Spatial and temporal analysis of marine invasions in California:

Morphological and molecular comparisons across habitats

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Chapter 1: Introduction

Overview

The Smithsonian Environmental Research Center (SERC) and Moss Landing Marine Laboratories (MLML) have undertaken 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 SB497.

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 controls.
4. Use other appropriate, existing data.
5. From these data, the following issues may be addressed: a) Determination of alternative discharge zones; b) identification of sensitive areas to avoid for uptake or discharge of ballast water; c) evaluation of long term effectiveness of discharge control measures; d) determination of risk zones where ballast water discharge should be prohibited; e) determine the rate and risks of establishment of NIS in California.

SERC and MLML have designed and implemented analyses to explicitly (a) assist CDFW in meeting requirements of SB497 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 was designed *a priori* to explicitly make formal quantitative (statistical) estimates and comparisons. 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, and also lays the 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 (species richness). We use a question-driven approach to inform and refine the sampling effort. We seek to evaluate (test) the (a) capacity and sensitivity of detection for NIS, (b) differences in species richness across habitats, geographic regions, and taxonomic groups, and (c) changes in invasion dynamics (NIS detection rate) over time associated with different transfer mechanisms (vectors) and management practices. More broadly, our approach is designed explicitly to establish a robust, quantitative baseline and implement a time-series of repeated measures, which serve to assess status and trends 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 an initial phase, we designed a 4-6 year 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). Of those NIS present on the outer coast of California (including those in recent CDFG 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.
- 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) will assess the extent to which NIS are spreading to outer coastal regions that are adjacent to bays. The current (initial) phase lays the groundwork and establishes the baseline to meet these goals.

Approach

In this initial phase, we are intensively sampling the invertebrate communities in 10 different estuaries in California. Within each estuary, we will sample 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 estuaries, (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, infauna, 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. 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, weather conditions.

The biota collected in surveys are analyzed to identify taxonomic composition, using morphological analyses and genetic analyses. 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 "field vouchers" are identified 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 collected from all habitats will be sequenced for mitochondrial Cytochrome c oxidase subunit I, 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 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, shift in vector operation, 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 analysis 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 of ballast water control methods by establishment of baseline for invasion of the holoplankton, and monitor plankton 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 public accessible database utilizing past and concurrent CDFG data, and other related data, utilizing SERC's National Exotic Marine and Estuarine Species Information System (NEMESIS) framework (Fofonoff *et al*. 2003).

Structure of this Report

In this report, we present results from the surveys and analyses for five estuaries in California, or approximately half of the initial phase of the Program. These estuaries include San Diego Bay, Mission Bay, Morro Bay, San Francisco Bay, and Bodega/Tomales Bay. Similar work is being completed for the remaining five estuaries now, under a separate project with CDFW. We expect to provide an overall synthesis of the data for all 10 estuaries after completing the latter. Thus, the current report represents the mid-way point in the initial phase.

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

- Part 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), zooplankton (Chapter 4), and outer coast (Chapter 5). For Chapters 2-4, we include data from each of the five bays, allowing comparisons among bays within community type.

- Part II provides the genetic analyses and results for benthic invertebrates and zooplankton conducted by MLML. These are organized into two chapters. Chapter 6 presents results from genetic analyses of voucher specimens collected in the benthic habitat surveys conducted by SERC. This chapter includes (a) direct comparison of morphological and genetic identification for the individual benthic invertebrate vouchers and (b) some initial metagenetic analyses of community samples. Chapter 7 describes results from metagenetic analyses for whole zooplankton community samples collected by SERC.

- Part III provides a brief conclusion, including 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 are being made accessible through CalNEMO, a California portal of NEMESIS. 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 x site for this specific study (report) will also be made available through NEMESIS.

Part I: Morphological Detection and Analysis of NIS by Habitat

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Chapter 2: Hard Substrate Communities

To detect the presence of non-native invertebrate taxa in hard substrate communities, we sampled five estuaries: San Diego Bay, Mission Bay, Morro Bay, San Francisco Bay, and Bodega/Tomales 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 at each site. In general, our goal was to analyze 50 plates per bay (10 sites x 5 plates). In Morro Bay, 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 here.

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.

Across the five estuaries, we collected and analyzed samples of the hard substrate invertebrate community from high salinity waters in one year each, on a total of approximately 250 plates (5 estuaries x 10 sites x 5 replicates). In addition, we sampled the high salinity portion of San Francisco Bay in identical fashion (10 sites x 5 replicates x 2 years = 100 additional plates) in two additional years, including one survey year (2011) before the current study. Finally, we also collected and analyzed another 25 plates (5 sites x 5 replicates) from low salinity waters in the San Francisco Bay Delta, as this is the only estuary in our study with a substantial low salinity area. Thus, our analyses include data from 375 plates across these estuaries from 2011-2013.

Results

A. Spatial Variation in High Salinity Habitats of Five Estuaries.

Our analyses indicate that our sampling program performed well at detecting and characterizing the NIS in the hard substrate community in the 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 2-2 (R Core Team 2015; Oksanen 2015). Species accumulation curves show rarefaction curves of species richness and the associated standard error. Species richness estimators reported are Chao, Jack1, Jack 2, and Boot values along with standard errors (Canning-Clode *et al*. 2008).

Total NIS Richness Among Estuaries.

When we combine all 5 estuaries, species accumulation for NIS approaches an asymptote (Figure 2.1). We observed (detected) 27 NIS for mobile invertebrates and 47 NIS for sessile invertebrates. The total estimated richness for these two groups was approximately 30 and 50 NIS, respectively (Table 2.1), indicating that we detected 90-94% of the estimated total pool of NIS. In contrast, the species accumulation curves for native and other species are further from their asymptotes, suggesting we have detected 70-80% of the total species pool (Table 2.1).

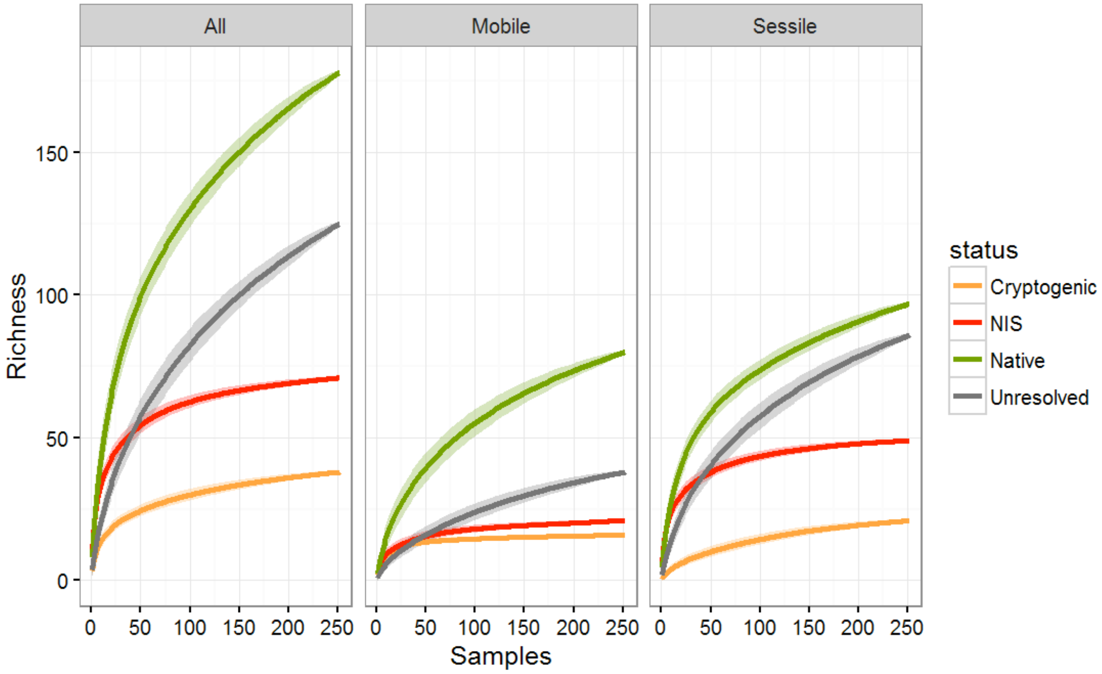


Figure 2.1. Species accumulation curves by invasion status for high salinity across all five estuaries (combined). Status is designated based on literature and SERC NEMESIS database. Samples (x-axis) represents settlement plates placed at 10 sites (5 plates per site) in each of 5 bays along the California coast (n = 250 plates total). NIS asymptotes agree strongly with species richness estimators (see Table 2.1). Bodega and San Francisco were sampled in 2012 while other bays were sampled in 2013.

Table 2.1. Species richness estimators by invasion status for high salinity sites across all five estuaries (combined).

Data	Status	Species	Chao	Chao SE	Jack1	Jack1 SE	Jack2	Boot	Boot SE	n
All	Cryptogenic	41	49.08482	8.984005	47.96429	2.988095	52.94041	43.0376	1.636203	252
	Introduced	74	78.3746	5.900805	79.96825	2.817203	82.96421	75.86404	1.634427	252
	Native	181	227.40714	17.323098	232.78571	9.025584	256.71381	203.56022	5.190632	252
	Unresolved	125	170.83118	18.493509	169.81349	8.034693	193.71392	144.1365	4.737243	252
Mobile	Cryptogenic	23	29.10069	6.055825	29.97222	2.635252	32.96422	26.28443	1.488137	252
	Introduced	51	52.59365	2.154372	54.98413	1.992063	54.01183	53.35165	1.291908	252
	Native	99	129.25446	15.560752	125.89286	5.547095	140.82124	110.91177	3.012578	252
	Unresolved	85	119.18523	16.384438	115.87698	6.380813	132.7974	98.81626	3.691286	252
Sessile	Cryptogenic	18	18.99603	2.291612	19.99206	1.408602	21.97619	18.75317	0.6940581	252
	Introduced	23	28.97619	7.238567	26.98413	1.992063	30.95238	24.51239	0.9924859	252
	Native	82	102.16964	10.150826	108.89286	5.895286	117.89257	94.64845	3.669933	252
	Unresolved	40	54.16578	9.307511	55.93651	4.22674	62.91652	47.32024	2.4185247	252

<u>Total NIS Richness Within Individual Estuaries</u>.

A similar pattern exists within the five 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 14-21 NIS per estuary, and we detected 23-35 NIS per estuary for sessile invertebrates (Table 2.2). It is also noteworthy that for sessile taxa in San Francisco Bay and San Diego Bay, our observed and estimated NIS richness were nearly identical.

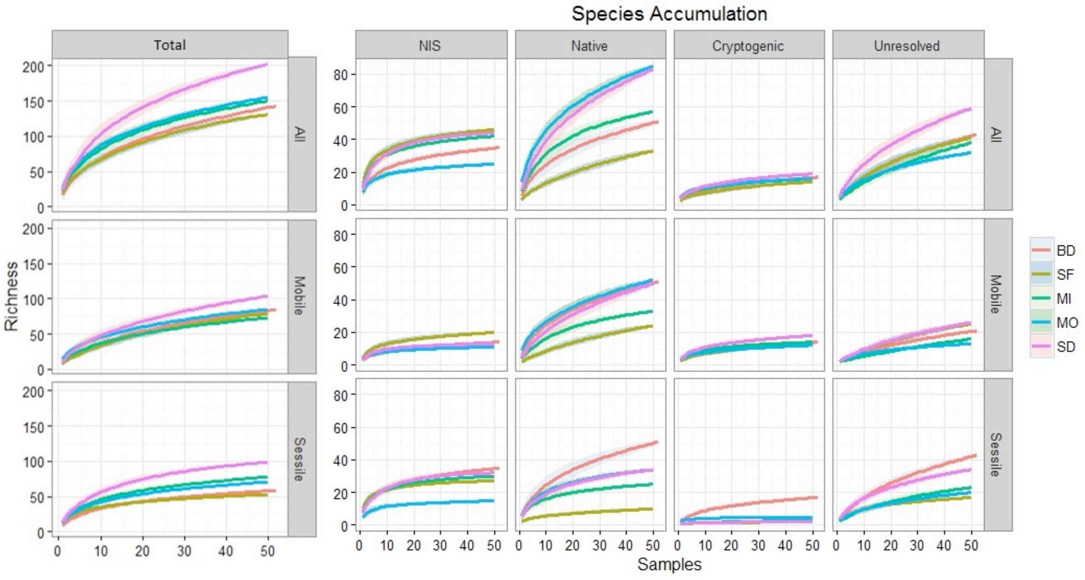


Figure 2.2. Species Accumulation curves separated by invasion status for high salinity sites in of each bay sampled. Status is designated based on literature and SERC NEMESIS database. Samples (x-axis) represents settlement plates placed at 10 sites (5 plates per site) in each of 5 bays along the California coast (n = 50 plates per bay; n = 250 plates total). NIS asymptotes agree strongly with species richness estimators (Table 2.2).

Table 2.2. Species richness estimators by invasion status for high salinity sites within five estuaries (separately).

	Bay	Status	Species	Chao	Chao SE	Jack1	Jack1 SE	Jack2	Boot	Boot SE	n
All	Bodega Bay	Cryptogenic	44	83.55769	24.184099	65.57692	5.56504	81.07466	53.07318	2.797642	52
		Native	52	111.33654	39.745348	73.57692	7.382963	90.96003	60.85058	3.934571	52
		NIS	18	23.88462	6.365007	23.88462	2.780825	26.82579	20.61259	1.469534	52
		Unresolved	36	55.86058	19.808827	44.82692	3.258637	51.5954	39.68447	1.705741	52
	Mission Bay	Cryptogenic	39	72.88	20.163333	60.56	5.734876	75.09714	48.04425	3.224473	50
		Native	58	73.73444	10.07447	74.66	5.481496	82.51633	65.63954	3.35383	50
		NIS	17	20.92	5.193226	20.92	1.96	22.87918	18.82426	1.157291	50
		Unresolved	43	47.41	4.715657	48.88	2.4005	50.87837	45.88955	1.520285	50
	Morro Bay	Cryptogenic	33	53.7025	16.116011	45.74	3.53344	54.45837	38.51167	1.967672	50
		Native	86	111.75562	12.598095	114.42	7.302849	127.21347	98.89875	3.885631	50
		NIS	17	18.47	2.251999	19.94	1.69741	19.99878	18.52803	1.003271	50
		Unresolved	26	35.8	10.14144	30.9	2.600385	35.7	27.91807	1.296864	50
	San Diego Bay	Cryptogenic	57	87.11273	15.876114	82.48	7.505358	97.09551	68.13575	4.043211	50
		Native	84	147.504	30.246719	119.28	9.561088	144.43592	98.8506	5.107417	50
		NIS	20	24.08333	4.793756	24.9	2.191347	26.87878	22.3177	1.289217	50
		Unresolved	45	46.568	2.125641	48.92	1.96	48.05796	47.43207	1.470393	50
	San Francisco Bay	Cryptogenic	42	59.64	11.016497	59.64	5.389545	68.45633	49.89649	2.975891	50
		Native	34	54.23	13.22836	50.66	5.481496	60.39714	41.21142	2.667455	50
		NIS	15	16.568	2.125641	18.92	1.96	18.05796	17.12157	1.43045	50
		Unresolved	47	53.0025	5.966454	53.86	3.26233	56.81837	50.24014	1.763024	50
Mobile	Bodega Bay	Cryptogenic	11	13.20673	3.336831	13.94231	1.698742	14.94155	12.37216	0.9469314	52
		Native	52	111.33654	39.745348	73.57692	7.382963	90.96003	60.85058	3.9345709	52
		NIS	11	13.94231	4.425042	13.94231	1.698742	16.82692	12.12936	0.8512893	52
		Unresolved	14	21.84615	11.444231	17.92308	2.410222	20.82655	15.68527	1.2455407	52
	Mission Bay	Cryptogenic	21	53.01333	25.815624	34.72	4.167205	45.33878	26.49319	2.0906785	50
		Native	44	123.05333	57.43219	65.56	5.903287	83.85878	52.73236	3.0614425	50
		NIS	14	17.92	5.193226	17.92	1.96	19.87918	15.73429	1.1357437	50
		Unresolved	32	32.735	1.243116	34.94	1.69741	32.17755	34.04445	1.3060196	50
	Morro Bay	Cryptogenic	20	33.23	12.221567	28.82	2.94	34.63878	23.69979	1.4777854	50
		Native	55	157.08333	72.409346	79.5	5.814637	100.67878	64.79253	2.7291043	50
		NIS	13	14.96	3.672166	14.96	1.385929	15.93959	13.87888	0.7617729	50
		Unresolved	23	24.47	2.251999	25.94	1.69741	25.99878	24.56768	1.0987379	50
	San Diego Bay	Cryptogenic	35	66.752	21.38766	52.64	4.813232	65.21796	42.42215	2.5502692	50
		Native	59	98.24083	19.137672	89.38	7.818721	107.8551	72.06936	4.3154912	50
		NIS	17	20.0625	3.593098	21.9	2.191347	22.93837	19.36338	1.2844203	50
		Unresolved	37	43.272	5.814515	44.84	3.10535	47.81796	40.88171	1.8991618	50
	San Francisco Bay	Cryptogenic	24	37.80167	10.205901	36.74	3.800684	43.57755	29.57947	2.0424773	50
		Native	19	35.33333	14.559202	28.8	3.099032	35.57878	23.09273	1.5753737	50
		NIS	10	22.25	16.803645	14.9	3.268333	18.75959	11.95589	1.6161341	50
		Unresolved	36	41.88	6.360377	41.88	2.4005	44.81878	38.75404	1.4509466	50
Sessile	Bodega Bay	Cryptogenic	35	79.25721	30.761835	53.63462	5.111009	68.13311	42.70101	2.622098	52
		Native	52	111.33654	39.745348	73.57692	7.382963	90.96003	60.85058	3.934571	52
		NIS	9	10.47115	2.253511	11.94231	1.698742	11.99887	10.48323	1.017916	52
		Unresolved	24	36.25962	16.816549	28.90385	2.193067	32.76885	25.99921	1.146249	52
	Mission Bay	Cryptogenic	29	49.90667	14.248753	44.68	4.609382	54.39755	35.65726	2.426856	50
		Native	49	69.23	13.22836	65.66	5.657455	75.39714	56.47596	3.632127	50
		NIS	15	21.125	7.409137	19.9	2.191347	22.81918	17.17133	1.254978	50
		Unresolved	42	46.00167	3.977566	48.86	2.592836	49.93755	45.55598	1.712543	50
	Morro Bay	Cryptogenic	29	34.44444	4.538148	38.8	3.099032	39.93633	33.9169	2.044197	50
		Native	76	110.92364	17.889635	103.44	6.524661	119.97551	87.98065	3.412707	50
		NIS	15	22.84	11.435524	18.92	1.96	21.81959	16.63473	1.013171	50
		Unresolved	24	27.0625	3.593098	28.9	2.191347	29.93837	26.35679	1.249388	50
	San Diego Bay	Cryptogenic	47	106.535	34.283666	73.46	7.569069	93.73755	57.69	3.851881	50
		Native	68	104.22231	17.505632	98.38	7.818721	115.91469	81.11023	4.422313	50
		NIS	15	17.205	3.334473	17.94	1.69741	18.93918	16.44982	1.12227	50
		Unresolved	42	46.00167	3.977566	48.86	2.592836	49.93755	45.58766	1.735973	50
	San Francisco Bay	Cryptogenic	35	47.25	8.303028	49.7	4.503998	55.63633	41.79087	2.560638	50
		Native	28	98.805	61.295108	44.66	5.481496	59.09918	34.58855	2.593064	50
		NIS	14	22.82	9.978868	19.88	2.4005	23.75918	16.49017	1.229295	50
		Unresolved	45	48.43	3.439985	51.86	2.946659	51.99714	48.5984	1.659678	50

Percent Contribution of NIS to Total Species Richness Per Estuary.

NIS contributed 20-50% of total observed species richness per estuary for sessile invertebrates. The percent contribution was highest in San Francisco Bay and lowest in Morro Bay (Figure 2.3). The same rank order in percent contribution by NIS is observed for mobile invertebrates and all species combined, ranging from 12-27% and 15-35% of species richness, respectively. The relative dominance of NIS in San Francisco Bay is driven by both relatively high NIS richness and low native species richness (see red and green respectively in Figure 2.3).

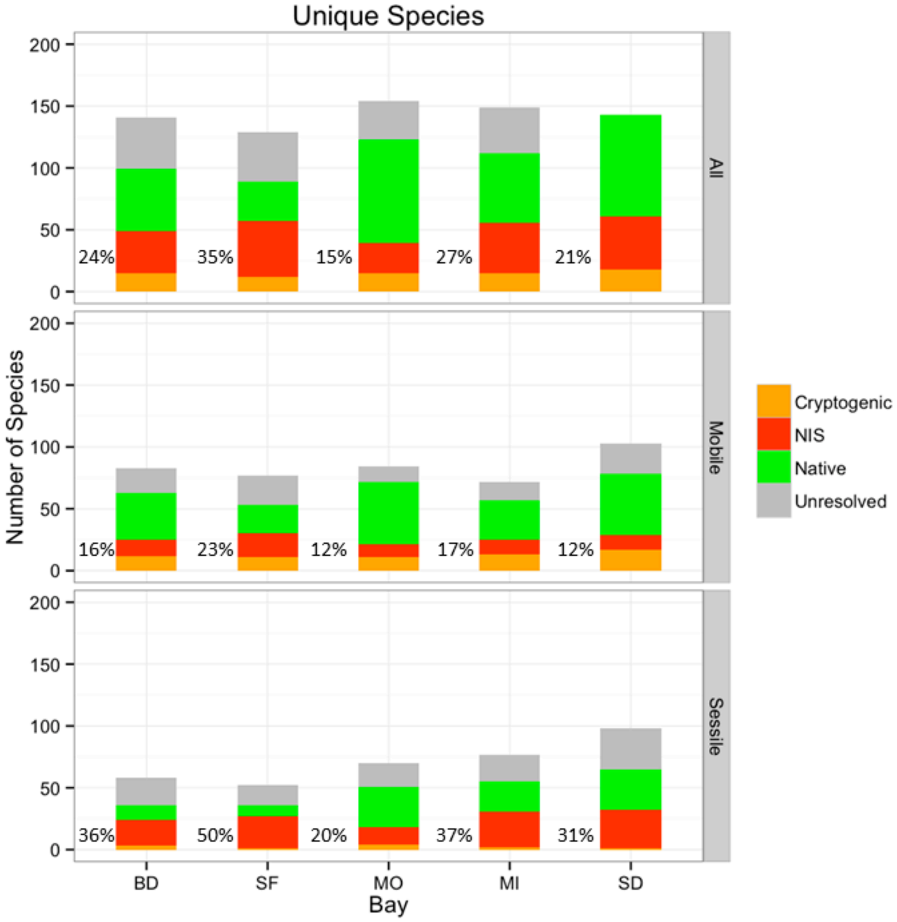


Figure 2.3. Number of unique species detected in each bay as a function of status. Status is designated based on literature and SERC NEMESIS database. The percent of all species in each bay that are NIS is listed to the left of each bar for that bay, and is calculated as the percentage of unique NIS out of the total unique species in that bay.

NIS Richness Per Plate Within Bays.

The observed mean NIS richness varies 2 to 3 fold among sites across the 5 different estuaries when combining mobile and sessile taxa (Figure 2.4). This is driven largely by variation in sessile species, and the sites in San Francisco Bay are at the highest end of the range. While this figure emphasizes differences among sites, the elevated NIS richness per plate in San Francisco Bay is also observed at the bay level when comparing mean NIS richness for all plates within each of the five bays (Figure 2.5). Thus, overall, San Francisco Bay has the highest per plate NIS richness within individual sites as well as for the entire bay. This pattern is driven by higher number of sessile invertebrates than the other four bays.

An additional analysis of total NIS richness per site (instead of plate) is also presented in Appendix 2.3.

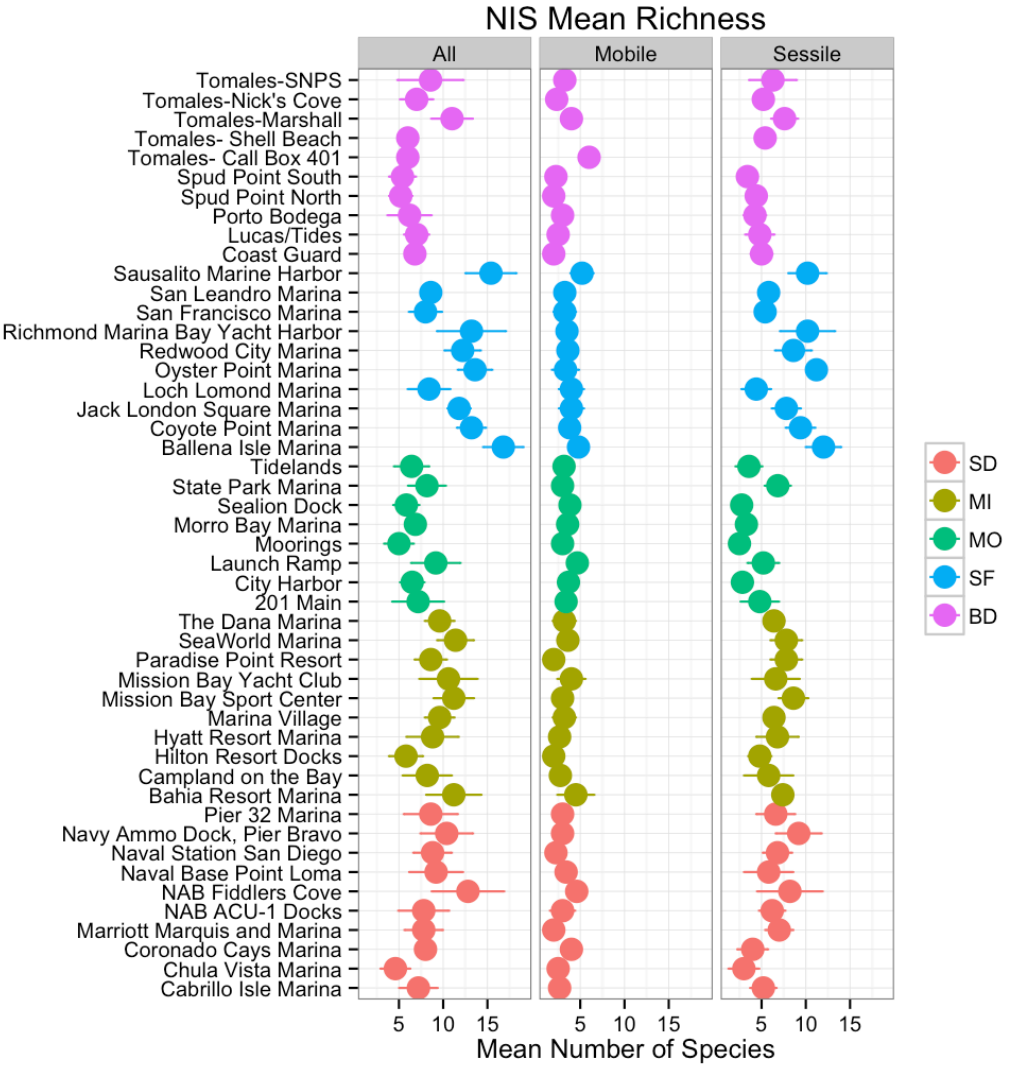


Figure 2.4. Mean number of NIS detected per plate averaged within sites. Error bars equal ±1SD.

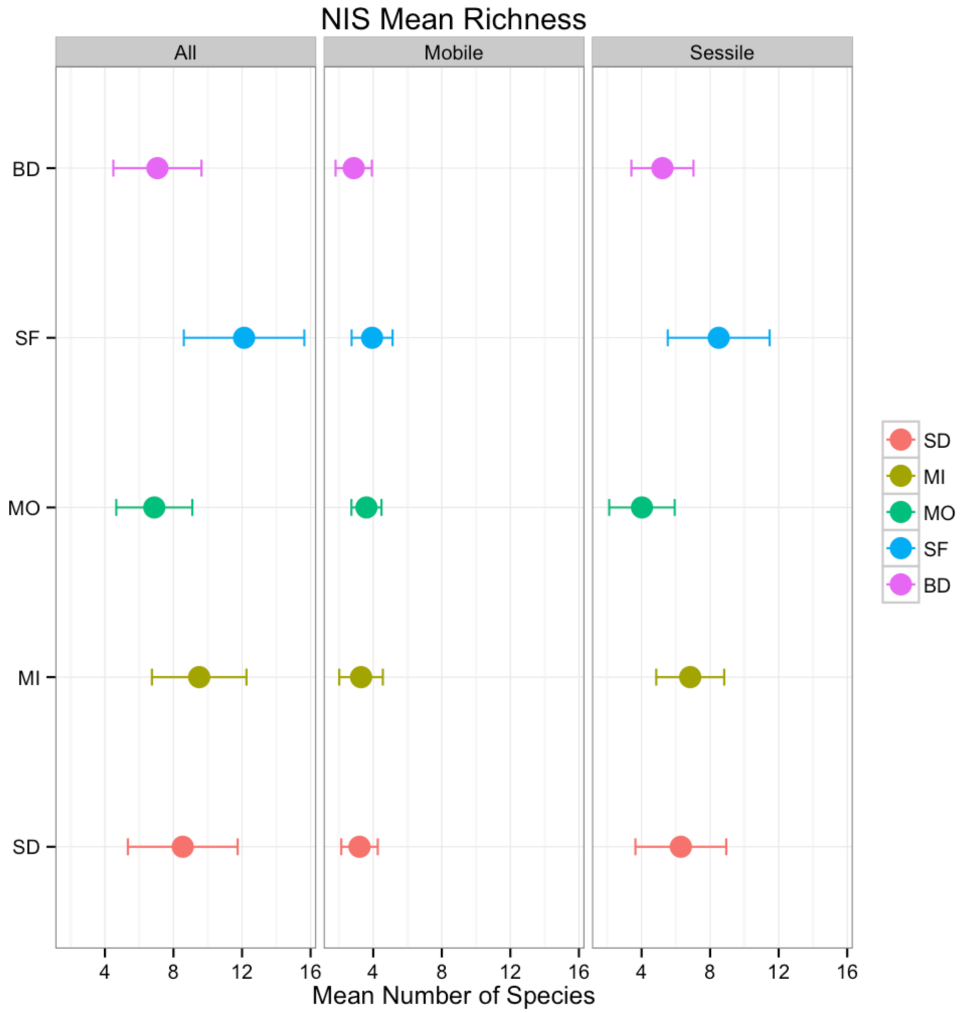


Figure 2.5. Mean number of NIS detected per plate averaged within bays. Error bars equal ±1SD.

Relative Abundance of 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 a mean of >50% of the points sampled per plate in three of the estuaries (Figure 2.6). Although San Francisco Bay had a relatively high NIS richness per plate (as discussed above), this did not translate into a higher relative abundance of NIS than in some of the other estuaries. In sharp contrast, however, San Francisco Bay had both the lowest native species richness and relative abundance (Figures 2.3 and 2.7) for the sessile invertebrates.

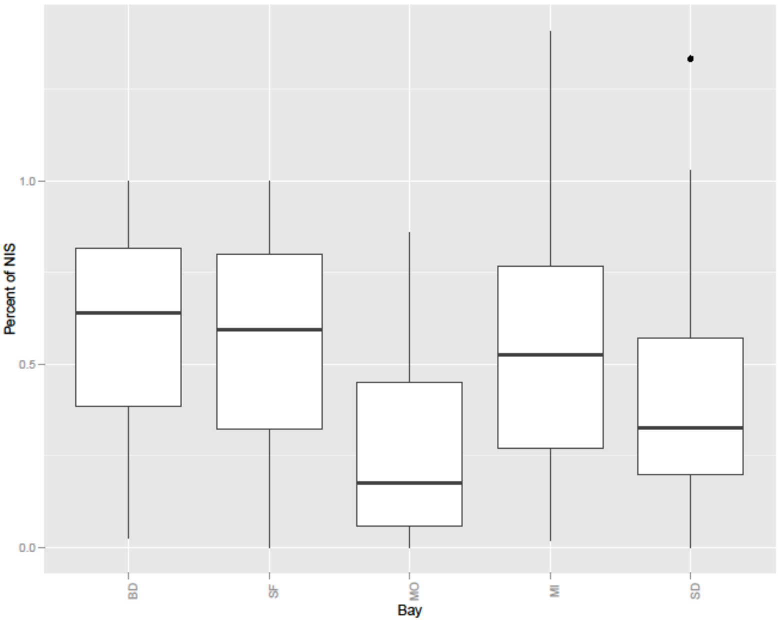


Figure 2.6. Mean percent cover of NIS (sessile taxa only) per plate in each bay based on point count measurements. Approximately 50 point count measurements were made per plate, identifying sessile invertebrates to species. Status is designated based on literature and SERC NEMESIS database. Error bars equal ±1SD.

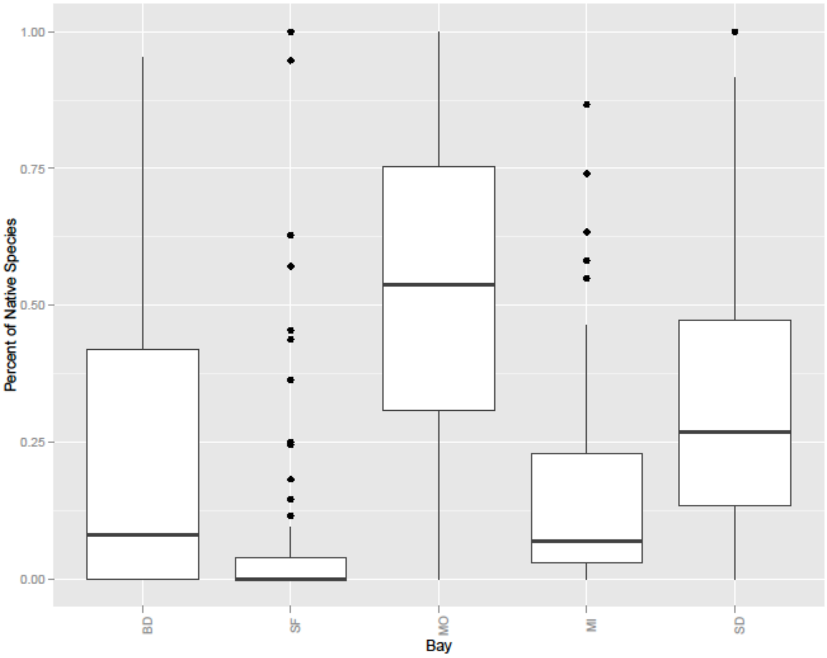


Figure 2.7. Mean percent cover of native species (sessile taxa only) per plate in each bay based on point count measurements. Approximately 50 point count measurements were made per plate, identifying sessile invertebrates to species. Status is designated based on literature and SERC NEMESIS database.

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 in San Francisco Bay. Figure 2.8 compares the species accumulation curves for NIS in 2012 (also shown in previous section in Figure 2.2) to that in 2011 and 2013 in San Francisco Bay, using only high salinity sites surveyed with identical methods. Figure 2.9 shows the total number of NIS observed in each year.

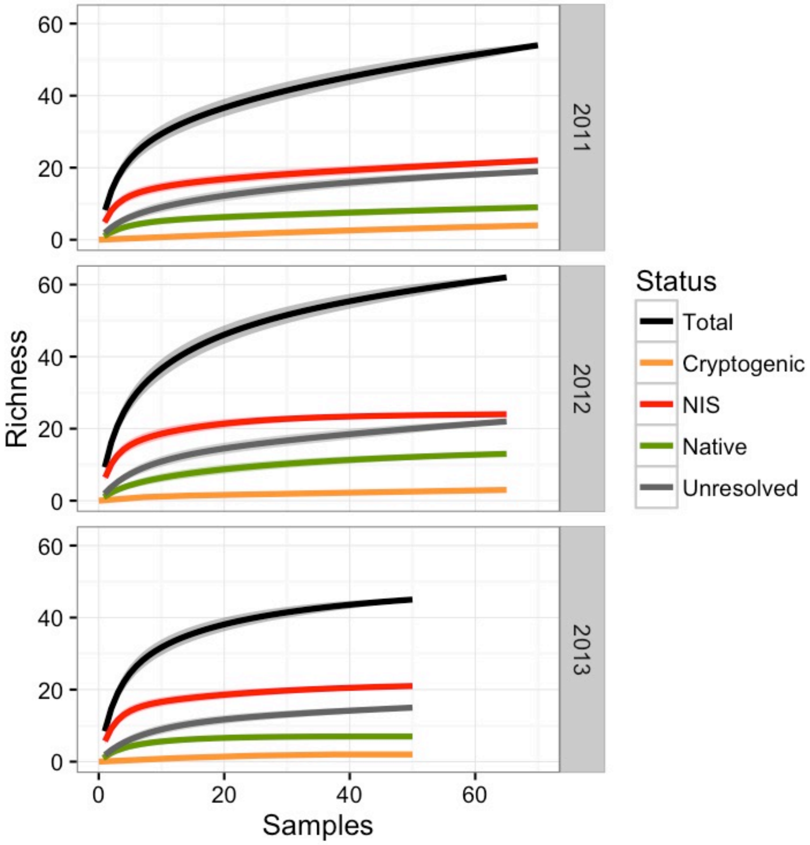


Figure 2.8. Species accumulation (sessile taxa only) curves by invasion status for high salinity sites across of San Francisco Bay in three different years. Status is designated based on literature and SERC NEMESIS database.

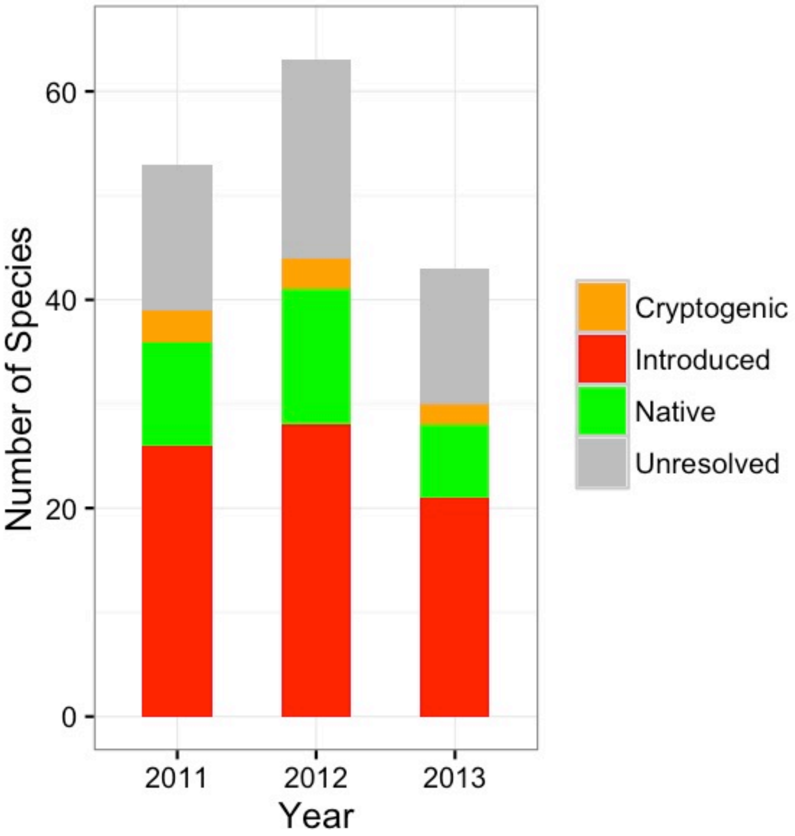


Figure 2.9. Number of unique species (sessile taxa only) detected in each year by invasion status for high salinity sites across San Francisco Bay in three different years. Status is designated based on literature and SERC NEMESIS database.

Total NIS Richness Among Years at Low Salinity Sites.

A similar pattern is observed among low salinity sites sampled in San Francisco Bay, in that (a) the number of detected NIS reach an asymptote quickly with increasing samples, especially relative to native and other taxa, (b) the total number detected in each of the two years shows high consistency. There are also two differences in NIS richness between the low and high salinity sites. First, the high salinity sites exhibit a longer tail (or more extended flattening out of the curve) than low salinity sites, reflecting simply the larger number of samples (and sites) surveyed in the high salinity region. Second, the overall species richness (non-native and total) is lower in the low salinity region.

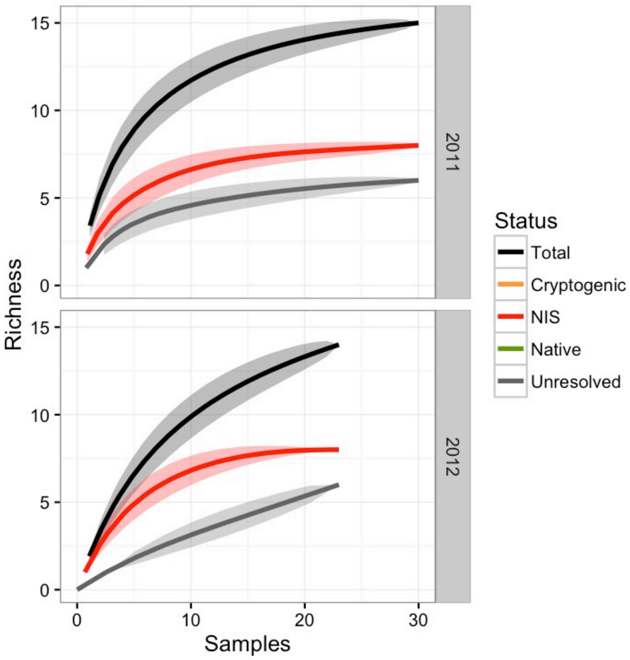


Figure 2.10. Species accumulation (sessile taxa only) curves by invasion status for low salinity sites across of San Francisco Bay in two years (freshwater sites were not sampled in 2013). Status is designated based on literature and SERC NEMESIS database. No cryptogenic or native species were found at low salinity sites.

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 2.11). 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 (Andrew Chang, unpublished data). 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. Also, as expected from our analysis of 2012 data in the previous section, the mean NIS richness per plate was consistently lower in both years at low salinity sites, compared to those of higher salinity.

