1	1	3	3					1	1	1		9					1		8	1	1	
<i>Victorella pavida</i>	I																					1									
<i>Watersipora</i> sp.	I	3	1			1						3	2								1	1	2				3				
<i>Watersipora subtorquata</i> complex	I	2	7	1 0	2	4	4	1	1	8	2	1	6	2	2	6	2	2	7	7	9	9	5	5	4	5	9	9	1 0	1 0	

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		CNIDARIA																													
Anthozoa																															
Actinaria	U										2															1	2		2	2	
Anthozoa	U				1			1			1																		1		
<i>Diadumene franciscana</i>	I																1									1					
<i>Diadumene leucolena</i>	I														3																
<i>Diadumene lineata</i>	I			1	1			1	1						2		3			1						4	1		3		
<i>Diadumene</i> sp.	U	2			5			3	1		5	1		1			1	8		5						5	8		1	5	3
Hydrozoa																															
Athecata	U		1			1		3			1				1		1						1			3	2		1		
<i>Bougainvillia</i> sp.	U				6	1		6			2			1																	
Bougainvilliidae	U													1	1		1									2					
<i>Cordylophora</i> sp.	U							1																							
<i>Ectopleura crocea</i>	U													2	1	1				1											
<i>Garveia franciscana</i>	U				2																										
Hydrozoa	U					2		1	1			1														1	1				
Hydrozoa sp. A	U												1					1			1					1					
Hydrozoa sp. B	U																										1				
Hydrozoa sp. D	U					2	2			1																2			1		
Hydrozoa sp. E	U											1				1											1				
<i>Obelia</i> sp.	U					1		1					2				1									8					
Thecata	U		2										1																		

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016				
ECHINODERMATA																																
Echinodermata																																
<i>Amphipholis squamata</i>	C										1	7		1						1						2						
Ophiuroidea	U										3																					
ENTOPROCTA																																
Kamptozoa																																
<i>Barentsia benedeni</i>	U	1			2	2								1			1		1							7	2					
<i>Barentsia</i> sp.	U					1																				1						
Kamptozoa	U									2				1				1								1	3	5				
MOLLUSCA																																
Bivalvia																																
Anomiidae	U	1																														
Bivalvia	U		1									2			1	2																
Heterodonta	U									1	3								1												1	
<i>Leptopecten latiauratus</i>	N																												1			
<i>Modiolus</i> sp.	N																			1												
<i>Musculista senhousia</i>	I	2	2		4	4		1		9	1	8		7	6	3	1	4	2	1	1		1	1			1					
Mytilidae	U				1	1		2				1		2				4			1			2								
<i>Mytilus galloprovincialis/trossulus</i> complex	U																						1									
<i>Mytilus</i> sp.	U																			1		4	1						7			
<i>Ostrea lurida</i>	N	7			9	6		8	3	1	0	4		9	6	1	2	3		6			7	6		3			1	3		
Ostreidae	U	4	3		1	3			1			4			4	3		1	1		1		3						2	1		
<i>Pododesmus</i> cf. <i>macrochisma</i>	N																												1			
Veneridae	U									1																						

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
GASTROPODA																															
<i>Alia carinata</i>	N																														
<i>Crepidula plana</i>	I									1			1																		
<i>Crepidula</i> sp.	U																														
Gastropoda	U		1																												
<i>Haminoea japonica</i>	I		1		1	1	2		1							2	3	1													
<i>Iselica ovoidea</i>	N											1																			
<i>Marseniopsis sharonae</i>	N																												1		
<i>Odostomia</i> sp.	U					1																									
<i>Odostomia</i> sp. SF1 Phillips	N							2					1																		
<i>Urosalpinx cinerea</i>	I											1																			
NUDIBRANCHIA																															
<i>Cuthona albocrusta</i>	N					1																									
<i>Cuthona perca</i>	I																														
<i>Dirona picta</i>	N																3												1		
<i>Eubranchus</i> sp.	U						1																								
<i>Flabellina verrucosa</i>	N																														
<i>Janolus barbarensis</i>	N																													1	
<i>Polycera atra</i>	N																													4	
<i>Polycera hedgpethi</i>	N									1	2		1																	1	

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
		Opisthobranchia																													
<i>Aplysiopsis enteromorphae</i>	N																														
<i>Placida dendritica</i>	C	1																													
NEMATODA																															
Nematoda																															
Nematoda	U					2						4			1			1			1			1	5						
Nemertea																															
Nemertea																															
<i>Cephalothrix</i> sp.	U					2	3		4			1	1		2						2			1	0	1		1	3		
<i>Lineus ruber</i>	N																										1				
Nemertea	U								1	2					1												1				
Tubulanidae	U					1																									
PLATYHELMINTHES																															
Platyhelminthes																															
<i>Acanthozoon lepidum</i>	N																					3						1			
<i>Acerotisa alba</i>	N		1																									4			
<i>Acerotisa californica</i>	N																				2	5	1					6			
<i>Cycloporus</i> sp.	N																											1			
<i>Eurylepta aurantiaca</i>	N					3	1		1			1	2		1	4															
Leptoplanidae	U																		2		1										
<i>Notocomplana atticola</i>	N											4																			
Platyhelminthes	U					1													1	1									5		

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016					
Prosthiostomidae	U																																
<i>Pseudoceros</i> sp.	N																																
PORIFERA																																	
Porifera																																	
<i>Euplectella</i> sp.	U																																
<i>Grantia</i> sp.	U							1		1																							
<i>Halichondria</i> sp.	U	5			6	1		8		1		1	1	3	2	9			8		1	3	1		6		5		2				
<i>Haliclona</i> sp.	U	2			2		2	5				1		2	2	3		1	2		1						2		1				
<i>Microciona prolifera</i>	U	1																															
Porifera	U	5	1					3			2	3	4			3		1	2			1											
Porifera sp. A	U		5	7		1	6		9	9			9	7		4	9		1	2	9		3		6	6		5	5				
Porifera sp. B	U					2	2		1	3			1	4		2			5		1	2											
Porifera sp. C	U											1	2		1	1					3		4			1		1					
<i>Sycon</i> sp.	U	7	1			2	2	9	1		3	6	1	5	6	1		7		3					1	5	1		2				
UROCHORDATA																																	
Tunicata																																	
<i>Aplidium</i> sp.	U	1													1																		
<i>Aplidium</i> sp. A	U							1																									
Aplousobranchia	U								1																								
<i>Ascidia</i> sp.	U				1							1				1	1		2		2							1	1				
<i>Ascidia zara</i>	I	1	9	1	7	9	9	7	1	5	1	9	6	9	1	1	0	1	0	8	9	6	4	8	1	4	2	7	1	5	5	3	4
Ascidiacea	U		1																														

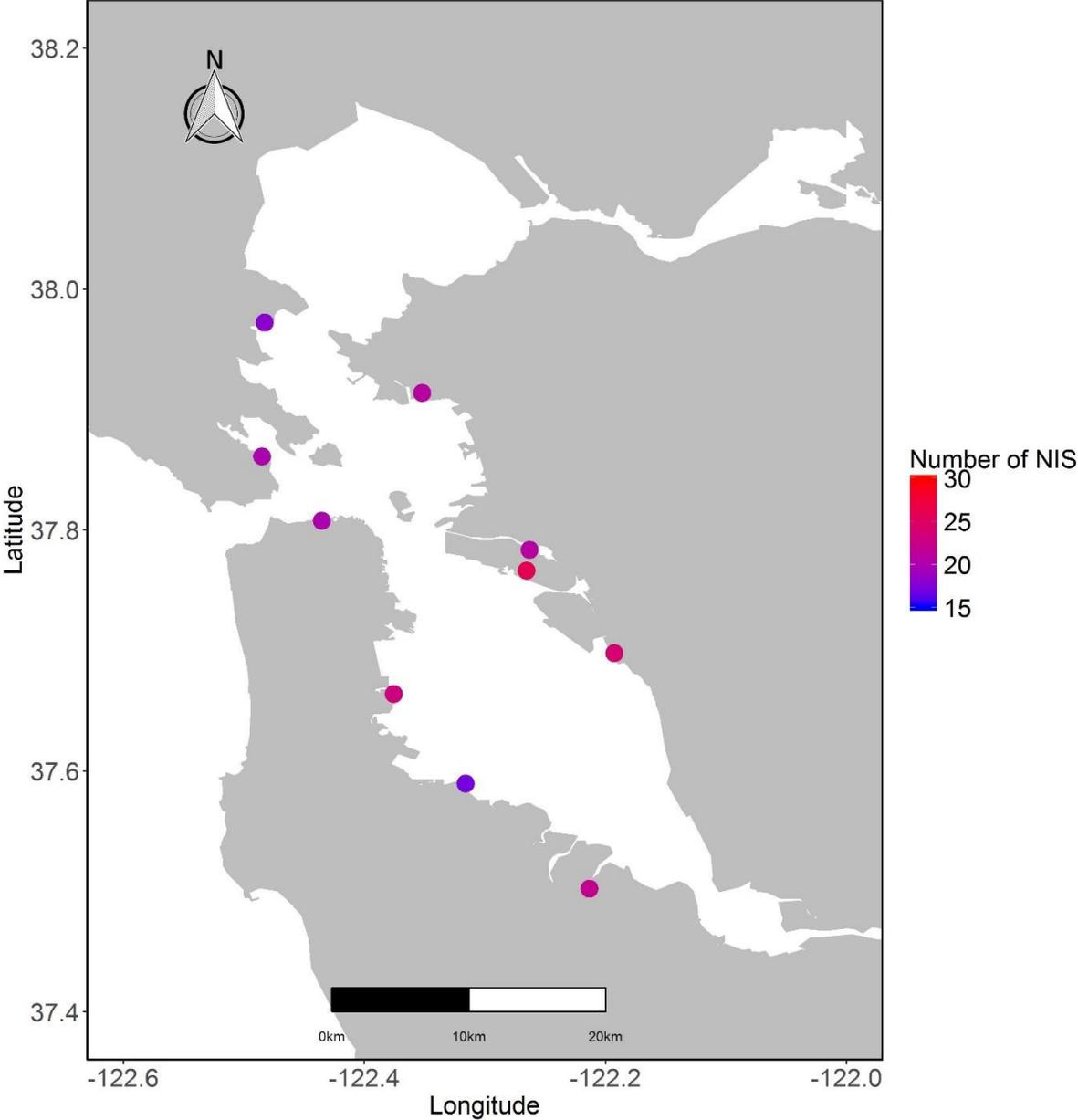
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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016		
		<i>Botryllinae</i>	I	6	8	2	4	7		3	7	4	7	8	7	7	8	3	2	9	6	7	8	1	5	9	3		5	1	8	8	1
<i>Botrylloides diegensis</i>	I	3	7	9		7	1		5	1		1	5		8	1	1	6	6		3	4	9		5	4	1	3	4	1	8	9	
<i>Botrylloides sp.</i>	I	3	1	2	4	7	1	1	3	3	1	4	1	2	7	2	2	1	3		4	2	7	2	2	5	1	1	1	3	8	3	
<i>Botrylloides violaceus</i>	I	1	3	5	7	3	4	8	4	1	4			5	3	3	1	1			9	2	6	7	6	3		3	1	7	5	9	
<i>Botryllus schlosseri</i>	I	8	6	7	5	8	6	3	6	1	4	9		1	9	9	1	4	4		9	5	1	5	6	2	2	3	2	4	7	1	
<i>Botryllus sp. A</i>	I	4									2			2			1				1						1						
<i>Ciona robusta</i>	I	3		3	5	3		4			8	9	3	7	6	4	9	5	3		1	0	4	7	3	5	2	3	1		7		2
<i>Ciona savignyi</i>	I	1	1	9	7	1	9	6	8	3	1	1	8	9	9	1	9	9	8		1	0	8	1	2	4	1	4	5	3	4	4	8
<i>Ciona sp.</i>	I	1	2	2	2	4	1	2		1	1		7	3	4	1		5	5			2	1			1	1	1	1	3	3		
Didemnidae	U	1		4	2	5	8		2	2		1	4	4	3	9		3	9			5	1		8	8		7	3		2	6	
<i>Didemnum vexillum</i>	I	5		1	7	3	3	1	1					1	1		1				1	0	1	9			3		1	1	8	5	
<i>Diplosoma listerianum</i>	I	9	1	1	7	4	1	3	6	1	8	1	1	3	6	1	7	9	1		1	0	1	9	8	8	7	2	7	3	8	0	1
<i>Distaplia occidentalis</i>	N	1					1																		7				1	3		1	0
<i>Distaplia sp.</i>	U						2														1			3	1				1			1	
<i>Microcosmus sp.</i>	U											1																					
<i>Microcosmus squamiger</i>	I											3																					
<i>Molgula ficus</i>	I						1					2				1																	
<i>Molgula manhattensis</i>	I	6	1	3	8	6	7	8	1	7	2	1		1	5	8	9	5	6		1	1	8	4	3	3	8	8	9			4	
<i>Molgula sp.</i>	U	2	1		1		1			3	1	1			1		1	1	3						1	1		1	1				1
<i>Perophora annectens</i>	N	3	4																														

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		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016			
<i>Perophora japonica</i>	I	1																													
<i>Perophora</i> sp.	U	1	1	1																				1							
Phlebobranchia	U						1					1												1							
Stolidobranchia	U		2	2									1	6											1		1	1			
<i>Styela canopus</i>	I												1																		
<i>Styela cf. canopus</i>	I												4																		
<i>Styela clava</i>	I		2			3	3	4	5	2															3	5	3	1			
<i>Styela</i> sp.	U	5				3	2	3	1	5		4	2	1	4	4	5	4		1	8		1	5	1		3	2	3	2	2
Styelidae	U		1				2			3			3			1				8		2			2			2			

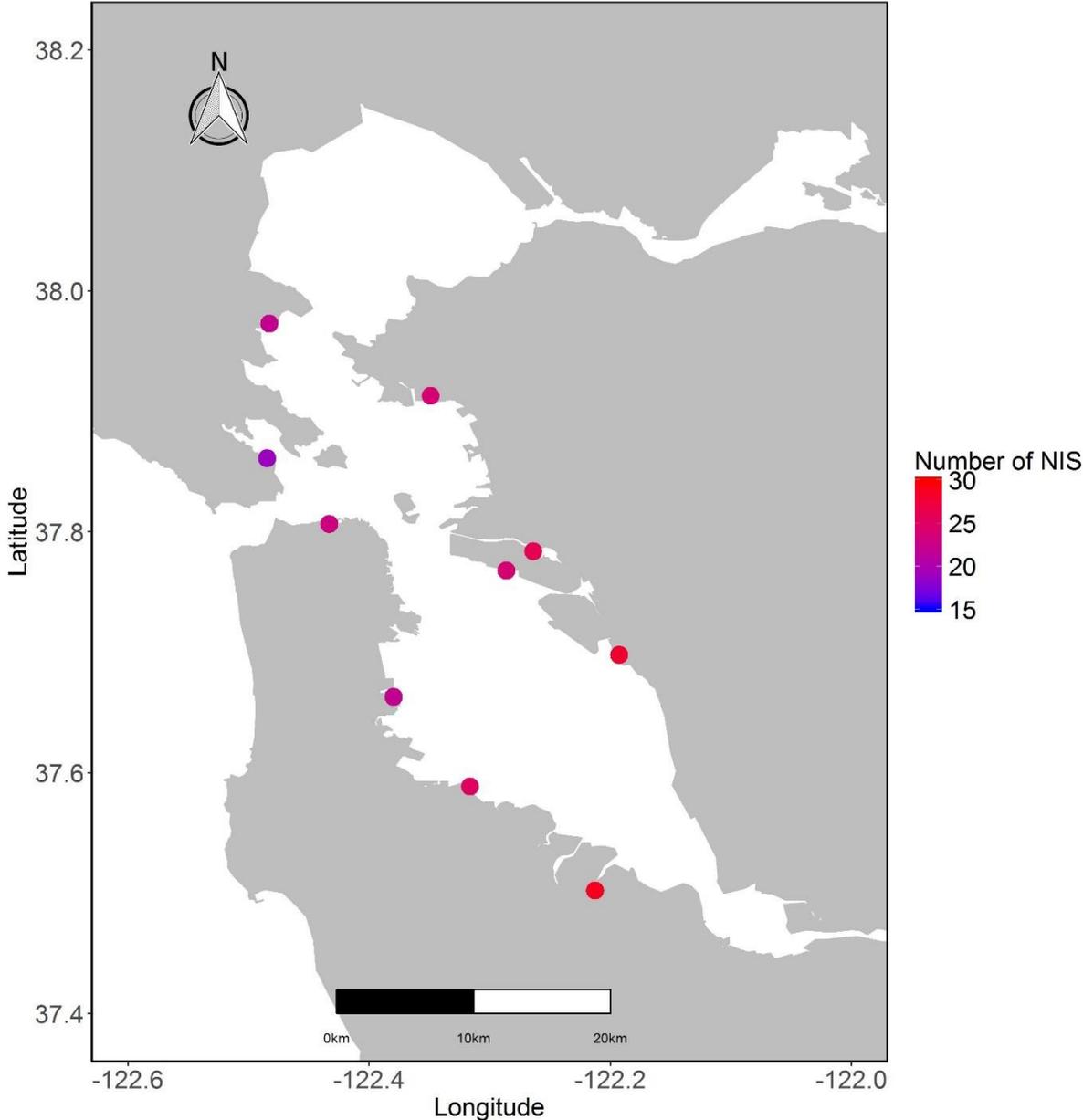
Appendix 2.3: Non-Native Species Richness by Estuary and Year

The heat maps below show non-native invertebrate species richness detected in hard substrate samples for each bay and year surveyed. Taxonomic identification is based on morphological characteristics.

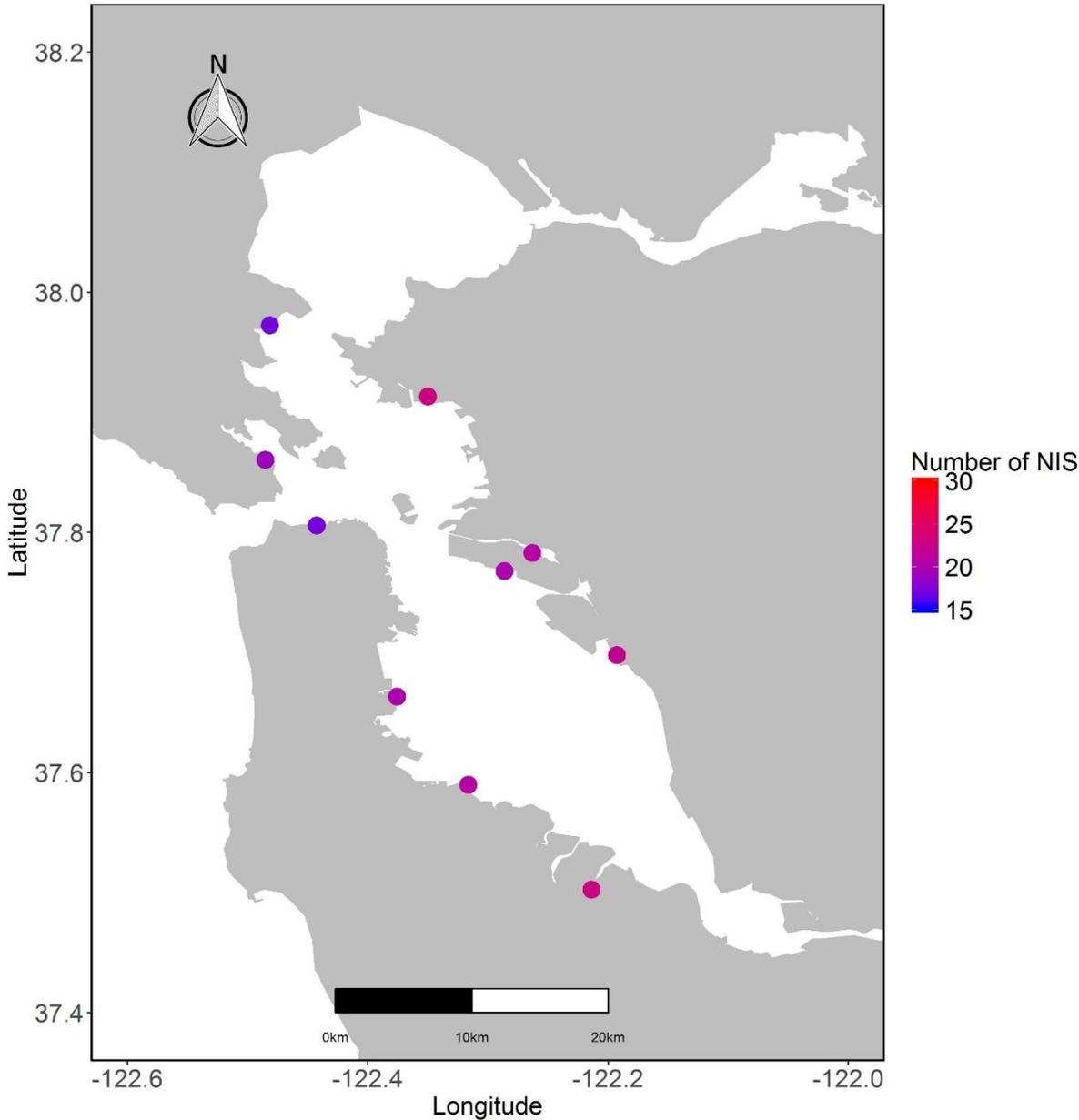
San Francisco Bay 2014



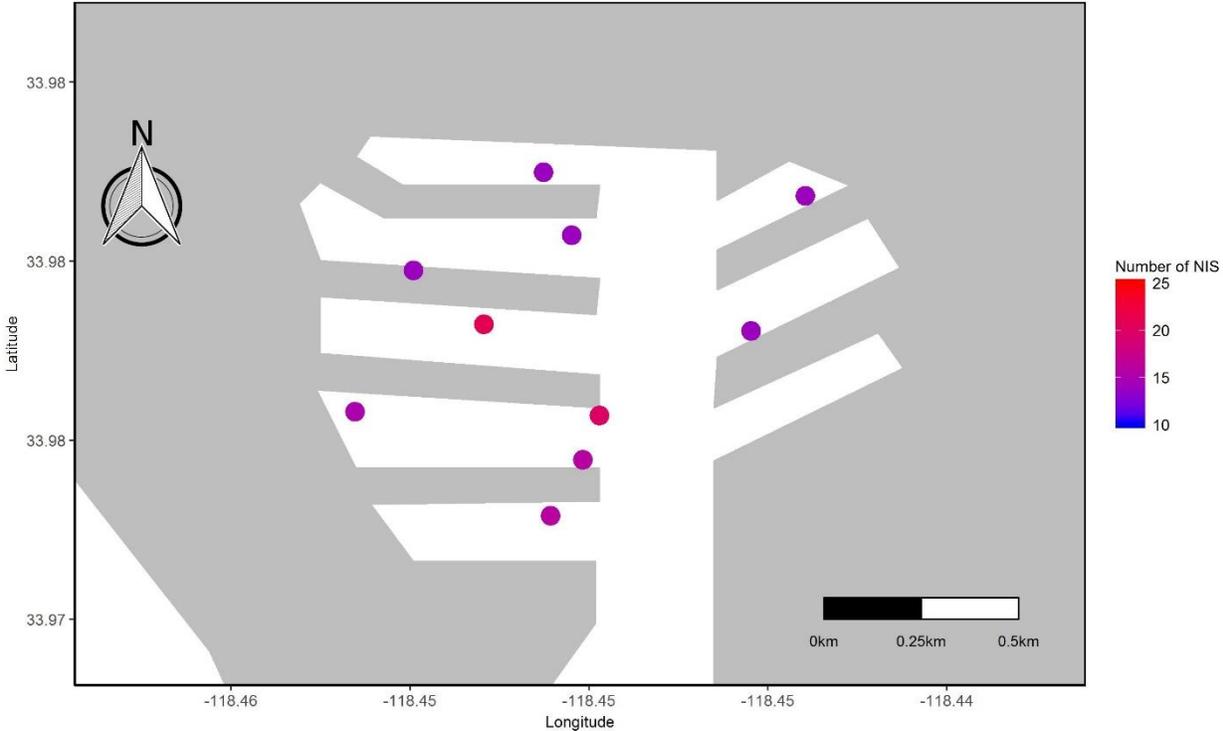
San Francisco Bay 2015



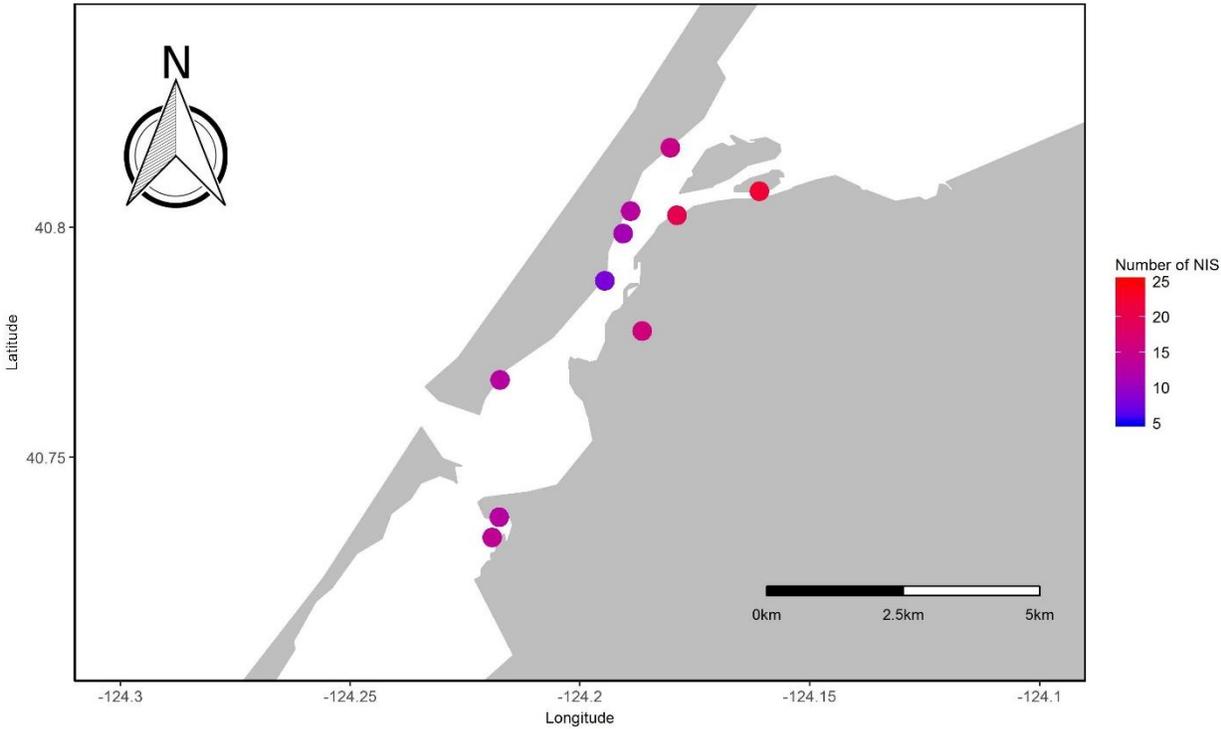
San Francisco Bay 2016



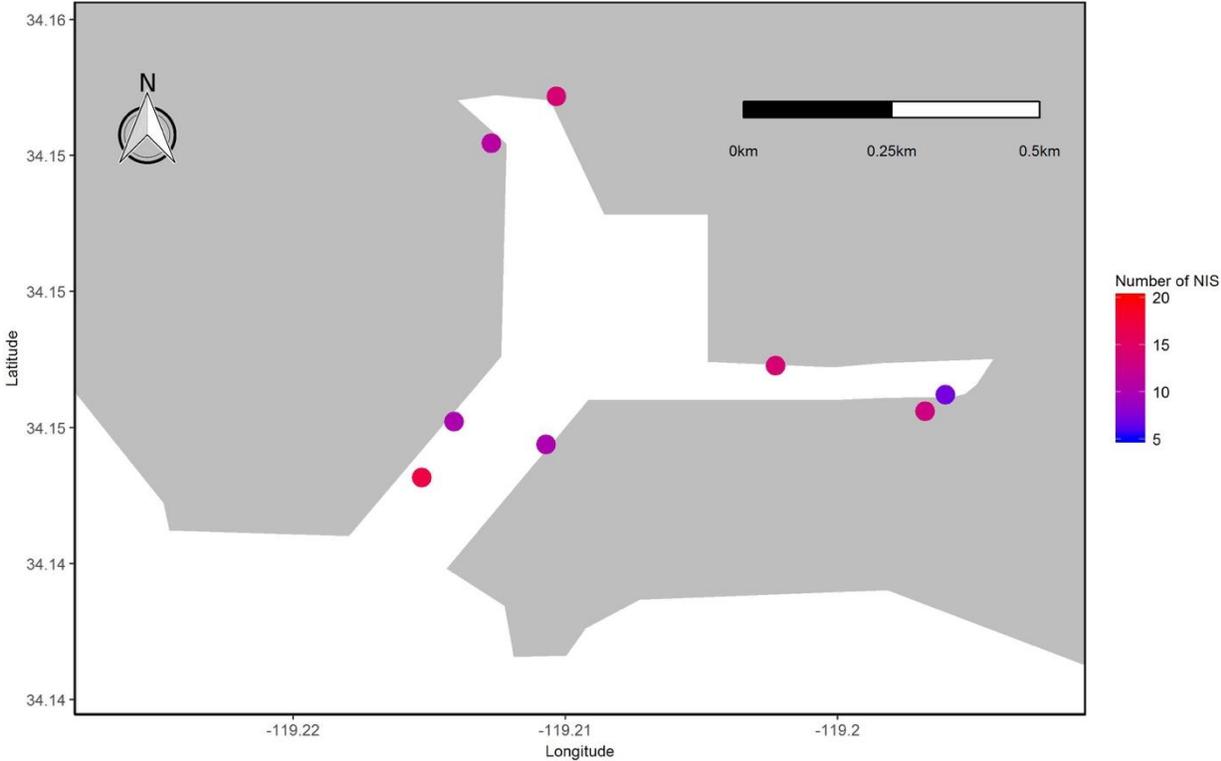
Marina del Rey 2015



Humboldt Bay 2015



Port Hueneme 2015



Chapter 3: Soft Sediment Communities

Introduction

A. Field Collections

Surveys of invertebrate communities in soft sediment habitats were conducted in San Francisco Bay (2014, 2015, 2016) and Humboldt Bay (2015). We used a stratified sampling scheme to sample at ten stations in the high salinity region in each Bay. At each station, five replicate grab samples were collected at 200m intervals at each depth sampled, as described below. Salinity and temperature were collected using a YSI Model 85 (Yellow Springs Instrument Co.) meter, and depth was recorded using a depth sensor on the boat.

In San Francisco Bay each year (2014, 2015, 2016), we sampled shallow subtidal depths (2m below MLLW) at ten stations in the higher salinity region of the Bay. Five replicate grab samples were collected at 200m intervals at each station and depth, generating a total of 50 samples (replicates x 10 stations).

In Humboldt Bay in 2015, we sampled five stations in the higher-salinity region of the Bay at intertidal, shallow subtidal (2m below MLLW), and deep (5m below MLLW) depths, and five additional stations at shallow subtidal (2m below MLLW) only. Five replicate grab samples were collected at 200m intervals at each station and depth generating 100 samples (5 replicates x 3 depths x 5 stations, and 5 replicates x 1 depth x 5 stations).

We used a standard Young-modified Van Veen grab (Dauer & Lane 2005, US EPA 2009) with shovels capturing grab samples with a surface area of 0.1m² deployed via hydraulic winch to collect all samples. The entire grab sample was sieved on a 1mm mesh screen, and the retained organisms were preserved in 95% ethanol (except for polychaetes and soft-bodied organisms that were preserved in 10% formalin).

B. Sample Analyses

Morphological analyses for soft sediment taxa proceeded through several steps, and all collected organisms were sorted and identified to the lowest taxonomic level, as follows:

Coarse sorting and removal of polychaete taxa in the field followed by examination under dissecting microscopes, with vouchers taken for genetics and preserved in 95% ethanol.

1. Laboratory sorting of grab samples using dissecting microscopes where necessary and identification by in-house experts to the lowest taxonomic level using California fauna identification keys (Kozloff 1996, Carlton 2007) and consultation with taxonomic experts.

2. Verification of morphological voucher identification. A subset of samples was selected randomly for independent verification, based on morphological characters, by recognized taxonomic experts. Unique or unusual specimens, or potential first records of a species, were subject to additional scrutiny, including additional examination of morphological characters, consultation with additional taxonomic experts, and targeted genetic analyses as possible to confirm or revise morphological identifications.

Voucher specimens of each morphotaxon were taken from each sample. Where possible, based on size and species constraints, the same organisms were split into a paired morphological and DNA sample, to provide direct comparisons of genetic and morphological identifications. All voucher specimens were labeled with a unique identification number, and genetic vouchers were sent to MLML for analyses.

C. Data Analyses

The morphological identifications of specimens produced a list of taxa identified to the lowest possible taxonomic level for each sample, along with their abundances (number of individuals). For each taxon, we classified the invasion status in the bay in question, based upon previous analyses and using a synthesis of information in the National Exotic Marine and Estuarine Species Information System (Fofonoff *et al.*, 2017). Four categories were used for this classification: NIS, native, cryptogenic (of uncertain status, *sensu* Carlton 1996), and undetermined (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 detected at each depth for each Bay and sampling year.

Results

A. Overall Summary

San Francisco Bay 2014

Seventy-five morphospecies were detected for the 2014 soft sediment community survey in San Francisco Bay: 37 native, 21 NIS, 5 cryptogenic and 12 unresolved species. Native species accounted for a total of 1713 individuals (27% of the community), NIS species for 4530 individuals (71% of the community), cryptogenic for a total of 39 individuals (0.61% of the community) and unresolved species for 119 individuals (2% of the community).

San Francisco Bay 2015

Sixty-three morphospecies were detected for the 2015 soft sediment community survey in San Francisco Bay: 29 native, 23 NIS, 5 cryptogenic and 6 unresolved species. Native species accounted for a total of 197 individuals (7% of the community), NIS species for 2356 individuals (88% of the community), cryptogenic for a total of 89 individuals (3% of the community) and unresolved species for 48 individuals (2% of the community).

San Francisco Bay 2016

Seventy-two morphospecies were detected for the 2016 soft sediment community survey in San Francisco Bay: 29 native, 23 NIS, 5 cryptogenic and 15 unresolved taxa. Native species accounted for a total of 1905 individuals (25% of the community), NIS for 5035 individuals (66% of the community), cryptogenic for a total of 54 individuals (0.7% of the community) and unresolved taxa for 635 individuals (8% of the community).

Humboldt Bay 2015

A total of 136 morphospecies were detected for soft sediment community survey in Humboldt Bay: 84 native, 13 NIS, 5 cryptogenic and 34 unresolved. Native species accounted for a total of 4927 individuals (57% of the community), NIS species for 910 individuals (13% of the community), cryptogenic for a total of 36 individuals (0.4% of the community) and unresolved for 2799 individuals (34% of the community).

B. Detection of NIS in San Francisco and Humboldt Bays.

Our analyses indicate that our sampling program performed well in detecting and characterizing the NIS in the soft sediment community in both San Francisco and Humboldt Bays. The figures below show the detection of species in the high salinity portion of both bays (2014–2016 for San Francisco, and 2015 for Humboldt). The accumulation curves show the rarefaction of species richness. Calculations were performed using the R package *vegan* 2.4 (R Core Team 2017; Oksanen *et al.* 2015).

Total NIS Richness in San Francisco and Humboldt Bays.

NIS richness approached an asymptote rapidly in shallow water subtidal habitats in San Francisco Bay in all three years sampled (Figures 3.1, 3.2, 3.3), and in Humboldt Bay in 2015 (Figure 3.3). We observed 22 NIS in San Francisco Bay in 2014 and 24 NIS in both 2014 and 2015, while we found a total of 14 NIS in Humboldt Bay in 2015. The accumulation curves suggest that we have detected > 90% of the estimated total NIS in Humboldt Bay and 84-100% of the estimated total NIS in San Francisco Bay across each of the three years (Table 3.1).

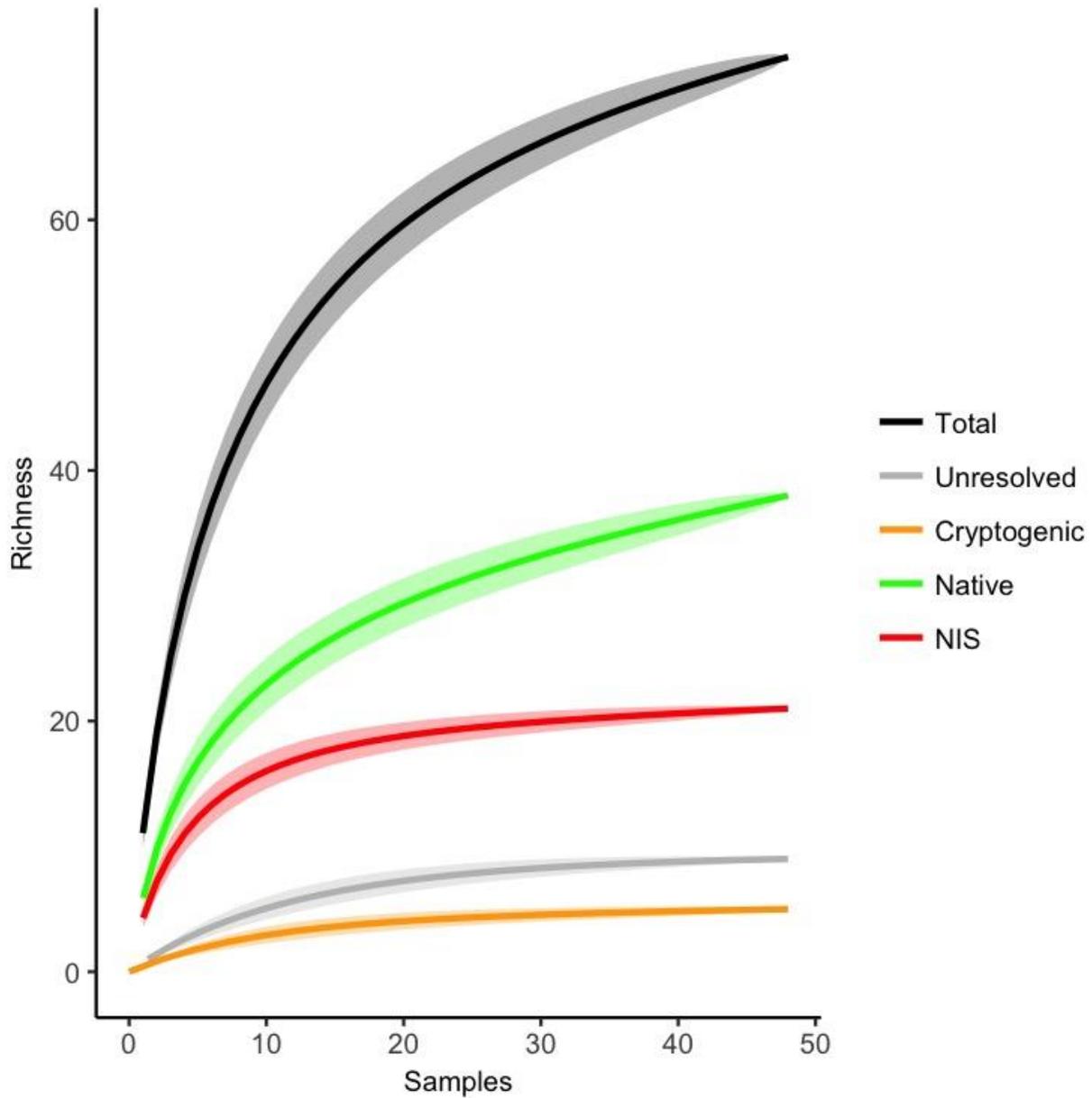


Figure 3.1. Species accumulation curves by invasion status for San Francisco Bay, 2014. Status is designated based on literature and SERC NEMESIS database. Here, a sample represents a grab taken at 5 locations in each of 10 sites in the high salinity region of the Bay (n=50 grabs total). NIS asymptote agrees with species richness estimators (see Table 3.1). Shading around each line represents 1SD.

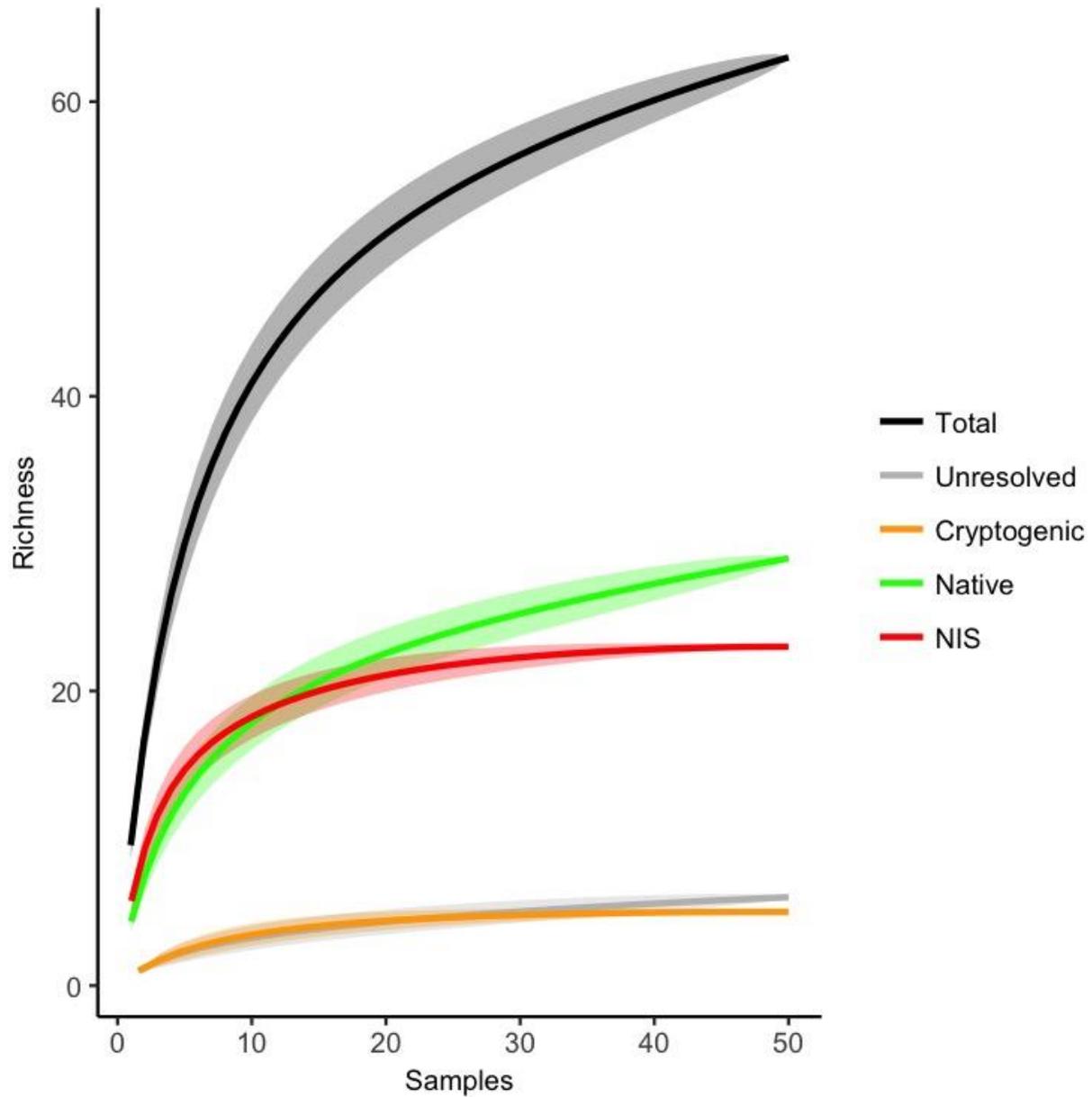


Figure 3.2. Species accumulation curves by invasion status for San Francisco Bay, 2015. Status is designated based on literature and SERC NEMESIS database. Here, a sample represents a grab taken at 5 locations in each of 10 sites in the high salinity region of the Bay (n=50 grabs total). NIS asymptote agrees with species richness estimators (see Table 3.1). Shading around each line represents 1SD.

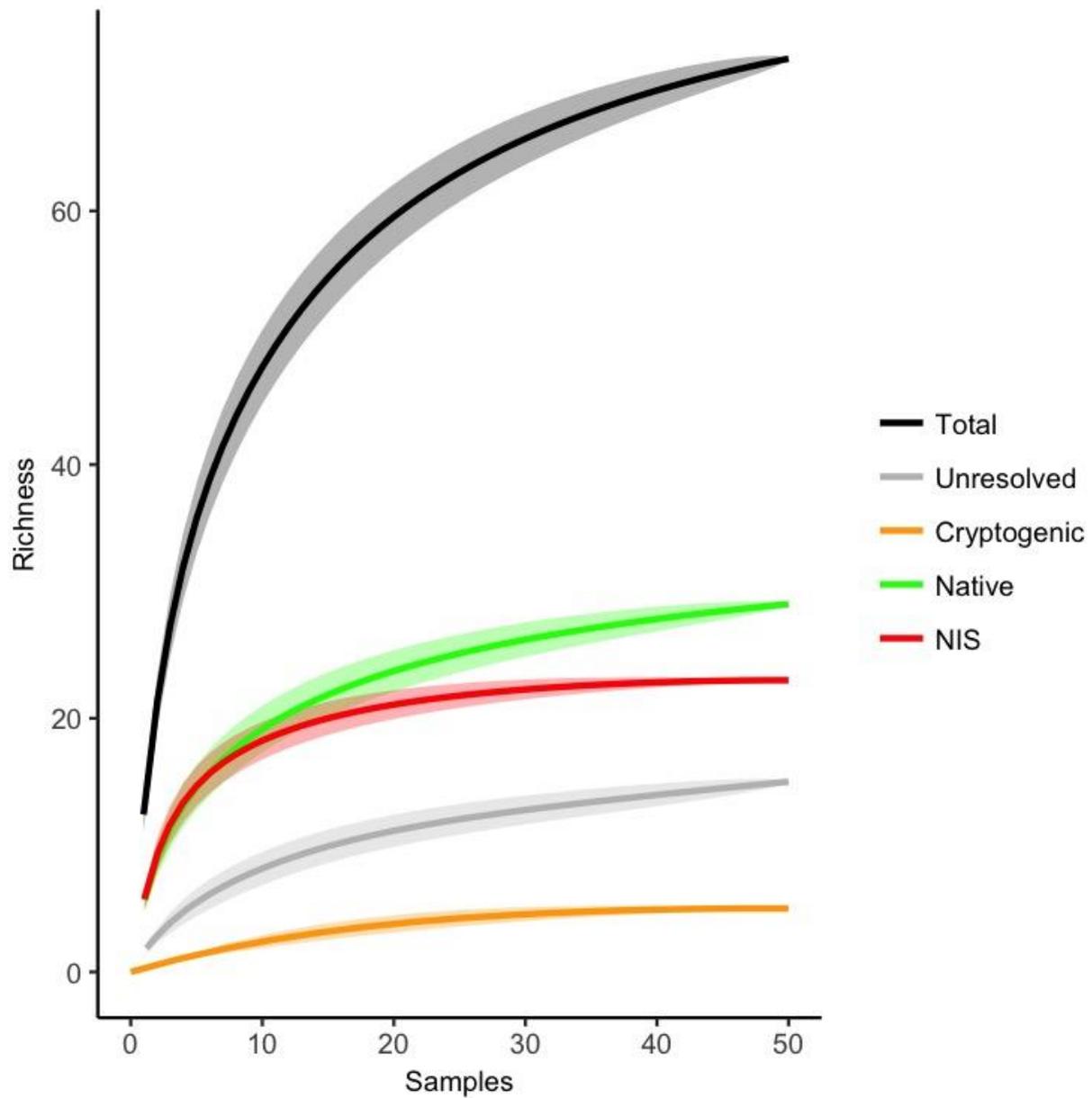


Figure 3.3. Species accumulation curves by invasion status for San Francisco Bay, 2016. Status is designated based on literature and SERC NEMESIS database. Here, a sample represents a grab taken at five locations in each of 10 sites in the high salinity region of the Bay (n=50 grabs total). NIS asymptote agrees with species richness estimators (see Table 3.1). Shading around each line represents 1SD.

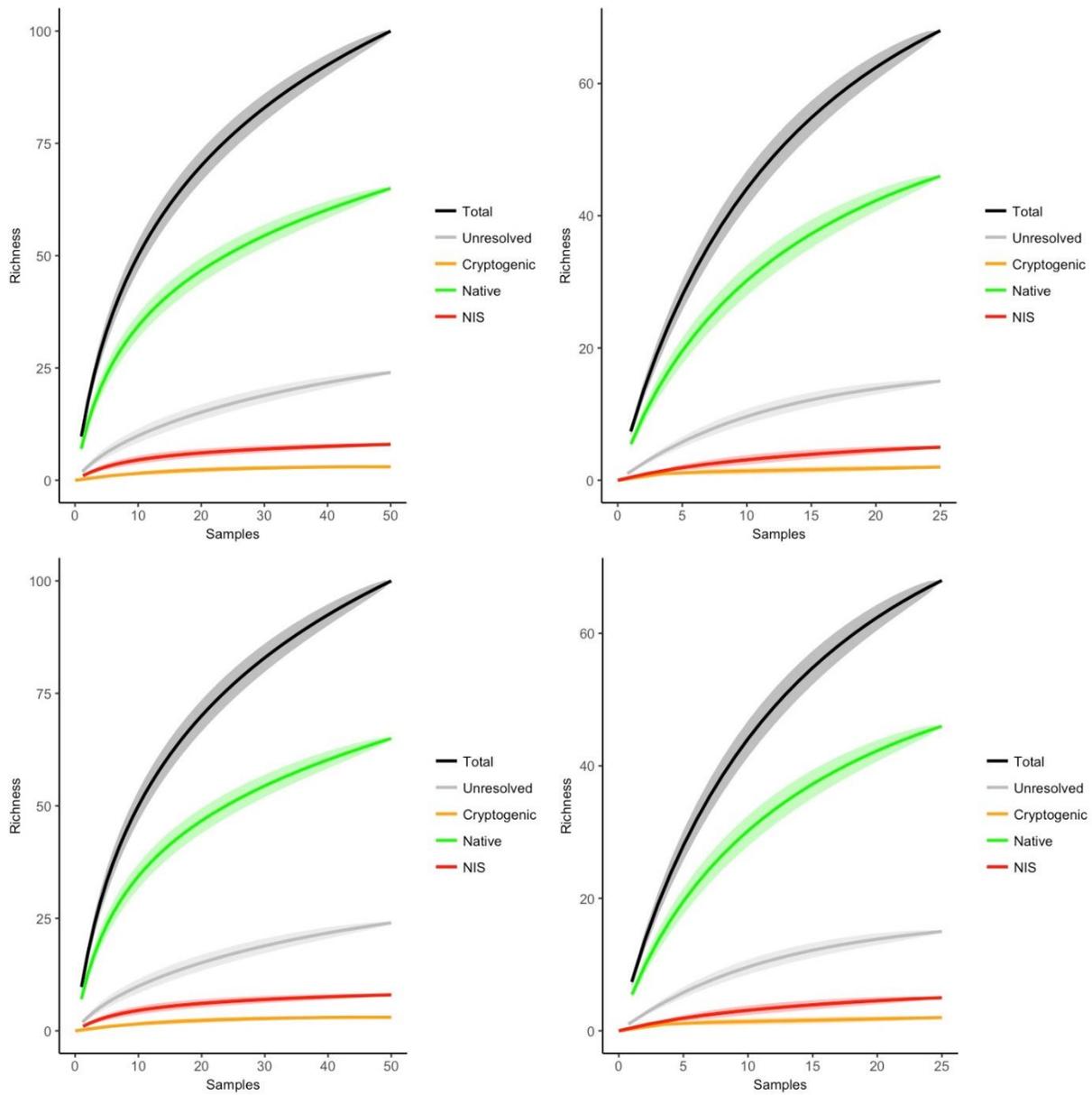


Figure 3.4. Species accumulation curves by invasion status for Humboldt Bay, 2015, across all depths (top left), intertidal (top right), shallow subtidal (bottom left), and deep depths (bottom right). Status is designated based on literature and SERC NEMESIS database. NIS asymptote across all depths agrees with species richness estimators (see Table 3.1). Shading around each line represents 1SD.

Table 3.1. Species richness estimators by invasion status for Humboldt Bay in 2015 and San Francisco Bay in 2014, 2015, and 2016.

Bay	Year	Status	Species	Chao	Chao SE	Jack1	Jack1 SE	Jack2	Boot	Boot SE	n
HB	2015	Cryptogenic	8	8.49	1.31	8.99	0.99	9	8.52	0.62	100
HB	2015	Native	87	121.38	18.83	111.75	5.69	127.52	97.86	3.07	100
HB	2015	NIS	15	15.16	0.53	15.99	0.99	14.06	15.93	0.85	100
HB	2015	Total	137	169.49	13.84	174.62	8.18	190.52	154.7	4.83	100
HB	2015	Unresolved	36	42.66	5.26	46.89	3.57	48.94	41.39	2.14	100
SF	2014	Cryptogenic	8	8	0.46	8.98	0.98	9.94	8.46	0.56	50
SF	2014	Native	41	60.75	17.07	51.77	4.72	59.5	45.58	2.4	50
SF	2014	NIS	24	24.98	1.84	25.96	1.38	26	25.01	0.93	50
SF	2014	Total	76	91.74	10.82	90.69	5.11	98.5	82.75	2.84	50
SF	2014	Unresolved	12	12.24	0.72	12.98	0.98	12.06	12.71	0.72	50
SF	2015	Cryptogenic	8	8	0	8	0	7.06	8.18	0.4	50
SF	2015	Native	32	42.45	10.08	39.84	3.41	44.7	35.35	1.84	50
SF	2015	NIS	26	28.2	3.33	28.94	1.7	29.94	27.44	1.02	50
SF	2015	Total	66	77.83	8.65	78.74	4.29	84.64	71.83	2.43	50
SF	2015	Unresolved	9	10.96	3.67	10.96	1.39	11.94	9.87	0.76	50
SF	2016	Cryptogenic	8	8	0	8	0	5.18	8.42	0.65	50
SF	2016	Native	32	35.06	3.59	36.9	2.19	37.94	34.44	1.35	50
SF	2016	NIS	26	26	0	26	0	22.24	26.58	0.77	50
SF	2016	Total	75	79.08	3.48	84.8	3.1	83.12	80.44	2.47	50
SF	2016	Unresolved	18	30.25	16.8	22.9	2.19	26.76	20	1.13	50

Percent Contribution of NIS to Total Species Richness in San Francisco and Humboldt Bays.

San Francisco Bay had a much higher average proportion of NIS making up total species richness relative to Humboldt Bay (Figures 3.5 to 3.8). NIS contributed up to 36.5% of total observed species richness in San Francisco Bay (Figures 3.5 to 3.7), but just 10% of total observed species richness in Humboldt Bay (Figure 3.8). The difference between the two Bays is due to opposite patterns of native and NIS richness: Humboldt had a high number of native species and few NIS, whereas San Francisco Bay had fewer native species and more NIS.

Across depths in Humboldt Bay, NIS made up a relatively small percentage of overall species richness. In general, NIS were slightly more prevalent at shallower depths than deeper in Humboldt Bay, with intertidal sites having the greatest proportion of NIS (Figure 3.8). NIS were absent entirely from the shallow and deep sites at one location (US Coast Guard). Unresolved taxa made up a fairly constant proportion of overall species richness across sites and depths.

The percent contribution of NIS to overall richness was relatively constant among sites in San Francisco Bay in 2014 and 2016. In 2015, NIS made up a disproportionately larger percentage of overall richness, with greater variation among sites, a change that may have been related to a marine heat wave that occurred that year (Cavole *et al.* 2016).

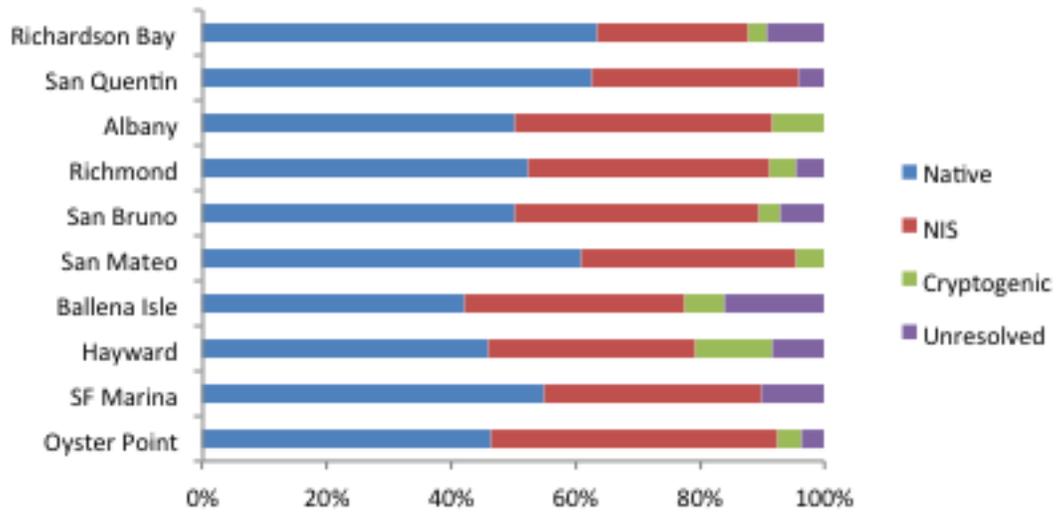


Figure 3.5. San Francisco Bay, 2014. Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site and depth.

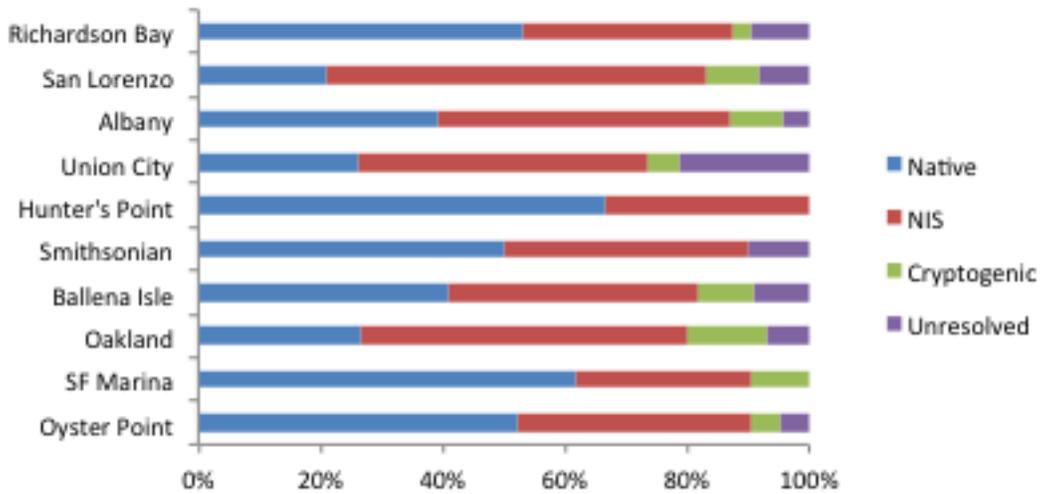


Figure 3.6. San Francisco Bay, 2015. Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species for low salinity (bottom) and high salinity (top) sites.

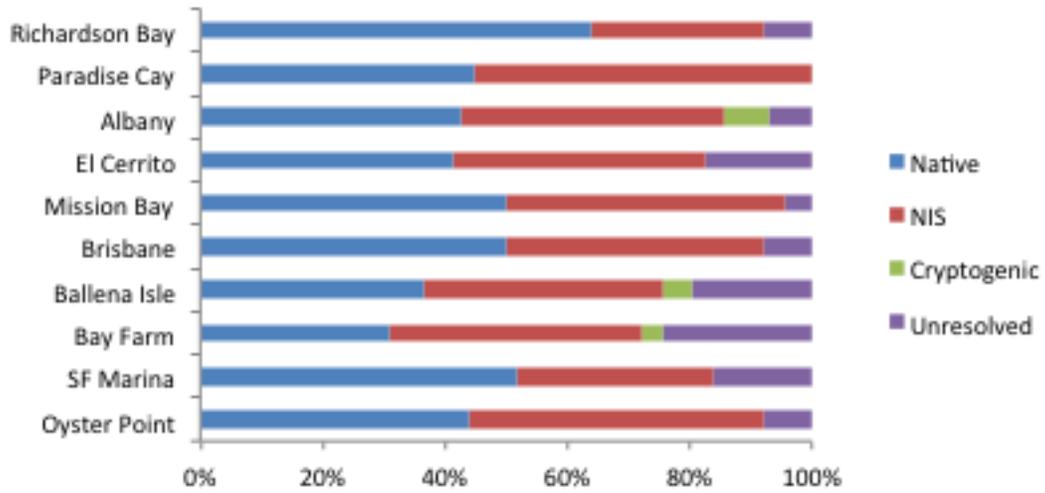


Figure 3.7. San Francisco Bay, 2016. Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site.



Figure 3.8. Humboldt Bay, 2015 Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site.

Relative Abundance of NIS per Site in San Francisco and Humboldt Bays. Overall, NIS made up a large percentage of the individuals found in San Francisco Bay, but not Humboldt Bay. In San Francisco Bay, NIS made up a much larger percentage of individuals in our grab samples than native species, reaching a maximum abundance of 93% of all individuals (Figures 3.9 to 3.12).

There was significant variation among sites and years in San Francisco Bay in the relative abundance of NIS, which ranged from 29% to 93% (Figures 3.9 to 3.11).

Although NIS made up a relatively small proportion of all individuals sampled in Humboldt Bay, we found higher NIS abundance at sites closer to the back portion of the Bay. In contrast, NIS abundance was very low at sites closer to the mouth of the Bay (Figure 3.12). We also found that NIS abundance in Humboldt Bay was greatest at intertidal sites and declined with depth, making up the smallest percentage of all organisms at deep locations (Figure 3.12).

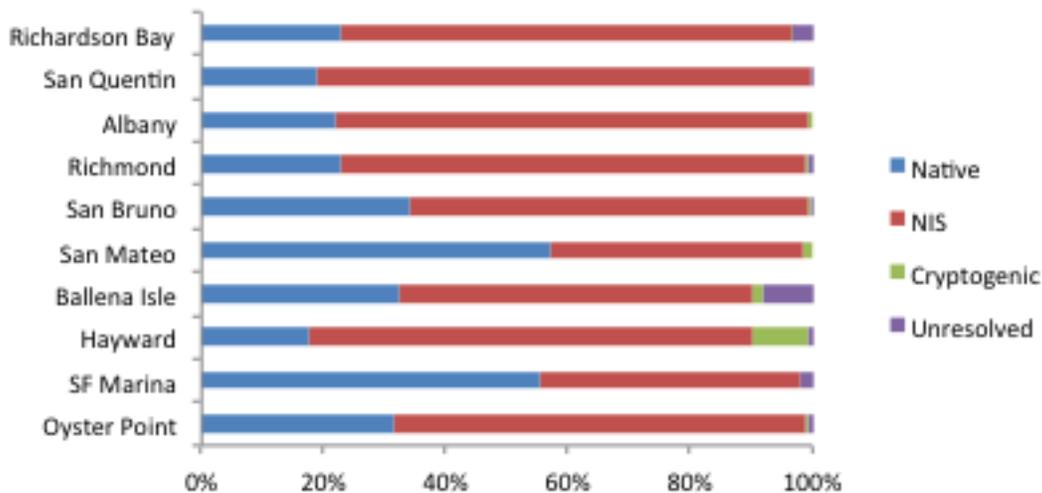


Figure 3.9. San Francisco Bay, 2014. Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site and depth.

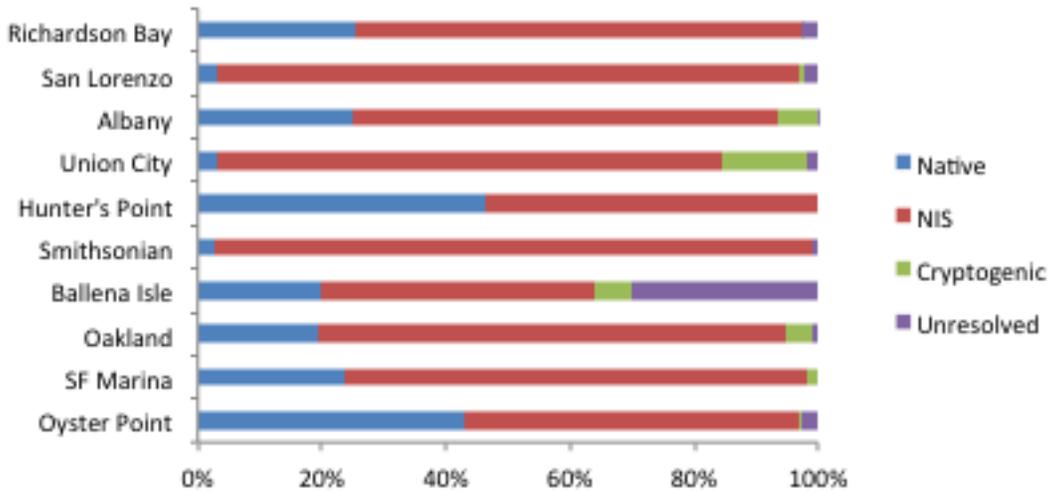


Figure 3.10. San Francisco Bay, 2015. Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species for low salinity (bottom) and high salinity (top) sites .

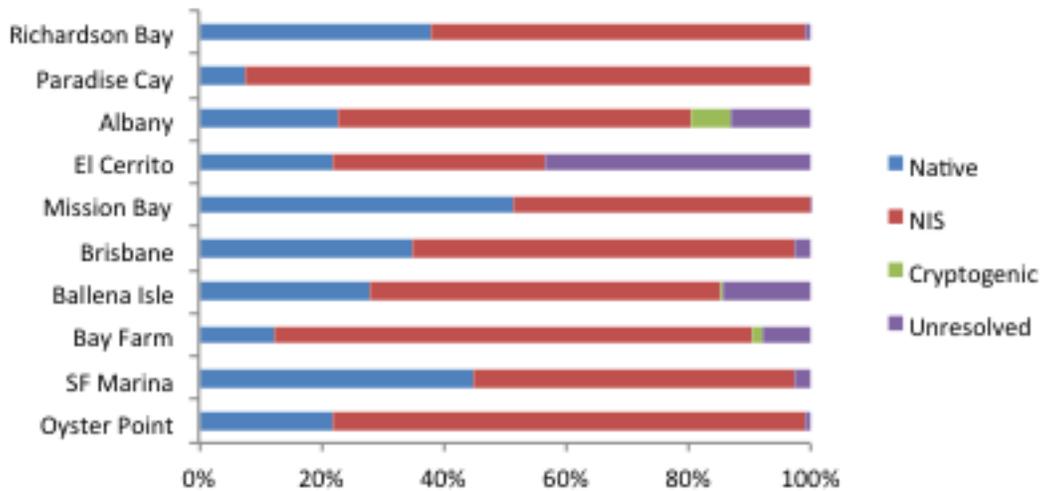


Figure 3.11. San Francisco Bay, 2016. Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site.

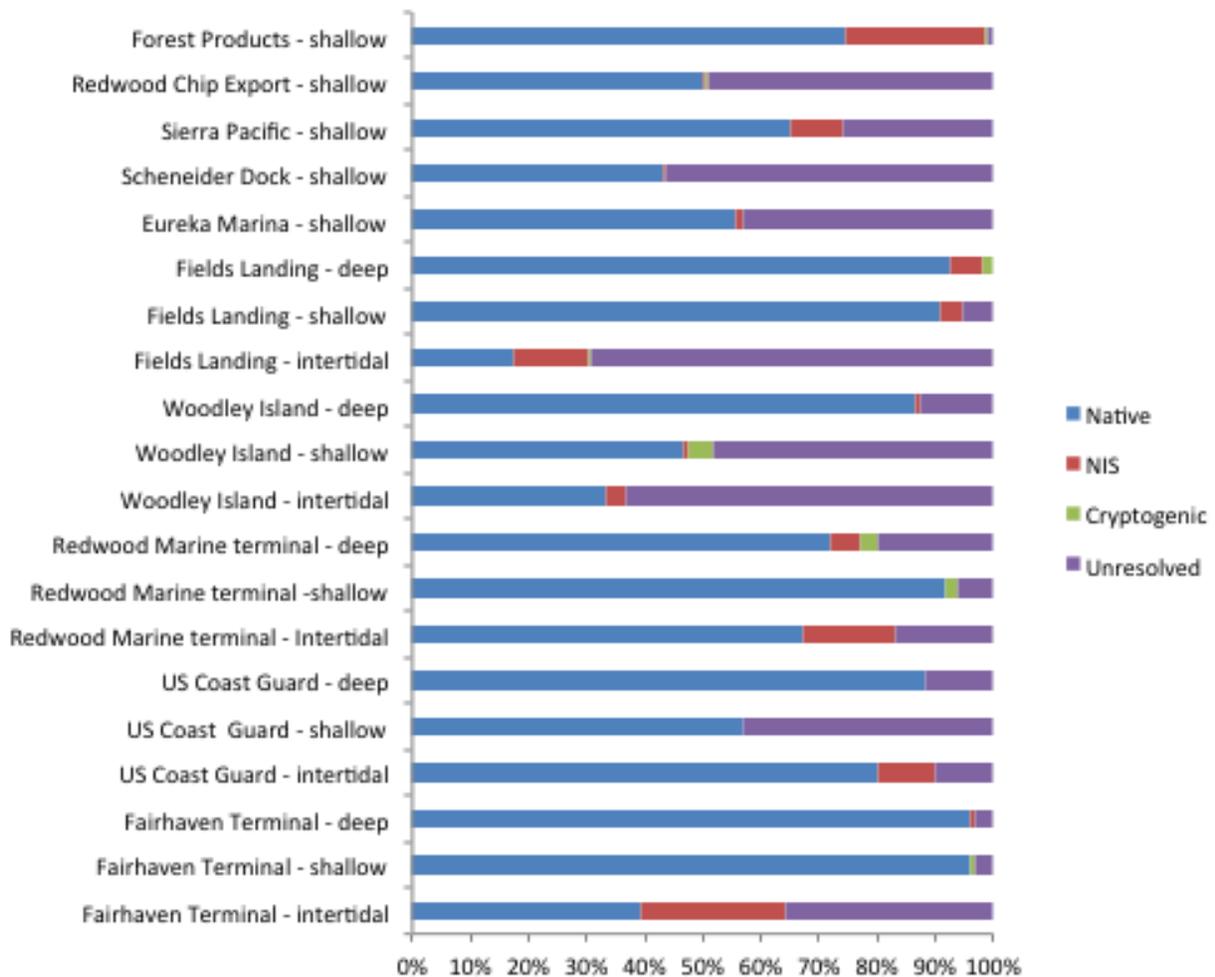


Figure 3.12. Humboldt Bay, 2015 Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site.

C. Newly Detected NIS across Bays and Years for the Soft Sediment Community

A total of 2 taxa were detected that appear to be new records for Humboldt Bay in 2015, using morphological taxonomy in the present study, and no new species were detected in the San Francisco Bay surveys (shallow subtidal zone surveyed in three years, 2014–2016). Importantly, no taxa new to California were detected morphologically in soft sediment habitats across these bays during the present study.

We found the Asian cephalaspidean gastropods *Philine auriformis* and *P. orientalis* in Humboldt Bay in grab samples from 2 and 3 out of 10 sites, respectively. Each species occurred in low abundance. *P. auriformis* was previously recorded in Coos Bay (1998). *P. orientalis* was previously found in Bodega Bay in 1998, and in San Francisco and Tomales Bays in 2004.

We consider these records to be tentative new records for Humboldt Bay, pending further confirmation, with caution engendered by *Philine*'s tendency to fluctuate greatly in population size, and its occurrence in deeper waters subtidally and offshore. In that context, it seems likely that neither record represents a significant range expansion along the coast.

The relative lack of new records is surprising given the spatial and temporal scale of these sampling efforts, including three years in San Francisco Bay, and detailed morphological analyses. The results suggest that the rate of invasion or detection may be quite variable over time, or perhaps that a shift in invasion rates has occurred since previous analyses of invasion detection encompassing soft sediment were performed in the late 1990s. Repeated sampling over time in each Bay will help determine whether this is a lasting or ephemeral pattern.

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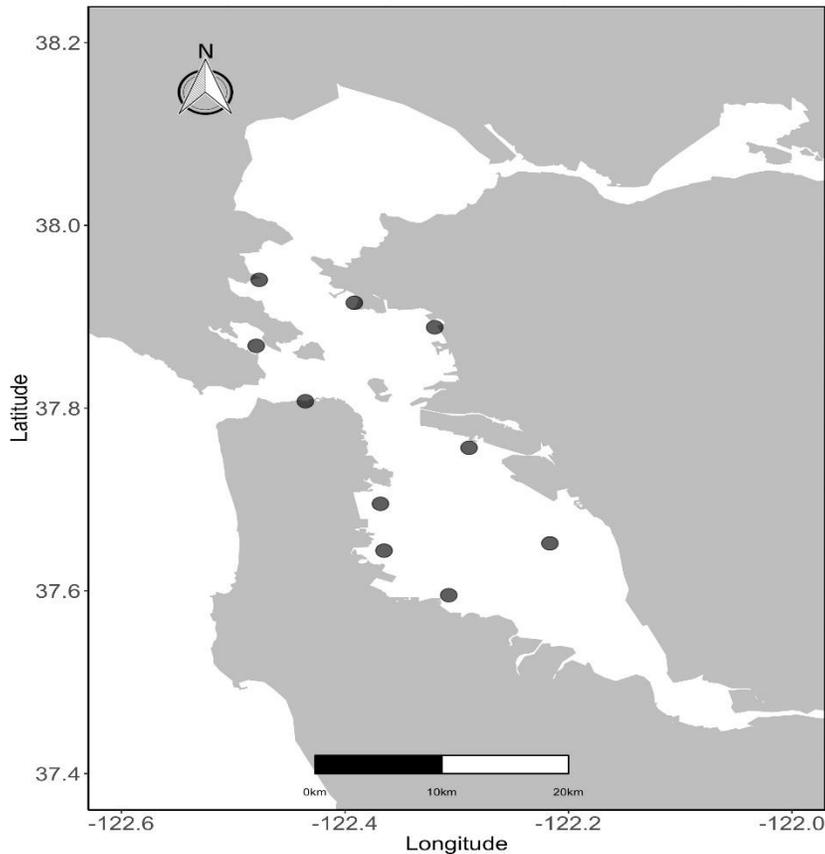
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Appendix 3.1: Survey Locations by Estuary and Year

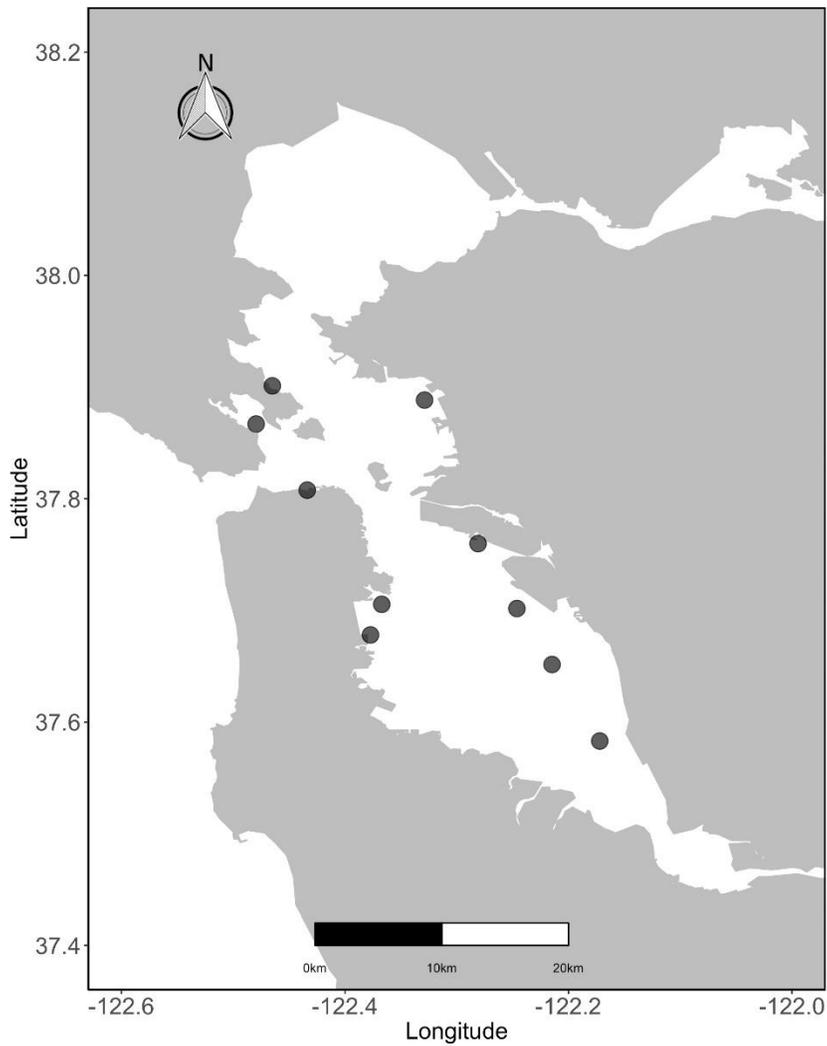
The maps and tables below indicate locations and dates for soft sediment surveys for each estuary and year.

San Francisco Bay 2014



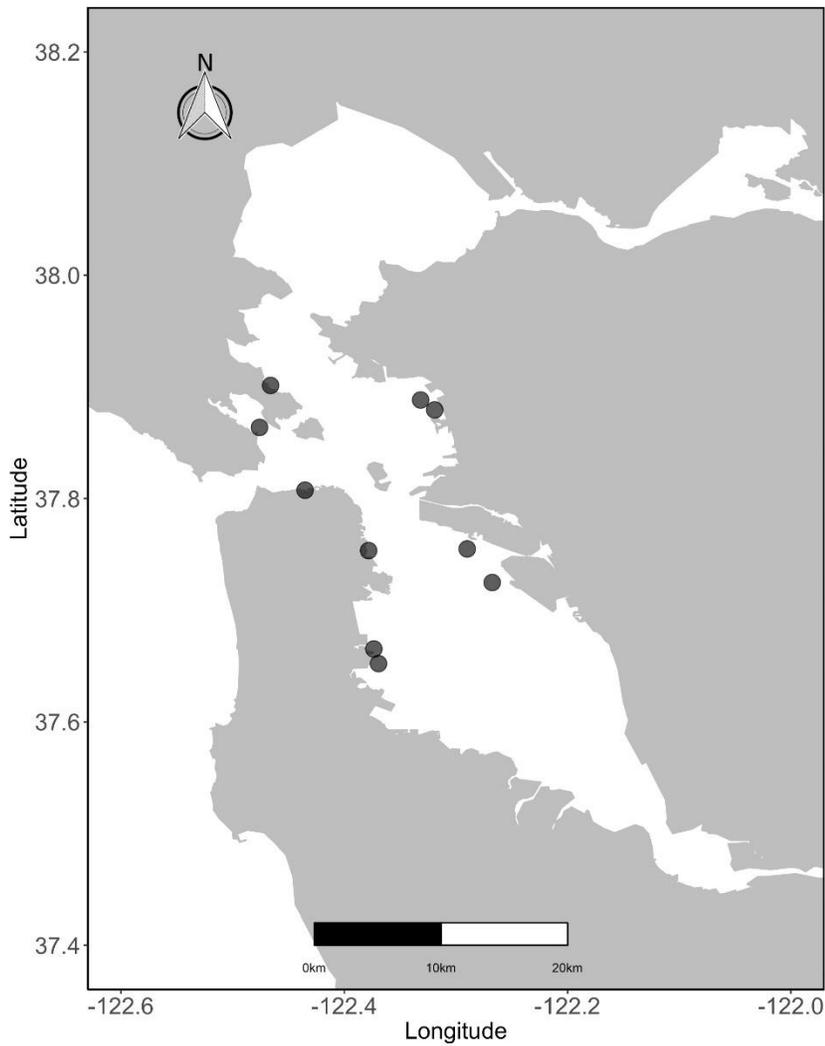
Site	Latitude	Longitude	Depth (m)	Salinity (‰)	Temperature (°C)
San Mateo	37°35.715'N	122°18.431'W	2.3	31.10	19.13
San Bruno	37°38.642'N	122°21.906'W	1.9	31.29	18.80
Albany	37°53.304'N	122°19.187'W	2.0	29.93	19.14
Hayward	37°39.122'N	122°13.003'W	1.8	30.85	19.94
San Quentin	37°56.424'N	122°28.608'W	2.0	29.18	18.47
Ballena Isle	37°45.395'N	122°17.342'W	2.3	31.07	20.72
Richmond	37°54.913'N	122°23.503'W	1.8	29.83	20.87
Oyster Point	37°41.718'N	122°22.098'W	2.0	31.29	17.77
San Francisco Marina	37°48.450'N	122°26.130'W	1.4	32.23	16.72
Richardson Bay	37°52.090'N	122°28.770'W	1.8	32.06	19.48

San Francisco Bay 2015



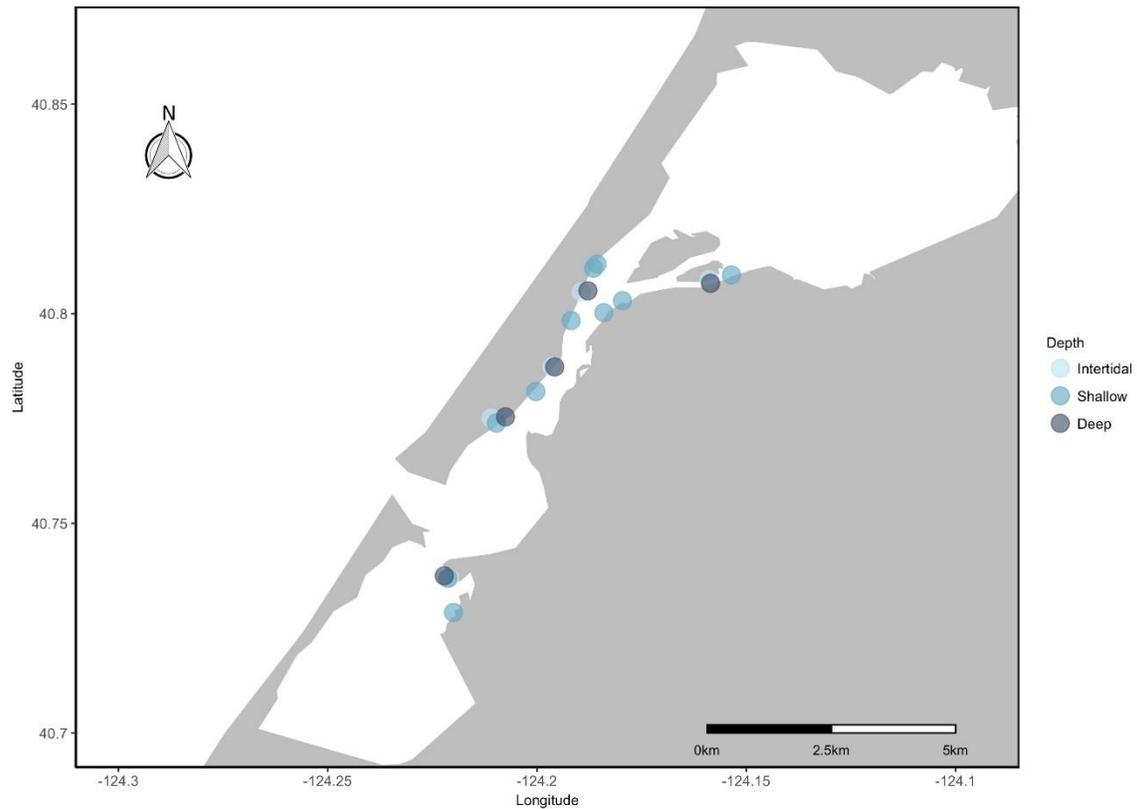
Site	Latitude	Longitude	Depth (m)	Salinity (‰)	Temperature (°C)
Union City	37°34.982'N	122°10.325'W	1.9	30.11	21.22
San Lorenzo	37°39.093'N	122°12.886'W	2.1	30.37	21.78
Albany	37°53.303'N	122°19.729'W	2.6	28.92	20.13
Hunter's Point	37°42.327'N	122°22.030'W	3.3	30.99	19.19
Oakland	37°42.100'N	122°14.773'W	2.0	30.31	18.19
Ballena Isle	37°45.589'N	122°16.859'W	2.3	30.69	20.01
Smithsonian	37°54.065'N	122°27.903'W	2.3	29.36	19.22
Oyster Point	37°40.681'N	122°22.634'W	3.5	30.86	18.40
San Francisco Marina	37°48.462'N	122°26.032'W	1.7	32.06	15.74
Richardson Bay	37°52.014'N	122°28.774'W	2.0	31.57	17.59

San Francisco Bay 2016



Site	Latitude	Longitude	Depth (m)	Salinity (‰)	Temperature (°C)
Brisbane	37.6522°N	122.3694°W	2.1	28.06	17.45
Bay Farm	37.7248°N	122.2676°W	2.1	24.58	18.64
Albany	37.8794°N	122.3191°W	2.8	27.68	17.36
Mission Bay	37.7534°N	122.3783°W	2.0	28.52	16.74
El Cerrito	37.8883°N	122.3317°W	2.2	27.68	17.36
Ballena Isle	37.7549°N	122.2900°W	2.0	25.00	18.46
Paradise Cay	37.9013°N	122.4661°W	1.9	21.90	18.62
Oyster Point	37.6653°N	122.3736°W	2.3	28.06	17.62
San Francisco Marina	37.8075°N	122.4352°W	2.0	29.18	16.00
Richardson Bay	37.8637°N	122.4760°W	2.4	29.28	15.86

Humboldt Bay 2015



Depth	Site	Latitude	Longitude	Depth (m)	Salinity (‰)	Temperature (°C)
Intertidal	Woodley Island	40.80816°N	124.15886°W	0.0	33.97	18.47
	Redwood Marine Terminal	40.80524°N	124.18944°W	0.0	34.06	17.10
	Fairhaven Terminal	40.78738°N	124.19643°W	0.0	33.52	16.54
	US Coast Guard	40.77512°N	124.21095°W	0.0	33.88	16.62
	Fields Landing	40.73701°N	124.22095°W	0.0	33.60	15.38
Shallow	Eureka Marina	40.80313°N	124.17957°W	2.2	34.17	18.70
	Schneider Dock	40.80027°N	124.18404°W	2.0	33.66	16.56
	Sierra Pacific	40.81184°N	124.18567°W	2.1	33.66	15.86
	Woodley Island	40.80928°N	124.15356°W	2.0	34.28	20.52
	Redwood Marine Terminal	40.81088°N	124.18650°W	2.0	34.38	19.24
	Redwood Chip Export	40.79839°N	124.19184°W	2.0	33.76	17.24
	Fairhaven Terminal	40.78147°N	124.20026°W	2.0	34.18	17.32
	US Coast Guard	40.77396°N	124.20970°W	2.0	34.36	17.62
	Fields Landing	40.73694°N	124.22128°W	2.0	33.98	15.64
	Forest Products	40.72877°N	124.21996°W	2.0	33.78	15.30
Deep	Woodley Island	40.80726°N	124.15854°W	4.8	34.60	20.76
	Redwood Marine Terminal	40.80548°N	124.18783°W	5.0	34.54	19.50
	Fairhaven Terminal	40.78732°N	124.19575°W	5.0	34.16	17.90
	US Coast Guard	40.77542°N	124.20753°W	5.0	34.42	17.90
	Fields Landing	40.73750°N	124.22215°W	5.0	34.00	16.84

Appendix 3.2. Taxa Identified Morphologically by Estuary and Year

San Francisco Bay 2014, 2015, 2016: Number of grabs (out of five replicates per site) in which each taxon was found, along with invasion status assigned based on literature and SERC's NEMESIS database.

	SPECIES STATUS	Albany			Ballena Isle			Bay Farm	Brisbane	El Cerrito	Hayward	Hunter's Point	Mission Bay	Oakland	Oyster Point			Paradise Cay	Richardson Bay			Richmond	San Bruno	San Francisco Marina			San Lorenzo	San Mateo	San Quentin	Smithsonian	Union City	
		2014	2015	2016	2014	2015	2016	2016	2016	2016	2014	2015	2016	2015	2014	2015	2016	2016	2014	2015	2016	2014	2014	2014	2015	2016	2015	2014	2014	2015	2015	
ANNELIDA																																
Capitellidae																																
Capitellidae	U				1																											
<i>Heteromastus filiformis</i> complex	I				1																				3							
<i>Heteromastus</i> sp.	C				1	3							1											1								
<i>Mediomastus</i> sp.	C						1																									
Cirratulidae																																
<i>Aphelochaeta monilaris</i>	N																															
<i>Cirriformia moorei</i>	N	4	4	5	4	4	4	2	5	4	5	4	5	4	5	5	5	4	2	1	1	1	5	4	1	2	3	3	1	2	2	
<i>Cirriformia</i> sp.	N																															
<i>Cirriformia spirabrancha</i>	N			1			1																									
Dorvilleidae																																
<i>Schistomeringos annulata</i>	N		3		1	5		1	1		1		3	3		1	4	4	2	1		1			1	1	1	3		1		
<i>Schistomeringos</i> sp.	N			3	4		5			3	1		3						1		2			1	1	1	3	3				
Eunicidae																																
<i>Marphysa</i> sp. C Harris	C				1			2			4			4													5	2				5
Glyceridae																																
<i>Glycera americana</i>	N					1			1				1					4		1	1				1	1			1	3	1	
<i>Glycera</i> cf. <i>oxvephala</i>	U						1																									
Goniadidae																																
<i>Glycinde picta</i>	N	5	4	4	3	5	3	1	4	1	2	3			1	5	3	5	4	3	5	1		5	4	5	5		3	4	1	
<i>Glycinde</i> sp.	U				1					1																						
Lumbrineridae																																
<i>Scotetoma tetraura</i> complex	N	1		2	1	3	3	2	5		1	4	5			4	5	5			1	2		5	3	4		1	2			
Maldanidae																																
<i>Sabaco elongatus</i>	I	5	5	5	3	3	2	1	5	5	2	4	1		5	5	4	5	4	2	1	5	5				1	1	4		2	
Nephtyidae																																
<i>Nephtys caecoides</i>	N	1	3	2				1												2	3	1			2	3						
<i>Nephtys ferruginea</i>	N																															
Nereididae																																
<i>Onuphis iridescens</i>	N																	2	1										2			
<i>Platynereis bicanaliculata</i>	N																		2	2	2								2			
Opheliidae																																
<i>Armandia brevis</i>	N										1																					
Orbiniidae																																
<i>Leitoscoloplos pugetensis</i>	N	5	5	5	3	2	5	1	1	5	3		5			2	5	4	2	3	5	4	1	4	4	5	3	3	3	5		
Phyllodoceidae																																
<i>Eumida bifoliata</i>	N										1																					
<i>Phyllococe longipes</i>	C																		2				1									
Polynoidae																																
<i>Harmothoe imbricata</i> complex	N	3	5	4	5	4	5	3	5	5	5	3	3	5	1	5	5	5	5	2	2	4	3	3	1	2	5	3	5	2	2	
<i>Hesperoneo adventor</i>	N																															
<i>Hesperoneo</i> sp.	N														1																	
<i>Malmgreniella</i> sp. SF1	U																				1				1							
Sabellidae																																
<i>Euchone limnicola</i>	N	4				1		3			4	5			3	3	2	4	5	4		2		1	2			5	2			
Syllidae																																
<i>Megasyllis nipponica</i>	I	2	1	3		3									1	5		1	1		1	1	1	2	4	2		4	2			
Terebellidae																																
<i>Amaeana occidentalis</i>	N																							2	5	3		1				
<i>Amaeana</i> sp. A Harris	I	1			1						4	4			4	3						4		2	2	2		1	2	5		
<i>Neoamphitrite</i> sp. A Harris	N	1	2	4		3				1						1						1										
<i>Polycirrus californicus</i> complex	N																															
<i>Polycirrus</i> sp.	U				5																			3			5					
<i>Polycirrus</i> sp. SF2	U					3	2																									
ARTHROPODA																																
Amphipoda																																
<i>Americhelidium pectinatum</i>	N			3		1			2			3	1			3		4	5	5	1											
<i>Ampelisca abdita</i>	I	1	3			4		1	5	5	1		3	5		2	5	5	4	2	1	5	5	5	5	5	5	1	5	2		
<i>Ampithoe laceriosa</i>	N			1																												
<i>Ampithoe valida</i>	I																															
Cumacean	U																															
<i>Grandidierella japonica</i>	I	1		4					4	5			1	2		5	5			1		3			3	4	1	1	1	1		
<i>Liljeborgia geminata</i> complex	C																							2	2	2						
<i>Listriella</i> cf. <i>goleta</i>	N																															
<i>Monocorophium acherusicum</i>	I		1			5	3																	3			5					
Oedicerotidae	U																															
<i>Paradexamine</i> sp.	U			4						1																						
<i>Photis brevipes</i>	N																															
<i>Sinocorophium alienense</i>	I			2		1			3	1			2						5	4	1	1					1		1			
<i>Sinocorophium heteroceratum</i>	I	4	5	4	3	3			5	5		4	5		5	5	5	2	5	4	5	5	5	5	5	5	5	5	5	5	3	
Caprellidae																																
<i>Caprella californica</i>	N																			5	2											
<i>Caprella mutica</i>	I																															
<i>Caprella scaura</i>	I	1	1	5						5								1	3													

	SPECIES STATUS	Albany			Ballena Isle			Bay Farm	Brisbane	El Cerrito	Hayward	Hunter's Point	Mission Bay	Oakland	Oyster Point			Paradise Cuy	Richardson Bay			Richmond	San Bruno	San Francisco Marina			San Lorenzo	San Mateo	San Quentin	Smithsonian	Union City				
		2014	2015	2016	2014	2015	2016	2016	2016	2016	2014	2015	2016	2016	2015	2014	2015	2016	2016	2014	2015	2016	2014	2014	2014	2015	2016	2015	2014	2014	2015	2015			
Decapoda																																			
<i>Cancer productus</i>	N			1					1							1				1															
<i>Cancer sp.</i>	U								1																										
<i>Crangon nigricauda</i>	N	1	2		1	2	1	1		1	3			2					2	1	1		3									4			
<i>Glebocarcinus oregonensis</i>	N				1	2	1				1			1					2	1	3						1			3		4			
<i>Hemigrapsus oregonensis</i>	N	2			3	1	1			2	1			1					5	1		4	4									1			
<i>Heptacarpus simpsoni</i>	N																																		
<i>Lophopanopeus bellus</i>	N						2																												
<i>Metacarcinus magister</i>	N										2																								
<i>Palaemon macrodactylus</i>	I				1					1				2													1								
<i>Pinnixa franciscana</i>	N					1								1																					
<i>Pyromaia tuberculata</i>	N					1								1		3																			
<i>Scleroplax granulata</i>	N				1		1				2			1		2						1	2												
<i>Upogebia pugettensis</i>	N								1																	1									
Isopoda																																			
Cirolanidae	U																						1												
<i>Idotea resicata</i>	N																																		
<i>Paranthura japonica</i>	I		1	3			4	2												4	1	3						3							
Pycnosonida																																			
<i>Ammonothea hilgendorfi</i>	C	1	3	4																															
Tanaidacea																																			
<i>Leptochelia sp.</i>	U		1			1																													
BRYOZOA																																			
Bryozoa																																			
<i>Anguinella palmata</i>	I												1	1																					
<i>Aspidelectra melolontha</i>	I					1																													
Calloporidae	U				1	1				2																									1
<i>Celeporella hyalina</i>	C					2																													
<i>Conopeum sp.</i>	U					5		1																											
<i>Cryptosula pallasiana</i>	I					1		1			2																								
<i>Electra sp.</i>	U					4																													
<i>Electra venturaensis</i>	N					3			2																										
<i>Hincsiina sp.</i>	U					1			2																										1
<i>Schizoporella errata</i>	I					2		1					2																					4	
<i>Smittioidea prolifica</i>	I					3		2	1				1																					4	
CNIDARIA																																			
Anthozoa																																			
Actinaria	U				4	5	3	4	5				2		4	3				2															1
<i>Stylatula elongata</i>	N		1																																
<i>Zoalutis actius</i>	N	3			5					3	3	5	2	1	4	3				5	1	2	1	5	4	1	2	1	3						
Ctenophora																																			
Ctenophora	U		3			5	1	2	4																										
Hydrozoa																																			
Hydrozoa	U				1										3																				
ECHINODERMATA																																			
Asterozoa																																			
<i>Amphipholis squamata</i>	C				3	2				1				2																					
<i>Amphipodia cf. urtica</i>	U					1																													
Amphiuridae	U						1																												
MOLLUSCA																																			
Bivalvia																																			
<i>Cryptomya californica</i>	N	2			2	1	5	1	5	1	1		2		1	4	3				3		2												1
<i>Leukoma staminea</i>	N																																		
<i>Lyonsia californica</i>	N	2	1	2	3			1	2						1																				
<i>Macoma inquinata</i>	N																																		
<i>Macoma petalum</i>	I																																		
<i>Musculista senhousia</i>	I		2	4	4	4	5	1	4	1	4	2	1	3		5	4			2			3												
<i>Ostrea lurida</i>	N										2																								
<i>Tagelus subteres</i>	N																																		
<i>Theora lubrica</i>	I	1	3	5		1	3		3	4		4	3		5	1	5			2	5		1			1	2	4	1				3		
<i>Venerupis philippinarum</i>	I				4	3	5	3	5	1	5	1	1	5	2	4	3	1		3	1	1	2			3	3	3	5	2				5	
Gastropoda																																			
<i>Crepidula convexa</i>	I				4	4	4	2					3																						
<i>Crepidula plana</i>	I				2	1	3	1																											
<i>Philine orientalis</i>	I	2	1	2	1	2	4		2	2					2	1	4																		
<i>Phyllaplysia taylora</i>	N																																		
NEMERTEA																																			
Nemertea																																			
Nemertea	U				2																														
UROCHORDATA																																			
Tunicata																																			
<i>Molgula manhattensis</i>	I	1	3	4	5	2							1	1																					

Humboldt Bay 2015: Number of grabs (out of five replicates per site and depth) in which each taxon was found, along with invasion status assigned based on literature and SERC's NEMESIS database.

	SPECIES STATUS	Eureka Marina			Fairhaven Terminal			Fields Landing			Forest Products	Redwood Chip Export	Redwood Marina			Schneider Dock	Sierra Pacific	US Coast Guard			Woodley Island		
		Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow		
																						Shallow	Deep
ANNELIDA																							
Arenicolidae																							
Arenicolidae	U	1																					
Capitellidae																							
Heteromastus filobranchus	N	3				3	4		2	5		1		5	1	1							
Heteromastus cf. filobranchus	N	1																					
Heteromastus sp.	N		1	2	1											3		5			1		
Cirratulidae																							
Aphelocheata cf. glandaria	U									1													
Cirratulidae	U								1														
Cirratulus sp.	U						1	1				2			2					1	4	2	
Cirriformia sp.	N																						
Polycirrus sp.	U											1											
Dorvilleidae																							
Dorvillea longicoris	N												1										
Dorvillea sp.	U	1										3	1		3								
Glyceridae																							
Glycera americana	N		1		1										1								
Glycera tenuis	N		1		3											5		3					
Gonianidae																							
Glycinde picta	N	4			1	1	3	4	2	5		4	5	3	2	1		1	1	1	3		
Lumbrineridae																							
Drilonereis sp.	U											2		4							2		
Lumbrineris californiensis	N		1																				
Scotetoma luti	N						1			2					1								
Scotetoma sp.	U											2											
Nephtyidae																							
Nephtys ferruginea	N														1								
Nephtys caecoides	N		2		1	2	3	2	4	4		1	3	3	1	1	3		4	2	4		
Nereididae																							
Nereis sp.	U													2									
Nereis vexillosa	N				1							1											
Platynereis bicanaliculata	N	1		1				2	4	2	1	1	3		2						1		
Syllidae	U											1											
Orbiniidae																							
Letoscoloplos pugettensis	N		1		4	5	1	5	5	3	5	4	4	4	5	1		2	4		2		
Oweniidae																							
Owenia collaris	N								3														
Pectinariidae																							
Pectinaria californiensis	N											1	1										
Phyllodoctidae																							
Eteone cf. balboensis	N																				1		
Eteone spilotus	N																				2		
Eumida longicornuta	N							2					1										
Phyllodoce hartmanae	N	3			1		1						2	2									
Pilargidae																							
Pilargis berkeleyi	N														2								
Pilargis maculata	N												1										
Polynoidae																							
Halosydna brevisetosa	N											1						1					
Harmothoe imbricata complex	N																						
Hesperone laevis	N	1																					
Malmgreniella barsei	N	2								1				2							1		
Malmgreniella macginitiei	N													1									
Sabellidae																							
Euchone limnicola	N											1	2										

	SPECIES STATUS	Eureka Marina	Fairhaven Terminal			Fields Landing			Forest Products	Redwood Chip Export	Redwood Marina			Schneider Dock	Sierra Pacific	US Coast Guard			Woodley Island			
		Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow	
Spionidae																						
<i>Boccardia proboscidea</i>	N							1	2	3										4	2	
<i>Dipolydora caulleryi</i>	C																					
<i>Paraprionospio alata</i>	C	1																				
<i>Pygospio californica</i>	C																	1				
<i>Scolecopsis squamata complex</i>	C																					
Terebellidae																						
<i>Amaeona occidentalis</i>	N	2	1					2	4			3	1	3	4	3					2	
<i>Polycirrus sp.</i>	U																			1	1	
Trochochaetidae																						
<i>Trochochaeta franciscanum</i>	N												1									
ARTHIROPODA																						
Amphipoda																						
<i>Allorchestes angusta</i>	N					1								1							1	
<i>Americhelidium shoemakeri</i>	N																					
<i>Amphioe lacertosa</i>	N					2	1		1			1			1							
<i>Amphioe valida</i>	I											3									2	
<i>Aogammarus pugettensis</i>	N											1										
<i>Eohaustorius washingtonianus</i>	N		1																			
<i>Grandidierella japonica</i>	I						5	1	4			1	4	1	5					1	1	1
<i>Maera similis</i>	N											1										
<i>Megamoera subtener</i>	N					1			1			4										
<i>Monocorophium acherusicum</i>	I								1				1									
<i>Monocorophium insidiosum</i>	I						1		4			1			1						1	
<i>Paracorophium lucasi</i>	I	2				2	4	3	3			3	3			4						
<i>Pontogeneia rostrata</i>	N						1		3													
<i>Protomedea prudens</i>	N	1			2	1	2	3				1						1				
<i>Rhepoxynetus sp.</i>	U						1	2														
Caprellidae																						
<i>Caprella californica</i>	N				1	2	5	4	4	1	1	1			5					1	1	
<i>Caprella cf. alaskana</i>	I														1							
<i>Caprella septentrionalis</i>	N									1												
<i>Caprella sp. B</i>	U		1			3												1				
Cirripedia																						
<i>Balanus crenatus</i>	N						1					2		2		2			3		1	
Cumacea																						
<i>Lamprops augustinenis</i>	N																2		1			
Decapoda																						
<i>Cancer productus</i>	N					1			2	1	3									1		
<i>Cancer sp.</i>	U																			1		
<i>Crangon nigricauda</i>	N	3				2	1	2	1					2	1					1	1	
<i>Emerita analoga</i>	N																					
<i>Hemigrapsus oregonensis</i>	N	1							2			3			2			1			1	
<i>Heptacarpus simpsoni</i>	N													1								
Hippolytidae	U								1	1												
<i>Lophopanopeus bellus</i>	N												2									
<i>Metacarcinus magister</i>	N					2														1		
<i>Neotrypaea californiensis</i>	N				1																	
<i>Neotrypaea sp.</i>	U											1	1									
<i>Pagurus sp.</i>	U						1															
<i>Pinnixa shmitti</i>	U	3	1				1					1		1	3							
Pinnotheridae	U				1																	
<i>Pugettia gracilis</i>	N						1		1													
<i>Romaleon antennarium</i>	N					1	1	1	3	1			1		2		2				1	
<i>Scleroplax granulata</i>	N											1	1		1		1				1	
Isopoda																						

	SPECIES STATUS	Eureka Marina	Fairhaven Terminal			Fields Landing			Forest Products	Redwood Chip Export	Redwood Marina			Schneider Dock	Sierra Pacific	US Coast Guard			Woodley Island		
		Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow
Isopoda																					
	<i>Exirolana linguifrons</i>	N						3			2				3						
	<i>Idotea rufescens</i>	N																			
	<i>Paracercis cordata</i>	N							1					1							
Tamidaea																					
	<i>Leptochelia sp.</i>	U				3								1						1	
BRYOZOA																					
Brvozoa																					
	<i>Celleporella hyalina</i>	C				2					4										
	<i>Electra sp.</i>	U					1				3										
	<i>Microporella californica</i>	N				2					4										
	<i>Parasmittina collifera</i>	N									1										
	<i>Parasmittina sp.</i>	U									2										
	<i>Tegella sp.</i>	U									1										
	<i>Watersipora subtorquata</i>	I	3							1											
CNIDARIA																					
Anthozoa																					
	Anthozoa	U								1	1										
CTENOPHORA																					
Ctenophora																					
	Ctenophora	U			1																
ECHINODERMATA																					
Asterozoa																					
	<i>Amphipholis squamata</i>	C								1											
	<i>Amphipoda cf. occidentalis</i>	U						1													
	<i>Amphipoda digitata</i>	N	1										2								
	<i>Amphipoda occidentalis</i>	C			1			2				1									
	<i>Ophiotrix spiculata</i>	N	1																		
Echinozoa																					
	<i>Dendroaster excentricus</i>	N																		1	
	<i>Paracaudina chilensis</i>	I			3																
MOLLUSCA																					
Bivalvia																					
	<i>Clinocardium nuttalli</i>	N					1	1			1			2	2		2		2		
	<i>Cryptomya californica</i>	N								1	3					1					
	<i>Leukoma staminea</i>	N					1	2	2										2		
	<i>Lyonsia californica</i>	N	1				1		1			3	1	2					1	2	
	<i>Macoma nasuta</i>	N	5	3	2	4	5	4	5	5	3	5	5	4	5	2		2	3	3	
	<i>Macrotoma sp.</i>	U					3	3	4	2	1		2	1				3	1	2	
	<i>Musculista senhousia</i>	I		1							1							1			
	<i>Mytilus trossulus</i>	N							1												
	<i>Saxidomus nuttalli</i>	N							3									1		1	
	<i>Saxidomus sp.</i>	U								1											
	<i>Solen sicarius</i>	N	1										3	1					1	1	
	<i>Tellina bodegensis</i>	N								2			2	1					2		
	<i>Venerupis philippinarum</i>	I				1	1										1				
Gastropoda																					
	<i>Alia carinata</i>	N			1				4	2		3		3		1	1				
	<i>Cylichna attonsa</i>	N	1																1		
	<i>Dirona picta</i>	N								2			1						1		
	<i>Lacuna marmorata</i>	N																	1		
	<i>Nassarius mendicis</i>	N	3	2				1	2	2	3	1	2	1	3		1	2		1	
	<i>Olivella biplicata</i>	N		3													1	1	1		
	<i>Olivella pycna</i>	N	1	3				1						4	1		1	1			
	<i>Philine auriformis</i>	I	1											1				1			
	<i>Philine orientalis</i>	N	1															1			

	SPECIES STATUS	Eureka Marina	Fairhaven Terminal			Fields Landing			Forest Products	Redwood Chip Export	Redwood Marina			Schneider Dock	Sierra Pacific	US Coast Guard			Woodley Island		
		Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow	Shallow	Shallow	Deep	Intertidal	Shallow	Shallow	Deep	Intertidal	Shallow	Deep	Intertidal	Shallow	
<i>Phyllaplysia taylori</i>	N																				
<i>Rictaxis punctocaelatus</i>	N	5									2										
<i>Turbonilla sp.</i>	U				1			1				1								2	
Nudibranchia																					
Nudibranchia	U						1				1	1									
Octopodidae																					
Octopodidae	N							1													
NEMERTEA																					
Nemertea																					
Nemertea	U	1		1					2	2			1	2			1				
PHORONIDA																					
Phoronida																					
Phoronida	U	4					4			4		3		4	1		1			1	
PORIFERA																					
Porifera																					
Porifera	U										2						1			1	

Chapter 4: Macro-Zooplankton Communities

Introduction

To detect the presence of non-native invertebrate taxa within macro-zooplankton assemblages, we sampled four estuaries including Humboldt Bay, Marina del Rey, Port Hueneme, and San Francisco Bay. For the first three estuaries, we sampled 10 sites per estuary in one year (2015), using both pump sampling and plankton net tows at each site (see below). For San Francisco Bay, we sampled 30-32 sites in each of three years (2014-2016), using plankton net tows. The specific locations and dates are indicated in Appendix 4.1.

For pump samples, a modified trash pump (North Star S106120 model; Honda GX160 gas motor) coupled to a plankton net assembly (0.75m diameter net; 80 μ m mesh size) was used to collect and filter zooplankton at 1m depth over 10 minutes, totaling 5m³ water volume filtered per sample. Five replicate pump samples were collected across 3-5 random locations within each site (Table 4.1). Samples were preserved in either 95% ethanol or 10% formalin in preparation for taxonomic identification of zooplankton species through genetic or morphological techniques, respectively. At each randomly-selected location within a site where pump sampling took place, latitude and longitude were recorded using a handheld GPS unit, and water temperature, dissolved oxygen, and salinity were measured at 1m depth.

For the three bays with pump sampling, we also collected two replicate vertical net tows in open water adjacent to each pump site (Table 4.1). A weighted plankton net (0.50m diameter; 80 μ m mesh size; 5-10lb weight) was deployed to 5m depth and pulled vertically up through the water column to collect the sample. The first tow sample was preserved in 95% ethanol and the second tow sample was preserved in 10% formalin in preparation for taxonomic identification of zooplankton species through genetic or morphological techniques, respectively. Latitude and longitude were recorded for the deployment location of the two replicate tows using a handheld GPS unit. Additionally, water temperature, dissolved oxygen, and salinity were measured at 1m and 5m depths.

For San Francisco Bay, we sampled 30-32 sites in each of the three years using vertical net tow as described above. For each site, two samples were collected for genetic analyses, including a vertical tow from each 5m and 10m depth. An identical pair for samples was collected for morphological samples from each station, along with an additional sample (5m depth) for voucher specimens. All samples were preserved as described above for each sample type.

Table 4.1. Summary of zooplankton samples collected in California (2014-2015). Samples were collected for both genetic and morphological analysis from four different estuaries, using pump and vertical net tow methods. Shown are the number of sampled in each bay and year per method, and the number of these samples allocated for each genetic and morphological analyses.

Bay	Year	Month	Method	Total Sites	Total Samples	Metagenetics	Morphology
San Francisco	2014	June	Tow	30	180	60	120
San Francisco	2015	June	Tow	32	192	64	128
Humboldt	2015	June	Pump	10	50	30	20
			Tow	10	20	10	10
Marina Del Rey	2015	July	Pump	10	50	30	20
			Tow	10	20	10	10
Port Hueneme	2015	July	Pump	10	50	30	20
			Tow	10	20	10	10
San Francisco	2016	July	Tow	32	192	64	128

Thus, across the four estuaries, we collected a total of 774 zooplankton samples for analysis (Table 4.1). Once collected, plankton samples were shipped to SERC to be curated and organized, then shipped to collaborating laboratories for morphological and genetic analyses as follows:

- **Morphological Analyses.** In general, for each of the pump sample sites, two replicates were sent for morphological analyses to Jeff Cordell, University of Washington. This included a formalin-preserved sample for identification of macro-zooplankton species present, and an ethanol-preserved sample for collection of identified voucher specimens of each taxon for DNA barcoding by MLML. For the net tow samples, the formalin-preserved samples were also sent to Jeff Cordell for identification of macro-zooplankton species present.
- **Genetic Analyses.** The remaining ethanol preserved samples (including pump samples and net tow samples) per site were sent directly to MLML for whole community analysis using next generation sequencing (see Chapter 5).

Results

The morphological analyses of zooplankton revealed only NIS that were previously detected in California. NIS detected in morphological analyses were restricted primarily to the copepods (Table 4.2), which were already known to be present in the respective bays, based upon previous studies by our research group and others. While other NIS are likely present (see Chapter 6), most of these are meroplankton, and species-level identifications are often not possible for these larval forms, which lack diagnostic morphological characteristics.