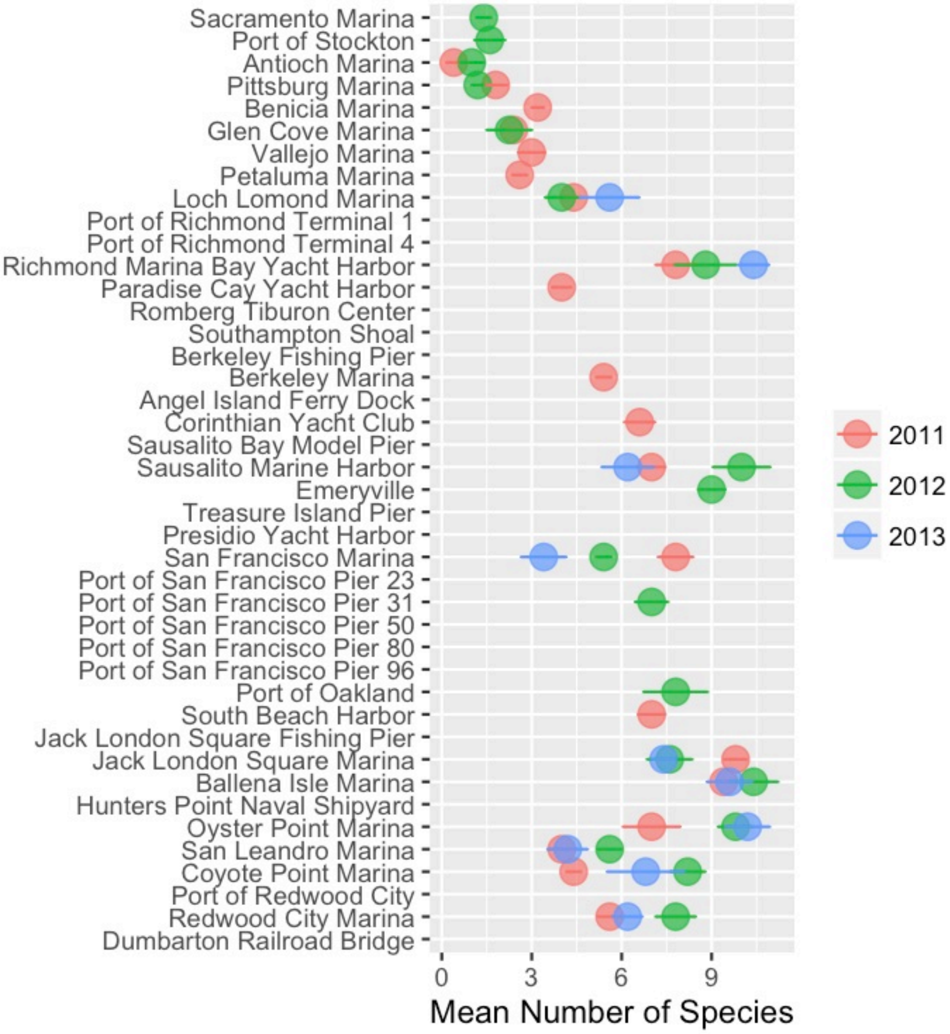


Figure 2.11. Mean number of NIS (sessile taxa only) detected per plate averaged within site per year. Error bars equal ±1SD. This combines low salinity (top) and high salinity sites (below Loch Lomond Marina).

Relative Abundance of NIS Per Plate Among Years and Sites.

The percent cover of NIS at high salinity sites was usually > 50% for most sites, across years (Figure 2.12). Although NIS relative abundance was also high at some brackish water sites (Benicia Marina, Glen Cove Marina, Vallejo, Petaluma), it was generally lower at the few freshwater sites sampled.

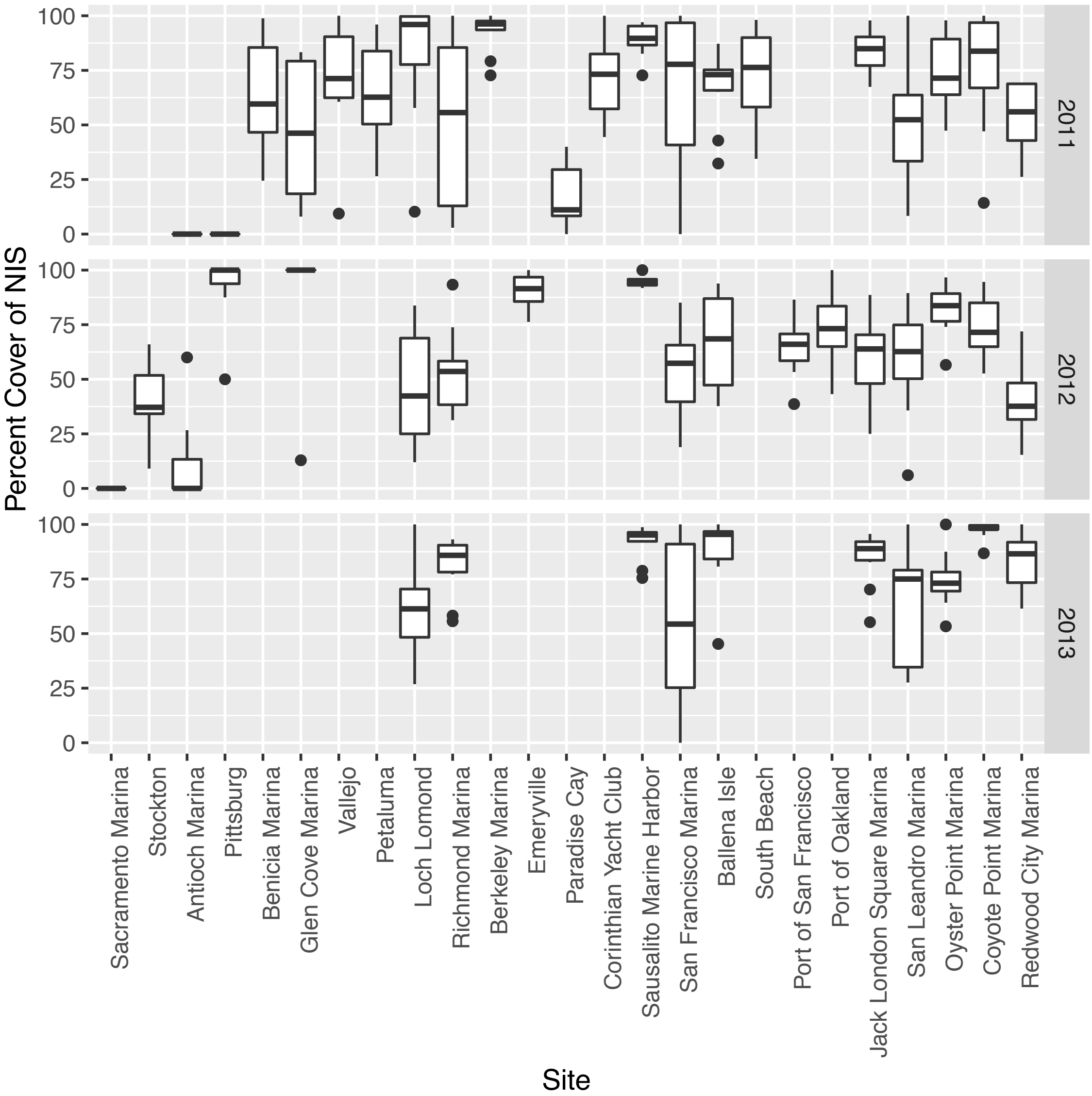


Figure 2.12. Mean percent cover of NIS (sessile taxa only) per plate within sites in each year based on point count measurements. Approximately 50 point count measurements were made per plate, identifying sessile invertebrates to species. Status is designated based on literature and SERC NEMESIS database. Error bars equal ±1SD.

C. Newly Detected NIS Across Bays and Years for the Hard Substrate Community

Only two new taxa were detected morphologically in our combined surveys of these five estuaries and the repeated measures (in multiple years) for San Francisco Bay. The bryozoan *Watersipora subovoidea* was detected in high salinity waters at 3 sites in San Diego Bay, 1 site in Mission Bay, and 1 site in Morro Bay, and the bryozoan *Fredericella indica* was found at 3 low salinity sites in San Francisco Bay (Appendix 2.2). Genetic analyses from MLML appear to confirm the identification of *W. subovoidea* (Jonathon Geller, personal communication), and we are not aware of other prior records of this taxon in California. It is also noteworthy that this species was detected in 3 different estuaries, separated by considerable distances, suggesting that the species may have been present (but undetected) for some time, during which it has spread coastwise among these estuaries. The second bryozoan may have been detected in earlier surveys in San Francisco Bay (Andrew Chang, unpublished data), and we are now reviewing archived specimens for verification.

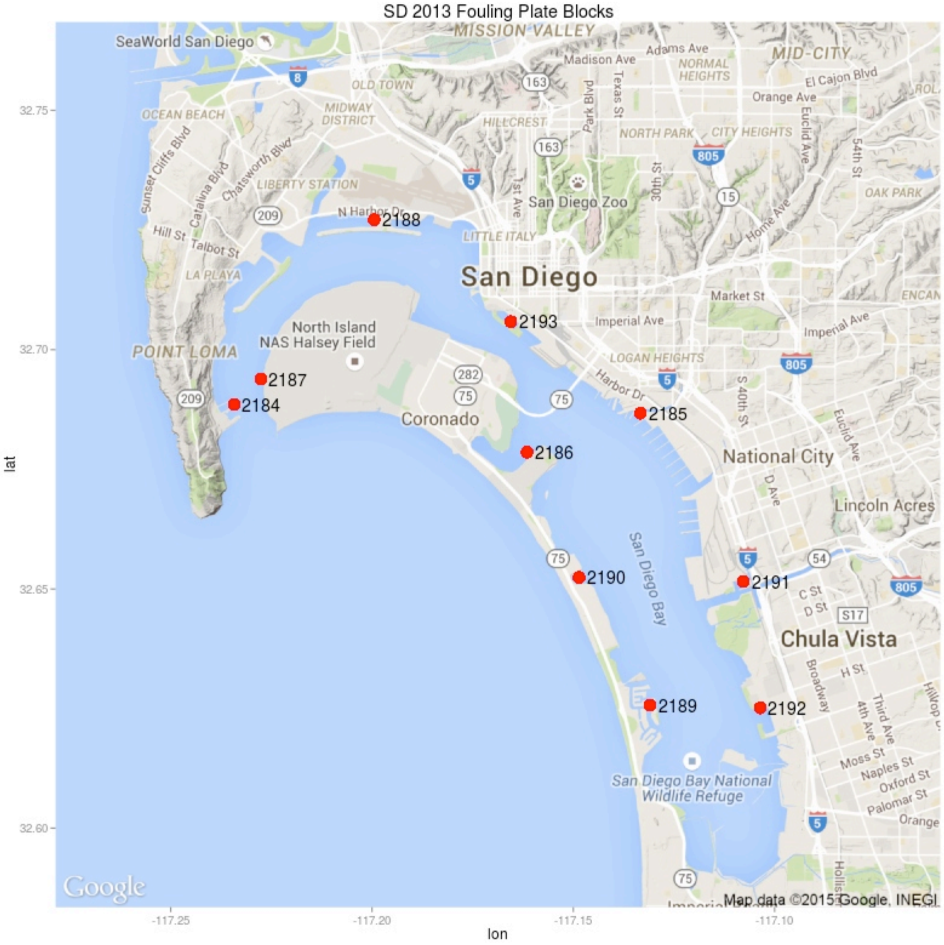
The paucity of new NIS records from these extensive surveys is surprising, given (a) the detailed analysis of literally thousands of macroinvertebrates collected from 375 hard substrate community samples (plates) across 5 estuaries, from San Diego Bay to Bodega Bay and (b) the inclusion of mobile biota at all sites that has arguably received much less recent attention in NIS surveys than sessile invertebrates.

These results suggest either the rate of invasion or detection is highly variable in time, or there has been a shift (slow down) in the invasion dynamics from that reported in the literature, especially for San Francisco Bay. The repeated measures for San Francisco Bay over the next several years, during the initial phase of the Program, are designed to explicitly test this hypothesis.

Appendix 2.1: Survey Locations by Estuary and Year

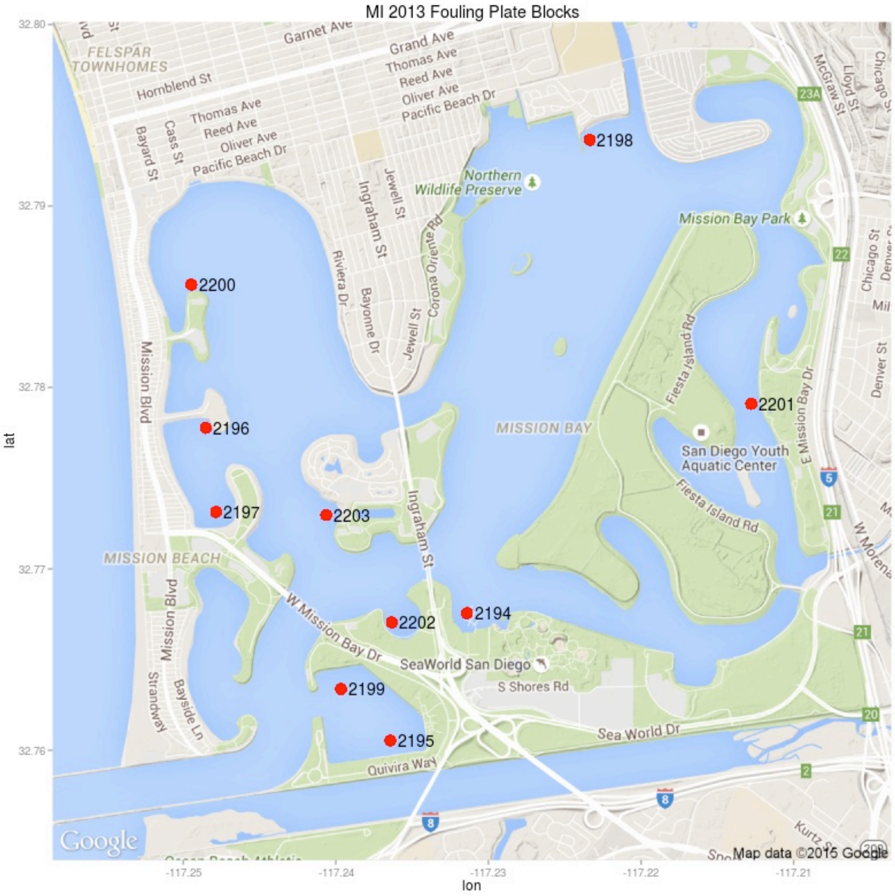
The maps and tables below indicate locations and dates for hard substrate surveys for each estuary and year.

San Diego Bay 2013



Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2184	Naval Base Point Loma	32.68855	-117.234	4/23/2013	7/16/2013
2185	Naval Station San Diego	32.68673	-117.133	4/23/2013	7/17/2013
2186	NAB ACU-1 Docks	32.67859	-117.162	4/24/2013	7/24/2013
2187	Navy Ammo Dock, Pier Bravo	32.69389	-117.228	4/24/2013	7/25/2013
2188	Cabrillo Isle Marina	32.72717	-117.199	4/25/2013	7/21/2013
2189	Coronado Cays Marina	32.6257	-117.131	4/25/2013	7/22/2013
2190	NAB Fiddlers Cove	32.65242	-117.149	4/25/2013	7/18/2013
2191	Pier 32 Marina	32.65156	-117.108	4/26/2013	7/26/2013
2192	Chula Vista Marina	32.62515	-117.104	4/26/2013	7/20/2013
2193	Marriott Marquis and Marina	32.70587	-117.166	4/26/2013	7/28/2013

Mission Bay 2013

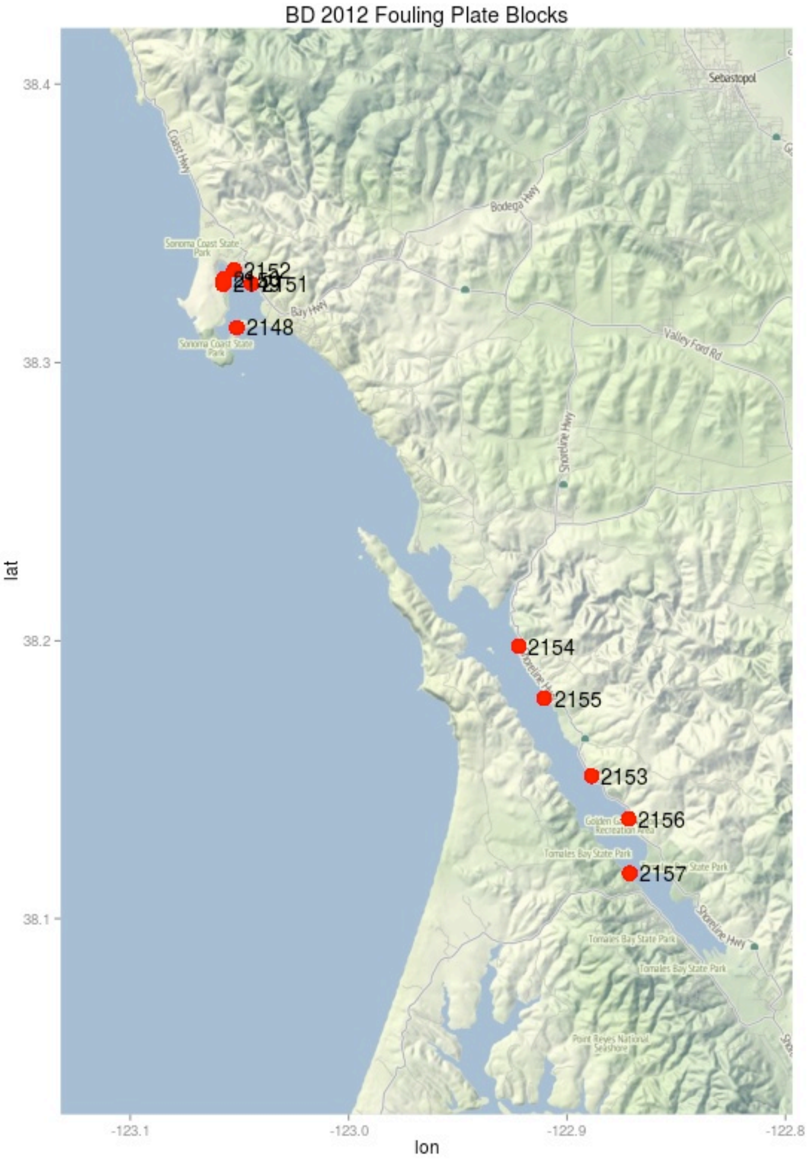


Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2194	SeaWorld Marina	32.76756	-117.231	4/24/2013	7/19/2013
2195	Marina Village	32.76054	-117.236	4/23/2013	7/23/2013
2196	Mission Bay Yacht Club	32.77777	-117.249	4/27/2013	7/29/2013
2197	Bahia Resort Marina	32.77314	-117.248	4/27/2013	8/4/2013
2198	Campland on the Bay	32.79364	-117.223	4/29/2013	7/31/2013
2199	Hyatt Resort Marina	32.76338	-117.24	4/29/2013	8/1/2013
2200	Mission Bay Sport Center	32.78567	-117.249	4/29/2013	8/3/2013
2201	Hilton Resort Docks	32.7791	-117.213	4/29/2013	7/30/2013
2202	The Dana Marina	32.76705	-117.236	4/30/2013	8/2/2013
2203	Paradise Point Resort	32.77297	-117.241	4/30/2013	7/5/2013

Morro Bay 2013

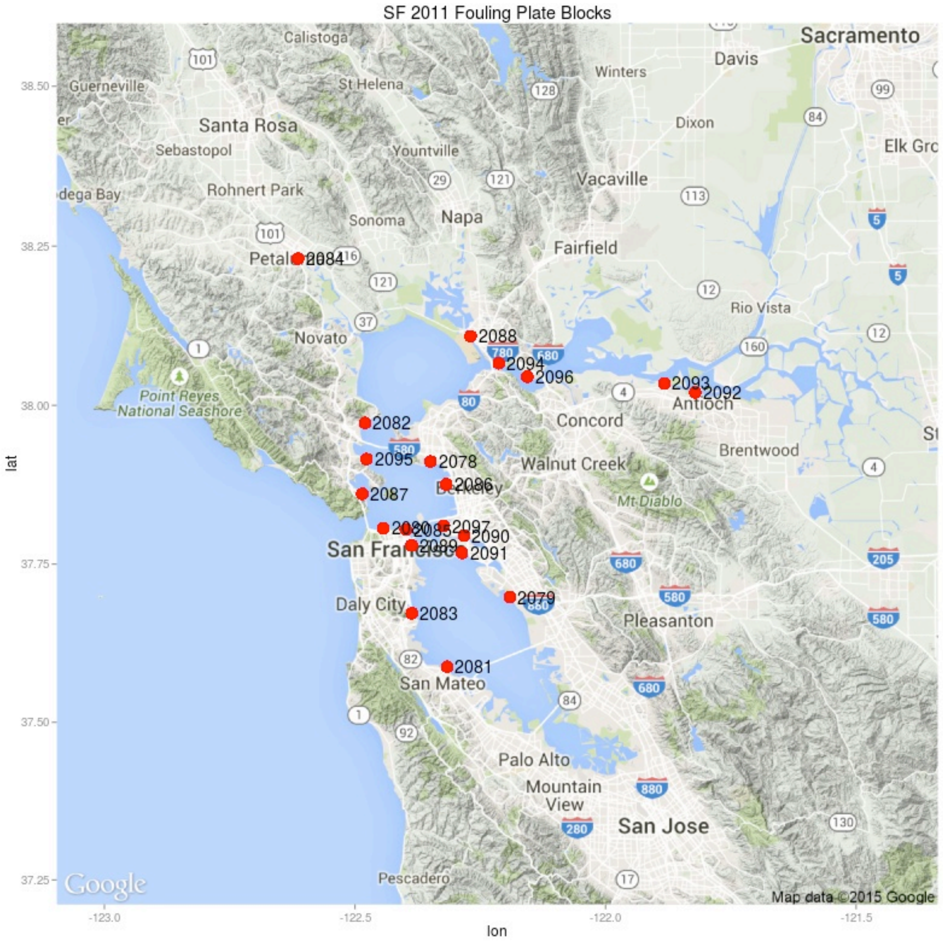
Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2214	201 Main	35.35635	NA	5/29/2013	8/30/2013
2215	City Harbor	35.37091	-120.858	5/28/2013	8/27/2013
2216	Launch Ramp	35.35773	-120.851	5/28/2013	9/4/2013
2217	Moorings	35.3619	-120.855	5/29/2013	8/29/2013
2218	Morro Bay Marina	35.36408	-120.853	5/20/2013	8/31/2013
2219	Grassy Bay Oyster Farm	35.33044	-120.852	5/29/2013	NA
2220	Sealion Dock	35.36581	-120.856	5/29/2013	8/28/2013
2221	State Park Marina	35.34589	-120.842	5/20/2013	9/2/2013
2222	Tidelands	35.36016	-120.852	5/28/2013	9/3/2013

Bodega / Tomales Bay 2012



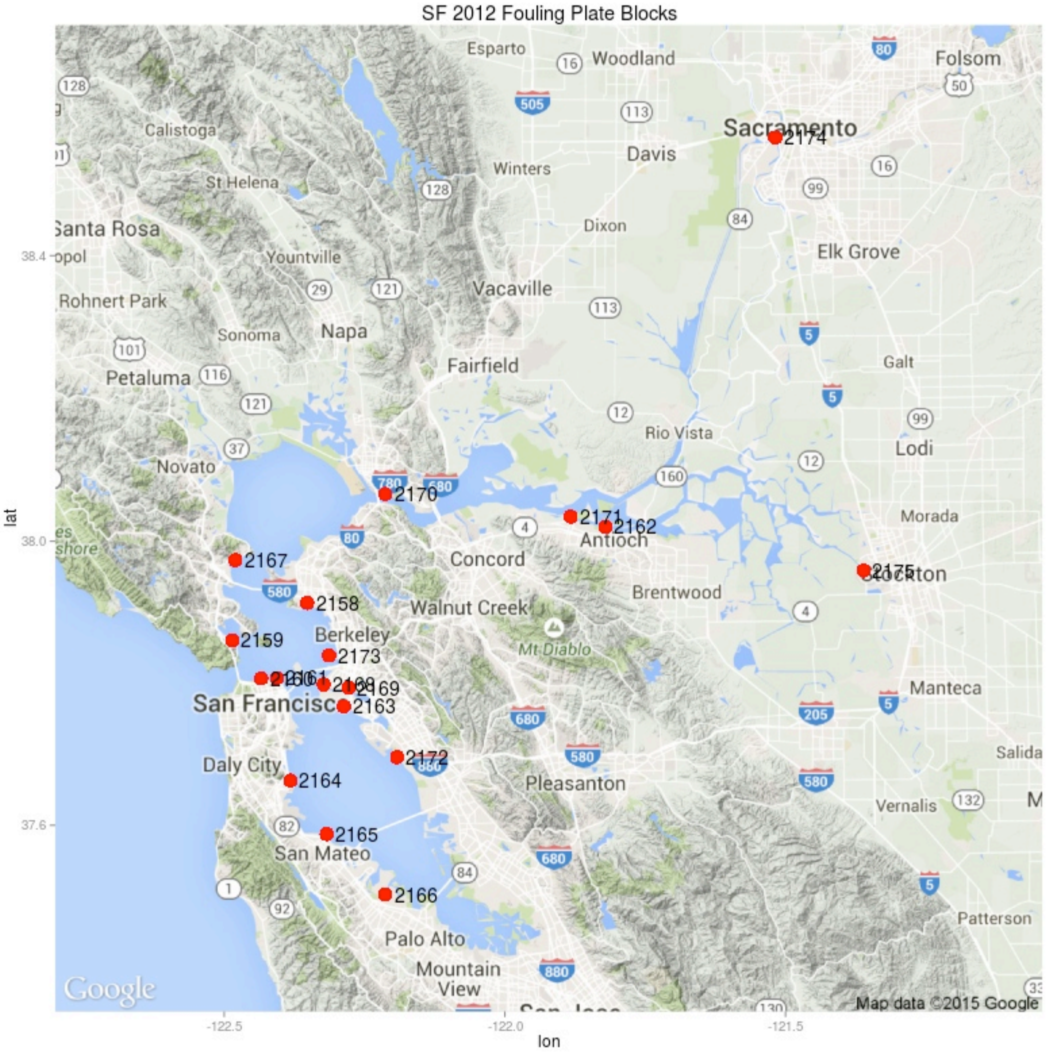
Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2148	Coast Guard	38.31256	-123.051	5/14/2012	8/13/2012
2149	Spud Point South	38.32808	-123.057	5/16/2012	8/14/2012
2150	Spud Point North	38.33009	-123.057	5/16/2012	8/14/2012
2151	Lucas/Tides	38.32835	-123.045	5/17/2012	8/20/2012
2152	Porto Bodega	38.33332	-123.052	5/17/2012	8/20/2012
2153	Tomales-Marshall	38.15136	-122.889	5/21/2012	8/22/2012
2154	Tomales-Nick's Cove	38.19802	-122.922	5/21/2012	8/21/2012
2155	Tomales- Call Box 401	38.17926	-122.91	5/22/2012	8/14/2012
2156	Tomales-SNPS	38.13592	-122.872	5/23/2012	8/15/2012
2157	Tomales- Shell Beach	38.11631	-122.871	5/24/2012	8/17/2012

San Francisco Bay 2011



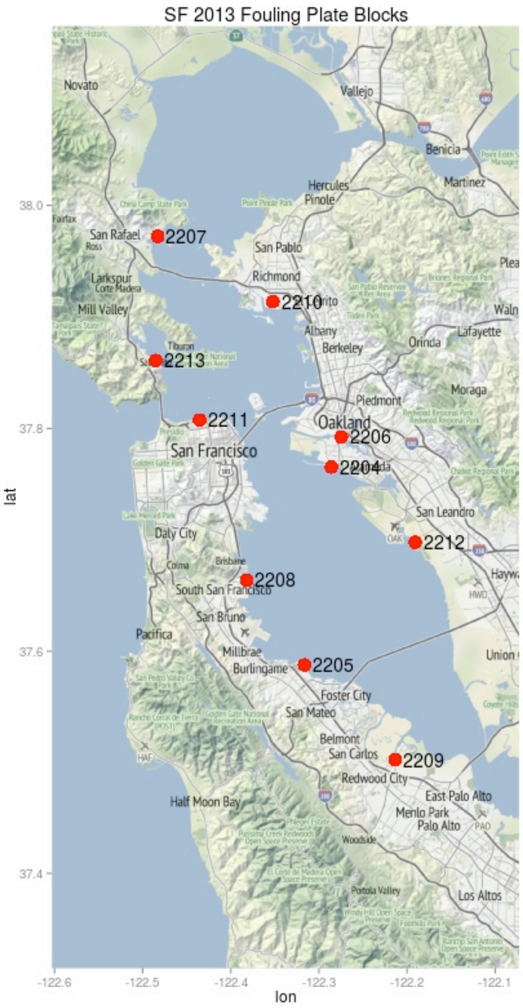
Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2078	Richmond Marina Bay Yacht Harbor	37.91172	-122.349	6/16/2011	9/15/2011
2079	San Leandro Marina	37.69787	-122.191	6/14/2011	9/20/2011
2080	San Francisco Marina	37.8067	-122.443	6/15/2011	9/20/2011
2081	Coyote Point Marina	37.588	-122.316	6/13/2011	9/14/2011
2082	Loch Lomond Marina	37.9724	-122.48	6/25/2011	9/16/2011
2083	Oyster Point Marina	37.6725	-122.386	6/30/2011	9/13/2011
2084	Petaluma Marina	38.23042	-122.614	6/15/2011	9/27/2011
2085	Redwood City Marina	37.80456	-122.399	6/24/2011	9/13/2011
2086	Berkeley Marina	37.87583	-122.318	6/25/2011	9/15/2011
2087	Sausalito Marine Harbor	37.86092	-122.485	6/25/2011	9/19/2011
2088	Vallejo Marina	38.10861	-122.269	6/28/2011	9/27/2011
2089	South Beach Harbor	37.77969	-122.387	6/25/2011	9/21/2011
2090	Jack London Square Marina	37.7947	-122.282	6/29/2011	9/20/2011
2091	Ballena Isle Marina	37.76764	-122.287	7/1/2011	9/22/2011
2092	Antioch Marina	38.02026	-121.821	7/1/2011	9/26/2011
2093	Pittsburg Marina	38.03457	-121.883	7/1/2011	9/26/2011
2094	Glen Cove Marina	38.06629	-122.213	7/2/2011	9/28/2011
2095	Paradise Cay Yacht Harbor	37.91556	-122.477	7/5/2011	9/12/2011
2096	Benicia Marina	38.04528	-122.156	7/6/2011	9/28/2011
2097	Corinthian Yacht Club	37.81025	-122.323	7/5/2011	9/12/2011

San Francisco Bay 2012



Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2158	Richmond Marina Bay Yacht Harbor	37.91342	-122.352	5/29/2012	8/24/2012
2159	Sausalito Marine Harbor	37.86092	-122.485	5/29/2012	8/23/2012
2160	San Francisco Marina	37.80713	-122.434	5/31/2012	8/28/2012
2161	Port of San Francisco Pier 31	37.80781	-122.406	5/31/2012	8/27/2012
2162	Antioch Marina	38.02026	-121.821	6/6/2012	8/31/2012
2163	Ballena Isle Marina	37.76764	-122.287	6/11/2012	9/11/2012
2164	Oyster Point Marina	37.66329	-122.382	6/1/2012	8/30/2012
2165	Coyote Point Marina	37.58774	-122.317	6/1/2012	8/29/2012
2166	Redwood City Marina	37.50225	-122.213	6/6/2012	9/4/2012
2167	Loch Lomond Marina	37.97359	-122.48	6/6/2012	9/6/2012
2168	Port of Oakland	37.79874	-122.323	6/7/2012	9/5/2012
2169	Jack London Square Marina	37.79398	-122.279	6/7/2012	9/7/2012
2170	Glen Cove Marina	38.06629	-122.213	6/8/2012	8/31/2012
2171	Pittsburg Marina	38.03457	-121.883	6/8/2012	9/10/2012
2172	San Leandro Marina	37.69616	-122.192	6/11/2012	9/13/2012
2173	Emeryville	37.83957	-122.313	6/12/2012	9/12/2012
2174	Sacramento Marina	38.56479	-121.518	6/15/2012	9/10/2012
2175	Port of Stockton	37.95889	-121.361	6/15/2012	9/13/2012

San Francisco Bay 2013



Block_ID	BlockName	LAT	LONG	Deployment	Retrieval
2204	Ballena Isle Marina	37.76559	-122.286	5/16/2013	8/14/2013
2205	Coyote Point Marina	37.58765	-122.316	5/14/2013	8/20/2013
2206	Jack London Square Marina	37.79256	-122.275	5/16/2013	8/22/2013
2207	Loch Lomond Marina	37.97231	-122.483	5/13/2013	8/23/2013
2208	Oyster Point Marina	37.66392	-122.382	5/13/2013	8/13/2013
2209	Redwood City Marina	37.50242	-122.213	5/14/2013	8/14/2013
2210	Richmond Marina Bay Yacht Harbor	37.91377	-122.352	5/15/2013	8/19/2013
2211	San Francisco Marina	37.80777	-122.435	5/13/2013	8/12/2013
2212	San Leandro Marina	37.69803	-122.191	5/16/2013	8/21/2013
2213	Sausalito Marine Harbor	37.86108	-122.485	5/13/2013	8/16/2013

Appendix 2.2. Taxa Identified Morphologically by Estuary and Year

San Diego Bay 2013

San Diego Bay, 2013	Status	Site	Cabrillo Isle Marina	Chula Vista Marina	Coronado Cays Marina	Marriott Marquis and Marina	NAB ACU-1 Docks	NAB Fiddlers Cove	Naval Base Point Loma	Naval Station San Diego	Navy Ammo Dock, Pier Bravo	Pier 32 Marina
Annelida												
Polychaeta												
Capitellidae												
Capitella capitata complex	C		1									
Cirratulidae												
Cirratulidae	U									1		
Cirriformia sp.	N						1			2		
Protocirrineris sp. 1	N		1									
Dorvilleidae												
Dorvillea schistomeringos sp.	U			2								
Ophryotrocha sp.	C		1									
Flabelligeridae												
Pherusa capulata	N				1							
Lumbrineridae												
Lumbrineris perkinsi	I		2	3	2		1					
Nereididae												
Neanthes acuminata complex	C		2									
Nereididae	U										1	
Nereis latescens	N			1								
Nereis mediator	N								1			
Nereis vexillosa	N		1									
Platynereis bicanaliculata	N								2		2	
Opheliidae												
Armandia brevis	N			1	1						1	
Polyophthalmus pictus complex	C								1		1	
Phyllodocidae												
Eulalia quadrioculata	N								1			
Pterocirrus burtoni	N								2		3	
Polynoidae												
Halosydna johnsoni	N										1	
Sabellidae												
Branchiomma sp. 2	U						4		1	4		
Branchiomma sp. 3	U											2
Paradialychone ecaudata	N								1			
Parasabella pallida	N					4	2			1		1
Sabellidae	U							1				
Serpulidae												
Hydroides gracilis	N								3		2	
Hydroides sp.	U					2						
Salmacina tribranchiata	N					3				3		
Syllidae												
Amblyosyllis speciosa D	I									1		
Branchiosyllis cf. exilis	C			1								1
Branchiosyllis sp.	U			1								
Megasyllis nipponica	I		1		1			1				
Odontosyllis phosphorea	N			1				1				1
Syllis sp.	U								1			
Trypanosyllis sp. 1	N		1	1		1						2
Typosyllis sp.	U										1	
Terebellidae												
Eupolymnia heterobranchia	N						1	1		1		
Eupolymnia sp. B Harris	NA							2				
Nicolea cf. amnis	U		1			1			4	1	5	
Streblosoma sp.	U							1				
Streblosoma uncinatus	N							1				
Thelepus setosus	N				2							
Arthropoda												
Eucarida												
Decapoda												
Cancer jordani	N								2		2	
Cancer sp.	U								1			
Cancer sp. 1	U								1		1	
Decapoda	U					1						
Eualus sp.	N										1	
Heptacarpus brevirostris	N										1	
Heptacarpus sp.	N										3	

San Diego Bay, 2013	Status	Site	Cabrillo Isle Marina	Chula Vista Marina	Coronado Cays Marina	Marriott Marquis and Marina	NAB ACU-1 Docks	NAB Fiddlers Cove	Naval Base Point Loma	Naval Station San Diego	Navy Ammo Dock, Pier Bravo	Pier 32 Marina
Hippolyte californiensis	N									1		
Lophopanopeus sp. 1	N					1	1		2	1	4	
Pachycheles sp.	N								1			
Pyromaia tuberculata	N										1	
Synalpheus lockingtoni	N										1	
Peracarida												
Amphipoda												
Ampithoe plumulosa	C		1	2	1	1				2		
Ampithoe sp.	U							1				
Aoroides secunda	I		3		1	2	2	4	3	5		3
Apolochus picadurus	N			1	3							1
Colomastix sp.	C			1								
Dissiminassa dissimilis	N			3	2	1	2	5		5		
Dulichiella spinosa	N								2			
Elasmopus bampo	C		5	4	5	5	5	5	2	5		4
Elasmopus sp. 1	U								3			
Ericthonius brasiliensis	C			1	2		1		5	5	3	1
Eusiroidea	U				1							
Gammaropsis shoemakeri	N								3		5	
Gammaropsis sp.	N				1							
Grandidierella japonica	I				1		2	4				
Jassa slatteryi	C								3		1	
Jassa sp.	U								2	2	2	
Laticorophium baconi	N		1	1			1		5	5	3	
Leucothoe alata	C		4	4	1	1	4	3	1	4		2
Liljeborgia geminata complex	C						1					
Lysianassoidea	U											1
Monocorophium acherusicum	I				3	1	1	5	1			
Monocorophium sp.	U							1				
Paradexamine sp. 1	I			4	2			4		1	1	3
Pleustidae	U								1		1	1
Podocerus brasiliensis	C			1			1		4	3	4	1
Podocerus cristatus	C							1		1	2	3
Pontogeneia sp.	N											1
Protohyale sp. 1	U							2				
Quadrimaera reishi	N						1	1	1	3		
Stenothoe valida	I							1	5		1	
Stenothoidae	U								3		3	
Caprellidae												
Aciconula acanthosoma	N										1	
Caprella californica	N						1		4		5	
Caprella equilibra	C								2		1	
Caprella mutica	I								2			1
Caprella scaura	I								1			
Caprella simia	I					1			5	2	4	1
Caprella verrucosa	N								1			
Isopoda												
Ianiropsis sp.	U								1			
Janirilata sp. 1	N								2			
Joeropsis dubia dubia	N								3	1		
Joeropsis sp.	N						1		1			
Paracerceis sculpta	N		1	4	4	3	3	4		4	1	4
Paranthura elegans	N									2		
Paranthura japonica	I		4	1	2		2	4		1		2
Tanaidae												
Apseudidae	N								1		1	
Leptochelia sp. 2	U										1	
Tanaidae	U									1	1	
Zeuxo sp.	U		3		2	2	1	5	1	5		3
Cirripedia												
Balanidae	U								1			
Balanus trigonus	N								3	1	4	
Megabalanus californicus	N								2		1	
Pycnogonida												
Anoplodactylus californicus	C				1							
Nymphon pixellae	N				1							

San Diego Bay, 2013												
	Status	Site	Cabrillo Isle Marina	Chula Vista Marina	Coronado Cays Marina	Marriott Marquis and Marina	NAB ACU-1 Docks	NAB Fiddlers Cove	Naval Base Point Loma	Naval Station San Diego	Navy Ammo Dock, Pier Bravo	Pier 32 Marina
Bryozoa												
Gymnolaemata												
Cheilostomatida												
Bugula neritina	I				1	1		1	5		5	
Bugula sp.	U								1			
Bugula sp. C	U						1		2			
Bugulina longirostrata	N										2	
Bugulina stolonifera	I					1			1		2	2
Celleporaria brunnea	N		2	1	2	5	4	4	5	2	5	4
Celleporina robertsoniae	N									1		
Celleporina sp.	U								1			
Cradoscrupocellaria tenuirostris	N							1				
Cryptosula pallasiana	I									1	1	2
Hippopodina iririkiensis	I						2	2				
Licornia diegensis	N								3		5	
Microporella californica	N					1				3		
Pomocellaria californica	N								1			
Rhynchozoon sp.	U								2		1	
Rhynchozoon spicatum	N								1			
Rhynchozoon tumulosum	N										1	
Schizoporella occidentalae	N						1		1	1	1	1
Scruparia ambigua	C								2		1	
Scrupocellaria sp.	U								1			
Smittinidae	U						1					
Smittoidea prolifica	N						1			2	1	
Thalamoporella californica	N			2				1			1	
Watersipora arcuata	I						1		2	1	3	
Watersipora sp.	I						1					1
Watersipora subovoidea	I			1		1						1
Watersipora subtorquata	I		1	1	1	3	3	3	4	5	5	3
Ctenostomatida												
Amathia dichotoma	I										3	
Anguinella palmata	I										1	
Bowerbankia sp.	U								2		3	
Zoobotryon verticillatum	I		2	5	4	4	1	4		1		5
Stenolaemata												
Cyclostomatida												
Crisia occidentalis	N								2		4	
Crisia sp. A	U			1		1	1	2	1	2	1	1
Crisulipora occidentalis	N		3	1	3	5	5	5	1	5	1	5
Disporella buskiana	N							1				1
Entalophoroecia sp.	U							1				
Filicrisia franciscana	N										1	
Tubulipora aliciae	N								1			
Tubulipora pacifica	N					1			4	1	5	
Tubulipora sp.	U								1			
Chordata												
Ascidiacea												
Aplousobranchia												
Aplidium californicum	N		1					1			1	
Aplidium sp.	U		3									
Aplidium sp. A	U			1		1						
Aplousobranchia	U		1							1		
Didemnidae	U									1		
Didemnum vexillum	I								5	4	4	
Diplosoma listerianum	I		4	3	1	3	5	4	4	4	3	1
Distaplia occidentalis	N		2				3	1	3		4	
Phlebobranchia												
Ascidia ceratodes	N					1						1
Ascidia sp.	U					2			1		1	2
Ascidia zara	I				1	2			1			2
Ciona intestinalis	I		5	1		3	4	5	3	5	3	2
Ciona savignyi	I		2		1	1	4	3		3		3
Ciona sp.	U					4	1	1	3	2	2	
Phlebobranchia	U					1						
Perophora annectens	N			2		2	4	3		3		5

San Diego Bay, 2013												
	Status	**Site**	**Cabrillo Isle Marina**	**Chula Vista Marina**	**Coronado Cays Marina**	**Marriott Marquis and Marina**	**NAB ACU-1 Docks**	**NAB Fiddlers Cove**	**Naval Base Point Loma**	**Naval Station San Diego**	**Navy Ammo Dock, Pier Bravo**	**Pier 32 Marina**
Perophora sp.	U			2	1		3	2		2		1
Stolidobranchia												
Botryllinae	I							1		2	1	
Botrylloides giganteum	I										3	
Botrylloides sp.	I										2	
Botrylloides violaceus	I										2	
Botryllus schlosseri	I		4	1		4	2	3		3		4
Microcosmus squamiger	I			1		1	2	3				2
Molgula ficus	I					1		2				3
Molgula manhattensis	I											1
Molgula sp.	U				1	1		2		1		1
Polyandrocarpa zorritensis	I		4	1	2	5	5	4		1	4	1
Styela canopus	I			1	2	2		3		1		
Styela clava	I								2		1	
Styela plicata	I		3			3			1			
Styela sp.	U		1	2	2	4		2	3	3	2	5
Styelidae	U				1	4	1			2		2
Symplegma reptans	I		1					1		3	3	
Symplegma sp.	I						1		1			
Echinodermata												
Echinodermata	U									1		1
Ophiuroidea												
Amphipholis squamata	C			2	4	1	2		1	2		
Ophiactis simplex	N									1		
Ophiothrix spiculata	N								1			
Mollusca												
Gastropoda												
Gastropoda												
Amphissa sp.	N										1	
Astyris aurantiaca	N		1									
Crepipatella lingulata	N										1	
Nudibranchia												
Doto form A of Goddard	N										1	
Hermissenda crassicornis	N										1	
Polycera atra	N										2	
Bivalvia												
Lasaeidae	U										1	
Leptopecten latiauratus	N										1	
Musculista senhousia	I							2				
Mytilidae	U								1	1		
Ostrea lurida	N			5	5	4	5	4	5	4	4	4
Ostreidae	U					2						
Veneridae	U										1	
Platyhelminthes												
Hoploplana californica	N								1			
Phaenoplana longipenis	NA			1								
Prosthiostomum latocelis	NA									1	1	