Table 4.2: List of zooplankton taxa detected by morphological analysis per bay. NIS detected are indicated by an asterisk (*) in each column, and species names are also highlighted in grey.

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
ANNELIDA				
Clitellata				
Oligochaeta				
Oligochaeta	8	0	0	0
Polychaeta				
Oweniidae	0	7	0	0
Polychaeta	10	20	13	15
<i>Polygordius</i> sp.	0	1	0	0
Syllidae	3	0	1	0
BRYOZOA				
Bryozoa	0	2	10	16
CHAETOGNATHA				
Chaetognatha	1	20	11	5
CHELICERATA				
Arachnida				
Acarina	2	0	0	0
CHORDATA				
Actinopterygii				
Teleostei	4	17	14	20
Tunicata				
Appendicularia				
<i>Fritillaria</i> sp.	0	3	0	0
<i>Oikopleura dioica</i>	4	14	10	16

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
Asciidiacea				
Asciidiacea	7	1	8	19
<i>Botryllus/Botrylloides</i> spp.	7	3	6	7
Doliolida				
Doliolida	0	2	1	0
CILIOPHORA				
Tintinnida				
Tintinnida	3	10	10	13
CNIDARIA				
Hydrozoa				
<i>Aglantha digitale</i>	0	0	3	0
Hydrozoa	3	10	10	5
<i>Obelia</i> sp.	0	3	0	3
Siphonophora	0	3	1	0
CRUSTACEA				
Amphipoda				
Caprellidae	2	2	0	4
Corophiidae	3	2	0	1
Gammaridea	5	10	2	7
Hyperidea	0	1	0	0

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
Branchiopoda				
Cladocera				
<i>Bosmina</i> sp. (FW)	2	0	0	0
<i>Ceriodaphnia</i> sp. (FW)	2	0	0	0
Chydoridae (FW)	1	0	0	0
<i>Daphnia</i> spp. (FW)	1	0	0	0
<i>Diaphanosoma</i> sp. (FW)	2	0	0	0
<i>Evadne nordmanni</i>	0	18	0	0
<i>Evadne spinifera</i>	0	0	6	0
<i>Holopedium</i> <i>gibberum</i> (FW)	1	0	0	0
<i>Penilia avirostris</i>	0	0	10	0
<i>Pleopsis</i> <i>polyphemoides</i>	0	0	1	1
<i>Pleuroxus</i> sp. (FW)	1	0	0	0
<i>Podon leuckarti</i>	0	16	0	0
<i>Pseudevadne</i> <i>tergestina</i>	0	0	11	2
<i>Sida crystallina</i> (FW)	2	0	0	0
<i>Simocephalus</i> sp. (FW)	2	0	0	0
Cirripedia				
Cirripedia	9	20	12	19

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
Copepoda				
Calanoida				
<i>Acartia (Acartiura) hudsonica</i>	4	12	0	16
<i>Acartia (Acartiura) sp.</i>	1	3	0	0
<i>Acartia californiensis</i>	9	8	9	7
<i>Acartia danae</i>	0	0	0	8
<i>Acartia spp.</i>	5	20	17	20
<i>Acartia tonsa</i>	4	9	12	15
<i>Acartiella sinensis*</i>	2*	0	0	0
Calanoida	2	4	2	1
<i>Calanus pacificus</i>	0	4	0	0
<i>Calanus sp.</i>	0	5	2	2
<i>Calocalanus sp.</i>	0	0	0	1
<i>Calocalanus styliremis</i>	0	0	0	1
<i>Calocalanus tenuis</i>	0	0	0	1
<i>Centropages abdominalis</i>	0	4	0	0
<i>Centropages bradyi</i>	0	0	1	0
<i>Centropages spp.</i>	0	5	0	0
<i>Clausocalanus furcatus</i>	0	0	9	1
<i>Clausocalanus sp.</i>	0	0	9	14
<i>Ctenocalanus vanus</i>	0	1	0	0
Eucalanidae	0	2	0	0
<i>Eurytemora americana</i>	0	10	0	0

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
<i>Eurytemora pacifica</i>	0	0	0	3
<i>Eurytemora</i> sp.	0	14	0	4
<i>Lucicutia gemina</i>	0	1	0	0
<i>Mecynocera clausi</i>	0	0	0	1
<i>Metridia</i> sp.	0	1	0	0
<i>Microcalanus</i> sp.	0	1	0	0
<i>Osphranticum labronectum</i> (FW)	1	0	0	0
<i>Paracalanus indicus</i>	0	0	0	1
<i>Paracalanus quasimodo</i>	1	0	0	0
<i>Paracalanus</i> sp.	6	11	14	20
Pontellidae	0	3	5	2
<i>Pontellopsis</i> sp.	0	0	1	0
<i>Pseudocalanus mimus</i>	1	8	0	0
<i>Pseudocalanus</i> sp.	1	13	0	0
<i>Pseudodiaptomus euryhalinus</i>	0	0	0	0
<i>Pseudodiaptomus forbesi</i> *	4*	0	0	0
<i>Pseudodiaptomus marinus</i> *	2*	1*	0	0
<i>Pseudodiaptomus euryhalinus</i>	0	0	0	1
<i>Pseudodiaptomus</i> sp.	10	2	10	8
<i>Sinocalanus doerrii</i> (FW)	1	0	0	0
<i>Skistodiaptomus pallidus</i> (FW)	1	0	0	0

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
<i>Stephos pacificus*</i>	1*	0	0	0
<i>Stephos</i> sp.	1	0	0	0
<i>Tortanus discaudatus</i>	1	1	0	0
<i>Tortanus</i> sp.	6	7	0	1
Caligoida				
Caligoida	4	5	0	3
Canuelloida				
<i>Coullana canadensis</i>	2	0	0	0
<i>Longipedia</i> sp.	0	8	1	2
Clausidiidae				
Clausidiidae	8	15	0	2
Cyclopoida				
<i>Acanthocyclops</i> sp. (FW)	1	0	0	0
cf. <i>Tropocyclops</i> sp. (FW)	1	0	0	0
<i>Corycaeus</i> <i>amazonicus</i>	0	0	3	1
<i>Corycaeus anglicus</i>	0	9	3	9
<i>Corycaeus</i> cf. <i>erythraeus</i>	0	0	0	1
<i>Corycaeus</i> cf. <i>latus</i>	0	0	3	1
<i>Corycaeus</i> sp.	0	11	10	9
Cyclopidae (FW)	8	1	1	3
Cyclopoida (Parasitic)	1	0	0	0
<i>Diacyclops thomasi</i> (FW)	1	0	0	0
<i>Dioithona oculata</i>	0	2	17	20

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
<i>Eucyclops</i> sp.	2	0	0	0
<i>Farranula curta</i>	0	0	0	2
<i>Limnoithona sinensis</i> (FW)*	1*	0	0	0
<i>Limnoithona</i> <i>tetraspina</i> *	4*	0	0	0
<i>Macrocyclus</i> <i>albidus</i> (FW)	1	0	0	0
<i>Mesocyclops</i> sp. (FW)	1	0	0	0
<i>Oithona atlantica</i>	0	3	3	2
<i>Oithona davisae</i> *	10*	3*	17*	7*
<i>Oithona nana</i>	0	0	1	0
<i>Oithona plumifera</i>	0	0	0	2
<i>Oithona similis</i>	2	18	10	20
<i>Oithona</i> sp.	0	0	0	1
<i>Oncaea</i> sp.	0	10	1	5
<i>Paroithona</i> sp.	0	1	0	0
Thaumatopsyllidae	0	0	0	1
Harpacticoida				
Ectinosomatidae	0	1	0	0
<i>Emerita analoga</i>	0	0	4	0
<i>Euterpina acutifrons</i>	10	12	18	14
Harpacticoida	10	15	3	7
<i>Harpacticus</i> sp.	0	5	0	10
<i>Microsetella</i> <i>norvegica</i>	1	4	1	5
<i>Microsetella rosea</i>	0	3	0	1
<i>Microsetella</i> sp.	0	0	1	0

Taxon		2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
	<i>Neotachidius triangularis</i>	1	0	0	0
	<i>Porcellidium</i> sp.	0	3	0	2
	<i>Pseudobradya</i> sp.	3	5	0	0
	Tachidiidae	5	4	0	0
	<i>Tisbe</i> spp.	4	1	0	1
	<i>Zaus</i> sp.	0	2	0	0
Monstrilloida					
	Monstrilloida	0	0	0	1
Poecilostomatoida					
	Ergasilidae	1	0	0	0
	Poecilostomatoida	6	8	2	6
	Copepoda	14	20	20	20
	Copepoda (Parasitic)	0	2	2	1
Cumacea					
	Cumacea	0	2	0	2
Decapoda					
Brachyura					
	Cancridae	1	4	0	0
	<i>Fabia subquadrata</i>	0	3	0	0
	Grapsidae	4	0	4	3
	<i>Lophopanopeus</i> sp.	1	0	0	0
	Pinnotheridae	2	16	0	0
	<i>Rhithropanopeus harrisi</i> *	1*	0	0	0
	Xanthidae	0	2	0	0

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
Callianassidae				
<i>Neotrypaea</i> sp.	1	1	0	0
Caridea				
Caridea	4	11	3	1
Hippolytidae	0	1	0	0
Pandalidae	0	0	0	1
Crangonoidea				
Crangonidae	1	3	1	0
Paguridae				
Paguridae	0	2	0	0
Penaecidae	0	0	8	0
Porcellanidae				
Porcellanidae	1	0	0	0
Euphausiacea				
Euphausiacea	0	1	4	0
<i>Thysanoessa</i> sp.	0	0	0	0
Facetotecta				
Facetotecta	0	1	0	0
Isopoda				
Cirolanidae	0	1	0	1
Isopoda	6	5	2	1
Munnidae	1	0	0	0
Sphaeromatidae	2	0	0	0
Mysidacea				
Mysidacea	1	0	0	1

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
Mysidae				
<i>Alienacanthomysis macropsis</i>	0	1	0	0
Ostracoda				
Ostracoda	7	4	1	3
Tanaidacea				
Tanaidacea	3	1	0	2
CTENOPHORA				
Ctenophora	0	4	0	0
<i>Pleurobrachia</i> sp.	0	4	0	0
DINOFLAGELLATA				
<i>Noctiluca</i> sp.	0	18	0	0
ECHINODERMATA				
Echinodermata	0	6	5	10
FORAMINIFERA				
Foraminifera	0	0	0	1
INSECTA				
Chironomidae	1	0	0	0
MOLLUSCA				
Bivalvia				
Bivalvia	9	18	11	10
Gastropoda				
Gastropoda	3	18	11	18
<i>Littorina</i> sp.	0	3	0	2
Pteropoda	0	0	9	1

Taxon	2014-2016 San Francisco Bay	2015 Humboldt Bay	2015 Marina del Rey	2015 Port Hueneme
NEMATODA				
Nematoda	8	18	0	3
Nemertea	0	1	2	0
PHORONIDA				
Phoronida	1	13	0	0
PLATYHELMINTHES				
Turbellaria	9	11	14	10
ROTIFERA				
Rotifera	6	16	1	19
UNIDENTIFIED				
Unidentified	5	6	8	13

Appendix 4.1: Survey Locations by Bay and Year

The tables below indicate locations and dates for macro-zooplankton surveys for each bay and year. A separate table is shown for each bay and year, indicating the sampling date, method, location, and number of replicate samples (for total, metagenetic analysis, and morphological analysis).

San Francisco Bay 2014

Bay	Year	Month	Method	Site	Landmark	Latitude	Longitude	Total Replicates	Metagenetics	Morphology
San Francisco	2014	June	Tow	SFHS-01	North of Dumbarton Bridge	37.5111	-122.1295	6	2	4
San Francisco	2014	June	Tow	SFHS-02	Dumbarton Bridge	37.5349	-122.1710	6	2	4
San Francisco	2014	June	Tow	SFHS-03	Between Dumbarton and San Mateo Bridges	37.5519	-122.1955	6	2	4
San Francisco	2014	June	Tow	SFHS-04	Between Dumbarton and San Mateo Bridges	37.5720	-122.2322	6	2	4
San Francisco	2014	June	Tow	SFHS-05	San Mateo Bridge	37.5805	-122.2518	6	2	4
San Francisco	2014	June	Tow	SFHS-06	San Mateo Bridge	37.5866	-122.2608	6	2	4
San Francisco	2014	June	Tow	SFHS-07	North of San Mateo Bridge	37.6039	-122.2842	6	2	4
San Francisco	2014	June	Tow	SFHS-08	Burlingame	37.6296	-122.3168	6	2	4
San Francisco	2014	June	Tow	SFHS-09	San Francisco International Airport	37.6574	-122.3141	6	2	4
San Francisco	2014	June	Tow	SFHS-10	South San Francisco	37.6861	-122.3184	6	2	4
San Francisco	2014	June	Tow	SFHS-11	South San Francisco	37.7085	-122.3596	6	2	4
San Francisco	2014	June	Tow	SFHS-12	Richmond Marina	37.7355	-122.3603	6	2	4
San Francisco	2014	June	Tow	SFHS-13	Across from Alameda	37.7656	-122.3775	6	2	4
San Francisco	2014	June	Tow	SFHS-14	Bay Bridge	37.7999	-122.3912	6	2	4
San Francisco	2014	June	Tow	SFHS-15	Fisherman's Wharf	37.8126	-122.4027	6	2	4
San Francisco	2014	June	Tow	SFHS-16	Golden Gate	37.8098	-122.4477	6	2	4
San Francisco	2014	June	Tow	SFHS-17	Treasure Island	37.8326	-122.3836	6	2	4
San Francisco	2014	June	Tow	SFHS-18	Angel Island	37.8556	-122.4178	6	2	4
San Francisco	2014	June	Tow	SFHS-19	Between Tiburon and Angel Island	37.8717	-122.4465	6	2	4
San Francisco	2014	June	Tow	SFHS-20	Tiburon	37.8830	-122.4340	6	2	4
San Francisco	2014	June	Tow	SFHS-21	San Quentin Prison	37.9187	-122.4582	6	2	4
San Francisco	2014	June	Tow	SFHS-22	Richmond	37.9304	-122.4379	6	2	4
San Francisco	2014	June	Tow	SFHS-23	Southern San Pablo Bay	37.9638	-122.4379	6	2	4
San Francisco	2014	June	Tow	SFHS-24	Bay Bridge					
San Francisco	2014	June	Tow	SFHS-25	San Pablo					
San Francisco	2014	June	Tow	SFHS-26	San Pablo	38.0316	-122.3693	6	2	4
San Francisco	2014	June	Tow	SFHS-27	Northeast San Pablo Bay	38.0495	-122.3315	6	2	4
San Francisco	2014	June	Tow	SFHS-28	Carquinez Strait, Highway 80 bridge	38.0634	-122.2227	6	2	4
San Francisco	2014	June	Tow	SFHS-29	Carquinez Strait, Benecia	38.0453	-122.1767	6	2	4
San Francisco	2014	June	Tow	SFHS-30	Carquinez Strait, Highway 680 bridge	38.0364	-122.1279	6	2	4
San Francisco	2014	June	Tow	SFHS-31	Suisun Bay, Cacheco Creek	38.0524	-122.0943	6	2	4
San Francisco	2014	June	Tow	SFHS-32	Suisun Bay, Roe Island	38.0761	-122.0291	6	2	4

San Francisco Bay 2015

Bay	Year	Month	Method	Site	Landmark	Latitude	Longitude	Total Replicates	Metagenetics	Morphology
San Francisco	2015	June	Tow	SFHS-01	North of Dumbarton Bridge	37.5160	-122.1304	6	2	4
San Francisco	2015	June	Tow	SFHS-02	Dumbarton Bridge	37.5321	-122.1650	6	2	4
San Francisco	2015	June	Tow	SFHS-03	Between Dumbarton and San Mateo Bridges	37.5515	-122.1948	6	2	4
San Francisco	2015	June	Tow	SFHS-04	Between Dumbarton and San Mateo Bridges	37.5717	-122.2142	6	2	4
San Francisco	2015	June	Tow	SFHS-05	San Mateo Bridge	37.5852	-122.2423	6	2	4
San Francisco	2015	June	Tow	SFHS-06	San Mateo Bridge	37.5970	-122.2604	6	2	4
San Francisco	2015	June	Tow	SFHS-07	North of San Mateo Bridge	37.6070	-122.2730	6	2	4
San Francisco	2015	June	Tow	SFHS-08	Burlingame	37.6232	-122.2901	6	2	4
San Francisco	2015	June	Tow	SFHS-09	San Francisco International Airport	37.6594	-122.3221	6	2	4
San Francisco	2015	June	Tow	SFHS-10	South San Francisco	37.6853	-122.3258	6	2	4
San Francisco	2015	June	Tow	SFHS-11	South San Francisco	37.7174	-122.3477	6	2	4
San Francisco	2015	June	Tow	SFHS-12	Richmond Marina	37.7326	-122.3595	6	2	4
San Francisco	2015	June	Tow	SFHS-13	Across from Alameda	37.7634	-122.3768	6	2	4
San Francisco	2015	June	Tow	SFHS-14	Bay Bridge	37.7977	-122.3915	6	2	4
San Francisco	2015	June	Tow	SFHS-15	Fisherman's Wharf	37.8131	-122.3994	6	2	4
San Francisco	2015	June	Tow	SFHS-16	Golden Gate	37.8091	-122.4468	6	2	4
San Francisco	2015	June	Tow	SFHS-17	Treasure Island	37.8321	-122.3887	6	2	4
San Francisco	2015	June	Tow	SFHS-18	Angel Island	37.8474	-122.4042	6	2	4
San Francisco	2015	June	Tow	SFHS-19	Between Tiburon and Angel Island	37.8669	-122.4476	6	2	4
San Francisco	2015	June	Tow	SFHS-20	Tiburon	37.8770	-122.4273	6	2	4
San Francisco	2015	June	Tow	SFHS-21	San Quentin Prison	37.9087	-122.4577	6	2	4
San Francisco	2015	June	Tow	SFHS-22	Richmond	37.9304	-122.4367	6	2	4
San Francisco	2015	June	Tow	SFHS-23	Southern San Pablo Bay	37.9633	-122.4368	6	2	4
San Francisco	2015	June	Tow	SFHS-24	Bay Bridge	38.0093	-122.4207	6	2	4
San Francisco	2015	June	Tow	SFHS-25	San Pablo	38.0203	-122.4064	6	2	4
San Francisco	2015	June	Tow	SFHS-26	San Pablo	38.0396	-122.3661	6	2	4
San Francisco	2015	June	Tow	SFHS-27	Northeast San Pablo Bay	38.0548	-122.3171	6	2	4
San Francisco	2015	June	Tow	SFHS-28	Carquinez Strait, Highway 80 bridge	38.0610	-122.2216	6	2	4
San Francisco	2015	June	Tow	SFHS-29	Carquinez Strait, Benecia	38.0419	-122.1668	6	2	4
San Francisco	2015	June	Tow	SFHS-30	Carquinez Strait, Highway 680 bridge	38.0368	-122.1281	6	2	4
San Francisco	2015	June	Tow	SFHS-31	Suisun Bay, Cacheco Creek	38.0484	-122.0967	6	2	4
San Francisco	2015	June	Tow	SFHS-32	Suisun Bay, Roe Island	38.0653	-122.0527	6	2	4

Humboldt Bay 2015

Bay	Year	Month	Method	Site	Landmark	Latitude	Longitude	Total Replicates	Metagenetics	Morphology
Humboldt	2015	July	Pump	HB-P01	Woodley Island Marina	40.8075	-124.1631	5	3	2
Humboldt	2015	July	Pump	HB-P02	Schneider Dock	40.7973	-124.1866	5	3	2
Humboldt	2015	July	Pump	HB-P03	Eureka Public Marina	40.8034	-124.1780	5	3	2
Humboldt	2015	July	Pump	HB-P04	Redwood Marine Terminal Berth A	40.8165	-124.1814	5	3	2
Humboldt	2015	July	Pump	HB-P05	Redwood Marine Terminal Berth B	40.8037	-124.1883	5	3	2
Humboldt	2015	July	Pump	HB-P06	CA Redwood Chip Export	40.7981	-124.1906	5	3	2
Humboldt	2015	July	Pump	HB-P07	Fairhaven Terminal	40.7885	-124.1947	5	3	2
Humboldt	2015	July	Pump	HB-P08	US Coast Guard	40.7679	-124.2157	5	3	2
Humboldt	2015	July	Pump	HB-P09	E2 Landing Marina	40.7383	-124.2226	5	3	2
Humboldt	2015	July	Pump	HB-P10	HB Forest Products Terminal	40.7325	-124.2191	5	3	2
Humboldt	2015	July	Tow	HB-T01	Woodley Island Marina	40.7338	-124.2199	2	1	1
Humboldt	2015	July	Tow	HB-T02	Schneider Dock	40.7366	-124.2216	2	1	1
Humboldt	2015	July	Tow	HB-T03	Eureka Public Marina	40.7684	-124.2148	2	1	1
Humboldt	2015	July	Tow	HB-T04	Redwood Marine Terminal Berth A	40.7912	-124.1914	2	1	1
Humboldt	2015	July	Tow	HB-T05	Redwood Marine Terminal Berth B	40.7999	-124.1892	2	1	1
Humboldt	2015	July	Tow	HB-T06	CA Redwood Chip Export	40.8038	-124.1787	2	1	1
Humboldt	2015	July	Tow	HB-T07	Fairhaven Terminal	40.7972	-124.1874	2	1	1
Humboldt	2015	July	Tow	HB-T08	US Coast Guard	40.8022	-124.1886	2	1	1
Humboldt	2015	July	Tow	HB-T09	E2 Landing Marina	40.8168	-124.1802	2	1	1
Humboldt	2015	July	Tow	HB-T10	HB Forest Products Terminal	40.8066	-124.1640	2	1	1

Marina del Rey 2015

Bay	Year	Month	Method	Site	Landmark	Latitude	Longitude	Total Replicates	Metagenetics	Morphology
Marina Del Rey	2015	July	Pump	MDR-P01	Waves MDR Apartments A	33.9729	-118.4495	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P02	Waves MDR Apartments B	33.9744	-118.4512	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P03	Neptune Marina	33.9752	-118.4568	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P04	ESPRIT MDR Apartments	33.9762	-118.4495	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P05	Dolphin Marina	33.9787	-118.4495	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P06	Holiday Marina	33.9801	-118.4537	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P07	Del Rey Yacht Club A	33.9806	-118.4514	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P08	Del Rey Yacht Club B	33.9827	-118.4527	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P09	Catalina Yacht Anchorage	33.9824	-118.4439	5	3	2
Marina Del Rey	2015	July	Pump	MDR-P10	Anchorage 47	33.9793	-118.4438	5	3	2
Marina Del Rey	2015	July	Tow	MDR-T01	Coastal north of bay mouth	33.9690	-118.4690	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T02	Coastal north of bay mouth	33.9683	-118.4737	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T03	Coastal north of bay mouth	33.9661	-118.4730	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T04	Coastal north of bay mouth	33.9713	-118.4709	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T05	Coastal north of bay mouth	33.9686	-118.4716	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T06	Coastal south of bay mouth	33.9532	-118.4569	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T07	Coastal south of bay mouth	33.9506	-118.4552	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T08	Coastal south of bay mouth	33.9480	-118.4593	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T09	Coastal south of bay mouth	33.9511	-118.4617	2	1	1
Marina Del Rey	2015	July	Tow	MDR-T10	Coastal south of bay mouth	33.9504	-118.4584	2	1	1

Port Hueneme 2015

Bay	Year	Month	Method	Site	Landmark	Latitude	Longitude	Total Replicates	Metagenetics	Morphology
Port Hueneme	2015	July	Pump	PH-P01	Berth 1	34.1473	-119.2093	5	3	2
Port Hueneme	2015	July	Pump	PH-P02	Berth 4	34.1488	-119.2054	5	3	2
Port Hueneme	2015	July	Pump	PH-P03	Channel piles	34.1464	-119.2105	5	3	2
Port Hueneme	2015	July	Pump	PH-P04	Berth 5	34.1488	-119.2026	5	3	2
Port Hueneme	2015	July	Pump	PH-P05	Port ops building	34.1463	-119.2117	5	3	2
Port Hueneme	2015	July	Pump	PH-P06	Port mouth south jetty	34.1443	-119.2115	5	3	2
Port Hueneme	2015	July	Pump	PH-P07	Desalination station	34.1477	-119.2108	5	3	2
Port Hueneme	2015	July	Pump	PH-P08	Wharf 3	34.1498	-119.2062	5	3	2
Port Hueneme	2015	July	Pump	PH-P09	Navy boat ramps	34.1533	-119.2099	5	3	2
Port Hueneme	2015	July	Pump	PH-P10	Wharfinger floating dock	34.1482	-119.2020	5	3	2
Port Hueneme	2015	July	Tow	PH-T01	Berth 1	34.1485	-119.2021	2	1	1
Port Hueneme	2015	July	Tow	PH-T02	Berth 4	34.1481	-119.2046	2	1	1
Port Hueneme	2015	July	Tow	PH-T03	Channel piles	34.1483	-119.2063	2	1	1
Port Hueneme	2015	July	Tow	PH-T04	Berth 5	34.1522	-119.2090	2	1	1
Port Hueneme	2015	July	Tow	PH-T05	Port ops building	34.1530	-119.2101	2	1	1
Port Hueneme	2015	July	Tow	PH-T06	Port mouth south jetty	34.1499	-119.2084	2	1	1
Port Hueneme	2015	July	Tow	PH-T07	Desalination station	34.1486	-119.2093	2	1	1
Port Hueneme	2015	July	Tow	PH-T08	Wharf 3	34.1468	-119.2106	2	1	1
Port Hueneme	2015	July	Tow	PH-T09	Navy boat ramps	34.1452	-119.2114	2	1	1
Port Hueneme	2015	July	Tow	PH-T10	Wharfinger floating dock	34.1433	-119.2141	2	1	1

San Francisco Bay 2016

Bay	Year	Month	Method	Site	Landmark	Latitude	Longitude	Total Replicates	Metagenetics	Morphology
San Francisco	2016	July	Tow	SFHS-01	North of Dumbarton Bridge	37.5089	-122.1219	6	2	4
San Francisco	2016	July	Tow	SFHS-02	Dumbarton Bridge	37.5169	-122.1501	6	2	4
San Francisco	2016	July	Tow	SFHS-03	Between Dumbarton and San Mateo Bridges	37.5508	-122.1881	6	2	4
San Francisco	2016	July	Tow	SFHS-04	Between Dumbarton and San Mateo Bridges	37.5704	-122.2237	6	2	4
San Francisco	2016	July	Tow	SFHS-05	San Mateo Bridge	37.5823	-122.2488	6	2	4
San Francisco	2016	July	Tow	SFHS-06	San Mateo Bridge	37.5899	-122.2572	6	2	4
San Francisco	2016	July	Tow	SFHS-07	North of San Mateo Bridge	37.6072	-122.2806	6	2	4
San Francisco	2016	July	Tow	SFHS-08	Burlingame	37.6366	-122.3095	6	2	4
San Francisco	2016	July	Tow	SFHS-09	San Francisco International Airport	37.6641	-122.3241	6	2	4
San Francisco	2016	July	Tow	SFHS-10	South San Francisco	37.6831	-122.3291	6	2	4
San Francisco	2016	July	Tow	SFHS-11	South San Francisco	37.7035	-122.3438	6	2	4
San Francisco	2016	July	Tow	SFHS-12	Richmond Marina	37.7257	-122.3530	6	2	4
San Francisco	2016	July	Tow	SFHS-13	Across from Alameda	37.7628	-122.3711	6	2	4
San Francisco	2016	July	Tow	SFHS-14	Bay Bridge	37.7938	-122.3863	6	2	4
San Francisco	2016	July	Tow	SFHS-15	Fisherman's Wharf	37.8127	-122.4012	6	2	4
San Francisco	2016	July	Tow	SFHS-16	Golden Gate	37.8094	-122.4464	6	2	4
San Francisco	2016	July	Tow	SFHS-17	Treasure Island	37.8334	-122.3810	6	2	4
San Francisco	2016	July	Tow	SFHS-18	Angel Island	37.8516	-122.4136	6	2	4
San Francisco	2016	July	Tow	SFHS-19	Between Tiburon and Angel Island	37.8665	-122.4468	6	2	4
San Francisco	2016	July	Tow	SFHS-20	Tiburon	37.8842	-122.4362	6	2	4
San Francisco	2016	July	Tow	SFHS-21	San Quentin Prison	37.9154	-122.4701	6	2	4
San Francisco	2016	July	Tow	SFHS-22	Richmond	37.9300	-122.4336	6	2	4
San Francisco	2016	July	Tow	SFHS-23	Southern San Pablo Bay	37.9627	-122.4410	6	2	4
San Francisco	2016	July	Tow	SFHS-24	Bay Bridge	38.0020	-122.4346	6	2	4
San Francisco	2016	July	Tow	SFHS-25	San Pablo	38.0201	-122.3996	6	2	4
San Francisco	2016	July	Tow	SFHS-26	San Pablo	38.0215	-122.3678	6	2	4
San Francisco	2016	July	Tow	SFHS-27	Northeast San Pablo Bay	38.0548	-122.3114	6	2	4
San Francisco	2016	July	Tow	SFHS-28	Carquinez Strait, Highway 80 bridge	38.0603	-122.2242	6	2	4
San Francisco	2016	July	Tow	SFHS-29	Carquinez Strait, Benecia	38.0407	-122.1693	6	2	4
San Francisco	2016	July	Tow	SFHS-30	Carquinez Strait, Highway 680 bridge	38.0403	-122.1274	6	2	4
San Francisco	2016	July	Tow	SFHS-31	Suisun Bay, Cacheco Creek	38.0475	-122.1000	6	2	4
San Francisco	2016	July	Tow	SFHS-32	Suisun Bay, Roe Island	38.0635	-122.0624	6	2	4

Section II: Genetic Detection and Analysis of NIS by DNA Sequencing of Voucher Specimens and Plankton.

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Chapter 5: High Throughput Sequencing of Fouling and Infaunal Organisms from California Bays

Introduction

This chapter reports the results of DNA sequencing of specimens (also referred to as “vouchers,” although physical specimens were not always kept). Genetic identification of specimens used the concept of DNA barcoding, the use of species-specific DNA sequence to aid or confirm identification of specimens. For most animal subjects, DNA barcoding uses a fragment of the Cytochrome c oxidase subunit I (COI) mitochondrial gene. Additional loci, such as the 28S rRNA gene (equivalently, large subunit, or LSU, rRNA) can supplement or substitute for COI when interspecific variation in COI is low. In this study, vouchers were examined by expert taxonomists or parataxonomists (*i.e.*, those trained in species identification), and DNA sequencing served to evaluate accuracy and consistency of identification, as well as to further expand a library of DNA sequences for NIS. The combined morphological and molecular approaches have produced a set of identifications for which we can have very high confidence in correct identification, and provided information on which taxa are prone to misidentification. Conversely, the joint application of morphology and genetics has pointed to some weaknesses in the molecular approach, namely the sensitivity of the technique to exogenous DNA.

Joint morphological and genetic analysis of voucher specimens is not without drawbacks, in particular additive costs of sample collection and sorting, morphological identification, and sequencing. For these reasons, a purely genetic approach (omitting morphological identification) or a metagenetic analysis (equivalently “metabarcoding,” omitting sorting) could be applied to settling plates or other environmental samples in some situations. The extremely high abundance of planktonic organisms makes individual analysis prohibitive and metagenetics particularly attractive (Chapter 6). For settling plates, we addressed the cost and labor issue of genetic analysis through adoption of novel high-throughput sequencing methods.

High throughput voucher sequencing is the use of next-generation sequencing (NGS) technology to obtain, in parallel, genetic sequences from many DNA fragments. Applying this method to tissue vouchers allowed us to address specific goals from the project, such as the identification of individual epifaunal and infaunal specimens, comparisons of molecular and morphological results, and the expansion of the DNA barcode database in a faster and more cost-effective way than with traditional Sanger sequencing.

At the time of this report, traditional Sanger DNA sequencing (also called dideoxynucleotide chain termination sequencing) provides longer read lengths and lower sequencing error rate than NGS methods. When the number of samples is not prohibitive, Sanger sequencing produces higher quality and longer sequence data. However, the use of NGS allowed us to more quickly process many thousands of samples. The resulting gigabytes of NGS data can also be used to identify sequences

underrepresented in the database to be re-sequenced with the traditional Sanger method. In this study, species that we knew *a priori* were underrepresented in the reference database were also sequenced with Sanger as a first course of action and added to the database for the later analysis of the NGS voucher data.

High Throughput Voucher Sequencing Molecular Methods

Glossary of Terms

A glossary of technical terms used in description of molecular methods and data analysis is given at the end of this report.

Materials received for analysis.

Marine invertebrate tissue samples from specimens presumed to be purely from one individual organism were collected from PVC settlement plates by SERC personnel and stored in 90% ethanol at room temperature until they could be further processed. MLML periodically received shipments of specimens from SERC sorted to vials and assigned unique identifying codes.

DNA Isolation

DNA isolation was accomplished using either Qiagen DNeasy columns for unique tissues destined for Sanger Sequencing or Fisher MagJET Genomic DNA kits for tissues directed toward high throughput next generation sequencing. Briefly, a subsample of tissue, up to approximately the size of a grain of rice (around 25 mg), was rinsed of ethanol with distilled water (or removed directly from DMSO-based storage buffer (DNE) without rinsing), moved to a lysis solution in a 96-well block (or individual tubes for the DNeasy kit), and processed according to each manufacturers' instructions. In cases where provided tissue was smaller than the recommended size, we nonetheless carried the tissue through the extraction and downstream processes. Genomic DNA was suspended in nuclease-free water. Two wells (A01 and D05) were left empty of tissue as blanks to check for cross-contamination between wells. Genomic DNA was stored and cataloged in 96-well plate format (henceforth, genomic DNA plates).

PCR Amplification

The COI gene was amplified by polymerase chain reaction as described in Geller *et al.* (2013), using jgLCO1490 and jgHCO2198 primers for Sanger sequencing and substituting mICOLintF (Leray *et al.* 2013) for jgLCO1490 in NGS sequencing. The LSU gene amplification process was similar but used primers specific for that gene. From a genomic DNA plate, well D05 (void of tissue, containing only water) was carried through to detect potential cross-well contamination by genomic DNA, while well A01 was used as the PCR no-template control, substituting 1 μ L of nuclease-free water for template. Reactions were checked for PCR success on an agarose gel stained with ethidium bromide. Each primer pair contained extra nucleotides that coded for position on the 96-well PCR plate. Products for conventional sequencing were amplified with unmodified primers and sent to Elim Biopharmaceuticals (Hayward, CA) for sequencing.

Plate-indexing

Sequencing on the Ion Torrent PGM instrument required PCR templates to be pooled prior to additional preparation for sequencing. In order to later separate resulting sequences for each PCR product, PCR amplification products from the initial round of COI or 28S amplification (already containing well index tags) were re-amplified with primers containing extra nucleotides that signify the source PCR plate. PCR success was assessed with an agarose gel stained with ethidium bromide. Plates with a high proportion (>50%) of successfully amplified wells were then pooled into a single tube and purified using Agencourt AMPure beads according to the manufacturer's protocol. Plates with lower success were further optimized until a majority of templates were amplified.

Ion Torrent Library Preparation

The purified pool of PCR products was quantified and end-repaired using the Ion Fragment Library kit according to the manufacturer's protocol, except that half volumes were used in the end-repair reaction to save reagent costs. End-repair products were purified using Ampure beads.

Purified and end-repaired products were ligated with an IonXpress barcode adapter, unique to each plate, (*i.e.*, a plate index) and purified with Ampure beads. The resulting pool of fragmented, adapter-ligated PCR products was then size selected for a 400 bp (base-pair) library (490 bp target) using a Pippin prep instrument. The size selected sample was again purified with Ampure beads and PCR-amplified. Amplified libraries were purified with Ampure, and quality-checked on an Agilent Bioanalyzer 2100. The optimal template dilution for sequencing was determined with a TaqMan qPCR kit. Template was loaded onto an Ion 318 Chip V2 and sequenced with the Ion Torrent PGM using the Ion PGM Hi-Q View kit. All Ion Torrent reagent kits were purchased from Life Technologies.

Sequence Analysis

Voucher sequencing files were downloaded from the Torrent server as fastq files. Sequences were demultiplexed, quality filtered, and assembled into contiguous sequences (contigs). Contigs were searched using BLAST (Basic Local Alignment Search Tool) against two databases; our in-house database of reference sequences (MLML-refs) and COArbitrator (described below). The BLAST results were sorted and filtered to find the most likely genetic identification. These steps are more fully described in the following paragraphs.

Demultiplexing, Filtering, and Assembling of Ion Torrent Sequence Reads

An initial round of demultiplexing and filtering was done by the Ion Torrent within the default settings and sequences below the Phred quality score of Q20¹ were removed. The remaining sequences were separated by IonXpress barcodes (used to indicate plate of origin, see molecular methods section). QIIME version 1 was used to demultiplex (separate) reads for each well of the plate, and additional quality filtering steps were performed using custom scripts² to allow for parallel processing. Briefly, the

¹ Q20 indicates a 99% base call accuracy. For more information see: https://en.wikipedia.org/wiki/Phred_quality_score

² Custom scripts can be found here: <https://github.com/DidemnumVex/IonTorrentVoucher/blob/master/IonZipSplitv3.2.py>

custom script (IonZipSplitv3.2) unzips and renames the sequence files and those files are passed through:

1. *convert_fastaqual_fastq.py* to separate nucleotide sequence from quality scores into two different files (fasta and qual). These are input files for *split_libraries.py*
2. *split_libraries.py*³ with a minimum sequence length of 75, a quality score window of 50, minimum quality score (Q20), and *-disable_primers* set to 'True'. Primers were left in place to provide an anchor of conserved sequence for the assembler.
3. *convert_fastaqual_fastq.py* to merge the nucleotide and quality files into a single file (fastq).
4. The program MIRA was then used to assemble the sequences.

After voucher reads were assembled, they were combined into a single fasta file, which was then passed to Geneious v10 (Biomatters, Christchurch, New Zealand) for primer trimming. Because of the size of the file, it took Geneious several days to find and remove primers from these sequences.

BLAST Search Against MLML and COArbitrator Nucleotide Sequences

The assembled sequences were then searched using BLAST (Basic Local Alignment Search Tool) against two separate databases. The first database (COArbitrator) was curated by Philip Heller and contains COI nucleotide sequences after confirmation of COI amino acid motifs from the Conserved Domain Database (NCBI). This is to ensure that there are open reading frames in the sequences and that they can be translated correctly. The protein sequences themselves were not used for taxonomic assignment because of loss of genetic information in variable codon positions. The second database (MLML) was generated from in-house sequencing of voucher material identified by SERC associates. Sequences were searched with the *blastn* (BLAST nucleotide) default settings, with the exception that matches with an e-value below 0.01 were ignored. Sequences were searched in parallel using the python multiprocessing module, allowing 56 search threads to run simultaneously. This drastically reduced the time to run the assembled sequences through BLAST. The same search process was performed on the assembled sequences against the MLML reference database. Results were saved as separate xml files for later processing and filtering.

BLAST Result Filtering

Probable BLAST matches were chosen as follows:

All records returned by BLAST ("hits") below 94.5% pairwise similarities were ignored: this threshold assumes genetic similarity of 94.5% or less rejects a conspecific relationship between a reference and a novel sequence. (95% is a typical threshold value for species assignment; we adjusted this slightly to account for higher sequencing error rate by Ion Torrent, compared to Sanger sequencing). Assemblies were grouped by Vial ID and given the following concordance ranks (where the lowest value is the

³ http://qiime.org/scripts/split_libraries.html

strongest concordance between genetic and morphological taxonomic assignment) as follows for each Vial ID group:

1. Genus and species of reference and assigned morphospecies match, there was a long alignment, and the morphospecies was not a common contaminant
2. Genus match, long alignment, morphospecies not common contaminant
3. Genus match, long alignment, morphospecies is common contaminant
4. Genus and species match, long alignment, morphospecies is common contaminant
5. Family match, long alignment
6. Order match, long alignment
7. Class match, long alignment
8. Phylum match, long alignment
9. Genus and species match, short alignment, morphospecies not a common contaminant
10. Genus match, short alignment, morphospecies not common contaminant
11. Genus match, short alignment, morphospecies is common contaminant
12. Genus and species match, short alignment, morphospecies is common contaminant
13. Family match, short alignment
14. Order match, short alignment
15. Class match, short alignment

where “morphospecies” is the taxonomic assignment based on morphology, “short alignments” are alignments less than 280 bp and “long alignments” are longer than 280 bp. In practice, alignment length is a function of query sequence length, since references were all >280 bp, and database adequacy (vouchers will have shorter regions of alignment to more distantly related species). “Common contaminants” are species with sequences that were frequently found in wells with presumptive morphospecies that could not be mistaken for the other; phylum level mismatches were used as a proxy for such unmistakable mismatches. Results were then sorted ascending by the match rank, descending by database (MLML or Coarbitrator), and descending by percent pairwise identity, and the top hit was chosen. If no match could be assigned by these criteria, “No BLAST Hit” was assigned for that VialID. Morphological and molecular assignments that disagreed at the phylum level were included in the “No BLAST Hit” category.

The above ranking system favors long alignments over shorter alignments. Shorter alignments arise from short contigs. It is possible that, among assemblies from a vial, an incorrect long contig could be prioritized over a correct short contig. Generally, though, extraneous contigs are filtered out by other criteria (*e.g.*, they are common contaminants or phylum-level mismatches to morphospecies identifications).

Results and Discussion

Genetic Assignments

Of 21,109 vials processed, 56% of vials (11,826) were assigned a genetic identification. [Note: identification does not have an associated statistical probability but is based on the BLAST result ranking system (previous section) and the available reference databases]. Vials that could not be identified are discussed below. The resulting concordance between morphological versus genetic identification varied greatly by taxon (Table 5.1). For instance, within the Bryozoa, 80% were correctly identified to the species level, and 89% to the genus or species levels. Similarly, chordates were correctly identified to species 68% of the time and to the genus level for an additional 15% of vials. However, within the sponges, the correct species was never assigned and in 55% of the cases, the phylum was the lowest rank attempted by the morphologist. Cnidarians were similarly difficult to identify morphologically, with 26% identified to the class level only, though this is not surprising since hydroids and small anthozoans are reputedly difficult to identify in the field.

Overall, concordance of species-level assignments appeared relatively low, at best 80% for bryozoans and less for many frequently occurring taxa such as annelids and crustaceans. However, it should be noted that our analysis of concordance didn't differentiate between the *refinement* of a coarse identification (*e.g.*, morphological ID = *Mytilus* sp. and genetic ID = *Mytilus trossulus*) versus a *reassignment* (*e.g.*, morphological ID = *Mytilus galloprovincialis* and genetic ID = *Mytilus trossulus*). Both were considered *nonconcordant*. For present purposes, our definition of concordance was strict, requiring an exact match (but allowing for synonymy, when known). However, when *both* the genetic and morphological identifications were resolved only to the same higher taxon (*e.g.*, a genus such as *Mytilus* sp.), results were considered concordant at that level (in this case, the genus level).

Many morphological assignments were made only to the genus or higher levels. Table 5.1 shows the percent of vouchers where morphological and genetic assignment mismatched at the genus (and other higher levels). For generic assignments, there was much stronger agreement between genetics and morphology. For example, while 60.5% of arthropod vouchers agreed at the species level, 88.5% agreed at the genus level (species matches plus genus-only matches). This suggests that morphological assignments are relatively reliable at the genus level. However, this may not be adequate for NIS studies, as many genera have both native and introduced species.

Table 5.2 shows all genetic species assignments for all sampled bays. The set of vouchers that were sequenced was not a random sample of organisms on settling plates, therefore the abundance of specimens for each genetically identified species is not a reflection of relative population sizes. This table, however, may supplement geographic analyses based on morphological identification.

While not tallied, refinement (or coarsening) of identification is shown in Figures 5.1 through 5.12, which are matrices ("heat maps") in which genetic and morphological identification are paired in a square array with cells colored by frequency of results. In this document, matrices are static figures and

have been plotted for all (Figure 5.1 and 5.12) or individual phyla (Figs. 5.2 to 5.11). Dynamic (zoomable) matrices can be provided in html format.

Concordance of Morphological and Genetic Identification for Specimens with Species-level

Morphological Assignment.

Vouchers that were fully resolved by morphology to binomials are the set of specimens for which taxonomists and parataxonomist had highest confidence in identification. Concordance rate for all specimens, regardless of level of resolution, may unreasonably underestimate taxonomists' accuracy by including vouchers where there was implicit uncertainty. That is, by declining to provide a fully resolved name, a taxonomist was declaring uncertainty. DNA sequences may identify such specimens, but the unresolved name is not necessarily an error, but rather an inefficiency or ineffectiveness. While the ability to genetically identify a voucher that could not be morphologically identified is important to biotic surveys, we separately assessed concordance between genetic and morphological identifications for only fully resolved vouchers (Table 5.3). For vouchers given binomial names, there was an overall concordance of 82%. Some of the taxa with highest rates of misidentification (assuming the genetic ID to be correct) were the polychaetes *Capitella teleta* and *Eulalia levicor*, the barnacle *Megabalanus rosa*, the bryozoan *Bugula longirostrata*, and the chordate *Ascidia virginea*.

There were many species for which no specimens given binomial names could be verified. Examples are the ascidian *Botrylloides pizoni* and the hydrozoan *Bougainvillia muscus*. This, however, does not mean that none of these species were genetically identified among all specimens. Rather, they may have been identified from specimens not fully resolved by morphological identification, for example the polychaete *Eulalia levicor*, the amphipod *Ampelisca abdita*, or the barnacle *Megabalanus rosa*.

For many taxa, apparent misidentification was found but involved few specimens for each genetic reassignment. For example, eight specimens of the polychaete *Schistomeringos longicornis* were identified by both morphology and genetics, while another 19 were genetically identified as 12 other species. Many of these morphological-genetic mismatches were clearly due to implausible genetic assignments (discussed below), such as a morphological nudibranch that was genetically identified as a mussel. Thus, the total 18% overall discordance rate for fully resolved morphological assignments combines both true morphological misidentification and genetic misassignment. Our concern should focus on abundant, systematic misidentifications of the sort mentioned in the previous paragraph.

Finally, apparent discordance could be a result of unrecognized synonymy or errors of identification within Genbank. While some level of database curation has taken place within Coarbitrator, it is nearly impossible to manually verify that every sequence in Genbank was properly identified, thus it is important to investigate a reference's provenance before drawing conclusions based on genetic identification.

The results given here paint a picture of taxonomists' strengths and weaknesses and suggest that they might spend less time on certain taxonomic groups and more on others. It is evident that Porifera, Platyhelminthes, Nemertea, and Cnidaria present particular problems for morphological identification.

Identification of Porifera and Cnidaria rely on microscopical characters (spicules and nematocysts) that are inconvenient for field taxonomy, and tiny Platyhelminthes and Nemertea are relatively devoid of useful macroscopic characters. For these 'difficult' taxa, it is also imperative that references from reputable and verified sources be used for genetic identifications.

Failed Genetic Identification

Of 21,109 vials processed, 9,283 failed to yield results (Table 5.4) due to a combination of factors, including a) failure to make an assembly (2,993 vials), b) failure to find a BLAST hit (6,269 vials), c) human or bacterial contamination (2 vials) or d) because the resulting alignment was too short to confirm a blast hit (19 vials). The failed assemblies are actually fewer than we expected because of our use of pooled 96 well plates without manually removing weak PCR products. Because we did not choose only the strongest PCR products for sequencing, low quality PCR products were passed through to the sequencing stage. Ordinarily, we would not have attempted to use traditional Sanger sequencing on such PCR products. Some of these weak PCR products yielded usable sequence, but many did not. Sequencing failure rate was only 14%, attrition typical of other high-volume sequencing projects.

While only two vials were found to have failed due to bacterial or human DNA contamination, these only represent vials where there was also no plausible match was found within the assembled contigs. There were more assemblies that were evidently human or bacterial sequences (far more bacterial than human), but those vials also produced plausible assemblies. This was similarly true for short alignments. Short alignments were only taken into consideration when there was no plausible long alignment that matched. However, if there was no match and the resulting alignment was short, it was categorized to have failed due to alignment length. Lack of a BLAST match to any known reference was the largest category of failure. There are a few possible reasons for this result:

1. An absence of reference sequences exceeding the 94.5% similarity threshold. This threshold is meant to limit matches to conspecifics, and so will exclude closely related organisms even if there was a 'hit' at *e.g.*, 93%. This may also be affected by sequencing error for a haplotype already near that identity threshold (*i.e.*, error to a conspecific sequence 95% similar to a reference could push it below our threshold). These sequences have been retained and may later be identified as our database expands.
2. True 'No Hit' results. For a portion of the 'No Hit' category, BLAST returned 'No HIT' according to the default minimum criteria for match (*i.e.*, e-value was greater than 10). These may represent nonsense sequences or sequences for which there is not even a distant relative within the database. Given that our sequences could include bacterial COI sequences, it is plausible that there were many unknown bacterial sequences within the assemblies.
3. The only hits were implausible. If the only hit for an assembly was from phylum different than expected, the sorting algorithm categorized the result as "No-Hit". While these were the minority of the 'No Hit' category, exact numbers are not currently available. These result from poor quality sequences, chimeric sequences, or laboratory or symbiont contamination.

Contamination

Figure 5.12 shows genetic versus morphological identifications without filtering “extra” sequences from vouchers, low quality sequences, or implausible taxa (that is, belonging to a different phylum than expected). In this figure, the diagonal pattern indicates concordance, while the diffuse cloud of points reflects unexpected genetic assignments. Strong horizontal and vertical arrays of points represent patterns of likely contamination, either by symbiotic organisms (epibionts, gut contents) or physical transfer of genetic material (*i.e.*, SERC or MLML lab contamination). The word “contamination” is here used in a broad sense of any deviation from a single sequence from a voucher, be that for natural or artifactual causes. Sources of contamination are not yet understood. Examination of extraction no-tissue controls and PCR no-template controls showed a very low rate of laboratory contamination: of 259 plates extracted, 11 showed a positive signal in the no-tissue-added well. Only 3 of 259 PCR plates showed a positive signal in the no-template-added well. (PCR plates showing a positive signal were re-run to achieve no contamination). We conducted a series of experiments to determine if well-to-well movement of DNA occurred during extractions on mock extraction plates by filling wells that surrounded a tissue-free well (lysis buffer only) with tissue extractions. We found that empty wells could be contaminated during the heated, shaking steps of extraction when foil sealing film or press-on tube caps were used, but was eliminated when plates with wells using screw-on caps with o-rings were used. We therefore switched to plates using screw-on caps. Despite this change to the extraction procedure, an analysis of the number of sequences detected in a well over time showed only a small reduction (Figure 5.13). In summary, tissue and PCR contamination was rare, and we took effort to further minimize tissue contamination during extraction, yet we continued to observe multiple sequences from most vials.

Interestingly, the highest number of phyla/well was seen for specimens that came from bulk voucher vials (multiple specimens per vial). This suggests that, even though specimens were later sorted into separate extraction wells, being stored with other specimens resulted in DNA contamination. A previous study showed that ethanol from bulk collection jars could yield DNA (Hajibabaei *et al.* 2012), thus it is plausible that tissues were contaminated prior to extraction.

We examined the identity of extra DNA sequences in wells (Table 5.5) and found many that were among the most common organisms on settling plates (bryozoans and ascidians, for example). Encrusting organisms might be the most likely to be attached to other organisms when sampled. Other extra sequences were from common epibionts or commensals such as caprellid amphipods, scaleworms and syllids (*Halosydna* and *Megasyllis*) that could be clinging to targeted organisms. However, preliminary experiments suggested that environmental DNA is leaked into settling plate soak water during plate retrieval and processing. Some of the organisms in Table 5.5 would shed blood (molluscs, annelids, bryozoans, chordates and arthropods) or superficial cells (sponges and cnidarians) into the soak water if injured during processing. In short, there appear to be multiple routes for DNA to move from one specimen, vial, or well to another.

Lastly, certain taxa were more likely to be contaminated by bacterial sequences (data not shown). These included sponges, tunicates, and bryozoans. Sponge-associated bacteria are subjects of extensive

research and bacteria are known to compose a great deal of biomass within the tissues of certain Porifera species (Vacelet and Donadey 1977, Hentschel *et al.* 2006). Similarly, a bryozoan-associated bacteria called 'Endobugula' (Lim and Haygood 2004) was detected among the assemblies. Tunicates, with their mode of ultrafilter feeding, are enriched with ingested bacterial cells. For example, when we compared a *Ciona*-derived COI sequence from this project to Genbank, we discovered that there was a bacterial sequence within Genbank mislabeled as *Ciona intestinalis*. This may also indicate a *Ciona*-specific bacterial species, but more work would need to be done to know the host specificity.

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Appendix 5.1

Table 5.1. Concordance between morphological and genetic identifications by taxon rank. Vial count represents number of genetic versus morphological matches at the species (complete binomial) level or mismatches for the specific given rank. Percent of phylum total is the species match or higher taxon mismatch divided by the total for that phylum, as a percentage. Long alignments are alignments longer than 280 bp. Short alignments are shown separately to examine whether if concordance is affected by the length of the region of overlap between query (vial) and subject (reference).

		Vial Count			Percent of phylum total		
		Long Alignment	Short Alignment	All	Long Alignment	Short Alignment	All
Annelida	Species Match	442	159	601	31.19%	36.81%	32.50%
	Species Mismatch	211	94	305	14.89%	21.76%	16.50%
	Genus Mismatch	343	42	385	24.21%	9.72%	20.82%
	Family Mismatch	185	35	220	13.06%	8.10%	11.90%
	Order Mismatch	230	102	332	16.23%	23.61%	17.96%
	Class Mismatch	6	0	6	0.42%	0.00%	0.32%
Arthropoda	Species Match	797	91	888	66.14%	34.73%	60.53%
	Species Mismatch	303	106	409	25.15%	40.46%	27.88%
	Genus Mismatch	37	19	56	3.07%	7.25%	3.82%
	Family Mismatch	42	20	62	3.49%	7.63%	4.23%
	Order Mismatch	23	26	49	1.91%	9.92%	3.34%
	Class Mismatch	3	0	3	0.25%	0.00%	0.20%
Bryozoa	Species Match	2046	347	2393	80.05%	67.91%	78.02%
	Species Mismatch	254	89	343	9.94%	17.42%	11.18%
	Genus Mismatch	15	1	16	0.59%	0.20%	0.52%
	Family Mismatch	113	52	165	4.42%	10.18%	5.38%
	Order Mismatch	53	22	75	2.07%	4.31%	2.45%
	Class Mismatch	75	0	75	2.93%	0.00%	2.45%
Chordata	Species Match	1908	442	2350	68.46%	54.57%	65.33%
	Species Mismatch	429	175	604	15.39%	21.60%	16.79%
	Genus Mismatch	151	41	192	5.42%	5.06%	5.34%

		Vial Count			Percent of phylum total		
		Long Alignment	Short Alignment	All	Long Alignment	Short Alignment	All
	Family Mismatch	75	33	108	2.69%	4.07%	3.00%
	Order Mismatch	224	119	343	8.04%	14.69%	9.54%
Cnidaria	Species Match	35	5	40	8.18%	5.62%	7.74%
	Species Mismatch	188	37	225	43.93%	41.57%	43.52%
	Genus Mismatch	35	10	45	8.18%	11.24%	8.70%
	Family Mismatch	49	3	52	11.45%	3.37%	10.06%
	Order Mismatch	114	34	148	26.64%	38.20%	28.63%
	Class Mismatch	7	0	7	1.64%	0.00%	1.35%
Echinodermata	Order Mismatch	0	8	8		100.00%	100.00%
Entoprocta	Species Match	0	1	1	0.00%	100.00%	33.33%
	Class Mismatch	2	0	2	100.00%	0.00%	66.67%
Mollusca	Species Match	314	49	363	39.10%	42.98%	39.59%
	Species Mismatch	281	37	318	34.99%	32.46%	34.68%
	Genus Mismatch	126	14	140	15.69%	12.28%	15.27%
	Family Mismatch	30	8	38	3.74%	7.02%	4.14%
	Order Mismatch	41	6	47	5.11%	5.26%	5.13%
	Class Mismatch	11	0	11	1.37%	0.00%	1.20%
Nemertea	Species Mismatch	32	0	32	59.26%		59.26%
	Order Mismatch	1	0	1	1.85%		1.85%
	Class Mismatch	21	0	21	38.89%		38.89%
Platyhelminthes	Species Mismatch	3	1	4	5.00%	50.00%	6.45%
	Family Mismatch	2	1	3	3.33%	50.00%	4.84%
	Class Mismatch	55	0	55	91.67%	0.00%	88.71%
Porifera	Species Mismatch	70	11	81	27.24%	40.74%	28.52%
	Family Mismatch	7	8	15	2.72%	29.63%	5.28%
	Order Mismatch	38	8	46	14.79%	29.63%	16.20%

		Vial Count			Percent of phylum total		
		Long Alignment	Short Alignment	All	Long Alignment	Short Alignment	All
	Class Mismatch	142	0	142	55.25%	0.00%	50.00%
All		9569	2257	11826			

Table 5.2. Genetic Identification of vouchers by bay. BD = Bodega, HB = Humboldt, MDR = Marina Del Rey, MI = Mission, MO = Morro, MB = Monterey Bay, PH = Port Hueneme, SD = San Diego, SF = San Francisco. Unk=Unknown and indicates we don't have location information for those samples. Numbers refer to the number of tissue vials with a genetic identification (row) from a bay location (column). Bays highlighted in yellow were the focus of Part II of the initial phase.

		VialID											
Bay		BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total	
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826
<i>Polysiphonia</i>	<i>schneideri</i>		0	0	0	0	0	0	0	0	0	1	1
<i>Alitta</i>	<i>succinea</i>	Annelida	0	0	0	0	0	0	0	0	0	30	30
<i>Amphiduros</i>	<i>pacificus</i>		0	0	0	0	2	0	0	0	0	0	2
<i>Asabellides</i>			0	0	0	0	0	0	0	0	0	1	1
<i>Barantolla</i>	<i>americana</i>		0	2	0	0	0	0	0	0	0	0	2
<i>Bergstroemia</i>	<i>nigromaculata</i>		0	0	0	0	1	0	0	0	0	0	1
<i>Capitella</i>	<i>capitata</i>		0	0	0	0	0	0	0	0	0	11	11
	<i>teleta</i>		1	1	0	0	1	0	0	0	0	17	20
<i>Chone</i>	<i>magna</i>		7	0	0	1	11	0	0	0	0	0	19
<i>Cirratulus</i>			0	0	0	0	0	0	0	0	0	1	1
	<i>cirratus</i>		0	0	0	0	0	0	0	0	0	2	2
<i>Ctenodrilus</i>	<i>serratus</i>		1	0	0	0	0	0	0	0	0	9	10
<i>Eulalia</i>			0	0	0	0	0	0	0	0	1	0	1
	<i>levicor</i>		0	0	0	0	17	0	0	0	0	0	17
<i>Eupolyornia</i>	<i>heterobranchia</i>		29	0	0	0	9	0	0	1	0	0	39
<i>Ficopomatus</i>	<i>enigmaticus</i>		0	0	0	0	0	0	0	0	0	16	16
<i>Halosydna</i>	<i>brevisetosa</i>		0	8	0	0	14	0	0	0	0	0	22
<i>Harmothoe</i>	<i>imbricata</i>		36	11	0	10	3	2	0	0	0	221	283
<i>Hydroides</i>	<i>elegans</i>		0	0	27	9	0	0	0	0	1	0	37
<i>Leitoscoloplos</i>	<i>pugettensis</i>		0	4	0	0	0	0	0	0	0	0	4
<i>Lumbrineris</i>	<i>perkinsi</i>		0	0	0	0	0	0	0	0	7	0	7
<i>Marphysa</i>	<i>sanguine</i>		0	1	0	0	0	0	0	0	0	9	10
<i>Megalomma</i>	<i>splendida</i>		0	0	0	0	1	0	0	0	0	0	1
<i>Megasyllis</i>	<i>nipponica</i>		9	0	2	11	0	1	0	8	11	248	290
<i>Myrianida</i>	<i>convoluta</i>		1	0	0	0	0	0	0	0	0	0	1
	<i>pachycera</i>		0	0	0	3	0	0	0	0	0	0	3
	<i>pentadentata</i>		1	0	0	2	0	0	0	0	5	4	12

		VialID											
Genus (or lowest rank)	species	Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total
Phylum			1061	954	313	987	1203	60	39	278	958	5973	11826
	<i>Myxicola</i>		2	0	0	0	0	0	0	0	0	1	3
	<i>Nainereis</i>		0	0	0	2	0	0	0	0	0	0	2
	<i>Naineris dendritica</i>		0	0	0	0	0	0	0	0	0	4	4
	<i>Neanthes acuminata</i>		0	0	0	1	0	0	0	0	2	13	16
	<i>Nereis vexillosa</i>		0	6	0	0	0	0	0	0	0	5	11
	<i>Ophryotrocha labronica</i>		0	0	0	0	0	0	0	0	1	11	12
	<i>Oxydromus pugettensis</i>		13	0	0	0	2	0	0	0	0	1	16
	<i>Parasabella</i>		0	0	8	2	0	0	0	0	13	3	26
	<i>Phyllodoce medipapillae</i>		0	0	0	0	1	0	0	0	0	0	1
	<i>Platynereis</i>		91	58	0	2	50	0	0	3	7	86	297
	<i>bicaniculata</i>		0	1	0	0	0	0	0	0	0	1	2
	<i>Polycirrus</i>		4	0	0	0	0	1	0	0	0	0	5
	<i>Polydora cornuta</i>		0	0	0	0	0	0	0	0	0	4	4
	<i>Protodorvillea gracilis</i>		0	0	0	0	0	1	0	0	0	3	4
	<i>Schistomeringos longicornis</i>		4	6	0	4	0	1	0	0	0	47	62
	<i>Schizobranhia insignis</i>		0	1	0	0	6	0	0	0	0	0	7
	<i>Serpula columbiana</i>		0	0	0	0	0	0	0	0	0	1	1
	<i>Streblosoma uncinatus</i>		0	0	0	0	0	0	0	0	5	0	5
	<i>Streblospio benedicti</i>		0	0	0	0	0	0	0	0	0	1	1
	<i>Syllis alternata</i>		10	0	0	1	2	0	0	0	2	0	15
	<i>elongata</i>		2	0	0	0	1	0	0	0	0	0	3
	family_Capitellidae		2	0	0	4	0	0	0	0	7	1	14
	family_Chrysopetalidae		0	32	0	0	43	0	0	0	3	10	88
	family_Nereidae		0	0	0	0	0	0	0	0	1	0	1
	family_Nereididae		11	0	0	0	6	1	0	0	0	17	35
	family_Opheliidae		11	0	0	0	4	0	0	0	1	7	23
	family_Syllidae		2	0	0	0	18	0	0	0	1	5	26
	family_Terebellidae		0	9	0	5	2	0	0	3	24	280	323
	<i>Amathia</i>	Arthropoda	1	6	0	0	0	0	0	0	0	15	22
	<i>Americorophium</i>		0	0	0	0	0	0	0	0	0	10	10
	<i>Ampelisca abdita</i>		2	0	0	0	0	0	0	0	0	4	6

		VialID										Total	
Bay		BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF		
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826
<i>Amphibalanus</i>	<i>amphitrite</i>		0	0	0	0	0	0	0	0	2	11	13
	<i>improvisus</i>		0	1	0	0	0	0	0	0	0	75	76
<i>Ampithoe</i>			0	0	0	0	0	0	0	0	0	63	63
	<i>lacertosa</i>		7	0	0	0	0	0	0	0	0	5	12
	<i>sectiman</i>		1	0	0	0	0	0	0	0	0	0	1
	<i>valida</i>		0	0	0	0	0	0	0	0	0	3	3
<i>Aoroides</i>			6	0	0	1	2	0	0	3	2	38	52
	<i>columbiae</i>		11	0	0	0	29	0	0	0	0	0	40
<i>Aruga</i>	<i>holmesii</i>		3	0	0	0	0	0	0	0	0	4	7
<i>Balanus</i>	<i>crenatus</i>		32	61	0	0	38	0	0	0	0	70	201
	<i>glandula</i>		2	29	0	0	0	0	0	2	0	0	33
	<i>trigonus</i>		0	0	0	1	6	0	0	13	1	0	21
<i>Cancer</i>	<i>antennarius</i>		2	4	0	0	1	0	0	0	4	0	11
<i>Caprella</i>			0	0	0	0	8	0	0	0	2	0	10
	<i>californica</i>		8	0	0	0	17	0	0	6	4	7	42
	<i>equilibra</i>		2	0	0	0	4	0	0	5	0	2	13
	<i>mutica</i>		5	2	0	0	31	0	0	3	2	60	103
	<i>scaura</i>		0	0	0	0	0	0	0	0	0	1	1
	<i>simia</i>		0	0	0	0	34	0	0	6	7	52	99
<i>Deutella</i>	<i>californica</i>		1	4	0	0	5	0	0	0	0	0	10
<i>Erichthonius</i>			3	0	0	0	11	0	0	1	2	4	21
<i>Gammaropsis</i>	<i>shoemakeri</i>		0	0	0	0	5	0	0	1	6	0	12
	<i>thompsoni</i>		2	0	0	0	0	0	0	0	1	0	3
<i>Gammarus</i>	<i>daiberi</i>		0	0	0	0	0	0	0	0	0	6	6
<i>Gnorimosphaeroma</i>	<i>oregonensis</i>		0	0	0	0	0	0	0	0	0	12	12
<i>Grandidierella</i>	<i>japonica</i>		0	0	0	0	0	0	0	0	0	12	12
<i>Hemigrapsus</i>	<i>oregonensis</i>		3	0	0	0	0	0	0	0	0	7	10
<i>Hyaella</i>	<i>azteca</i>		0	0	0	0	0	0	0	0	0	1	1
<i>Ianiropsis</i>	<i>serricaudis</i>		14	0	0	0	0	0	0	0	0	10	24
<i>Jassa</i>	<i>marmorata</i>		1	0	0	0	0	0	0	0	0	0	1
	<i>slatteryi</i>		10	1	0	0	21	1	0	7	2	7	49

Genus (or lowest rank)	species	Phylum	VialID										Total
			Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	
			1061	954	313	987	1203	60	39	278	958	5973	11826
	<i>staudei</i>		1	5	0	0	6	0	0	0	0	0	12
<i>Leptocheilia</i>			5	0	0	0	1	0	0	0	0	3	9
<i>Leucothoe</i>	<i>alata</i>		2	0	3	0	0	1	0	0	2	83	91
<i>Liljeborgia</i>			0	0	0	1	0	0	0	0	0	7	8
<i>Megabalanus</i>	<i>rosa</i>		0	3	0	1	3	0	0	10	5	0	22
<i>Melitida</i>	<i>rylovae</i>		1	0	0	0	0	0	0	0	0	6	7
<i>Metacarcinus</i>	<i>magister</i>		0	0	0	0	0	0	0	0	0	1	1
<i>Monocorophium</i>			5	0	0	0	0	1	0	3	2	12	23
	<i>acherusicum</i>		9	5	0	0	44	0	0	1	1	17	77
	<i>insidiosum</i>		1	3	0	0	2	0	0	0	0	5	11
	<i>uenoi</i>		4	0	0	0	0	0	0	0	0	0	4
<i>Munna</i>	<i>japonica</i>		1	0	0	0	0	0	0	0	0	5	6
<i>Nebalia</i>			1	0	0	0	0	0	0	0	0	1	2
<i>Pachycheles</i>			0	2	0	0	0	0	0	0	0	0	2
<i>Pachygrapsus</i>	<i>crassipes</i>		0	0	0	0	0	0	0	1	0	0	1
<i>Paracerceis</i>	<i>cordata</i>		9	0	0	0	0	0	0	0	0	7	16
	<i>sculpta</i>		0	0	0	0	5	0	0	0	5	2	12
<i>Paradexamine</i>			0	0	0	0	0	0	0	3	0	12	15
<i>Paranthura</i>			9	0	1	0	1	0	0	0	0	48	59
<i>Pentidotea</i>	<i>resecata</i>		3	0	0	0	0	0	0	0	0	0	3
<i>Podocerus</i>			2	0	0	0	31	0	0	0	0	1	34
	<i>cristatus</i>		2	5	0	0	2	0	0	0	0	0	9
<i>Polycheria</i>	<i>osborni</i>		0	0	0	0	0	0	0	0	0	1	1
<i>Pontogeneia</i>			5	0	0	0	0	0	0	0	0	1	6
	<i>rostrata</i>		0	0	0	0	1	0	0	0	0	0	1
<i>Pugettia</i>	<i>producta</i>		0	0	0	0	1	0	0	0	0	0	1
<i>Synidotea</i>	<i>laevidorsalis</i>		0	0	0	0	0	0	0	0	0	4	4
<i>Uromunna</i>			0	0	0	0	0	0	0	0	0	1	1
<i>Zeuxo</i>			0	0	0	1	0	0	0	4	1	0	6
family_Anthuridae			0	0	0	0	0	0	0	0	0	1	1
family_Balanidae			0	0	0	0	2	0	0	0	0	0	2

		VialID											
Genus (or lowest rank)	species	Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826
family_Caprellidae			0	0	0	0	0	0	0	0	0	1	1
family_Gammaridae			0	0	5	0	1	0	0	1	10	0	17
order_Amphipoda			0	0	0	0	1	0	0	0	0	0	1
order_Decapoda			1	0	0	0	0	0	0	0	0	0	1
Alcyonidium		Bryozoa	0	51	0	0	0	0	0	0	0	0	51
Amathia			0	0	0	0	0	0	0	0	0	17	17
	<i>gracilis</i>		3	0	0	1	0	0	0	2	2	0	8
	<i>tertia</i>		0	0	0	0	0	0	0	0	0	3	3
	<i>verticillata</i>		0	0	0	55	0	4	0	0	45	57	161
	<i>vidovici</i>		1	0	0	18	17	0	0	0	2	0	38
Anguinella	<i>palmata</i>		1	1	0	0	0	0	0	0	0	21	23
Aspidelectra			0	0	0	0	0	0	0	0	0	3	3
Bugula	<i>flabellata</i>		0	0	0	0	0	0	0	0	6	0	6
	<i>longirostrata</i>		1	36	0	7	51	0	0	6	1	14	116
	<i>neritina</i>		28	13	9	85	41	4	0	19	28	339	566
	<i>pacifica</i>		18	15	0	0	5	1	0	0	0	17	56
	<i>stolonifera</i>		22	19	9	42	13	2	0	8	24	218	357
Bugulina	<i>stolonifera</i>		0	2	0	0	0	0	0	0	0	1	3
Buskia	<i>seriata</i>		0	0	0	0	0	0	0	0	0	3	3
Caulibugula	<i>ciliata</i>		2	0	0	0	0	0	0	0	0	6	8
Celleporaria	<i>brunnea</i>		0	0	29	37	13	2	1	7	38	21	148
Celleporella	<i>hyalina</i>		1	0	0	0	0	0	0	0	0	7	8
Conopeum			37	2	0	0	0	0	0	0	0	15	54
	<i>reticulatum</i>		1	0	0	0	0	0	0	0	0	1	2
	<i>tenuissimum</i>		0	10	0	0	0	0	0	0	0	25	35
Crisia	<i>occidentalis</i>		0	19	0	8	19	0	2	21	9	0	78
Cryptosula	<i>pallasiana</i>		3	5	15	20	7	2	0	2	11	87	152
Electra			0	0	0	0	0	0	0	0	0	3	3
Fenestrulina	<i>delicia</i>		0	9	0	0	17	0	0	0	0	5	31
Filicrisia	<i>franciscana</i>		0	15	0	0	11	0	0	5	0	1	32
Fredericella			0	0	0	0	0	0	0	0	0	2	2

		VialID												
		Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total	
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826	
<i>Membranipora</i>	<i>chesapeakeensis</i>	Chordata	0	0	0	0	0	0	0	0	0	33	33	
	<i>membranacea</i>		0	0	0	0	4	0	0	0	0	0	4	
<i>Nolella</i>	0		0	0	0	0	0	0	0	0	1	0	1	
<i>Parasmittina</i>	0		0	0	0	6	0	0	0	0	1	0	7	
<i>Pectinatella</i>	<i>magnifica</i>		0	0	0	0	0	0	0	0	0	28	28	
<i>Schizoporella</i>	<i>errata</i>		3	3	0	0	0	0	0	0	0	1	10	17
	<i>japonica</i>		42	15	0	1	39	0	0	15	1	19	132	
<i>Scruparia</i>	0		1	0	0	4	0	0	2	7	0	14		
<i>Scrupocellaria</i>	<i>diegensis</i>		0	23	0	4	24	0	0	0	9	9	69	
<i>Smittoidea</i>	<i>prolifera</i>		0	5	0	0	0	0	0	0	0	42	47	
<i>Tricellaria</i>	<i>occidentalis</i>		2	0	0	0	0	0	0	0	0	49	51	
<i>Victorella</i>	0		0	0	0	1	0	0	0	4	0	5		
<i>Watersipora</i>			10	4	0	0	26	1	11	0	0	8	60	
	<i>arcuata</i>		0	0	0	7	4	0	0	0	30	7	48	
	<i>subovoidea</i>		0	0	2	4	0	0	0	0	10	0	16	
	<i>subtorquata</i>	40	50	19	120	32	2	25	39	72	160	559		
<i>family Plumatellidae</i>	0	0	0	0	0	0	0	0	0	0	12	12		
<i>Aplidium</i>	0	0	12	42	0	2	0	0	14	2	72			
<i>Ascidia</i>	<i>ceratodes</i>	39	0	0	8	14	1	0	3	13	1	79		
	<i>virginea</i>	0	0	0	0	0	1	0	0	18	0	19		
	<i>zara</i>	3	0	1	1	0	2	0	0	17	308	332		
<i>Botrylloides</i>	<i>leachii</i>	9	5	7	25	4	1	0	1	18	321	391		
	<i>pizoni</i>	0	0	0	12	0	0	0	0	9	0	21		
	<i>violaceus</i>	91	49	11	16	41	0	0	10	7	283	508		
<i>Botryllus</i>	<i>schlosseri</i>	70	17	41	96	10	8	0	7	71	444	764		
<i>Ciona</i>	<i>intestinalis</i>	18	0	36	31	0	1	0	3	43	150	282		
	<i>savigni</i>	0	0	0	1	0	0	0	0	1	2	4		
	<i>savignyi</i>	1	13	2	19	0	1	0	3	25	180	244		
<i>Corella</i>	<i>inflata</i>	0	6	0	0	0	0	0	0	0	0	6		
<i>Didemnum</i>	<i>vexillum</i>	6	13	0	0	2	0	0	2	0	57	80		
<i>Diplosoma</i>	<i>listerianum</i>	28	12	13	17	7	0	0	1	10	84	172		

		VialID												
		Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total	
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826	
<i>Distaplia</i>	<i>occidentalis</i>	Cnidaria	1	1	0	5	3	0	0	0	13	0	23	
<i>Microcosmus</i>	<i>squamiger</i>		0	0	1	3	0	0	0	0	9	0	13	
<i>Molgula</i>	<i>manhattensis</i>		12	2	0	0	0	0	0	0	0	191	205	
<i>Perophora</i>	<i>japonica</i>		10	0	0	0	0	0	0	0	0	0	2	12
	<i>viridis</i>		0	0	0	1	0	0	0	0	0	15	0	16
<i>Polyandrocarpa</i>	<i>zorritensis</i>		0	0	14	50	0	3	0	0	55	0	122	
<i>Styela</i>	<i>canopus</i>		0	0	0	0	0	0	0	0	0	3	0	3
	<i>clava</i>		0	0	0	13	0	0	0	0	9	51	73	
	<i>plicata</i>		0	0	14	33	0	2	0	0	12	0	61	
class_Ascidiacea			2	4	0	11	38	0	0	2	1	31	89	
family_Styelidae			0	0	0	0	0	0	0	0	6	0	6	
<i>Anthopleura</i>	<i>elegantissima</i>		0	0	0	0	1	0	0	0	0	0	0	1
<i>Aurelia</i>			0	0	0	2	0	0	0	0	0	0	0	2
	<i>aurita</i>		0	0	0	2	0	0	0	0	1	1	4	
	<i>labiata</i>	1	0	0	0	0	0	0	0	0	0	0	1	
<i>Bougainvillia</i>		0	0	0	0	0	0	0	0	0	16	8	24	
	<i>muscus</i>	0	0	9	23	0	0	0	2	9	4	47		
<i>Cordylophora</i>		0	0	0	0	0	0	0	0	0	24	24		
	<i>caspia</i>	0	0	0	0	0	0	0	0	0	2	2		
<i>Diadumene</i>		4	0	10	5	1	1	0	2	9	103	135		
	<i>franciscana</i>	0	0	0	0	3	0	0	1	0	23	27		
	<i>leucolena</i>	2	0	0	0	0	0	0	0	0	24	26		
	<i>lineata</i>	0	0	0	0	0	0	0	0	0	8	8		
<i>Ectopleura</i>	<i>crocea</i>	4	0	5	4	10	0	0	5	5	10	43		
<i>Gonothyrea</i>	<i>loveni</i>	2	0	0	0	0	0	0	0	0	0	2		
<i>Hydra</i>	<i>hymanae</i>	0	0	0	0	0	0	0	0	0	1	1		
	<i>vulgaris</i>	0	0	0	0	0	0	0	0	0	4	4		
<i>Laomedea</i>	<i>calceoli</i>	0	0	0	0	0	0	0	0	0	4	4		
	<i>calceolifera</i>	1	3	0	0	4	0	0	0	0	3	11		
<i>Metridium</i>	<i>senile</i>	0	23	0	0	7	0	0	0	0	7	37		
<i>Moerisia</i>	<i>inkermanica</i>	0	0	0	0	0	0	0	0	0	8	8		

		VialID												
		Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total	
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826	
<i>Obelia</i>			0	0	0	0	1	0	0	0	0	0	1	
	<i>bidentata</i>		0	10	0	0	0	0	0	0	0	0	2	12
	<i>dichotoma</i>		0	1	0	0	2	0	0	0	0	0	1	4
	<i>longissima</i>		33	28	0	0	0	0	0	0	0	0	3	64
<i>Stauridiosarsia</i>	<i>cliffordi</i>		0	0	0	0	1	0	0	0	0	0	1	
class_Hydrozoa			3	9	0	4	0	0	0	3	5	0	24	
<i>Amphipholis</i>	<i>squamata</i>	Echinodermata	0	0	0	1	0	0	0	0	0	5	6	
<i>Pisaster</i>	<i>ochraceus</i>		0	1	0	0	0	0	0	0	0	0	1	
<i>Strongylocentrotus</i>	<i>purpuratus</i>		0	0	0	0	1	0	0	0	0	0	1	
<i>Barentsia</i>	<i>benedeni</i>	Entoprocta	0	0	0	0	0	0	0	0	0	1	1	
phylum_Entoprocta			0	0	0	0	0	0	0	0	0	2	2	
<i>Acanthodoris</i>	<i>nanaimoensis</i>	Mollusca	0	1	0	0	0	0	0	0	0	0	1	
	<i>rhodoceras</i>		0	1	0	0	0	0	0	0	0	0	1	
<i>Aeolidia</i>			0	0	0	0	1	0	0	0	0	0	1	
<i>Alia</i>	<i>carinata</i>		1	1	0	0	14	0	0	0	1	1	18	
<i>Amphissa</i>	<i>reticulata</i>		0	1	0	0	9	0	0	0	0	0	10	
<i>Caprella</i>			1	6	0	0	0	0	0	0	0	0	7	
<i>Crepidula</i>	<i>nummaria</i>		0	0	0	1	0	0	0	0	0	0	1	
	<i>plana</i>		1	0	0	0	0	0	0	0	0	4	5	
<i>Crepidatella</i>	<i>lingulata</i>		0	0	0	0	3	0	0	0	2	0	5	
<i>Cuthona</i>			0	6	0	0	9	0	0	0	1	0	16	
<i>Dendronotus</i>	<i>venustus</i>		13	8	0	0	3	0	0	0	0	0	24	
<i>Diaphorodoris</i>	<i>lirulatocauda</i>		0	4	0	0	1	0	0	0	0	0	5	
<i>Dirona</i>	<i>picta</i>		0	0	0	0	0	0	0	0	2	1	3	
<i>Doto</i>			0	0	0	1	4	0	0	1	1	0	7	
<i>Flabellina</i>	<i>trilineata</i>		2	5	0	0	0	0	0	0	0	0	7	
	<i>verrucosa</i>		1	4	0	0	0	0	0	0	0	0	5	
<i>Geukensia</i>	<i>demissa</i>		0	0	0	0	0	0	0	0	0	2	2	
<i>Haminoea</i>	<i>japonica</i>		6	0	0	0	0	0	0	0	0	33	39	
<i>Hermisenda</i>			1	31	0	2	21	0	0	0	4	0	59	
<i>Hiatella</i>	<i>arctica</i>		0	5	0	0	8	0	0	0	0	0	13	

Genus (or lowest rank)	species	Phylum	VialID									Total	
			Bay	BD	HB	MDR	MI	MO	Unk	MB	PH		SD
			1061	954	313	987	1203	60	39	278	958	5973	11826
<i>Lacuna</i>			0	0	0	0	3	0	0	0	0	1	4
<i>Littorina</i>	<i>littorea</i>		0	0	0	0	0	0	0	0	0	1	1
<i>Musculista</i>	<i>senhousia</i>		13	0	0	2	0	0	0	0	3	41	59
<i>Mya</i>	<i>arenaria</i>		0	0	0	0	0	0	0	0	0	1	1
<i>Mytilus</i>	<i>edulis</i>		0	0	0	0	1	0	0	0	0	0	1
	<i>galloprovincialis</i>		7	0	2	8	25	1	0	14	5	5	67
	<i>trossulus</i>		3	80	0	0	4	0	0	0	0	42	129
<i>Onchidoris</i>	<i>bilamellata</i>		0	8	0	0	0	0	0	0	0	1	9
<i>Ostrea</i>	<i>lurida</i>		1	2	2	44	0	5	0	1	33	108	196
<i>Placida</i>	<i>dendritica</i>		1	0	0	0	0	0	0	0	0	0	1
<i>Pododesmus</i>	<i>macrochisma</i>		0	0	0	0	36	0	0	0	0	0	36
<i>Polycera</i>	<i>atra</i>		0	1	0	6	26	1	0	0	8	3	45
	<i>hedgpethi</i>		0	1	0	4	7	0	0	0	1	7	20
<i>Ruditapes</i>	<i>philippinarum</i>		0	0	0	0	0	0	0	0	0	7	7
<i>Tenellia</i>			0	0	0	0	0	0	0	0	5	5	10
<i>Triopha</i>	<i>maculata</i>		0	0	0	0	4	0	0	0	0	0	4
<i>Urosalpinx</i>	<i>cinerea</i>		0	0	0	0	0	0	0	0	0	2	2
class_Bivalvia			4	0	0	0	7	1	0	0	0	0	12
class_Gastropoda			0	0	0	0	0	0	0	0	0	1	1
family_Fionidae			0	2	0	0	0	0	0	0	2	0	4
family_Terebellidae			6	4	0	0	0	0	0	0	6	57	73
family_Veneridae			1	0	0	0	0	0	0	0	0	0	1
order_Nudibranchia			0	0	0	0	1	0	0	0	1	3	5
<i>Cephalothrix</i>	<i>simula</i>	Nemertea	1	0	1	0	0	0	0	0	0	43	45
phylum_Nemertea			4	0	0	0	1	0	0	1	1	2	9
<i>Euplana</i>	<i>gracilis</i>	Platyhelminthes	1	0	0	0	0	0	0	0	0	3	4
<i>Pseudoceros</i>			0	0	0	0	5	0	0	0	0	0	5
phylum_Platyhelminthes			18	12	0	4	14	0	0	0	2	3	53
<i>Callyspongia</i>	<i>siphonella</i>	Porifera	0	0	0	2	1	0	0	0	1	6	10
<i>Eunapius</i>	<i>fragilis</i>		0	0	0	0	0	0	0	0	0	63	63
<i>Grantia</i>			0	0	0	0	0	0	0	0	0	22	22

		VialID											
		Bay	BD	HB	MDR	MI	MO	Unk	MB	PH	SD	SF	Total
Genus (or lowest rank)	species	Phylum	1061	954	313	987	1203	60	39	278	958	5973	11826
<i>Halichondria</i>	<i>sitiens</i>		6	4	3	15	2	1	0	5	9	136	181
<i>Haliclona</i>			0	0	0	0	0	0	0	0	0	1	1
	<i>cinerea</i>		0	0	0	0	0	0	0	0	1	0	1
	<i>tubifera</i>		0	0	0	0	0	0	0	0	0	1	1
	<i>xena</i>		0	0	0	0	1	0	0	0	0	0	1
<i>Terpios</i>	<i>hoshinota</i>		0	0	0	0	1	0	0	0	0	0	1
phylum_Porifera			0	1	0	2	0	0	0	0	0	0	3

Table 5.3. Concordance of morphological identifications (Morpho-genus and Morpho-species) and molecular identifications (Molec-genus and Molec-species) for vouchers, limited to vouchers given morphological binomial names. Morpho-species highlighted in red were not confirmed by molecular analysis. Molec-species in green were confirmation of at least one Morpho-species. Percent-mismatch values in yellow are highlighted as high frequency mismatches between morphological and molecular identifications, with both numerous specimens and high rate of mismatch. Most cases of discordance involved few specimens and were improbable misidentifications; these are likely due to contamination of DNA templates or database entries.

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
All					6636	1500	82	18
Annelida	<i>Alitta</i>	<i>succinea</i>	<i>Alitta</i>	<i>succinea</i>	18		69	
			<i>Capitella</i>	<i>capitata</i>		1		4
			<i>Cirriformia</i>	<i>moorei</i>		2		8
			<i>Glycinde</i>	<i>picta</i>		1		4
			<i>Nereis</i>	<i>latescens</i>		1		4
			<i>Notomastus</i>	<i>lineatus</i>		1		4
			<i>Sabaco</i>	<i>elongatus</i>		1		4
			<i>Scoloplos</i>	<i>acmeceps</i>		1		4
	<i>Barantolla</i>	<i>americana</i>	<i>Cirriformia</i>	<i>moorei</i>		1		50
			<i>Pectinaria</i>	<i>californiensis</i>		1		50
	<i>Bergstroemia</i>	<i>nigromaculata</i>	<i>Pterocirrus</i>	<i>montereyensis</i>		1		100
	<i>Capitella</i>	<i>capitata</i>	<i>Capitella</i>	<i>capitata</i>	10		100	
		<i>teleta</i>	<i>Capitella</i>	<i>capitata</i>		18		95
			<i>Megasyllis</i>	<i>nipponica</i>		1		5
	<i>Chone</i>	<i>magna</i>	<i>Euchone</i>	<i>limnicola</i>		1		14
			<i>Megasyllis</i>	<i>nipponica</i>		1		14
			<i>Paradialychone</i>	<i>ecaudata</i>		4		57
			<i>Pseudochitinopoma</i>	<i>occidentalis</i>		1		14
	<i>Ctenodrilus</i>	<i>serratus</i>	<i>Harmothoe</i>	<i>imbricata</i>		3		75
			<i>Polydora</i>	<i>cornuta</i>		1		25
	<i>Eulalia</i>	<i>levicor</i>	<i>Eulalia</i>	<i>quadrioculata</i>		13		87
			<i>Halosydna</i>	<i>brevisetosa</i>		1		7
			<i>Odontosyllis</i>	<i>phosphorea</i>		1		7
<i>Eupolymnia</i>	<i>heterobranchia</i>	<i>Eupolymnia</i>	<i>heterobranchia</i>	18		95		
		<i>Pseudochitinopoma</i>	<i>occidentalis</i>		1		5	

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
	<i>Ficopomatus</i>	<i>enigmaticus</i>	<i>Ficopomatus</i>	<i>enigmaticus</i>	15		100	
	<i>Halosydna</i>	<i>brevisetosa</i>	<i>Eulalia</i>	<i>quadrioculata</i>		1		7
			<i>Halosydna</i>	<i>brevisetosa</i>	13		93	
	<i>Harmothoe</i>	<i>imbricata</i>	<i>Branchiomma</i>	<i>nigromaculatum</i>		1		
			<i>Cirriformia</i>	<i>moorei</i>		1		
			<i>Euchone</i>	<i>limnicola</i>		1		
			<i>Harmothoe</i>	<i>imbricata</i>	260		1	
			<i>Lumbrineris</i>	<i>californiensis</i>		1		
			<i>Malmgreniella</i>	<i>macginitiei</i>		1		
			<i>Megasyllis</i>	<i>nipponica</i>		3		1
			<i>Nephtys</i>	<i>californiensis</i>		1		
			<i>Polydora</i>	<i>cornuta</i>		1		
			<i>Sabaco</i>	<i>elongatus</i>		4		1
	<i>Hydroides</i>	<i>elegans</i>	<i>Hydroides</i>	<i>elegans</i>	30		94	
				<i>gracilis</i>		1		3
			<i>Salmacina</i>	<i>tribranchiata</i>		1		3
	<i>Leitoscoloplos</i>	<i>pugettensis</i>	<i>Nephtys</i>	<i>caecoides</i>		1		20
				<i>ferruginea</i>		1		20
			<i>Pilargis</i>	<i>berkeleyae</i>		1		20
			<i>Scoletoma</i>	<i>tetraura</i>		1		20
			<i>Lumbrineris</i>	<i>perkinsi</i>	<i>Lumbrineris</i>	<i>perkinsi</i>	3	
			<i>Salmacina</i>	<i>tribranchiata</i>		1		25
	<i>Marphysa</i>	<i>sanguine</i>	<i>Harmothoe</i>	<i>imbricata</i>		1		100
	<i>Megasyllis</i>	<i>nipponica</i>	<i>Branchiomma</i>	<i>boholense</i>		1		
				<i>nigromaculatum</i>		4		2
			<i>Capitella</i>	<i>capitata</i>		2		1
			<i>Glycinde</i>	<i>picta</i>		2		1
<i>Harmothoe</i>			<i>imbricata</i>		10		4	
<i>Hydroides</i>			<i>elegans</i>		1			
			<i>gracilis</i>		1			
<i>Leitoscoloplos</i>			<i>pugettensis</i>		1			
<i>Megasyllis</i>			<i>nipponica</i>			226		85

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
			<i>Neanthes</i>	<i>acuminata</i>		7		3
			<i>Nephtys</i>	<i>caecoides</i>		2		1
			<i>Odontosyllis</i>	<i>phosphorea</i>		1		
			<i>Parasabella</i>	<i>pallida</i>		4		2
			<i>Platynereis</i>	<i>bicanaliculata</i>		1		
			<i>Polydora</i>	<i>cornuta</i>		3		1
	<i>Myrianida</i>	<i>pachycera</i>	<i>Myrianida</i>	<i>pachycera</i>		3		100
		<i>pentadentata</i>	<i>Harmothoe</i>	<i>imbricata</i>		1		50
			<i>Megasyllis</i>	<i>nipponica</i>		1		50
	<i>Naineris</i>	<i>dendritica</i>	<i>Naineris</i>	<i>dendritica</i>		1	2	50
	<i>Neanthes</i>	<i>acuminata</i>	<i>Megasyllis</i>	<i>nipponica</i>		3		23
			<i>Neanthes</i>	<i>acuminata</i>		7		54
			<i>Nereis</i>	<i>vexillosa</i>		2		15
			<i>Platynereis</i>	<i>bicanaliculata</i>		1		8
	<i>Nereis</i>	<i>vexillosa</i>	<i>Alitta</i>	<i>succinea</i>		5		45
			<i>Eulalia</i>	<i>quadrioculata</i>		1		9
			<i>Nereis</i>	<i>vexillosa</i>		4		36
			<i>Sabaco</i>	<i>elongatus</i>		1		9
	<i>Ophryotrocha</i>	<i>labronica</i>	<i>Polydora</i>	<i>cornuta</i>		1		50
			<i>Salmacina</i>	<i>tribranchiata</i>		1		50
	<i>Oxydromus</i>	<i>pugettensis</i>	<i>Megasyllis</i>	<i>nipponica</i>		1		20
	<i>Platynereis</i>	<i>bicanaliculata</i>	<i>Platynereis</i>	<i>bicanaliculata</i>		2		100
	<i>Polydora</i>	<i>cornuta</i>	<i>Harmothoe</i>	<i>imbricata</i>		1		33
			<i>Megasyllis</i>	<i>nipponica</i>		1		33
			<i>Polydora</i>	<i>cornuta</i>		1		33
	<i>Protodorvillea</i>	<i>gracilis</i>	<i>Capitella</i>	<i>capitata</i>		1		25
	<i>Schistomeringos</i>	<i>longicornis</i>	<i>Branchiomma</i>	<i>boholense</i>		1		4
			<i>Cirriiformia</i>	<i>moorei</i>		2		7
			<i>Ficopomatus</i>	<i>enigmaticus</i>		2		7
			<i>Glycinde</i>	<i>picta</i>		1		4
			<i>Leitoscoloplos</i>	<i>pugettensis</i>		1		4
			<i>Malmgreniella</i>	<i>nigralba</i>		1		4
			<i>Nephtys</i>	<i>caecoides</i>		3		11

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens		
					Match	Mismatch	Match	Mismatch	
			<i>Pherusa</i>	<i>neopapillata</i>		1		4	
			<i>Platynereis</i>	<i>bicanaliculata</i>		1		4	
			<i>Pseudopolydora</i>	<i>kempi</i>		1		4	
			<i>Sabaco</i>	<i>elongatus</i>		2		7	
			<i>Schistomeringos</i>	<i>longicornis</i>	8		30		
			<i>Scoletoma</i>	<i>tetraura</i>		3		11	
	<i>Schizobranhia</i>	<i>insignis</i>	<i>Halosydna</i>	<i>brevisetosa</i>		1		50	
			<i>Schizobranhia</i>	<i>insignis</i>	1		50		
	<i>Streblosoma</i>	<i>uncinatus</i>	<i>Capitella</i>	<i>capitata</i>		1		25	
			<i>Streblosoma</i>	<i>uncinatus</i>	3		75		
	<i>Streblospio</i>	<i>benedicti</i>	<i>Scoloplos</i>	<i>acmeceps</i>		1		100	
	<i>Syllis</i>	<i>alternata</i>	<i>Halosydna</i>	<i>brevisetosa</i>		1		33	
			<i>Megasyllis</i>	<i>nipponica</i>		1		33	
			<i>Pseudochitinopoma</i>	<i>occidentalis</i>		1		33	
					1		33		
Arthropoda	<i>Ampelisca</i>	<i>abdita</i>	<i>Gnorimosphaeroma</i>	<i>oregonensis</i>		1		25	
			<i>Grandidierella</i>	<i>japonica</i>		2		50	
			<i>Photis</i>	<i>brevipes</i>		1		25	
	<i>Amphibalanus</i>	<i>amphitrite</i>	<i>Amphibalanus</i>	<i>amphitrite</i>	10		77		
				<i>improvisus</i>		2		15	
				<i>Balanus</i>	<i>crenatus</i>		1		8
		<i>improvisus</i>	<i>Amphibalanus</i>	<i>amphitrite</i>		1		1	
				<i>improvisus</i>	69		92		
	<i>Balanus</i>	<i>crenatus</i>		5		7			
	<i>Ampithoe</i>	<i>lacertosa</i>	<i>Ampithoe</i>	<i>lacertosa</i>	7		70		
				<i>valida</i>		1		10	
				<i>Photis</i>	<i>brevipes</i>		1		10
				<i>Stenothoe</i>	<i>valida</i>		1		10
				<i>sectiman</i>	<i>Pugettia</i>	<i>producta</i>		1	
<i>valida</i>		<i>Ampithoe</i>	<i>valida</i>	3		100			
<i>Aoroides</i>	<i>columbiae</i>	<i>Aoroides</i>	<i>columbiae</i>	38		97			
			<i>secunda</i>		1		3		
<i>Aruga</i>	<i>holmesi</i>	<i>Aoroides</i>	<i>secunda</i>		2		33		
		<i>Aruga</i>	<i>holmesi</i>	2		33			

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
			<i>Colidotea</i>	<i>rostrata</i>	1		17	
			<i>Synidotea</i>	<i>laticauda</i>	1		17	
	<i>Balanus</i>	<i>crenatus</i>	<i>Amphibalanus</i>	<i>improvisus</i>	4		2	
				<i>Balanus</i>	<i>crenatus</i>	194		97
			<i>nubilus</i>	3		1		
		<i>glandula</i>	<i>Balanus</i>	<i>crenatus</i>	1		3	
				<i>glandula</i>	30		94	
			<i>nubilus</i>	1		3		
	<i>trigonus</i>	<i>Balanus</i>	<i>trigonus</i>	21		100		
	<i>Cancer</i>	<i>antennarius</i>	<i>Ampithoe</i>	<i>valida</i>	1		9	
			<i>Cancer</i>	<i>jordani</i>	6		55	
			<i>Pachycheles</i>	<i>rudis</i>	1		9	
			<i>Romaleon</i>	<i>branneri</i>	3		27	
	<i>Caprella</i>	<i>californica</i>	<i>Caprella</i>	<i>californica</i>	4	1	78	2
			<i>Caprella</i>	<i>equilibra</i>	13		100	
		<i>mutica</i>	<i>Caprella</i>	<i>mutica</i>	102		99	
			<i>Gammaropsis</i>	<i>thompsoni</i>	1		0	1
		<i>scaura</i>	<i>Caprella</i>	<i>scaura</i>	1		100	
		<i>simia</i>	<i>Amphibalanus</i>	<i>improvisus</i>	1		1	
				<i>Caprella</i>	<i>californica</i>	3		3
			<i>equilibra</i>		1		1	
			<i>simia</i>		92		93	
	<i>Dissiminassa</i>		<i>dissimilis</i>	1		1		
	<i>Paranthura</i>	<i>japonica</i>	1		1			
	<i>Deutella</i>	<i>californica</i>	<i>Deutella</i>	<i>californica</i>	10		20	
	<i>Gammaropsis</i>	<i>shoemakeri</i>	<i>Elasmopus</i>	<i>bampo</i>	1		9	
			<i>Gammaropsis</i>	<i>shoemakeri</i>	10		91	
		<i>thompsoni</i>	<i>Gammaropsis</i>	<i>thompsoni</i>	2		100	
	<i>Gammarus</i>	<i>daiberi</i>	<i>Gammarus</i>	<i>daiberi</i>	5		100	
	<i>Gnorimosphaeroma</i>	<i>oregonensis</i>	<i>Gnorimosphaeroma</i>	<i>oregonensis</i>	12		67	
<i>Grandidierella</i>	<i>japonica</i>	<i>Gnorimosphaeroma</i>	<i>oregonensis</i>	2		1		
		<i>Grandidierella</i>	<i>japonica</i>	10		5		

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
	<i>Hemigrapsus</i>	<i>oregonensis</i>	<i>Deutella</i>	<i>californica</i>		1		6
			<i>Gammaropsis</i>	<i>thompsoni</i>		2		11
			<i>Gammarus</i>	<i>daiberi</i>		2		11
			<i>Hemigrapsus</i>	<i>oregonensis</i>	1		6	
	<i>Hyaella</i>	<i>azteca</i>	<i>Hyaella</i>	<i>azteca</i>	1		100	
	<i>Ianiropsis</i>	<i>serricaudis</i>	<i>Heptacarpus</i>	<i>paludicola</i>		1		6
			<i>Ianiropsis</i>	<i>analoga</i>		3		19
				<i>montereyensis</i>		3		19
				<i>serricaudis</i>	5		31	
			<i>Incisocalliope</i>	<i>derzhavini</i>		1		6
			<i>Janiralata</i>	<i>occidentalis</i>		1		6
			<i>Jassa</i>	<i>slatteryi</i>		1		6
			<i>Laticorophium</i>	<i>baconi</i>		1		6
	<i>Jassa</i>	<i>marmorata</i>	<i>Jassa</i>	<i>marmorata</i>		1		100
		<i>slatteryi</i>	<i>Ericthonius</i>	<i>punctatus</i>		1		3
			<i>Ianiropsis</i>	<i>analoga</i>		1		3
			<i>Incisocalliope</i>	<i>derzhavini</i>		1		3
			<i>Jassa</i>	<i>marmorata</i>		1		3
				<i>slatteryi</i>	35		88	
		<i>Metopa</i>	<i>cistella</i>		1		3	
		<i>staudei</i>	<i>Jassa</i>	<i>marmorata</i>		1		9
			<i>staudei</i>	10		91		
	<i>Leucothoe</i>	<i>alata</i>	<i>Ampithoe</i>	<i>lacertosa</i>		1		1
			<i>Incisocalliope</i>	<i>derzhavini</i>		1		1
			<i>Jassa</i>	<i>slatteryi</i>		1		1
			<i>Laticorophium</i>	<i>baconi</i>		1		1
			<i>Leucothoe</i>	<i>alata</i>	82		93	
			<i>Paranthura</i>	<i>japonica</i>		1		1
			<i>Sinacorophium</i>	<i>heteroceratum</i>		1		1
	<i>Megabalanus</i>	<i>rosa</i>	<i>Amphibalanus</i>	<i>improvisus</i>		1		6
			<i>Balanus</i>	<i>nubilus</i>		1		6
<i>trigonus</i>					1		6	
<i>Megabalanus</i>			<i>californicus</i>		14		82	

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens		
					Match	Mismatch	Match	Mismatch	
	<i>Melitida</i>	<i>rylovae</i>	<i>Melita</i>	<i>rylovae</i>	5		100	0	
	<i>Metacarcinus</i>	<i>magister</i>	<i>Gammarus</i>	<i>daiberi</i>		1		100	
	<i>Monocorophium</i>	<i>acherusicum</i>		<i>Ampithoe</i>	<i>valida</i>		1		1
				<i>Laticorophium</i>	<i>baconi</i>		8		11
				<i>Metopa</i>	<i>cistella</i>		1		1
				<i>Monocorophium</i>	<i>acherusicum</i>	56		78	
				<i>Monocorophium</i>	<i>insidiosum</i>		2		3
				<i>Monocorophium</i>	<i>uenoi</i>		2		3
				<i>Photis</i>	<i>brevipes</i>		1		1
		<i>Podocerus</i>	<i>cristatus</i>		1		1		
		<i>insidiosum</i>	<i>Monocorophium</i>	<i>acherusicum</i>	<i>acherusicum</i>		1		9
				<i>insidiosum</i>	<i>insidiosum</i>	7		64	
	<i>uenoi</i>			<i>uenoi</i>		3		27	
	<i>uenoi</i>	<i>Monocorophium</i>	<i>uenoi</i>	<i>uenoi</i>	4		100		
	<i>Munna</i>	<i>japonica</i>	<i>Monocorophium</i>	<i>acherusicum</i>		1		1	
	<i>Pachygrapsus</i>	<i>crassipes</i>	<i>Hemigrapsus</i>	<i>oregonensis</i>		1		100	
	<i>Paracerceis</i>	<i>cordata</i>		<i>Ampithoe</i>	<i>valida</i>		1		8
				<i>Paracerceis</i>	<i>cordata</i>	10		83	
				<i>Photis</i>	<i>brevipes</i>		1		8
		<i>sculpta</i>	<i>Paracerceis</i>	<i>sculpta</i>	12		100		
	<i>Pentidotea</i>	<i>resecata</i>	<i>Idotea</i>	<i>resecata</i>	1		100		
	<i>Podocerus</i>	<i>cristatus</i>	<i>Podocerus</i>	<i>brasiliensis</i>	<i>brasiliensis</i>		2		22
				<i>cristatus</i>	<i>cristatus</i>	7		78	
<i>Polycheria</i>	<i>osborni</i>	<i>Polycheria</i>	<i>osborni</i>	1		100			
<i>Pontogeneia</i>	<i>rostrata</i>	<i>Pontogeneia</i>	<i>rostrata</i>	1		100			
<i>Synidotea</i>	<i>laevidorsalis</i>		<i>Caprella</i>	<i>californica</i>		1		25	
			<i>Caprella</i>	<i>equilibra</i>		1		25	
			<i>Idotea</i>	<i>rufescens</i>		1		25	
			<i>Stenothoe</i>	<i>valida</i>		1		25	
Bryozoa	<i>Amathia</i>	<i>gracilis</i>	<i>Amathia</i>	<i>verticillata</i>		1		25	
			<i>Fenestrolina</i>	<i>delicia</i>		1		25	
			<i>Thalamoporella</i>	<i>californica</i>		1		25	
			<i>Alcyonidium</i>	<i>polyoum</i>		2		1	

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens		
					Match	Mismatch	Match	Mismatch	
			<i>Amathia</i>	<i>verticillata</i>	123		79		
			<i>Bugula</i>	<i>neritina</i>		2		1	
				<i>stolonifera</i>		1		1	
			<i>Celleporaria</i>	<i>brunnea</i>		2		1	
			<i>Cradoscrupocellaria</i>	<i>tenuirostris</i>		2		1	
			<i>Crisulipora</i>	<i>occidentalis</i>		8		5	
			<i>Disporella</i>	<i>buskiana</i>		1		1	
			<i>Hippopodina</i>	<i>feegeensis</i>		1		1	
			<i>Schizoporella</i>	<i>errata</i>		1		1	
			<i>Scrupocellaria</i>	<i>bertholettii</i>		2		1	
			<i>Smittoidea</i>	<i>prolifica</i>		1		1	
			<i>Thalamoporella</i>	<i>californica</i>		7		5	
			<i>Tubulipora</i>	<i>pacifica</i>		1		1	
			<i>Watersipora</i>	<i>subtorquata</i>	1	1			
		<i>vidovici</i>	<i>Amathia</i>	<i>verticillata</i>	1	50			
			<i>Bugulina</i>	<i>longirostrata</i>	1	50			
	<i>Anguinella</i>	<i>palmata</i>	<i>Anguinella</i>	<i>palmata</i>	22	96			
			<i>Bugula</i>	<i>stolonifera</i>			1	4	
	<i>Bugula</i>	<i>flabellata</i>	<i>Amathia</i>	<i>verticillata</i>	1	33			
				<i>Bugula</i>	<i>flabellata</i>	1	33		
				<i>stolonifera</i>	1			33	
		<i>longirostrata</i>	<i>Bugula</i>	<i>flabellata</i>	1	1			
					<i>neritina</i>	5	4		
					<i>stolonifera</i>	18	16		
				<i>Bugulina</i>	<i>longirostrata</i>	77	69		
				<i>Celleporaria</i>	<i>brunnea</i>			1	1
				<i>Celleporella</i>	<i>hyalina</i>			1	1
				<i>Cheilopora</i>	<i>praelonga</i>			1	1
				<i>Crisularia</i>	<i>pacifica</i>			2	2
				<i>Cryptosula</i>	<i>pallasiana</i>			1	1
				<i>Licornia</i>	<i>diegensis</i>			1	1
				<i>Pomocellaria</i>	<i>varians</i>			1	1
			<i>Schizoporella</i>	<i>japonica</i>	1			1	

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens				
					Match	Mismatch	Match	Mismatch			
			<i>Watersipora</i>	<i>subtorquata</i>		2		2			
		<i>neritina</i>	<i>Bugula</i>	<i>neritina</i>	526		92	3			
				<i>stolonifera</i>		4			1		
			<i>Bugulina</i>	<i>longirostrata</i>		2					
			<i>Celleporaria</i>	<i>brunnea</i>		2					
			<i>Crisulipora</i>	<i>occidentalis</i>		3					1
			<i>Cryptosula</i>	<i>pallasiana</i>		5					1
			<i>Smittoidea</i>	<i>prolifca</i>		1					
			<i>Thalamoporella</i>	<i>californica</i>		2					
			<i>Tricellaria</i>	<i>occidentalis</i>		4					1
			<i>Tubulipora</i>	<i>pacifica</i>		1					
			<i>Watersipora</i>	<i>subtorquata</i>		3					1
		<i>pacifica</i>	<i>Amathia</i>	<i>verticillata</i>	50	1	91	2			
			<i>Bugula</i>	<i>neritina</i>		1			2		
				<i>stolonifera</i>		1			2		
				<i>Crisularia</i>		<i>pacifica</i>					
			<i>Tricellaria</i>	<i>occidentalis</i>		1			2		
		<i>Watersipora</i>	<i>subtorquata</i>	1		2					
		<i>stolonifera</i>	<i>Amathia</i>	<i>verticillata</i>	307	1	88				
			<i>Anguinella</i>	<i>palmata</i>		1					
			<i>Bugula</i>	<i>californica</i>		1					
				<i>neritina</i>		11			3		
				<i>stolonifera</i>							
			<i>Bugulina</i>	<i>longirostrata</i>		2			1		
			<i>Celleporaria</i>	<i>brunnea</i>		3			1		
			<i>Celleporella</i>	<i>hyalina</i>		1					
			<i>Conopeum</i>	<i>reticulum</i>		1					
			<i>Crisia</i>	<i>occidentalis</i>		1					
			<i>Crisularia</i>	<i>pacifica</i>		2			1		
			<i>Cryptosula</i>	<i>pallasiana</i>		4			1		
			<i>Pomocellaria</i>	<i>varians</i>		1					
		<i>Schizoporella</i>	<i>japonica</i>	1							
		<i>Scrupocellaria</i>	<i>bertholettii</i>	1							

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
			<i>Smittoidea</i>	<i>prolifca</i>		2		1
			<i>Thalamoporella</i>	<i>californica</i>		2		1
			<i>Tricellaria</i>	<i>occidentalis</i>		5		1
			<i>Watersipora</i>	<i>subtorquata</i>		2		1
	<i>Bugulina</i>	<i>stolonifera</i>	<i>Bugulina</i>	<i>longirostrata</i>		2		1
			<i>Watersipora</i>	<i>subtorquata</i>		1		
	<i>Buskia</i>	<i>seriata</i>	<i>Buskia</i>	<i>seriata</i>		2		100
	<i>Caulibugula</i>	<i>ciliata</i>	<i>Caulibugula</i>	<i>ciliata</i>		7		100
	<i>Celleporaria</i>	<i>brunnea</i>	<i>Celleporaria</i>	<i>brunnea</i>	138			93
			<i>Celleporella</i>	<i>hyalina</i>		2		1
			<i>Crisulipora</i>	<i>occidentalis</i>		1		1
			<i>Hippopodina</i>	<i>feegeensis</i>		1		1
			<i>Schizoporella</i>	<i>errata</i>		1		1
				<i>japonica</i>		1		1
			<i>Thalamoporella</i>	<i>californica</i>		2		1
			<i>Watersipora</i>	<i>subtorquata</i>		2		1
	<i>Celleporella</i>	<i>hyalina</i>	<i>Celleporella</i>	<i>hyalina</i>		8		100
	<i>Conopeum</i>	<i>reticulatum</i>	<i>Conopeum</i>	<i>tenuissimum</i>		1		100
		<i>tenuissimum</i>	<i>Bugula</i>	<i>stolonifera</i>		1		5
			<i>Conopeum</i>	<i>reticulum</i>		1		5
				<i>tenuissimum</i>	15			75
			<i>Cryptosula</i>	<i>pallasiana</i>		3		15
	<i>Crisia</i>	<i>occidentalis</i>	<i>Crisia</i>	<i>occidentalis</i>	63			43
			<i>Crisulipora</i>	<i>occidentalis</i>	11			7
			<i>Licornia</i>	<i>diegensis</i>		1		1
	<i>Cryptosula</i>	<i>pallasiana</i>	<i>Celleporaria</i>	<i>brunnea</i>		3		2
			<i>Celleporella</i>	<i>hyalina</i>		1		1
			<i>Cryptosula</i>	<i>pallasiana</i>	138			93
			<i>Hippopodina</i>	<i>feegeensis</i>		2		1
			<i>Schizoporella</i>	<i>japonica</i>		1		1
			<i>Scrupocellaria</i>	<i>bertholettii</i>		1		1
			<i>Smittoidea</i>	<i>prolifca</i>		1		1
		<i>Thalamoporella</i>	<i>californica</i>		1		1	