Mission Bay 2013

Mission Bay, 2013	Status	Site	Bahia Resort Marina	Campland on the Bay	Hilton Resort Docks	Hyatt Resort Marina	Marina Village	Mission Bay Sport Center	Mission Bay Yacht Club	Paradise Point Resort	SeaWorld Marina	The Dana Marina
Annelida												
Polychaeta												
Dorvilleidae												
Dorvillea schistomeringos sp.	U								1			
Dorvilleidae	U				1							
Eunicidae												
Lysidice sp.	N			1								
Flabelligeridae												
Pherusa capulata	N			1								
Nereididae												
Platynereis bicanaliculata	N								1	1		
Polynoidae												
Harmothoe imbricata complex	C					1					1	
Sabellidae												
Branchiomma sp. 2	U					4			1		3	4
Serpulidae												
Hydroides elegans	I					1	1					1
Hydroides gracilis	N		1				1			4		
Hydroides sp.	U						1			1		
Salmacina tribranchiata	N						1					2
Serpulidae	U		1									
Syllidae												
Amblyosyllis speciosa D	I		1									
Branchiosyllis cf. exilis	C				1							
Megasyllis nipponica	I					3	2					
Myrianida pachycera	I				2							
Odontosyllis phosphorea	N			1	1				2	1	1	
Salvatoria sp.	N										1	
Syllis gracilis complex	C							1				
Syllis sp.	U										1	
Trypanosyllis sp. 1	N			4								
Typosyllis sp.	U									2		
Typosyllis sp. 29	U					1						
Terebellidae												
Nicolea cf. amnis	U					1	1	1				
Pista alata	N							1				
Arthropoda												
Eucarida												
Decapoda												
Hemigrapsus oregonensis	N									1		
Lophopanopeus sp. 1	N								1	5		
Peracarida												
Amphipoda												
Ampithoe plumulosa	C			2			1					1
Aoridae	U					1						
Aoroides secunda	I		4	4		3	4	1	3		5	5
Apolochus picadurus	N			1						4	1	
Dissiminassa dissimilis	N					1	3	1	2		2	
Dulichiella spinosa	N									3		
Elasmopus bampo	C		5	5	5	5	5	5	5	5	5	5
Elasmopus sp. 1	U								1	1		
Ericthonius brasiliensis	C		5			4	1	1	5	5	5	3
Gammaropsis shoemakeri	N									5		
Gammaropsis sp.	N										1	
Gnathopleustes pugettensis	N							1				
Grandidierella japonica	I		1				1		3		1	1
Jassa sp.	U									1		
Laticorophium baconi	N		3	1					1		4	
Leucothoe alata	C		3	3	2	4	4	1	2		3	4
Liljeborgia geminata complex	C						1					2
Maera sp. 1	N								1	1		1
Metopa cistella	N									2		
Microjassa sp.	N									1		
Monocorophium acherusicum	I		1	4		1	2		4	1	3	4
Monocorophium insidiosum	I										1	
Monocorophium sp.	U										1	
Paradexamine sp. 1	I		1					1		1	4	1
Podocerus brasiliensis	C		1			2	3			2	3	
Podocerus cristatus	C		1		3			3	1	4	1	3
Protohyale sp. 1	N			2								1
Quadrimaera reishi	N								2	2	1	
Quadrimaera sp. 1	N		2						1	3		
Stenothoe valida	I		1						1			
Stenothoidae	U							1				
Caprellidae												
Caprella californica	N		1					1	1	3		1
Caprella equilibra	C									1		
Caprella mutica	I		3				1	4	4			1
Caprella simia	I		4			3	4	3	5	2	2	4
Caprella sp.	U									1		
Isopoda												
Paracerceis sculpta	N		5	5	5		2	5	5	5	4	4
Paranthura japonica	I		3	4	3		2	4			2	
Tanaidae												
Zeuxo sp.	U		2			4	4	2	2	3	4	3

Mission Bay, 2013	**Status**	**Site**	**Bahia Resort Marina**	**Campland on the Bay**	**Hilton Resort Docks**	**Hyatt Resort Marina**	**Marina Village**	**Mission Bay Sport Center**	**Mission Bay Yacht Club**	**Paradise Point Resort**	**SeaWorld Marina**	**The Dana Marina**
Cirripedia												
Balanus trigonus	N						1			1		
Megabalanus sp.	N									1		
Pycnogonida												
Achelia chelata	N									1		
Anoplodactylus californicus	C			3								
Nymphon pixellae	N		2						1			
Tanystylum occidentalis	N						1					
Bryozoa												
Gymnolaemata												
Cheilostomatida												
Bugula neritina	I		5	2		1	1	3	5	5	3	2
Bugula sp.	U						1					
Bugulina longirostrata	N									1		1
Bugulina stolonifera	I		2			1		5	1	3	3	
Celleporaria brunnea	N		5	2		4	4	2	4	5	5	5
Celleporidae	U											1
Cradoscrupocellaria tenuirostris	N			1				5		1		
Cryptosula pallasiana	I		1		2	2	1	3	1	1	1	5
Fenestrulina delicia	C									2		
Fenestrulina sp.	U									2		
Lagenicella punctulata	N									1		
Licornia diegensis	N									5		
Rhynchozoon sp. A	U									1		
Rhynchozoon tumulosum	N									5		
Schizoporella japonica	I				1							
Schizoporella occidentalae	N									2	1	4
Schizoporella sp.	U									1		
Smittoidea prolifica	N										2	
Thalamoporella californica	N		4	3	5	1	2		1	1	2	1
Watersipora arcuata	I				1			1		4		
Watersipora sp.	I						2					
Watersipora subovoidea	I						1					
Watersipora subtorquata	I		3	2	5	3	3	4	3	5	5	5
Ctenostomatida												
Amathia dichotoma	I		3	1	3			1				
Amathia sp.	U				1			1				
Bowerbankia sp.	U										1	1
Zoobotryon verticillatum	I		4	5	5	2		2	4		4	2
Stenolaemata												
Cyclostomatida												
Crisia occidentalis	N									1		
Crisia sp. A	U							1				
Crisulipora occidentalis	N		4	1		2	2	2	5	2	5	5
Cyclostomatida	U					1						
Disporella buskiana	N		4	1	2			4	3	5	2	
Lichenoporidae	U							1				
Tubulipora pacifica	N					1	3		2	3	2	3
Chordata												
Ascidiacea												
Aplousobranchia												
Aplidium californicum	N		3			3		3				
Aplidium sp.	U		1	2		1	1	3			1	
Aplidium sp. A	U					1	2	1				
Aplousobranchia	U			1								
Didemnum vexillum	I							5	1	5	5	
Diplosoma listerianum	I		5	1	3	5	5	4	5	3	5	4
Distaplia occidentalis	N					1		2	1	1		
Phlebobranchia												
Ascidia ceratodes	N											1
Ascidia zara	I					1	1		1			1
Ciona intestinalis	I		1	1	1	5	4	1	1	1		1
Ciona savignyi	I			2		3	4					1
Ciona sp.	U						1					
Perophora annectens	N			5								1
Stolidobranchia												
Botryllinae	I		1	1		2	3		2	2		2
Botrylloides diegensis	I			1								
Botrylloides giganteum	I									3		
Botrylloides sp.	I			1					1		1	
Botrylloides violaceus	I					2	1		1			
Botryllus schlosseri	I		2	3	1	1	3	2	2	2	5	3
Microcosmus squamiger	I		1	2					1		1	
Molgula ficus	I			2								
Polyandrocarpa zorritensis	I		3	5	1	1		5		1	3	
Polyzoa translucida	N							1				
Stolidobranchia	U									1		
Styela clava	I		1					1	1	1		1
Styela plicata	I				1	4	2	2				
Styela sp.	U		2		1		1			2		
Styelidae	U			1		1		1				1
Symplegma reptans	I		4					4	3	3	3	4
Echinodermata												
Ophiuroidea												
Amphipholis squamata	C			1				1	1		1	2

Mission Bay, 2013	**Status**	**Site**	**Bahia Resort Marina**	**Campland on the Bay**	**Hilton Resort Docks**	**Hyatt Resort Marina**	**Marina Village**	**Mission Bay Sport Center**	**Mission Bay Yacht Club**	**Paradise Point Resort**	**SeaWorld Marina**	**The Dana Marina**
Amphiuridae	U							1			1	
Ophiactis simplex	N							1				1
Mollusca												
Gastropoda												
Gastropoda												
Calyptraeidae	U									1		
Crepidula onyx	N									2		
Nudibranchia												
Hermissenda crassicornis	N								1			
Polycera atra	N									1		
Bivalvia												
Hiatella arctica	C									1		
Musculista senhousia	I		1									
Ostrea lurida	N		4	5	5			4	4	2	2	5
Ostreidae	U								1	1	1	
Nemertea												
Palaeonemertea	U				1							
Platyhelminthes												
Hoploplana californica	N									1		1
Pseudoceros mexicanus	N							1			1	

Morro Bay 2013

Morro Bay, 2013										
	Status	**Site**	**201 Main**	**City Harbor**	**Launch Ramp**	**Moorings**	**Morro Bay Marina**	**Sealion Dock**	**State Park Marina**	**Tidelands**
Annelida										
Polychaeta										
Chrysopetalidae										
Paleanotus bellis	N		6	6	6	6	5	5		7
Hesionidae										
Amphiduros sp.	U		1	2						
Oxydromus pugettensis	N				1					
Nereididae										
Nereis eakini	N							1		
Nereis latescens	N		2	1	1		3			2
Platynereis bicanaliculata	N		6	5	6	6	7	4	3	7
Phyllodocidae										
Eulalia quadrioculata	N		6	6	5	6	6	6		6
Phyllodoce longipes	C						2			1
Phyllodoce medipapillata	N		1							
Pterocirrus montereyensis	N						1			
Polynoidae										
Halosydna brevisetosa	N		1	5	3	6	4	5		2
Halosydna johnsoni	N		4	6	3	4	5	3		4
Halosydna sp.	N			1						
Sabellidae										
Paradialychone ecaudata	N			1	3					
Pseudopotamilla occelata	N				1					
Sabellidae	U			1						1
Serpulidae										
Hydroides gracilis	N			1	1		1		2	1
Hydroides sp.	U									2
Pseudochitinopoma occidentalis	N			1			3			2
Serpulidae	U						1			
Spionidae										
Polydora narica	N				1	1				
Spionidae	U				1					
Syllidae										
Eusyllis habei	C			1						
Exogone lourei	C				1					
Odontosyllis phosphorea	N						2			
Syllidae	U			1						
Syllis elongata	N							1		
Terebellidae										
Eupolymnia heterobranchia	N			4		1		1		1
Arthropoda										
Eucarida										
Decapoda										
Cancer jordani	N									1
Hemigrapsus oregonensis	N				1					
Heptacarpus paludicola	N								2	
Pugettia sp.	N		1			1				
Peracarida										
Amphipoda										
Allorchestes angusta	N		1		1		1			1
Ampithoe valida	I					3				
Aoridae	U		1		1				1	
Aoroides columbiae	N		3	6	5	4	6	6		7
Aoroides secunda	I			1					1	2
Elasmopus bampo	C		3	1	1			2	6	
Ericthonius brasiliensis	C				1	2	6	1		2
Gammaropsis shoemakeri	N				3	2	4			4
Jassa marmorata	I			1			1	2		
Jassa slatteryi	C			4	3	1	6	4		4
Jassa sp.	U		2	4	2	3	6	2		5

Morro Bay, 2013										
	Status	**Site**	**201 Main**	**City Harbor**	**Launch Ramp**	**Moorings**	**Morro Bay Marina**	**Sealion Dock**	**State Park Marina**	**Tidelands**
Jassa staudei	N					5	1	5		
Laticorophium baconi	N		1							
Liljeborgia geminata complex	C								1	1
Microjassa sp.	N			1						
Monocorophium acherusicum	I		5	5	6	6	7	6	4	6
Monocorophium insidiosum	I		2	3	4		2			1
Photis brevipes	N		3		2	3	1			3
Podocerus brasiliensis	C		4	3	6	5	7	6		5
Podocerus cristatus	C				1		1			
Pontogeneia rostrata	N									2
Protohyale frequens	N			1	1			1		
Protohyale sp. 1	N					1				1
Stenothoe valida	I				1					
Stenothoidae	U					1	1	3		3
Caprellidae										
Caprella californica	N		3	2	2		4	3	5	6
Caprella equilibra	C					3	2	4		2
Caprella mutica	I		4	6	6	6	7	6	3	5
Caprella simia	I		5	6	6	2	7	5		5
Deutella californica	N			1			1	2		1
Tritella sp.	N			1						
Isopoda										
Ianiropsis serricaudis	I				1					
Ianiropsis sp.	U		4	2	3		2		3	1
Paracerceis cordata	N				2					
Paracerceis sculpta	N		1			1			4	
Paranthura japonica	I		2		4	1	1	4		1
Tanaidae										
Leptochelia sp.	C		1							
Zeuxo sp.	U			1	1	1				
Cirripedia										
Balanus crenatus	N		6	6	6	6	7	6	1	7
Balanus trigonus	N		1		3		1			
Megabalanus sp.	U		2		2	2		1		1
Menesiniella aquila	N						1			
Pycnogonida										
Pycnogonum stearnsi	N						1			
Bryozoa										
Gymnolaemata										
Cheilostomatida										
Aetea pseudoanguina	N				1					1
Bugula neritina	I		5	6	2	6	3	5	6	3
Bugulina californica	N							1		
Bugulina longirostrata	N		2	5	4	1	6		5	6
Bugulina stolonifera	I				2	1	1		2	2
Celleporaria brunnea	N			2	2		4			3
Celleporella hyalina	C		5	1	5	1	1			4
Celleporina robertsoniae	N						3			
Crisularia pacifica	N			3						
Cryptosula pallasiana	I		3	1	3		1		1	4
Fenestrulina delicia	C		2		1		2			2
Fenestrulina sp.	U		1				1			1
Licornia diegensis	N		4	1	4		7			2
Membranipora villosa	N					1				
Microporella californica	N		1							
Parasmittina collifera	N		1		1					1
Pomocellaria californica	N			2				2		
Primavelans insculpta	N			1						1
Rhynchozoon sp.	U				1					
Rhynchozoon spicatum	N				1					

Morro Bay, 2013										
	Status	**Site**	**201 Main**	**City Harbor**	**Launch Ramp**	**Moorings**	**Morro Bay Marina**	**Sealion Dock**	**State Park Marina**	**Tidelands**
Schizoporella japonica	I		4	2	5		6		1	6
Schizoporella sp.	U									1
Scruparia ambigua	C		1		1					
Scruparia sp.	U									1
Smittoidea prolifica	N				1					
Tegella circumclathrata	N		1							
Watersipora arcuata	I								1	
Watersipora sp.	I							1		
Watersipora subovoidea	I									1
Watersipora subtorquata	I		6	6	5		7	3	6	7
Ctenostomatida										
Amathia dichotoma	I				4		1		6	
Bowerbankia sp.	U		4	6	5	6	5	3	1	7
Ctenostomatida	U		1							
Stenolaemata										
Cyclostomatida										
Crisia occidentalis	N		1	4	1		3	3		1
Filicrisia franciscana	N			2	2		3			1
Chordata										
Ascidiacea										
Aplousobranchia										
Phlebobranchia										
Stolidobranchia										
Aplousobranchia	U		1	1						
Ascidia ceratodes	N								1	
Ascidia sp.	U							1		
Botryllinae	I				2				1	2
Botrylloides sp.	I								1	
Botrylloides violaceus	I		2						6	
Botryllus schlosseri	I								4	
Didemnidae	U		1	1						2
Didemnum vexillum	I		3	1	4	2	4	3	1	1
Diplosoma listerianum	I		2	1		3			5	
Diplosoma sp.	U		1							
Distaplia occidentalis	N		2	4	1	5	1	3		1
Distaplia sp.	U		1	1			1		1	
Stolidobranchia	U		1				1			
Styela sp.	U		2	2	2	2	2	2		4
Styela truncata	N			1	1	4	2	1		
Echinodermata										
Asteroidea										
Asteroidea	U			1				2		
Echinoidea										
Echinoidea	U							1		
Strongylocentrotus sp.	N			2						
Mollusca										
Gastropoda										
Gastropoda										
Alia carinata	N			1	1		5	2		2
Alia tuberosa	N			1			1			
Amphissa sp.	N		1	2		1	2	2		
Crepidula sp.	U				1					
Crepipatella lingulata	N				1					
Marseniopsis sharonae	N		2							
Nudibranchia										
Cuthona albocrusta	N							1		
Dendronotus venustus	N							1		1
Doto form A of Goddard	N							1		
Hermissenda crassicornis	N			4	1	1	1	2		2
Janolus barbarensis	N		1							

Morro Bay, 2013										
	Status	**Site**	**201 Main**	**City Harbor**	**Launch Ramp**	**Moorings**	**Morro Bay Marina**	**Sealion Dock**	**State Park Marina**	**Tidelands**
Okenia angelensis	N		1							
Polycera atra	N		1	3				1	3	
Polycera hedgpethi	N			2						
Triopha maculata	N		1							
Bivalvia										
Crassadoma gigantea	N			2						
Hiatella arctica	C		5	6	5	5	6	6	2	5
Leptopecten latiauratus	N			2	1		1			
Modiolus sp.	N		1	5	1	1	4	3		1
Mytilus sp.	U		4	2	1	1	2			1
Pododesmus cf. macrochisma	N		4	4	3		4	3		2
Platyhelminthes										
Leptoplanidae	U			1						
Polycladida	U					1				
Pseudoceros sp.	N			1						
Stylochoplana sp.	N			1						1
Stylochus franciscanus	N					1				

Bodega / Tomales Bay 2012

Bodega and Tomales Bays, 2012	Status	Site	Coast Guard	Lucas / Tides	Porto Bodega	Spud Point North	Spud Point South	Tomales- Call Box 401	Tomales- Shell Beach	Tomales- Marshall	Tomales- Nick's Cove	Tomales- SNPS
Annelida												
Polychaeta												
Capitellidae												
Capitella capitata complex	C			2								
Chrysopetalidae												
Paleanotus bellis	N		1									
Cirratulidae												
Ctenodrilus serratus	C									1		
Dorvilleidae												
Dorvillea schistomeringos longicornis	N										1	
Hesionidae												
Oxydromus pugettensis	N								1	1	2	
Nereididae												
Nereididae	U								1			
Nereis latescens	N			1							1	
Nereis sp.	N									1		
Platynereis bicanaliculata	N		5	5	3	4	2	1	1	5	5	
Opheliidae												
Armandia brevis	N				2					2		
Phyllodocidae												
Eteone balboensis	N										1	
Eulalia quadrioculata	N		1									
Eulalia sp.	U		1									
Eumida longicornuta	N				1							
Polynoidae												
Harmothoe imbricata complex	C		4	1	2	2	2		2	2		2
Sabellidae												
Myxicola sp. A	C		1							1		
Paradialychone ecaudata	N		2		1						1	
Spionidae												
Dipolydora socialis	C			1							1	
Dipolydora sp.	U			1								
Polydora cf. websteri	C										1	
Polydora narica	N								1	2	3	
Polydora nuchalis	N			2								
Polydora sp.	U			1						1	1	
Syllidae												
Megasyllis nipponica	I								1	1		2
Myrianida sp.	U			1								
Odontosyllis phosphorea	N		1									
Syllidae	U		2									1
Syllis sp.	U										1	
Typosyllis aciculata	N										1	
Typosyllis sp.	U					1						
Terebellidae												
Eupolymnia heterobranchia	N		2	2	1	1	1			2	4	
Neoamphitrite sp. A	I								1			
Streblosoma sp. B	U											3
Terebellidae	U		1								1	
Polychaeta	U		2									
Arthropoda												
Eucarida												
Decapoda												
Cancer jordani	N		1									
Heptacarpus paludicola	N							1				
Heptacarpus sp.	N		1									
Pachycheles sp.	N		1									
Pugettia producta	N		1									
Peracarida												
Amphipoda												
Allorchestes angusta	N		1									
Ampithoe lacertosa	C		4	1	2	1	1					
Ampithoe sectimana	N		1									
Ampithoe sp.	U		1		1							
Ampithoe valida	I								2			
Aoroides columbiae	N		5									
Aoroides secunda	I			1	3	1	3			4		5
Aoroides sp.	U		1	1	1	3	1	1				1

Bodega and Tomales Bays, 2012	Status	Site	Coast Guard	Lucas / Tides	Porto Bodega	Spud Point North	Spud Point South	Tomales-Call Box 401	Tomales-Shell Beach	Tomales-Marshall	Tomales-Nick's Cove	Tomales-SNPS
Ericthonius brasiliensis	C		3					1				
Gammaropsis thompsoni	N		4									
Grandidierella japonica	I			1								
Jassa marmorata	I										1	
Jassa slatteryi	C						1	1		4	2	2
Jassa sp.	U						1				3	2
Jassa staudei	N		1									
Laticorophium baconi	N					1	1	2		3	1	
Melitidae	U									1		
Monocorophium acherusicum	I		3	4	4			2		4	2	
Monocorophium sp.	U		1				1	1			1	
Monocorophium uenoi	I		1	1			2	1				
Photis brevipes	N		2				1					
Photis sp.	N		3				1					
Podocerus cristatus	C		5									
Pontogeneia rostrata	N		1					2				
Stenothoe valida	I							1				
Caprellidae												
Caprella californica	N		2	4	3	1	3			4	5	
Caprella equilibra	C							1				
Caprella mutica	I		5	4	5	3	5	2		5	5	3
Caprella simia	I							1				
Caprellidae	U											1
Deutella californica	N		1									
Isopoda												
Ianiropsis serricaudis	I				1						1	1
Ianiropsis sp.	U		1	1	1	1				1	1	
Idotea sp.	N		2	1								
Janiralata occidentalis	N		1									
Paracerceis cordata	N		3	3	2	1	3		2	5	3	3
Paranthura japonica	I							1		3		2
Tanaidae												
Leptochelia sp.	C		3		1							
Zeuxo normani	C			1	1	1		1	1	2	5	6
Zeuxo sp.	U										1	
Cirripedia												
Balanus crenatus	N		5	3	3	2	1				2	
Balanus glandula	N										2	
Crustacea												
Copepoda	U							1				
Bryozoa												
Gymnolaemata												
Cheilostomatida												
Bugula neritina	I		5			1				5		4
Bugula sp.	U					1						
Bugulina stolonifera	I								3	5	1	
Conopeum sp.	U								5	2	1	4
Conopeum tenuissimum	I									1		4
Crisularia pacifica	N		4			2	1					
Cryptosula pallasiana	I		1	4	1					1		
Parasmittina sp.	U		4									
Primavelans insculpta	N								5			7
Schizoporella errata	I								1			
Schizoporella japonica	I		3	3	4	4	4		2			6
Schizoporella sp.	U									1		
Watersipora sp.	I			1								
Watersipora subtorquata	I		4	4	3	5	5		5	1	2	4
Ctenostomatida												
Bowerbankia sp.	U		4	1	1	2				1	1	2
Nolella sp.	U		1									
Echinodermata												
Ophiuroidea												
Amphipholis squamata	C											1
Mollusca												
Gastropoda												
Gastropoda												
Haminoea japonica	I											3
Lacuna sp.	N		1									

Bodega and Tomales Bays, 2012	**Status**	**Site**	**Coast Guard**	**Lucas / Tides**	**Porto Bodega**	**Spud Point North**	**Spud Point South**	**Tomales-Call Box 401**	**Tomales-Shell Beach**	**Tomales-Marshall**	**Tomales-Nick's Cove**	**Tomales-SNPS**
Nudibranchia												
Dendronotus venustus	N		3			3						
Flabellina trilineata	N			1								
Hermissenda crassicornis	N		1	1								
Nudibranchia	U				2							
Sacoglossa												
Placida dendritica	C									1		
Bivalvia												
Modiolinae	N						1					
Musculista senhousia	I									3	1	1
Mytilus sp.	U									1		
Simomactra sp.	U		1		1							
Chordata												
Ascidiacea												
Aplousobranchia												
Aplidium sp. A	U											1
Aplousobranchia	U					1						
Didemnidae	U											1
Didemnum sp.	U											1
Didemnum vexillum	I								5	1	4	5
Diplosoma listerianum	I		4		3	3	2			5	5	4
Distaplia occidentalis	N		5	4	4	5	3			4	4	1
Distaplia sp.	U		1				1			1		
Phlebobranchia												
Ascidia ceratodes	N		2			1				2	2	
Ascidia sp.	U		3	2	1	1				1		2
Ascidia zara	I		1						2		1	
Ciona intestinalis	I								1	4	2	1
Ciona sp.	U								1	2		1
Perophora annectens	N											1
Perophora japonica	I											2
Perophora sp.	U					1	1					1
Phlebobranchia	U			1								1
Stolidobranchia												
Botryllinae	I		4	1	1	2	1		2	1		4
Botrylloides sp.	I			1		1				1		2
Botrylloides violaceus	I		3	5	4	5	5		3	5	5	5
Botryllus schlosseri	I			5	2	1			1	4	5	2
Botryllus sp.	I									1		
Molgula manhattensis	I								1			
Molgula sp.	U											1
Nemertea												
Nemertea	U		1									
Palaeonemertea	U								1			
Zygonemertes virescens	C				1							
Platyhelminthes												
Acanthozoon lepidum	N			1	1						1	
Acerotisa californica	N		1			1					1	
Acotylea	U					1						
Polycladida	U					1						
Pseudoceros sp.	N		3	2	1	2					1	

San Francisco Bay 2011-2013

San Francisco Bay, 2011–2013	Status	Site	Antioch Marina		Ballena Isle Marina			Benicia Marina	Berkeley Marina	Corinthian Yacht Club	Coyote Point Marina			Emeryville	Glen Cove Marina		Jack London Square Marina			Loch Lomond Marina			Oyster Point Marina			Paradise Cay Yacht Harbor	Petaluma Marina	Pittsburg Marina		Port of Oakland	Port of San Francisco Pier 31	Port of Stockton	Redwood City Marina			Richmond Marina Bay Yacht			Sacramento Marina	San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor			South Beach Harbor	Vallejo Marina
	N/I/C/U	Year	11	12	11	12	13	11	11	11	11	12	13	12	11	12	11	12	13	11	12	13	11	12	13	11	11	11	12	12	12	12	11	12	13	11	12	13	12	11	12	13	11	12	13	11	12	13	11	11
Annelida																																																		
Polychaeta																																																		
Capitellidae																																																		
Capitella capitata complex	C													1																				1	3			4												
Capitellidae	U																																											1						
Chrysopetalidae																																																		
Paleanotus bellis	N																																								2	2								
Cirratulidae																																																		
Cirratulidae	U																							1																				1						
Cirratulus dillonensis	N																																	1										1						
Cirriformia cf. moorei	N												1					1					1																											
Cirriformia sp.	N					1							1										1	1											1	2			3						1						
Cirriformia sp. SD2	N																																	1	2									3	1					
Ctenodrilus serratus	C					2								3																								1							1						
Dorvilleidae																																																		
Dorvillea schistomeringos longicornis	N					2																																	4												
Dorvillea schistomeringos sp.	U					1																												1				1												
Ophryotrocha sp.	U																						1												1															
Glyceridae																																																		
Glycera americana	C																																				1													
Nereididae																																																		
Alitta succinea	I																														1																			
Neanthes acuminata complex	C																			1																														
Nereis latescens	N																	1													3																			
Nereis vexillosa	N																							1							3						1													
Platynereis bicanaliculata	N						2																														1		4				2						3		
Orbiniidae																																																		
Naineris dendritica	N																																						2												
Phyllodocidae																																																		
Phyllodocidae	U																																	1																
Polychaeta																																																		
Polychaeta	U																	1																										1						
Polynoidae																																																		
Halosydna johnsoni	N																								1											1														
Harmothoe imbricata complex	C					5	4		1			2	5	3		1	3	4	4		1	4	1	4	2					5	3			4	4		5	5					1	5	1		4	3	1	
Harmothoe sp.	C																							1							1																			
Serpulidae																																																		
Ficopomatus enigmaticus	I							5		1					5	2				5	3	1					5						1	1									2							5
Hydroides sp.	U									3																																								
Pseudochitinopoma occidentalis	N									1																														1										
Serpulidae	U															1																1																	1	
Spionidae																																																		
Dipolydora socialis	C																															1														1		2		
Dipolydora sp.	U																							1																										
Polydora cornuta	I						1					1		1		2	1														2											1								
Polydora narica	N																														1																1			
Polydora sp.	U													1											1																									
Syllidae																																																		
Autolytinae	U																								1																									
Exogone lourei	C						3																		1																									
Exogonidae	U																						1																											
Megasyllis nipponica	I					5	4					4	5					5	4			4		3	3					4				4	5		2	5			2	1		3	1		4	3	1	
Syllidae	U													1																																				
Syllis sp.	U													1																																				
Typosyllis sp.	U													1					1			1		1																										

San Francisco Bay, 2011–2013	Status	Site	Antioch Marina		Ballena Isle Marina			Benicia Marina	Berkeley Marina	Corinthian Yacht Club	Coyote Point Marina			Emeryville	Glen Cove Marina		Jack London Square Marina			Loch Lomond Marina			Oyster Point Marina			Paradise Cay Yacht Harbor	Petaluma Marina	Pittsburg Marina		Port of Oakland	Port of San Francisco Pier 31	Port of Stockton	Redwood City Marina			Richmond Marina Bay Yacht H			Sacramento Marina	San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor			South Beach Harbor	Vallejo Marina
	N/I/C/U	Year	11	12	11	12	13	11	11	11	11	12	13	12	11	12	11	12	13	11	12	13	11	12	13	11	11	11	12	12	12	12	11	12	13	11	12	13	12	11	12	13	11	12	13	11	12	13	11	11
Terebellidae																																																		
Eupolymnia heterobranchia	N					2																																												
Neoamphitrite sp. A	U					1	1						1	1				2				1		1	1					1				3	4			2						2	1					
Nicolea cf. amnis	U					3	3							1				5	5																			1												
Terebellidae	U					1																		1																										
Oligochaeta																																																		
Oligochaeta	U																																						1											
Arthropoda																																																		
Eucarida																																																		
Decapoda																																																		
Hemigrapsus sp.	U																				1											1																				
Heptacarpus sp.	N																															2											1									
Palaemon macrodactylus	I																				1	1								1																						
Peracarida																																																		
Amphipoda																																																		
Americorophium spinicorne	N		3	5												1													3	4			4																		
Amphipoda	U															1																																		
Ampithoe lacertosa	C					2	3						3					4	2					1	1										3	2			2			3	1			1					
Ampithoe valida	I														1	2					3					1																1								
Aoroides secunda	I						1					2							1																							2	4					5	5		
Aoroides sp.	U											1		1																												1					1				
Aruga holmesi	C																														1																				
Corophiidae	U																																				1														
Elasmopus bampo	C																																										1								
Ericthonius brasiliensis	C																															2										1	4								
Gammarus daiberi	I			1																									1	3			5																		
Grandidierella japonica	I					1		1				2	1			2		1		1	5			1						1					2	4			3						2	1					
Hadzioidea	U					1																																												
Hyalella azteca	C			2																																														
Incisocalliope derzhavini	I																				5																													
Jassa marmorata	I																														1																				
Jassa slatteryi	C																														2	3									1		2	2								
Jassa sp.	U															1															2	3											1						1			
Laticorophium baconi	N											4	1								4				1						2				1		1						1	2		1			1	1		
Leucothoe alata	C					1	5						5					5	5					4	2						1				4	5			4							1					
Liljeborgia geminata complex	C																	2	2																															
Lysianassoidea	U																	1																																
Melitidae	U																																						1											
Metopa cistella	N																																										1								
Monocorophium acherusicum	I											3	1			1					3				2	1					1	2			2		2					1	3		1	1		1	1		
Monocorophium insidiosum	I												1								1	4			2											2			1										1		
Monocorophium sp.	U					1																												1			1	1				2									
Monocorophium uenoi	I											1						1			1	3													1										1			1			
Paradexamine sp.	I					1																																										3	1		
Photis brevipes	N																															1										1	1								
Photis sp.	N																															1										1									
Podocerus brasiliensis	C																															2																			
Polycheria osborni	N																															1																			
Protohyale frequens	N																																									1									
Stenothoe valida	I																														2	3																			
Caprellidae																																																		
Caprella californica	N																														1	4									1	1									
Caprella equilibra	C								1	1																					2	2																			
Caprella mutica	I				4	4	1		3	2			3	5			3	4	3				2	2	1						5	4			4	4	5	5	5		1	4	4		2		2	3	5	1	
Caprella penantis	C																																										1								

San Francisco Bay, 2011–2013	Status	Site	Antioch Marina		Ballena Isle Marina			Benicia Marina	Berkeley Marina	Corinthian Yacht Club	Coyote Point Marina			Emeryville	Glen Cove Marina		Jack London Square Marina			Loch Lomond Marina			Oyster Point Marina			Paradise Cay Yacht Harbor	Petaluma Marina	Pittsburg Marina		Port of Oakland	Port of San Francisco Pier 31	Port of Stockton	Redwood City Marina			Richmond Marina Bay Yacht H			Sacramento Marina	San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor			South Beach Harbor	Vallejo Marina
	N/I/C/U	Year	11	12	11	12	13	11	11	11	11	12	13	12	11	12	11	12	13	11	12	13	11	12	13	11	11	11	12	12	12	12	11	12	13	11	12	13	12	11	12	13	11	12	13	11	12	13	11	11
Caprella scaura	I																																												1			3				
Caprella simia	I					4	5			1		2	3	1			2	2	4					4	3					4	3						3	3		4	4	4					2	4	2	
Caprella sp.	U				1							1												1							1										1									
Caprella sp. 3	U																													2																				
Caprellidae	U																				1																													
Deutella californica	N																														1																			
Isopoda																																																		
Colidotea rostrata	N																				1																													
Gnorimosphaeroma oregonensis	N															4					5									2							1													
Ianiropsis analoga	N																	1													1																			
Ianiropsis serricaudis	I																	1																																
Ianiropsis sp.	U					2												1	4												1								1										1	
Idotea rufescens	N																				1																													
Paranthura japonica	I					5	4					5	5	5				5	4			4		2	3					3		1		5	5		4	3						2	3		4	1		
Sphaeroma quoianum	I																					1													1		1													
Synidotea laevidorsalis	I															1					1										1																			
Uromunna sp.	U			2												2		2												4	1		2																	
Tanaidae																																																		
Leptochelia sp.	C																																								1						1			
Sinelobus sp.	I							4							5	2		1									5		5																					4
Zeuxo sp.	U					1																																		1										
Cirripedia																																																		
Amphibalanus amphitrite	I																3			1		1													1															
Amphibalanus improvisus	I				3	2	5	5	5	1		3	2		5	2	3	1	2	5			4	3	5	2	5		1		2		5	1	3	5	1	3					1						2	5
Amphibalanus sp.	U																												1																					
Balanidae	U									1																																								
Balanus crenatus	N				1	1	4		1	4		1					1	1	1					4	4	3					5					3	1	1		3	5	5				5	5	5	2	
Crustacea																																																		
Copepoda	U					1						1														1																								
Daphniidae	U																																						2											
Nebalia gerkenae	U												1																																					
Insecta																																																		
Chironomidae	U																															1							2											
Insecta	U		1																													1																		
Pycnogonida																																																		
Ammothea hilgendorfi	N																	1																										1						
Ammothella sp.	U																																											2						
Anopolodactylus sp.	U																														3																			
Callipallenidae	U																																											2						
Phoxichilidium sp.	U																																											3						
Pycnogonidae	U																																											1						
Bryozoa																																																		
Gymnolaemata																																																		
Cheilostomatida																																																		
Bugula neritina	I				5	5	5				1	3	4	5			5	5	5		1	5	3	5	5					1			5	5	5	1	5	5					3	5	4	1	4	3		
Bugula sp.	U					1																									1					2													1	
Bugulina longirostrata	N																														5																			
Bugulina stolonifera	I				5	2	3		5	5	5	4	1	4			5	2	2	1	5	4	5	4	5	2				2			5	5	3	5	4	4		4			4	5	4	5	5	3	5	
Caulibugula ciliata	N																														4																			
Celleporella hyalina	C																														5									1	2	1						1		
Cheilopora praelonga	N																														1																			
Conopeum reticulum	C																														1																			
Conopeum sp.	U					3		5							2		1			3	1	1	1		1	1								1		1	1	1						4						3
Conopeum tenuissimum	I					1		1												3			1								1			1		1								3						3
Crisularia pacifica	N				3		1			2	1	1									2	1	4		3	3					5					2				1						1				

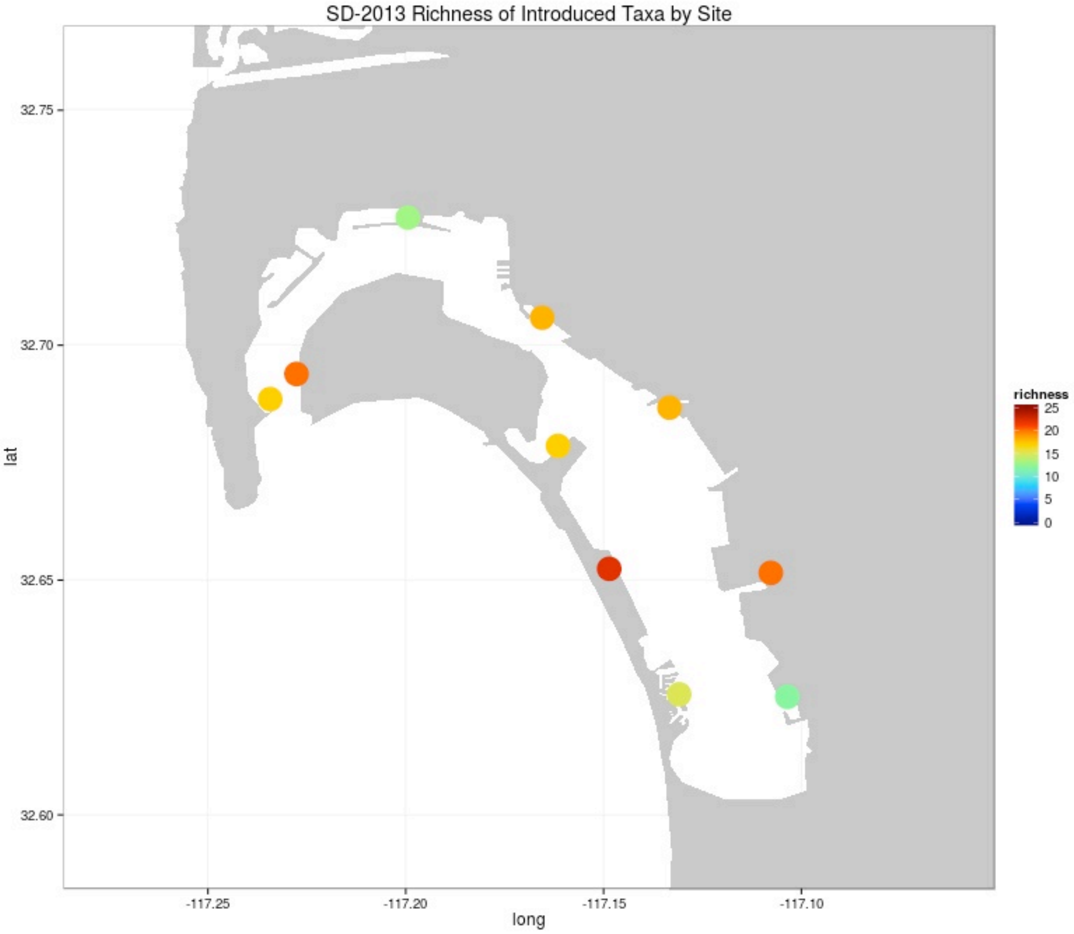
San Francisco Bay, 2011–2013	Status	Site	Antioch Marina		Ballena Isle Marina			Benicia Marina	Berkeley Marina	Corinthian Yacht Club	Coyote Point Marina			Emeryville	Glen Cove Marina		Jack London Square Marina			Loch Lomond Marina			Oyster Point Marina			Paradise Cay Yacht Harbor	Petaluma Marina	Pittsburg Marina		Port of Oakland	Port of San Francisco Pier 31	Port of Stockton	Redwood City Marina			Richmond Marina Bay Yacht			Sacramento Marina	San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor			South Beach Harbor	Vallejo Marina
	N/I/C/U	Year	11	12	11	12	13	11	11	11	11	12	13	12	11	12	11	12	13	11	12	13	11	12	13	11	11	11	12	12	12	12	11	12	13	11	12	13	12	11	12	13	11	12	13	11	12	13	11	11
Cryptosula pallasiana	I				5	4	2		5	5			2	4			3	1			1	2	2	3	1	4										5	3	3		3	4	3	1	2		5	4	3	5	
Cryptosula sp.	U													1																																				
Einhornia crustulenta	N														2																																			
Electra sp.	U																			1																														3
Electra sp. B	U														2	1																													1				
Membranipora chesapeakensis	I							5							2					1										5																				2
Schizoporella errata	I																														5																	1	
Schizoporella japonica	I									5																								1					5		1				3	4	1	2	
Scrupocellaria sp.	U																												1																				
Smittoidea prolifica	N				1				2				2				3	2	1	1	1	3	5	3	5	1				1						4	2						1	1					2	
Tricellaria occidentalis	N				3	1						5		2			5	3	2		2	2	1	1	3						1						5	3					1							
Watersipora sp.	I																												1																				
Watersipora subtorquata	I				4	4	1		1	5				3			4	3	1					1	2					3	5					1	3	5		5	5	3				5	5	4	3	
Ctenostomatida																																																		
Alcyonidium sp.	U																													2																			
Anguinella palmata	I																												1	2													2						
Bowerbankia sp.	U							4							4					3				2	1	5	2			1	4			3						1	4	2	2	5	5	2	2			4
Victorella pavida	I																									3	1																						
Phylactolaemata																																																		
Fredericella indica	I			1																							5				2																		
Fredericella sp.	I		2	3																							2				3							2											
Pectinatella magnifica	I																										1				3							5											
Plumatella repens	U		5	1																							3																						
Plumatellida	U			1																											1							2											
Stenolaemata																																																		
Filicrisia franciscana	N																													1									1										
Chordata																																																		
Ascidiacea																																																		
Aplousobranchia																																																		
Didemnidae	U						1					1									1		1		1												2	1		4		1			4		1			
Didemnum sp.	U																																		1		1		1									1	
Didemnum vexillum	I					3						3	5											4	3					3	4			2			3	3			5	1		2	1		4	5		
Diplosoma listerianum	I					4	5					5	2	5				4	5					2	5					2					1		5	5				3						5		
Distaplia occidentalis	N																													2										1	1						3		
Distaplia sp.	U																																								2								
Phlebobranchia																																																		
Ascidia sp.	U											2		1				1			1									1				1					1				1					2	
Ascidia zara	I				5	5	5			4	2	5	3	5			5	5	5			4	5	5	5	3				5			2	5	2	5	4	3		2				3	5	2	3		4	
Ciona intestinalis	I					5	3					4					1	5	3					4	3					5				5	4		4	3											
Ciona savignyi	I				4	3	5			2			1	5			5	5	4			1	2	4	3	2				5	1			5	5	1	3	1								2	4		1	
Ciona sp.	I				1	2	2			2		3	2	2			1		2			1	1	2	1	1					3						1	2		3	2	1			2				2	
Corella inflata	I																																							1		2					5	1		
Perophora annectens	N																																																1	
Phlebobranchia	U																	1																																
Stolidobranchia																																																		
Botryllinae	I				4	3	4		3	1	4	2	4				5	1	4		1	1	5	3	3	1					1		3	2	3	3	3	5		3		4	2	1	3	2	1	1	1	
Botrylloides diegensis	I						1					1													2												1													
Botrylloides sp.	I				1	5	2			1		3	1	3			2				1			4	2					3	1			2	2		3	1												
Botrylloides violaceus	I				5	3	3		2		4	4	1	2			4				2	2	4	2	3					3	5				1	5	2	5		5	5	3				5	5	5		
Botryllus schlosseri	I				5	3	4		5		5	1	3	4			4	2	2	1	4	2	5	3	4	1				4	3		5	4		5	2	5		5	4		5	1		5	5	1	5	
Botryllus sp. A	I												1						1																			1										1		
Molgula manhattensis	I				5	5	4		4	3	5	5	5	5		3	5	3	4	5	4	5	3	5	4	5				2	4		5	5	5	3	4	5		5			4	5	5	2	2		4	
Molgula sp.	U																				1				1																									
Stolidobranchia	U															1																																		
Styela clava	I					1							3											2	2					1						2				1										