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
			<i>Tubulipora</i>	<i>pacifica</i>		1		1
	<i>Fenestulina</i>	<i>delicia</i>	<i>Fenestulina</i>	<i>delicia</i>	22		100	
	<i>Filicrisia</i>	<i>franciscana</i>	<i>Crisulipora</i>	<i>occidentalis</i>		1		2
			<i>Filicrisia</i>	<i>franciscana</i>	25		61	
	<i>Membranipora</i>	<i>chesapeakeensis</i>	<i>Membranipora</i>	<i>chesapeakeensis</i>	32		100	
		<i>membranacea</i>	<i>Membranipora</i>	<i>villosa</i>		2		100
	<i>Pectinatella</i>	<i>magnifica</i>	<i>Pectinatella</i>	<i>magnifica</i>	23		100	
	<i>Schizoporella</i>	<i>errata</i>	<i>Schizoporella</i>	<i>errata</i>	14		93	
			<i>japonica</i>			1		7
		<i>japonica</i>	<i>Bugula</i>	<i>neritina</i>		1		1
			<i>Cryptosula</i>	<i>pallasiana</i>		2		1
			<i>Schizoporella</i>	<i>japonica</i>	124		66	
	<i>Scrupocellaria</i>	<i>diegensis</i>	<i>Celleporaria</i>	<i>brunnea</i>		1		1
			<i>Crisia</i>	<i>occidentalis</i>		2		3
			<i>Licornia</i>	<i>diegensis</i>	64		94	
			<i>Tricellaria</i>	<i>occidentalis</i>		1		1
	<i>Smittoidea</i>	<i>prolifica</i>	<i>Smittoidea</i>	<i>prolifica</i>	45		100	
	<i>Tricellaria</i>	<i>occidentalis</i>	<i>Bugula</i>	<i>neritina</i>		1		1
			<i>stolonifera</i>			5		3
			<i>Tricellaria</i>	<i>occidentalis</i>	44		30	
			<i>Watersipora</i>	<i>subtorquata</i>		1		1
	<i>Watersipora</i>	<i>arcuata</i>	<i>Bugula</i>	<i>neritina</i>		3		7
			<i>stolonifera</i>			1		2
			<i>Celleporaria</i>	<i>aperta</i>		1		2
			<i>Crisulipora</i>	<i>occidentalis</i>		1		2
			<i>Cryptosula</i>	<i>pallasiana</i>		1		2
			<i>Thalamoporella</i>	<i>californica</i>		2		4
			<i>Tricellaria</i>	<i>occidentalis</i>		1		2
			<i>Watersipora</i>	<i>arcuata</i>		33		72
			<i>subtorquata</i>		3		7	
		<i>subovoidea</i>	<i>Watersipora</i>	<i>subovoidea</i>		1		13
		<i>subtorquata</i>			7		88	
	<i>subtorquata</i>		<i>Bugula</i>	<i>neritina</i>		3		1

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
				<i>stolonifera</i>		2		
				<i>Celleporella</i>		1		
				<i>Crisia</i>		2		
				<i>Crisulipora</i>		6		1
				<i>Cryptosula</i>		5		1
				<i>Fenestrulina</i>		1		
				<i>Licornia</i>		4		1
				<i>Schizoporella</i>		1		
				<i>Smittoidea</i>		1		
				<i>Thalamoporella</i>		5		1
				<i>Tubulipora</i>		4		1
				<i>Watersipora</i>		1		
				<i>subtorquata</i>	495		93	
Chordata	Ascidia	ceratodes	Ascidia	<i>ceratodes</i>	56		89	
				<i>zara</i>	5		8	
			<i>Ciona</i>	1		2		
			<i>Distaplia</i>	1		2		
		virginea	Ascidia	<i>ceratodes</i>	12		67	
				<i>zara</i>	4		22	
			<i>Ciona</i>	2		11		
		zara	Ascidia	<i>ceratodes</i>	8		3	
				<i>zara</i>	223		70	
			Botrylloides	<i>diegensis</i>	2		1	
				<i>violaceus</i>	5		2	
			<i>Botryllus</i>	<i>schlosseri</i>	9		3	
			Ciona	<i>intestinalis</i>	4		1	
				<i>savignyi</i>	8		3	
			<i>Corella</i>	<i>inflata</i>	1			
<i>Didemnum</i>	<i>vexillum</i>		18		6			
<i>Diplosoma</i>	<i>listerianum</i>		2		6			
<i>Distaplia</i>	<i>occidentalis</i>	3		1				
Molgula	<i>ficus</i>	1						
	<i>manhattensis</i>	6		2				

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens		
					Match	Mismatch	Match	Mismatch	
			<i>Perophora</i>	<i>annectens</i>		1			
			<i>Styela</i>	<i>clava</i>		11		3	
	<i>Botrylloides</i>	<i>leachii</i>	<i>Ascidia</i>	<i>zara</i>	200	1	70		
			<i>Botrylloides</i>	<i>diegensis</i>					
				<i>perspicuus</i>		1			
				<i>violaceus</i>		54		19	
				<i>Botryllus</i>		<i>schlosseri</i>		22	8
				<i>Ciona</i>		<i>intestinalis</i>		1	
				<i>Didemnum</i>		<i>vexillum</i>		1	
				<i>Diplosoma</i>		<i>listerianum</i>		4	1
				<i>Distaplia</i>		<i>occidentalis</i>		1	
				<i>Styela</i>		<i>clava</i>		2	1
			<i>pizoni</i>	<i>Botrylloides</i>	<i>diegensis</i>	2	13		
					<i>perspicuus</i>	4	27		
					<i>violaceus</i>	4	27		
					<i>Botryllus</i>	<i>schlosseri</i>	1	7	
				<i>Diplosoma</i>	<i>listerianum</i>	1	7		
				<i>Styela</i>	<i>plicata</i>	1	7		
			<i>Symplegma</i>	<i>reptans</i>	2	13			
		<i>violaceus</i>	<i>Ascidia</i>	<i>ceratodes</i>	346	1	76		
			<i>Botrylloides</i>	<i>diegensis</i>		12		3	
				<i>violaceus</i>					
				<i>Botryllus</i>		<i>schlosseri</i>		27	6
				<i>Ciona</i>		<i>intestinalis</i>		2	0
						<i>savignyi</i>		4	1
				<i>Corella</i>		<i>inflata</i>		2	
				<i>Didemnum</i>		<i>vexillum</i>		2	4
				<i>Diplosoma</i>		<i>listerianum</i>		11	2
				<i>Distaplia</i>		<i>occidentalis</i>		18	4
				<i>Molgula</i>		<i>manhattensis</i>		3	1
				<i>Polyandrocarpa</i>		<i>zorritensis</i>		1	
			<i>Styela</i>	<i>clava</i>	2				
				<i>truncata</i>	3	1			

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens		
					Match	Mismatch	Match	Mismatch	
			<i>Symplegma</i>	<i>reptans</i>		1			
	<i>Botryllus</i>	<i>schlosseri</i>	<i>Ascidia</i>	<i>ceratodes</i>		1			
				<i>zara</i>		1			
			<i>Botrylloides</i>	<i>diegensis</i>		2			
				<i>perspicuus</i>		1			
				<i>violaceus</i>		13		2	
			<i>Botryllus</i>	<i>schlosseri</i>		697		94	
			<i>Ciona</i>	<i>savignyi</i>			2		
			<i>Didemnum</i>	<i>vexillum</i>			3		
			<i>Diplosoma</i>	<i>listerianum</i>			6		1
			<i>Distaplia</i>	<i>occidentalis</i>			4		1
			<i>Microcosmus</i>	<i>squamiger</i>			3		
			<i>Molgula</i>	<i>ficus</i>			1		
				<i>manhattensis</i>			3		
			<i>Styela</i>	<i>canopus</i>			3		
	<i>clava</i>				2				
	<i>Ciona</i>	<i>intestinalis</i>	<i>Ascidia</i>	<i>ceratodes</i>		2		1	
				<i>zara</i>		5		2	
			<i>Botrylloides</i>	<i>violaceus</i>		1			
			<i>Botryllus</i>	<i>schlosseri</i>		1			
			<i>Ciona</i>	<i>intestinalis</i>		252		94	
			<i>savignyi</i>			5		2	
		<i>Diplosoma</i>	<i>listerianum</i>		1				
		<i>Styela</i>	<i>clava</i>		1				
		<i>savignyi</i>	<i>Ascidia</i>	<i>zara</i>		1			
				<i>Botrylloides</i>	<i>violaceus</i>		1		
	<i>Botryllus</i>		<i>schlosseri</i>		1				
	<i>Ciona</i>		<i>intestinalis</i>		3		1		
		<i>savignyi</i>		235		97			
	<i>Corella</i>	<i>inflata</i>	<i>Corella</i>	<i>inflata</i>		5		83	
			<i>Diplosoma</i>	<i>listerianum</i>		1		17	
	<i>Didemnum</i>	<i>vexillum</i>	<i>Didemnum</i>	<i>vexillum</i>		36		97	
			<i>Diplosoma</i>	<i>listerianum</i>		1		3	

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
	<i>Diplosoma</i>	<i>listerianum</i>	<i>Botryllus</i>	<i>schlosseri</i>		1		1
			<i>Ciona</i>	<i>savignyi</i>		1		1
			<i>Didemnum</i>	<i>vexillum</i>		1		1
			<i>Diplosoma</i>	<i>listerianum</i>	163		87	9
			<i>Microcosmus</i>	<i>squamiger</i>		1		1
			<i>Molgula</i>	<i>manhattensis</i>		2		1
			<i>Styela</i>	<i>clava</i>		1		1
	<i>Distaplia</i>	<i>occidentalis</i>	<i>Didemnum</i>	<i>vexillum</i>		14		9
			<i>Diplosoma</i>	<i>listerianum</i>		2		1
			<i>Distaplia</i>	<i>occidentalis</i>	4		3	0
			<i>Symplegma</i>	<i>reptans</i>		2		1
	<i>Microcosmus</i>	<i>squamiger</i>	<i>Microcosmus</i>	<i>squamiger</i>	10		77	0
			<i>Styela</i>	<i>canopus</i>		3		23
	<i>Molgula</i>	<i>manhattensis</i>	<i>Ascidia</i>	<i>zara</i>		1		0
			<i>Botrylloides</i>	<i>violaceus</i>		2		1
			<i>Botryllus</i>	<i>schlosseri</i>		1		5
			<i>Ciona</i>	<i>intestinalis</i>		2		1
				<i>savignyi</i>		6		3
			<i>Didemnum</i>	<i>vexillum</i>		12		6
			<i>Diplosoma</i>	<i>listerianum</i>		5		2
			<i>Distaplia</i>	<i>occidentalis</i>		1		0
			<i>Molgula</i>	<i>manhattensis</i>	161		79	0
			<i>Styela</i>	<i>clava</i>		3		1
	<i>Perophora</i>	<i>japonica</i>	<i>Didemnum</i>	<i>vexillum</i>		1		1
			<i>Perophora</i>	<i>annectens</i>		7		4
		<i>viridis</i>	<i>Microcosmus</i>	<i>squamiger</i>		3		25
			<i>Perophora</i>	<i>annectens</i>		6		50
	<i>Polyandrocarpa</i>	<i>zorritensis</i>	<i>Botryllus</i>	<i>schlosseri</i>		1		1
			<i>Didemnum</i>	<i>vexillum</i>		2		2
			<i>Diplosoma</i>	<i>listerianum</i>		5		4
			<i>Distaplia</i>	<i>occidentalis</i>		1		1
			<i>Molgula</i>	<i>ficus</i>		5		4

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens	
					Match	Mismatch	Match	Mismatch
				<i>manhattensis</i>	93	1	78	1
			<i>Polyandrocarpa</i>	<i>zorritensis</i>		9		8
			<i>Styela</i>	<i>canopus</i>		1		1
				<i>clava</i>		1		1
			<i>Symplegma</i>	<i>reptans</i>		1		1
			<i>Styela</i>	<i>canopus</i>	<i>Microcosmus</i>	<i>squamiger</i>	2	67
					<i>Styela</i>	<i>canopus</i>	1	33
				<i>clava</i>	<i>Microcosmus</i>	<i>squamiger</i>	1	2
					<i>Molgula</i>	<i>manhattensis</i>	1	2
					<i>Styela</i>	<i>canopus</i>	9	14
		<i>clava</i>		54	83			
	<i>plicata</i>	<i>Botryllus</i>		<i>schlosseri</i>	1	2		
		<i>Ciona</i>		<i>intestinalis</i>	2	4		
				<i>savignyi</i>	1	2		
				<i>Didemnum</i>	<i>vexillum</i>	1	2	
		<i>Diplosoma</i>	<i>listerianum</i>	4	7			
		<i>Distaplia</i>	<i>occidentalis</i>	3	5			
		<i>Microcosmus</i>	<i>squamiger</i>	1	2			
		<i>Molgula</i>	<i>ficus</i>	1	2			
		<i>Polyandrocarpa</i>	<i>zorritensis</i>	2	4			
<i>Styela</i>		<i>clava</i>	1	2				
	<i>plicata</i>	37	65					
<i>Symplegma</i>	<i>reptans</i>	3	5					
Cnidaria	<i>Bougainvillia</i>	<i>muscus</i>	<i>Garveia</i>	<i>franciscana</i>	11		100	
	<i>Diadumene</i>	<i>franciscana</i>	<i>Diadumene</i>	<i>franciscana</i>	4	10	5	
				<i>leucolena</i>	2	22		
			<i>Zaolutus</i>	<i>actius</i>	9			
		<i>leucolena</i>	<i>Diadumene</i>	<i>franciscana</i>	4	57		
				<i>leucolena</i>	1	14		
		<i>Zaolutus</i>	<i>actius</i>	2	29			
		<i>lineata</i>	<i>Diadumene</i>	<i>franciscana</i>	4	67		
		<i>lineata</i>	2	33				
<i>Ectopleura</i>	<i>crocea</i>	<i>Ectopleura</i>	<i>crocea</i>	24	100			

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens			
					Match	Mismatch	Match	Mismatch		
	<i>Metridium</i>	<i>senile</i>	<i>Diadumene</i>	<i>franciscana</i>	10	59				
				<i>leucolena</i>					2	12
			<i>Zaolutus</i>	<i>actius</i>					5	29
Entoprocta	<i>Barentsia</i>	<i>benedeni</i>	<i>Barentsia</i>	<i>benedeni</i>	1	100				
Mollusca	<i>Acanthodoris</i>	<i>nanaimoensis</i>	<i>Acanthodoris</i>	<i>nanaimoensis</i>	1	100				
		<i>rhodoceras</i>	<i>Acanthodoris</i>	<i>rhodoceras</i>	1	100				
	<i>Alia</i>	<i>carinata</i>	<i>Astyris</i>	<i>aurantiaca</i>	5	45				
			<i>Haminoea</i>	<i>japonica</i>					1	9
			<i>Hermisenda</i>	<i>crassicornis</i>					1	9
			<i>Leptopecten</i>	<i>latiauratus</i>					1	9
			<i>Urosalpinx</i>	<i>cinerea</i>					3	27
<i>Amphissa</i>	<i>reticulata</i>	<i>Alia</i>	<i>carinata</i>	1	20					
		<i>Astyris</i>	<i>aurantiaca</i>	4	80					
<i>Crepidula</i>	<i>nummaria</i>	<i>Crepidula</i>	<i>convexa</i>	1	100					
	<i>plana</i>	<i>Crepidula</i>	<i>plana</i>	4	80					
			<i>Ostrea</i>	<i>lurida</i>	1	20				
<i>Crepidatella</i>	<i>lingulata</i>	<i>Crepidula</i>	<i>plana</i>	1	100					
<i>Dendronotus</i>	<i>venustus</i>	<i>Dendronotus</i>	<i>frondosus</i>	2	29					
		<i>Eubbranchus</i>	<i>doriae</i>	4	57					
		<i>Flabellina</i>	<i>trilineata</i>	1	14					
<i>Diaphorodoris</i>	<i>lirulatocauda</i>	<i>Acanthodoris</i>	<i>hudsoni</i>	1	33					
			<i>rhodoceras</i>	1	33					
		<i>Diaphorodoris</i>	<i>lirulatocauda</i>	1	33					
<i>Dirona</i>	<i>picta</i>	<i>Dirona</i>	<i>picta</i>	1	50					
		<i>Okenia</i>	<i>plana</i>	1	50					
<i>Flabellina</i>	<i>trilineata</i>	<i>Flabellina</i>	<i>trilineata</i>	6	86					
		<i>Hermisenda</i>	<i>crassicornis</i>	1	14					
	<i>verrucosa</i>	<i>Flabellina</i>	<i>trilineata</i>	3	60					
		<i>Hermisenda</i>	<i>crassicornis</i>	2	40					
<i>Geukensia</i>	<i>demissa</i>	<i>Geukensia</i>	<i>demissa</i>	2	100					
<i>Haminoea</i>	<i>japonica</i>	<i>Haminoea</i>	<i>japonica</i>	38	20					
		<i>Okenia</i>	<i>plana</i>	1	1					
<i>Hiatella</i>	<i>arctica</i>	<i>Hiatella</i>	<i>arctica</i>	13	100					

Phylum	Morpho-genus	Morpho-species	Molec-genus	Molec-species	Specimen Count		Percent of Specimens		
					Match	Mismatch	Match	Mismatch	
	<i>Musculista</i>	<i>senhousia</i>	<i>Arcuatula</i>	<i>senhousia</i>	55		100		
	<i>Mya</i>	<i>arenaria</i>	<i>Mya</i>	<i>arenaria</i>	1		100		
	<i>Mytilus</i>	<i>edulis</i>	<i>Doto</i>	<i>amyra</i>		1		100	
			<i>galloprovincialis</i>	<i>Hiatella</i>	<i>arctica</i>		2		50
				<i>Mytilus</i>	<i>californianus</i>		1		25
		<i>trossulus</i>	<i>Ostrea</i>	<i>lurida</i>		1		25	
			<i>Arcuatula</i>	<i>senhousia</i>		2		22	
			<i>Crassadoma</i>	<i>gigantea</i>		1		11	
			<i>Modiolus</i>	<i>modiolus</i>		5		56	
			<i>Mytilus</i>	<i>californianus</i>		1		11	
	<i>Onchidoris</i>	<i>bilamellata</i>	<i>Acanthodoris</i>	<i>rhodoceras</i>		2		33	
			<i>Okenia</i>	<i>plana</i>		1		17	
			<i>Onchidoris</i>	<i>bilamellata</i>		3		50	
	<i>Ostrea</i>	<i>lurida</i>	<i>Clinocardium</i>	<i>nuttallii</i>		1		1	
			<i>Macoma</i>	<i>petalum</i>		1		1	
			<i>Ostrea</i>	<i>lurida</i>		167		99	
	<i>Placida</i>	<i>dendritica</i>	<i>Placida</i>	<i>dendritica</i>	1		25		
	<i>Pododesmus</i>	<i>macrochisma</i>	<i>Crepidula</i>	<i>plana</i>		1		50	
			<i>Ostrea</i>	<i>lurida</i>		1		50	
	<i>Polycera</i>	<i>atra</i>	<i>Mytilus</i>	<i>californianus</i>		1		2	
			<i>hedgpethi</i>	<i>Polycera</i>	<i>atra</i>		43		96
				<i>Polycera</i>	<i>hedgpethi</i>		1		2
		<i>hedgpethi</i>	<i>Doto</i>	<i>amyra</i>		1		5	
			<i>Polycera</i>	<i>atra</i>		1		5	
		<i>hedgpethi</i>		<i>hedgpethi</i>		17		89	
	<i>Ruditapes</i>	<i>philippinarum</i>	<i>Ruditapes</i>	<i>philippinarum</i>	7		100		
	<i>Triopha</i>	<i>maculata</i>	<i>Triopha</i>	<i>maculata</i>	3		100		
	<i>Urosalpinx</i>	<i>cinerea</i>	<i>Urosalpinx</i>	<i>cinerea</i>	2		100		

Table 5.4. Failed Identifications. 9283 vouchers, listed by their morphological assignment, were not genetically identified for the following causes: (A) Failed assemblies, n=2993 vials. A failed assembly results from poor quality sequencing data which is typically due to a poor PCR results; (B) Bacterial or human origin, n=2 vials; (C) No BLAST hit, n=6269 vials. (D) Alignment too short; n=19 vials. If no species name for a voucher is shown, none was provided. Taxonomy here is based on prior morphological assessment.

(A) Failed assemblies.

Phylum	Genus	Species	Count
None given			59
Annelida	None given		128
	<i>Alitta</i>	<i>succinea</i>	1
	<i>Amaeana</i>		2
	<i>Amblyosyllis</i>		4
	<i>Armandia</i>	<i>brevis</i>	1
	<i>Bispira</i>		3
	<i>Branchiomma</i>		17
		<i>boholense</i>	5
	<i>Branchiosyllis</i>		4
	<i>Capitella</i>	<i>capitata</i>	6
	<i>Chone</i>	<i>gracilis</i>	1
	<i>Cirriformia</i>		3
		<i>moorei</i>	14
	<i>Dipolydora</i>	<i>caulleryi</i>	1
	<i>Dorvillea</i>		4
	<i>Euchone</i>	<i>limnicola</i>	7
	<i>Eulalia</i>		1
		<i>quadrioculata</i>	14
	<i>Eumida</i>		2
	<i>Eupolymnia</i>		1
	<i>Ficopomatus</i>	<i>enigmaticus</i>	12
	<i>Glycera</i>	<i>tenuis</i>	1
	<i>Glycinde</i>		1
		<i>picta</i>	5
	<i>Halosydna</i>		1
		<i>brevisetosa</i>	7
		<i>johnsoni</i>	1
	<i>Harmothoe</i>	<i>imbricata</i>	30
	<i>Heteromastus</i>		2
		<i>filobranthus</i>	1
	<i>Hydroides</i>		3
		<i>elegans</i>	5
		<i>gracilis</i>	13
	<i>Leitoscoloplos</i>	<i>pugettensis</i>	6
	<i>Lumbrineris</i>	<i>perkinsi</i>	3
	<i>Marphysa</i>		5
<i>Megasyllis</i>	<i>nipponica</i>	36	

Phylum	Genus	Species	Count
	<i>Micronereis</i>		1
	<i>Myrianida</i>	<i>pachycera</i>	1
	<i>Myxicola</i>	<i>infundibulum</i>	1
	<i>Naineris</i>		2
	<i>Neanthes</i>	<i>acuminata</i>	2
	<i>Neoamphitrite</i>		11
	<i>Nephtys</i>	<i>caecoides</i>	11
	<i>Nereis</i>	<i>laticens</i>	13
		<i>vexillosa</i>	1
	<i>Nicolea</i>		25
	<i>Odontosyllis</i>	<i>phosphorea</i>	12
	<i>Ophryotrocha</i>		9
	<i>Paleanotus</i>	<i>bellis</i>	7
	<i>Parasabella</i>		13
		<i>pallida</i>	13
	<i>Pectinaria</i>	<i>californiensis</i>	1
	<i>Pettiboneia</i>		4
	<i>Platynereis</i>		13
		<i>bicanaliculata</i>	26
	<i>Polycirrus</i>		2
	<i>Polydora</i>		15
		<i>cornuta</i>	2
	<i>Pseudochitinopoma</i>	<i>occidentalis</i>	3
	<i>Pseudopolydora</i>	<i>paucibranchiata</i>	2
	<i>Pterocirrus</i>	<i>montereyensis</i>	2
	<i>Sabaco</i>	<i>elongatus</i>	28
	<i>Salmacina</i>	<i>tribranchiata</i>	21
	<i>Scoletoma</i>	<i>tetraura</i>	2
	<i>Serpula</i>		1
	<i>Streblosoma</i>		3
		<i>uncinatus</i>	1
	<i>Timarete</i>		6
	<i>Trypanosyllis</i>		3
	Arthropoda	None given	
<i>Aciconula</i>		<i>acanthosoma</i>	1
<i>Americorophium</i>			5
<i>Ammothea</i>		<i>hilgendorfi</i>	1
<i>Ampelisca</i>			1
<i>Amphibalanus</i>		<i>amphitrite</i>	6
		<i>improvisus</i>	31
<i>Ampithoe</i>		<i>lacertosa</i>	16
		<i>plumulosa</i>	3
		<i>sectimana</i>	1
		<i>valida</i>	4
<i>Aoroides</i>		5	

Phylum	Genus	Species	Count
		<i>columbiae</i>	3
		<i>secunda</i>	22
	<i>Apolochus</i>	<i>picadurus</i>	2
	<i>Aruga</i>	<i>holmesi</i>	2
	<i>Balanus</i>	<i>crenatus</i>	23
		<i>nubilus</i>	1
		<i>trigonus</i>	5
	<i>Calanus</i>	<i>pacificus</i>	1
	<i>Cancer</i>	<i>jordani</i>	2
	<i>Caprella</i>		4
		<i>californica</i>	13
		<i>equilibra</i>	1
		<i>mutica</i>	12
		<i>scaura</i>	1
		<i>simia</i>	35
	<i>Clausocalanus</i>	<i>parapergens</i>	1
	<i>Deutella</i>	<i>californica</i>	1
	<i>Diaphanosoma</i>		1
	<i>Dissiminassa</i>	<i>dissimilis</i>	7
	<i>Ditrichocorycaeus</i>	<i>anglicus</i>	1
	<i>Dulichia</i>		1
	<i>Elasmopus</i>		1
		<i>bampo</i>	52
	<i>Erichthonius</i>	<i>punctatus</i>	35
	<i>Eurylana</i>		1
	<i>Gammaropsis</i>	<i>shoemakeri</i>	4
		<i>thompsoni</i>	1
	<i>Gammarus</i>	<i>daiberi</i>	1
	<i>Gnorimosphaeroma</i>	<i>oregonensis</i>	9
	<i>Grandidierella</i>	<i>japonica</i>	9
	<i>Heptacarpus</i>		1
	<i>Ianiropsis</i>		4
		<i>analoga</i>	1
	<i>Jassa</i>	<i>slatteryi</i>	2
	<i>Laticorophium</i>	<i>baconi</i>	3
	<i>Leptochelia</i>		1
	<i>Leucothoe</i>		2
	<i>Leucothoe</i>	<i>alata</i>	25
	<i>Liljeborgia</i>	<i>geminata</i>	1
	<i>Lophopanopeus</i>		1
	<i>Maera</i>		1
	<i>Megabalanus</i>	<i>californicus</i>	10
	<i>Melita</i>	<i>nitida</i>	1
	<i>Monocorophium</i>		1
		<i>acherusicum</i>	16

Phylum	Genus	Species	Count
		<i>insidiosum</i>	3
	<i>Nippoleucon</i>	<i>hinumensis</i>	1
	<i>Oithona</i>	<i>similis</i>	1
	<i>Pachycheles</i>		3
	<i>Palaemon</i>	<i>macrodactylus</i>	2
	<i>Paracalanus</i>	<i>parvus</i>	1
	<i>Paracerceis</i>	<i>cordata</i>	1
		<i>sculpta</i>	37
	<i>Paradexamine</i>		13
	<i>Paranthura</i>	<i>japonica</i>	49
	<i>Podocerus</i>	<i>brasiliensis</i>	7
		<i>cristatus</i>	10
	<i>Protohyale</i>		1
		<i>frequens</i>	1
	<i>Pyromaia</i>	<i>tuberculata</i>	1
	<i>Quadrimaera</i>	<i>reishi</i>	2
	<i>Sinelobus</i>		2
	<i>Sinocalanus</i>		1
	<i>Sphaeroma</i>	<i>quoianum</i>	1
	<i>Stenothoe</i>	<i>valida</i>	3
	<i>Synidotea</i>	<i>laticauda</i>	4
	<i>Zeuxo</i>		2
		<i>normani</i>	1
Bryozoa	None given		8
	<i>Amathia</i>		11
		<i>verticillata</i>	10
	<i>Anguinella</i>	<i>palmata</i>	3
	<i>Bugula</i>	<i>flabellata</i>	1
		<i>neritina</i>	110
		<i>stolonifera</i>	30
	<i>Bugulina</i>	<i>longirostrata</i>	5
	<i>Caulibugula</i>	<i>ciliata</i>	3
	<i>Celleporaria</i>		1
		<i>aperta</i>	2
		<i>brunnea</i>	21
	<i>Celleporella</i>	<i>hyalina</i>	4
	<i>Celleporina</i>		1
	<i>Conopeum</i>		11
		<i>reticulum</i>	2
		<i>tenuissimum</i>	2
	<i>Cradoscrupocellaria</i>	<i>tenuirostris</i>	1
	<i>Crisia</i>	<i>occidentalis</i>	8
	<i>Crisularia</i>	<i>pacifica</i>	4
	<i>Crisulipora</i>	<i>occidentalis</i>	9
	<i>Cryptosula</i>	<i>pallasiana</i>	28

Phylum	Genus	Species	Count	
	<i>Cyclostomella</i>		1	
	<i>Electra</i>	<i>monostachys</i>	1	
	<i>Fenestrulina</i>	<i>delicia</i>	3	
	<i>Filicrisia</i>			2
			<i>franciscana</i>	6
	<i>Hippopodina</i>	<i>feegeensis</i>	3	
	<i>Lichenopora</i>		4	
	<i>Licornia</i>	<i>diegensis</i>	3	
	<i>Membranipora</i>	<i>chesapeakeensis</i>	4	
	<i>Nolella</i>		1	
	<i>Pacificincola</i>		4	
	<i>Parasmittina</i>		8	
	<i>Pectinatella</i>	<i>magnifica</i>	3	
	<i>Schizoporella</i>			1
			<i>errata</i>	2
			<i>japonica</i>	11
	<i>Scruparia</i>		2	
	<i>Scrupocellaria</i>	<i>bertholettii</i>	1	
	<i>Smittoidea</i>	<i>prolifica</i>	16	
	<i>Thalamoporella</i>	<i>californica</i>	6	
	<i>Tricellaria</i>	<i>occidentalis</i>	17	
	<i>Tubulipora</i>	<i>pacifica</i>	7	
	<i>Watersipora</i>			4
		<i>arcuata</i>	5	
		<i>subtorquata</i>	77	
Chlorophyta	<i>Bryopsis</i>		1	
	<i>Enteromorpha</i>		1	
	<i>Ulva</i>		1	
Chordata	None given		19	
	<i>Aplidium</i>		7	
	<i>Ascidia</i>			1
			<i>ceratodes</i>	15
			<i>zara</i>	19
	<i>Botrylloides</i>		<i>diegensis</i>	23
			<i>perspicuus</i>	3
			<i>violaceus</i>	44
	<i>Botryllus</i>	<i>schlosseri</i>	56	
	<i>Ciona</i>			3
			<i>intestinalis</i>	28
			<i>savignyi</i>	42
	<i>Corella</i>	<i>inflata</i>	6	
	<i>Didemnum</i>	<i>vexillum</i>	24	
	<i>Diplosoma</i>	<i>listerianum</i>	87	
	<i>Distaplia</i>			14
		<i>occidentalis</i>	66	

Phylum	Genus	Species	Count
	<i>Microcosmus</i>	<i>squamiger</i>	5
	<i>Molgula</i>	<i>ficus</i>	3
		<i>manhattensis</i>	54
	<i>Perophora</i>		5
		<i>annectens</i>	3
	<i>Polyandrocarpa</i>	<i>zorritensis</i>	5
	<i>Styela</i>		15
		<i>canopus</i>	5
		<i>clava</i>	27
		<i>plicata</i>	11
		<i>truncata</i>	3
<i>Symplegma</i>	<i>reptans</i>	10	
Cnidaria	None given		34
	<i>Aglaophenia</i>		4
	<i>Bougainvillia</i>		1
	<i>Clytia</i>		1
	<i>Cordylophora</i>		16
	<i>Coryne</i>		1
	<i>Diadumene</i>		9
		<i>franciscana</i>	10
		<i>leucolena</i>	5
		<i>lineata</i>	3
	<i>Ectopleura</i>	<i>crocea</i>	5
	<i>Garveia</i>	<i>franciscana</i>	12
	<i>Gonothyraea</i>		2
	<i>Hydra</i>		1
	<i>Obelia</i>		31
	<i>Pennaria</i>		1
	<i>Phialidium</i>		2
	<i>Polysiphonia</i>		1
<i>Zaolutus</i>	<i>actius</i>	3	
Echinodermata	None given		18
Entoprocta	None given		5
	<i>Barentsia</i>	<i>benedeni</i>	6
"Lophophorata"	None given		1
Mollusca	None given		37
	<i>Alia</i>	<i>carinata</i>	6
	<i>Anomia</i>		4
	<i>Arcuatula</i>	<i>senhousia</i>	22
	<i>Astyris</i>	<i>aurantiaca</i>	8
	<i>Crepidula</i>	<i>convexa</i>	5
	<i>Crepidatella</i>	<i>lingulata</i>	1
	<i>Cryptomya</i>	<i>californica</i>	1
	<i>Cuthona</i>		3
<i>albocrusta</i>		1	

Phylum	Genus	Species	Count
	<i>Dendronotus</i>		2
		<i>frondosus</i>	1
	<i>Diaphorodoris</i>	<i>lirulatocauda</i>	1
	<i>Doto</i>		1
		<i>amyra</i>	3
	<i>Eubranchus</i>	<i>misakiensis</i>	1
	<i>Flabellina</i>		1
		<i>trilineata</i>	1
	<i>Gemma</i>	<i>gemma</i>	1
	<i>Haminoea</i>	<i>japonica</i>	10
	<i>Hermisenda</i>	<i>crassicornis</i>	9
	<i>Hiatella</i>	<i>arctica</i>	16
	<i>Lacuna</i>	<i>unifasciata</i>	3
	<i>Leptopecten</i>	<i>latiauratus</i>	2
	<i>Littorina</i>	<i>saxatilis</i>	1
	<i>Lyonsia</i>	<i>californica</i>	1
	<i>Mytilus</i>		79
		<i>californianus</i>	15
	<i>Okenia</i>	<i>angelensis</i>	1
	<i>Ostrea</i>	<i>lurida</i>	48
	<i>Philine</i>	<i>orientalis</i>	5
	<i>Polycera</i>	<i>atra</i>	12
		<i>hedgpethi</i>	4
<i>Ruditapes</i>	<i>philippinarum</i>	1	
<i>Triopha</i>	<i>maculata</i>	1	
<i>Urosalpinx</i>	<i>cinerea</i>	4	
Nematoda	None given		1
Nemertea	None given		10
Platyhelminthes	None given		18
	<i>Eurylepta</i>	<i>aurantiaca</i>	2
	<i>Hoploplana</i>		3
	<i>Pseudoceros</i>		34
	<i>Stylochus</i>		6
Porifera	None given		86
	<i>Grantia</i>		10
	<i>Halichondria</i>		13
	<i>Haliclona</i>		12
Porifera	<i>Leucosolenia</i>		1
	<i>Sycon</i>		19

(B) Human or bacterial Origin

Phylum	Genus	Species	Count
Bryozoa	<i>Bugula</i>	<i>neritina</i>	1
	<i>Tubulipora</i>		1

(C) No BLAST hit.

Phylum	Genus	Species	Count
None given			262
<i>Annelida</i>	None given		342
	<i>Alitta</i>	<i>succinea</i>	11
	<i>Amaeana</i>		26
		<i>occidentalis</i>	3
	<i>Amblyosyllis</i>		4
	<i>Armandia</i>	<i>brevis</i>	12
	<i>Boccardiella</i>	<i>ligerica</i>	1
	<i>Branchiomma</i>		32
		<i>nigromaculatum</i>	1
	<i>Branchiosyllis</i>		18
	<i>Branchiura</i>	<i>sowerbyi</i>	1
	<i>Capitella</i>	<i>capitata</i>	7
	<i>Chone</i>	<i>gracilis</i>	1
	<i>Cirriformia</i>		5
		<i>moorei</i>	13
	<i>Crucigera</i>	<i>zygophora</i>	2
	<i>Dipolydora</i>	<i>caulleryi</i>	2
		<i>socialis</i>	1
	<i>Dorvillea</i>		11
	<i>Drilonereis</i>		2
	<i>Euchone</i>	<i>limnicola</i>	19
	<i>Eulalia</i>		9
		<i>quadrioculata</i>	25
	<i>Eumida</i>		4
	<i>Eupolymnia</i>		2
		<i>heterobranchia</i>	1
	<i>Ficopomatus</i>	<i>enigmaticus</i>	67
	<i>Glycera</i>	<i>americana</i>	50
		<i>tenuis</i>	1
	<i>Glycinde</i>		8
		<i>picta</i>	25
	<i>Halosydna</i>		15
		<i>brevisetosa</i>	20
<i>johnsoni</i>		6	
<i>leius</i>		1	
<i>Harmothoe</i>	<i>imbricata</i>	51	
<i>Hesperonoe</i>		3	

Phylum	Genus	Species	Count	
		<i>laevis</i>	1	
	<i>Heteromastus</i>		11	
	<i>Hobsonia</i>	<i>florida</i>	2	
	<i>Hydroides</i>			10
			<i>elegans</i>	5
			<i>gracilis</i>	54
	<i>Laonome</i>		1	
	<i>Leitoscoloplos</i>	<i>pugettensis</i>	37	
	<i>Lumbrineris</i>	<i>perkinsi</i>	4	
	<i>Malmgreniella</i>		<i>bansei</i>	1
			<i>nigralba</i>	1
	<i>Marenzelleria</i>	<i>viridis</i>	6	
	<i>Marphysa</i>		28	
	<i>Mediomastus</i>	<i>acutus</i>	1	
	<i>Megalomma</i>	<i>pigmentum</i>	2	
	<i>Megasyllis</i>	<i>nipponica</i>	35	
	<i>Micronereis</i>	<i>nanaimoensis</i>	1	
	<i>Myrianida</i>	<i>pachycera</i>	1	
Annelida	<i>Neanthes</i>		1	
		<i>acuminata</i>	13	
	<i>Neoamphitrite</i>		3	
	<i>Neosabellaria</i>	<i>cementarium</i>	1	
	<i>Nephtys</i>		1	
		<i>caecoides</i>	37	
		<i>californiensis</i>	6	
	<i>Nereis</i>		2	
		<i>latescens</i>	1	
		<i>vexillosa</i>	1	
	<i>Nicolea</i>		8	
	<i>Notomastus</i>	<i>lineatus</i>	1	
		<i>magnus</i>	1	
	<i>Odontosyllis</i>	<i>phosphorea</i>	17	
	<i>Ophryotrocha</i>		8	
	<i>Paleanotus</i>	<i>bellis</i>	2	
	<i>Paraprionospio</i>	<i>alata</i>	1	
	<i>Parasabella</i>		12	
		<i>pallida</i>	48	
	<i>Petaloproctus</i>	<i>neoborealis</i>	1	
	<i>Phyllodoce</i>	<i>williamsi</i>	1	
	<i>Piromis</i>		8	
	<i>Pista</i>	<i>brevibranchiata</i>	6	
	<i>Platynereis</i>		8	
		<i>bicanaliculata</i>	17	
	<i>Polycirrus</i>		4	
	<i>Polydora</i>		55	
		<i>cornuta</i>	8	

Phylum	Genus	Species	Count
	<i>Pseudochitinopoma</i>	<i>occidentalis</i>	41
	<i>Pseudopolydora</i>	<i>paucibranchiata</i>	6
	<i>Pterocirrus</i>	<i>montereyensis</i>	2
	<i>Sabaco</i>	<i>elongatus</i>	67
	<i>Salmacina</i>	<i>tribranchiata</i>	36
	<i>Schistomeringos</i>	<i>longicornis</i>	1
	<i>Scoletoma</i>	<i>tetraura</i>	16
	<i>Scoloplos</i>	<i>acmeceps</i>	33
	<i>Serpula</i>		1
	<i>Streblosoma</i>		3
		<i>uncinatus</i>	1
	<i>Syllis</i>		2
	<i>Thormora</i>	<i>johnstoni</i>	2
	<i>Timarete</i>		1
	<i>Trypanosyllis</i>		24
Arthropoda	None given		31
	<i>Allorchestes</i>	<i>angusta</i>	2
	<i>Ampelisca</i>		4
	<i>Amphibalanus</i>		4
		<i>amphitrite</i>	7
		<i>improvisus</i>	48
	<i>Ampithoe</i>		1
		<i>lacertosa</i>	1
		<i>plumulosa</i>	1
		<i>valida</i>	6
	<i>Aoroides</i>		8
		<i>columbiae</i>	2
		<i>inermis</i>	5
		<i>secunda</i>	5
	<i>Apothyale</i>	<i>anceps</i>	1
	<i>Apolochus</i>	<i>picadurus</i>	2
	<i>Aruga</i>	<i>holmesi</i>	4
	<i>Balanus</i>	<i>crenatus</i>	10
		<i>nubilus</i>	25
		<i>trigonus</i>	13
	<i>Cancer</i>	<i>jordani</i>	1
		<i>magister</i>	1
	<i>Caprella</i>		12
		<i>californica</i>	9
		<i>equilibra</i>	2
		<i>mutica</i>	20
<i>scaura</i>		16	
<i>simia</i>		5	
<i>verrucosa</i>	1		
<i>Chthamalus</i>		1	
<i>Crangon</i>	<i>nigricauda</i>	3	

Phylum	Genus	Species	Count
	<i>Deutella</i>	<i>californica</i>	2
	<i>Dissiminassa</i>	<i>dissimilis</i>	4
	<i>Elasmopus</i>		2
	<i>Erichthonius</i>		1
		<i>punctatus</i>	22
	<i>Exopalaemon</i>	<i>modestus</i>	1
	<i>Gammaropsis</i>	<i>thompsoni</i>	2
	<i>Gammarus</i>	<i>daiberi</i>	2
	<i>Gnorimosphaeroma</i>	<i>oregonensis</i>	2
	<i>Grandidierella</i>	<i>japonica</i>	13
	<i>Hemigrapsus</i>	<i>oregonensis</i>	3
	<i>Heptacarpus</i>		1
		<i>paludicola</i>	4
	<i>Heteropleustes</i>	<i>setosus</i>	1
	<i>Hourstonius</i>	<i>vilordes</i>	1
	<i>Hyaella</i>	<i>azteca</i>	1
	<i>Ianiropsis</i>		9
		<i>analoga</i>	1
	<i>Idotea</i>	<i>resecata</i>	2
	<i>Incisocalliope</i>	<i>derzhavini</i>	7
	<i>Ischyrocerus</i>		1
	<i>Janiralata</i>	<i>occidentalis</i>	1
	<i>Jassa</i>		5
		<i>marmorata</i>	5
		<i>slatteryi</i>	4
	<i>Joeropsis</i>		1
		<i>dubia</i>	1
	<i>Laticorophium</i>	<i>baconi</i>	6
	<i>Leptocheilia</i>		2
	<i>Leptodiptomus</i>		1
	<i>Leucothoe</i>	<i>alata</i>	7
	<i>Liljeborgia</i>		1
	<i>Lophopanopeus</i>		4
	<i>Maera</i>		1
	<i>Megabalanus</i>	<i>californicus</i>	6
	<i>Melita</i>	<i>nitida</i>	6
	<i>Mesocyclops</i>	<i>edax</i>	1
	<i>Metopa</i>	<i>cistella</i>	3
	<i>Monocorophium</i>		4
		<i>acherusicum</i>	7
		<i>uenoi</i>	5
	<i>Nebalia</i>	<i>gerkenae</i>	1
	<i>Nippoleucon</i>	<i>hinumensis</i>	3
	<i>Palaemon</i>	<i>macrodactylus</i>	1
	<i>Paracerceis</i>	<i>sculpta</i>	1
	<i>Paramicrodeutopus</i>	<i>schmitti</i>	1

Phylum	Genus	Species	Count
	<i>Paranthura</i>	<i>japonica</i>	23
	<i>Photis</i>		8
		<i>brevipes</i>	20
	<i>Podocerus</i>	<i>brasiliensis</i>	12
		<i>cristatus</i>	1
	<i>Polycheria</i>	<i>osborni</i>	1
	<i>Pseudodiptomus</i>	<i>forbesi</i>	1
	<i>Pugettia</i>	<i>producta</i>	1
	<i>Pyromaia</i>	<i>tuberculata</i>	2
	<i>Quadrimaera</i>	<i>reishi</i>	2
	<i>Romaleon</i>	<i>branneri</i>	2
	<i>Scleroplax</i>	<i>granulata</i>	3
	<i>Sinelobus</i>		9
		<i>stanfordi</i>	4
	<i>Sinocalanus</i>		2
	<i>Sinocorophium</i>	<i>alienense</i>	1
		<i>heteroceratum</i>	6
	<i>Sphaeroma</i>	<i>quoianum</i>	4
	<i>Stenothoe</i>	<i>valida</i>	16
	<i>Synidotea</i>	<i>laticauda</i>	4
	<i>Uromunna</i>		6
	<i>Zeuxo</i>		1
		<i>normani</i>	12
Bryozoa	None given		61
	<i>Aetea</i>		2
	<i>Amathia</i>		124
		<i>gracilis</i>	1
		<i>verticillata</i>	7
	<i>Anguinella</i>	<i>palmata</i>	1
	<i>Aspidelectra</i>		1
	<i>Bugula</i>		2
		<i>flabellata</i>	1
		<i>neritina</i>	88
		<i>stolonifera</i>	12
	<i>Bugulina</i>	<i>longirostrata</i>	4
	<i>Caulibugula</i>	<i>ciliata</i>	1
	<i>Celleporaria</i>		3
		<i>aperta</i>	10
		<i>brunnea</i>	51
	<i>Celleporella</i>	<i>hyalina</i>	68
	<i>Celleporina</i>		5
	<i>Conopeum</i>		9
	<i>Crisia</i>	<i>occidentalis</i>	8
<i>Crisularia</i>	<i>pacifica</i>	1	
<i>Crisulipora</i>	<i>occidentalis</i>	83	
<i>Cryptosula</i>	<i>pallasiana</i>	57	

Phylum	Genus	Species	Count	
	<i>Cyclostomella</i>		1	
	<i>Electra</i>	<i>crustulenta</i>	1	
	<i>Fenestrulina</i>			1
			<i>delicia</i>	3
	<i>Filicrisia</i>		11	
	<i>Hippopodina</i>	<i>feegeensis</i>	11	
	<i>Lichenopora</i>		18	
	<i>Licornia</i>	<i>diegensis</i>	4	
	<i>Pacificincola</i>		16	
	<i>Parasmittina</i>		26	
	<i>Pectinatella</i>	<i>magnifica</i>	2	
	<i>Schizoporella</i>			8
			<i>errata</i>	8
			<i>japonica</i>	9
	<i>Scruparia</i>		1	
	<i>Scrupocellaria</i>			1
			<i>bertholettii</i>	14
	<i>Smittoidea</i>	<i>prolifera</i>	25	
	<i>Tegella</i>	<i>circumclathrata</i>	3	
	<i>Thalamoporella</i>	<i>californica</i>	29	
	<i>Tricellaria</i>	<i>occidentalis</i>	23	
	<i>Tubulipora</i>	<i>pacifica</i>	33	
	<i>Watersipora</i>			2
		<i>arcuata</i>	1	
		<i>subtorquata</i>	47	
Chlorophyta	<i>Bryopsis</i>		1	
	<i>Enteromorpha</i>		2	
	<i>Ulva</i>		1	
Chordata	None given		36	
	<i>Aplidium</i>		12	
	<i>Ascidia</i>	<i>ceratodes</i>	2	
		<i>zara</i>	15	
	<i>Botrylloides</i>			1
			<i>diegensis</i>	6
			<i>perspicuus</i>	2
			<i>violaceus</i>	15
	<i>Botryllus</i>	<i>schlosseri</i>	43	
	<i>Ciona</i>			2
			<i>intestinalis</i>	34
			<i>savignyi</i>	37
	<i>Corella</i>	<i>inflata</i>	16	
	<i>Didemnum</i>	<i>vexillum</i>	158	
	<i>Diplosoma</i>	<i>listerianum</i>	129	
	<i>Distaplia</i>			16
			<i>occidentalis</i>	102
<i>Metandrocarpa</i>		4		

Phylum	Genus	Species	Count
	<i>Microcosmus</i>	<i>squamiger</i>	26
	<i>Molgula</i>	<i>ficus</i>	19
		<i>manhattensis</i>	30
	<i>Perophora</i>		5
		<i>annectens</i>	40
	<i>Polyandrocarpa</i>	<i>zorritensis</i>	6
	<i>Styela</i>		25
		<i>canopus</i>	19
		<i>clava</i>	27
		<i>montereyensis</i>	7
		<i>plicata</i>	4
	<i>truncata</i>	10	
<i>Symplegma</i>	<i>reptans</i>	40	
<i>Ciliophora</i>	<i>Folliculina</i>		1
Cnidaria	None given		146
	<i>Aglaophenia</i>		10
	<i>Alcyonidium</i>		2
	<i>Bougainvillia</i>		21
	<i>Clytia</i>		9
	<i>Cordylophora</i>		25
		<i>caspia</i>	1
	<i>Diadumene</i>	<i>franciscana</i>	8
		<i>leucolena</i>	2
		<i>lineata</i>	3
	<i>Ectopleura</i>	<i>crocea</i>	20
	<i>Garveia</i>	<i>franciscana</i>	21
	<i>Gonothyraea</i>		11
	<i>Hydra</i>		7
	<i>Hydractinia</i>		3
	<i>Metridium</i>		2
	<i>Monostaechas</i>		1
	<i>Obelia</i>		62
	<i>Pennaria</i>		2
	<i>Phialidium</i>		3
	<i>Plumularia</i>		4
	<i>Polysiphonia</i>		6
<i>Stylatula</i>	<i>elongata</i>	3	
<i>Zaolutus</i>	<i>actius</i>	7	
Echinodermata	None given		47
Entoprocta			12
	<i>Barentsia</i>	<i>benedeni</i>	14
"Lophophorata"	None given		9
Mollusca			70
	<i>Alia</i>	<i>carinata</i>	2
	<i>Anomia</i>		13
	<i>Arcuatula</i>	<i>senhousia</i>	29

Phylum	Genus	Species	Count
	<i>Astyris</i>	<i>aurantiaca</i>	4
	<i>Corbula</i>	<i>amurensis</i>	6
	<i>Crassadoma</i>	<i>gigantea</i>	3
	<i>Crepidula</i>	<i>convexa</i>	4
		<i>plana</i>	8
	<i>Crepidatella</i>	<i>lingulata</i>	8
	<i>Cryptomya</i>	<i>californica</i>	2
	<i>Cuthona</i>		2
	<i>Dendronotus</i>		4
		<i>frondosus</i>	6
	<i>Diaphorodoris</i>	<i>lirulatocauda</i>	2
	<i>Dirona</i>	<i>picta</i>	1
	<i>Doto</i>	<i>amyra</i>	4
		<i>columbiana</i>	2
	<i>Eubranchus</i>	<i>doriae</i>	1
		<i>misakiensis</i>	1
	<i>Flabellina</i>	<i>verrucosa</i>	1
	<i>Gemma</i>	<i>gemma</i>	3
	<i>Geukensia</i>	<i>demissa</i>	1
	<i>Haminoea</i>	<i>japonica</i>	6
	<i>Hermisenda</i>	<i>crassicornis</i>	4
	<i>Hiatella</i>	<i>arctica</i>	43
	<i>Ilyanassa</i>	<i>obsoleta</i>	1
	<i>Janolus</i>	<i>fuscus</i>	1
	<i>Lacuna</i>	<i>unifasciata</i>	1
	<i>Leptopecten</i>	<i>latiauratus</i>	24
	<i>Littorina</i>		6
	<i>Lyonsia</i>	<i>californica</i>	1
	<i>Macoma</i>	<i>petalum</i>	2
	<i>Marsenina</i>	<i>rhombica</i>	1
	<i>Marseniopsis</i>		8
	<i>Modiolus</i>	<i>modiolus</i>	3
	<i>Montereina</i>	<i>nobilis</i>	1
	<i>Mya</i>	<i>arenaria</i>	3
	<i>Mytilus</i>		16
		<i>californianus</i>	28
	<i>Okenia</i>	<i>angelensis</i>	12
		<i>plana</i>	4
	<i>Onchidoris</i>	<i>bilamellata</i>	1
	<i>Ostrea</i>		1
		<i>lurida</i>	50
	<i>Philine</i>	<i>orientalis</i>	17
	<i>Polycera</i>	<i>atra</i>	1
		<i>hedgpethi</i>	2
	<i>Potamopyrgus</i>	<i>antipodarum</i>	1
	<i>Ruditapes</i>	<i>philippinarum</i>	5

Phylum	Genus	Species	Count
	<i>Tenellia</i>	<i>adpersa</i>	1
	<i>Theora</i>	<i>lubrica</i>	3
	<i>Urosalpinx</i>	<i>cinerea</i>	3
Nematoda			12
Nemertea			64
	<i>Cephalothrix</i>		11
	<i>Lineus</i>	<i>ruber</i>	1
	<i>Tubulanus</i>		1
Platyhelminthes			104
	<i>Eurylepta</i>	<i>aurantiaca</i>	28
	<i>Hoploplana</i>		44
	<i>Notocomplana</i>	<i>acticola</i>	17
	<i>Pseudoceros</i>		121
	<i>Stylochus</i>		25
Porifera	None given		368
	<i>Euplectella</i>		1
	<i>Grantia</i>		43
	<i>Halichondria</i>		110
	<i>Haliclona</i>		154
	<i>Leucosolenia</i>		14
	<i>Microciona</i>	<i>prolifera</i>	3
	<i>Sycon</i>		142
Rhodophyta	<i>Ceramium</i>		1
"Xenacoelomorpha"	None given		1

(D) Short alignments.

Phylum	Genus	Species	Count
Annelida	<i>Eupolytmia</i>	<i>heterobranchia</i>	1
	<i>Halosydna</i>	<i>brevisetosa</i>	1
	<i>Hydroides</i>		1
	<i>Paradialychone</i>	<i>ecaudata</i>	1
	<i>Parasabella</i>	<i>pallida</i>	2
	<i>Pterocirrus</i>	<i>burtoni</i>	1
Arthropoda	<i>Balanus</i>	<i>trigonus</i>	1
Bryozoa	<i>Crisulipora</i>	<i>occidentalis</i>	1
	<i>Watersipora</i>	<i>subtorquata</i>	1
Chordata			3
	<i>Diplosoma</i>	<i>listerianum</i>	1
	<i>Perophora</i>		2

Table 5.5. List of species considered common contaminants. DNA sequences identified as these species appeared in >1000 assemblies from vials of unrelated vouchers. Source of each contamination result is not known, however impure tissue samples, exchange of tissue between vials, cross-sample movement of lysate during extraction or of DNA during PCR set up are possible. Evidence also exists for eDNA in settlement plate holding tank water during morphological analyses.

Annelida	<i>Megasyllis nipponica</i>
	<i>Halosydna brevisetosa</i>
	<i>Ficopomatus enigmaticus</i>
	<i>Polydora cornuta</i>
Arthropoda	<i>Amphibalanus amphitrite</i>
	<i>Balanus glandula</i>
	<i>Caprella penantis</i>
	<i>Balanus crenatus</i>
	<i>Jassa marmorata</i>
	<i>Leucothoe alata</i>
	<i>Caprella scaura</i>
	<i>Caprella simian</i>
Bryozoa	<i>Watersipora subtorquata</i>
	<i>Bugula neritina</i>
	<i>Cryptosula pallasiana</i>
	<i>Amathia verticillata</i>
	<i>Schizoporella japonica</i>
Chordata	<i>Celleporaria brunnea</i>
	<i>Botrylloides violaceus</i>
	<i>Ciona intestinalis</i>
	<i>Botryllus schlosseri</i>
	<i>Ascidia zara</i>
	<i>Molgula manhattensis</i>
	<i>Botrylloides leachii</i>
<i>Styela plicata</i>	
Cnidaria	<i>Diadumene leucolena</i>
Mollusca	<i>Ostrea stentina</i>
	<i>Ostrea lurida</i>
	<i>Mytilus galloprovincialis</i>
	<i>Mytilus trossulus</i>
Porifera	<i>Halichondria sitiens</i>

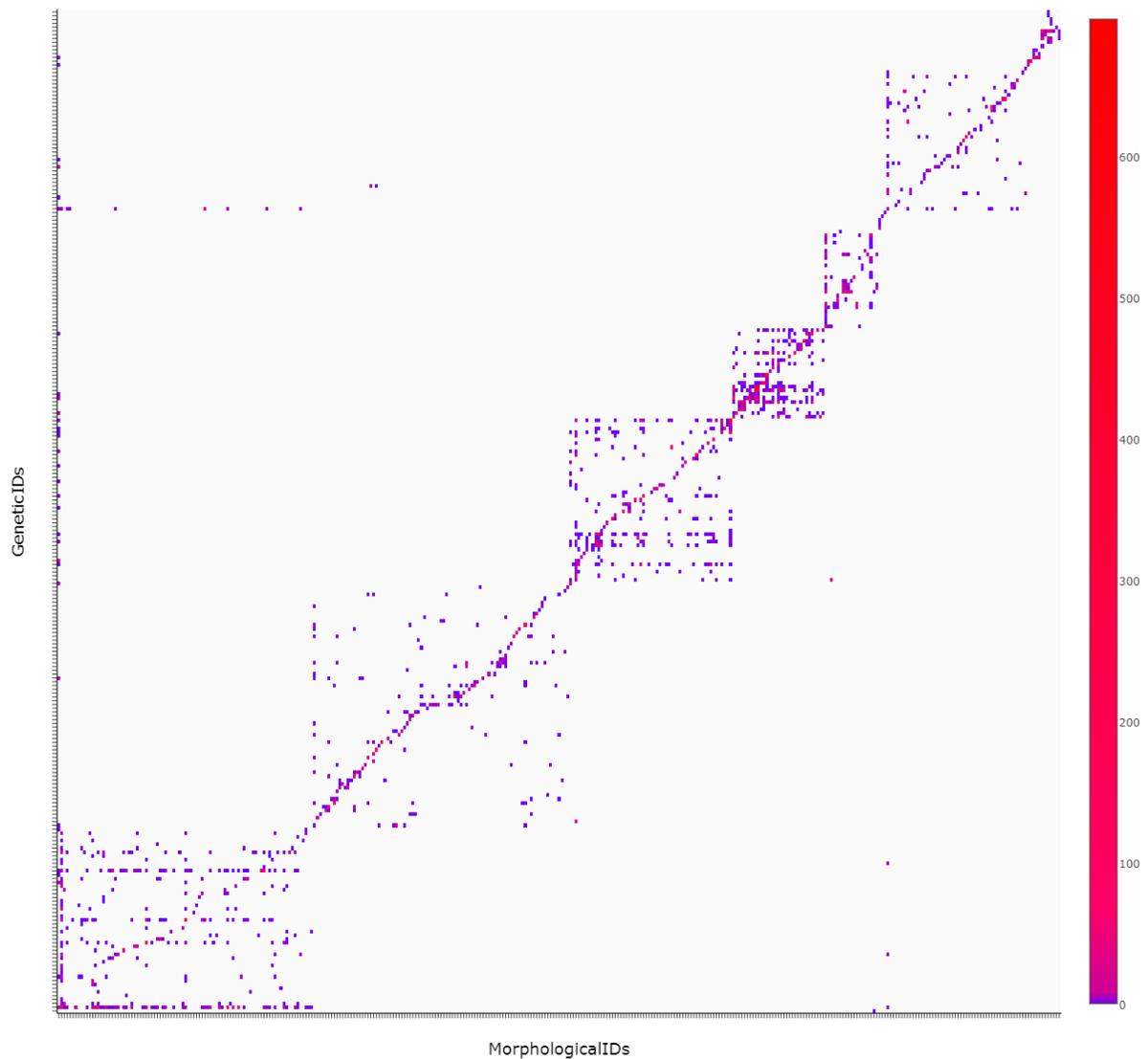


Figure 5.1. Matrix of all provisional genetic identifications versus morphological identifications. Each colored cell represents the number of vials with that morphological and genetic identification combination, where the intensity of red represents count. Scale is set to log for ease of viewing. Species are arranged by phylum, such that clusters of related species can be seen as blocks. Perfect agreement of morphological and genetic identification would produce a single diagonal line; points off the diagonal represent disagreement. There is not a perfect diagonal line because some taxonomic names were not found genetically, or vice versa. The phyla are, in order: Annelida, Arthropoda, Bryozoa, Chordata, Cnidaria, Entoprocta, Mollusca, Nemertea, Platyhelminthes, and Porifera (left to right, bottom to top). The subplots 5.2 through 5.11 show each phylum in more detail.

GeneticIDs Versus MorphologicalIDs

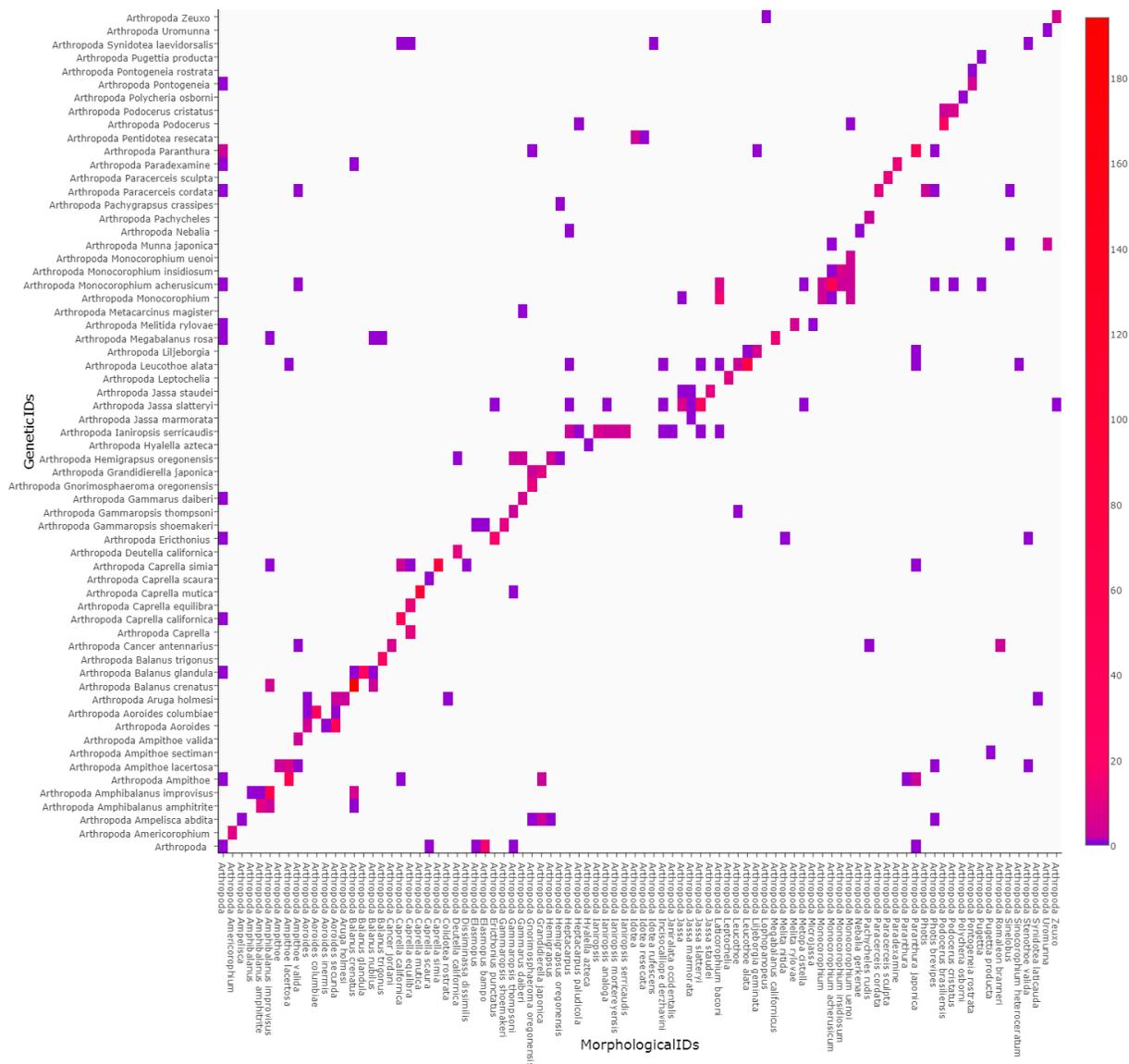


Figure 5.3. Matrix of genetic vs. morphological ID for phylum Arthropoda. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

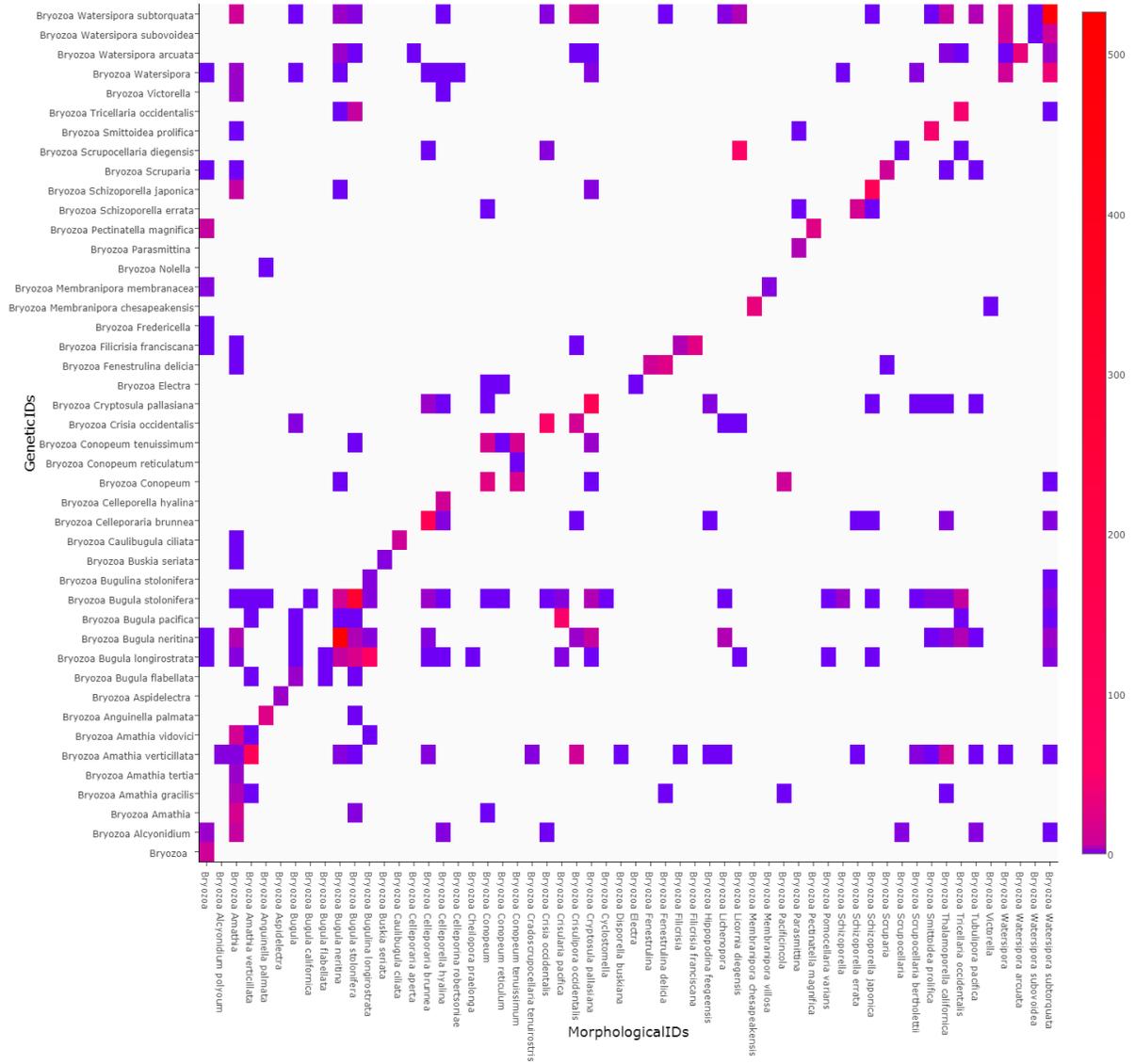


Figure 5.4. Matrix of genetic vs. morphological ID for phylum Bryozoa. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

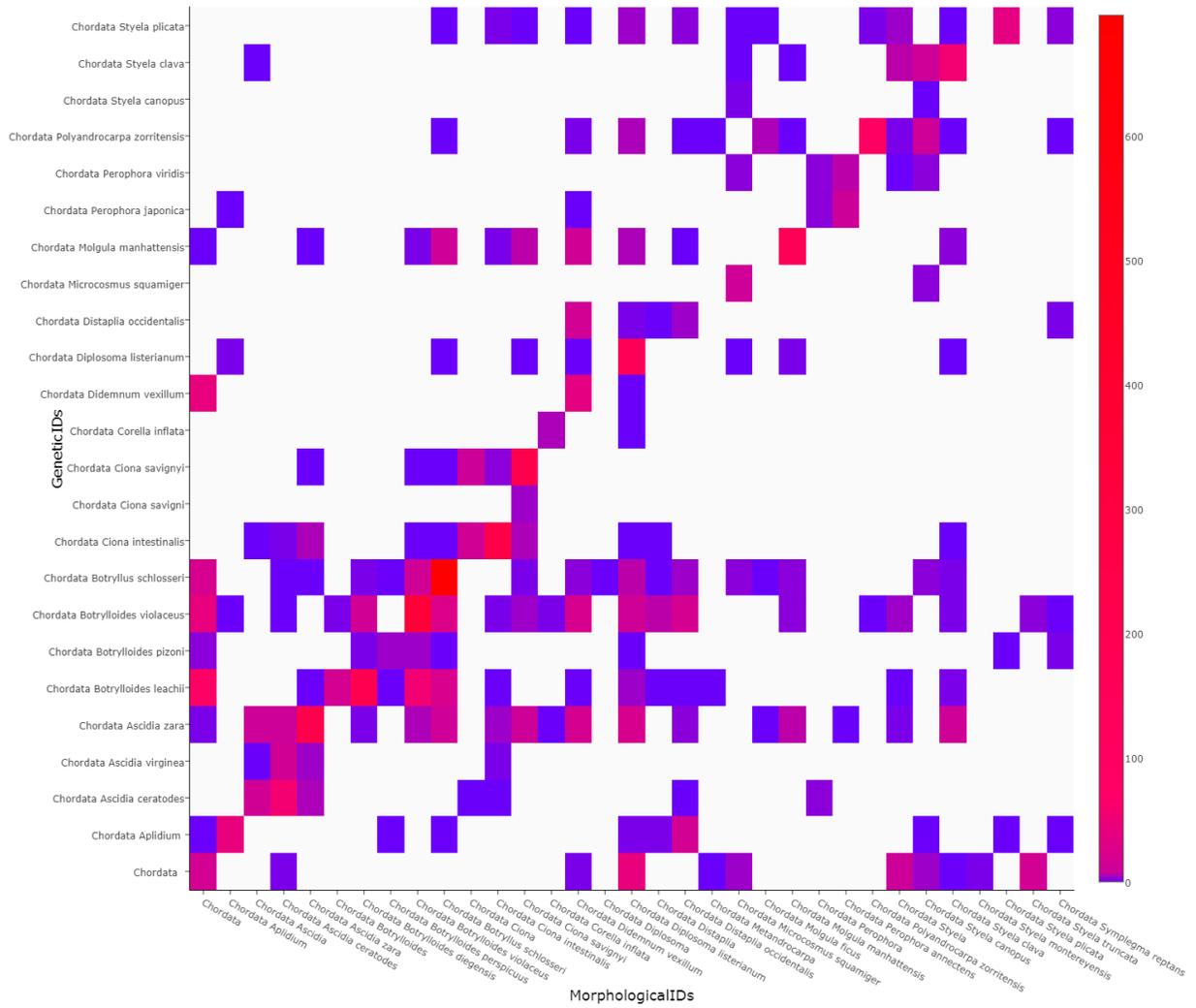


Figure 5.5. Matrix of genetic vs. morphological ID for phylum Chordata. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

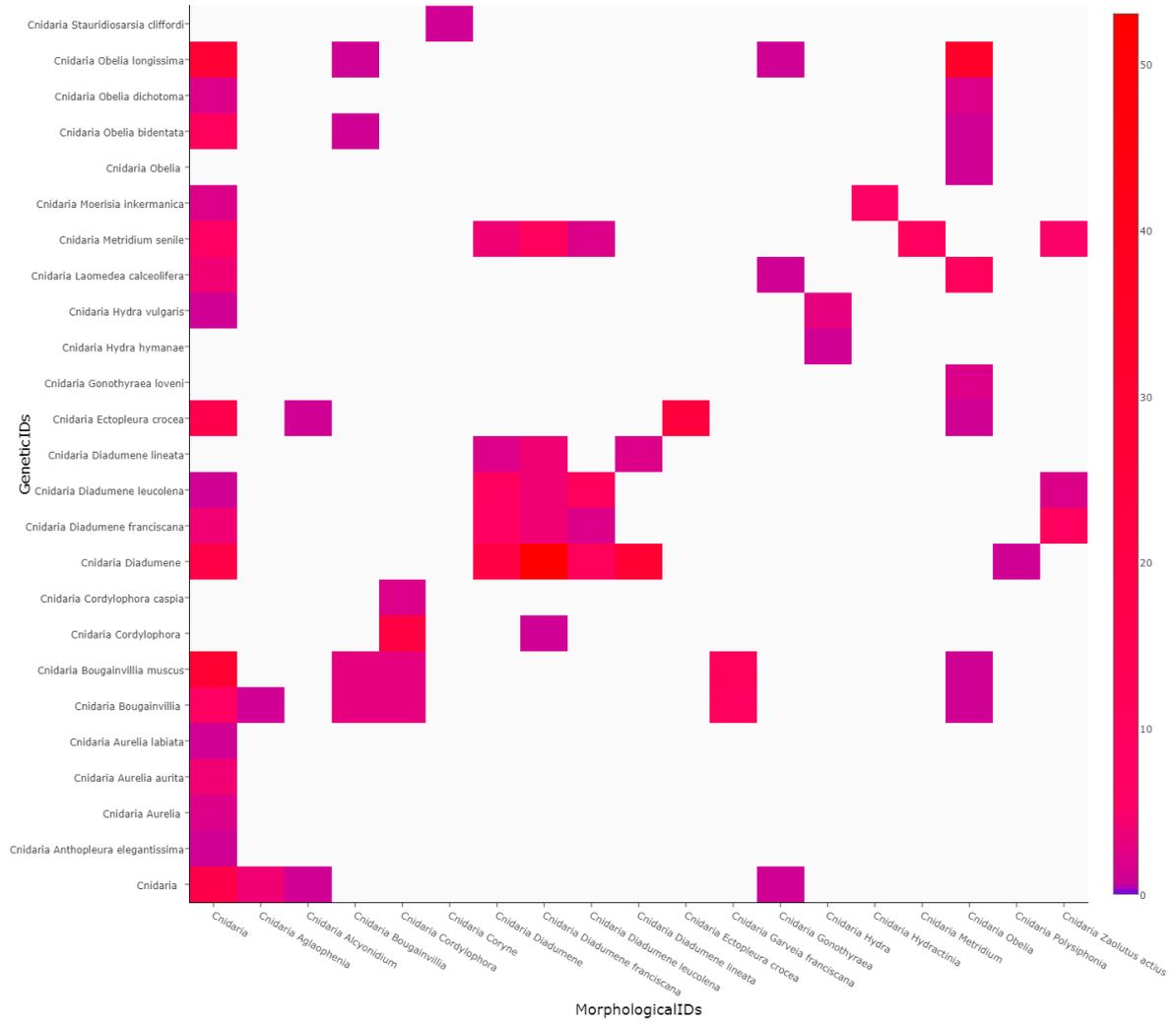


Figure 5.6. Matrix of genetic vs. morphological ID for phylum Cnidaria. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

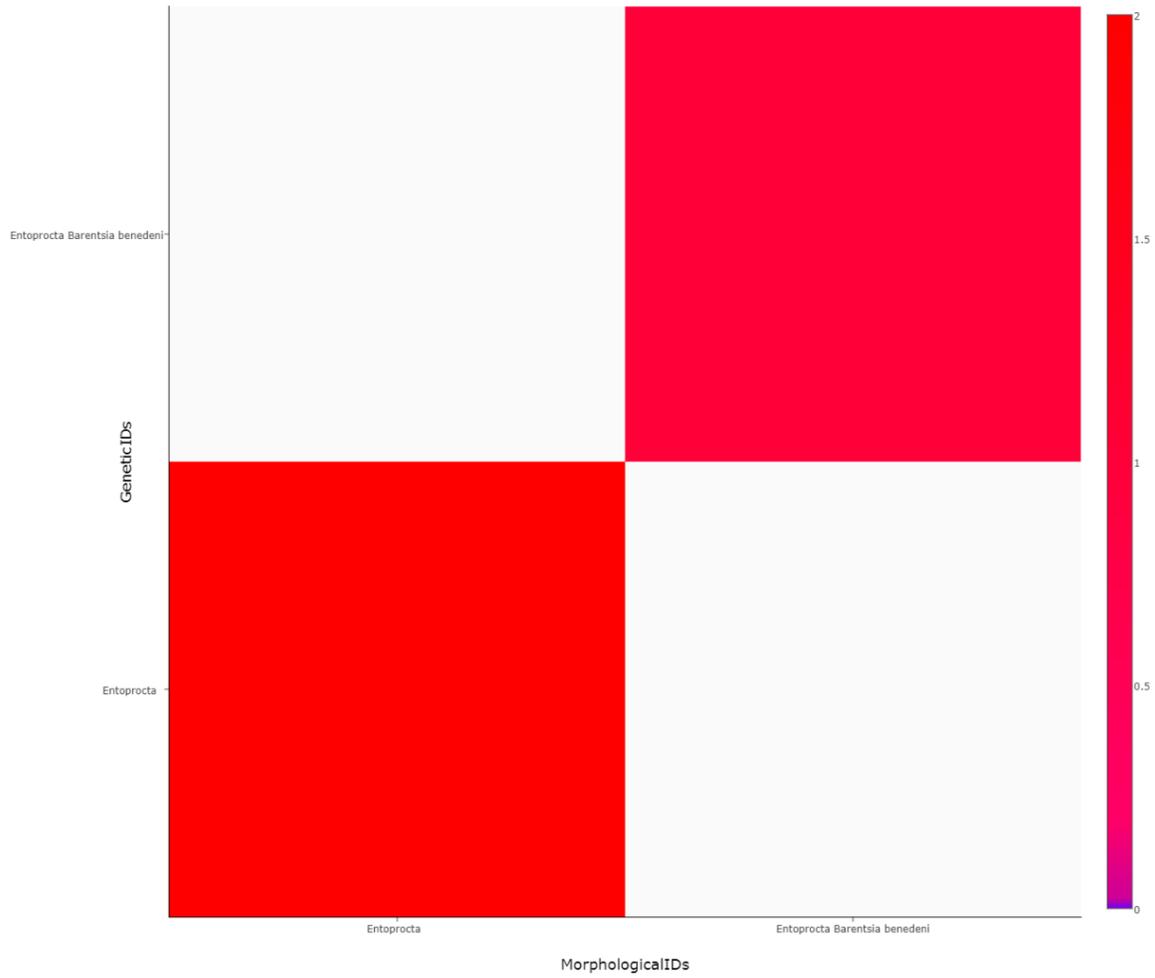


Figure 5.7. Matrix of genetic vs. morphological ID for phylum Entoprocta. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

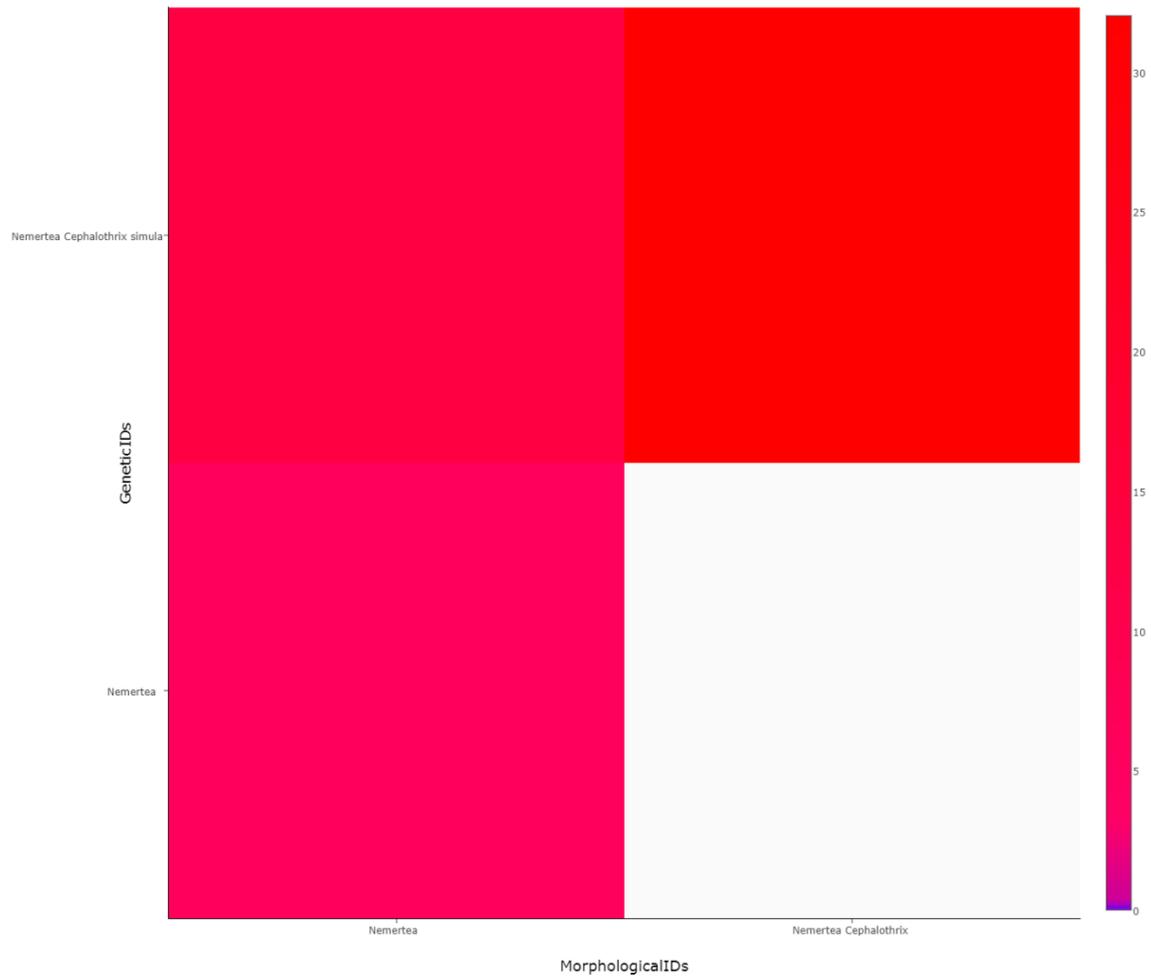


Figure 5.9. Matrix of genetic vs. morphological ID for phylum Nemertea. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

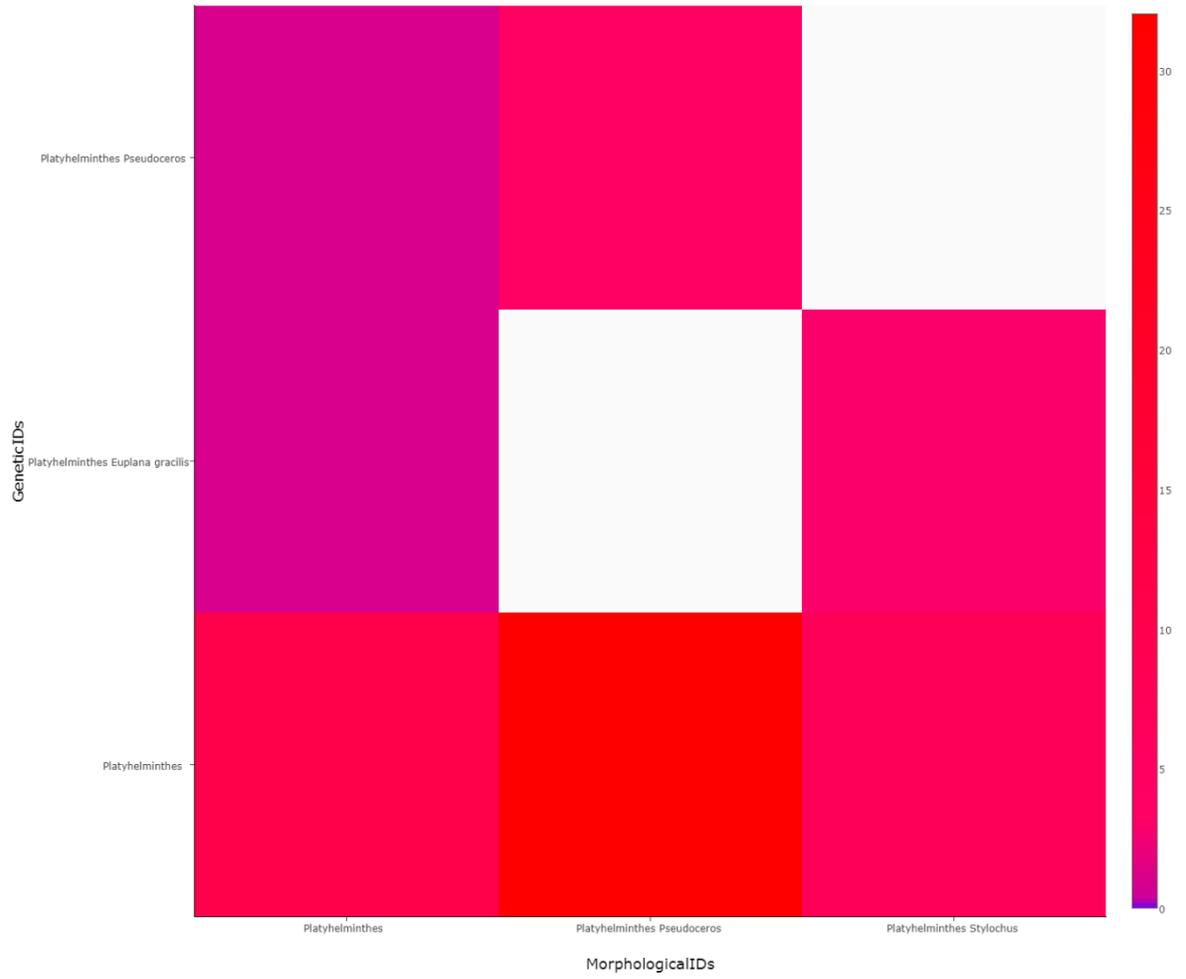


Figure 5.10. Matrix of genetic vs. morphological ID for phylum Platyhelminthes. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

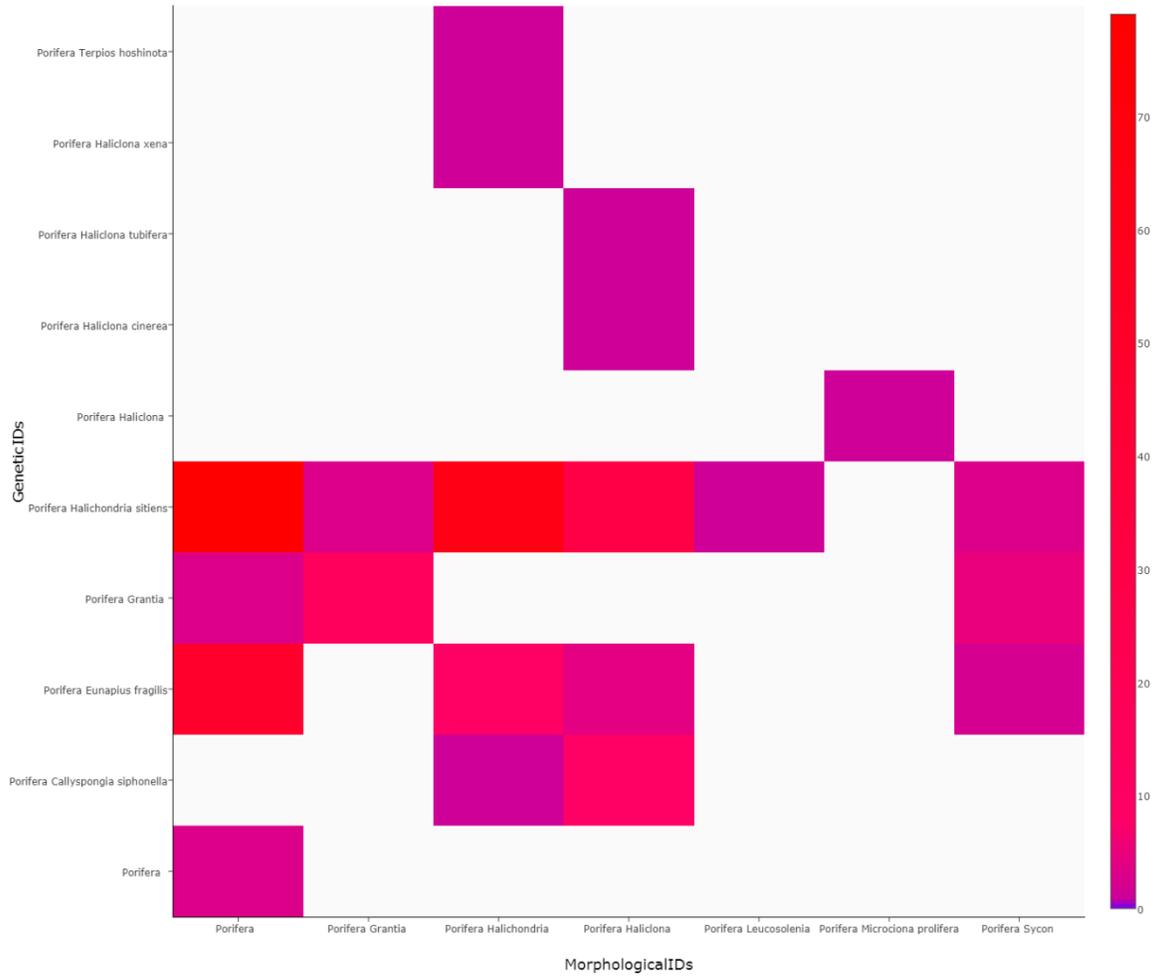


Figure 5.11. Matrix of genetic vs. morphological ID for phylum Porifera. See the caption of Figure 5.1 for more detail.

GeneticIDs Versus MorphologicalIDs

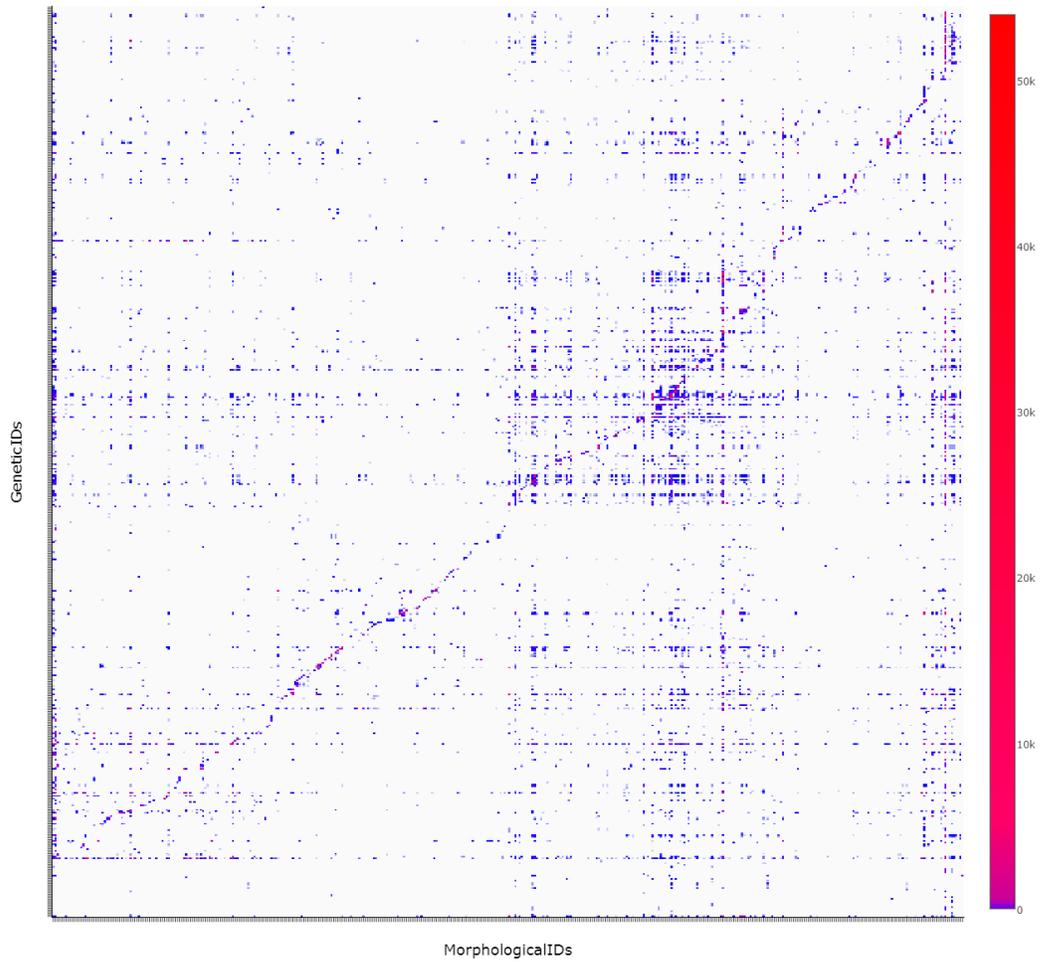


Figure 5.12. Matrix of genetic vs morphological ID for all taxa without filtering of implausible taxa or multiple assemblies from individual vials. See the caption of Figure 5.1 for more detail.

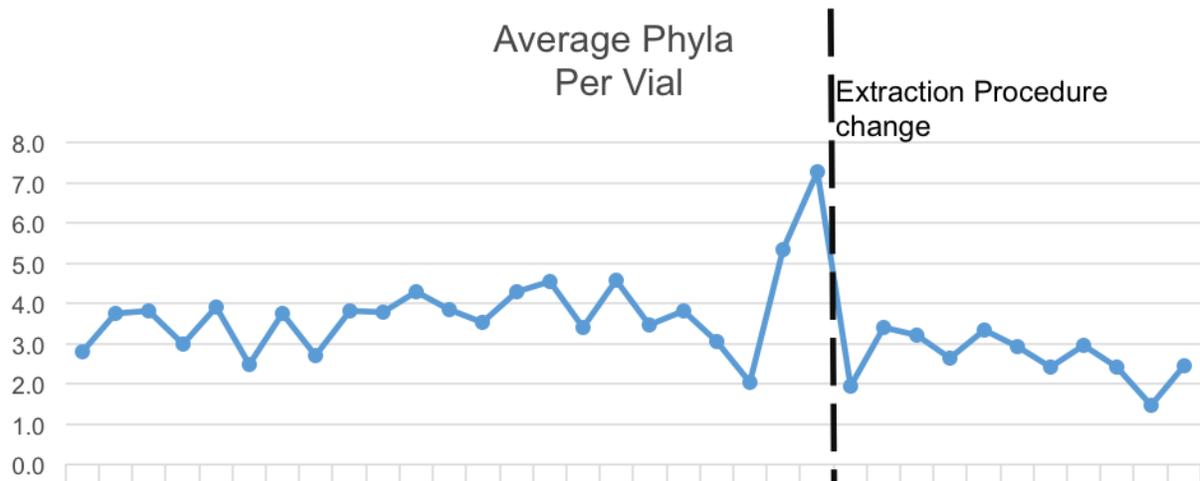


Fig 5.13. Average number of Phyla detected per vial in a series of 96 well extraction plates, ordered chronologically. The dashed vertical line denotes a change to extraction plates with screw-capped wells.

Chapter 6: Metagenetic Analysis of Plankton

Introduction

To inventory marine NIS, many sampling strategies may be used, and each will have strengths and weaknesses. Qualitative sampling (*e.g.*, "bioblitz" campaigns) may garner a large proportion of actual diversity but effort may vary among sites or personnel, and data are less amenable to statistical analysis. Quadrat based quantitative sampling can provide more consistency in effort, but different habitats may be difficult to compare. Settling plates make uniform effort and habitat but select for primarily epibenthic species among the total biota. Sampling of plankton can complement these approaches by targeting both holoplanktonic NIS (completely missed in benthic sampling) and meroplankton (the larvae of benthic species). Meroplankton are contributed to the plankton by organisms in all habitats and thus expand the effective habitat space sampled in a NIS survey. Not all taxa produce planktonic larvae, and larval abundance is episodic, therefore plankton sampling for NIS is complementary to methods targeting benthic adults. Further, for many species, larvae are morphologically undescribed or simply difficult to identify. Genetic analysis is therefore ideally suited for species identification in plankton.

Organisms in plankton can be extraordinarily abundant, and even sorting into morphospecies groups, as a prelude to DNA sequencing, can be prohibitively laborious. Metagenetics, also known as metabarcoding, is the simultaneous sequencing of genetic loci from mixed template samples, such as a DNA extraction from a plankton sample. (Metagenomics is the sequencing of mixed genomic DNA and thus potentially includes all loci). Sequences derived from metagenetic sequencing can be compared to reference DNA barcode sequence databases to detect the presence of specific sequences that represent species of interest. Metagenetics is thus an attractive approach to genetic analysis of plankton.

In this chapter, we present results of metagenetic analysis of 617 cumulative plankton samples from Parts I and II of the California bays monitoring program. We attempted to identify all known non-indigenous species (NIS) present in each sample, potential NIS previously unknown in California, as well as native species represented in available sequence database. We also examined variation in total and NIS community structure across California bays.

Methods

Collections

Samples were collected by either pumping surface (1 m depth) water through a 80 μm mesh, or by vertical tows from 5 or 10 m below the surface with 80 μm mesh net (Table 6.1). Samples were preserved in 95% ethanol and shipped to Moss Landing Marine Laboratories.

Table 6.1. Samples included in this study, by (A) bay and year, (B) method, and (C) depth. Some samples were repeated, bringing the total number of sequenced samples to 617.

A. Site and year	n
Humboldt Bay 2015	40
Bodega/Tomales Bay 2014	40
San Francisco Bay 2013	45
San Francisco Bay 2014	60
San Francisco Bay 2015	64
San Francisco Bay 2016	64
Morro Bay 2013	40
Mission Bay 2013	40
Marina Del Rey 2015	40
Port Hueneme 2015	40
San Pedro 2015	40
Newport Bay 2015	40
San Diego Bay 2013	40
B. Method	n
Pump	315
Vertical Tow	278
C. Depth	
1 meter below surface	315
5 meters below surface	198
10 meters below surface	80

DNA extraction.

Prior to extraction, each plankton sample was sieved through a clean 80- μ m mesh (retaining the storage ethanol for each sample) and rinsed well with 1X TE (Tris-EDTA) buffer. Total sample weight was recorded, and approximately 0.25 grams (wet weight) of each sample was added to the PowerBead tube of a MoBio PowerSoil extraction kit. When total sample weight did not exceed 0.25 grams, the entire sample was used. Remaining material was stored in the original bottle with the 95% ethanol retained after sieving the sample. The extraction continued by following the manufacturer's protocol, except that samples were eluted into 80 μ L of the provided elution buffer. A 20- μ L aliquot of each DNA sample was transferred to a 96-well plate for downstream applications.

Library preparation

Genomic DNA was quantified using picogreen, according to the manufacturer's protocol and standardized to 5 ng μL^{-1} . The COI gene was amplified, in triplicate, using primers with adapters for Nextera barcode indices (Table 6.2).

Table 6.2. Primers used for PCR amplification and Illumina sequencing.

Leray LCO forward primer [Nextera adapter]: [TCGTCCGGCAGCGTCAGATGTGTATAAGAGACAG]-GGWACWGGWTGAACWGTWTAYCCYCC
JG HCO reverse primer [Nextera adapted]: [GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG]-TAIACYTCIGGRTGICCRARAAYCA

2.5 ng genomic DNA was amplified in a PCR cocktail comprising a final concentration of 1 x Kapa Robust Hot Start Ready Mix, 0.2 mg mL^{-1} BSA, 2 mM MgCl_2 , and 0.4 μM of each primer in a 25 μL reaction. Reaction conditions consisted of an initial 3 min melt at 95° C, followed by 27 cycles of a 1 min at 95° C, 45 sec at 47° C, and 1 min at 72° C with a final 72° C hold for 5 min. PCR amplicons were viewed on a 2% agarose gel stained with ethidium bromide. Triplicates were pooled and purified with 1.4 x the sample concentration of Agencourt Ampure beads, according to the manufacturer's protocol.

To attach the Nextera barcodes, 2.5 μL of pooled and purified amplicons were amplified in a PCR cocktail comprising a final concentration of 1 x Kapa Robust Hot Start Ready Mix, 0.2 mg mL^{-1} BSA, 0.2 μM each forward and reverse barcode, and 2 mM MgCl_2 in a final volume of 25 μL . Reaction conditions consisted of an initial 3 min melt at 95° C, followed by 8 cycles of a 30 sec at 95° C, 30 sec at 55° C, and 30 sec at 72° C with a final 72° C hold for 5 min. PCR products were viewed on a 2% agarose gel stained with ethidium bromide. Amplicons were purified with 1.4 x the sample concentration of Agencourt Ampure beads, according to the manufacturer's protocol. Purified samples were quantified using picogreen, according the manufacturer's protocol. Barcoded amplicons were pooled evenly according to their concentration in ng μL^{-1} .

The library was denatured and diluted to a concentration of 20 pM and run on an Illumina MiSeq using a 600 cycle v3 cartridge according to the manufacturer's recommended protocols.

Bioinformatic analysis

There were six Illumina sequencing runs for Phase 2 [BZS04, 05, 06, 07, 08, 09; BZS=Baywide Zooplankton Survey]. For each run, de-multiplexed forward and reverse read files were generated by the Illumina software and analyzed using USEARCH 9.0 and 10.0 (Edgar 2010). First, forward and reverse fastq files were merged, allowing for a maximum of 12 differences in the overlap and only keeping aligned reads between 352 and 376 base pairs (-fastq_mergepairs). At this time, sequences were also renamed by their sample name to facilitate downstream processing. Merged reads were then filtered (-fastq_filter) using a maximum expected error (max_ee) of 0.5, which is a stringent filtering criteria allowing for <1 error per read. Primers were removed from all merged reads (-fastx_truncate), which were dereplicated (-fastx_uniques).

In USEARCH v10.0, there is an algorithm that allows for the 'denoising' of amplicon-based Illumina reads (Edgar 2016), which generated zero-radius OTUs (or ZOTUs) that are biologically relevant sequences. This same algorithm includes a stringent chimera filter. We used the latest version of this algorithm to generate ZOTUs (-unoise3) for each run using the default parameters. To determine that all the ZOTUs were in fact COI sequences, we generated alignments for each run using the MAFFT plug-in (Kato and Kuma 2002) in Geneious v10.2.3 (Biomatters, Ltd., San Francisco, California). Any sequences that did not appear to be COI in the alignment (*i.e.*, those sequences that did not align well with others) were extracted from the aligned set and blasted against NCBI GenBank through Geneious. Based on these results, if a sequence was identified as COI, it remained in the alignment. If it was not identified as COI, then it was removed from the alignment. If sequences were removed from the alignment, the alignment was remade with the new set of sequences and visually inspected for any sequences that did not align well until all sequences were confirmed to be COI either through proper alignment or identification via GenBank.

The ZOTUs were renamed with a unique identifier for each run to differentiate ZOTUs across runs. Then, in order to assign the same ZOTU across all runs in perpetuity, we created a Master ZOTU list using sequence data generated in Phase 1 and Phase 2. (Note that Phase 1 sequences were processed using the same pipeline as described above for each Phase 2 sequencing run.) To create the Master ZOTU list, we started with Phase 1 sequences as the first set and then mapped BZS04 sequences to them at 100% identity (-usearch_global). Those sequences that did not match at 100% were then appended to the Phase 1 sequence fasta file, which was renamed Master_ZOTU_list_v1. This process continued until all Phase 2 runs had individually mapped to the latest version of the Master ZOTU list and all sequences that did not match those on the list already had been appended. At the time of writing, the latest version of this list is v6.

To create a ZOTU table with all the runs from Phase 2, the merged, filtered, trimmed reads from each run were concatenated into a single file, which was then mapped to the v6 of the Master ZOTU list at 97% identity, as recommended in the software documentation (-usearch_global).

To determine the species-level clusters, all ZOTUs were reclustered into OTUs at a 95% similarity level using `-usearch cluster_smallmem`. Hereafter, “OTU” refers to these 95% clusters unless otherwise specified. Raw reads were then mapped to OTUs. To examine community-level analyses of ZOTUs across bays, reads for each sample were rarefied to 1000 and 10,000; the lower number retains the most sites, while the larger number results in loss of samples with fewer than 10,000 reads. Rarefied reads were then mapped to the 95%-OTUs and used in the software package PRIMER to produce nonmetric multiple dimensional scaling (nMDS) plots to illustrate patterns of similarity among OTU composition and abundance within and between bays. These data were also used for permutational analysis of variance (PERMANOVA) tests of differences among bays.

To identify taxa, OTUs were BLASTed against the MLML reference database and a curated COI database called CO-Arbitrator (Heller *et al.*, in review). We retained matches that exceeded 94.5% similarity and had 90-100% sequence length overlap with our query sequence. A similarity of 95% is commonly used as a threshold for biological species under the assumption of an interspecific “barcode gap.” (We chose 94.5% to allow for up to 0.5% sequencing error). However, some sequences that are 95% similar may belong to >1 species if divergence is low in a genus (for example if divergence is recent or rate of substitution is low). The converse error is possible too. Phylogenetic analysis, when possible, is superior to threshold analysis to detect reciprocally monophyletic clades - whether such clades represent species will remain to be determined independently.

Ecological patterns were investigated by PERMANOVA tests of differentiation of OTU composition among bays and the effect of additional factors of temperature and salinity. Patterns were also explored by nMDS. Both analyses used the software package PRIMER. An important caveat is that these analyses treat read abundance as equivalent to organismal abundance. PCR efficiency for different species and how this varies for different plankton samples (due to differences in species composition, for example) is not presently known. In addition, clustering of reads into OTUs may split some species that are highly genetically variable and lump some that are more conserved than typical. It may be best to consider these analyses as addressing community OTU composition in a strict sense of genetic clusters, rather than biological species.

We repeated ecological analyses while confining the analysis to the species known to be established NIS in California. NIS status was determined by reference to a list provided by SERC (Appendix 6.1).

Results

For the six Illumina runs that were performed for Phase 2, we generated 55,040,497 sequences. After merging and filtering out low quality sequences, the final dataset contained 28,605,589 sequences. These clustered into 14,635 ZOTUs and 3719 95%-threshold OTUs.

For Phase 1 and 2 combined (a total 617 samples), we retained ~94% of samples with rarefaction at 1,000, while at 10,000 we retained ~63% of samples (*i.e.*, 6% and 37% of samples, respectively, contained too few reads at those rarefaction levels). For Phase 2 only (a total 440 samples), we retained ~93% of samples with rarefaction at 1,000, and at depth of 10,000, we retained ~75% of samples.

Community composition differences among sites

At both the 1,000-read and 10,000 read levels of rarefaction, the OTU composition was significantly different across bays (Tables 6.3 and 6.4). The differentiation among sites is conspicuous in nMDS plots (Figure 6.1), although it is difficult to attribute community differences to latitude because of environmental differences among sites (below).

Table 6.3. One-way PERMANOVA results of community composition for all bays when reads were rarefied to 1,000.

Factor	df	SS	MS	Pseudo-F	P(perm)
Bay	9	64068	71187	32.403	0.001
Residual	553	1214900	2196.9		
Total	562	1855600			

Table 6.4. One-way PERMANOVA results of community composition for all bays when reads were rarefied to 10,000 reads.

Factor	df	SS	MS	Pseudo-F	P(perm)
Bay	9	450780	50087	25.272	0.001
Residual	381	755110	1981.9		
Total	390	1205900			

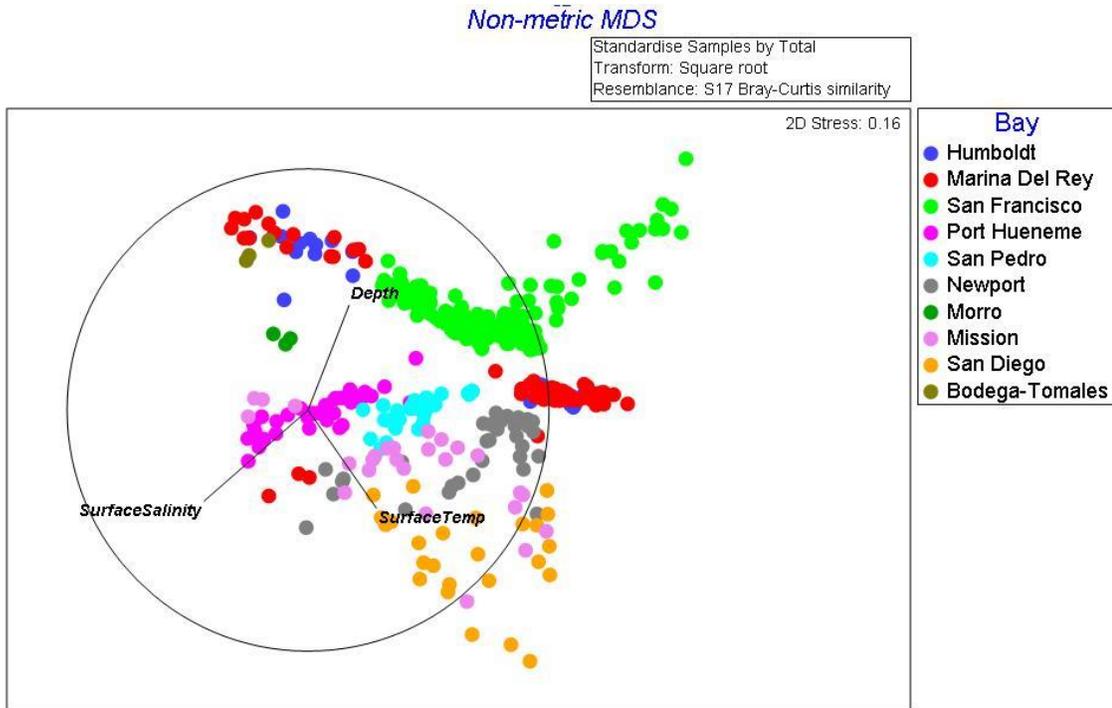


Figure 6.1 nMDS plot of all samples and sites in Phase I and Phase II, with reads rarefied to 10,000 and with overlay of environmental data. Site was a significant factor in community differentiation. Site effects include environmental differences (overlay) as well as biogeographic and invasion history.

With data rarefied to 1000 reads, samples were ~29-45% similar within sites (Table 6.5). Humboldt Bay samples showed the least internal consistency (28.77% similarity). Between sites, samples were ~12-29% similar, and every site was significantly different from any other. However, Humboldt Bay and Marina Del Rey were only marginally statistically different from each other.

Table 6.5. Percent similarity within and between sites when rarefaction level = 1,000. All pairs were significantly different ($p < 0.008$).

	Humboldt	Marina Del Rey	San Francisco	Port Hueneme	San Pedro	Newport
Humboldt	28.77					
Marina Del Rey	28.96	40.17				
San Francisco	17.21	20.41	35.95			
Port Hueneme	11.84	11.97	14.58	43.70		
San Pedro	14.35	18.45	22.87	28.28	44.87	
Newport	15.15	22.79	20.15	16.14	24.85	42.68

With data rarefied to 10,000 reads, samples were ~33-50% similar within sites (Table 6.6). Humboldt Bay samples again the least internal consistency (33.75% similarity). Between sites, samples were ~14-36% similar, and Humboldt Bay and Marina Del Rey were not significantly different, presumably due to the low internal consistency in Humboldt Bay.

Table 6.6. Percent similarity within and between sites when rarefaction level = 10,000. All pairs were significantly different except Humboldt and Marina Del Rey (p=0.06, ns).

	Humboldt	Marina Del Rey	San Francisco	Port Hueneme	San Pedro	Newport
Humboldt	33.75					
Marina Del Rey	36.26,ns	43.76				
San Francisco	19.95	22.48	38.12			
Port Hueneme	13.61	13.35	15.45	47.60		
San Pedro	17.52	20.73	24.19	31.18	49.50	
Newport	18.53	23.73	20.40	18.97	27.54	44.04

Effects of environmental variables.

Community composition was analyzed with respect to the influence of environmental factors. Salinity was binned into categories of <20, >20≤25, >25≤30, and >30 ppt, while temperature was binned into categories of 10≤13, >13≤16, >16≤19, >19≤22, >22≤25, and >25° C. Not all samples were provided with temperature and salinity data, so not all interaction terms could be evaluated. Temperature, or the interaction of temperature and bays, appeared to have a significant effect on community composition for data rarefied to both 1,000 and 10,000 reads (Table 6.7 and 6.8). The effect of salinity is ambiguous, with no significant effect when more samples were included by rarefying to a lower level (1000 reads); when more reads were included, which necessarily reduced the number of samples, a marginally non-significant effect of salinity was observed (p=0.087).

Table 6.7. PERMANOVA results of community composition for all bays when reads were rarefied to 1,000 per sample. Unrepresented data bins precluded tests for some interactions.