San Francisco Bay, 2011–2013	Status	Site	Antioch Marina		Ballena Isle Marina			Benicia Marina	Berkeley Marina	Corinthian Yacht Club	Coyote Point Marina			Emeryville	Glen Cove Marina		Jack London Square Marina			Loch Lomond Marina			Oyster Point Marina			Paradise Cay Yacht Harbor	Petaluma Marina	Pittsburg Marina		Port of Oakland	Port of San Francisco Pier 31	Port of Stockton	Redwood City Marina			Richmond Marina Bay Yacht			Sacramento Marina	San Francisco Marina			San Leandro Marina			Sausalito Marine Harbor			South Beach Harbor	Vallejo Marina
	N/I/C/U	Year	11	12	11	12	13	11	11	11	11	12	13	12	11	12	11	12	13	11	12	13	11	12	13	11	11	11	12	12	12	12	11	12	13	11	12	13	12	11	12	13	11	12	13	11	12	13	11	11
Styela sp.	U				1					1	2								1				1		3	3							2	1		3	1	1		2				3			3		2	
Cnidaria																																																		
Anthozoa																																																		
Actiniaria	U																						1																											
Diadumene leucolena	I																	1																																
Hydrozoa																																																		
Gonothyraea clarki	C										1																																							
Mollusca																																																		
Gastropoda																																																		
Alia carinata	N																														1																			
Haminoea japonica	I					4																																					2						
Lacuna sp.	N																														2																			
Odostomia sp.	U																	1																																
Planorbidae	N			1																																														
Urosalpinx cinerea	I																																											1					
Nudibranchia																																																		
Ancula pacifica	N																														1																			
Catriona rickettsi	I																														2																			
Cumanotus sp.	N																													1																				
Cuthona albocrusta	N								1																																									
Eubranchus misakiensis	I								1																																									
Okenia plana	I																																																1
Sacoglossa																																																		
Elysia hedgpethi	N																								1																									
Placida dendritica	C				1				1																																									
Bivalvia																																																		
Hiatella arctica	C																																						2										
Modiolus sp.	N																																						1						1				
Musculista senhousia	I																	1	2																					1								1	
Mytilidae	U									1							1		2																								1						
Mytilus sp.	U				1				1	4					1			1		2		1				5				1	4					2	1			5	2	4				4	1		4	
Ostrea lurida	N						1			1		1	4						4			2			5	2					1					1				3		3							3	
Ostreidae	U				2					1	1	1					2	2			1		2			3										1				2	1								2	
Pododesmus cf. macrochisma	N																														1									1										
Veneridae	U																	1																						1									1	
Polyplacophora																																																		
Chitonida	N																																													1			
Nemertea																																																		
Nemertea	U																																											1					
Palaeonemertea	U																																													1			
Zygonemertes virescens	C																																							1						1			
Platyhelminthes																																																		
Acerotisa californica	N																																													1	2		
Polycladida	U																							1																									
Pseudoceros sp.	N																	1																															
Stylochus franciscanus	N																							1																1									
Stylostomum lentum	N					1						1																																					

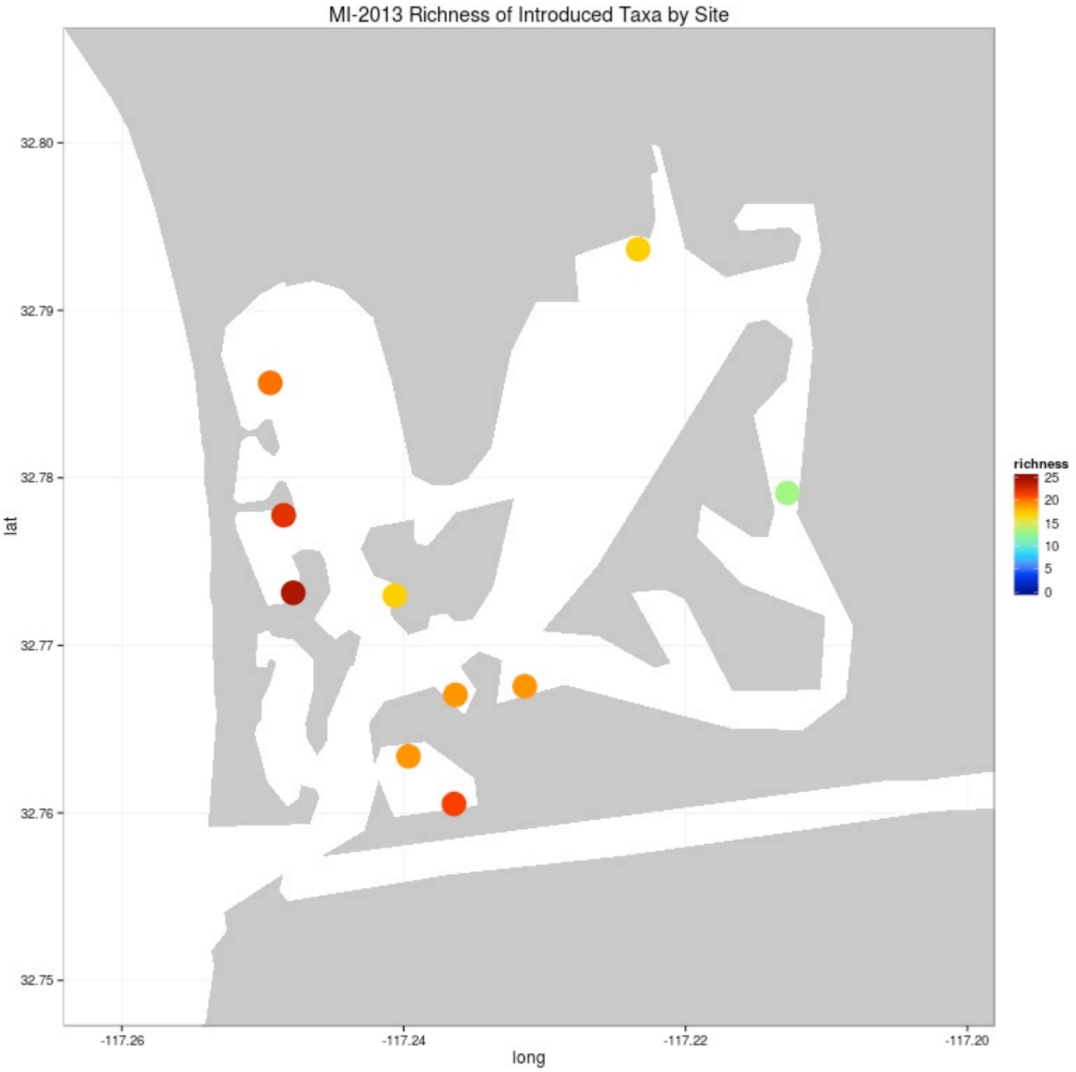
Appendix 2.3: Non-Native Species Richness by Estuary and Year

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.

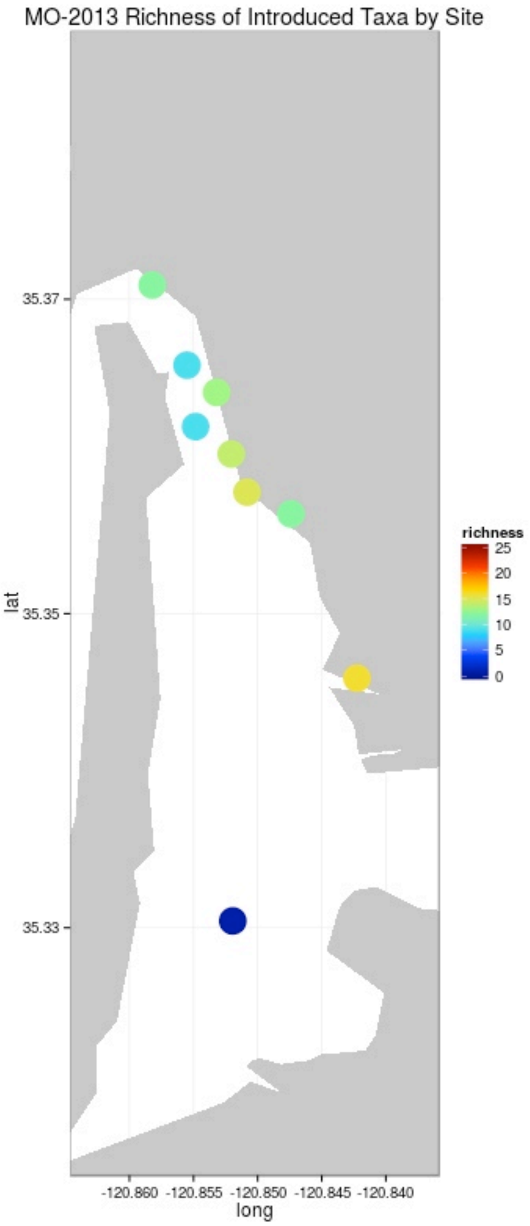
San Diego Bay 2013



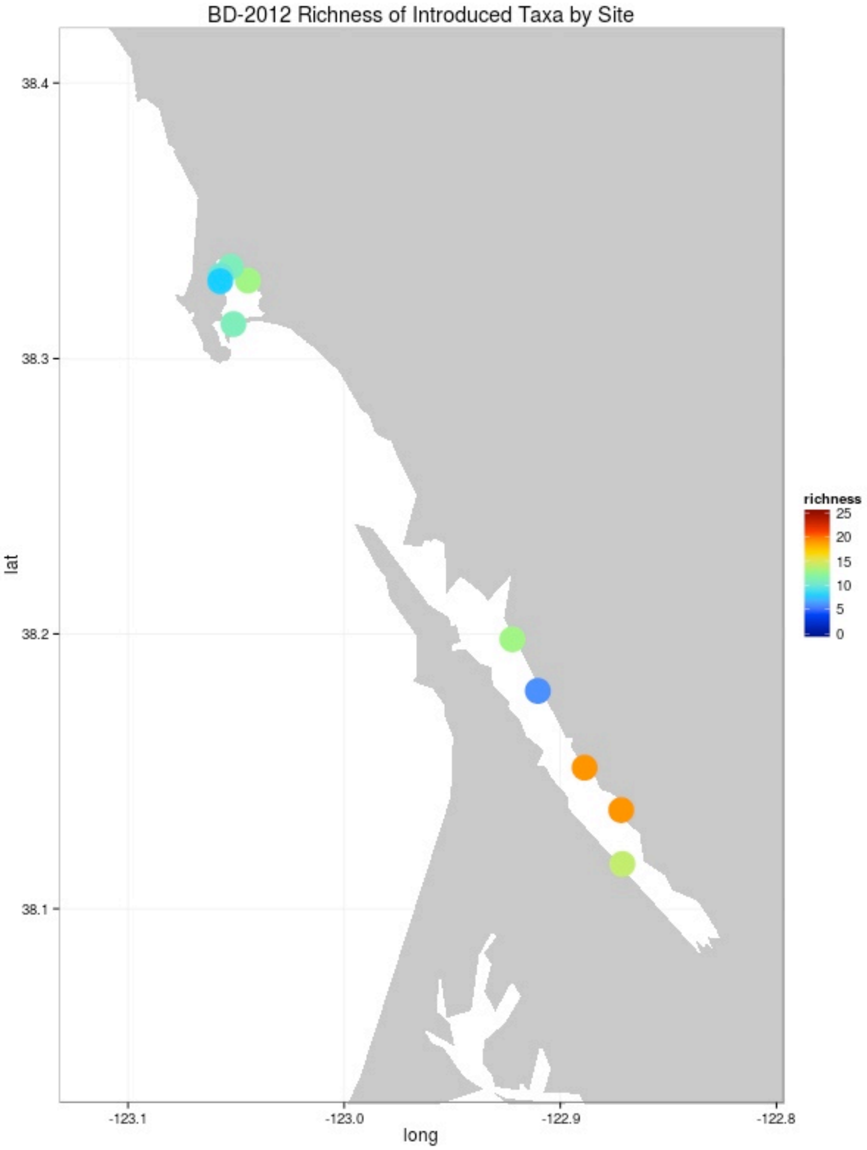
Mission Bay 2013



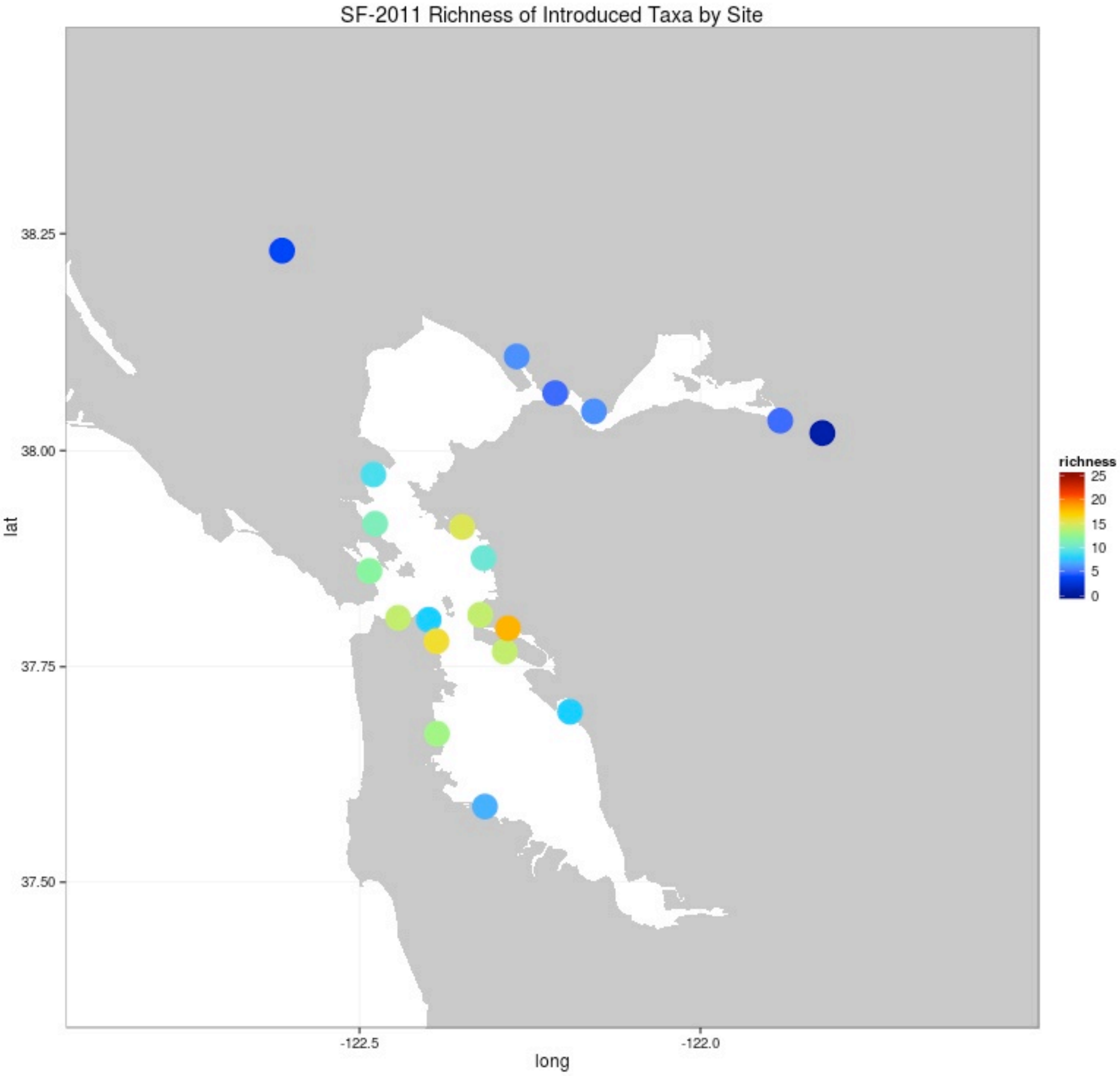
Morro Bay 2013



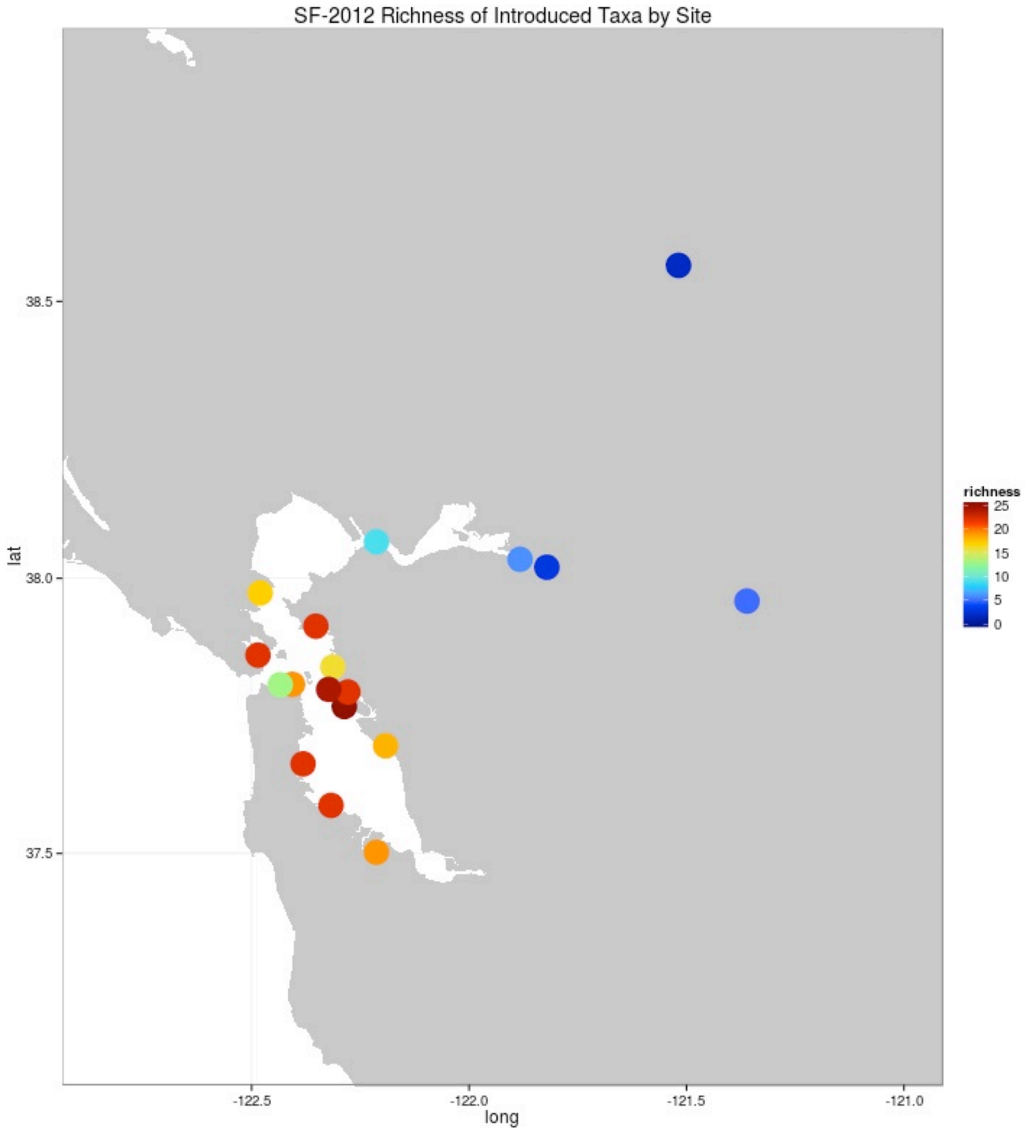
Bodega / Tomales Bay 2012



San Francisco Bay 2011

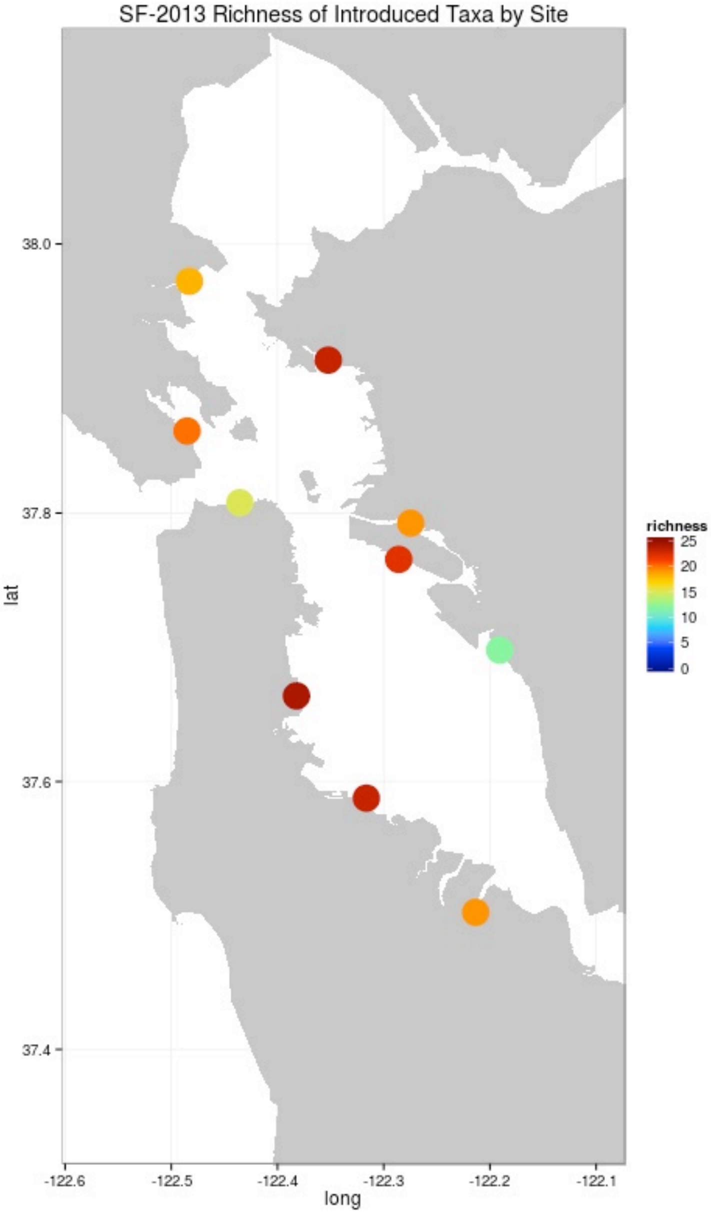


San Francisco Bay 2012



San Francisco Bay

2013



Chapter 3: Soft Sediment Communities

Introduction

A. Field Collections

Surveys of invertebrate communities in soft sediment habitats were conducted in two major embayments, San Francisco Bay and San Diego Bay. San Francisco Bay was sampled in both 2012 and 2013, while San Diego Bay was sampled in 2013. We used a stratified sampling scheme to sample at ten stations in the high salinity region in each Bay, with an additional five stations in the low salinity region in San Francisco Bay in 2013. 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 85 (Yellow Springs Instruments), and depth was recorded using a depth sensor on the boat.

In San Francisco Bay in 2012, we sampled ten stations in the higher-salinity region of the Bay at intertidal, shallow (2m below MLLW), and deep (5m below MLLW) depths. Five replicate grab samples were collected at 200m intervals at each station and depth generating a total of 100 samples (5 replicates x 1 depth x 5 stations) + (5 replicates x 3 depths x 5 stations).

In San Francisco Bay in 2013, we sampled shallow depths (2m below MLLW) at ten stations in the higher salinity region and five stations in the brackish-to-freshwater region. Five replicate grab samples were collected at 200m intervals at each station and depth generating a total of 75 samples (5 replicates x 5 stations) + (5 replicates x 10 stations).

In San Diego Bay in 2013, we sampled shallow depths (2m below MLLW) at ten stations throughout the Bay. Five replicate grab samples were collected at 200m intervals at each station, generating a total of 50 samples (5 replicates x 10 stations).

We used a standard Young-modified Van Veen grab (Dauer 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:

1. Coarse sorting and removal of polychaete taxa in the field following examination under dissecting microscopes, with vouchers taken for genetics.

2. Laboratory sorting of grab samples using dissecting microscopes where necessary and identification by in-house experts to the lowest taxonomic level (species level in 72% of cases)

using broad California fauna identification keys (Kozloff 1996, Carlton 2007), more specialized keys for specific groups, and consulting taxonomic experts.

3. Verification of morphological voucher identification. A subset of samples were 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 to confirm or revise morphological identifications.

Voucher specimens of each morphotaxon were taken from each sample. Where possible, the same organisms were split into a morphological and a DNA sample to provide direct comparisons of genetic and morphological identifications. All voucher specimens were labeled with a unique identification number.

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.*, 2003; Ruiz *et al.*, 2011). Four categories were used for this classification: native, introduced (NIS), 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 date, and separately for freshwater and marine sites in San Francisco Bay in 2013.

Results

A. Overall Summary

San Francisco Bay 2012

75 morphospecies were recorded: 34 native, 28 NIS, 4 cryptogenic and 9 unresolved species. Native species accounted for a total of 1,837 individuals (17% of the community), NIS species for 8,714 individuals (82% of the community), cryptogenic for a total of 78 individuals (1% of the community) and unresolved species for 62 individuals (1% of the community).

San Francisco Bay 2013

74 morphospecies were recorded: 29 native, 25 NIS, 4 cryptogenic and 16 unresolved species. Native species accounted for a total of 3,619 individuals (25% of the community), NIS species for 10,421 individuals (72% of the community), cryptogenic for a total of 73 individuals (1% of the community) and unresolved species for 327 individuals (2% of the community).

San Diego Bay 2013

88 morphospecies were recorded: 51 native, 8 NIS, 4 cryptogenic and 25 unresolved. Native species accounted for a total of 4,017 individuals (58% of the community), NIS species for 2,103 individuals (30% of the community), cryptogenic for a total of 129 individuals (2% of the community) and unresolved for 711 individuals (10% of the community).

B. Detection of NIS in San Francisco and San Diego 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 San Diego Bays. The figures below show the detection of species in the high salinity portion of both bays (2012 and 2013 for San Francisco, and 2013 for San Diego), and in the low salinity portion of San Francisco Bay in 2012. The accumulation curves show the rarefaction of species richness. Calculations were performed using the R package vegan 2.3 (R Core Team 2015; Oksanen 2015).

Total NIS Richness In San Francisco and San Diego Bays.

NIS richness approached an asymptote rapidly in shallower habitats in San Francisco Bay in both 2012 (Figure 3.1b, d) and 2013 (Figure 3.2b, d), but not in deeper habitats (Figure 3.1f). In San Francisco Bay, the rapid leveling off of the accumulation curve in shallower depths, particularly the intertidal, indicates that this habitat was sufficiently sampled to capture all NIS predicted to occur in this region. We observed 28 NIS in San Francisco Bay in 2012 and 25 in 2013, while we found a total of just 8 NIS in San Diego Bay in 2013. NIS richness also approached an asymptote in both high salinity and lower salinity regions of San Francisco Bay during the 2012 surveys (Figure 3.2b, d). In San Diego Bay, NIS richness did not appear to be approaching an asymptote (Figure 3.3), suggesting that a greater proportion of the estimated total pool of NIS remains undetected, but this is likely influenced by the low number of NIS detected in San Diego Bay.

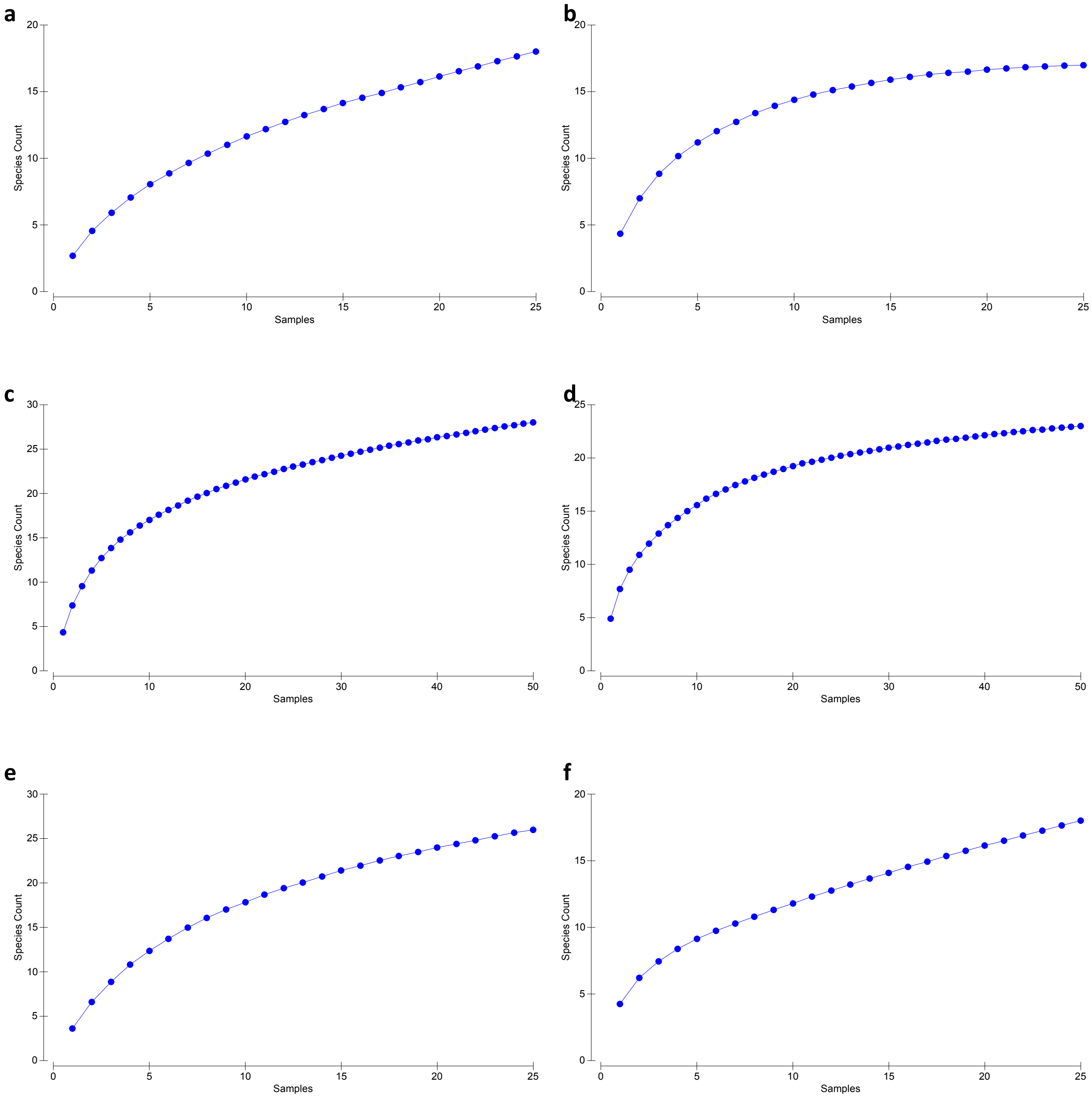


Figure 3.1. San Francisco Bay, 2012. Species accumulation curves for intertidal natives (a) and NIS (b), shallow natives (c) and NIS (d), deep natives (e) and NIS (f). Status is designated based on literature and SERC NEMESIS database. Here a sample represents a grab taken at 5 locations in each of 10 sites in the high salinity region of the Bay (n=250 grabs total).

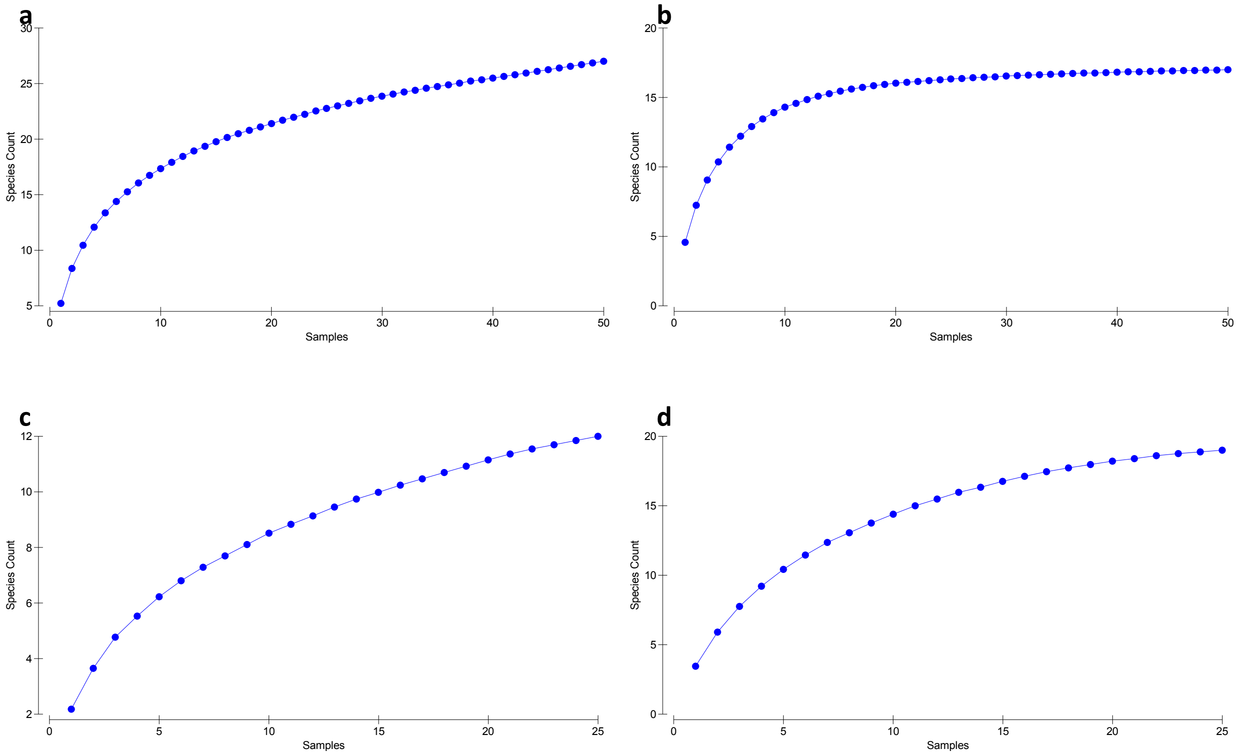


Figure 3.2. San Francisco Bay, 2013. High salinity natives (a) and NIS (b), low salinity natives (c) and NIS (d) species accumulation plots.

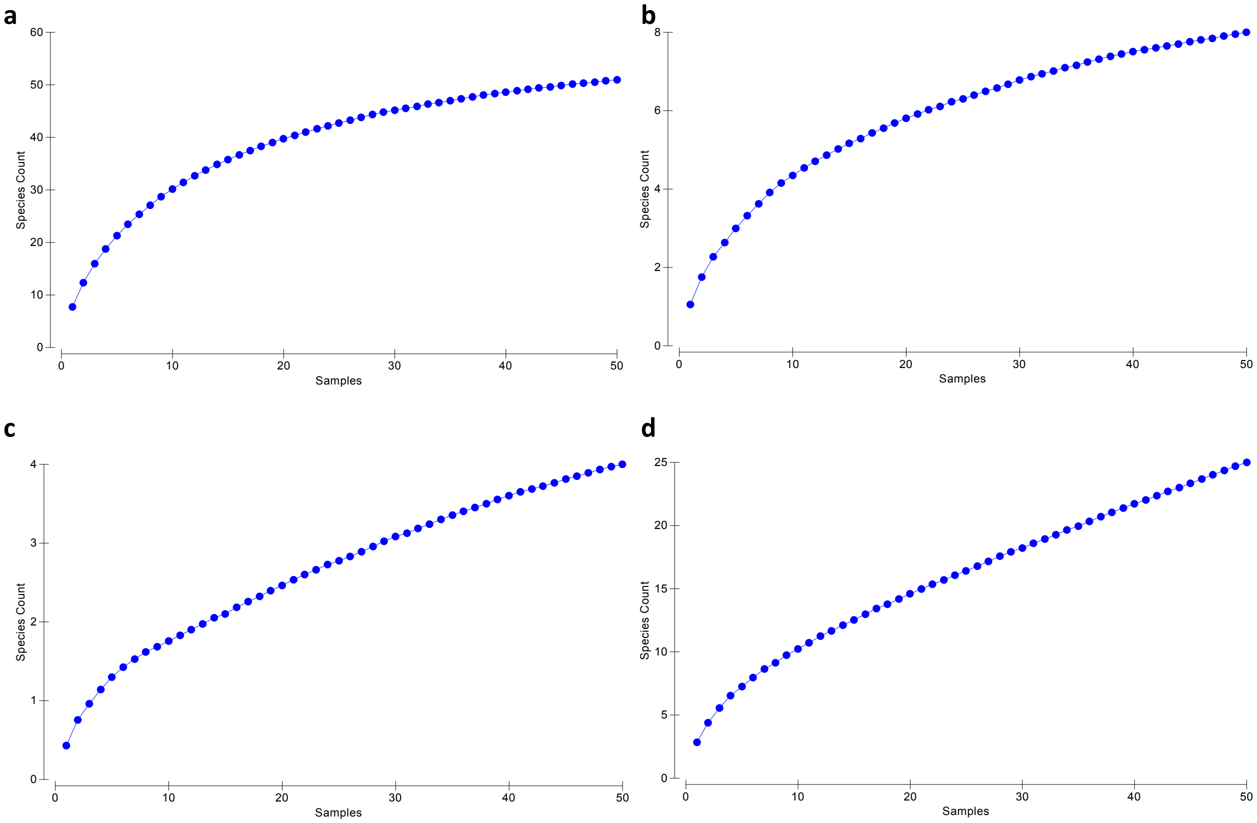


Figure 3.3. San Diego Bay, 2013. Native (a), NIS (b), cryptogenic (c) and unresolved (d) species accumulation plots.

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

NIS contributed up to 78% of total observed species richness in each bay, with San Francisco Bay having a much higher average proportion of NIS making up total species richness relative to San Diego Bay (Figures 3.4 to 3.6). NIS contributed 14 to 78% of total richness in San Francisco Bay (Figures 3.4 and 3.5), and 0% to 18% in San Diego Bay (Figure 3.6). The difference between the two Bays is due largely to the high number of native species in San Diego Bay and the low native species richness in San Francisco Bay. The percent contribution of NIS to overall richness was relatively constant among sites in each Bay, with additional variation in 2013 in San Francisco Bay due to the influence of freshwater sites (Figure 3.5). Although NIS in the freshwater sites varied much more in their contributions to total species richness per site, this larger influence is also driven by the lower overall richness (7 to 23 species per site, compared to 17 to 27 species per site in the marine region of San Francisco Bay).

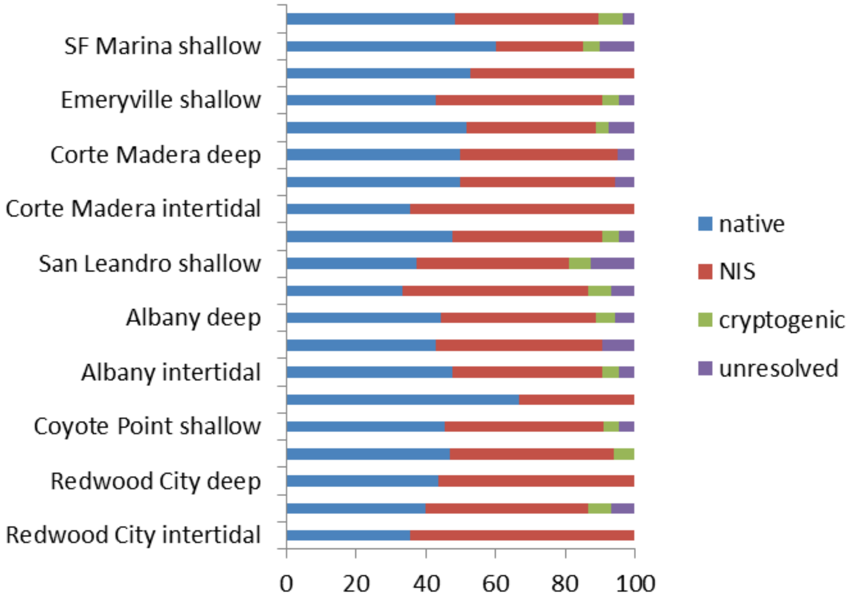


Figure 3.4. San Francisco Bay, 2012. Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site and depth.

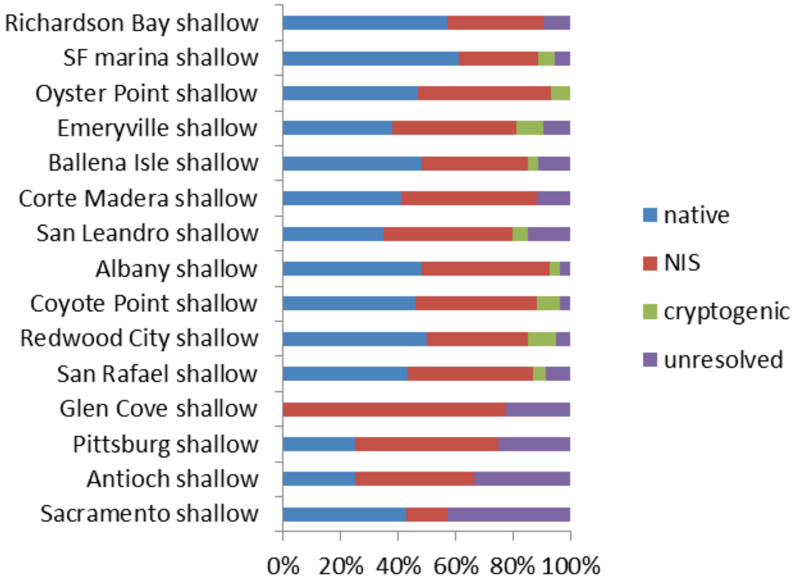


Figure 3.5. San Francisco Bay, 2013. Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species for low salinity (bottom) and high salinity (top) sites .

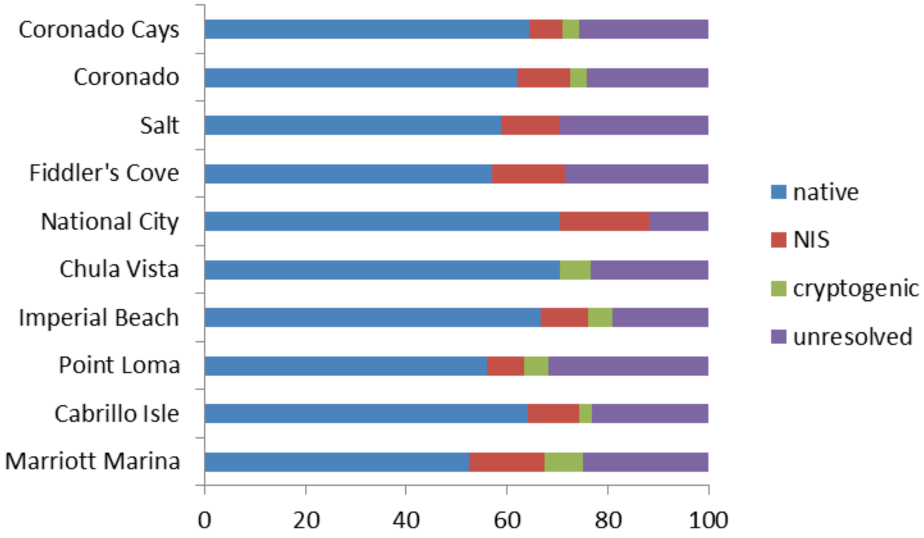


Figure 3.6. San Diego Bay, 2013. Total species richness contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site.

Relative Abundance of NIS Per Site in San Francisco and San Diego Bays.

Overall, NIS made up a significant percentage of the individuals found in both Bays. 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 98% of all individuals (Figures 3.7 and 3.8).

There was significant variation among sites in both bays in the relative abundance of NIS. In San Francisco Bay average NIS abundances ranged from 10% to 98%, while in San Diego Bay, average NIS abundance ranged from 0% to 51% (Figures 3.7 to 3.9).

In San Diego Bay, a striking geographic pattern of NIS abundance was evident, with higher NIS abundance at sites closer to the back portion of the bay, and particularly on the western side, where NIS made up as much as 51% of all individuals found per grab sample (Figure 3.9). This pattern is made more remarkable by the low number of NIS in San Diego Bay (8 NIS total, out of 88 species, with 51 native species).

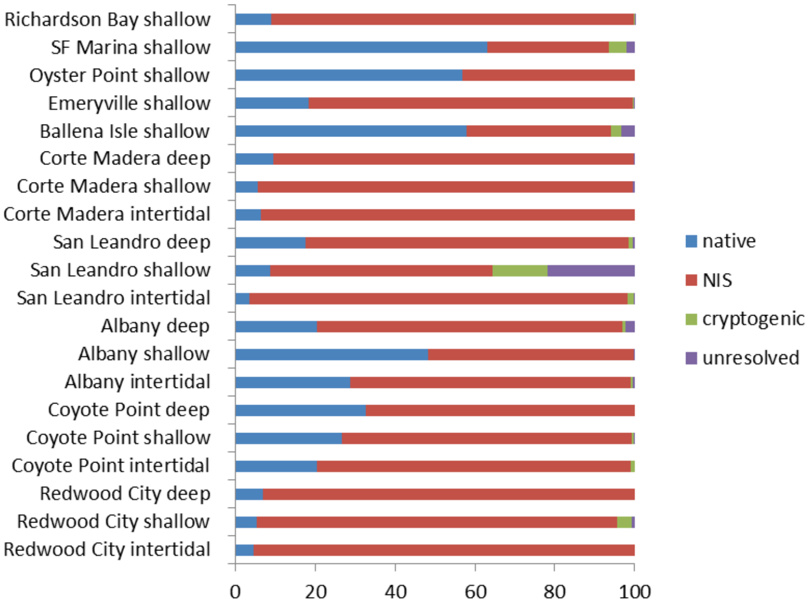


Figure 3.7. San Francisco Bay, 2012. Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site and depth.

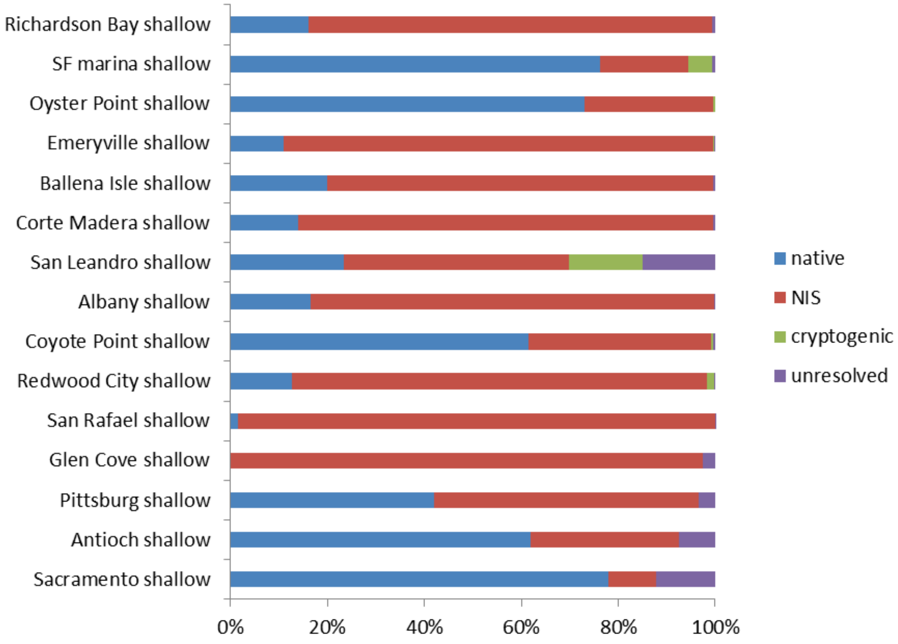


Figure 3.8. San Francisco Bay, 2013. Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species for low salinity (bottom) and high salinity (top) sites .

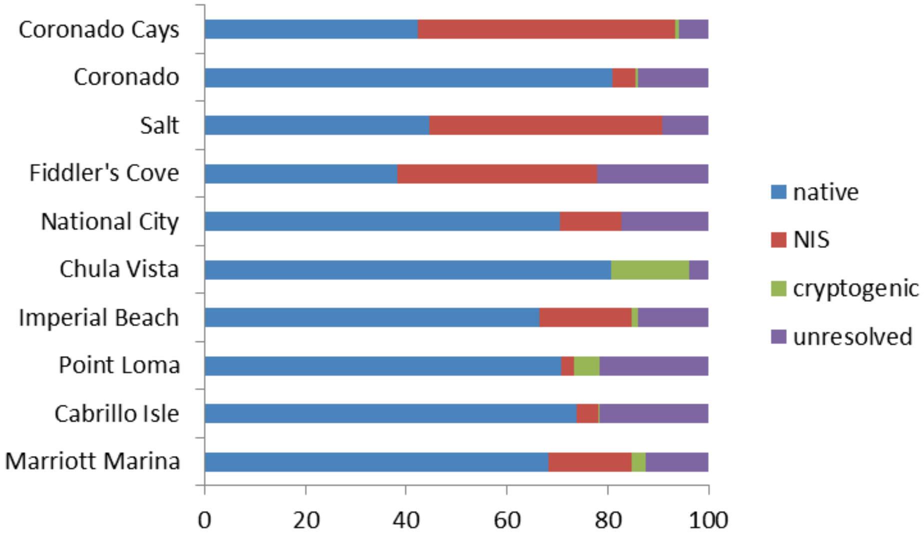


Figure 3.9. San Diego Bay, 2013. Total abundance contribution percentages for native (blue), NIS (red), cryptogenic (green) and unresolved (purple) species per site.

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

Only one new taxon was detected using morphological taxonomy during the two years of soft sediment surveys in the present study, across San Diego Bay (one year) and San Francisco Bay (two years), spanning a range of depths and salinity regions (in San Francisco Bay). We found the Japanese cephalaspidean gastropod *Haminoea japonica* in grab samples from 5 out of the 10 sites surveyed in San Diego Bay, where it appeared to be in low-to-moderate abundance. This taxon has been found at numerous locations in San Francisco Bay, where it was first recorded in 1999 (Gosliner 2006), but it has not previously been reported elsewhere in California. This species has been found to be exclusively associated with a schistosome parasite that causes cercarial dermatitis in humans (Brant 2010). Final confirmation of this identification via genetic analyses is pending.

For San Francisco Bay, we detected several taxa that have previously been recorded, but not reported in the literature, including the polychaetes *Marphysa* sp C Harris, *Amaeana* sp A Harris, which were both first recorded in 2004, and *Neoamphitrite* sp A Harris, which was first recorded in 1997. Final confirmation of these taxa via genetic analyses is pending.

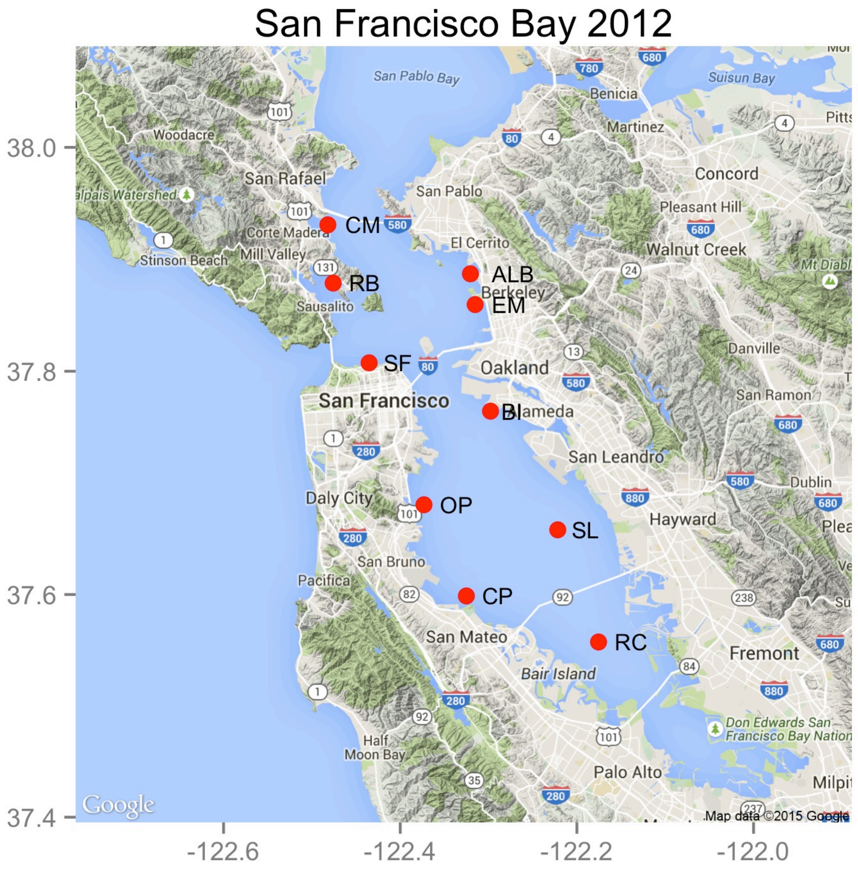
The dearth of new records is surprising given (a) the extensive sampling effort and detailed morphological analyses used to examine 225 samples across San Francisco and San Diego Bays, including both high and low salinity regions of San Francisco Bay, and several depths, and (b) the absence of recent (from the previous approximately 20 years) comprehensive surveys from either location.

As with the hard substrate surveys, these results suggest that the rate of invasion or detection may be highly variable over time, or that a fundamental shift in invasion rates has occurred, particularly for San Francisco Bay. Repeated sampling in San Francisco Bay for the Program over the next several years will explicitly test this hypothesis.

Appendix 3.1: Survey Locations by Estuary and Year

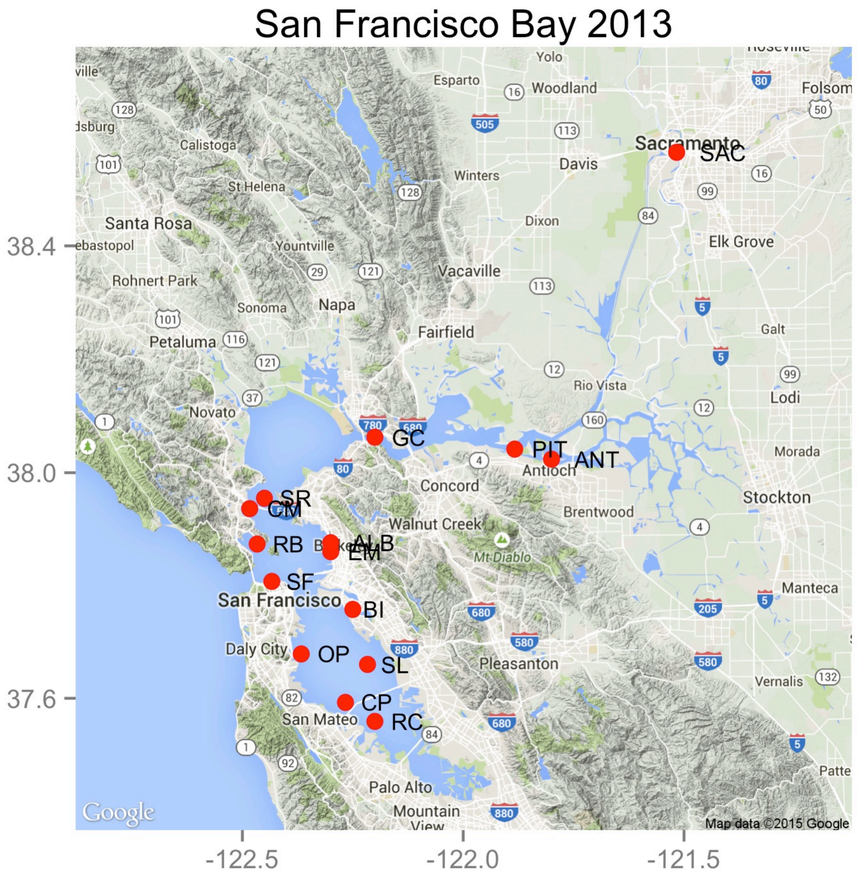
The maps tables below indicate locations and dates for soft sediment surveys for each estuary and year.

San Francisco Bay 2012



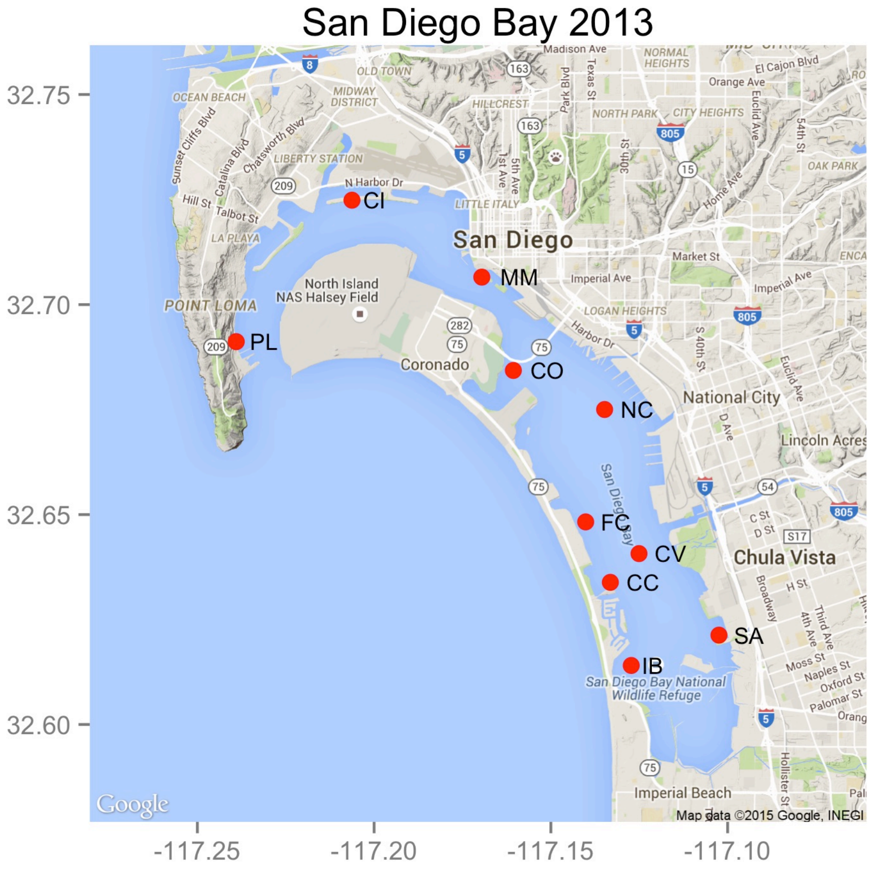
Site	Site Code	Latitude	Longitude	Depth	Salinity (‰)	Temperature (°C)
Redwood City	RC	37°35'32.95''N	122°20'03.93''W	intertidal	30.72	21.37
		37°33'26.61''N	122°10'31.76''W	shallow	28.50	19.70
		37°19'54.09''N	122°6'37.11''W	deep	28.50	20.50
Coyote Point	CP	37°35'21.51''N	122°19'43.35''W	intertidal	30.50	19.56
		37°35'55.37''N	122°19'30.55''W	shallow	30.26	19.35
		37°37'49.75''N	122°19'17.21''W	deep	29.20	20.79
Albany	ALB	37°53'05.84''N	122°18'57.50''W	intertidal	30.38	18.16
		37°53'13.48''N	122°19'14.01''W	shallow	30.23	17.72
		37°52'06.74''N	122°23'13.18''W	deep	29.66	17.05
San Leandro	SL	37°41'56.16''N	122°11'33.58''W	intertidal	30.35	18.81
		37°39'28.82''N	122°13'18.06''W	shallow	29.52	19.78
		37°40'58.11''N	122°16'09.89''W	deep	29.54	20.30
Corte Madera	CM	37°55'47.90''N	122°29'49.39''W	intertidal	30.15	18.91
		37°55'51.11''N	122°28'55.00''W	shallow	27.88	17.25
		37°55'24.54''N	122°27'53.06''W	deep	27.90	17.51
Ballena Isle	BI	37°45'51.31''N	122°17'52.05''W	shallow	30.41	20.28
Emeryville	EM	37°51'34.61''N	122°18'54.66''W	shallow	30.20	20.45
Oyster Point	OP	37°40'49.65''N	122°22'23.22''W	shallow	30.71	19.00
San Francisco Marina	SF	37°48'27.02''N	122°26'06.77''W	shallow	31.42	17.03
Richardson Bay	RB	37°52'43.60''N	122°28'33.26''W	shallow	30.60	18.53

San Francisco Bay 2013



Salinity Region	Site	Site Code	Latitude	Longitude	Depth (m)	Salinity (‰)	Temperature (°C)
Low	Sacramento	SAC	38°33.862'N	121°31.311'W	3.0	0.07	19.34
Low	Antioch	ANT	38°1.433'N	121°48.983'W	2.6	0.58	18.95
Low	Pittsburg	PIT	38°2.501'N	121°53.768'W	2.6	1.54	19.09
Low	Glen Cove	GC	38°3.766'N	122°12.183'W	2.0	13.39	18.35
Low	San Rafael	SR	37°57.287'N	122°27.714'W	2.5	25.87	17.22
High	Redwood City	RC	37°33.528'N	122°12.561'W	2.2	30.03	20.72
High	Coyote Point	CP	37°35.557'N	122°16.912'W	2.3	29.82	20.20
High	Albany	ALB	37°52.561'N	122°18.950'W	1.5	29.76	21.24
High	San Leandro	SL	37°39.609'N	122°13.057'W	1.9	29.98	19.40
High	Corte Madera	CM	37°56.181'N	122°29.480'W	2.2	30.44	15.79
High	Ballena Isle	BI	37°45.468'N	122°15.980'W	2.0	30.27	19.49
High	Emeryville	EM	37°51.611'N	122°18.858'W	1.5	30.23	19.21
High	Oyster Point	OP	37°40.726'N	122°22.941'W	2.0	30.59	17.62
High	San Francisco Marina	SF	37°48.452'N	122°26.121'W	2.5	30.09	20.38
High	Richardson Bay	RB	37°52.419'N	122°28.661'W	2.1	30.33	16.75

San Diego Bay 2013



San Diego Bay 2013

Site	Site Code	Latitude	Longitude	Depth (m)	Salinity (‰)	Temperature (°C)
Cabrillo Isle	CI	32.72485°N	117.206265°W	2.7	33.2	21.4
Marriott Marina	MM	32.70654°N	117.16954°W	1.9	33.7	21.9
Point Loma	PL	32.69118°N	117.23900°W	2.0	33.3	19.9
Imperial Beach	IB	32.61406°N	117.12727°W	2.0	35.3	24.5
Salt	SA	32.62129°N	117.10246°W	2.7	35.2	25.6
National City	NC	32.67503°N	117.13480°W	2.9	33.8	23.9
Chula Vista	CV	32.64071°N	117.12507°W	2.4	34.5	24.9
Coronado	CO	32.68433°N	117.16061°W	2.3	34.0	23.4
Fiddler's Cove	FC	32.64826°N	117.14016°W	2.7	34.7	24.3
Coronado Cays	CC	32.63387°N	117.13318°W	2.1	34.9	24.6

Appendix 3.2. Taxa Identified Morphologically by Estuary and Year

San Francisco Bay 2012

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.

	Status	Redwood City intertidal	Redwood City shallow	Redwood City deep	Coyote Point intertidal	Coyote Point shallow	Coyote Point deep	Albany intertidal	Albany shallow	Albany deep	San Leandro intertidal	San Leandro shallow	San Leandro deep	Corte Madera intertidal	Corte Madera shallow	Corte Madera deep	Ballena Isle shallow	Emeryville shallow	Oyster Point shallow	SF Marina shallow	Richardson Bay shallow
Annelida																					
Capitellidae																					
Heteromastus filobranchus	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Heteromastus sp	C	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4	0
Notomastus sp	N	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	1
Cirratulidae																					
Cirriformia moorei	N	1	1	1	2	5	3	0	0	0	0	1	2	0	0	1	5	0	5	2	5
Cirriformia sp	N	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0
Dorvilleidae																					
Schistomeringos sp	N	0	0	1	0	0	0	0	0	0	0	1	1	0	0	5	3	1	0	0	3
Eunicidae																					
Marphysa sp C. Harris	C	0	5	0	3	2	0	0	0	0	3	4	4	0	0	0	0	0	0	0	0
Flabelligeridae																					
Pherusa neopapillata	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Goniadidae																					
Glycinde picta	N	4	0	1	3	2	0	4	3	3	1	0	1	5	3	4	2	4	4	4	4
Glycinde sp.	U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Lumbrinereidae																					
Scoletoma tetraura complex	N	0	1	0	0	2	5	0	0	0	0	0	5	0	2	0	0	0	5	1	0
Maldanidae																					

	Status	Redwood City intertidal	Redwood City shallow	Redwood City deep	Coyote Point intertidal	Coyote Point shallow	Coyote Point deep	Albany intertidal	Albany shallow	Albany deep	San Leandro intertidal	San Leandro shallow	San Leandro deep	Corte Madera intertidal	Corte Madera shallow	Corte Madera deep	Ballena Isle shallow	Emeryville shallow	Oyster Point shallow	SF Marina shallow	Richardson Bay shallow
Sabaco elongatus	I	1	5	5	4	5	5	1	5	4	5	2	4	4	5	4	1	5	4	0	4
Nephtyidae																					
Nephtys caecoides	N	1	0	0	0	0	0	4	4	2	0	0	0	0	1	0	0	1	2	2	1
Nephtys sp	U	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
Nereididae																					
Neanthes succinea	I	5	0	2	2	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	3
Orbiniidae																					
Leitoscoloplos pugettensis	N	0	0	0	1	0	0	0	1	2	0	0	0	0	1	0	1	0	4	1	0
Polychaeta																					
Polychaeta (unidentified)	U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Polynoidae																					
Harmothoe imbricata complex	N	0	5	3	4	5	3	0	5	1	2	3	0	5	2	1	5	5	0	1	4
Hesperonoe sp	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Sabellidae																					
Euchone limnicola	N	0	1	1	0	5	4	0	4	0	0	0	0	0	0	0	0	5	0	0	3
Spionidae																					
Dipolydora branchycephala	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Scolelepis squamata complex	C	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Syllidae																					
Megasyllis nipponica	I	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	2	0	0	1	3
Terebellidae																					
Amaeana occidentalis	N	0	0	0	0	1	1	1	0	0	0	0	1	0	1	0	1	1	0	1	0
Amaeana sp A Harris	I	0	0	0	0	1	0	0	0	1	0	0	1	0	0	5	1	0	1	3	0
Neoamphitrite sp A Harris	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	1
Polycirrus sp	U	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0

	Status	Redwood City intertidal	Redwood City shallow	Redwood City deep	Coyote Point intertidal	Coyote Point shallow	Coyote Point deep	Albany intertidal	Albany shallow	Albany deep	San Leandro intertidal	San Leandro shallow	San Leandro deep	Corte Madera intertidal	Corte Madera shallow	Corte Madera deep	Ballena Isle shallow	Emeryville shallow	Oyster Point shallow	SF Marina shallow	Richardson Bay shallow
Trochochetidae																					
Trochochaeta franciscana	N	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Arthropoda																					
Ampeliscidae																					
Ampelisca abdita	I	0	5	5	4	5	4	2	5	2	0	0	2	5	3	5	0	4	3	0	5
Amphitoidae																					
Ampithoe valida	I	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0
Aoridae																					
Grandidierella japonica	I	3	0	0	0	0	0	3	1	0	1	0	0	5	0	1	0	4	2	0	3
Balanidae																					
Balanus crenatus	N	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Callianassidae																					
Neotrypaea californiensis	N	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Caprellidae																					
Caprella ferrea	N	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3
Caprella simia	I	0	0	0	0	3	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Caridea																					
Palaemon macrodactylus	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Corophiidae																					
Corophium heteroceratum	I	1	5	3	2	1	0	0	4	5	0	0	3	1	5	4	1	5	3	3	5
Monocorophium acherusicum	I	0	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	4
Crangonidae																					
Crangon nigricauda	N	0	2	0	1	1	1	1	1	0	0	1	0	1	0	4	1	0	1	1	2
Hyalidae																					
Allorchestes sp.	U	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

	Status	Redwood City intertidal	Redwood City shallow	Redwood City deep	Coyote Point intertidal	Coyote Point shallow	Coyote Point deep	Albany intertidal	Albany shallow	Albany deep	San Leandro intertidal	San Leandro shallow	San Leandro deep	Corte Madera intertidal	Corte Madera shallow	Corte Madera deep	Ballena Isle shallow	Emeryville shallow	Oyster Point shallow	SF Marina shallow	Richardson Bay shallow
Idoteidae																					
Idotea rufescens	N	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Synidotea laticauda	I	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inachoididae																					
Pyromaia tuberculata	N	0	0	0	0	0	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Isaeidae																					
Photis brevipes	N	0	0	0	0	0	0	2	1	0	0	0	0	0	0	3	0	0	0	0	3
Oedicerotidae																					
Oedicerotidae (unidentified)	U	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paranthuridae																					
Paranthura japonica	I	0	3	1	2	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	1
Pinnotheridae																					
Pinnixa franciscana	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Scleroplax granulata	N	1	0	0	0	2	1	0	0	0	0	0	1	0	0	0	0	0	2	0	0
Pycnogonidae																					
Pycnogonum rickettsi	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Upogebiidae																					
Upogebia pugettensis	N	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Varunidae																					
Cancer magister	N	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemigrapsus oregonensis	N	0	0	0	5	0	0	0	2	0	1	1	0	2	1	1	4	5	0	0	3
Chordata																					
Molguidae																					
Molgula manhattensis	I	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	4	0	0	0	0
Cnidaria																					

	Status	Redwood City intertidal	Redwood City shallow	Redwood City deep	Coyote Point intertidal	Coyote Point shallow	Coyote Point deep	Albany intertidal	Albany shallow	Albany deep	San Leandro intertidal	San Leandro shallow	San Leandro deep	Corte Madera intertidal	Corte Madera shallow	Corte Madera deep	Ballena Isle shallow	Emeryville shallow	Oyster Point shallow	SF Marina shallow	Richardson Bay shallow
Isanthidae																					
Zaolutus actius	N	0	0	2	2	2	3	0	4	0	0	4	2	1	0	2	3	3	1	0	0
Virgulariidae																					
Stylatula elongata	N	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	3	0
Ectoprocta																					
Electridae																					
Aspidelectra melolontha	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Hincksinidae																					
Hincksina sp	U	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Schizoporelloidea																					
Schizoporella errata	I	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Schizoporella sp	U	0	0	0	0	0	0	0	0	0	1	4	0	0	0	0	2	0	0	0	0
Smittinidae																					
Smittoidea prolifica	I	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Watersiporidae																					
Watersipora subtorquata	I	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Mollusca																					
Calyptreiadae																					
Crepidula convexa	I	0	0	0	0	0	0	0	0	0	2	5	0	0	0	0	2	0	0	0	0
Crepidula plana	I	0		0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cardiidae																					
Clinocardium nuttallii	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Columbellidae																					
Alia carinata	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Corbulidae																					

	Status	Redwood City intertidal	Redwood City shallow	Redwood City deep	Coyote Point intertidal	Coyote Point shallow	Coyote Point deep	Albany intertidal	Albany shallow	Albany deep	San Leandro intertidal	San Leandro shallow	San Leandro deep	Corte Madera intertidal	Corte Madera shallow	Corte Madera deep	Ballena Isle shallow	Emeryville shallow	Oyster Point shallow	SF Marina shallow	Richardson Bay shallow
Corbula amurensis	I	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lyonsiidae																					
Lyonsia californica	N	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Myidae																					
Cryptomya californica	N	2	0	0	0	1	1	2	0	1	1	0	0	0	0	0	2	1	1	0	1
Mytilidae																					
Musculista senhousia	I	2	0	1	0	4	0	0	0	0	3	2	0	1	0	0	1	0	1	0	1
Philinidae																					
Philine orientalis	I	1	2	0	1	5	1	0	3	0	0	0	2	0	1	2	2	5	0	0	0
Semelidae																					
Theora lubrica	I	0	5	4	1	5	5	0	2	0	0	0	4	4	2	1	0	4	5	0	3
Tellinidae																					
Macoma petalum	I	3	0	0	0	0	0	2	0	1	1	0	1	0	0	0	0	1	0	2	0
Veneridae																					
Gemma gemma	I	0	0	0	0	0	0	2	0	1	4	0	0	0	0	0	0	0	0	0	0
Venerupis philippinarum	I	3	5	2	4	0	5	1	2	3	5	4	2	4	1	1	5	1	1	2	0
Nemertea																					
Nemertea																					
Nemertea (unidentified)	U	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	1	1	0	2	0

San Francisco Bay 2013

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.

	Status	Sacramento	Antioch	Pittsburg	Glen Cove	San Rafael	Redwood City	Coyote Point	Albany	San Leandro	Corte Madera	Ballena Isle	Emeryville	Oyster Point	SF Marina	Richardson Bay
Annelida																
Capitellidae																
Capitellidae (unidentified)	U	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Heteromastus sp	C	0	0	0	0	0	0	0	0	0	0	0	2	0	3	0
Notomastus sp	N	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Cirratulidae																
Cirriformia moorei	N	0	0	0	0	1	4	5	1	1	0	4	0	5	1	1
Cirriformia sp	N	0	0	0	0	2	0	0	0	0	0	0	0	0	4	0
Dorvilleidae																
Schistomeringos sp	N	0	0	0	0	3	0	5	0	1	0	0	0	0	1	2
Eunicidae																
Marphysa sp C. Harris	C	0	0	0	0	0	2	1	0	5	0	0	0	0	0	0
Goniadidae																
Glycinde picta	N	0	0	0	0	1	0	3	2	4	3	4	4	4	5	5
Glycinde sp	U	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Lumbrinereidae																
Scoletoma tetraura complex	N	0	0	0	0	0	2	2	0	0	1	0	1	5	0	0
Maldanidae																
Sabaco elongatus	I	0	0	0	0	3	5	2	5	1	4	2	5	5	0	3
Nephtyidae																
Nephtys caecoides	N	0	0	0	0	0	3	0	1	0	4	4	0	1	3	2
Nereididae																
Neanthes succinea	I	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0

	Status	Sacramento	Antioch	Pittsburg	Glen Cove	San Rafael	Redwood City	Coyote Point	Albany	San Leandro	Corte Madera	Ballena Isle	Emeryville	Oyster Point	SF Marina	Richardson Bay
Nereididae (unidentified)	U	1	3	3	0	0	0	0	0	0	0	0	0	0	0	0
Orbiniidae																
Leitoscoloplos pugettensis	N	0	0	0	0	0	2	5	2	2	3	1	5	5	3	4
Pectinariidae																
Pectinaria californiensis?	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Phyllodocidae																
Phyllodoce medipapillata?	U	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Polynoidae																
Harmothoe imbricata complex	N	0	0	0	0	4	4	4	3	5	1	2	5	3	0	1
Sabellidae																
Euchone limnicola	N	1	2	5	0	3	1	0	4	0	3	3	5	0	0	1
Spionidae																
Marenzelleria viridis	I	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0
Pseudopolydora kempi	C	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Syllidae																
Megasyllis nipponica	I	0	0	0	0	2	0	1	1	0	2	2	2	2	2	0
Typosyllis sp	U	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Terebellidae																
Amaeana occidentalis	N	0	0	0	0	0	0	2	0	1	0	0	0	0	1	3
Amaeana sp A Harris	I	0	0	0	0	2	0	0	1	0	5	0	0	1	2	0
Neoamphitrite sp A Harris	C	0	0	0	0	0	1	1	1	0	0	1	1	1	0	0
Polycirrus sp	U	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Oligochaeta																
Oligochaeta (unidentified)	U	5	4	1	0	0	0	0	0	0	0	1	0	0	0	0
Arthropoda																
Ampeliscidae																
Ampelisca abdita	I	0	1	0	5	5	4	2	5	2	5	3	5	5	0	4

	Status	Sacramento	Antioch	Pittsburg	Glen Cove	San Rafael	Redwood City	Coyote Point	Albany	San Leandro	Corte Madera	Ballena Isle	Emeryville	Oyster Point	SF Marina	Richardson Bay
Amphitoidae																
Ampithoe sp	U	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Aoridae																
Grandidierella japonica	I	0	0	1	5	0	1	1	2	1	0	2	2	2	0	1
Caprellidae																
Caprella ferrea	N	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Caprella mutica	I	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Caprella sp A	U	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Caridea																
Palaemon macrodactylus	I	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0
Corophiidae																
Americorophium spinicorne	N	5	5	4	0	0	0	0	0	0	0	0	0	0	0	0
Americorophium stimpsoni	N	4	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Corophium heteroceratum	I	0	0	0	2	4	5	1	5	1	3	2	4	4	4	4
Monocorophium acherusicum	I	0	0	0	0	0	0	1	0	1	1	2	1	0	0	2
Monocorophium insidiosum	I	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Sinocorophium alienense	I	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Crangonidae																
Crangon nigricauda	N	0	0	0	0	1	1	0	1	0	1	2	0	1	0	3
Gammaridae																
Gammarus daiberi	I	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Haustoriidae																
Eohaustorius brevicuspis	N	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Inachoididae																
Pyromaia tuberculata	N	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0
Isaeidae																
Photis brevipes	N	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3

	Status	Sacramento	Antioch	Pittsburg	Glen Cove	San Rafael	Redwood City	Coyote Point	Albany	San Leandro	Corte Madera	Ballena Isle	Emeryville	Oyster Point	SF Marina	Richardson Bay
Leptocheliidae																
Leptochelia sp	U	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0
Liljeborgiidae																
Listriella cf. goleta	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Paranthuridae																
Paranthura japonica	I	0	0	0	0	0	0	0	2	0	0	0	4	0	0	0
Pinnotheridae																
Pinnixa franciscana	N	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Scleroplax granulata	N	0	0	0	0	0	0	2	1	0	0	2	0	0	0	0
Upogebiidae																
Upogebia pugettensis	N	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Varunidae																
Cancer magister	N	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0
Hemigrapsus oregonensis	N	0	0	0	0	0	0	2	3	0	0	0	4	0	0	0
Cumacea																
Cumacea (unidentified)	U	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Chordata																
Molgulidae																
Molgula manhattensis	I	0	0	0	0	1	0	4	1	0	0	0	0	0	0	0
Cnidaria																
Isanthidae																
Zaolutus actius	N	0	0	0	0	5	1	5	4	5	0	3	4	0	0	1
Virgulariidae																
Stylatula elongata	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
Echinodermata																
Amphiuridae																
Amphiuridae (unidentified)	U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

	Status	Sacramento	Antioch	Pittsburg	Glen Cove	San Rafael	Redwood City	Coyote Point	Albany	San Leandro	Corte Madera	Ballena Isle	Emeryville	Oyster Point	SF Marina	Richardson Bay
Mollusca																
Calyptreiadae																
Crepidula convexa	I	0	0	0	0	0	0	5	0	5	0	0	0	0	0	0
Crepidula plana	I	0	0	0	0	0	0	4	0	3	0	0	0	0	0	0
Corbulidae																
Corbicula fluminea	I	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
Corbula amurensis	I	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0
Lyonsiidae																
Lyonsia californica	N	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Myidae																
Cryptomya californica	N	0	0	0	0	0	4	5	0	0	0	0	1	0	0	0
Mytilidae																
Musculista senhousia	I	0	0	0	1	0	0	4	2	1	0	0	0	0	0	0
Philinidae																
Philine orientalis	I	0	0	0	0	2	2	4	4	0	0	1	4	0	1	0
Semelidae																
Theora lubrica	I	0	0	0	0	0	3	0	4	0	2	1	5	4	0	1
Tellinidae																
Macoma petalum	I	0	0	0	3	1	0	0	1	0	0	2	0	0	0	0
Veneridae																
Venerupis philippinarum	I	0	0	0	0	0	3	0	0	5	0	3	0	0	1	3
Nemertea																
Nemertea (unidentified)	U	0	0	0	0	1	0	0	1	0	1	0	4	0	1	0
Plathelminthes																
Plathelminthes (unidentified)	U	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Sipuncluidae																
Sipunculus sp	U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

	Status	Sacramento	Antioch	Pittsburg	Glen Cove	San Rafael	Redwood City	Coyote Point	Albany	San Leandro	Corte Madera	Ballena Isle	Emeryville	Oyster Point	SF Marina	Richardson Bay
Sponge																
Sponge (unidentified)	U	2	0	0	0	0	1	2	0	0	0	0	0	0	0	0

San Diego Bay 2013

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.