Factor	df	SS	MS	Pseudo-F	P(perm)
Bay-Year	6	161250	26875	15.497	0.001
Surface Temperature	2	6665.2	3332.6	1.9216	0.022
Surface Salinity	1	1604	1604	0.92486	0.451
Bay-Year x Surface Temperature *	8	35069	4383.7	2.5277	0.001
Surface Temperature x Surface Salinity*	3	5017.5	1672.5	0.96438	0.484
Residues	282	489060	1734.3		
Total	313	1060300			

*indicates uneven sample sizes

Table 6.8. PERMANOVA results of community composition for all bays when reads were rarefied to 10,000 reads. Unrepresented data bins precluded tests for some.

Factor	df	SS	MS	Pseudo-F	P(perm)
Bay-Year	6	122960	20494	13.374	0.001
Surface Temperature	2	4934.8	2467.4	1.6102	0.071
Surface Salinity	2	4836.7	2418.3	1.5782	0.087
Bay x Surface Temperature*	6	27252	4541.9	2.9641	0.001
Residual	191	292670	1532.3		
Total	214	670320			

*indicates uneven sample sizes

San Francisco Bay

Focus on the most often sampled site, San Francisco Bay, facilitates consideration of environmental drivers apart from confounding historical factors, and year-to-year variation. Moreover, environmental metadata were more complete for San Francisco Bay. Main effects of year, surface temperature, and salinity all had significant effects on community composition in plankton samples, but almost all interactive terms were also significant (Table 6.9 and 6.10). nMDS plots indicate segregation of low salinity sites (Figure 6.2 and 6.3), and it appears that low salinity sites were more prevalent in 2015 and 2016 (Figure 6.4).

Table 6.9. PERMANOVA results of community composition in San Francisco when a rarefaction level of 1000 reads was used. Temperature and salinity data were binned into levels, therefore sample sizes for some combinations of year, temperature and salinity varied.

Factor	df	SS	MS	Pseudo-F	P(perm)
Year	1	6611.4	6611.4	6.2394	0.001
Surface Temperature	2	11249	5624.7	5.3082	0.001
Surface Salinity	1	3262.3	3262.3	3.0787	0.001
Year x Surface Temperature*	5	15650	3129.9	2.9538	0.001
Year x Surface Salinity**	6	16705	2784.2	2.6275	0.001
Surface Temperature x Surface Salinity*	2	3791.9	1896	1.7893	0.017
Year x Surface Temperature x Surface Salinity**	3	6189.5	2063.2	1.9471	0.003
Residual	163	172720	1059.6		
Total	190	430190			

*indicates uneven sample sizes

Table 6.10 PERMANOVA results of community composition in San Francisco when a rarefaction level of 10,000 reads was used. Temperature and salinity data were binned into levels, therefore sample sizes for combinations of year, temperature and salinity varied.

Factor	df	SS	MS	Pseudo-F	P(perm)
Year	1	6365.4	6365.4	8.02	0.001
Surface Temperature	2	8174.1	4087.1	5.1494	0.001
Surface Salinity	1	4025.5	4025.5	5.0718	0.001
Year x Surface Temperature*	3	6237.5	2079.2	2.6196	0.001
Year x Surface Salinity*	5	12338	2467.6	3.109	0.001
Surf Temp x Surface Salinity*	1	1030.7	1030.7	1.2986	0.152
Year x Surface Temperature x Surface Salinity**	2	3841.5	1920.7	2.42	0.002
Residues	111	88100	793.7		
Total	133	277170			

*indicates uneven sample sizes

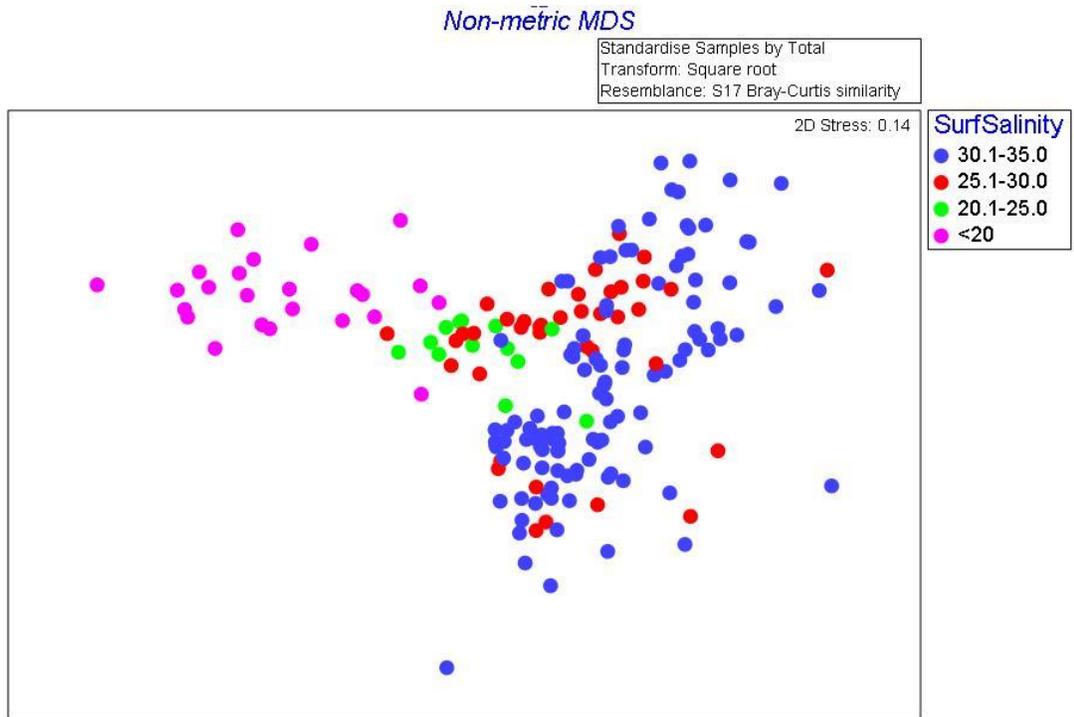


Figure 6.2. nMDS plot representing community composition of plankton samples in San Francisco Bay, coded by salinity, based on data rarefied to 1,000 reads per sample.

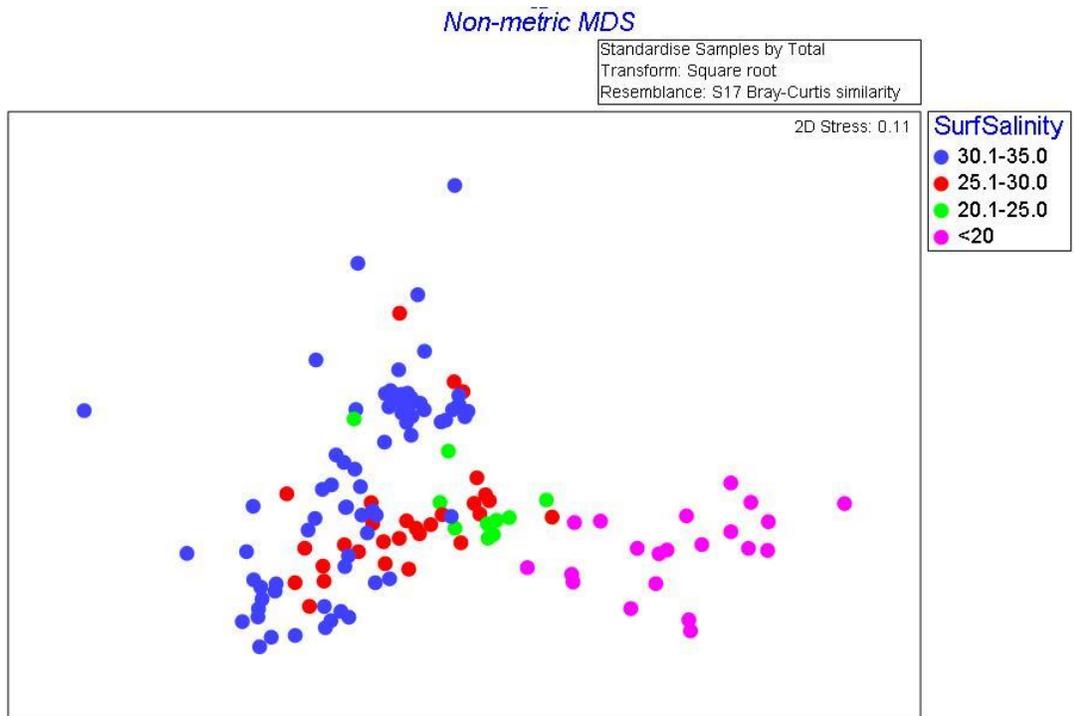


Figure 6.3. nMDS plot representing community composition of plankton samples in San Francisco Bay, coded by salinity, based on data rarefied to 10,000 reads per sample.

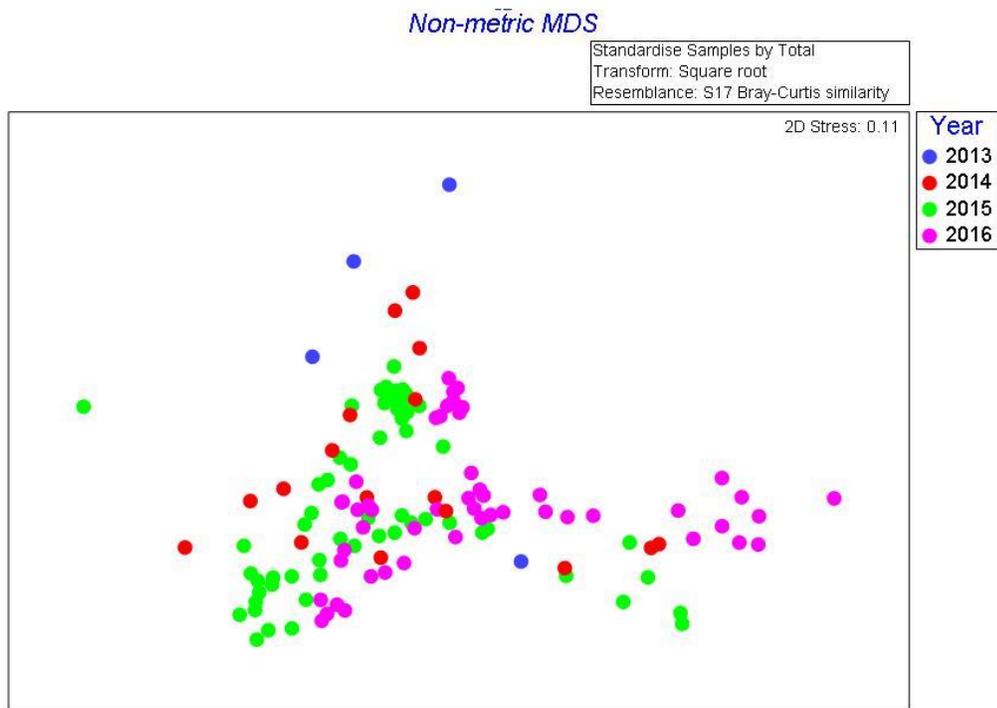


Figure 6.4. nMDS from Phases I and II from San Francisco Bay, rarefied to 10,000 reads and coded by year.

Plankton samples were differentiated by depth and method of collection (Figures 6.5 and 6.6). These were in part similar ways to bin data, as the pump method was used only at the surface, while vertical tows were used for both 5-meter and 10-meter depths. No obvious differences are seen in the 5- and 10-meter samples. The nature of the vertical tow will include 5-meter water in the 10-meter sample. This may contribute to the similarity of the 5- and 10-meter samples.

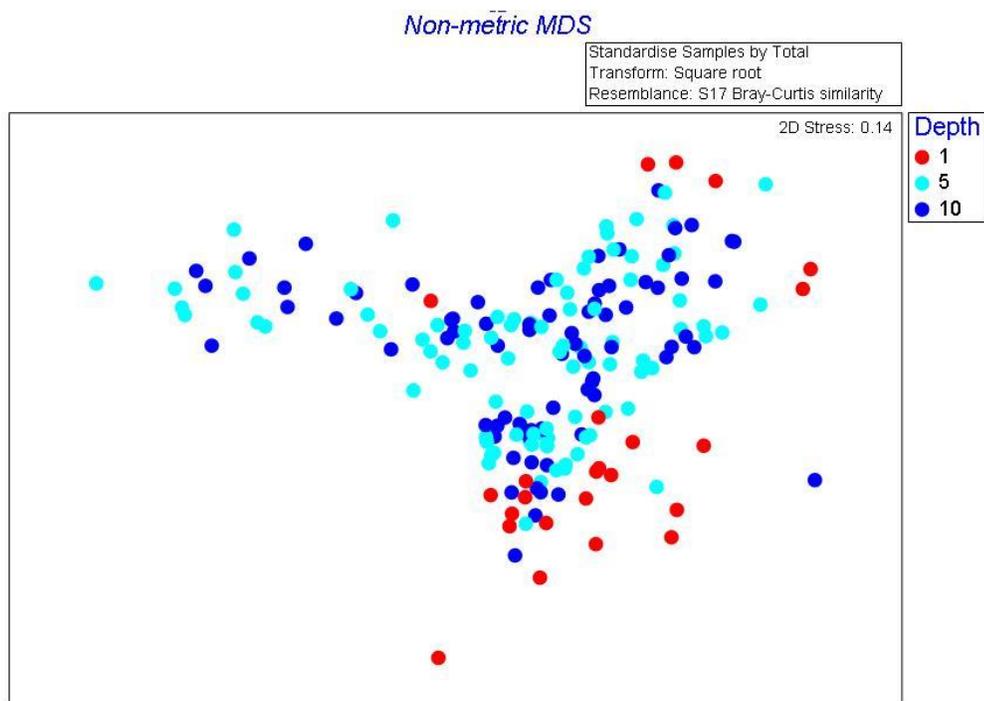


Figure 6.5. nMDS from Phases I and II from San Francisco Bay, rarefied to 10,000 reads and coded by depth.

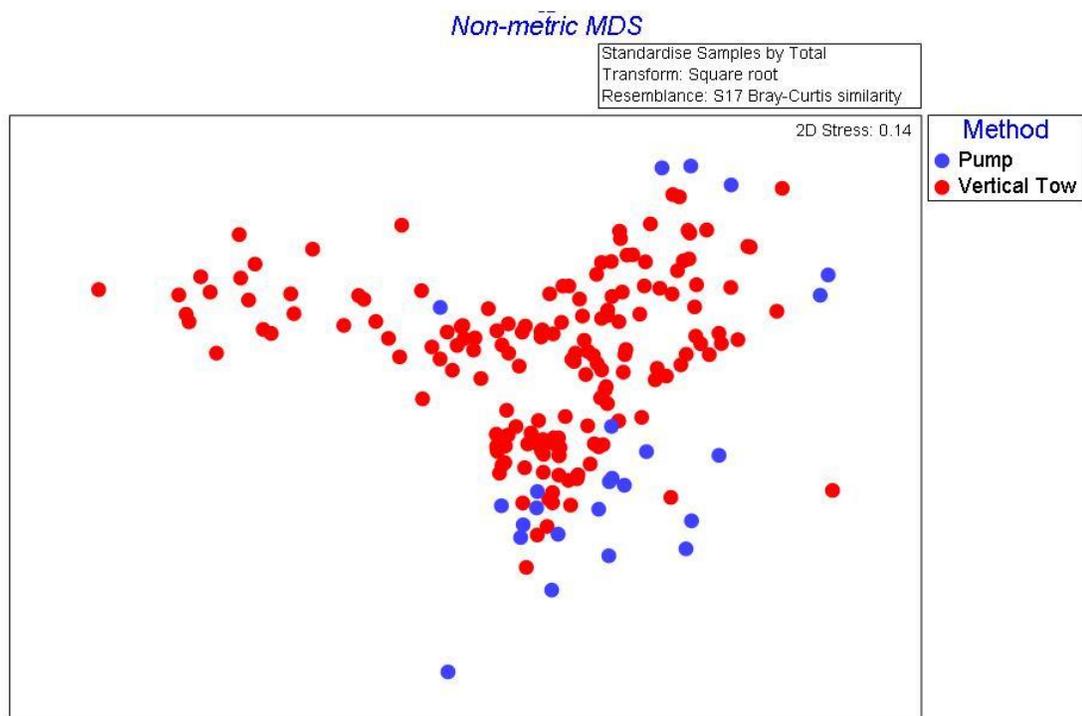


Figure 6.6 nMDS from Phases I and II from San Francisco Bay, rarefied to 1,000 reads and coded by sample method.

NIS in plankton Samples

We found 69 out of 252 marine invertebrate species from the SERC NIS list (Appendix 6.1), and two algal species (Table 6.11) (algae were not a focus, but were inevitably captured in plankton nets), using the top BLAST hit exceeding 95% similarity in reference databases. Some OTU may have had matches >95% to reference sequences from more than one taxon due to misidentification within Genbank, or lack of differentiation of COI within a group of closely related species. Therefore, it is possible that the single top hit may not be the correct identity of an OTU, leading to false positives and negatives in Table 6.11. Distribution of read counts of NIS among bays was highly uneven. Three species were detected in all 13-sampling site-dates: the barnacle *Amphibalanus amphitrite*, the copepod *Pseudodiaptomus marinus*, and the mussel *Musculista senhousia* (Figure 6.7). While every bay, as expected, contained NIS, a small minority of individual samples lacked NIS altogether (Table 6.12). Surprisingly absent from Table 6.11 is the mussel *Mytilus galloprovincialis*. It is possible that ZOTU of the *Mytilus galloprovincialis* were combined into the *M. trossulus* or *M. edulis* OTU by the 95% similarity criterion, and these species do not appear in the SERC NIS list as the former is native and the latter is unknown in California

TABLE 6.11. NIS from SERC list (Appendix 6-1) found in 94.5% BLAST table.

OTU Name	Binomial Name	Phylum	Class	San Francisco 2013	Morro 2013	Missio n 2013	San Diego 2013	Bodega Tomales 2014	San Francisco 2014	Humboldt 2015	San Francisco 2015	Port Hueneme 2015	Marina Del Rey 2015	San Pedro 2015	Newport 2015	San Francisco 2016
OTU_BZS05_135	<i>Alitta succinea</i>	Annelida	Polychaeta	9	0	0	0	0	1175	0	924	0	0	0	504	4798
OTU_BZS05_230	<i>Epigamia toroi</i>	Annelida	Polychaeta	0	0	0	0	0	0	0	0	38	0	0	0	0
OTU_BZS06_400	<i>Ficopomatus nigmaticus</i>	Annelida	Polychaeta	0	0	0	0	0	1	1	0	0	0	19	0	0
OTU_BZS08_220	<i>Marenzelleria neglecta</i>	Annelida	Polychaeta	0	0	0	0	0	1	0	0	0	0	0	0	22
OTU_BZS09_529	<i>Streblospio benedicti</i>	Annelida	Polychaeta	16	3	0	0	1	16	18	4	4	14	0	0	17
OTU_Phase1_14	<i>Myrianida pachycera</i>	Annelida	Polychaeta	0	0	67	1	0	0	6	0	0	23	44	7	0
OTU_Phase1_53	<i>Polydora cornuta</i>	Annelida	Polychaeta	899	0	6	183	0	7001	20	19474	30	373	839	314	41907
OTU_Phase1_71	<i>Megasyllis hipponica</i>	Annelida	Polychaeta	239	0	68	84	18	146	0	50	7	0	18	0	44
OTU_Phase1_91	<i>Myrianida pentadentata</i>	Annelida	Polychaeta	1	0	1	126	0	1519	0	89	0	0	7	0	11
OTU_Phase1_15	<i>Amphibalanus amphitriti</i>	Arthropod	Hexanauplia	106	3	8120	9676	2	16	2173	295	2642	19343	58618	168	639
OTU_Phase1_23	<i>Amphibalanus amphitriti</i>	Arthropod	Hexanauplia	0	0	39	9	0	0	1	0	0	106	186	16	0
OTU_Phase1_93	<i>Amphibalanus improvisus</i>	Arthropod	Hexanauplia	2932	1	1	0	0	75906	6	72661	172	11	40	0	42319
OTU_BZS05_253	<i>Rhithropanopeus harrisi</i>	Arthropod	Malacostraca	0	0	0	0	0	0	0	36	0	0	0	0	40
OTU_BZS06_182	<i>Ampelisca abdita</i>	Arthropod	Malacostraca	2	0	0	0	0	13	0	135	0	0	0	0	3330
OTU_BZS08_153	<i>Grandidierella japonica</i>	Arthropod	Malacostraca	0	26	0	0	2	13	2	16	3	11	0	2	26
OTU_BZS08_209	<i>Ampithoe valida</i>	Arthropod	Malacostraca	0	0	0	0	0	32	0	0	0	0	0	0	1474
OTU_BZS08_703	<i>Carcinus maenas</i>	Arthropod	Malacostraca	0	0	0	0	0	0	0	0	0	0	0	0	155
OTU_Phase1_11	<i>Paradexamine sp</i>	Arthropod	Malacostraca	0	0	2142	1144	0	0	0	1	1196	0	0	1064	0
OTU_Phase1_11	<i>Caprellia simia</i>	Arthropod	Malacostraca	74	38	201	38	1	0	0	0	148	0	130	0	15
OTU_Phase1_18	<i>Caprellia nutica</i>	Arthropod	Malacostraca	92	21	128	979	20	0	0	0	0	0	7	1	0
OTU_Phase1_18	<i>Orthione griffenis</i>	Arthropod	Malacostraca	1	143	2	45	60	41	40	26	0	9	0	30	73
OTU_Phase1_23	<i>Palaemon macrodactylus</i>	Arthropod	Malacostraca	28	0	0	26	0	1123	0	4151	1	4	0	0	6303
OTU_Phase1_25	<i>Monocorophium insidiosus</i>	Arthropod	Malacostraca	20	14	0	1	0	1	18	35	0	6	0	0	0
OTU_Phase1_76	<i>Monocorophium cherus</i>	Arthropod	Malacostraca	25	17	62	131	3	0	7	898	0	16	0	1	3449
OTU_BZS05_283	<i>Acartia sinensis</i>	Arthropod	Maxillopoda	22	0	0	0	0	5741	0	1586	0	0	0	0	42550
OTU_BZS08_206	<i>Mytilicola orientalis</i>	Arthropod	Maxillopoda	0	0	0	0	0	6	0	3	0	0	0	0	50
OTU_BZS08_249	<i>Acartia sinensis</i>	Arthropod	Maxillopoda	0	0	0	0	0	18	0	1	0	0	0	0	406
OTU_Phase1_21	<i>Pseudodiaptomus marinus</i>	Arthropod	Maxillopoda	3656	10	23539	16005	16	115858	2988	197958	25742	93227	38493	25941	530570
OTU_BZS06_225	<i>Watersipora arcuata</i>	Bryozoa	Gymnolaemata	0	0	0	15	0	0	0	0	0	0	53	0	0
OTU_BZS08_601	<i>Anguinella palmata</i>	Bryozoa	Gymnolaemata	19	0	0	0	0	3	0	1	0	0	0	0	237
OTU_BZS09_490	<i>Cryptosula pallasiana</i>	Bryozoa	Gymnolaemata	0	0	0	0	0	0	0	0	0	0	12	0	0
OTU_Phase1_13	<i>Watersipora sp</i>	Bryozoa	Gymnolaemata	3	28	0	0	55	5	9	402	0	1	0	0	0
OTU_Phase1_25	<i>Bugula heritina</i>	Bryozoa	Gymnolaemata	7	2	0	7	0	0	0	0	14	0	2	10	0
OTU_Phase1_28	<i>Watersipora subtorquata</i>	Bryozoa	Gymnolaemata	0	0	17	0	0	0	0	0	0	0	11	0	0
OTU_Phase1_39	<i>Amathia verticillata</i>	Bryozoa	Gymnolaemata	0	4	10112	6550	3	0	0	0	2	207	66	284	0
OTU_Phase1_43	<i>Watersipora subtorquata</i>	Bryozoa	Gymnolaemata	3	1	394	783	88	0	3	0	59	1	88	9	0
OTU_Phase1_58	<i>Bugula heritina</i>	Bryozoa	Gymnolaemata	112	18	163	128	29	0	3	0	29	2	79	6	0
OTU_Phase1_60	<i>Watersipora subovoidea</i>	Bryozoa	Gymnolaemata	0	0	118	189	0	0	0	0	0	6	232	32	0
OTU_BZS05_101	<i>Tridentiger barbatus</i>	Chordata	Actinopterygii	0	0	0	0	0	5192	0	7199	1	0	0	0	14757
OTU_BZS05_111	<i>Tridentiger trigonocephalus</i>	Chordata	Actinopterygii	0	0	0	23	0	586	0	474	0	0	13	0	2083
OTU_Phase1_12	<i>Molgula manhattensis</i>	Chordata	Ascidacea	82	0	0	0	0	12	0	11	0	0	0	0	17

OTU_Phase1_13	<i>Microcosmus squamiger</i>	Chordata	Ascidacea	0	0	68	60	0	0	1	1	17	23	1261	708	0
OTU_Phase1_16	<i>Corella inflata</i>	Chordata	Ascidacea	44	0	0	0	0	1	1	0	0	0	0	0	0
OTU_Phase1_16	<i>Didemnum vexillum</i>	Chordata	Ascidacea	83	1	0	0	25	5	31	176	180	0	0	0	1
OTU_Phase1_28	<i>Botrylloides violaceus</i>	Chordata	Ascidacea	57	1	97	432	186	1	133	17	65	3	7	0	7
OTU_Phase1_31	<i>Ascidia zara</i>	Chordata	Ascidacea	1094	0	126	717	0	18	56	71	104	661	309	1198	377
OTU_Phase1_40	<i>Styela plicata</i>	Chordata	Ascidacea	0	0	47	978	0	1	11	0	41	729	4868	300	0
OTU_Phase1_41	<i>Botryllus schlosseri</i>	Chordata	Ascidacea	16	1	557	226	9	0	11	0	37	16	83	146	0
OTU_Phase1_58	<i>Ciona savignyi</i>	Chordata	Ascidacea	83	0	12	664	0	2	3	11	5722	54	3518	42	17
OTU_Phase1_64	<i>Diplosoma austerianum</i>	Chordata	Ascidacea	53	1	17	0	109	0	0	0	7	5	4	1	4
OTU_Phase1_74	<i>Polyandrocarpa zorritensis</i>	Chordata	Ascidacea	1	1	948	5066	0	0	392	0	96	474	215	188	5
OTU_Phase1_12	<i>Diadumene sp</i>	Cnidaria	Anthozoa	0	0	13	122	0	0	0	0	0	0	0	0	0
OTU_Phase1_47	<i>Diadumene leucolea</i>	Cnidaria	Anthozoa	0	1	3957	5	1	213	0	226	0	0	0	0	12990
OTU_BZS04_199	<i>Ectopleura crocea</i>	Cnidaria	Hydrozoa	0	2	0	0	0	7	16	1541	39	0	3	15	118
OTU_BZS05_336	<i>Blackfordia virginica</i>	Cnidaria	Hydrozoa	7	0	0	0	0	215	0	169	0	0	0	0	1064
OTU_Phase1_27	<i>Barentsia benedeni</i>	Entoprocta		22	0	0	0	0	2	0	9	0	0	0	0	0
OTU_BZS05_496	<i>Corbula tumensis</i>	Mollusca	Bivalvia	9	0	0	0	0	581	0	24488	1	0	1	4	8051
OTU_BZS06_472	<i>Macoma petalum</i>	Mollusca	Bivalvia	5	0	0	0	0	0	0	38	0	0	0	0	54
OTU_Phase1_18	<i>Crassostrea gigas</i>	Mollusca	Bivalvia	0	1	11	54	1	0	4	0	16	55	129	130	0
OTU_Phase1_23	<i>Geukensia demissa</i>	Mollusca	Bivalvia	163	0	0	0	0	0	0	18	3	0	23	2021	13
OTU_Phase1_26	<i>Mya arenaria</i>	Mollusca	Bivalvia	5	0	0	0	15	0	45	19	14	37	0	0	0
OTU_Phase1_29	<i>Musculista senhousia</i>	Mollusca	Bivalvia	1	1	635	76	0	9	0	3	0	0	0	666	3
OTU_Phase1_73	<i>Musculista senhousia</i>	Mollusca	Bivalvia	30	1	5172	649	132	9	4	320	25	2	42	1478	105
OTU_BZS05_106	<i>Sakuraeolis nasimensis</i>	Mollusca	Gastropoda	0	0	0	0	0	87	0	846	0	0	0	0	258
OTU_BZS05_179	<i>Spurwinkia salsa</i>	Mollusca	Gastropoda	1	0	0	0	0	4300	0	7880	0	0	0	0	2642
OTU_Phase1_18	<i>Crepidula plana</i>	Mollusca	Gastropoda	47	0	0	0	0	7926	0	52211	0	4	1	1	3478
OTU_Phase1_67	<i>Haminoea japonica</i>	Mollusca	Gastropoda	191	0	0	0	0	0	0	0	0	0	0	0	0
OTU_BZS06_119	<i>Grateloupia turuturu</i>	Rhodophyta	Florideophyceae	0	0	12	4	0	0	1	0	6	0	173	0	0
OTU_BZS08_248	<i>Acrochaetium secundatum</i>	Rhodophyta	Florideophyceae	0	0	0	0	0	0	0	0	0	0	0	0	21
OTU_Phase1_20	<i>Schizymenia lubyi</i>	Rhodophyta	Florideophyceae	0	0	2	46	0	0	0	0	3	0	3	0	0
OTU_Phase1_35	<i>Lomentaria nakodatensis</i>	Rhodophyta	Florideophyceae	28	0	7	349	0	0	0	1	26	0	76	0	0
Total read number				10288	340	56861	45591	776	227802	6004	394475	36490	115423	109673	35287	724500

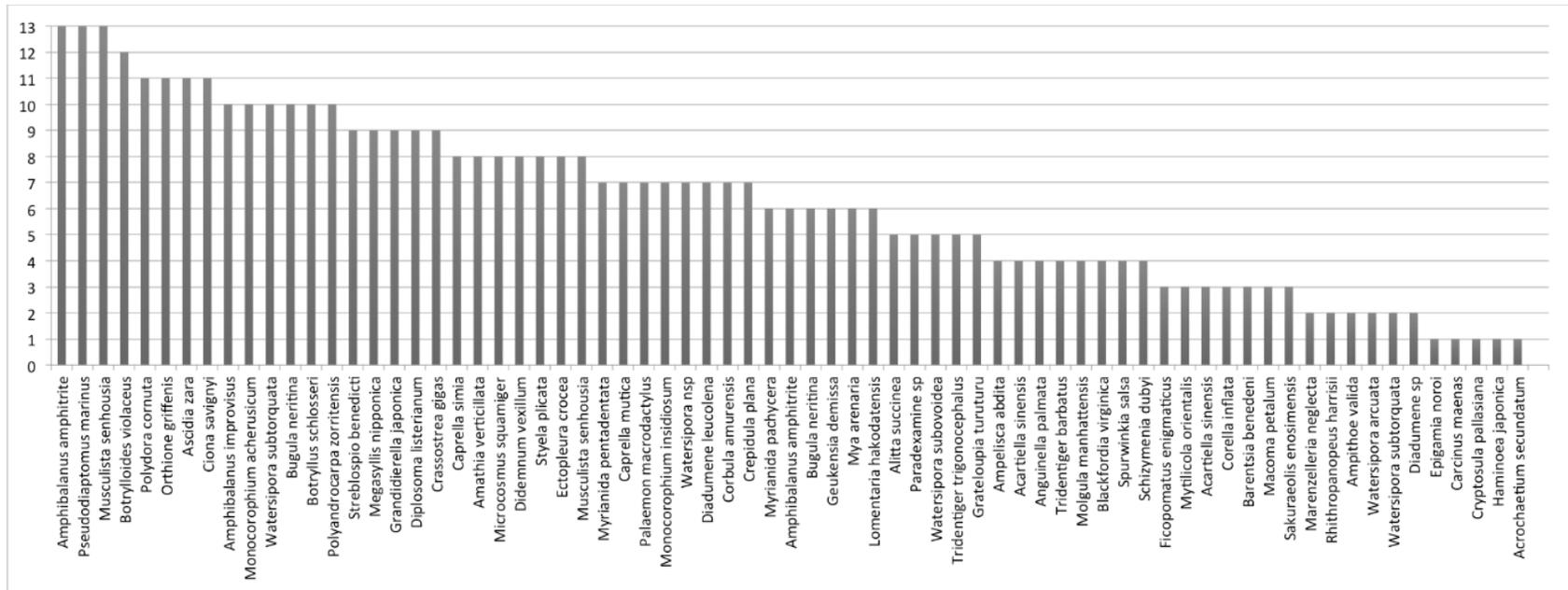


Figure 6.7. Distribution of NIS detected in metagenetic analysis among site-dates in ten California bays. San Francisco Bay was sampled in three years.

Table 6.12. CalNIS Phase 1 and 2 plankton samples that contained no NIS on the SERC list (Appendix 6.1).

Sample ID	Bay	Site	Year	Method	Depth (m)
BZS000575	San Francisco	SF-P12	2013	Pump	1
BZS000581	San Francisco	SF-P15	2013	Pump	1
BZS000582	San Francisco	SF-P15	2013	Pump	1
BZS000601	Morro	MO-P02	2013	Pump	1
BZS000619	Morro	MO-T03	2013	Vertical Tow	5
BZS000633	Morro	MO-P08	2013	Pump	1
BZS000654	Morro	MO-T09	2013	Vertical Tow	5
BZS000663	Morro	MO-T04	2013	Vertical Tow	5
BZS000672	San Diego	SD-P01	2013	Pump	1
BZS001357	Bodega-Tomales	BT-T05	2014	Vertical Tow	5
BZS001359	Bodega-Tomales	BT-T01	2014	Vertical Tow	5
BZS001361	Bodega-Tomales	BT-T02	2014	Vertical Tow	5
BZS001377	Bodega-Tomales	BT-T10	2014	Vertical Tow	5
BZS001417	Humboldt	HB-P03	2015	Pump	1
BZS001426	Humboldt	HB-P05	2015	Pump	1
BZS001427	Humboldt	HB-P05	2015	Pump	1
BZS001445	Humboldt	HB-P09	2015	Pump	1
BZS001658	San Francisco	SFHS-31	2015	Vertical Tow	5
BZS001754	Marina Del Rey	MDR-P04	2015	Pump	1
BZS001757.1	Marina Del Rey	MDR-P05	2015	Pump	1
BZS001759.2	Marina Del Rey	MDR-P05	2015	Pump	1
BZS001764.3	Marina Del Rey	MDR-P06	2015	Pump	1
BZS001764.4	Marina Del Rey	MDR-P06	2015	Pump	1
BZS001772.3	Marina Del Rey	MDR-P08	2015	Pump	1
BZS001777.1	Marina Del Rey	MDR-P09	2015	Pump	1
BZS001791	Marina Del Rey	MDR-T03	2015	Vertical Tow	5
BZS001795	Marina Del Rey	MDR-T05	2015	Vertical Tow	5
BZS001848rD	San Pedro	SP-P09	2015	Pump	1
BZS001943	Newport	NP-P05	2015	Pump	1
BZS002061	San Francisco	SFHS-05	2016	Vertical Tow	10

Differences in NIS composition among Bays.

We observed significant differences in NIS composition and abundance among samples (Table 6.13 and 6.14; Figures 6.8 and 6.9). When year of sampling was also a significant factor for differences among plankton samples (Table 6.14), however, year is confounded by site, since

only San Francisco Bay was sampled in multiple years (Figure 6.10). San Francisco Bay was sharply different in 2015 and 2016.

Table 6.13. PERMANOVA testing differences in NIS composition and abundance among all plankton samples across years with reads per sample rarified to 1,000.

Factor	df	SS	MS	Pseudo-F	P(perm)
Plankton sample	10	189870	18987	19.739	0.001
Residues	216	207780	961.94		
Total	226	397650			

Table 6.14. PERMANOVA testing differences in NIS composition and abundance among all plankton samples across years with reads per sample rarified to 10,000.

Factor	df	SS	MS	Pseudo-F	P(perm)
Plankton Sample	4	23765	5941.3	7.2837	0.001
Residues	45	36706	815.7		
Total	49	60471			

Table 6.15. PERMANOVA testing differences in NIS composition and abundance among plankton samples and years with reads per sample rarified to 1,000. Only San Francisco Bay was sampled in multiple years therefore the interaction of Bay and Year was not included.

Factor	df	SS	MS	Pseudo-F	P(perm)
Bay	7	102980	14711	15.293	0.001
Year	3	21400	7133.4	7.4157	0.001
Residues	216	207780	961.94		
Total	226	397650			

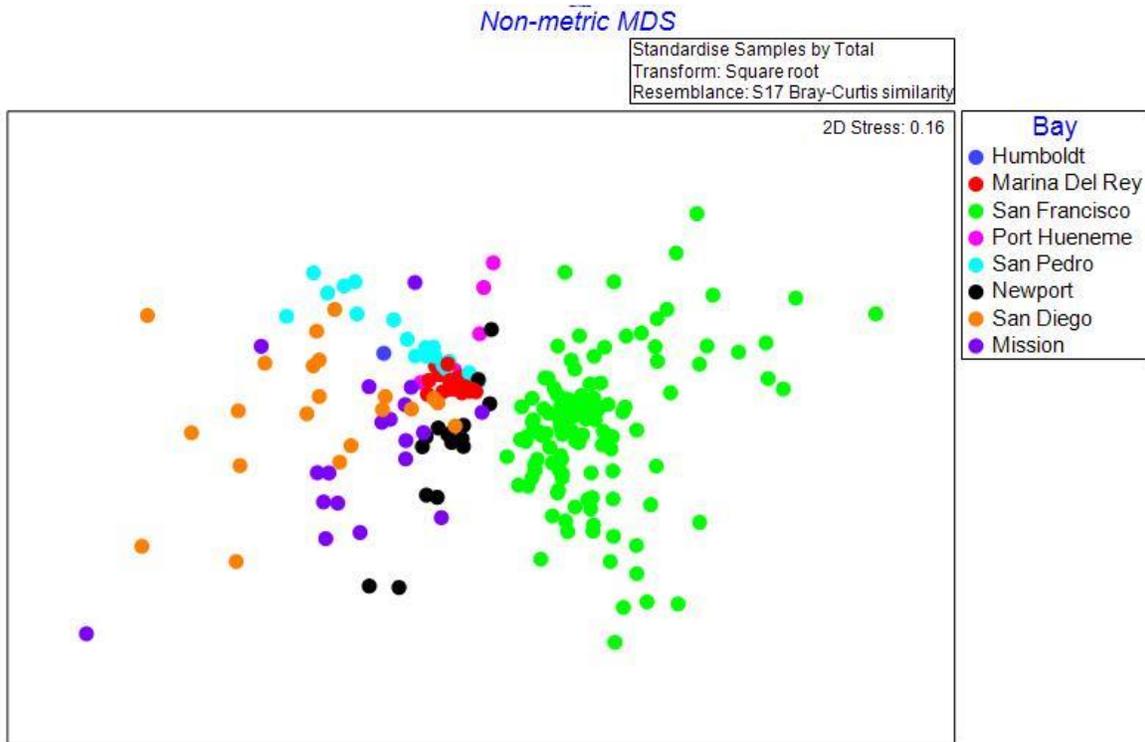


Figure 6.8. nMDS of NIS assemblages in California bays using 1000 rarefied reads.

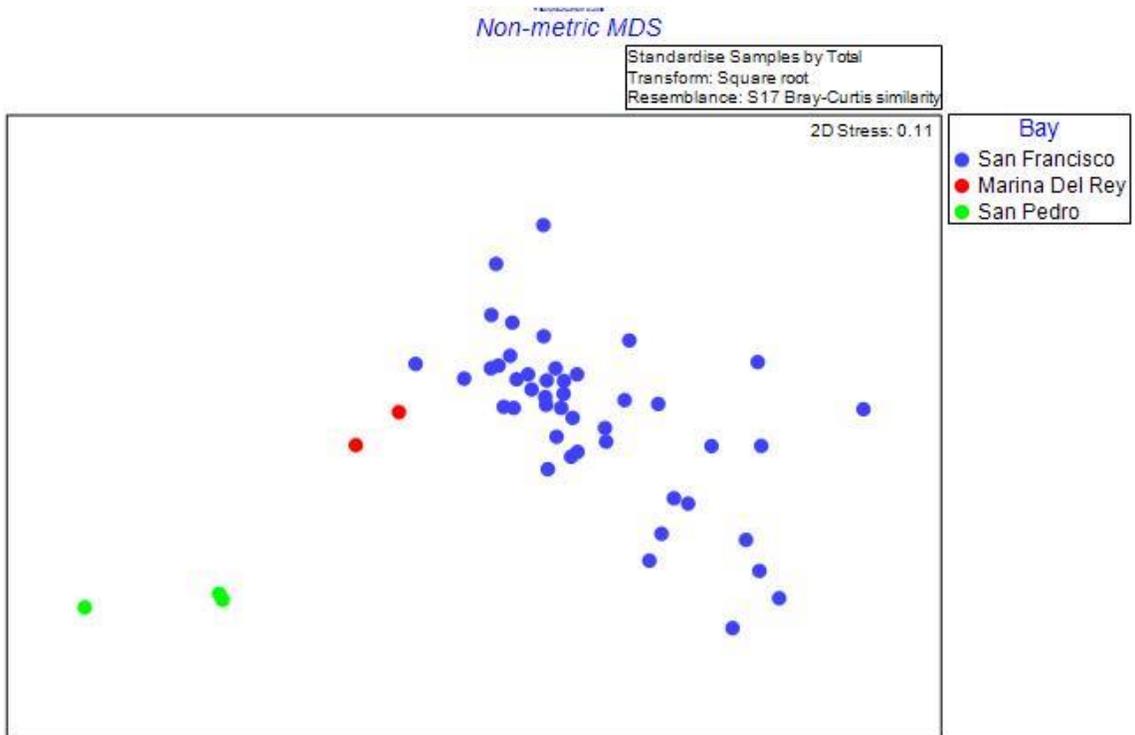


Figure 6.9. nMDS of NIS assemblages in California bays using 10,000 rarefied reads. Fewer bays are represented than in Figure 6.8 due to insufficient number of reads outside of the three bays shown.

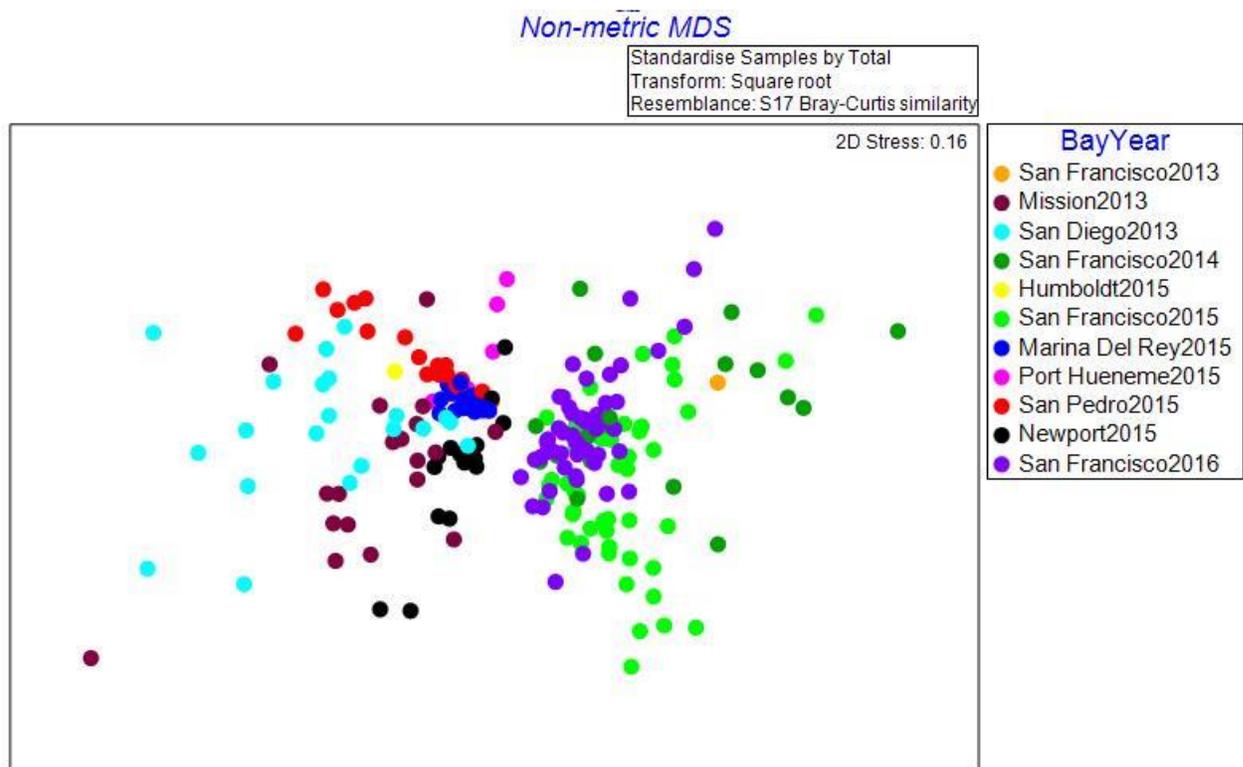


Figure 6.10. nMDS of NIS assemblages in California bays separated by year and using 1,000 rarefied reads. Only San Francisco Bay was repeatedly sampled and shows variation by years (c.f., 2015 v. 2016).

Role of environmental factors for NIS composition and abundance.

Salinity had a strong impact on NIS plankton communities (Table 6.16) and had significant interactions with year and surface temperature (Figures 6.11 and 6.12). Rainfall increased significantly in 2016, which probably explains the interaction with year. Loss of samples due to rarefaction to 10,000 reads per sample reduced statistical power but the same pattern was evident (Table 6.17).

Table 6.16. PERMANOVA including environmental factors for composition of non-indigenous invertebrate species (NIS) in all bays across years after rarefaction to 1000 reads per sample.

Factor	df	SS	MS	Pseudo-F	P(perm)
BayYear	7	68790	9827.1	15.894	0.001
Surface Temperature	2	1493.8	746.91	1.208	0.271
Surface Salinity	1	2161.9	2161.9	3.4967	0.003
BayYear x Surface Temperature*	8	12258	1532.2	2.4782	0.001
BayYear x Surface Salinity*	4	7042.2	1760.5	2.8475	0.001
Surface Temperature x Surface Salinity*	2	1230	615.02	0.99471	0.444
BayYear x Surface Temperature x Surface Salinity**	2	2122.2	1061.1	1.7162	0.069
Residues	188	116240	618.29		
Total	226	397650			

*i indicates uneven sample sizes

Table 6.17. PERMANOVA including environmental factors for composition of non-indigenous invertebrate species (NIS) in all bays across years after rarefaction to 10,000 reads per sample.

Factor	df	SS	MS	Pseudo-F	P(perm)
BayYear	2	8021.8	4010.9	9.4445	0.001
Surface Temperature	2	936.6	468.3	1.1027	0.333
Surface Salinity	1	877.37	877.37	2.0659	0.086
BayYear x Surface Salinity*	4	3487.3	871.83	2.0529	0.008
Surface Temperature x Surface Salinity*	1	339.75	339.75	0.8	0.499
Residues	32	13590	424.68		
Total	49	60471			

*i indicates uneven sample sizes

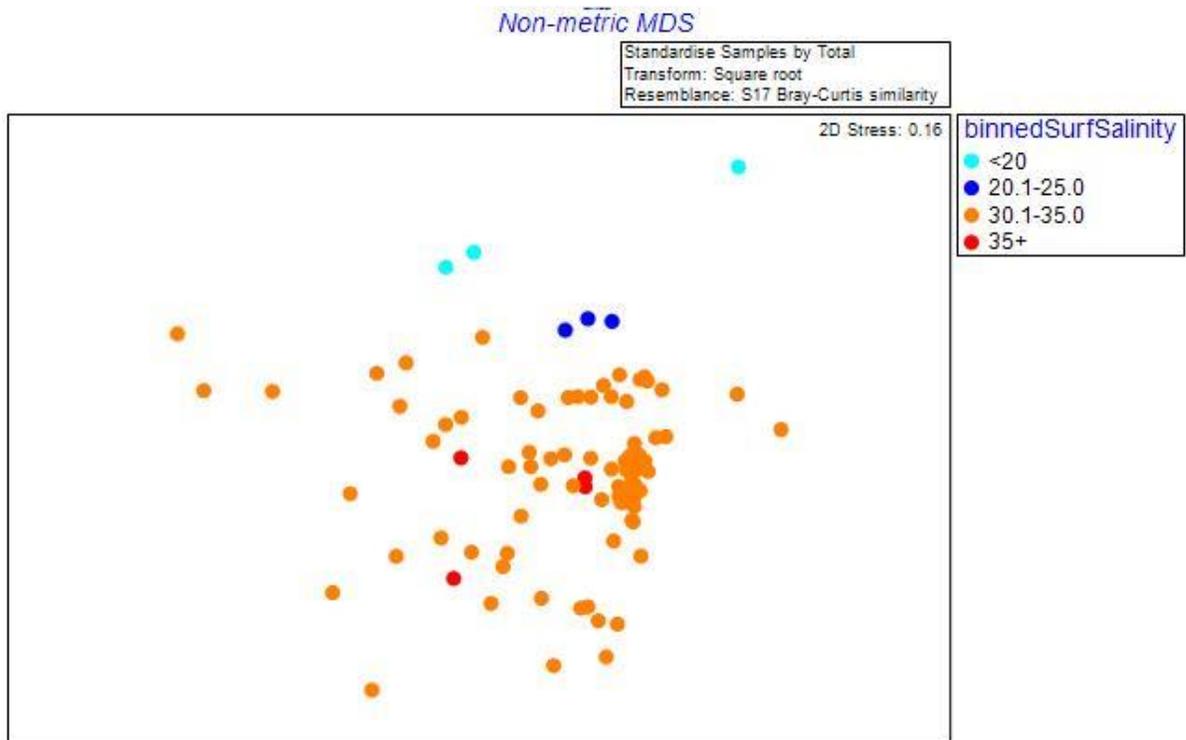


Figure 6.11. nMDS of NIS assemblages in California bays binned into surface salinity groups, using 1000 rarefied reads.

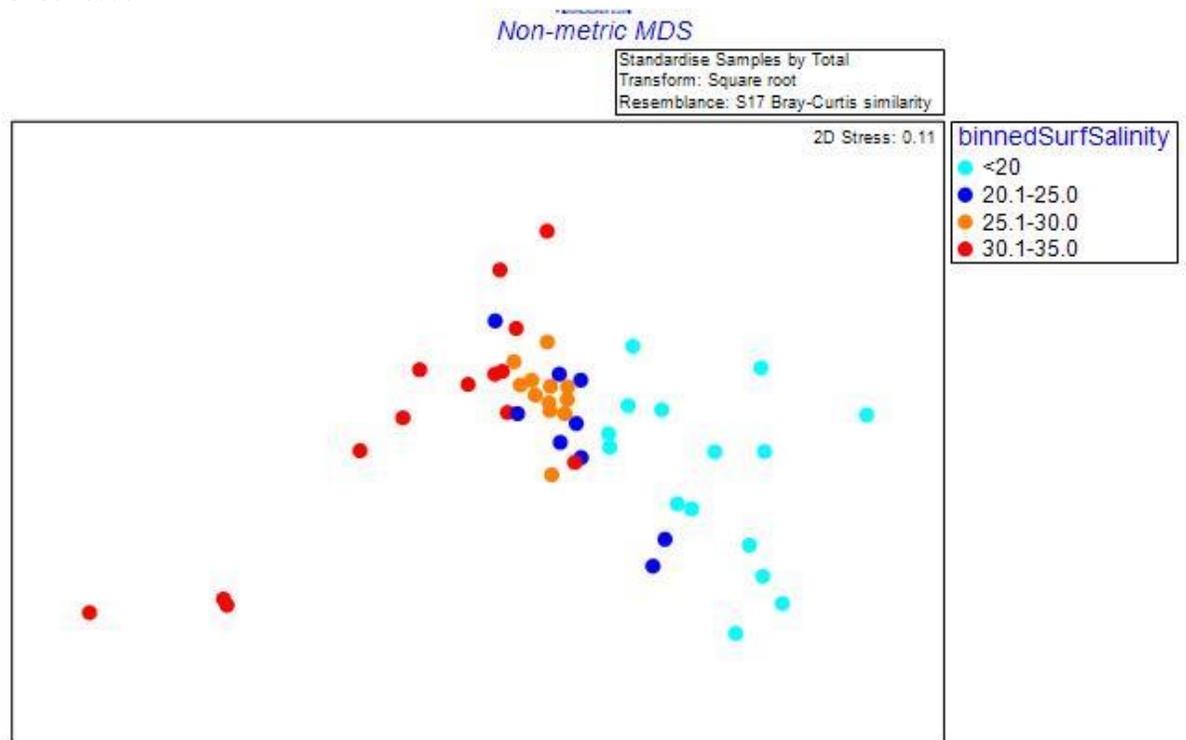


Figure 6.12. nMDS of NIS assemblages in California bays binned into surface salinity groups, using 10,000 rarefied reads

Differences in NIS composition within San Francisco Bay.

Data restricted to San Francisco Bay allows a better focus on sources of variation in NIS plankton composition and abundance (Figures 6.13 to 6.16). As for all sites, NIS varied across years in San Francisco Bay and interacted significantly with surface salinity (Table 6.18 and 6.19).

Temperature was marginally not significant as a driver of NIS assemblages at the 1000 read rarefaction level, and too few samples had enough reads to test that effect more robustly at the 10,000 read level.

Table 6.18. PERMANOVA results of community composition of non-indigenous invertebrate species (NIS) for San Francisco samples when reads were rarified to 1000.

Factor	df	SS	MS	Pseudo-F	P(perm)
Year	1	3129.7	3129.7	5.3883	0.001
Surface Temperature	1	1041.1	1041.1	1.7924	0.123
Surface Salinity	1	2001	2001	3.445	0.006
Year x Surface Temperature *	3	2822.1	940.69	1.6196	0.055
Year x Surface Salinity *	4	7042.2	1760.5	3.0311	0.001
Surface Temperature x Surface Salinity*	1	269.24	269.24	0.46354	0.832
Year x Surface Temperature x Surface Salinity*	2	2122.2	1061.1	1.8269	0.058
Residues	101	58664	580.83		
Total	121	146430			

*i indicates that sample size was not equal across factors.

Table 6.19. PERMANOVA results of community composition of non-indigenous invertebrate species (NIS) for San Francisco samples when reads were rarified to 10,000. Insufficient data were available to test the interaction of Year and Surface Temperature.

Factor	df	SS	MS	Pseudo-F	P(perm)
Year	1	2454.5	2454.5	5.5878	0.001
Surface Temperature	1	755.69	755.69	1.7204	0.146
Surface Salinity	1	877.37	877.37	1.9974	0.091
Year x Surface Salinity *	4	3487.3	871.83	1.9848	0.02
Surface Temperature x Surface Salinity*	1	339.75	339.75	0.77345	0.512
Residues	30	13178	439.26		
Total	44	41611			

*i indicates that sample size was not equal across factors.

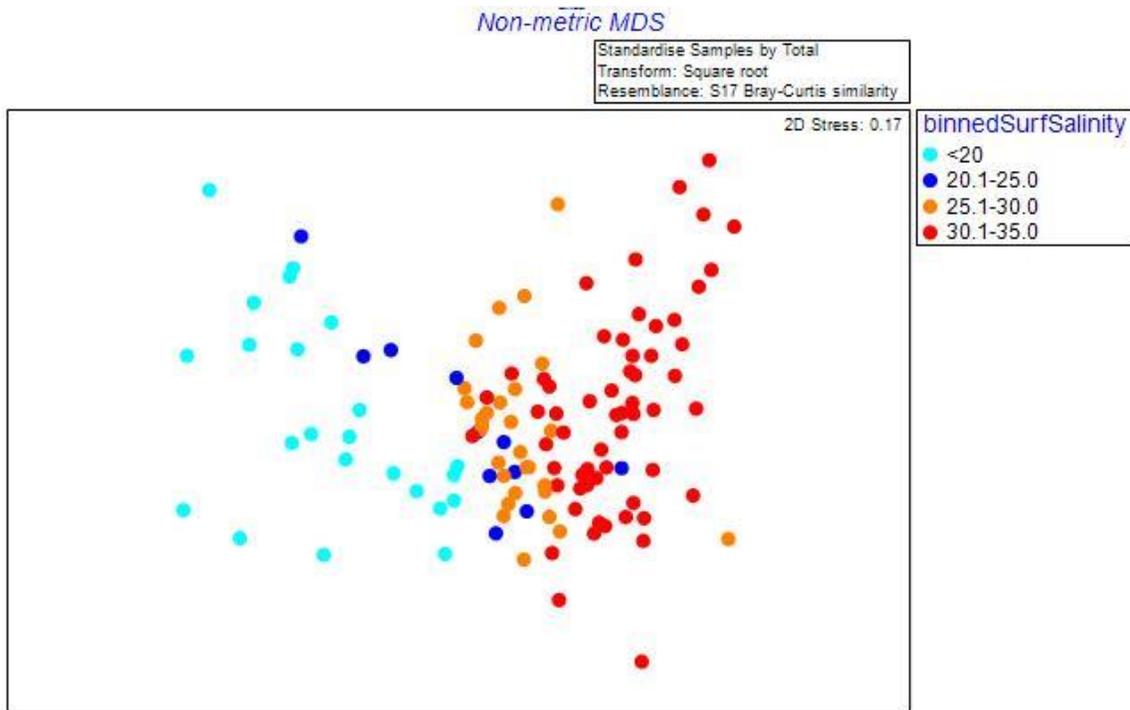


Figure 6.13 nMDS of NIS assemblages in San Francisco bay binned into surface salinity groups, using 1000 rarefied reads per sample.