	Status	Marriott Marina	Cabrillo Isle	Point Loma	Imperial Beach	Chula Vista	National City	Fiddler's Cove	Salt	Coronado	Coronado Cays
Annelida											
Capitellidae											
Notomastus lineatus complex	C	0	0	2	0	0	0	0	0	0	0
Notomastus sp	U	0	0	1	0	0	0	0	0	0	0
Flabelligeridae											
Piromis capulata	N	0	0	0	1	0	0	0	1	0	1
Glyceridae											
Glycera americana	N	3	4	5	2	3	5	1	0	4	5
Lumbrinereidae											
Drilonereis sp	U	1	0	0	0	0	0	0	0	0	0
Lumbrinereidae (unidentified)	U	3	5	4	5	2	5	5	4	5	5
Maldanidae											
Petaloproctus neoborealis	N	2	2	0	0	0	0	0	0	0	0
Nephtyidae											
Nephtys caecoides	N	0	4	4	0	1	0	0	0	2	0
Nephtys californiensis	N	2	0	0	0	0	0	0	0	0	0
Nereididae											
Neanthes acuminata	N	0	0	0	2	0	0	0	1	0	1
Nereis latescens	N	0	0	2	0	0	0	0	0	0	0
Oenonidae											
Lumbrineris erecta	N	1	0	0	0	0	0	0	0	0	0
Onuphidae											
Onuphis iridescens	N	0	0	1	0	0	0	0	0	0	0
Orbiniidae											
Leitoscoloplos pugettensis	N	1	1	0	0	0	0	0	5	0	0
Scoloplos acmeceps	N	3	4	5	4	3	3	1	0	5	5

	Status	Marriott Marina	Cabrillo Isle	Point Loma	Imperial Beach	Chula Vista	National City	Fiddler's Cove	Salt	Coronado	Coronado Cays
Phyllodocidae											
Eumida longicornuta?	N	0	0	1	0	0	0	0	0	0	0
Polynoidae											
Harmothoe hirsuta	N	0	0	0	0	0	0	0	0	1	2
Harmothoe imbricata complex	N	2	1	0	0	0	0	0	0	1	1
Malmgreniella macginitiei	N	0	0	0	0	0	0	0	0	1	0
Sabellidae											
Megalomma pigmentum	N	0	0	0	2	0	1	0	3	1	0
Sigalionidae											
Sthenelais fusca	N	0	0	1	1	0	0	0	0	0	0
Terebellidae											
Pista brevibranchiata	N	0	1	0	0	0	0	1	0	0	0
Arthropoda											
Alpheidae											
Alpheus californiensis	N	1	1	0	2	0	0	1	2	0	4
Ampeliscidae											
Ampelisca sp	U	0	0	1	0	0	0	0	0	0	0
Ampithoidae											
Ampithoe sp	U	0	0	0	0	0	0	0	0	0	1
Aoridae											
Grandidierella japonica	I	1	0	0	0	0	0	0	1	0	0
Callianassidae											
Neotrypaea gigas	N	0	4	2	0	0	0	0	0	0	0
Caprellidae											
Caprella californica	N	0	0	2	0	0	0	0	0	0	2
Caprella sp	U	0	0	1	0	0	0	0	0	0	0
Phtisica marina	C	1	0	0	0	0	0	0	0	0	0
Corophiidae											
Monocorophium acherusicum	I	1	0	0	0	0	0	0	0	0	0
Gammaropsidae											
Gammaropsis sp	U	0	1	0	0	0	0	0	0	0	0

	Status	Marriott Marina	Cabrillo Isle	Point Loma	Imperial Beach	Chula Vista	National City	Fiddler's Cove	Salt	Coronado	Coronado Cays
Gammaropsis thompsoni	N	2	3	4	0	1	3	0	0	2	0
Hyalidae											
Protohyale canalina	N	0	1	0	0	0	0	0	0	0	1
Hyppolytidae											
Hippolyte californiensis	N	0	0	0	0	0	0	0	0	0	1
Kalliapseudidae											
Mesokalliapseudes crassus	N	4	4	1	0	0	0	0	0	0	0
Leptocheliidae											
Leptochelia sp	U	0	0	1	0	0	0	0	0	0	0
Leucothoidae											
Leucothoe alata	N	0	0	0	1	0	0	0	0	0	0
Leucothoe sp. complex	U	0	0	0	0	1	0	0	0	0	0
Oedicerotidae											
Oedicerotidae (unidentified)	U	0	0	0	0	0	0	0	0	0	1
Panopeidae											
Lophopanopeus bellus	N	1	3	0	0	0	0	0	0	1	2
Paranthuridae											
Paranthura japonica	I	1	1	0	0	0	0	0	0	0	0
Phoxichilidiidae											
Anoplodactylus erectus	N	0	0	0	1	1	0	0	1	0	0
Phoxocephalidae											
Phoxocephalidae (unidentified)	U	1	1	5	0	0	0	0	0	1	0
Phoxocephalidae											
Heterophoxus sp	U	5	4	5	2	0	0	0	0	3	2
Pinnotheridae											
Pinnixa longipes	N	1	0	0	0	0	0	0	0	0	0
Scleroplax granulata	N	0	1	1	0	0	1	0	0	0	0
Podoceridae											
Podocerus cristatus complex	N	0	1	0	0	0	0	0	0	0	3
Serolidae											
Heteroserolis carinata	N	0	1	2	0	1	0	0	0	0	0

	Status	Marriott Marina	Cabrillo Isle	Point Loma	Imperial Beach	Chula Vista	National City	Fiddler's Cove	Salt	Coronado	Coronado Cays
Sphaeromatidae											
Paracereis sculpta	N	0	0	0	0	0	0	0	0	0	3
Squillidae											
Stomatopoda (unidentified)	U	2	2	1	4	0	2	3	2	4	4
Tryphosinae											
Hippomedon sp	U	0	0	0	0	1	0	0	0	0	0
Varunidae											
Cancer jordani	N	0	0	2	0	0	0	0	0	0	0
Cancer sp	U	0	0	1	0	0	0	0	0	0	0
Amphipoda											
Amphipoda (unidentifiable)	U	0	3	0	0	0	0	0	0	0	0
Cnidaria											
Alcampidae											
Halcampa sp?	U	0	0	0	0	0	0	4	4	3	3
Actiniaria											
Anemone (unidentified)	U	1	0	0	0	0	0	0	2	0	1
Echinodermata											
Amphiuridae											
Amphiodia (Amphispina) digitata	N	0	1	0	0	0	0	0	0	0	0
Amphipholis squamata	C	3	1	4	2	5	0	0	0	1	2
Dendrasteridade											
Dendraster excentricus	N	0	0	2	0	0	0	0	0	0	0
Ophiactidae											
Ophiactis simplex	C	1	0	0	0	0	0	0	0	0	0
Synaptidae											
Leptosynapta clarki	N	2	1	1	4	0	2	0	0	0	0
Insecta											
Insect larvae	U	0	0	1	0	0	0	0	0	0	0
Mollusca											
Calyptreiadae											
Crepidula convexa	I	1	0	0	0	0	0	0	0	0	0

	Status	Marriott Marina	Cabrillo Isle	Point Loma	Imperial Beach	Chula Vista	National City	Fiddler's Cove	Salt	Coronado	Coronado Cays
Crucibulum spinosum	N	0	0	0	0	0	0	0	0	2	0
Haminoeidae											
Haminoea japonica	I	3	1	1	0	0	4	0	0	2	0
Lottiidae											
Lottia depicta	N	0	1	1	0	0	0	0	0	1	4
Lucinidae											
Epilucina californica	N	1	1	1	0	0	0	0	0	0	0
Lyonsiidae											
Lyonsia californica	N	3	0	0	0	5	4	0	2	4	3
Myidae											
Cryptomya californica	N	1	3	2	0	0	0	0	0	0	0
Mytilidae											
Musculista senhousia	I	3	1	2	3	0	1	4	5	5	5
Nassariidae											
Nassarius tegula	N	0	0	0	1	2	3	1	0	0	2
Ostreidae											
Ostrea sp	U	0	1	0	0	0	0	0	0	0	0
Pectinidae											
Leptopecten latiauratus	N	0	0	0	0	0	0	0	0	1	0
Periplomatidae											
Periploma discus	N	0	1	0	0	0	0	0	0	0	0
Scaphandridae											
Acteocina inculta	N	0	0	0	0	5	1	0	4	3	4
Semelidae											
Theora lubrica	I	0	0	1	1	0	1	1	0	1	2
Solecurtidae											
Tagelus subteres	N	3	0	4	2	1	4	3	0	1	2
Solenidae											
Solen sicarius	N	4	3	2	4	4	4	5	2	2	4
Thraciidae											
Asthenothaerus diegensis	N	5	5	3	5	5	5	5	2	5	5

	Status	Marriott Marina	Cabrillo Isle	Point Loma	Imperial Beach	Chula Vista	National City	Fiddler's Cove	Salt	Coronado	Coronado Cays
Veneridae											
Chione cf. californiensis	U	1	2	3	0	0	0	0	0	2	0
Chione undatella	N	3	0	1	0	0	0	0	0	2	0
Venerupis philippinarum	I	0	3	0	0	0	0	0	0	0	0
Platyhelminthes											
Plathelminthes 1	U	1	0	0	0	0	0	0	0	0	0
Plathelminthes 2	U	0	0	1	0	0	0	0	0	0	0
Sipuncula											
Sipunculidae											
Sipunculida (unidentified)	U	1	0	0	0	0	0	0	0	0	0
Sipunculus sp	U	1	0	0	0	0	0	0	0	0	0
Themiste pyroides	N	1	2	0	0	0	0	0	0	0	0

Chapter 4: Macro-Zooplankton Communities

To detect the presence of non-native invertebrate taxa within macro-zooplankton assemblages, we sampled five estuaries including San Diego Bay, Mission Bay, Morro Bay, San Francisco Bay, and Bodega/Tomales Bay. For each estuary, we sampled 8-10 sites per estuary, and the specific locations and dates are indicated in Appendix 4.1.

We used two methods to collect macro-zooplankton samples, including pump samples and net tow samples. A modified trash pump (North Star S106120 model; Honda GX160 gas motor) coupled to a plankton net assembly (0.75m diameter net; 80µm mesh size) was used to collect and filter zooplankton at 1m depth over 10 minutes, totaling 5m^3 water volume filtered per sample. Five replicate pump samples were collected across 3-5 random locations within each site. 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 most sites, two replicate vertical net tows were collected in open water adjacent to each pump site. A weighted plankton net (0.50m diameter; 80µm 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.

Thus, across the five estuaries, we collected a total of approximately 250 pump samples (5 estuaries x 10 sites x 5 replicates) from high salinity waters. We also collected another 25 samples (5 sites x 5 replicates) from low salinity waters in San Francisco Bay Delta, as the only estuary in our study with a substantial low salinity area. In addition, we collected approximately 100 net tow samples (5 estuaries x 10 sites x 2 replicates).

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, a formalin-preserved sample was also sent to Jeff Cordell for identification of macro-zooplankton species present.

- **Genetic Analyses.** The remaining ethanol preserved samples (generally 3 pump samples and 1 net tow sample) per site were sent directly to MLML for whole community analysis using next generation sequencing.

Results

The morphological analyses of zooplankton revealed only NIS that were previously detected in California, although we note that taxonomic resolution was limited for many taxa of meroplankton, for which species-level identifications are often not possible for these larval forms (which lack diagnostic morphological characteristics). Those NIS detected in morphological analyses were restricted primarily to the copepods (see Appendix 4.2).

Many of the non-native copepods we detected were also found in previous analyses (studies) by our lab and others, and are known to be present in the respective estuaries. One noteworthy new record, however, is *Stephos pacificus,* a small hyperbenthic calanoid described from Japan. We found this species in pump samples from San Francisco Bay. Although previously known from southern California and Washington state (Cordell, personal communication), our sample appears to be the first reported occurrence of the species in San Francisco Bay.

It is not clear whether this record of *S. pacificus* in San Francisco Bay represents a recent invasion or one that has been overlooked. In this regard, it is notable that this copepod was detected in pump samples surrounding marinas, which may have been under-sampled historically. Most previous work on macro-zooplankton in San Francisco Bay has been in more open water or neritic habitats.

Appendix 4.1: Survey Locations by Estuary and Year

The maps and tables below indicate locations and dates for macro-zooplankton surveys for each estuary and year.

Bay	Collection Date	Site Code	Marina	Latitude	Longitude
San Francisco Bay					
	7/1/2013	SF-P01	Port of Redwood City Marina	37.5027	-122.2123
	7/2/2013	SF-P02	Coyote Point Marina	37.5883	-122.3180
	7/2/2013	SF-P03	Oyster Point Marina	37.6641	-122.3792
	7/3/2013	SF-P04	San Leandro Marina	37.6983	-122.1901
	7/3/2013	SF-P05	Ballena Isle Marina	37.7664	-122.2872
	6/21/2013	SF-P06	San Francisco Marina East	37.8072	-122.4337
	6/27/2013	SF-P07	Emeryville Marina	37.8411	-122.3116
	6/19/2013	SF-P08	Bridgeway Marine Corp (a.k.a., Sausalito Marina)	37.8614	-122.4853
	6/26/2013	SF-P09	Richmond Marina	37.9134	-122.3529
	6/18/2013	SF-P10	Loch Lomond Marina	37.9723	-122.4798
	7/20/2013	SF-P11	Glen Cove Marina	37.0678	-122.2133
	7/17/2013	SF-P12	Pittsburgh Marina	37.0347	-122.8835
	7/15/2013	SF-P13	Antioch Marina	37.0199	-122.8214
	7/19/2013	SF-P14	River Point Landing Marina	37.9772	-122.3756
	7/18/2013	SF-P15	Sacramento marina	37.5662	-122.5179
Morro Bay					
	8/20/2013	MO-P01	Morro Bay State Park Marina	35.3454	-120.8407
	8/20/2013	MO-P02	Fuel Dock	35.3562	-120.8482
	8/20/2013	MO-P03	Coastal Boatworks	35.3570	-120.8491
	8/22/2013	MO-P04	Tidelands Park South	35.3576	-120.8508
	8/21/2013	MO-P05	Tidelands Park North	35.3601	-120.8521
	8/21/2013	MO-P06	Yacht Club	35.3628	-120.8528
	8/20/2013	MO-P07	Morro Marina	35.3643	-120.8533
	8/21/2013	MO-P08	Giovanni's Fish Market	35.3674	-120.8543
	8/21/2013	MO-P09	City Harbor South	35.3692	-120.8556
	8/21/2013	MO-P10	City Harbor North	35.3704	-120.8578
Mission Bay					
	8/23/2013	MI-P01	Marina Village Marina	32.7607	-117.2356
	8/28/2013	MI-P02	Hyatt Regency Hotel	32.7636	-117.2393
	8/29/2013	MI-P03	The Dana Hotel	32.7668	-117.2355
	8/26/2013	MI-P04	Sea World	32.7677	-117.2309
	8/28/2013	MI-P05	Hilton Resort and Spa Hotel	32.7787	-117.2126
	8/27/2013	MI-P06	Campland on the Bay	32.7936	-117.2236
	8/27/2013	MI-P07	Mission Bay Sport Center	32.7854	-117.2499
	8/26/2013	MI-P08	Mission Bay Yacht Club	32.7778	-117.2494
	8/27/2013	MI-P09	Bahia Resort	32.7732	-117.2483
	8/26/2013	MI-P10	Paradise Point Resort Spa and Marina	32.7732	-117.2410
San Diego Bay					
	8/24/2013	SD-P01	Harbor Police Transient Docks	32.7101	-117.2343
	8/24/2013	SD-P02	Heritage Yacht Sales	32.7179	-117.2257
	8/24/2013	SD-P03	Shelter Cove Marina	32.7201	-117.2218
	8/27/2013	SD-P04	Cabrillo Isle Marina	32.7263	-117.2005
	8/30/2013	SD-P05	Sunroad Marina	32.7261	-117.1907
	8/30/2013	SD-P06	Marriott Hotel Marina	32.7055	-117.1652
	8/28/2013	SD-P07	Glorietta Marina	32.6791	-117.1740
	8/25/2013	SD-P08	Pier 32 Marina	32.6517	-117.1080
	8/25/2013	SD-P09	Chula Vista Yacht Club	32.6265	-117.1309
	8/25/2013	SD-P10	Chula Vista Marina	32.6248	-117.1051
Bodega/Tomales Bay					
	7/14/2014	BT-P01	Spud Point A	38.3302	-123.0575
	7/14/204	BT-P02	Spud Point B	38.3292	-123.0567
	7/15/2014	BT-P03	Porto Bodega	38.3340	-123.0514
	8/5/2014	BT-P04	Mason's Marina	38.3323	-123.0591
	7/14/2014	BT-P05	North of Lucas Wharf	38.3263	-123.0416
	7/14/2014	BT-P06	Yacht Club Dock - Abandoned	38.3246	-123.0402
	8/5/2014	BT-P07	Bodega Harbor public boat ramp	38.3230	-123.0547
	8/5/2014	BT-P08	US Coast Guard dock	38.3127	-123.0514
	7/15/2014	BT-P09	Marshals Boatworks	38.1514	-123.8885
	8/5/2014	BT-P10	Nick's Cove public boat ramp	38.1996	-122.9219

Appendix 4.2: List of Zooplankton Taxa Detected Morphologically by Estuary

Taxon	Status	San Francisco	Morro	Mission	San Diego
Acanthocyclops sp.		X			
Acarina		X			
Acartia (Acartiura) hudsonica		X	X	X	
Acartia (Acartiura) sp.		X			
Acartia californiensis		X	X	X	
Acartia spp.		X	X	X	X
Acartia tonsa		X	X	X	X
Acartiella sinensis	NIS	X			
Agonidae			X		
Appendicularia			X		
Ascidiacea		X		X	X
Bivalvia		X	X	X	X
Bosmina longirostris		X			
Bosmina sp.		X			
Botryllus/Botrylloides		X	X	X	X
Brachyura			X		X
Bryozoa			X		
Calanoida		X			
Calanus pacificus			X		
Calanus sp.			X	X	
Caligidae		X	X		
Calocalanus tenuis				X	
Cancridae		X	X		
Caprellidea		X	X	X	
Caridea		X	X	X	X
Ceriodaphnia sp.		X			
Chaetognatha		X	X	X	X
Chironomidae		X			
Chydoridae		X			
Cirripedia		X	X	X	X
Clausidiidae		X	X		
Copepoda		X	X	X	X
Copepoda, parasitic					X
Corophiidae		X	X	X	
Corycaeus amazonicus			X		
Corycaeus anglicus			X		
Corycaeus sp.			X	X	X
Coullana canadensis	NIS	X			
Crangonidae		X		X	
Ctenocalanus vanus			X		
Cyclopidae		X	X		X
Cyclopoida		X			
Cyclopoida, parasitic		X			
Daphnia spp.		X			
Diacyclops thomasi		X			
Diaphanosoma sp.		X			
Dioithona oculata			X	X	X
Emerita analoga			X		

Taxon	Status	San Francisco	Morro	Mission	San Diego
Ergasilidae		X			
Eucalanus bungii v. californicus			X		
Eucalanus sp.			X		
Eucyclops sp.		X			
Euphausiacea			X		
Euterpina acutifrons		X	X	X	X
Evadne nordmanni			X	X	
Gammaridea		X	X	X	X
Gastropoda		X	X	X	X
Grapsidae		X			
Harpacticoida		X	X	X	X
Holopedium gibberum		X			
Hydrozoa		X			
Isopoda		X	X		
Limnoithona sinensis		X			
Limnoithona tetraspina	NIS	X			
Littorina sp.			X		
Lophopanopeus sp.		X			
Lucicutia sp.			X		
Macrocyclops albidus		X			
Mesocyclops sp.		X			
Microsetella norvegica		X			
Microsetella rosea			X		
Microsetella spp.			X	X	
Munnidae		X			
Mysidacea		X			
Nematoda		X	X	X	X
Neotachidius triangularis		X			
Neotrypaea sp.		X	X		
Oikopleura dioica		X	X	X	
Oithona davisae	NIS	X		X	X
Oithona similis		X	X	X	X
Oithona spp.					X
Oligochaeta		X	X		
Oncaea spp.			X	X	
Osphranticum labronectum		X			
Ostracoda		X	X	X	X
Pachygrapsus crassipes		X	X	X	X
Paracalanus quasimodo		X	X		
Paracalanus sp.		X	X	X	
Parvocalanus crassirostris					X
Phoronida		X			
Pinnotheridae		X	X		
Pleuroxus sp.		X			
Podon polyphemoides			X		X
Poecilostomatoida		X	X		X
Polychaeta		X	X	X	X
Pontellidae		X	X	X	

Taxon	Status	San Francisco	Morro	Mission	San Diego
Porcellanidae		X	X		
Pseudobradya sp.		X	X	X	X
Pseudocalanus mimus		X			
Pseudocalanus spp.		X			X
Pseudodiaptomus euryhalinus		X	X		
Pseudodiaptomus forbesi	NIS	X			
Pseudodiaptomus marinus	NIS	X			
Pseudodiaptomus spp.		X	X	X	X
Rhithropanopeus harrisii	NIS	X			
Rotifera		X	X	X	X
Sida crystallina		X			
Simocephalus sp.		X			
Sinocalanus doerrii		X			
Skistodiaptomus pallidus		X			
Sphaeromatidae		X		X	X
Stephos pacificus	NIS	X			X
Stephos sp.		X			
Syllidae		X	X	X	X
Tachidiidae		X			X
Tanaidacea		X			X
Teleosti		X	X	X	X
Thysanoessa sp.		X			
Tintinnida		X	X	X	X
Tisbe spp.		X	X	X	X
Tortanus discaudatus		X			
Tortanus sp.		X			
Tropocyclops?		X			
Turbellaria		X	X	X	X

Chapter 5: Outer Coast Communities

To examine the potential colonization of outer coast regions by NIS, we conducted intertidal and subtidal surveys to detect the presence of the bryozoan *Watersipora* spp. *Watersipora* has been reported previously on the outer coast of California, but recent observations suggest the bryozoans may have spread to new sites and increased in abundance, including within marine protected areas. There are multiple species of *Watersipora* known to be introduced and established in California. Since *Watersipora* spp. are conspicuous in color and morphology (i.e., easily identified visually in the field), and also known to be present on the outer coast, surveys focused on this species provide an important model for evaluation of "spill-over" of NIS from estuaries and colonization of outer coastal habitats. Here, we report on initial surveys to detect *Watersipora*, assess its current distribution and abundance, and identify the species present at multiple outer coast sites in California.

A. Survey Sites

We surveyed ten rocky intertidal sites and eight subtidal sites in the counties of Marin, San Mateo, Santa Cruz and Monterey in Central California for *Watersipora* in winter 2014 and spring-summer 2015 (Appendix 5.1, Tables 5A.1 and 5A.2).

Intertidal sites were visited during minus tides in December 2014 and May and June 2015. We took several factors into account in selecting sites. First, we sought to include (sample) areas with high natural resource value, including sites within the National Marine Sanctuary system, the National Parks system and California's Marine Protected Areas system. Secondly, we wanted to distribute sampling effort along our broader study area (Marin to Monterey counties). Finally, we explicitly included sites where *Watersipora* had been noticed recently by us and colleagues (including new occurrence locations in the past two years).

Subtidal surveys were carried out at several popular recreational dive sites on and near the Monterey Peninsula (Appendix 5.1, Table 5A.2) in October-November 2014. This site was selected because colleagues had suggested *Watersipora* had been spreading and increasing in abundance in the area, but a more extensive survey was not available to characterize the current status. Moreover, the site allowed easy access for diving and included marine protected areas. Focusing on this smaller area also allowed us to compare abundance and distribution of target species both (a) along a wave-exposure gradient and (b) with increasing distance from a potential source population in the Monterey Harbor.

B. Survey Methods

For both intertidal and subtidal surveys, we surveyed 30 x 2 m belt transects to characterize the distribution and abundance of *Watersipora*. Transects were delineated by placing a 30 m transect tape on the substrate; researchers visually surveyed for the target species within 1 m on each side of the tape, on both horizontal and vertical surfaces, in cracks and crevices and under rocks where applicable. Each researcher visually divided the width of the transect into two 0.5-m^2 segments (A, B for the side closest the water for and C, D for the shoreward side for intertidal transects and A, B, on the left of transect and C, D on the right for subtidal transects, oriented from 0 to 30 m), providing geographically explicit information which could be mapped along the resulting transect grid system.

When a colony was encountered, we estimated abundance within a 0.5-m^2 quadrat, placed within the belt transect in the 0.5 m x 0.5 m grid. Abundance was estimated visually as percent cover to the nearest 5%, with the exception of extremely low cover (one or two small patches or individuals present) which we categorized as 1% cover. For some subtidal surveys locations where *Watersipora* was present in most quadrats, divers simply noted presence at the 0.5 m marks and only collected detailed data at the 1 m marks.

At intertidal sites, we placed transects at high, mid- and low-tide levels, running these parallel to shore, and surveyed the sites more broadly by walking for ~20 minutes. Constraints of tide, daylight and shoreline slope limited our ability to use these methods at every site (see Appendix 5.1, Table 5A.1). At subtidal sites, we ran 2 to 4 transects depending on the size of the site (see Appendix 5.1, Table 5A.2), generally perpendicular to shore, in depths from ~3 to ~8 m. In most cases, there was little depth change between the start and end of a transect survey. An effort was made to cover distinct habitat areas (sub-locations) within a dive site, where this was applicable. All dives were beach entries.

In addition to estimates of percent cover for each survey, we also noted the primary substrate type, depth and major species cover of the transect, as well as the substrate type (natural rock, rip rap, artificial substrate) and orientation (vertical versus horizontal) on which *Watersipora* was growing in each quadrat.

C. Data Analyses

We analyzed distribution data at three levels: across sites (presence/absence), across transects at each site (% of transects in which taxa were found), and across a total of 240 x 0.5 m^2 quadrats, which were sampled within the 2 x 30 m belt transects. For abundance estimates, we used quadrat-level data to calculate mean percent cover for entire transects, excluding any portions of a transect that were inappropriate habitat for our target species (i.e., sand patches for obligate hard-substrate organisms). Any given value of mean cover at the transect level could result from many quadrats all with similar cover levels or from fewer quadrats with very high cover; to look for differences in abundance across smaller scales we calculated means and variance of cover for quadrats in which *Watersipora* was found. In addition, we examined the data for patterns in substrate type and orientation.

Results

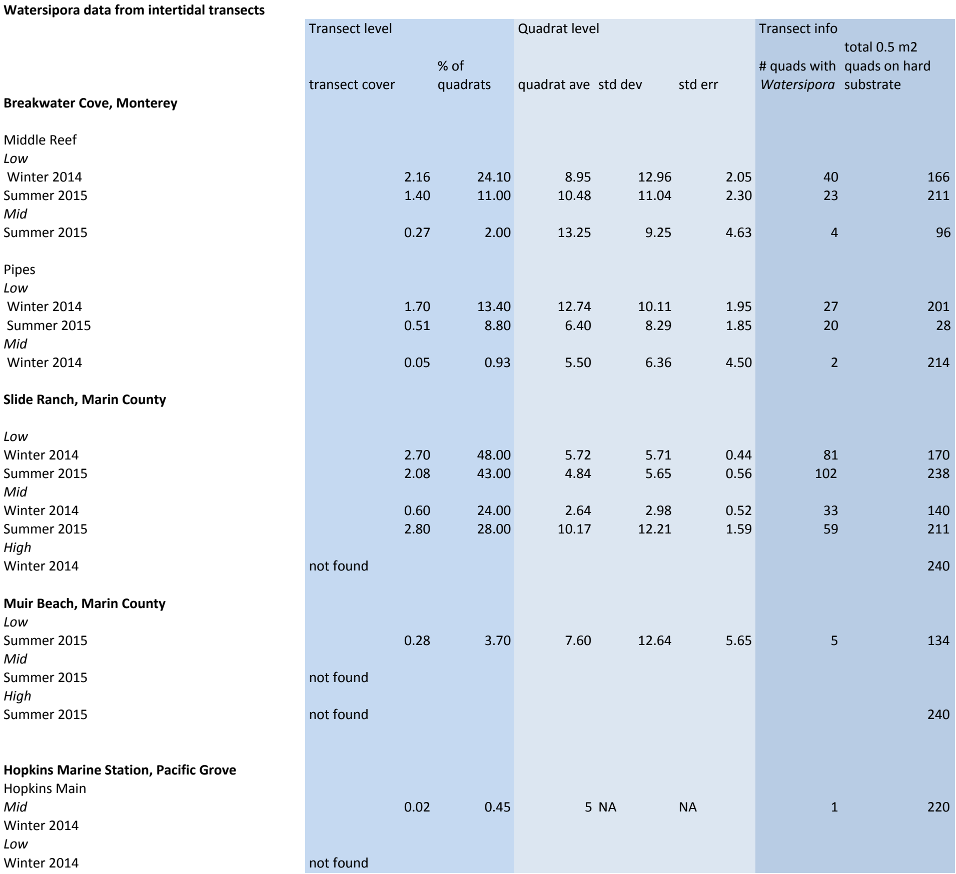
Intertidal Patterns of Distribution and Abundance

Watersipora was found at four of our 10 survey sites, in mid- and low intertidal transects (Figure 5.1). Contemporary with our study, *Watersipora* was also found in 2014 during an additional intertidal survey near Santa Maria Creek in Pt. Reyes National Park, but no abundance data were collected (Kathy Ann Miller, personal communication).

Figure 5.1. Intertidal survey locations. Red stars indicate sites where *Watersipora* was detected. The Pt. Reyes occurrence was reported by KA Miller (unpublished data).

In our surveys, *Watersipora* was most abundant and broadly distributed at Slide Ranch, Marin County and Breakwater Cove, Monterey County (Table 5.1). At Slide Ranch, it was more widely distributed in the low than the mid zone. In winter 2014 and summer 2015, *Watersipora* was found in 48% and 42% of quadrats in the low zone, respectively. By contrast, we detected the organism in only 24% and 28% of quadrats surveyed in the mid-zone in winter and summer. In the mid-intertidal zone at Slide Ranch, there was also a difference between the two sampling dates: in the summer, cover was 2.8% compared with 0.6% in winter. This was not the case for the low transects, which did not change appreciatively between winter (2.7%) and summer (2.1%). In contrast, at Breakwater Cove, *Watersipora* was ~1.5 to 2 times more common in the low intertidal transects in winter 2014 than summer 2015 (Middle Reef transect: -- 24% of quadrats, 2.2% cover in winter versus 11% of quadrats, 1.4% in summer; Pipes transect: 13.4% of quadrats, 1.7% cover vs. 8.8% of quadrats, 0.51% cover). Both the intertidal frequency of occurrence and percent cover were lower at the Muir Beach and Hopkins Marine Station than the other two sites Table 5.1).

Table 5.1. Frequency of Occurrence and Percent Cover of *Watersipora* Detected in Intertidal Surveys. For each site and date, shown are (a) percent of transect *Watersipora* cover and percent quadrats occupied (Transect Level), (b) mean percent *Watersipora* cover and associated measures of variation within quadrats (Quadrat Level), and (c) total number of quadrats where *Watersipora* was detected and total surveyed.



Watersipora data from intertidal transects

	Transect level		Quadrat level			Transect info	
	transect cover	% of quadrats	quadrat ave	std dev	std err	# quads with *Watersipora*	total 0.5 m2 quads on hard substrate
Breakwater Cove, Monterey							
Middle Reef							
Low							
Winter 2014	2.16	24.10	8.95	12.96	2.05	40	166
Summer 2015	1.40	11.00	10.48	11.04	2.30	23	211
Mid							
Summer 2015	0.27	2.00	13.25	9.25	4.63	4	96
Pipes							
Low							
Winter 2014	1.70	13.40	12.74	10.11	1.95	27	201
Summer 2015	0.51	8.80	6.40	8.29	1.85	20	28
Mid							
Winter 2014	0.05	0.93	5.50	6.36	4.50	2	214
Slide Ranch, Marin County							
Low							
Winter 2014	2.70	48.00	5.72	5.71	0.44	81	170
Summer 2015	2.08	43.00	4.84	5.65	0.56	102	238
Mid							
Winter 2014	0.60	24.00	2.64	2.98	0.52	33	140
Summer 2015	2.80	28.00	10.17	12.21	1.59	59	211
High							
Winter 2014	not found						240
Muir Beach, Marin County							
Low							
Summer 2015	0.28	3.70	7.60	12.64	5.65	5	134
Mid							
Summer 2015	not found						
High							
Summer 2015	not found						240
Hopkins Marine Station, Pacific Grove							
Hopkins Main							
Mid	0.02	0.45	5	NA	NA	1	220
Winter 2014							
Low							
Winter 2014	not found						

Watersipora colonies were typically small in the intertidal zone, representing 1-10% cover in most quadrats where it was present, with occasional larger patches (Table 5.1). Quadrat cover averages ranged from 5.5% (standard deviation (SD) = 6.36) to 13.5% (+/- 9.25 SD) at Breakwater Cove, and at Slide Ranch between 2.64% (+/- 2.98 SD) and 10.17% (+/- 12.21 SD). At these sites, *Watersipora* was growing nearly exclusively on vertically oriented surfaces, attached to a wide variety of substrates, including natural rock and rip-rap, fleshy red and brown algae, upright and encrusting coralline algae and other bryozoans. As with frequency of occurrence, percent cover was relatively low at the other two intertidal sites

Subtidal Patterns of Distribution and Abundance

We found *Watersipora* at four of our eight subtidal survey sites: Breakwater Cove, McAbee Beach, Hopkins Marine Station, and Lovers Point. In addition to our surveys, several other historical occurrence records in the region were noted during our study:

- Steve Lonhart photographed *Watersipora* on Jan 11 2007 at Eric's Pinnacle, May 11 2007 at the Moss Landing Power Plant outfall, and Nov 17 2008 at Ventura Rocks near Point Sur.
- Chela Zabin reported a single colony from pilings on the Santa Cruz Municipal Wharf in 1998.
- James Watanabe reported that *Watersipora* had slowly spread along Cannery Row to near Pt. Pinos by 2002. No abundance data were collected from these earlier sightings.

Across the sites surveyed, *Watersipora* exhibited an increase in frequency and abundance with proximity to Breakwater Cove Marina and Monterey Harbor (Figure 5.2, Table 5.2), which is separated from Breakwater Cove by the Coast Guard Pier (a long cement and rip-rapped breakwater). The bryozoan was the most frequently detected at Breakwater Cove, where it was found in all four transects and in 23% of all quadrats across these transects. At McAbee Beach, it was found in both transects and in 10% of quadrats. At Hopkins Marine Station, it was found in all three transects and 8% of quadrats, and at Lovers Point South in two of three transects and 1% of quadrats. Averaging across transects, cover was estimated at 2.5% at Breakwater Cove, 1.3% at McAbee, 0.8% at Hopkins and 0.17% at Lovers Point. At the transect level, Middle Reef 1 and Middle Reef 2 had the highest cover estimates at 3.9% and 3.6%, respectively. McAbee East transect had the third highest cover at 2.3%.

Subtidal quadrats typically had greater *Watersipora* cover than did intertidal quadrats (Tables 5.1 and 5.2). Within quadrats where it was present, *Watersipora* cover ranged from an average of 5% (± 4.42 SD) to 19.2% (± 17.11 SD) at Breakwater Cove; typical cover at Hopkins Marine Station was 13% (± 12.37) , while quadrats in one Lovers Point transect averaged 23% (± 32.13 SD) cover.

Watersipora was found on both vertical and horizontal surfaces, but large foliose colonies were found only on vertical surfaces. Colonies were attached to a wide variety of surfaces, including natural rock and rip-rap, rebar and old metal pipes, fleshy and encrusting algae, barnacles, tubeworms and other bryozoans. We also observed it on the carapaces of decorator crabs. *Watersipora* was also being used as substrate/habitat by other species. For example, at Lover's Point, we observed many brittle star legs extending from large foliose colonies.

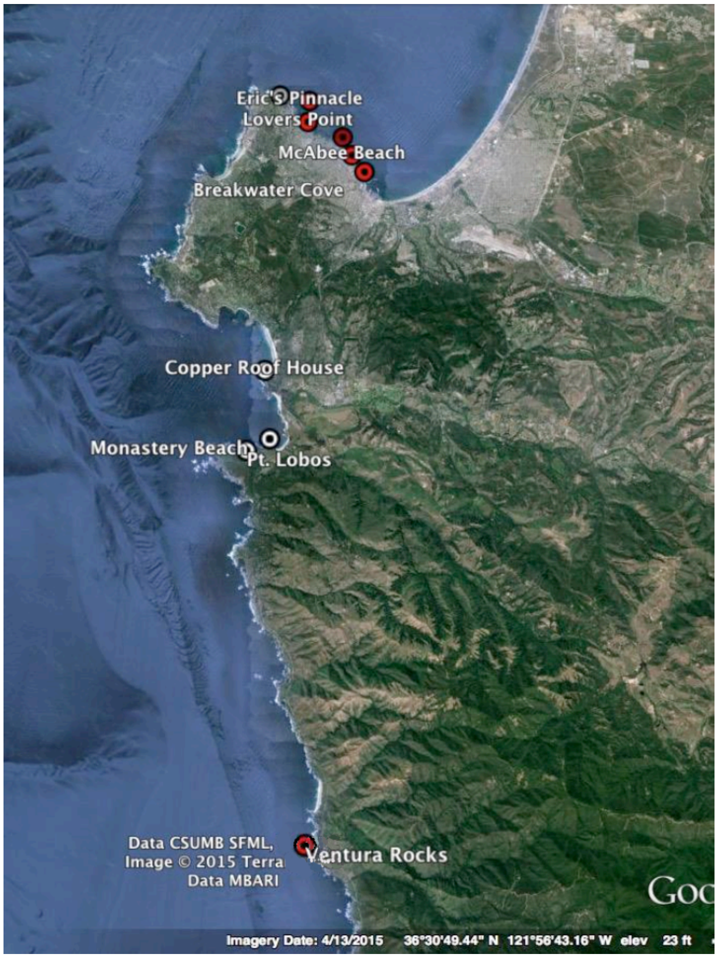


Figure 5.2. Subtidal survey locations. Red circles indicate sites where *Watersipora* was detected. The Eric's Pinnacle and Ventura Rocks occurrence records reported from S Lonhart (unpublished data; see text for information on this and additional occurrence records outside of our survey area).

Table 5.2. Frequency of Occurrence and Percent Cover of *Watersipora* Detected in Subtidal Surveys. For each site and date, shown are (a) percent of transect *Watersipora* cover and percent quadrats occupied (Transect Level), (b) mean percent *Watersipora* cover and associated measures of variation within quadrats (Quadrat Level), and (c) total number of quadrats where *Watersipora* was detected and total surveyed.

	Transect level		Quadrat level			Transect info	
	transect cover	% of quadrats	quadrat ave	std dev	std err	# quads with *Watersipora*	total 0.5 m2 quads on hard substrate
Breakwater Cove, Monterey							
Breakwater Transect	0.83	5.00	16.50	11.92	0.99	12	240
Middle Reef Transect 1	3.87	33.75	11.70	14.80	1.65	81	240
Middle Reef Transect 2	3.60	18.75	19.18	17.11	2.55	45	240
Pipes	1.71	34.17	5.00	4.42	0.69	41	120
Site average (+/-std err)	2.5 (+/-0.76)	22.92	13.09				
McAbee Beach							
West Transect	0.21	6.67	3.27	2.74	0.68	16	240
East Transect	2.34	12.77	10.48	12.18	2.19	30	235
Site average (+/-std err)	1.27 (+/-1.07)	9.72	6.88				
Hopkins Marine Station							
110 m Transect	1.70	14.17	13.40	12.37	3.00	17	120
130 m Transect	0.54	3.75	14.44	9.59	3.20	9	240
90 m Transect	0.29	4.58	13.60	12.30	7.10	11	240
Site average (+/-std err)	0.84 (+/-0.44)	7.50	13.81				
Lovers Point							
Northwest Transect	0.48	2.08	23.00	32.13	16.07	5	240
Near Point Transect 1	0.03	0.83	2.33	2.31	1.33	2	240
Near Point Transect 2	0.00	0.00	0.00			0	240
Site average (+/-std err)	0.17 (+/-0.16)	0.97	8.44				

Synthesis and Historical Perspective

We report new and previously unpublished occurrence records of *Watersipora* for multiple locations, some dating back many years prior to this survey. It's clear that these bryozoans have occurred on the outer coast in Monterey for some time, and we are now compiling this historical record. For example, student surveys at Hopkins Marine Station found that percent cover of *Watersipora* within a 40 x 40 m study plot varied from a high of 4% (SD 10.1%, N = 33) in August 2009 to a low of 0.7% (SD 1.8%, N = 32) in August 2011. During this same timeframe, *Watersipora* was found to vary in frequency of occurrence (detection) among years at the site, occurring in 25-56% of quadrats sampled at various times. Our surveys in the same vicinity had a frequency of occurrence below 15%, suggesting considerable temporal (inter- or intra-annual variation).

Overall, our surveys (and the additional records collected) now extend the known geographic distribution of *Watersipora*, indicating that the organism is common at many outer coast locations. The full extent of its current and potential future distribution is presently unclear. While these new records are consistent with increased colonization of outer coast habitats in recent years, the temporal dynamics in the outer coast distribution (spread) and abundance (percent cover) remains uncertain. However, this study provides baseline measures against which we can test for temporal changes in the distribution and abundance of this invader. More broadly, *Watersipora* represents one of the few NIS that has successfully established populations on the outer coast, providing a useful model system to understand invasion dynamics and spillover from estuaries to outer coastal habitats.

Appendix 5.1: Survey Locations of Outer Coast Sites

Table 5A.1. Intertidal Survey Locations Sampled in the Current Study.

Site name	County	Date of survey(s)	Transects	GPS coordinates
Duxbury Reef, Bolinas	Marin	Dec 4 2014	1 high, 2 mid, 1 low	37°53’40 N 122°42’30 W
Slide Ranch, Marin Headlands	Marin	Dec 3, 20 2014, June 4 2015	1 high, 1 mid, 1 low 1 mid, 1 low	37°52’25 N 122°36’00 W
Muir Beach, Marin Headlands	Marin	May 19 2015	1 low, 1 mid, 1 high	37°51’28 N 122°34’27 W
Fitzgerald Marine Preserve, Half Moon Bay	San Mateo	Dec 8 2014	1 low, 2 mid	37°31’29 N 122°31’07 W
Mavericks, Half Moon Bay	San Mateo	Dec 7 2014	1 high, 1 mid	37°29’43 N 122°29’55 W
Davenport Landing, Davenport	Santa Cruz	Dec 23 2014	1 mid, 1 low	37°01’20 N 122°12’57 W
Natural Bridges, Santa Cruz	Santa Cruz	June 16 2015	1 high, 1 mid	36°56’55 N 122°03’49 W
Soquel Point, Santa Cruz	Santa Cruz	Dec 21 2014, June 17 2015	2 high 1 high, 1 low	36°57’17 N 121°58’19 W
Breakwater Cove, Monterey	Monterey	Dec 6 2014, June 2015	2 low, 1 mid 2 low, 1 mid	36°38’45 N 121°53’43 W
Hopkins Marine Station, Pacific Grove	Monterey	December 2014	1 high, 2 mid, 1 low	36°37’17 N 121°37’13 W

Table 5A.2. Subtidal Survey Locations Sampled in the Current Study.

Location	**Sub location(s)**	**Date**	**# of transects**	**Substrate**	**GPS coordinates**
Breakwater Cove, Monterey	Breakwater	10/6/2014	1	breakwater structure (rip-rap)	36°36’34 N 121°53’36 W
Breakwater Cove, Monterey	Middle Reef	10/6/2014	2	natural rocky reef, sand	36°36’36 N 121°53’42 W
Breakwater Cove, Monterey	Pipes (to Metridium Field)	10/6/2014	1	primarily metal pipes, some natural rock, sand	36°36’42 N 121°53’47 W
McAbee Beach, Monterey	Kelp forest out from beach, SE and SW headings	10/6/2014	2	primarily rocky reef, sand, some pipes	36°36’59 N 121°53’53 W
Hopkins Marine Station, Pacific Grove	inner reef, perpendicular, centered on permanent transect at 90, 110 and 130 m marks	10/8/2014	3	rocky reef, sand	36°37’17 N 121°37’13 W
Lovers Point, Pacific Grove	South, kelp forest, SE and SW headings	10/8/2014	3	rocky reef, sand	36°37’39 N 121°55’04 W
Coral Street, Pacific Grove	Kelp forest at point	10/8/2014	3	rocky reef	36°38’10 N 121°55’36 W
Copper Roof House, Carmel	Kelp forest below house	10/8/2014	3	rocky reef, sand	36°32’46 N 121°55’59 W
Monastery Beach south (MonoLobos), Carmel	Kelp forest, roughly parallel to cliff, one inshore and one deeper	10/7/2014	2	rocky reef, sand	36°31’26 N 121°55’47 W
Pt. Lobos State Park, Big Sur	Whalers Cove, Middle Reef	11/6/2014	3	rocky reef	36°31’16 N 121°56’22 W

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Part II. Molecular Genetic Detection and Confirmation of NIS

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Chapter 6: Benthic Invertebrates

Certain procedures and goals of the joint MLML-SERC effort are specific to the Molecular Ecology laboratory at MLML. These are:

1. Expansion of a DNA Barcode database of *Cytochrome c oxidase subunit I* (COI) and *Large Subunit rRNA* (LSU) DNA sequences. These sequences derived from voucher specimens that have been expertly identified as narrowly as possible serve as references with which to compare new specimens that are unidentified or identified in quick surveys with a lower level of confidence. Results are presented in Appendices 6.1 and 6.2.

2. Sequence Detection. To identify individual specimens, MLML will use next generation sequencing to generate DNA sequences to compare to DNA barcode databases.

3. Data Collection, Management, and Access. MLML will maintain a relational database to track samples, disposition of individual specimens (chain of custody and archived vouchers), compare results of molecular- and morphologically-based organism identifications, and other pertinent information (such as physical data recorded at the time of collection). MLML genetic identifications will be accessible to SERC to enable cross-referencing with the new (NEMESIS portal) MISP database. Database operations are described in Section IIF.
4. Maintain Voucher Collections. MLML will maintain DNA extractions as molecular voucher collections to complement specimen voucher collections at SERC.

This chapter presents the analysis of benthic invertebrates using molecular genetic methods to detect NIS and confirm species identifications, using samples collected by SERC as described in Chapter 2. In Chapter 7, we present genetic results from plankton community analyses.

***A. Voucher materials received for analysis*.**

Marine invertebrate tissue samples were collected from PVC settlement plates and soft-sediment cores 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.

B. DNA Isolation.

DNA isolation was accomplished using either Promega SV Wizard kits or Fisher MagJET Genomic DNA kits. Briefly, a subsample of tissue, approximately the size of a grain of rice, was rinsed of ethanol with distilled water, crushed with a pestle in a 96 deep-well block, and processed according to the manufacturer's instructions. In cases where provided tissue was smaller than the recommended size, we nonetheless carried the tissue through the extraction process. 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).

C. PCR Amplification

The COI gene was amplified by polymerase chain reaction as described in Geller et al., 2013. LSU gene amplification 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 genomic DNA contamination, while well A01 was used as the PCR no template control, substituting 1 uL 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. Routine sequencing was performed on the Ion Torrent PGM platform.

D. Well-indexing.

Sequencing on the Ion Torrent PGM instrument requires PCR templates to be pooled prior to additional preparation for sequencing. In order to separate resulting sequences for each PCR plate, PCR amplification products from the initial round of COI or 28S amplification were diluted 1:50 and 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.

E. 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 pooled. We employed a novel approach to the plate indexing, allowing us double the read lengths for the sequences, a method for which we are currently preparing a manuscript. Products were fragmented chemically using the Ion Shear kit or mechanically using a Covaris ultrasonic shearing instrument to reduce the fragment size for the limits of the sequencer. An adapter needed for the Ion Torrent sequencing kit was ligated to fragments, which were then purified with Ampure beads. The resulting pool of fragmented, adapter-ligated PCR products was then size selected for a 400 bp 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 optimum 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 PGM400 kit. All Ion Torrent reagent kits were purchased from Life Technologies.

F. Bioinformatic analyses.

Metadata for vouchers was entered into a MySQL database at MLML. This includes metadata provided by SERC as well as information to track processing for each vial received. Two reference databases of COI sequences were created to support assessment of samples. The first database (MLML-COI Reference) contains 133 manually curated, high quality records of species (and in some cases haplotype groups within species) previously collected in California waters. These sequences were primarily derived from conventional Sanger (dideoxynucleotide chain termination) sequencing. The second database (CO-ARBitrator DB), which is curated by software, contains 382,010 records collected from the GenBank repository (Benson, 2005) by an adaptation of the ARBitrator algorithm (Heller et al., 2014): records from GenBank were accepted on the basis of high sequence similarity to known metazoan COI sequences, high similarity to the COX-1 conserved domain, and relatively low similarity to any other conserved domain.

Similarly, specimens were also assigned names by comparison to databases of the Large Subunit Ribosomal RNA (LSU) barcode fragment. As above, 2 databases of the barcode gene were collected. The first (MLML-LSU Reference) is a manually-curated collection of 102 species or (haplotypes within species) with high quality collected in California waters. The second database is a larger collection consisting of 7525 animal LSU sequences extracted from the SILVA LSURef version 123 database (www.arb-silva.de; Quast et al., 2013; Yilmaz et al., 2014). Reads were then processed by the Coastline algorithm with modified thresholds: the similarity requirements for the three steps were 85%, 97%, and 97% respectively.

To evaluate the plausibility of genetic assignments, a table of 9 morphological characters was compiled for the 382 most commonly observed organisms (Table 6.I). When an original morphological assignment and a genetic assignment computed by the pipeline (see below) differ in at least 1 of these characters, the reassignment is considered implausible.

Table 6.1. Character codes for plausibility assessment. Composite codes delineate broad morphological categories that are thought to be unmistakable during morphological identification. Examples are given.

Characters:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	8	**9**	
1	Sessile 1	Solitary	Zooids connected directly	Wormlike	Shelled	Encrusting/ prostrate	Branching stalks	Jointed appendages	Crab-like	
2	Motile 2	Compound	Zooids Connected by stolons	Not wormlike	Not Shelled	Upright	Not branching	Non jointed appendages	Not crab-like	
3	N/A	N/A	NA	N/A	N/A	N/A	N/A	N/A	N/A	

Examples										**CODE**
Sea anemone	1	1	3	2	2	2	2	3	3	113222233
Hydroid (Obelia)	1	2	3	2	2	2	1	3	3	123222133
Errant Polychaete	2	1	3	1	2	3	3	2	3	213123323
Tube worm, feather duster, phoronid	1	1	3	1	2	3	3	2	3	113123323
Tube worm, muddy tube	1	1	3	1	2	3	3	2	3	113123323
Tube worm, calcareous tube	1	1	3	1	1	3	3	3	3	113113333
Compound ascidian	1	2	3	2	2	1	2	3	3	123221233
Solitary or social ascidian	1	1	1	2	2	3	2	3	3	111223233
Encrusting bryozoan	1	2	3	2	1	1	2	3	3	123211233
Branching bryozoan	1	2	3	2	1	2	1	3	3	123212133
Clam, mussel, oyster	1	1	3	2	1	3	3	3	3	113213333
Amphipod	2	1	3	2	1	3	3	1	2	213213312
Copepod	2	1	3	2	1	3	3	1	2	213213312
Crab	2	1	3	2	1	3	3	1	1	213213311
Barnacle	1	1	3	2	1	3	3	1	3	113213313
Nemertean	2	1	3	1	2	3	3	3	3	213123333
Platyhelminthes	2	1	3	2	2	3	3	3	3	213223333
Porifera	1	1	3	3	2	1	3	3	3	113321333

G. Coastline Bioinformatic Pipeline.

A software pipeline named “Coastline” (COI Assessment Pipeline) and written in Java by P. Heller was developed to provide automated assessment of genetic sequences with respect to their human-assigned taxonomic categories. In the following “prior” identification refers the morphological identification and “posterior” refers to the genetic identification generated by Coastline.

The Coastline algorithm begins with a filtering step to eliminate contaminant (non-metazoan) and nonsense reads. Reads that do not match any record in either database with ≥75% sequence similarity over ≥ 50 nucleotides are removed from the data set. Such reads are microbial in origin, spurious products of PCR, or artifacts of the Ion Torrent PGM (e.g., very low quality reads). After this filtering, samples that retain fewer than 50 reads have the outcome NOT_ENOUGH_READS and are given the posterior label of "No assignment."

Reads from remaining samples are compared to the MLML-COI Reference and Coarbitrator databases using the BLASTN (nucleotide BLAST) algorithm (Altschul et al., 1990), with thresholds of >95% sequence similarity over ≥ 50 bp. Outcomes from this BLAST search are:

CONFIRM SIMPLE. Samples where ≥ 30% of reads match a single reference with ≥ 95% sequence similarity and the margin to the next highest ranking reference is ≥ 10% are classified as CONFIRM SIMPLE if the taxon of the reference (the "posterior" assignment) is identical to the taxon assigned by SERC (the "prior" assignment).

CONFIRM REFINE. Samples where ≥ 30% of reads match a single reference with ≥ 95% sequence similarity and the margin to the next highest ranking reference is ≥ 10% are classified as confirmed with refinement when the posterior is a taxon included in the prior taxon (e.g., the prior is a family and the posterior is a genus or species).

REASSIGN. Samples where ≥30% of reads match a single reference with ≥ 95% sequence similarity and the margin to the next highest ranking reference is ≥ 10% are classified as reassigned when the posterior does not match the prior. These are putatively misidentified morphologically. Reassignments are further analyzed for plausibility, generating subcategories:

REASSIGN: PLAUSIBLE, where the plausibility codes match;

REASSIGN: IMPLAUSIBLE where the plausibility codes mismatch; and

REASSIGN: PLAUSIBILITY N/A where a plausibility code is not available for the prior or the posterior. This is frequently the case for low-resolution names (eg, “sp1”).

ASSIGN. Samples for which no prior taxonomic information is available and where ≥90% of reads match a single reference with >= 95% sequence similarity and the margin to the next highest ranking reference is ≥ 10% are classified as assigned to that single reference.

AMBIGUOUS. Samples for which multiple references are matched by significant numbers of reads are classified as ambiguous. A list of supported reference taxa is created in which the most strongly supported taxon is supported by at least 30% of reads, and each taxon is supported by at least 50 reads and is within 10% of the next-most supported reference taxon. The taxa within this list are then examined for inclusion of a plausible posterior. This produced subcategories:

AMBIGUOUS PLAUSIBLE, where a member of the list is plausible;

AMBIGUOUS IMPLAUSIBLE, where no members of the list are plausible; and

AMBIGUOUS PLAUSIBILITY N/A, where plausibility codes were not available for members of the list.

Finally, for ambiguous outcomes, any blast result supporting the prior is noted even when supported by fewer than 50 reads, generating the outcome:

AMBIGUOUS PRIOR DETECTED, where the majority of reads support posteriors other than the prior, yet there is some evidence for the presence of the prior in the sample vial.

Current limitations to Coastline.

Coastline was developed in order to avoid manual analysis of the very large data sets produced in this study. As such, it was implemented on actual data and iteratively improved. However, with each iteration, we have identified features of the algorithms that required revision, and this process is not complete. Two important processes that require attention are how reads are culled and plausibility analysis.

Reads which pass the 75% similarity threshold that culls microbial sequences but do not pass the 95% similarity threshold for assignment to references in the DNA sequence databases were discarded. These reads may in the future be assignable to newly acquired reference sequences. If most or all reads are discarded in this fashion, Coastline currently interprets the voucher as having too few reads. This may obscure "no call" of ample data in the category "not enough reads." If most or all reads are in the discarded 75-95% fraction, but >50 reads pass the 95% similarity threshold, Coastline will nominate the reference associated with those few reads and potentially mis-assign the voucher. These algorithmic limitations will be revised in future updates to Coastline.

Plausibility analysis is also incomplete because each new species or taxon encountered has to be coded manually. Names given only as higher taxonomic ranks are usually difficult to code as morphology may vary within that taxon. For example a taxon called Porifera sp1 could not be classified as branching or encrusting.

Because of these limitations, Coastline results are supplemented here by manual inspection of the data.

H. Testing the bioinformatics pipeline.

To validate Coastline's algorithm with simulated data, a statistical model of an Ion Torrent sequencing run was computed using a representative sample with a large number of reads and a genetic confirmation of the morphological assignment. Reads from the representative sample were blasted against the reference COI sequence of the associated organism, with a 98% similarity threshold. Four normal distributions were computed for starting position and length of reads aligning near the 5’ end of the subject reference and for starting position and length of reads aligning near the 3’ end. The fraction of reads aligning near the 5’ end was computed to be 46.2%. Lastly, a normal distribution for number of reads per well was computed from all wells in the data set. This model was used to simulate Ion Torrent sequencing data.

To test how well Coastline will classify newly discovered haplotypes of commonly encountered species, reads from 960 samples were simulated as follows. 10 reference sequences were selected: *Ascidia zara, Botrylloides violaceus, Botryllus schlosseri, Ciona intestinalis, Ciona savignyi, Diadumene leucolena*, *Diadumene lineata, Jassa slatteryi*, *Styela clava,* and *Tricellaria occidentalis.* Closely related species were included to challenge Coastline with similar sequences. For each reference, transitional mutations were generated at randomly selected 3rd-codon positions, until the haplotype sequence was 95% similar to the original reference; this was repeated 8 times for each reference sequence to represent intraspecific variation for each reference taxon. For each simulated haplotype, we simulated 12 sequencing runs in which the number of reads was drawn from the reads-per-well distribution, with a minimum of 500 reads, and 46.2% probability of containing the 5' terminus; otherwise they contained the 3’ end. Length and starting position in the simulated haplotype sequence were drawn from the corresponding normal distributions. Since the PGM sequencer tends to erroneously extend homopolymers (multiple runs of a nucleotide), up to 4 homopolymer sites were randomly chosen from each read, and extended by 1 (with 90% probability) or 2 (with 10% probability) nucleotides. Each simulated well was analyzed by Coastline, using the reference sequence from which reads were simulated as the morphological assignment.

Results.

A. Sample processing.

13,813 specimens were received, and vial numbers entered into a Microsoft Access database at MLML (this database has since been converted to a MySQL database in anticipation of merger with the SERC database). These specimens represented 528 morphotypes considered by SERC taxonomists as separate biological morphospecies; however, these specimens were often not identified morphologically to the species level. Extractions were performed on every specimen in a 96-well format, and the 96-well format was preserved through DNA sequencing. 16,157 PCR reactions were performed (some PCR reactions were repeated). PCR results were tabulated as strong, faint, smeary (i.e, producing both specific and non-specific PCR product), or failed (no apparent PCR product). Strong, faint, and smeary PCR product all produced sufficient reads on the Ion Torrent PGM sequencer for further analysis in >96% percent of cases. "Failed" PCR product produced sufficient reads in 72% of cases, suggesting that sub-detection levels of DNA amplification can still produce NGS data.

B. Testing of Coastline with simulated data.

Reference sequences were mutated to simulate novel haplotype variation of known species, and "sequenced" in-silico by generating "reads" that conform to our results from Ion Torrent

PGM sequencing, including sequencing error due to homopolymer extension. 100% of simulated haplotypes were correctly assigned to the proper taxon, with a CONFIRM SIMPLE outcome of Coastline. The mean fraction of reads that BLASTed to the correct reference was 99.8±0.1% (standard deviation). Most reads that did not match the proper reference did not match any reference. The only cases where reads matched an incorrect reference were simulated *Diadumene leucolena* reads BLASTed to *D. lineata*, and *D. lineata* reads BLASTed to *Metridium senile*. In both cases, the mean fraction of mismatched reads was 0.02%±0.1%. Anthozoans have low rate of mtDNA sequence divergence, so these sequence are intitially very similar in these simulations. These results shows the robustness of the DNA barcodes and the Coastline algorithm with idealized data.

***C. Updates to DNA barcode database*.**

Appendix 6.1 lists specimens sequenced by Sanger sequencing for COI and LSU used in the MLML reference database (described below). Appendix 6.2 lists specimens that have been sequenced but have not been completely curated; they will be added into the operational reference data and added to DNA database.

D. Coastline analysis of vouchers.

Table 6.2A breaks down results of Coastline analysis of vouchers sequenced for COI, based on the current local databases. Many (3406, or 28%) of vouchers were categorized as yielding too few reads for further analysis. While this is a large number, it is consistent with ~70% PCR success rate from our previous pilot study in SF Bay and other barcode projects (Geller et al. 2013). Of the vouchers that produced sufficient read numbers, 26% of genetic identifications agreed with morphological identification. This number, however, is an underestimate of actual concordance due to limitations of Coastline and differences in nomenclature used by SERC and MLML. This is discussed further below. Confirmation and plausible reassignments comprised 45% of vouchers that were assigned. However, low-resolution taxonomic names (i.e., "*Platynereis* sp1" or "Gastropod") were not encoded for plausibility analysis. Confirmations and all potentially plausible reassignments comprise 74% of vouchers that were assigned.

The Coastline analysis also indicates that 11% of the genetic identifications (8% of total vouchers in Table 6.2A) were discordant with morphological identifications and resulted in plausible reassignments across all taxa. This varies among taxa, and the implications for error rates are explored below.

Table 6.2A. Coastline results for Cytochrome c oxidase subunit I.

Outcome	Cases	Percent of Total	Percent of Called	Percent of Unambiguous
CONFIRM: SIMPLE	2193	18%	25%	45%
CONFIRM: REFINE	128	1%	1%	3%
ASSIGN: NO_PRIOR_IDENITIFICATION	135	1%	2%	3%
REASSIGN: PLAUSIBLE_REASSIGNMENT	951	8%	11%	20%
REASSIGN: IMPLAUSIBLE_REASSIGNMENT	548	5%	6%	11%
REASSIGN: UNKNOWN_PLAUSIBLITY_REASSIGNMENT	890	7%	10%	18%
AMBIGUOUS: PLAUSIBLE_POSTERIOR_DETECTED	316	3%	4%	
AMBIGUOUS: PRIOR_DETECTED	284	2%	3%	
AMBIGUOUS: IMPLAUSIBLE_POSTERIOR_DETECTED	1551	13%	18%	
AMBIGUOUS: UNKNOWN_PLAUSIBILITY_POSTERIOR_DETECTED	1622	13%	19%	
NO_CALL: NOT_ENOUGH_READS	3406	28%		
Total	12024	100%		
CONFIRM+PLAUSIBLE	3872	32%	45%	
CONFIRM+PLAUSIBLE+UNKNOWN_PLAUSIBILITY	6384	53%	74%	

Table 6.2B. Coastline results for Large Subunit rRNA, or LSU (also known as 28S rRNA)

Outcome	Cases	Percent of Total	Percent of Called	Percent of Unambiguous
CONFIRM: SIMPLE	2857	22%	27%	32%
CONFIRM: REFINE	656	5%	6%	10%
ASSIGN: NO_PRIOR_IDENITIFICATION	220	2%	2%	4%
REASSIGN: PLAUSIBLE_REASSIGNMENT	1926	15%	18%	24%
REASSIGN: IMPLAUSIBLE_REASSIGNMENT	945	7%	9%	14%
REASSIGN: UNKNOWN_PLAUSIBLITY_REASSIGNMENT	2237	17%	21%	27%
AMBIGUOUS: PLAUSIBLE_POSTERIOR_DETECTED	243	2%	2%	
AMBIGUOUS: PRIOR_DETECTED	379	3%	4%	
AMBIGUOUS: IMPLAUSIBLE_POSTERIOR_DETECTED	300	2%	3%	
AMBIGUOUS: UNKNOWN_PLAUSIBILITY_POSTERIOR_DETECTED	697	5%	7%	
NO_CALL: NOT_ENOUGH_READS	2364	18%		
Total	12824	100%		
CONFIRM+PLAUSIBLE	6061	47%	58%	
CONFIRM+PLAUSIBLE+UNKNOWN_PLAUSIBILITY	6758	53%	65%	

Table 6.3. Comparison of COI and LSU results from Coastline.

Outcome	COI	LSU
CONFIRM: SIMPLE	18%	22%
CONFIRM: REFINE	1%	5%
ASSIGN: NO_PRIOR_IDENITIFICATION	1%	2%
REASSIGN: PLAUSIBLE_REASSIGNMENT	8%	15%
REASSIGN: IMPLAUSIBLE_REASSIGNMENT	5%	7%
REASSIGN: UNKNOWN_PLAUSIBLITY_REASSIGNMENT	7%	17%
AMBIGUOUS: PLAUSIBLE_POSTERIOR_DETECTED	3%	2%
AMBIGUOUS: PRIOR_DETECTED	2%	3%
AMBIGUOUS: IMPLAUSIBLE_POSTERIOR_DETECTED	13%	2%
AMBIGUOUS: UNKNOWN_PLAUSIBILITY_POSTERIOR_DETECTED	13%	5%
NO_CALL: NOT_ENOUGH_READS	28%	18%

Results from from COI and 28S were broadly similar (Table 6.3, 6.4). LSU had a nominally higher rate of confirmation and plausible reassignment; this is due to a lower proportion of sequencing reactions with insufficient reads for analysis (18% vs. 28%). Also, LSU in general is less divergent among related species, therefore fewer taxa in total are found by analysis of LSU sequences (Table 6.4).

T**able 6.4A: Binomials identified by both COI and LSU.**

Amphibalanus improvisus
Anguinella palmata
Ascidia ceratodes
Ascidia zara
Balanus crenatus
Balanus glandula
Barentsia benedeni
Botrylloides violaceus
Botryllus schlosseri
Botrylloides diegensis
Bugula neritina
Bugula pacifica
Bugula stolonifera
Caprella mutica
Celleporaria brunnea
Celleporella hyalina
Cephalothrix simula
Ciona intestinalis
Ciona savignyi
Corella inflata
Cryptosula pallasiana
Diadumene leucolena
Diadumene lineata
Didemnum vexillum
Fenestrulina delicia
Laomedea calceolifera
Molgula manhattensis
Muscalista senhousi
Mytilus trossulus
Myxicola infundibulum
Obelia longissima
Ostrea conchaphila
Schizoporella japonica
Scrupocellaria digensis
Smittoidea prolifica
Styela clava
Tricellaria occidentalis
Watersipora subtorquata

Table 6.4B. Taxa found by COI only.

Alia carinata
Amathia gracilis
Amblypneustes pallidus
Ancistrocheirus lesueuri
Arcuatula senhousia
Aurelia labiata
Caprella californica
Caprella simia
Caulibugula ciliata
Conopeum tenuissimum
Crangon septemspinosa
Crepidula plana
Crepipatella lingulata
Cychrus kralianus
Monocorophium acherusicum
Mya arenaria
Myrianida pentadentata
Mytilus galloprovincialis
Naineris dendritica
Nereis vexillosa
Oligotoma nigra
Onchidoris bilamellata
Oscarella lobularis
Palaemon macrodactylus
Paralithodes camtschaticus
Pectinatella magnifica
Phoronis vancouverensis
Pododesmus machrochisma

Dendronotus venustus
Electra monostachys
Garra tana
Gonothyraea clarki
Grandidierella japonica
Haminoea japonica
Harmothoe imbricata
Hermissenda crassicornis
Ilyanassa obsoleta
Jassa marmorata
Jassa slatteryi
Kellia suborbicularis
Megalobrama terminalis
Membranipora membranacea
Microcosmus squamiger
Polyandrocarpa zorritensis
Polycera atra
Polycera hedgpethi
Polysiphonia brodiei
Pugettia producta
Schistomeringos longicornis
Schizoporella dunkeri
Schizoporella errata
Shizobranchia insignis
Styela plicata
Syllis alternata
Watersipora arcuata
Watersipora spN
Watersipora subovoidea
Zoobotryon verticillatum

Table 6.4C. Taxa found only by LSU.

Barentsia gracilis
Cirratulus cirratus
Conopeum reticulum
Diadumene cincta
Ectopleura crocea
Ficopomatus enigmatus
Halichondria bowerbanki
Haliclona xena
Megasyllis nipponica
Monia umbonata
Mycale macilenta
Obelia bidentata
Pachycordyle pusilla
Serpula columbiana
Triactis producta
Zeuxo holdichi

E. New identifications from genetic analysis.

Many taxa given low resolution morphological identifications remain to be identified and barcoded (see Appendix 6.2). Present results indicate that *Anomia* sp1 is the native rock jingle *Podosesmus machrochisma.* We also found that the morphological taxon "Amphipoda sp1" matched our database records for both *Aoroides crassicornis* and *A. secundus*.

F. Error rate of morphological identifications.

An estimate of error in morphological identification can be made from analysis of plausible reassignments. Appendix 6.3 lists categories of reassignment for COI; for example the first line shows that four specimens of *Amphibalanus improvisus* were genetically reassigned to *Balanus crenatus,* while 41 *Amphibalanus improvisus* were correctly identified, therefore the error rate was 4/45, or 9%. Error rates varied from 1 to 31% for species with more than 10 specimens reassigned or confirmed. The most common error was assigning different species of *Bugula* to *B. neritina* and assigning different species of *Botrylloides* to *B. violaceus*. For COI, plausible reassignments totaled 951 vouchers out of 3,272 plausible assignments, or an error rate of 21%.

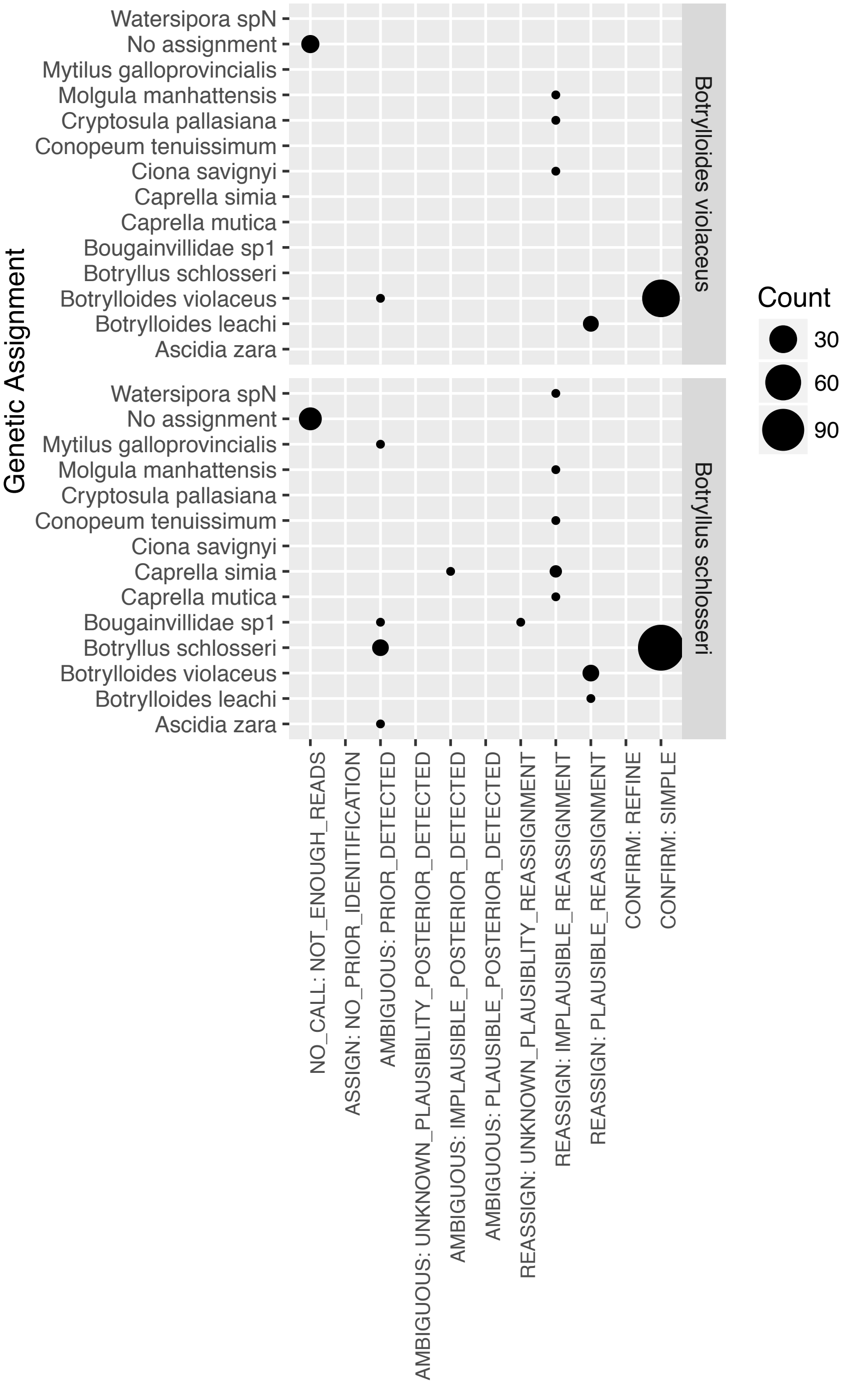

Figure 6.1. Comparisons between genetic and morphological identifications of two Botryllid species. Vouchers that had the morphological assignment of *Botrylloides violaceus* (**Top**) or *Botryllus schlosseri* (**Bottom**), all of their genetic assignments as well as their COASTLINE assignments.

Unfortunately, the high rate of ambiguous assignments in this study do not allow a comprehensive analysis of morphological error rates across all taxa.

An expanded and more detailed comparison of morphological and genetic analysis for botryllid ascidians is shown in Figure 6.1. The genetic identifications and classification by Coastline is indicated for each species, scaled to the number of vouchers that fall into each category. In addition to error in morphological identifications, other sources of error are discussed below.

G. Pseudo-reassignments due to asynchrony of MLML and SERC database updates.

Some mismatches between morphological and genetic identification reflect asynchrony in changes to nomenclature in MLML and SERC databases. These include genetic references that used vouchers with preliminary or provisional SERC names (i.e., those identified at only higher taxonomic levels). SERC later revised these placeholder names to Latin binomials in the SERC database. In the future, SERC will notify MLML that a provisional name had been updated. Too, MLML and SERC may have used synonyms for the same species by drawing on different authorities for valid nomenclature.

These database issues generally result in the complete "reassignment" of one name to another, causing 100% disagreement between morphological and genetic identifications. Currently, Coastline is not able to detect and report such one to one replacements.

Example 1: ***Platynereis bicanaliculata***

A voucher called *Platynereis* sp1, was sequenced and entered into the MLML database. Later, SERC provided 71 additional specimens of the same taxon but named *Platynereis bicanaliculata*. These were sequenced and reads were processed in Coastline, which found a match with *Platynereis* sp1. MLML has no independent data to refute SERC's use of the name *Platynereis bicanaliculata*, therefore *Platynereis* sp1 can be updated to *Platynereis bicanaliculata.* Coastline reassignment of 71 *Platynereis bicanaliculata* to *Platynereis* sp1 deflates the morphological-genetic confirmation rate because both names refer to the same species.

Although Coastline did not recognize the synonymy of *Platynereis bicanaliculata* and *Platynereis* sp1, genetic analysis indicated potentially misidentified specimens of polychaetes, only some of which are plausible misidentifications for *P. bicanaliculata*. Specimens morphologically identified as *Armandia brevis, Eulalia quadriculata, Halosydna brevisetosa, Halosydna johnsoni, Harmothoe imbricata, Hydroides gracilis, Micronereis nanaimoensis, Micronereis* sp1, *Neoamphitrite* sp1, *Neoamphitrite* spA, *Nereis latescens*, *Odontosyllis phosphorea*, i, *Paradialychone ecaudata*, *Platynereis* sp1, Polychaeta, *Polydora* sp1, Sabellidae, Serpulidae, Spionidae, Terebellidae, and *Trypanosyllis* sp1 were genetically assigned to *Platynereis* sp1 (i.e, *P. bicanaliculata*).

Example 2: ***Diplosoma listerianum***

164 *Diplosoma listerianum* were reassigned to a reference sequence called "*Diplosoma* 1" on the basis of an earlier voucher given this low-resolution name. As in the first example, the names *D. listerianum* and "*Diplosoma* 1" were unrecognized synonyms in the SERC and MLML database and were therefore counted falsely as reassignments. However, genetic analysis also uncovered many ascidians given other names that were reassigned to *Diplosoma* 1. These were *Distaplia* sp1, *Didemnum vexillum, Distaplia occidentalis, Symplegma reptans, Perophora annectens, Botryllus schlosseri, Microcosmus squamiger, Polyandrocarpa zorritensis, Aplidium* sp1, and *Aplidium californicum*. On the other hand, NGS sequencing is sensitive to contamination at the tissue or DNA levels, and various other vouchers were implausibly reassigned to *Diplosoma* 1. These included polychaetes, flatworms, bryozoans, solitary ascidians, hydroids, tanaids, and sponges. As discussed below, the true nature, as biological or artifactual, of implausible and ambiguous assignments of NGS sequences is not fully understood.

Example 3. *Harmothoe imbricata*

The original voucher for this species was morphologically identified as "Polynoidae sp1," sequenced, and entered in the MLML barcode database. Later, 123 subsequent samples were identified morphologically as *Harmathoe imbricata* but, as a consequence of the earlier voucher, identified by Coastline as Polynoidae sp1.

Additional examples of pseudo-reassignment due to database issues are given in Table 5.

Table 6.5. Pseudo-reassignments due to synonymy in MLML and SERC databases.

Final Morphological ID	**n**	**Known in MLML DNA barcode database as**
Cordylophora sp1	*5*	*Bougainvilliidae sp1 or sp2*
Ericthonius brasiliensis	*5*	*Gammaridae1 or Gammaridae2*
Garveia franciscana	*17*	*Bougainvilliidae sp1 or Bougainvilliidae Sp2*
Nicolea spA	*28*	*Terebellidae sp1*
Amphithoe lacertosa	*6*	*Ampithoe* sp1
Chrysopetalum occidentalis	29	Terebellidae sp2
Cirriformis sp1	8	Annelida sp1
Total	98	

The cases identified above are not exhaustive but sum to 456 COI reassignments in the Coastline result that are actually agreement between morphological and genetic analysis.

H. Pseudo-reassignments due to synonymy of binomial names.

In some cases, technicians at MLML attempted to update nomenclature in the DNA barcode database to conform with WoRMS, the World Register of Marine Species. For example, MLML changed the record for *Scrupocellaria diegensis* to *Licornia diegensis,* while SERC did not accept this change, resulting in 26 reassignments by Coastline.

I. Pseudo-reassignments due to misnamed MLML references.

Some records in the DNA barcode databases were initially named by matches to Genbank. For example, the MLML database record for *Botrylloides leachii* comes from earlier *Botrylloides* specimens that were unidentified or called *B. violaceus* but that best matched a Genbank record named *B. leachii (*or *"B. leachi")*. In this study, 76 vouchers that were morphologically identified as *Botrylloides diegensis* were consequently genetically reassigned to *Botrylloides leachii*. (There is no Genbank record for *B. diegensis*). Most likely, the MLML reference sequence is misnamed because the phylogenetic affinity to the Genbank record is overestimated in the absence of independent *B. diegensis* sequences, or the Genbank record of *B. leachii* is a mistake.

It appears likely that all of the botryllid vouchers reassigned to *B. leachii* were in fact *B. diegensis*. Note, however, that it was not only vouchers morphologically identified as *Botrylloides diegensis* that were reassigned by Coastline to *B. leachii*: 38 vouchers morphologically identified as *Botrylloides violaceus*, 30 vouchers identified as *Botryllus*, 17 vouchers identified as *Botrylloides* sp4, and 21 vouchers identified as *Botryllus schlosseri* were also reassigned to "*B. leachii*" and were thus actually *Botrylloides diegensis*.

J. Implausible reassignments and ambiguities.

Coastline attempted to analyze reassignments for plausibility: given that each voucher was examined by a technician with a high, or at least reasonable, level of training, some misidentifications were simply not plausible. For example, a bryozoan could never be mistaken for an amphipod. As previously noted, the plausibility analysis in Coastline is imperfect and many reassignments were classified as "unknown plausibility." Nonetheless a striking number of reassignments were categorized as implausible. Figure 6.2 presents all implausibly identifications, and it is clear that ~15 species comprise the majority of these reassignments. These were among the most abundant samples from each bay and site, and suggest that these taxa contaminated tissues, extractions, or PCR products. Sources of contamination are further discussed below. In addition to implausible reassignments, many vouchers resulted in an ambiguous outcome, where more than one taxon was found by Coastline from a putatively single specimen. Specific examples for *Bugula neritina*, cirripeds, and *Cryptosula pallasiana* are shown in Figs. 6.3 to 6.5.

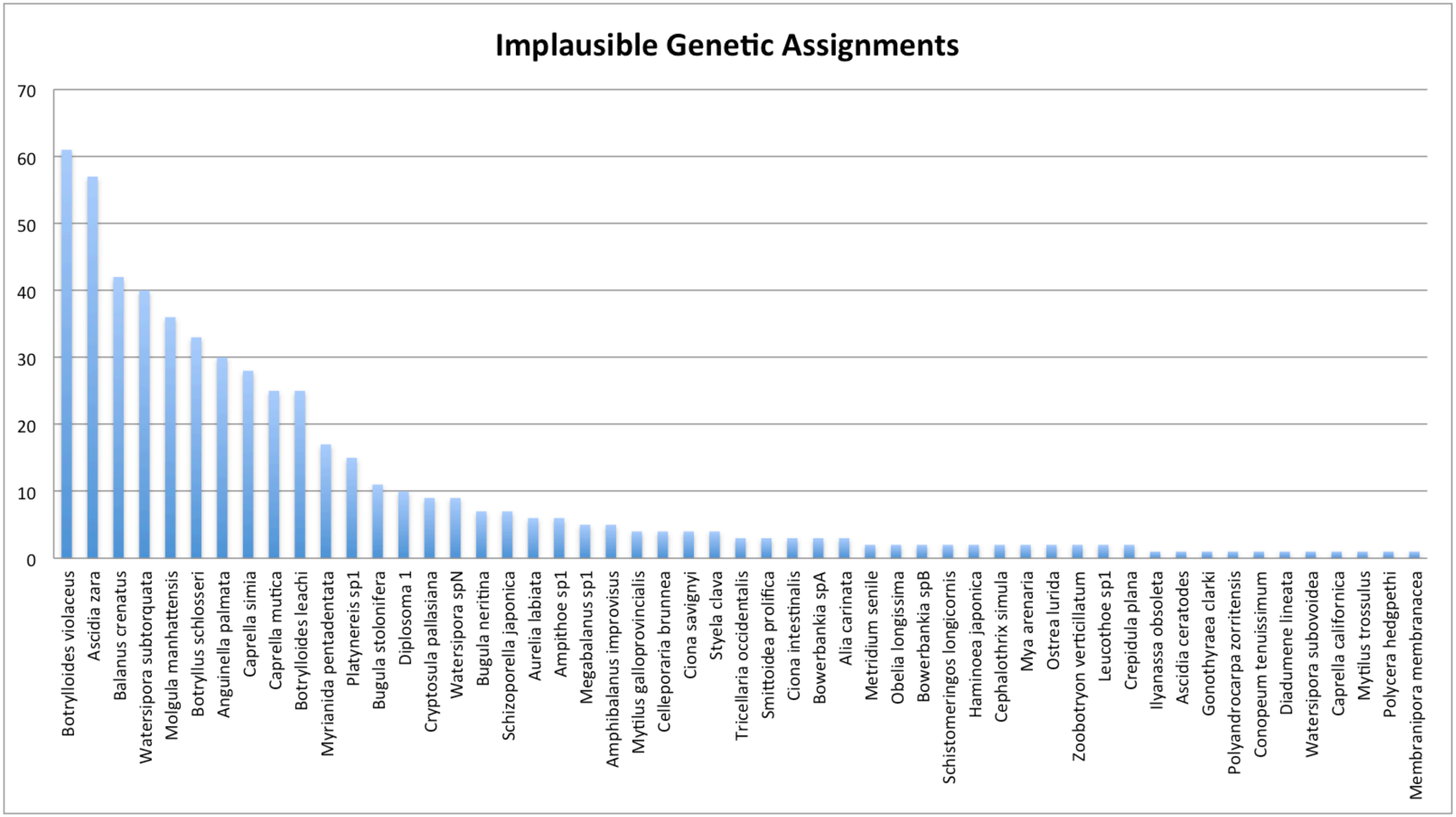

Figure 6.2. Implausible genetic assignments by Coastline analysis of COI for voucher specimens with prior morphological identification. The majority of reassignments are to the most abundant species, suggesting these may contaminate tissues, extractions or PCR products

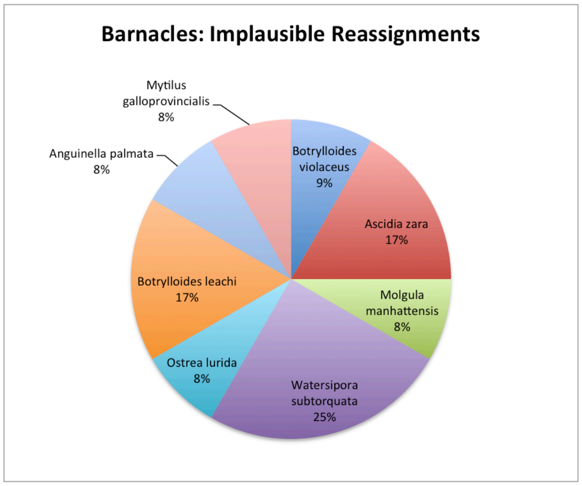

Figure 6.3. Implausible assignments of COI sequences from barnacle vouchers.

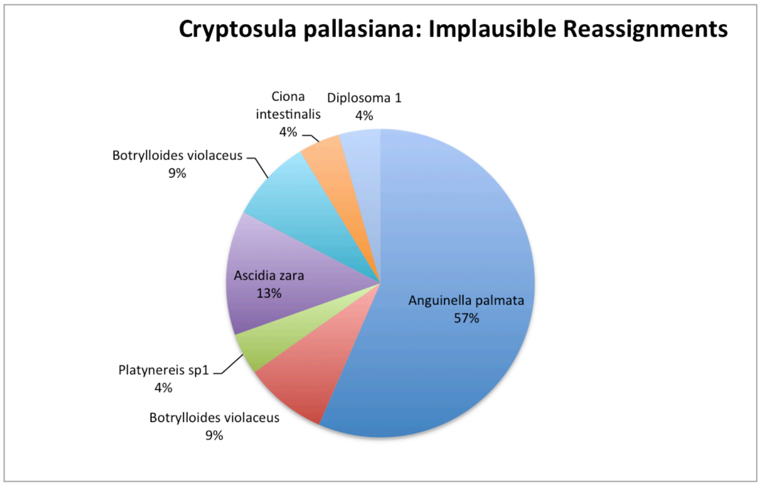

Figure 6.4. Implausible assignments of COI sequences from the bryozoan *Cryptosula pallasiana*.

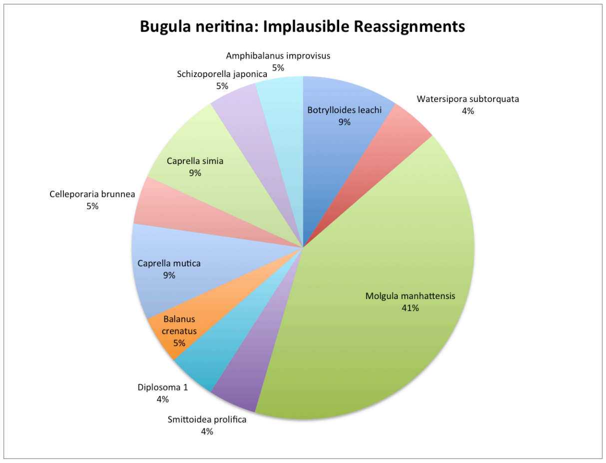

Figure 6.5. Implausible assignments of COI sequences from the bryozoan *Bugula neritina.*

The testing of Coastline in simulations, discussed earlier, suggests that it is unlikely that implausible reassignments were actually due to inadequate power of DNA sequences to distinguish individual species. Therefore, implausible genetic identifications are likely due to correct identifications of the wrong DNAs, and ambiguities were due to more than template receiving the same indexing molecular tag.

We suggest seven hypotheses that are not mutually exclusive for these outcomes:

1) Ecological contamination, where species in physical contact with a target organism contribute tissue or DNA to a vial. This could be due to epibiosis or trophic relationships.

2) eDNA, in which samples are soaked together in examination trays, allowing leached DNA to spread across samples.

3) Contamination in tissue handling by uncleaned forceps or mislabeling of vials.

4) Contamination during DNA extraction or PCR, in which wells of a 96 well plate may be subjected to droplets or aerosols containing genomic DNA from neighboring wells.

5) Contamination of indexing primers, such that a single well (with exactly one COI PCR product) becomes indexed with more than well-barcode (here, "barcode" refers to nucleotide sequences added to PCR product to identify the source sample). Sequences sorted in-silico would be mixtures of all PCR products that were inadvertently indexed with the same well-barcode. In other words, no physical contamination by tissue or PCR product had occurred, but a proportion of sequences would be mis-assigned to the wrong voucher specimen.

6) Sequencer error in the 5' region of templates can cause change to the sequence of indexing well-barcodes, resulting in mis-assignment.

7) The sensitivity of NGS increases the output of incorrect sequences because of trace production of spurrious PCR product below detection on gels is passed through our workflow and generates sequences. Species that are known to be difficult to extract and sequence are more likely to have contain spurrious products from contamination via the sources 1-4 above. For example, we know that *Didemnum vexillum* is difficult to extract and amplify with our primer sets. Many *D. vexillum* vouchers were assigned to other, mostly implausible taxa (Fig. 6.6); only 42 of 169 were confirmed by COI as *D. vexillum*. The majority of implausible assignments are to species common in our workflow that may "take over" primary or indexing PCR in the absence of competition from viable *D. vexillum* DNA.

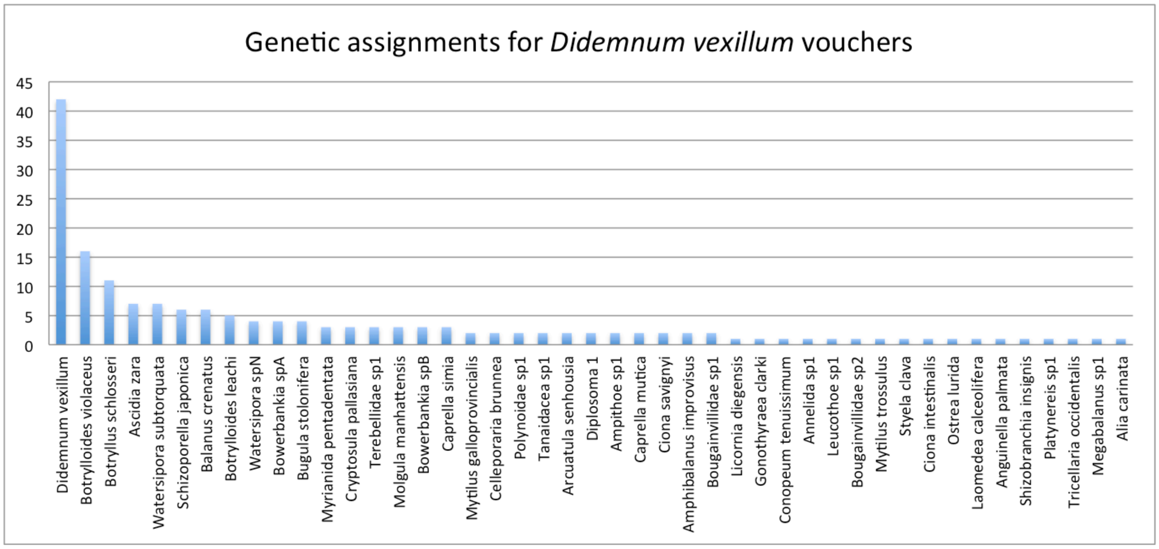

Figure 6.6. All genetic assignments, including confirmations reassignments, and ambiguous outcomes, for *Didemnum vexillum*. This species is known to be difficult to extract and amplify, and may therefore be especially prone to amplification of ecological or laboratory contaminant.