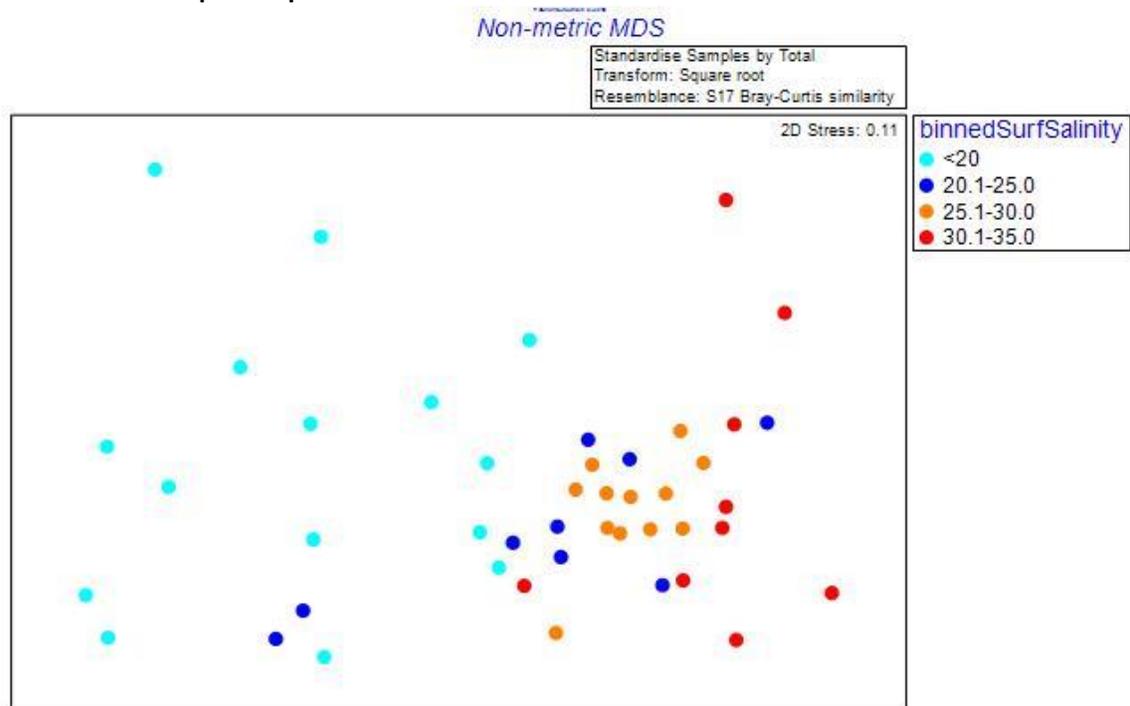


Figure 6.14 nMDS of NIS assemblages in San Francisco bay binned into surface salinity groups, using 10,000 rarefied reads per sample.

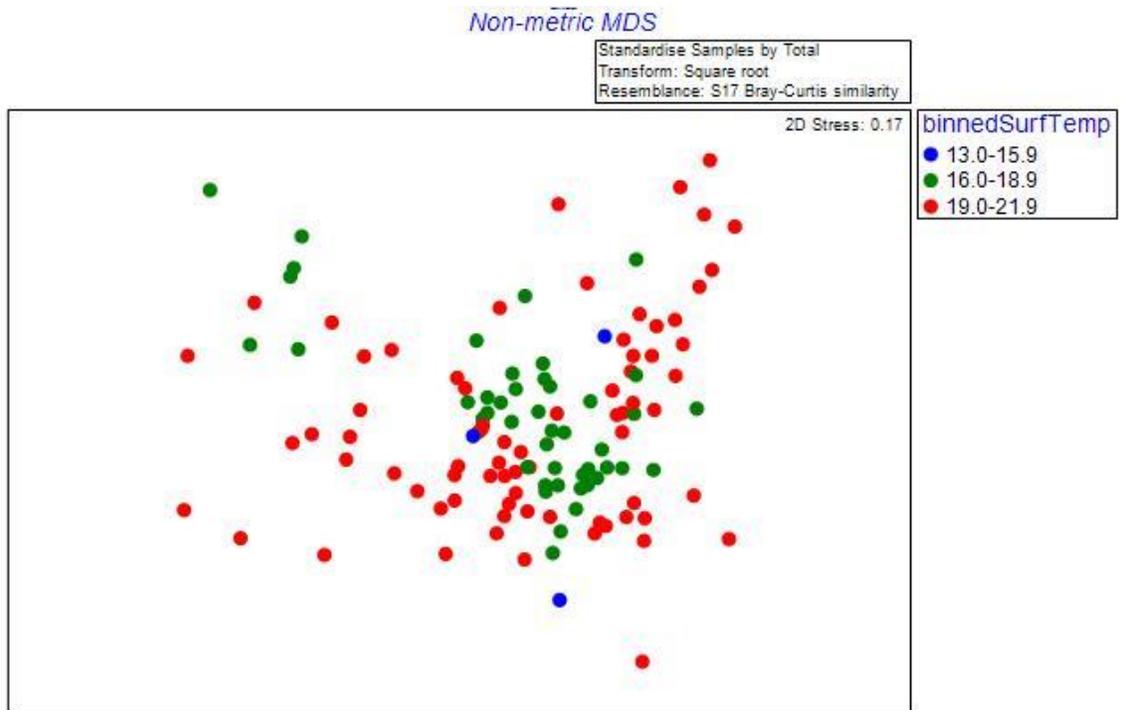


Figure 6.15 nMDS of NIS assemblages in San Francisco bay binned into temperature groups, using 1000 rarefied reads per sample.

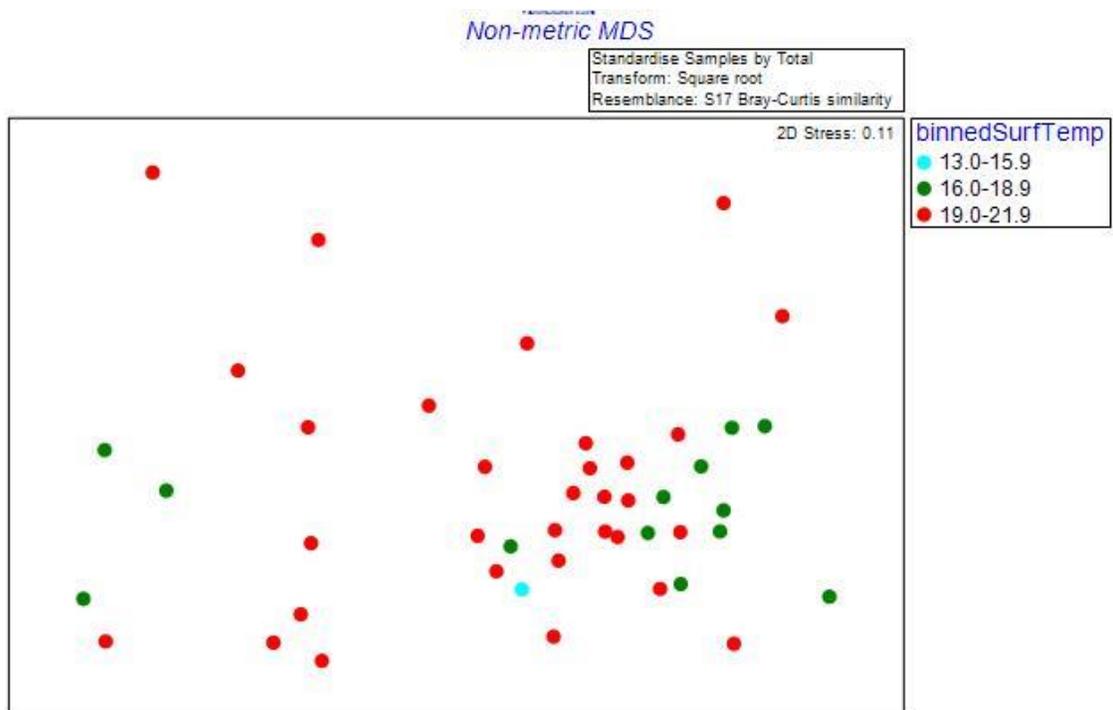


Figure 6.16. nMDS of NIS assemblages in San Francisco bay binned into temperature groups, using 10,000 rarefied reads per sample.

Additional NIS that potentially are contained in plankton samples

Table 6.11 includes all known marine NIS in California, according to Appendix 6.1, that were detected in plankton samples through metagenetic analysis. To find NIS not presently known in California, we reviewed all BLAST hits >94.5% for OTU representative sequences, including hits not top-ranked. We found 666 species by this criterion. Of these, 70 were on the SERC California NIS list, leaving 596 additional species. Most of these species are native. S. Foss (pers. com.) identified 21 species that are potential new NIS based on biogeographical information, after excluding synonyms and freshwater species (Table 6.20). This list undoubtedly contains false positives because each OTU is likely a single species even though its sequence may hit more than one database record. However, this list will contain fewer false negatives than if we used only the single top hit, as lower ranked hits may be the correct species.

Table 6.20. Potential NIS not present in Appendix 6.1. These species have COI sequences in Genbank that are >94.5% identical to OTU in California plankton samples. This is not considered unequivocal evidence of presence, but these species may be put on a watch list.

Binomial	Phylum	Class	Taxon	Status*
<i>Acantholobulus bermudensis</i>	Arthropoda	Malacostraca	Crab	Y
<i>Agalma elegans</i>	Cnidaria	Hydrozoa	Siphonophora	C
<i>Alderia modesta</i>	Mollusca	Gastropoda	Saccoglossa	C
<i>Alexandrium fundyense</i>	Myzozoa	Dinophyceae	Dinoflagellate	Y
<i>Amathia gracilis</i>	Bryozoa	Gymnolaemata	Bryozoan	Y
<i>Amathia vidovici</i>	Bryozoa	Gymnolaemata	Bryozoan	Y
<i>Ameritella versicolor</i>	Mollusca	Bivalvia	Clam	Y
<i>Amphibalanus reticulatus</i>	Arthropoda	Hexanauplia	Barnacle	Y
<i>Amphipholis squamata</i>	Echinodermata	Ophiuroidea	Brittlestar	C
<i>Ascidia virginea</i>	Chordata	Asciacea	Tunicate	Y
<i>Aurelia labiata</i>	Cnidaria	Scyphozoa	Jellyfish	C
<i>Botrylloides pizoni</i>	Chordata	Asciacea	Tunicate	Y
<i>Crassostrea virginica</i>	Mollusca	Bivalvia	Oyster	Y
<i>Halichondria panicea</i>	Porifera	Demospongiae	Sponge	C
<i>Megabalanus rosa</i>	Arthropoda	Hexanauplia	Barnacle	Y
<i>Membranipora membranacea</i>	Bryozoa	Gymnolaemata	Bryozoan	C
<i>Menippe adina</i>	Arthropoda	Malacostraca	Crab	Y
<i>Menippe mercenaria</i>	Arthropoda	Malacostraca	Crab	Y
<i>Onchidoris bilamellata</i>	Mollusca	Gastropoda	Nudibranch	C
<i>Perna viridis</i>	Mollusca	Bivalvia	Bivalve	Y
<i>Pfiesteria piscicida</i>	Myzozoa	Dinophyceae	Algae	Y

*Y=potential novel NIS; C=cosmopolitan or potential cryptic species complex.

Discussion and Conclusions.

Over 14,000 COI haplotypes clustered into over 3700 OTU were found in plankton samples drawn from California bays by metagenetic analysis of the barcoding fragment of the mitochondrial COI gene. This is much more diversity than would be expected from morphological analysis. Extremely high OTU diversity in marine metagenetic studies is a common feature in such studies, and the meaning of this diversity is still a question of active investigation. Direct comparison of our diversity estimates to morphological studies of bay or other coastal plankton is difficult because most studies have not identified samples to the species level.

Community analyses of plankton communities revealed striking geographic differences among bays, and the pattern was not simply latitudinal. Where environmental metadata were complete, we detected a strong effect of surface water salinity; it is not surprising that salinity should have a strong effect on plankton composition. We also detected inter-annual differences in plankton assemblages, especially in San Francisco Bay where sampling was annually repeated. A long drought broke in 2016, and differences between years might be cross-correlated with salinity. Indeed, we observed a significant interaction between year of sampling and salinity in statistical analyses. We note a caveat that these ecological analyses treat reads as numerical abundance and OTU as species. The relationship between read number and numerical abundance is likely not straightforward. Furthermore, OTU bins based on thresholds have the potential to split some species into haplotype groups and lump some species as one OTU if their sequences are quite similar. A particularly genetically diverse species might contain many haplotype groups, and haplotypes might be distributed differently among bays reflecting population genetic processes. In that scenario, differences in species composition among bays could be overestimated.

Sixty nine invertebrates and two algae of 252 known marine NIS were detected in our samples, though many were in low abundance and present in few bays. An important caveat to this study is that the relationship between read number and numerical abundance of any species is not yet understood, as mentioned in the preceding paragraph, therefore it may be more pragmatic to consider Table 6.11 as a list of species present. By this reasoning, however, we should be cautious in saying that a species is absent if it is not detected. Quantitative PCR (qPCR) studies may be useful to investigate actual numerical abundance in Illumina libraries and, by extension, in plankton samples. Similarly, species not detected may be probed for in DNA extractions using qPCR or end-point PCR with species-specific primers as a secondary assay for presence.

A comparison of species lists for bays based on plankton metagenetics and settling plate morphological analysis is not yet complete. In comparing benthic species, we should expect plankton samples to be less diverse, as only species with plankton larvae are likely to be well represented. (Some species lacking planktonic larvae may occur in plankton samples,

nonetheless, as broken fragments, shed mucus or tissues, or eDNA). Further, plankton samples are less time-averaged than settling plates. Meroplankton reflect recent reproductive activity of benthic adults and transport processes. Species composition in plankton is therefore likely to be more episodic than on settling plates and may explain why some samples had no NIS.

By relaxing criteria for annotating OTU, we found 27 additional species that could be NIS if they were confirmed in California estuaries. Many of these would be of great concern: oysters and other bivalves, predatory crabs, fouling bryozoans and sea squirts, and fish-killing protists. Uncertainty over actual ecological impact should any of these species become established highlights the challenges of such prediction.

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Edgar R.C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19), 2460-2461.

Edgar R.C. 2016. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon reads. Preprint (doi: <https://doi.org/10.1101/081257>)

Fofonoff P.W., Ruiz G.M., Steves B., Simkanin C., & Carlton J.T. 2017. California Non-native Estuarine and Marine Organisms (Cal-NEMO) System. <http://invasions.si.edu/nemesis/>

Heller et al. (in review)

Katoh M., & Kuma M. 2002 MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30:3059-3066

Appendix 6.1

List of Marine NIS in California (Fofonoff *et al.* 2017)

TXA_Group	TXA_Binomial
Algae	<i>Acrochaetium secundatum</i>
Algae	<i>Aglaothamnion tenuissimum</i>
Algae	<i>Attheya armata</i>
Algae	<i>Bryopsis</i> sp. 1
Algae	<i>Caulacanthus okamurae</i>
Algae	<i>Ceramium kondoi</i>
Algae	<i>Ceramium sungminbooi</i>
Algae	<i>Codium fragile</i>
Algae	<i>Colpomenia claytoniae</i>
Algae	<i>Dasya sessilis</i>
Algae	<i>Elachista nigra</i>
Algae	<i>Fucus spiralis</i>
Algae	<i>Gelidium vagum</i>
Algae	<i>Gracilaria vermiculophylla</i>
Algae	<i>Gracilariopsis chorda</i>
Algae	<i>Grateloupia turuturu</i>
Algae	<i>Lomentaria hakodatensis</i>
Algae	<i>Mutimo cylindricus</i>
Algae	<i>Neosiphonia japonica</i>
Algae	<i>Pachymeniopsis lanceolata</i>
Algae	<i>Polysiphonia denudata</i>
Algae	<i>Pterosiphonia tanakae</i>
Algae	<i>Pyropia suborbiculata</i>
Algae	<i>Sargassum horneri</i>
Algae	<i>Sargassum muticum</i>
Algae	<i>Schizymenia dubyi</i>
Algae	<i>Ulva australis</i>
Algae	<i>Ulva clathratioides</i>
Algae	<i>Undaria pinnatifida</i>
Amphibians-Frogs	<i>Lithobates catesbeiana</i>
Amphibians-Frogs	<i>Xenopus laevis</i>
Annelids-Leeches	<i>Myzobdella lugubris</i>
Annelids-Oligochaetes	<i>Branchiura sowerbyi</i>
Annelids-Oligochaetes	<i>Cambarincola pamela</i>
Annelids-Oligochaetes	<i>Chaetogaster diaphanus</i>
Annelids-Oligochaetes	<i>Eukerria saltensis</i>
Annelids-Oligochaetes	<i>Limnodriloides monotheucus</i>
Annelids-Oligochaetes	<i>Potamothrix bavaricus</i>
Annelids-Oligochaetes	<i>Tubificoides apectinatus</i>
Annelids-Oligochaetes	<i>Tubificoides brownae</i>
Annelids-Oligochaetes	<i>Tubificoides diazi</i>
Annelids-Oligochaetes	<i>Tubificoides wasselli</i>
Annelids-Oligochaetes	<i>Varichaetadrilus angustipenis</i>
Annelids-Polychaetes	<i>Alitta succinea</i>
Annelids-Polychaetes	<i>Amblyosyllis speciosa</i> form 4
Annelids-Polychaetes	<i>Boccardiella ligerica</i>
Annelids-Polychaetes	<i>Crucigera websteri</i>
Annelids-Polychaetes	<i>Diplocirrus SD1</i>

Annelids-Polychaetes	<i>Epigamia noroi</i>
Annelids-Polychaetes	<i>Ficopomatus enigmaticus</i>
Annelids-Polychaetes	<i>Hediste diadroma</i>
Annelids-Polychaetes	<i>Heteromastus filiformis</i> species complex
Annelids-Polychaetes	<i>Hobsonia florida</i>
Annelids-Polychaetes	<i>Hydroides dirampha</i>
Annelids-Polychaetes	<i>Hydroides elegans</i>
Annelids-Polychaetes	<i>Laonome</i> cf. <i>calida</i>
Annelids-Polychaetes	<i>Marenzelleria neglecta</i>
Annelids-Polychaetes	<i>Megasyllis nipponica</i>
Annelids-Polychaetes	<i>Myrianida convoluta</i>
Annelids-Polychaetes	<i>Myrianida pachycera</i>
Annelids-Polychaetes	<i>Myrianida pentadentata</i>
Annelids-Polychaetes	<i>Neodexiospira brasiliensis</i>
Annelids-Polychaetes	<i>Polydora cornuta</i>
Annelids-Polychaetes	<i>Polydora hoplura</i>
Annelids-Polychaetes	<i>Proceraea okadai</i>
Annelids-Polychaetes	<i>Pseudopolydora paucibranchiata</i>
Annelids-Polychaetes	<i>Pseudopolydora</i> cf. <i>kempi</i>
Annelids-Polychaetes	<i>Sabaco elongatus</i>
Annelids-Polychaetes	<i>Streblospio benedicti</i>
Arthropoda-Insects	<i>Anisolabis maritima</i>
Arthropoda-Insects	<i>Neochetina bruchi</i>
Arthropoda-Insects	<i>Neochetina eichhorniae</i>
Arthropoda-Insects	<i>Prokelisia marginata</i>
Arthropoda-Insects	<i>Pselactus spadix</i>
Arthropoda-Insects	<i>Thambemyia borealis</i>
Arthropoda-Insects	<i>Trigonotylus uhleri</i>
Bacteria	<i>Teredinibacter turnerae</i>
Bacteria	<i>Xenohalotis californiensis</i>
Bryozoans	<i>Aeverillia armata</i>
Bryozoans	<i>Amathia verticillata</i>
Bryozoans	<i>Anguinella palmata</i>
Bryozoans	<i>Aspidelectra melolontha</i>
Bryozoans	<i>Bugula neritina</i>
Bryozoans	<i>Bugulina fulva</i>
Bryozoans	<i>Bugulina stolonifera</i>
Bryozoans	<i>Conopeum chesapeakeensis</i>
Bryozoans	<i>Conopeum tenuissimum</i>
Bryozoans	<i>Cryptosula pallasiana</i>
Bryozoans	<i>Pectinatella magnifica</i>
Bryozoans	<i>Schizoporella errata</i>
Bryozoans	<i>Schizoporella japonica</i>
Bryozoans	<i>Victorella pavida</i>
Bryozoans	<i>Watersipora arcuata</i>
Bryozoans	<i>Watersipora</i> n. sp.
Bryozoans	<i>Watersipora subtorquata</i> complex
Cnidarians-Anthozoans	<i>Bunodeopsis</i> sp. A
Cnidarians-Anthozoans	<i>Diadumene franciscana</i>
Cnidarians-Anthozoans	<i>Diadumene leucolena</i>
Cnidarians-Anthozoans	<i>Diadumene lineata</i>

Cnidarians-Anthozoans *Diadumene* sp. 1
 Cnidarians-Anthozoans *Nematostella vectensis*
 Cnidarians-Hydrozoans *Bimeria vestita*
 Cnidarians-Hydrozoans *Blackfordia virginica*
 Cnidarians-Hydrozoans *Cladonema pacificum*
 Cnidarians-Hydrozoans *Clava multicornis*
 Cnidarians-Hydrozoans *Climacocodon ikarii*
 Cnidarians-Hydrozoans *Cordylophora caspia*
 Cnidarians-Hydrozoans *Corymorpha* sp. A
 Cnidarians-Hydrozoans *Craspedacusta sowerbii*
 Cnidarians-Hydrozoans *Ectopleura crocea*
 Cnidarians-Hydrozoans *Garveia franciscana*
 Cnidarians-Hydrozoans *Gonionemus vertens*
 Cnidarians-Hydrozoans *Laomedea calceolifera*
 Cnidarians-Hydrozoans *Maeotias marginata*
 Cnidarians-Hydrozoans *Moerisia lyonsi*
 Cnidarians-Scyphozoans *Aurelia coerulea*
 Cnidarians-Scyphozoans *Phyllorhiza punctata*
 Crustaceans-Amphipods *Ampelisca abdita*
 Crustaceans-Amphipods *Ampithoe valida*
 Crustaceans-Amphipods *Aoroides secunda*
 Crustaceans-Amphipods *Caprella drepanochir*
 Crustaceans-Amphipods *Caprella mutica*
 Crustaceans-Amphipods *Caprella scaura*
 Crustaceans-Amphipods *Caprella simia*
 Crustaceans-Amphipods *Chelura terebrans*
 Crustaceans-Amphipods *Corophium alienense*
 Crustaceans-Amphipods *Corophium heteroceratum*
 Crustaceans-Amphipods *Crangonyx floridanus*
 Crustaceans-Amphipods *Eochelidium* sp. A
 Crustaceans-Amphipods *Gammarus daiberi*
 Crustaceans-Amphipods *Grandidierella japonica*
 Crustaceans-Amphipods *Incisocalliope derzhavini*
 Crustaceans-Amphipods *Jassa marmorata*
 Crustaceans-Amphipods *Leucothoe nagatai*
 Crustaceans-Amphipods *Melita nitida*
 Crustaceans-Amphipods *Melita rylovae*
 Crustaceans-Amphipods *Microdeutopus gryllotalpa*
 Crustaceans-Amphipods *Monocorophium acherusicum*
 Crustaceans-Amphipods *Monocorophium insidiosum*
 Crustaceans-Amphipods *Monocorophium uenoi*
 Crustaceans-Amphipods *Paracorophium* sp.
 Crustaceans-Amphipods *Paradexamine* sp. SD1
 Crustaceans-Amphipods *Stenothoe valida*
 Crustaceans-Amphipods *Transorchestia enigmatica*
 Crustaceans-Barnacles *Amphibalanus amphitrite*
 Crustaceans-Barnacles *Amphibalanus eburneus*
 Crustaceans-Barnacles *Amphibalanus improvisus*
 Crustaceans-Cladocerans *Daphnia lumholtzi*
 Crustaceans-Copepods *Acartiella sinensis*
 Crustaceans-Copepods *Eurytemora carolleeae*

Crustaceans-Copepods	<i>Harpacticella paradoxa</i>
Crustaceans-Copepods	<i>Lernaea cyprinacea</i>
Crustaceans-Copepods	<i>Limnoithona sinensis</i>
Crustaceans-Copepods	<i>Limnoithona tetraspina</i>
Crustaceans-Copepods	<i>Mytilicola orientalis</i>
Crustaceans-Copepods	<i>Oithona davisae</i>
Crustaceans-Copepods	<i>Pseudodiaptomus forbesi</i>
Crustaceans-Copepods	<i>Pseudodiaptomus inopinus</i>
Crustaceans-Copepods	<i>Pseudodiaptomus marinus</i>
Crustaceans-Copepods	<i>Sinocalanus doerrii</i>
Crustaceans-Copepods	<i>Tortanus dextrilobatus</i>
Crustaceans-Crabs	<i>Carcinus maenas</i>
Crustaceans-Crabs	<i>Eriocheir sinensis</i>
Crustaceans-Crabs	<i>Rhithropanopeus harrisi</i>
Crustaceans-Crayfish	<i>Orconectes virilis</i>
Crustaceans-Crayfish	<i>Pacifastacus leniusculus</i>
Crustaceans-Crayfish	<i>Procambarus clarkii</i>
Crustaceans-Cumaceans	<i>Nippoleucon hinumensis</i>
Crustaceans-Isopods	<i>Asellus hilgendorffii</i>
Crustaceans-Isopods	<i>Caecidotea racovitzai</i>
Crustaceans-Isopods	<i>Caecijaera horvathi</i>
Crustaceans-Isopods	<i>Dynoides dentisinus</i>
Crustaceans-Isopods	<i>Eurylana arcuata</i>
Crustaceans-Isopods	<i>Gnorimosphaeroma rayi</i>
Crustaceans-Isopods	<i>lais californica</i>
Crustaceans-Isopods	<i>Ianiropsis serricaudis</i>
Crustaceans-Isopods	<i>Limnoria quadripunctata</i>
Crustaceans-Isopods	<i>Limnoria tripunctata</i>
Crustaceans-Isopods	<i>Orthione griffenis</i>
Crustaceans-Isopods	<i>Paranthura japonica</i>
Crustaceans-Isopods	<i>Pseudosphaeroma</i> sp. A
Crustaceans-Isopods	<i>Sphaeroma quoianum</i>
Crustaceans-Isopods	<i>Sphaeroma walkeri</i>
Crustaceans-Isopods	<i>Synidotea laticauda</i>
Crustaceans-Isopods	<i>Uromunna</i> sp. A
Crustaceans- Leptostacans	<i>Nebalia</i> sp A.
Crustaceans-Mysids	<i>Deltamysis holmquistae</i>
Crustaceans-Mysids	<i>Hyperacanthomysis longirostris</i>
Crustaceans-Mysids	<i>Neomysis japonica</i>
Crustaceans-Mysids	<i>Orientomysis aspera</i>
Crustaceans-Mysids	<i>Orientomysis hwanhaiensis</i>
Crustaceans-Ostracods	<i>Aspidoconcha limnoriae</i>
Crustaceans-Ostracods	<i>Eusarsiella zostericola</i>
Crustaceans-Ostracods	<i>Redekea californica</i>
Crustaceans-Ostracods	<i>Spinileberis quadriaculeata</i>
Crustaceans-Shrimp	<i>Palaemon kadiakensis</i>
Crustaceans-Shrimp	<i>Palaemon macrodactylus</i>
Crustaceans-Shrimp	<i>Palaemon modestus</i>
Crustaceans-Tanaids	<i>Sinelobus</i> cf. <i>stanfordi</i>
Entoprocts	<i>Barentsia benedeni</i>

Entoprocts	<i>Urnatella gracilis</i>
Fishes	<i>Acanthogobius flavimanus</i>
Fishes	<i>Alosa sapidissima</i>
Fishes	<i>Ameiurus catus</i>
Fishes	<i>Ameiurus melas</i>
Fishes	<i>Ameiurus nebulosus</i>
Fishes	<i>Carassius auratus</i>
Fishes	<i>Cyprinella lutrensis</i>
Fishes	<i>Cyprinus carpio</i>
Fishes	<i>Dorosoma petenense</i>
Fishes	<i>Gambusia affinis</i>
Fishes	<i>Gila orcuttii</i>
Fishes	<i>Hypomesus nipponensis</i>
Fishes	<i>Ictalurus furcatus</i>
Fishes	<i>Ictalurus punctatus</i>
Fishes	<i>Lepomis cyanellus</i>
Fishes	<i>Lepomis gibbosus</i>
Fishes	<i>Lepomis gulosus</i>
Fishes	<i>Lepomis macrochirus</i>
Fishes	<i>Lepomis microlophus</i>
Fishes	<i>Lucania goodei</i>
Fishes	<i>Lucania parva</i>
Fishes	<i>Menidia audens</i>
Fishes	<i>Micropterus coosae</i>
Fishes	<i>Micropterus dolomieu</i>
Fishes	<i>Micropterus punctulatus</i>
Fishes	<i>Micropterus salmoides</i>
Fishes	<i>Morone saxatilis</i>
Fishes	<i>Notemigonus crysoleucas</i>
Fishes	<i>Oreochromis mossambicus</i>
Fishes	<i>Percina macrolepida</i>
Fishes	<i>Pimephales promelas</i>
Fishes	<i>Poecilia latipinna</i>
Fishes	<i>Pomoxis annularis</i>
Fishes	<i>Pomoxis nigromaculatus</i>
Fishes	<i>Ptychocheilus grandis</i>
Fishes	<i>Rhinogobius brunneus</i>
Fishes	<i>Tridentiger barbatus</i>
Fishes	<i>Tridentiger bifasciatus</i>
Fishes	<i>Tridentiger trigonocephalus</i>
Fungi	<i>Claviceps purpurea</i> var. <i>spartinae</i>
Mammals	<i>Ondatra zibethicus</i>
Mollusks-Bivalves	<i>Corbicula fluminea</i>
Mollusks-Bivalves	<i>Corbula amurensis</i>
Mollusks-Bivalves	<i>Crassostrea gigas</i>
Mollusks-Bivalves	<i>Gemma gemma</i>
Mollusks-Bivalves	<i>Geukensia demissa</i>
Mollusks-Bivalves	<i>Laternula gracilis</i>
Mollusks-Bivalves	<i>Lyrodus pedicellatus</i>
Mollusks-Bivalves	<i>Macoma petalum</i>
Mollusks-Bivalves	<i>Mercenaria mercenaria</i>

Mollusks-Bivalves	<i>Musculista senhousia</i>
Mollusks-Bivalves	<i>Mya arenaria</i>
Mollusks-Bivalves	<i>Mytilus galloprovincialis</i>
Mollusks-Bivalves	<i>Neotrapezium liratum</i>
Mollusks-Bivalves	<i>Nuttallia obscurata</i>
Mollusks-Bivalves	<i>Petricolaria pholadiformis</i>
Mollusks-Bivalves	<i>Teredo bartschi</i>
Mollusks-Bivalves	<i>Teredo navalis</i>
Mollusks-Bivalves	<i>Theora lubrica</i>
Mollusks-Bivalves	<i>Venerupis philippinarum</i>
Mollusks-Gastropods	<i>Assimineia parasitologica</i>
Mollusks-Gastropods	<i>Babakina festiva</i>
Mollusks-Gastropods	<i>Batillaria attramentaria</i>
Mollusks-Gastropods	<i>Bellamyia chinensis</i>
Mollusks-Gastropods	<i>Boonea bisuturalis</i>
Mollusks-Gastropods	<i>Busycotypus canaliculatus</i>
Mollusks-Gastropods	<i>Catriona rickettsi</i>
Mollusks-Gastropods	<i>Cecina manchurica</i>
Mollusks-Gastropods	<i>Crepidula convexa</i>
Mollusks-Gastropods	<i>Crepidula fornicata</i>
Mollusks-Gastropods	<i>Crepidula plana</i>
Mollusks-Gastropods	<i>Cuthona perca</i>
Mollusks-Gastropods	<i>Haminoea japonica</i>
Mollusks-Gastropods	<i>Leostyletus misakiensis</i>
Mollusks-Gastropods	<i>Littoridinops monroensis</i>
Mollusks-Gastropods	<i>Littorina saxatilis</i>
Mollusks-Gastropods	<i>Melanochlamys ezoensis</i>
Mollusks-Gastropods	<i>Melanoides tuberculata</i>
Mollusks-Gastropods	<i>Myosotella myosotis</i>
Mollusks-Gastropods	<i>Ocenebra inornata</i>
Mollusks-Gastropods	<i>Okenia plana</i>
Mollusks-Gastropods	<i>Philine auriformis</i>
Mollusks-Gastropods	<i>Philine orientalis</i>
Mollusks-Gastropods	<i>Potamopyrgus antipodarum</i>
Mollusks-Gastropods	<i>Sakuraeolis enosimensis</i>
Mollusks-Gastropods	<i>Spurwinkia salsa</i>
Mollusks-Gastropods	<i>Tenellia adspersa</i>
Mollusks-Gastropods	<i>Tritia obsoleta</i>
Mollusks-Gastropods	<i>Urosalpinx cinerea</i>
Myxozoans	<i>Myxobolus koi</i>
Nematodes	<i>Capillaria catenata</i>
Nematodes	<i>Hysterothylacium brachyurum</i>
Nematodes	<i>Philometroides sanguineus</i>
Nemerteans	<i>Cephalothrix cf. simula</i>
Plants	<i>Agrostis gigantea</i>
Plants	<i>Alisma lanceolatum</i>
Plants	<i>Cakile edentula</i>
Plants	<i>Cakile maritima</i>
Plants	<i>Cotula coronopifolia</i>
Plants	<i>Egeria densa</i>
Plants	<i>Eichhornia crassipes</i>

Plants	<i>Iris pseudacorus</i>
Plants	<i>Juncus gerardii</i>
Plants	<i>Lepidium latifolium</i>
Plants	<i>Limosella australis</i>
Plants	<i>Lythrum salicaria</i>
Plants	<i>Myriophyllum aquaticum</i>
Plants	<i>Myriophyllum spicatum</i>
Plants	<i>Parapholis incurva</i>
Plants	<i>Polygonum patulum</i>
Plants	<i>Polypogon elongatus</i>
Plants	<i>Potamogeton crispus</i>
Plants	<i>Rorippa nasturtium-aquaticum</i>
Plants	<i>Salsola soda</i>
Plants	<i>Schinus terebinthifolius</i>
Plants	<i>Spartina alterniflora</i>
Plants	<i>Spartina anglica</i>
Plants	<i>Spartina densiflora</i>
Plants	<i>Spartina patens</i>
Plants	<i>Spergularia maritima</i>
Plants	<i>Typha angustifolia</i>
Plants	<i>Zostera japonica</i>
Platyhelminthes	<i>Alloglossidium corti</i>
Platyhelminthes	<i>Atractolytocestus huronensis</i>
Platyhelminthes	<i>Austrobilharzia variglandis</i>
Platyhelminthes	<i>Bothriocephalus cuspidatus</i>
Platyhelminthes	<i>Cercaria batillariae</i>
Platyhelminthes	<i>Corallobothrium fimbriatum</i>
Platyhelminthes	<i>Dactylogyrus extensus</i>
Platyhelminthes	<i>Gigantobilharzia</i> sp.
Platyhelminthes	<i>Himastha quissetensis</i>
Platyhelminthes	<i>Khawia japonensis</i>
Platyhelminthes	<i>Lepocreadium setiferoides</i>
Platyhelminthes	<i>Leptoplana limnoriae</i>
Platyhelminthes	<i>Ligictalurus pricei</i>
Platyhelminthes	<i>Maritrema arenaria</i>
Platyhelminthes	<i>Megathylacoides giganteum</i>
Platyhelminthes	<i>Microphallus similis</i>
Platyhelminthes	<i>Pisciamphistoma stunkardi</i>
Platyhelminthes	<i>Stephanostomum tenue</i>
Platyhelminthes	<i>Zoogonus lasius</i>
Protozoans	<i>Ancistrocoma pelseneeri</i>
Protozoans	<i>Ancistrum cyclidioides</i>
Protozoans	<i>Boveria teredinidi</i>
Protozoans	<i>Conidophrys pilisuctor</i>
Protozoans	<i>Cothurnia limnoriae</i>
Protozoans	<i>Lagenophrys cochinchensis</i>
Protozoans	<i>Lankesteria ascidiae</i>
Protozoans	<i>Lobochona prorates</i>
Protozoans	<i>Mirofolliculina limnoriae</i>
Protozoans	<i>Rhizodorus tagatzi</i>
Protozoans	<i>Sphenophrya dosinia</i>

Protozoans	<i>Trochammina hadai</i>
Reptiles-Turtles	<i>Trachemys scripta</i>
Sponges	<i>Chalinula loosanoffi</i>
Sponges	<i>Clathria prolifera</i>
Sponges	<i>Cliona</i> sp.
Sponges	<i>Halichondria bowerbanki</i>
Sponges	<i>Hymeniacidon sinapium</i>
Sponges	<i>Prosuberites</i> sp.
Tunicates	<i>Ascidia zara</i>
Tunicates	<i>Botrylloides giganteum</i>
Tunicates	<i>Botrylloides violaceus</i>
Tunicates	<i>Botryllus schlosseri</i>
Tunicates	<i>Ciona robusta</i>
Tunicates	<i>Ciona savignyi</i>
Tunicates	<i>Corella inflata</i>
Tunicates	<i>Didemnum vexillum</i>
Tunicates	<i>Diplosoma listerianum</i>
Tunicates	<i>Microcosmus squamiger</i>
Tunicates	<i>Molgula citrina</i>
Tunicates	<i>Molgula ficus</i>
Tunicates	<i>Molgula manhattensis</i>
Tunicates	<i>Perophora japonica</i>
Tunicates	<i>Polyandrocarpa zorritensis</i>
Tunicates	<i>Styela canopus</i>
Tunicates	<i>Styela clava</i>
Tunicates	<i>Styela plicata</i>
Tunicates	<i>Symplegma reptans</i>

Section III: Synthesis

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Chapter 7: Current Status of the Marine NIS Detection in California and Future Directions

Introduction

The CDFW Marine Invasive Species Program has implemented an intensive and long-term program to detect and evaluate NIS invasions in coastal marine waters throughout California, to assess the effectiveness of ballast water management (and other actions) in reducing the introduction and spread of NIS. To meet the goals of this Program, the CDFW has advanced biological surveys of marine communities for a diverse range of habitats and taxonomic groups, to both establish baseline measures of species distributions and detect new arrivals of NIS.

In 2012, CDFW launched a new, 3-part campaign of field-based surveys, representing a new initiative to evaluate the current status and trends of NIS in California. Led by SERC and MLML, this campaign focuses primarily on bays and estuaries, because these are hotspots for invasions in California and elsewhere around the globe and aims to significantly advance our current ability to detect new NIS and evaluate temporal changes in invasion dynamics in several ways. First, we use standardized (quantitative) measures of NIS distribution and abundance across multiple habitats, including benthic (hard substrate and soft-sediment) and planktonic assemblages, to allow formal (statistical) comparisons across space or time. Second, we have implemented repeated measures in San Francisco Bay, serving as a sentinel site to both provide a robust baseline and evaluate changes in species detection (*i.e.*, new invasions) across years. Third, we are combining both morphological and genetic analyses to cross-validate NIS identifications and improve methods for detection, increasing sensitivity and cost-efficiency.

This is the second report on progress and results for the current CDFW initiative. Here we summarize key findings presented in Chapters 1-6, combining information from the morphological and molecular analyses. We also highlight some initial comparisons across habitats and methods, discussing the expanded set of future comparisons planned for our next (third) report in this 3-part campaign. In addition, we provide conclusions and recommendations that stem from the results to date.

State of NIS in California Coastal Waters: Morphological Analyses

Our surveys detected surprisingly few new NIS records across the four bays based on morphological analysis. For the hard substrate habitats, only one NIS, the bryozoan *Cradosrupocellaria bertholletii* found in Humboldt Bay, was considered new to the state (see Chapter 2), and we are presently examining genetic data and the biogeography of this species to confirm this status. In addition, we detected 8 new NIS records for San Francisco Bay, representing probable coastwise spread from elsewhere in the state (where the species are known to occur). For three tunicate species, this spread was associated with unusually warm temperatures in 2015, possibly facilitating colonization from warmer waters in southern California, where the species occur. The other new records were polychaetes, which may either

be recent arrivals or simply overlooked to date, since polychaete diversity has not received as much attention as other taxonomic groups.

For soft-sediment surveys, morphological analysis revealed two new NIS records for Humboldt Bay, including the Asian cephalaspidean gastropods *Philine auriformis* and *P. orientalis*, both of which were known previously from the U.S. Pacific coast (Chapter 3). No new NIS were detected by morphological analysis of soft-sediment invertebrates for San Francisco Bay (Chapter 3) or zooplankton across the four different bays (Chapter 4).

Species accumulation curves indicated that our surveys detected a high percentage (> 90%) of the total pool of NIS estimated to be present in each bay per year. Moreover, the repeated surveys for San Francisco Bay (a sentinel site), across multiple years with different environmental (thermal and salinity) conditions, serve to measure temporal change in NIS composition, providing a robust baseline to detect and evaluate new invasions. Thus, despite the performance of spatially extensive surveys (4 bays) and temporal replication (3 years in San Francisco Bay), across several habitats, it is noteworthy that only one new NIS was detected for California in the current study, using morphological analyses.

Use of DNA Barcoding in California Surveys

We used DNA barcoding to confirm the morphological identification of NIS and to build a barcode library for multiple applications. As described in Chapter 5, voucher specimens of putative species (morphospecies) were collected during morphological analysis and processed to obtain DNA sequences. In general, the DNA barcode confirmed the presence of the NIS. When morphological identification yielded a species-level identification, these were concordant with the genetic results 82% of the time. For the other 18% where the genetic and morphological data were discordant at the species level, this was due to multiple causes, including (a) morphological misidentification, (b) sequences from one or more non-target organisms associated with the voucher specimen, (c) laboratory contamination due to handling and workflow by SERC or MLML teams, or true environmental DNA (eDNA). Extra DNA sequences were commonly observed and indicate that non-target DNA molecules had entered into the workflow for each vial. We evaluated laboratory contamination with no-tissue and no-template controls in extraction and PCR stages and found contamination at these stages to be rare. Therefore, we believe that the majority of excess sequences result from DNA introduced prior to DNA extraction steps.

The cases of discordance between morphological and genetic results have helped improve our sample processing and workflow, and also underscore some of the current limitations with each morphological and genetic component. For some taxonomic groups (*e.g.*, tunicates and bryozoans), species assignment by morphological analysis performed well, when large and high quality specimens are available. However, some specimens in these groups are low quality, in that they are small or damaged and lack key characters needed for identification. Moreover, other taxonomic groups are notoriously difficult to identify with confidence, even for skilled and

knowledgeable researchers, including sponges and hydroids, limiting the percentage of vouchers that are given species-level identifications. For these latter groups, even genus and higher-level identifications can be challenging. Our workflow now reflects these limitations, using morphological analyses to measure abundance and community composition at the species level for groups with high fidelity, and reviewing possible morphological traits to improve fidelity for additional groups. Where species-level identifications are currently not readily achieved (at the current time) by morphological analysis, occurrences are recorded at coarser taxonomic level, such as genus or family. In both cases, vouchers are collected for genetic analysis to obtain species-level identifications.

The MLML DNA barcode database version used herein contains 269 species and is continuing to grow, as we add additional voucher analyses. However, a fully genetic approach to voucher analysis is premature, at least by the next-generation sequencing approach used in this study. In our current study, genetic identification was hampered by the finding of multiple sequences for most specimens. Therefore, the prior knowledge of the type of organism was used to select the most plausible genetic result; without this prior knowledge, genetic identification by the methods used here would have been ineffective for specimens that yielded multiple sequences. Full morphological identification, however, would not be necessary in most cases, as the extra sequences are usually implausible for the type of organism. In other words, a crude identification would generally be sufficient to pinpoint a correct DNA sequence. With this approach, less expert technicians would be sufficient to process vouchers for sequencing.

Alternatively, vouchers could be sequenced solely with the Sanger method, for which multiple sequences per specimen are impossible: with that method, mixed templates produce unreadable results. The downside to Sanger sequencing, since mixed templates appear to be common, would be a higher rate of sequence failure, unless the lower sensitivity of Sanger negates superimposed sequences.

At the present time, we have maintained both morphological and genetic analysis of vouchers from field surveys to multiple ends. First, the paired samples are required to build a comprehensive NIS barcode library, to both confirm identifications and advance metagenetic approaches to detect species and evaluate community composition. Second, morphological analyses provide novel information on NIS species abundance and effects on community structure, which complement genetic data on species detection and occurrence. For this reason, the ongoing research (Part III of the current study) has established San Francisco Bay and Los Angeles/Long Beach as sentinel sites, to sustain both morphological and genetic measures to be repeated across years, combined with metagenetic analyses (see below) of additional bays throughout the state that focus on genetic detection of NIS.

Use of Metagenetics in California Surveys

We implemented metagenetic analyses in the current 3-part study to detect and evaluate the occurrence of NIS, focusing on both plankton and hard substrate communities. The current

report includes analysis of plankton communities (Chapter 6), and the metagenetic analysis of hard substrate panel communities is a focus of the current work, to be included in the next (Part 3) report.

We collected 593 plankton samples by pump and net tow from 10 bays spanning the coast of California from 2013-2015. In total, we found 3,719 operational taxonomic units (OTU) by clustering sequences at the 95% level. OTUs are roughly equivalent to biological species, although variation in molecular evolutionary rates may cause some species to be lumped or split. Too, some nominal species may actually be two or more cryptic species that are separated at the 95% threshold yet bear the same name. Thus, there is not perfect correspondence between OTUs and biological species. Community analyses showed strong differences in plankton and NIS communities across sites, but not strictly corresponding to geographic separation. Surface temperature and salinity were important drivers of community composition. Sequences were identified by BLAST using private and public databases. BLAST searches may produce more than one match that exceeds a 95% similarity threshold due to variation in molecular evolutionary rate, as noted above, or database errors. Because specimens are not linked to sequences in metagenetic analyses, we included all matches exceeding 95% as possibly present in our samples

Using this approach for plankton metagenetics, NIS were detected in every bay, and we found 69 of 252 marine NIS (excluding vertebrates and vascular plants) recognized in California. We also found sequences matching additional NIS not presently verified in California. We suggest those additional species be considered possible but not verified new NIS in California and be targets for discovery in bioblitz and other benthic surveys, to confirm current status.

It is also useful to recognize that this analysis provides a minimum estimate of NIS detection performance. More than 69 NIS may be detected eventually in these sequence data, since we still lack DNA barcodes for some known NIS. Thus, as our barcode library continues to grow, we predict additional NIS will be confirmed present in these samples.

Cross-Method Comparison for California Survey Data

We compared NIS found in plankton to those found on settlement plates in each bay (except San Pedro and Newport bays, for which panel data were not yet available). On average, we found 32.3 ± 6.6 (standard deviation) NIS per bay in plankton, 32.4 ± 7.7 from morphological analysis of settlement panels, and 14.4 ± 4.3 NIS found in both sample types (Table 7.1).

The plankton samples provide an integrative approach to detect species across multiple habitat types, since these include both waterborne stages of benthic (hard substrate and soft-sediment) species and holoplankton. Among plankton samples were infaunal and planktonic NIS, whereas plate samples do not include most infaunal and plankton species.

Our data indicate that high throughput sequencing was effective at detecting many NIS and finding community variation in Californian estuarine waters. However, the general episodic nature of larval production suggests a need for higher frequency of plankton sampling or supplementation with benthic surveys to detect additional NIS present. We also expect that some taxa will not be detected in plankton assemblages, due to (a) reproductive mode (including direct development and lecithotrophic larvae, which spend little-to-no time in the water column), (b) frequency of reproduction, and (c) larval behavior.

Next Steps

We will expand this cross-method comparison in the current work and next report, Part 3 of the current initiative, providing a more synthetic view of detection performance and gaps across measures for the 10 focal bays studied. This analysis will specifically examine the frequency of detection of NIS by habitat (plankton, hard substrate, and soft-sediment) and method (morphological and metagenetic analysis). Such analysis is not yet possible, until the metagenetic analyses of panel samples (now underway) are completed in Part 3 of the study.

Using the expanded cross-system comparison and synthesis, we will provide a comprehensive evaluation of status and trends of NIS in California waters and make recommendations to further optimize the CDFW program for detection and management of NIS. Based on our results to date from this research program, some of these recommendations are already implemented in the current (Part 3) study, including:

- Reduced individual voucher sequencing and increased metagenetic analysis of settling panels.
- Focused sequencing on taxonomic groups that have proven most difficult for morphological analysis and groups needed to fill database gaps, using bioblitz activities to augment survey collections.
- Use of Sanger sequencing for a lower volume of vouchers that serves to (a) reduce the level of technical difficulty in sample preparation and (b) minimize sequences from non-target taxa.
- Use of Illumina sequencing to replace Ion Torrent sequencing for metagenetics, due to higher sequence yield, simpler sample preparation, and lower per run cost. In addition, Life Technologies have phased out the current Ion Torrent PGM instrument, reagent and software product development has ceased, and legacy support will expire.
- Use of paired head-to-head comparisons of plankton and settlement panel metagenetic, to those from morphological analyses in sentinel bays to evaluate the performance of detection methods for individual NIS, habitats, and traits. This information can further refine relative sampling effort for each method in ongoing surveys, to meet CDFW program objectives.

Table 7.1. Number of NIS found in plankton and settlement panels in each bay where plankton was taken. NIS in plankton were identified by analysis of DNA sequences from bulk samples as described in Chapter 6. NIS on panels were identified by morphological analysis as described in Chapter 2. Because plankton samples were not examined morphologically, and vouchers from panels were not necessarily sequenced, these data assume that taxonomic names are used similarly. Additionally, some species found by molecular analysis are unlikely to be seen on panels (*i.e.*, holoplankton and infaunal species), and some species found on plates are unlikely to be found in plankton (species lacking planktonic larvae). Lastly, unresolved names (order, family, or genus only) were included in morphological species lists but not in molecular analysis.

	Found on Both	Found in Plankton Only	Found on Panels Only	Total in Plankton	Total on Panels	Total in Bay
San Francisco 2013	20	19	11	39	31	50
San Francisco 2014	17	21	22	38	39	60
San Francisco 2015	18	24	30	42	48	72
Morro Bay 2013	10	13	12	23	22	35
Mission Bay 2013	18	16	19	34	37	53
San Diego Bay 2013	19	17	19	36	38	55
Bodega-Tomales Bays 2014	12	9	19	21	31	40
Humboldt Bay 2015	12	17	18	29	30	47
Port Hueneme 2015	9	25	13	34	22	47
Marina del Rey 2015	10	19	17	29	27	46
Newport Bay 2015	10	20	21	30	31	51
Average	14.1	18.2	18.3	32.3	32.4	50.5
Standard Deviation	4.3	4.6	5.3	6.6	7.7	9.9

Selected Glossary of Barcoding and Metabarcoding Terminology

Amplicon – a product of the polymerase chain reaction (PCR), which amplifies a targeted region from a DNA template (see template). Loosely synonymous with “PCR product” though “amplicon” may refer to a single molecule, while “PCR product” usually refers to pool of amplified DNA.

Assembly – the alignment of multiple reads of the same or overlapping DNA sequence to produce a single consensus sequence called a “contig” (contiguous sequence).

Binning – grouping DNA sequences based on some criterion, for example a threshold of similarity.

BLAST – Basic Local Alignment Search Tool; a method to find similar sequences (subjects) in a database to a submitted sequence (query).

Chimera – two sequences merged into one during the PCR process.

Contig – see Assembly

Demultiplexing – in the context of metabarcoding, sorting a list of DNA sequences into groups corresponding to the biological sample of origin (for example, a plankton tow, or a voucher specimen)

Denosing – removal of sequencing errors that are predicted by statistical inference to occur due to known artifacts in the NGS platform.

Diversity – in the context of biological diversity, usually refers to both the number of species (richness) and their relative abundances (evenness) in a given area

E score – “The Expect value (E) is a parameter that describes the number of hits one can “expect” to see by chance when searching a database of a particular size.” (blast.ncbi.nlm.nih.gov). A barcode that matches a Genbank record will have an E score $\lll 0.01$.

End repair – an enzymatic process to remove overhangs in DNA fragments, used to facilitate blunt-end ligation of DNA fragments.

Evenness – how evenly abundant (*i.e.*, relative abundance) species or OTUs are in a given area of interest

FASTA – a standardized file format for DNA sequences readable by many software packages.

FASTQ – a file format containing sequence and quality scores for each base pair.

Library – in the context of metabarcoding, a PCR product to which relevant DNA adaptors have been ligated.

Locus (plural, loci) – a physical region of the genome, sometime used synonymously with “gene” (a coding region), or gene fragment, but not necessarily a coding region.

Ligation – enzymatic joining of two DNA fragments.

Non-metric multidimensional scaling (nMDS) – a method of visualizing (dis)similarities between different sampling units. It is an ordination technique that attempts to represent the multi-dimensional nature of similarities between samples (in this case, the richness and relative abundance of species or OTUs on panels, grabs, or tows) in two dimensions. The result is a visual representation of a matrix of pairwise similarity scores for all samples of interest.

Next generation sequencing (NGS) – a variety of methods for DNA sequencing that typically allow massively parallel (*i.e.*, simultaneous) sequencing of single molecule DNA templates. The term is falling out of usage because sequencing technology continues to advance, therefore “next” becomes a somewhat meaningless term. Sequencing methods are now often referred to the instrument and chemistry involved (eg, Illumina MiSeq).

OTU (operational taxonomic unit) – a group of specimens or, in the context of molecular systematics, DNA sequences thought to comprise a evolutionary lineage such as a species. In the context of DNA barcoding, a criterion is used to identify such groups, such as threshold of sequence similarity.

Phred score – a measure of quality of a base call in DNA sequencing, expressed as a Q, where Q is a logarithmic value. For example, Q20 is 1/100 probability of incorrect base call (or 99% base call accuracy).

Polymerase Chain Reaction (PCR) – an *in vitro*, enzymatic reaction to make sufficient copies of a targeted region on a DNA template for DNA sequencing or other procedures.

Primer – a short single stranded DNA molecule used to initiate (“prime”) the polymerase chain reaction. Primer DNA sequences are designed to bind to the DNA to be copied (see Template) at a specific nucleotide sequence; this allows specificity of DNA amplification.

Rarefaction (rarefy) – resampling of individual data points in a sample (in this case, DNA reads or species) to standardize the number of data points per sample. This is meant to account for differences in sampling effort (in this case, variation in sequencing depth, or different numbers of panels or samples per bay due to panel loss)

Richness – number of morphologically distinct species or OTUs found in a given sampling area

Sanger sequencing – *in vitro*, enzymatic reaction to determine the order of nucleotides on a single DNA template, in this case a pool of identical DNA molecules (see Template); Next-generation sequencing, in contrast, achieves this for many (millions) templates that are not necessarily

similar by tracking. Sanger sequencing is named for Frederick Sanger, its inventor, and is more technically called dideoxynucleotide chain termination reaction.

Species accumulation curve – a graph of the cumulative number of species found in a given area of interest as a function of the number of samples (panels, grabs, tows) examined

Template – a DNA molecule or pool of molecules that is acted on by enzymes; in the context of PCR, the DNA to be copied; in the context of DNA sequencing, the DNA to be sequenced.