A similar pattern is seen for vouchers morphologically identified only as Porifera. Of 527 specimens, only 10 were assigned to a sponge taxon. The majority (251) were unassigned due to too few reads (an indication of poor PCR) or implausibly assigned (Fig 6.7).

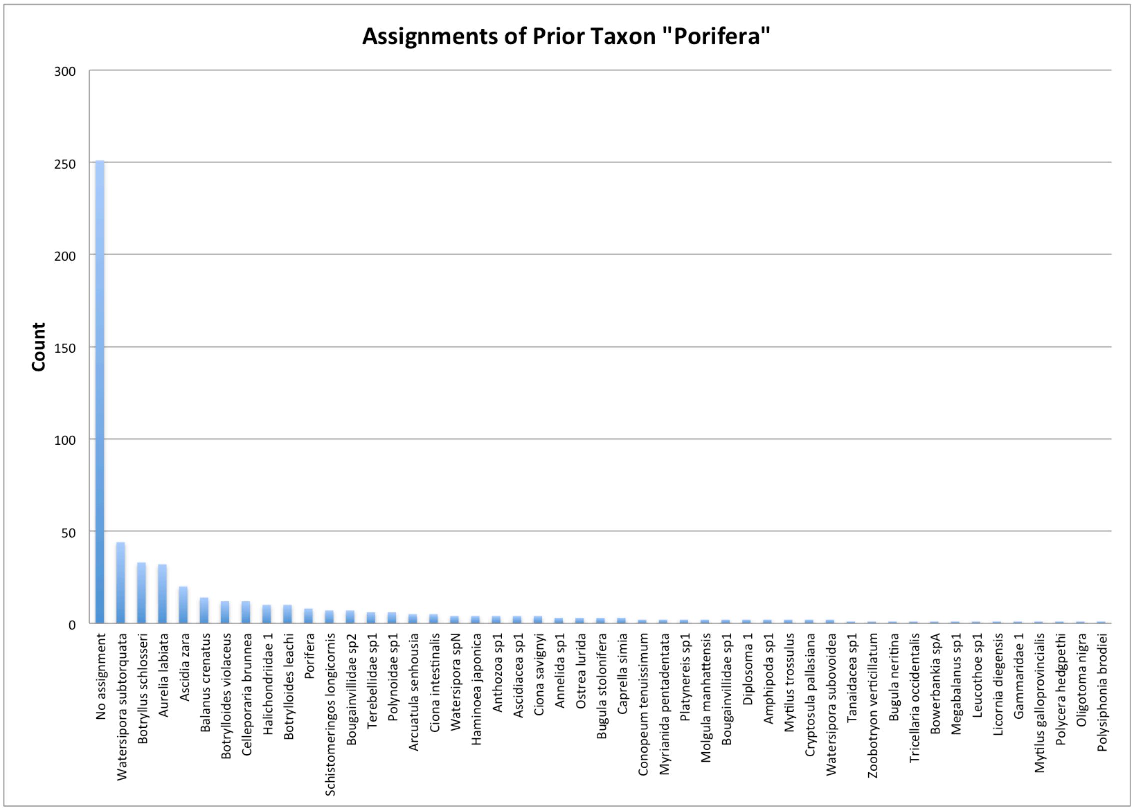

Figure 6.7. . All genetic assignments, including confirmations reassignments, and ambiguous outcomes, for the taxon identified only as "Porifera". Sponges contain secondary compounds that can interfere with PCR. Consequently, most specimens yielded too few reads for analysis; reads from other specimens appear to be a random collection of species in the fouling community.

Contamination of microbial metagenomic libraries is a well-known phenomenon (e.g., Strong et al. 2014) that bedevils both ecological and clinical studies. The issue is essentially unstudied for marine metazoan metagenetic studies.

K. Implausibles and ambiguous outcomes are evidence of presence.

The previous section describes many cases where database reference sequences that match DNA reads from a voucher are implausible identifications for morphologically known vouchers. We consider these cases to represent contamination from some source, which may include other organisms, gear, seawater, or DNA molecules that have contacted a specimen or its derivatives (DNA, PCR products). Unless the contamination is extraneous to this project, the DNA reads are evidence of presence of the novel organisms that were genetically identified. In these cases, we do not have a morphological voucher to verify these species in our sampling sites, but we would be imprudent to ignore them.

For example, Voucher specimens 154780, 178080 and 178412 were identified as *C. tenuissimum* but are assembled consensus sequences included some that were 98.7% and 97.8% similar to

Genbank records HM070022, HM070022 and HM070021 identified morphologically as *Electra* sp.

Cephalothrix simula were identified from reads derived from *Ciona intestinalis, Microcosmus squamiger*, Gastropoda, Ctenostomida, *Ficopomatus enigmaticus* and Nemertea vouchers. When assembled, these "extra" sequences matched Genbank COI record GU726640 for *Cephalothrix* sp. 6 at 98.5% similarity with 87% coverage. LSU sequences from these vouchers BLASTED to *C. hongkoniensis* (98.9% simalarity; HQ856840), *Procephalothrix simulus* (98.5% simalarity; AJ436891).

Some other examples are the shrimp *Crangon septemspinosa* sequenced from a vial morphologically identified as *Gammarus daiberi*; the sea urchin *Amblypneustes pallidus*, from the terebellid identified as *Streblosoma uncinatus*; the sponge *Oscarella lobularis* from *Diplosoma listerianum*, and the bryozoan *Schizoporella dunkeri* from *Doto amyra.*

L. Metagenetic analysis of plates.

If extractions from voucher specimens are collections of template DNA, sequencing becomes a mini-metagenetics problem. To investigate this concept, Ion Torrent data from four 96-well plates were combined, for a total of 1,112,733 reads. These were size filtered to >300 bp, stripped of PCR primers, and truncated to common length of 273 bp. Reads were then dereplicated, screened for chimera sequences, and clustered into OTUs with at least two reads, leaving 4990 OTU. These were BLASTED against the Coarbitrator (Genbank) and MLML databases, resulting in 67 named taxa that were ≥95% similar with ≤50% coverage (some may be duplicates due to synonyms in the databases) (Table 6.6). *Callyspongia siphonella* and *Terpios hoshinota* stand out as potentially novel species, however COI barcodes are least resolving for sponges so these results were not too striking. The echiuran *Metabonellia haswelli,* apparently an Australian species is also a peculiar hit; more detailed analysis of the OTU sequence however indicates that a better match was the native *Urechis caupo* (99% similarity to Genbank AY619711 with 100% coverage). *Urechis* was not contained in the reference databases.

The only taxa identified morphologically that was not found in the metagenetic analysis of these plates were: *Crisularia pacifica*, *Diadumene leucolena*, *Grantia*, *Perophera* sp., and *Polydora* sp. The conclusion is that metagenetics of pooled reads from four plates did a good job of generating a species list that is similar but more exhaustive than a morphological species list.

Table 6.6. Metagenetic analysis of 4 plates of voucher specimens (i.e, n=386).

Taxon Detected	**Database Source**	**Morphologically identified?**
Amathia1	MLML	yes
Amphibalanus improvisus	Both	yes
Amphipod1	MLML	no
Annelid1	MLML	no
Ascidia_ceratodes	MLML	yes
Ascidia zara	Both	yes
Balanus_crenatus	MLML	yes
Balanus glandula	Both	no
Botrylloides "leachii" (diegensis)	Both	yes
Botrylloides violaceus	Both	yes
Botryllus schlosseri	Both	yes
Bugula dentata	GB	no
Bugula neritina	Both	yes
Bugula pacifica	Both	no.
Bugula stolonifera	Both	yes
Callyspongia siphonella	GB	no
Campanulariidae1	MLML	no
Caprella californica	Both	no
Caprella mutica	Both	no
Caprella simia	MLML	no
Chone magna CMC01	GB	no.
Ciona intestinalis	Both	yes
Ciona savignyi	Both	yes
Conopeum tenuissimum	MLML	*Conepeum* sp.
Cryptosula pallasiana	Both	yes
Dendronotus venustus	GB	no
Didemnum vexillum	Both	yes
Diplosoma listerianum	GB	yes.
Ectopleura crocea	GB	no.
Eupolymnia heterobranchia	GB	no
Gonothyraea clarki	MLML	*Gonothyraea* sp.
Halichondriidae1	MLML	Halichondria sp.
Haliclona oculata	GB	*Haliclona* sp.
Haminoea japonica	Both	*Styela clava*
Hermissenda crassicornis	GB	yes
Leucothoe1	MLML	no
Maractis sp. SP-2014	MLML	no
Megalomma splendida	GB	no
"Metabonellia haswelli" (Urechis caupo)	GB	no
Metridium senile	MLML	no

Molgula manhattensis	Both	yes
Musculista senhousia	Both	yes
Mytilus edulis	GB	*Mytilus* sp.
Mytilus galloprovincialis	Both	*Mytilus* sp.
Mytilus trossulus	Both	*Mytilus* sp.
Obelia bidentata	GB	*Obelia* sp.
Obelia longissima	MLML	*Obelia* sp.
Obelia sp. MW-2012	GB	*Obelia* sp.
Obelia sp. RW-2010	GB	*Obelia* sp.
Ophiodromus pugettensis	GB	no
Platynereis1	MLML	yes
Platynereis sp. CMC02	GB	yes
Polycirrus sp. CMC03	GB	no
Polycirrus sp. CMC06	GB	no
Polychaeta1	MLML	no
Polynoidea1	MLML	no
Schistomeringos longicornis	Both	no
Schizoporella errata	Both	no
Schizoporella japonica	MLML	yes
Serpula columbiana	MLML	no
Syllid1	MLML	Syllidae
Syllis alternata CMC04	GB	Syllidae
Terpios hoshinota	GB	no
Terebellidae2	MLML	Terebellidae
Tricellaria occidentalis	MLML	yes
Watersipora sp. Santa Cruz Harbour	Both	*W. subtorquata*
Watersipora subtorquata	Both	yes

M. Identification of intraspecific diversity in Botryllus schlosseri.

While it was not a specific goal within the current contract to detect intraspecific variation, DNA sequence can be used in this manner. When intraspecific genetic divergence is associated with geographic regions, sources of invasions can be inferred. In general, we found that Ion Torrent PGM data were not conducive to this sort of analysis because of the computational effort of assembling consensus sequences from hundreds to thousands of read per voucher specimen. Also, the high error rate of Ion Torrent PGM sequences, creates the need for extensive manual editing of assemblies and alignments. However, we explored this approach for one case, the widely introduced ascidian *Botryllus schlosseri*.

Geographic structure within species is not uncommon among marine invertebrates. Yund et al. (2015) recently presented evidence for clades within *Botryllus schlosseri*, including one, called subclade Bs2, restricted to the Northwest Atlantic. We analyzed sequences from vouchers that were both morphologically and genetically confirmed as *Botryllus schlosseri* by assembling reads into a single consensus sequence for each specimen, aligning with sequences from the Yund et al. (2015) study, and generating a maximum likelihood tree. Clades found by Yund et al. (2015) were identified, and we noted the placement of specimens collected in this projectour voucher specimens. All *Botryllus schlosseri* that were confirmed by both genetics and morphology are were contained in the widespread Clade A and mostly within subclade Bs2, and were similar to haplotype Bs8 that that was previously noted on the Pacific coast (Fig. 6.8). Some of the haplotypes in the Bs2 subclade from vouchers in this study appear to be novel, however we recommend further resequencing to confirm this due to high error rate in the Ion Torrent PGM sequencing. We conclude that the majority of haplotypes can be traced to populations in the Northwest Atlantic, perhaps associated with oyster tranfers made in the late 19th and early 20th century. Others haplotypes are contained in Clade A but outside subclade Bs2 and are not unambiguously associated with a single region in the north Atlantic or Mediterranean Sea.

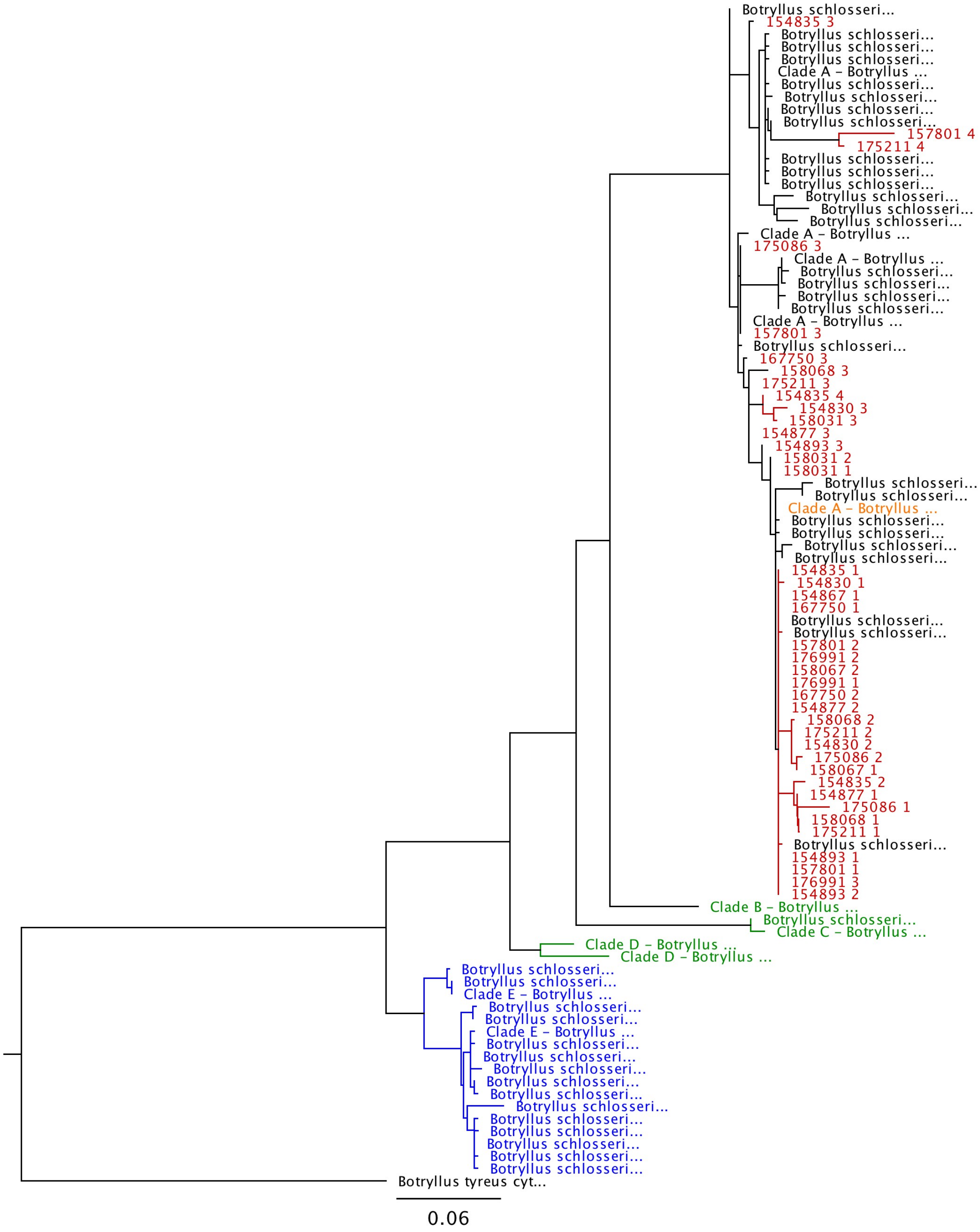

Figure 6.8. ***Botryllus schlosseri*****.** Widespread Clade A is shown in black, and subclade Bs2 is marked by the representative haplotype Bs2 in orange. Clade E from the Northeast Atlantic is shown in blue; Clades B, C, and D from Spain and France are shown in green. Specimens from the current study are shown in red, are all from Clade A, and are mostly nested in subclade Bs2.

N. Review of Methodology.

The approach taken herein was to create a reference library of full-length DNA barcode sequences with Sanger sequencing, and use NGS sequencing to generate shorter sequences that could be identified by querying that database. It was unexpected that NGS sequencing from single specimens should generate reads matching more than one DNA barcode. This introduced complexity in the data analysis because multiple results for each specimen had to be examined. Consequently, significant resources were devoted to development of Coastline outside the scope of the contract. Our analytical approach was to rank all references that received support by reads from an individual voucher, and apply thresholds to determine unambiguous and ambiguous selection of a single candidate for the genetic identification. Such thresholds were necessarily arbitrary, and uncertainty arose when the prior (morphological) identification was among lower ranked references.

Our assumption is that multiple taxa within DNA extraction ostensibly from a single voucher results contamination at some step in the processing workflow, from specimen sorting through in-silico analysis. (Reads from bacteria were expected, as most organisms are hosts to a rich microbiome). The high-volume methods for sample handling may have resulted in greater opportunity for contamination of tissue, extractions, or PCR products. For example, we process samples in a 96-well format in which samples are physically within millimeters of each other: opening and closing caps could create pervasive aerosols that contaminate neighboring wells. Also, even trace levels of contamination by indexing primers would confuse the indexing of individual wells.

Database issues also confounded automated data analysis within Coastline. In particular, asynchrony between MLML and SERC databases resulted in synonyms that Coastline did not recognize. Reassignments, whether ambiguous or unambiguous by Coastline thresholds, often appeared implausible. Automated plausibility analysis using morphological codes also proved difficult. For plausibility, it will be easier automatically compare the phylum of prior and posterior identifications, which should reveal the majority of implausible reassignments encountered. Coastline could also limit data analysis to strong PCR results: this should reduce the number of ambiguous outcomes. Finally, Coastline should retain reads that do not match the local databases, as these may be novel genotypes that do not appear in any database.

It is clear that improvements in sample processing and analysis are necessary if NGS is to be used for future voucher sequencing. Alternatively, Sanger sequencing should be reconsidered as the first option. Sanger sequencing is more expensive on a per-read basis, but is not subject to the ambiguous outcomes we experienced. This is because any significant contamination results in illegible DNA sequences that are discarded, whereas NGS is a single-molecule sequencing method and contamination does not prevent production of legible, distinct sequences. On the other hand, metagenetic analysis of pooled voucher data was straightforward and produced results that were congruent with morphological identification. In conclusion, the strength of metagenetic analysis is strongly supported (see also the plankton results, Chapter 6) while simplification to the physical and computational handling of voucher data is clearly necessary.

Appendix 6.1.

Taxa that have been sequenced by MLML and included in the MLML-COI and MLML-LSU databases. A Genbank entry of LSU was used for *Ciona savignyi*. Analyses were conducted under the previous contract ("SFNIS") or current contract ("CalNIS"). + = sequenced, - = not sequenced. Organisms that were not identified with a Latin binomial are given provisional names for use in database functions and analyses.

Organism	COI	LSU	Project
Alcyonidium1	-	+	SFNIS
Amphibalanus amphitrite amphitrite	+	-	CalNIS
Amphibalanus improvisus	+	-	SFNIS
Amphipod1	+	-	SFNIS
Amphipod2	-	+	SFNIS
Ampithoe lacertosa	+	-	CalNIS
Anguinella palmata	+	+	SFNIS
Annelid1	+	-	SFNIS
Anthozoa1	+	-	SFNIS
Aoroides columbiae	+	-	CalNIS
Ascidia ceratodes	+	+	SFNIS
Ascidia zara	+	+	SFNIS
Ascidiacae1	+	-	SFNIS
Aurelia labiata	+	-	SFNIS
Aurelia1	+	-	SFNIS
Balanus crenatus	+	+	SFNIS
Balanus glandula	+	+	SFNIS
Balanus improvisus	-	+	SFNIS
Barentsia benedeni	+	+	SFNIS
Barentsia gracilis	-	+	SFNIS
Bispira1	+	+	SFNIS
Bispira2	-	+	SFNIS
Bivalvia1	+	-	SFNIS
Botrylloides diegensis	-	+	SFNIS
Botrylloides leachi1	+	-	SFNIS
Botrylloides leachi2	+	-	SFNIS
Botrylloides violaceus1	+	+	SFNIS
Botrylloides violaceus2	+	-	SFNIS
Botryllus schlosseri	+	+	SFNIS
Bougainvillea sp.	+	-	CalNIS
Bougainvillia1	+	-	SFNIS
Bowerbankia gracilis	-	+	SFNIS
Bowerbankia1	+	+	SFNIS
Bowerbankia2	-	+	SFNIS
Bryozoa1	+	-	SFNIS
Bryozoa2	+	-	SFNIS
Bryozoa3	+	-	SFNIS
Buccinoidea1	-	+	SFNIS
Bugula neritina	+	+	SFNIS

Bugula pacific	+	+	SFNIS
Bugula stolonifera	+	+	SFNIS
Bugula1	-	+	SFNIS
Caenogastropod2	-	+	SFNIS
Caenogastropod1	-	+	SFNIS
Capitellidae1	-	+	SFNIS
Caprella californica	+	-	SFNIS
Caprella mutica	-	+	SFNIS
Caprella mutica1	+	-	SFNIS
Caprella mutica2	+	-	SFNIS
Caprella simia	+	-	CalNIS
Caulibugula ciliata	+	-	SFNIS
Celleporaria brunnea	+	+	SFNIS
Celleporella hyalina	+	+	SFNIS
Cephalothrix simula	+	-	SFNIS
Ciona intestinalis	+	+	SFNIS
Ciona savignyi	+	+	SFNIS
Cirratulidae1	+	-	SFNIS
Cirratulus cirratus	+	+	SFNIS
Cirriformia1	-	+	SFNIS
Clathria1	-	+	SFNIS
Collumbellidae1	+	-	SFNIS
Conopeum reticulum	-	+	SFNIS
Conopeum tenuissimum1	+	-	SFNIS
Conopeum tenuissimum2	+	-	SFNIS
Conopeum1	-	+	SFNIS
Conopeum2	-	+	SFNIS
Corella inflata	+	+	SFNIS
Corophium1	+	+	SFNIS
Crassadoma gigantea	+	-	SFNIS
Crepidula plana	+	-	SFNIS
Cryptosula pallasiana1	+	+	SFNIS
Cryptosula pallasiana2	+	-	SFNIS
Dendrobeania lichenoides	+	-	SFNIS
Diadumene cincta	-	+	SFNIS
Diadumene leucolena	-	+	SFNIS
Diadumene lineata	-	+	SFNIS
Diadumene1	+	-	SFNIS
Didemnum vexillum	+	+	SFNIS
Didemnum2	-	+	SFNIS
Diplosoma1	+	-	SFNIS
Distaplia1	+	+	SFNIS
Distaplia2	-	+	SFNIS
Ectopleura crocea	-	+	SFNIS
Electra1	+	-	SFNIS
Ericthonius brasiliensis	+	-	CalNIS

Fenestrulina delicia	+	+	SFNIS
Ficopomatus enigmatus	-	+	SFNIS
Flustrina1	-	+	SFNIS
Gammarid1	-	+	SFNIS
Gammaridae	+	-	SFNIS
Gammaridae ampithoe1	+	-	SFNIS
Gammarus daiberi	+	-	CalNIS
Gastropoda1	-	+	SFNIS
Gonothyraea clarki	+	-	SFNIS
Gonothyraea1	-	+	SFNIS
Halichondria bowerbanki	-	+	SFNIS
Halichondria2	-	+	SFNIS
Halichondriidae1	-	+	SFNIS
Haliclona xena	-	+	SFNIS
Haminoea callidegenita	+	-	SFNIS
Haminoea japonica	+	-	CalNIS
Hyalella azteca	+	-	CalNIS
Hydrozoa1	+	-	SFNIS
Hydrozoa2	+	-	SFNIS
Illyanassa obsoleta	+	-	SFNIS
Isopod1	-	+	SFNIS
Jassa slatteryi	+	-	SFNIS
Jassa staudei	+	-	CalNIS
Laomedea calceolifera	+	+	SFNIS
Leucosolenia1	-	+	SFNIS
Leucothoe1	+	-	SFNIS
Megabalanus1	+	-	SFNIS
Megasyllis nipponica	-	+	SFNIS
Membranipora chesapeakensis	+	-	SFNIS
Membranipora membranacea	+	-	SFNIS
Metridium senile	+	-	SFNIS
Molgula manhattensis	+	+	SFNIS
Monia umbonata	-	+	SFNIS
Monocorophium acherusicum	+	-	CalNIS
Monocorophium insidiosum1	+	-	CalNIS
Monocorophium insidiosum2	+	-	SFNIS
Musculista senhousia	+	+	SFNIS
Mya arenaria	+	-	SFNIS
Mycale macilenta	-	+	SFNIS
Myrianida pentadentata	+	-	SFNIS
Mytilus galloprovincialis	+	+	SFNIS
Mytilus trossolus	+	+	SFNIS
Mytilus1	-	+	SFNIS
Myxicola infundibulum	+	+	SFNIS
Naineris dendritica	+	-	SFNIS
Nebalia1	-	+	SFNIS

Neodexiospira1	-	+	SFNIS
Nereis vexillosa	+	-	CalNIS
Nudibranchia1	-	+	SFNIS
Nudibranchia2	+	-	SFNIS
Nudibranchia3	+	-	SFNIS
Obelia bidentata	-	+	SFNIS
Obelia longissima	+	+	SFNIS
Onchidoris bilamellata	+	-	SFNIS
Ostrea conchaphila	+	+	SFNIS
Ostreola equestris	+	-	SFNIS
Pachycordyle pusilla	+	-	SFNIS
Pectinatella magnifica	+	-	CalNIS
Phialella quadrata	-	+	SFNIS
Phoronis vancouverensis	+	-	SFNIS
Pinauay crocea	+	-	SFNIS
Platyhelminthes1	+	-	SFNIS
Platynereis1	+	-	SFNIS
Polycera atra	+	-	CalNIS
Polycera hedgpethi	+	-	CalNIS
Polychaeta1	+	-	SFNIS
Polychaeta2	-	+	SFNIS
Polydora1	-	+	SFNIS
Polynoidae1	-	+	SFNIS
Polynoidae2	+	-	SFNIS
Polysiphonia1	+	-	SFNIS
Procephalothrix simulus	-	+	SFNIS
Schistomeringos longicornis	+	-	SFNIS
Schizoporella dunkeri	+	-	SFNIS
Schizoporella errata	+	-	SFNIS
Schizoporella japonica	+	+	SFNIS
Scrupocellaria digensis	+	+	SFNIS
Serpula columbiana	+	+	SFNIS
Shizobranchia insignis	+	-	SFNIS
Smittoidea prolifica	+	+	SFNIS
Stramonita biserialis	+	-	SFNIS
Styela clava	+	+	SFNIS
Sycon1	-	+	SFNIS
Sycon1	-	+	SFNIS
Syllid1	+	-	SFNIS
Syllis1	+	-	SFNIS
Syllis2	+	-	SFNIS
Syllis3	+	-	SFNIS
Tanaidacea1	+	-	SFNIS
Terebellidae1	-	+	SFNIS
Terrebellidae2	+	-	SFNIS
Thaisella kiosquiformis	+	-	SFNIS

Triactis producta	-	+	SFNIS
Tricellaria occidentalis	+	+	SFNIS
Turbellaria1	-	+	SFNIS
Urosalpinx cinerea	+	-	CalNIS
Venerupis philippinarum	+	-	CalNIS
Watersipora subtorquata	+	+	SFNIS
Zeuxo holdichi	-	+	SFNIS

Appendix 6.2.

Taxa, as identified by SERC, that have been conventionally sequenced to be entered into MLML databases pending editing and quality verification. + = sequenced, - = not sequenced.

Organism	**COI**	**LSU**
Aetea 1	+	-
Americorophium spinicorne	+	+
Ammothea hilgendorfi	+	-
Ampithoe lacertosa	+	-
Ampithoe valida	-	+
Aoroides 1	-	+
Aoroides inermis	-	+
Aoroides2	+	-
Aphoyale anceps	-	+
Aplidium1	+	-
Aruga holmesi	+	-
Bryozoa1	-	+
Bugula flabellata	+	-
Calloporidae1	+	-
Cancer magister	-	+
Caprella equilibra	+	+
Caprella scaura	-	+
Caprella1	+	-
Caprella2	-	+
Caprella3	-	+
Caridean	+	-
Chrysopetalidae1	-	+
Ciona 1	+	-
Clytia1	-	+
Corbula amurensis	-	+
Crangon nigricauda	+	+
Crisia occidentalis	+	-
Cryptomya californica	-	+
Cuthona1	+	-
Dendronotus 1	-	+
Deutella californica	+	+
Dirona picta	+	-
Distaplia occidentalis	+	-
Dorvillea1	-	+
Eubranchus misakiensis	+	-
Eudistylia 1	+	-
Fenestrulina 1	+	-
Filicrisia 1	-	+
Filicrisia franciscana	+	-
Gammaropsis thompsoni	+	+

Gastropoda1	+	-
Gemma gemma	-	+
Gnorimosphaeroma oregonensis	+	+
Halosydna leius	-	+
Hemigrapsus 1	-	+
Hemigrapsus oregonensis	-	+
Heptacarpus 1	-	+
Heptacarpus paludicola	+	+
Heteropleustes setosus	-	+
Hippopodina feegeensis	-	+
Hydra 1	+	-
Hydroides 1	+	-
Hydrozoa1	+	-
Ianiropsis montereyensis	+	-
Idotea rufescens	-	+
isopod1	+	-
Jassa marmorata	-	+
Kamptozoa1	+	-
Kamptozoa2	-	+
Laticorophium baconi	-	+
Leptochelia 1	+	-
Leptopecten latiauratus	+	-
Leucothoe alata	+	-
Lichenopora1	+	-
Liljeborgia germinata	+	-
Liljeborgia1	-	+
Lumbrinereis perkinsi	-	+
Lyonsia californica	-	+
Macoma petalum	-	+
Micronereis nanaimoensis	+	-
Micronereis1	+	-
Monocorophium uenoi	+	+
Monocorophium1	+	-
Munna 1	+	-
Munna 2	-	+
Myrianida pachycera	-	+
Nebalia gerkenae	+	-
Nemertea1	+	-
Nemertea2	+	-
Nemertea3	-	+
Nenanthes accuminata	-	+
Nereididae1	+	-
Nubranchia	-	+
Nudibranchia1	-	+
Okenia plana	+	-

Oligochaeta1	+	-
Opheliidae1	+	-
Pachycheles1	+	-
Palaemon macrodactylus	-	+
Paracerceis sculpta	+	-
Parasmittina 1	+	-
Pennaria 1	-	+
Perophora 1	+	-
Photis 1	-	+
Platyhelminthes1	+	-
Platyhelminthes2	+	-
Plumularia 1	-	+
Podocerus brasiliensis	+	+
Podocerus cristatus	+	-
Polychaeta1	+	-
Polychaeta2	-	+
Polynoidae1	-	+
Polynoidae1	+	-
Pontogeneia rostrata	+	-
Porifera1	-	+
Pyromaia tuberculata	-	+
Scleroplax granulata	-	+
Scruparia1	+	+
Serpula 1	-	+
Serpulidae1	+	-
Sinocorophium heteroceratum	-	+
Sphaeroma quoianum	-	+
Spionid	+	-
Spionidae1	+	-
Spirorbid	+	-
Stenothoe valida	-	+
Streblosoma uncinatus	+	-
Streblosoma1	+	-
Styela 1	+	-
Stylatula elongata	-	+
Syllidae1	-	+
Syllidae2	+	-
Synidotea laticuada	-	+
Terebellidae1	+	-
Theora lubrica	-	+
Thormora johnstoni	-	+
Urosalpinx cinerea	+	-
Venerupis philippinarum	+	-
Victorella1	-	+
Zaolutus actius	-	+
Zeuxo normani	-	+

Appendix 6.3

Categories of all Coastlline reassignments of vouchers specimens to plausible posterior identifications. The first two columns are the category of reassignment and its count; the third and fourth columns are the number of confirmations for that prior identification. The fifth column is the percent of posteriors that were reassigments; i.e., the error rate of morphological identification for that posterior.

Reassignment (from the first to the second listed)	**Count**	**Confirmations of:**	**Count**	**Mis-ID Rate**
Amphibalanus improvisus to Balanus crenatus	4	*Amphibalanus improvisus*	41	9%
Ampithoe lacertosa to Ampithoe sp1	5			
Ascidia ceratodes to Ascidia zara	7	*Ascidia ceratodes*	46	15%
Ascidia ceratodes to Ciona intestinalis	1			
Ascidia sp1 to Ascidia ceratodes	5			
Ascidia sp1 to Ascidia zara	2			
Ascidia zara to Ascidia ceratodes	1	*Ascidia zara*	96	1%
Ascidia zara to Ciona intestinalis	3			
Astyris aurantiaca to Alia carinata	6			
Balanus crenatus to Amphibalanus improvisus	4	*Balanus crenatus*	106	5%
Balanus crenatus to Balanus glandula	1			
Balanus trigonus to Balanus crenatus	1			
Balanus trigonus to Megabalanus sp1	1			
Botrylloides diegensis to Botrylloides leachii	76	*(B. diegensis=B. leachii)*		
Botrylloides diegensis to Botrylloides violaceus	2			
Botrylloides diegensis to Botryllus schlosseri	1			
Botrylloides sp4 to Botrylloides leachi	16			
Botrylloides sp4 to Botrylloides violaceus	8			
Botrylloides spA to Botryllus schlosseri	1			
Botrylloides violaceus to Botrylloides leachi	36	*Botrylloides violaceus*	168	20%
Botrylloides violaceus to Botryllus schlosseri	5			
Botryllus schlosseri to Botrylloides leachi	20	*Botryllus schlosseri*	424	7%
Botryllus schlosseri to Botrylloides violaceus	11			
Botryllus schlosseri to Diplosoma 1	1			
Botryllus to Botrylloides leachi	30			
Botryllus to Botrylloides violaceus	29			
Bowerbankia sp1 to Bowerbankia spA	9			
Bowerbankia sp1 to Bowerbankia spB	9			
Bugula longirostrata to Bugula neritina	1			
Bugula longirostrata to Bugula stolonifera	1			

Bugula neritina to Bugula pacifica	2	*Bugula neritina*	22	31%
Bugula neritina to Bugula stolonifera	7			
Bugula neritina to Tricellaria occidentalis	1			
Bugula pacifica to Bugula stolonifera	2	*Bugula pacifica*	21	9%
Bugula sp1 to Bugula pacifica	1			
Bugula stolonifera to Bowerbankia spA	2	*Bugula stolonifera*	146	3%
Bugula stolonifera to Bugula pacifica	1			
Bugula stolonifera to Tricellaria occidentalis	2			
Caprella scaura to Caprella simia	1			
Caprella simia to Caprella mutica	1	*Caprella simia*	10	9%
Celleporella hyalina to Watersipora subtorquata	1	*Celleporella hyalina*	3	25%
Ciona intestinalis to Ascidia ceratodes	1	*Ciona intestinalis*	116	4%
Ciona intestinalis to Ciona savignyi	3			
Ciona intestinalis to Molgula manhattensis	1			
Ciona savignyi to Ascidia zara	3	*Ciona savignyi*	106	5%
Ciona savignyi to Ciona intestinalis	2			
Ciona sp1 to Ciona intestinalis	3			
Conopeum sp1 to Conopeum tenuissimum	23			
Cryptosula pallasiana to Conopeum tenuissimum	3	*Cryptosula pallasiana*	66	6%
Cryptosula pallasiana to Smittoidea prolifica	1			
Diadumene franciscana to Diadumene lineata	3			
Diadumene leucolena to Diadumene lineata	1	*Diadumene leucolena*	1	80%
Diadumene leucolena to Metridium senile	3			
Diadumene sp1 to Diadumene lineata	2			
Diplosoma listerianum to Botrylloides violaceus	5			
Diplosoma listerianum to Botryllus schlosseri	2			
Diplosoma listerianum to Diplosoma 1	76			
Distaplia occidentalis to Botrylloides violaceus	8			
Distaplia occidentalis to Botryllus schlosseri	2			
Distaplia sp1 to Botrylloides violaceus	4			
Fenestrulina delicia to Schizoporella japonica	1			
Gonothyraea sp1 to Gonothyraea clarki	1			
Halosydna brevisetosa to Platynereis sp1	1			
Harmothoe imbricata to Myrianida pentadentata	2			
Harmothoe imbricata to Platynereis sp1	2			
Heptacarpus sp1 to Jassa slatteryi	1			
Hiatella arctica to Mytilus galloprovincialis	1			

Hippopodina feegeensis to Celleporaria brunnea	1			
Hippopodina feegeensis to Cryptosula pallasiana	2			
Lacuna unifasciata to Alia carinata	1			
Leucothoe alata to Leucothoe sp1	14			
Megabalanus californicus to Megabalanus sp1	10			
Megabalanus sp to Balanus crenatus	1			
Megabalanus sp to Megabalanus sp1	2			
Membranipora villosa to Membranipora membranacea	1			
Metridium sp1 to Metridium senile	1			
Microcosmus squamiger to Styela clava	1			
Micronereis nanaimoensis to Platynereis sp1	3			
Micronereis sp1 to Platynereis sp1	4			
Micronereis sp1 to Schistomeringos longicornis	1			
Modiolus modiolus to Mytilus trossulus	2			
Molgula ficus to Ascidia zara	1			
Molgula manhattensis to Ascidia zara	3			
Molgula manhattensis to Styela clava	1			
Monocorophium acherusicum to Corophium sp1	1			
Monocorophium sp1 to Monocorophium acherusicum	1			
Monocorophium uenoi to Corophium sp1	4			
Mytilus californianus to Mytilus galloprovincialis	1			
Mytilus californianus to Mytilus trossulus	1			
Mytilus sp1 to Mytilus galloprovincialis	33			
Mytilus sp1 to Mytilus trossulus	31			
Naineris sp1 to Naineris dendritica	2			
Neanthes accuminata to Myrianida pentadentata	1			
Nereis latescens to Platynereis sp1	1			
Obelia sp1 to Gonothyraea clarki	2			
Obelia sp1 to Laomedea calceolifera	4			
Obelia sp1 to Obelia longissima	30			
Odontosyllis phosphorea to Myrianida pentadentata	1			
Okenia plana to Onchidoris bilamellata	1			
Ostrea sp1 to Ostrea lurida	2			
Pettiboneia sanmatiensis to Schistomeringos longicornis	4			
Platynereis bicanaliculata to Platynereis sp1	71			

Platynereis bicanaliculata to Schistomeringos longicornis	2			
Platynereis to Schistomeringos longicornis	2			
Polyandrocarpa zorritensis to Microcosmus squamiger	1	*Polyandrocarpa zorritensis*	4	20%
Schizoporella errata-like to Schizoporella errata	5			
Schizoporella japonica to Schizoporella errata	1	*Schizoporella japonica*	81	1%
Schizoporella sp2 to Schizoporella japonica	1			
Scrupocellaria bertholetti to Bugula stolonifera	1			
Smittoidea prolifica to Cryptosula pallasiana	1	*Smittoidea prolifica*	22	4%
Styela canopus to Styela clava	8			
Styela clava to Ascidia zara	3	*Styela clava*	36	12%
Styela clava to Molgula manhattensis	2			
Styela sp1 to Ascidia zara	1			
Styela sp1 to Styela clava	5			
Symplegma reptans to Ascidia zara	1			
Tricellaria occidentalis to Bugula stolonifera	4	*Tricellaria occidentalis*	17	19%
Tubulipora pacifica to Watersipora subtorquata	1			
Typosyllis nipponica to Myrianida pentadentata	125			
Urosalpinx cinerea to Alia carinata	3			
Watersipora sp1 to Watersipora subovoidea	4			
Watersipora sp1 to Watersipora subtorquata	12			
Watersipora subovoidea to Watersipora subtorquata	1	*Watersipora subovoidea*	2	50%
Watersipora subtorquata to Conopeum tenuissimum	1			
Watersipora subtorquata to Watersipora spN	46	*Watersipora subtorquata*	263	17%
Watersipora subtorquata to Watersipora subovoidea	6			
Zoobotryon verticillatum to Watersipora subtorquata	1	*Zoobotryon verticillatum*	3	25%

Chapter 7: Zooplankton Metagenetics

This chapter presents the analysis of zooplankton using molecular genetic methods to detect NIS and native species, and to compare species composition among sites. In contrast to voucher specimen analysis (Chapter 6), individual specimens were not genetically analyzed. Rather, entire plankton samples were characterized by next generation sequencing of the COI barcode DNA fragment. Millions of short reads were clustered into operational taxonomic units (OTUs), and identified to species, where possible, using Genbank as a reference database.

A. Samples.

Zooplankton samples (n=212) were received from San Francisco (collection year: 2013), Mission Bay (2013), San Diego Bay (2013), Morro Bay (2013), and Bodega/Tomales Bays (2014). Each bottle was preserved in 95% ethanol and labeled with a unique 6-digit identification number. One extraction was performed for each bottle received. Table 7.1 below summarizes information from each bay sampled.

Table 7.1. Plankton samples processed. Details of sample sites are given in Appendix 7.1.

Bay	Number Bottles Received and Extracted	Sampling Year
Bodega/Tomales	40	2014
Mission	41	2013
Morro	40	2013
San Diego	40	2013
San Francisco	51	2013

B. DNA extraction.

Prior to extraction, each plankton sample was sieved through a clean 80-µm 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 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 µL of the provided elution buffer. A 20-µL aliquot of each DNA sample was transferred to a 96-well plate for downstream applications.

C. 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 (below).

Leray LCO forward primer [Nextera adapter]: **[TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]**-GGWACWGGWTGAACWGTWTAYCCYCC
JG HCO reverse primer [Nextera adapted]: **[GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG]**-TAI ACYTCIGGRTGICCRAARAAYCA

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^{o} C, followed by 27 cycles of a 1 min at 95^{o} C, 45 sec at 47^{o} C, and 1 min at 72^{o} C with a final 72^{o} 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^{o} C, followed by 8 cycles of a 30 sec at 95^{o} C, 30 sec at 55^{o} C, and 30 sec at 72^{o} C with a final 72^{o} 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.

D. Data analysis

17,215,061 read pairs from all samples were pooled using Geneious 9.1.2; pairs of reads were merged using to generate 7,913,588 sequences representing the full amplicon length. PCR primer sequences were then trimmed. These sequences were size filtered to produce 6,771,458 sequences between 350 and 380 bp. Further analysis was performed with the 64 bit version of USEARCH 1.861 (Edgar 2015). The filtered full-length sequences were dereplicated and checked for chimera, then clustered at a threshold of 97%. Centroid sequences from each cluster was selected and reclustered into 4,318 Operational Taxonomic Units (OTUs) at the 95% threshold that is typically used for DNA barcode analysis. The two-step clustering approach is intended to minimize false clusters due to undetected chimeras. Singleton OTU were discarded as likely representing sequencing error. The 4,318 OTU were BLASTED against the Coarbitrator database of COI sequences culled from Genbank, and sorted by query coverage and pairwise identity to database reference sequences. 300 OTU had no match in the database. Matches to the database that exceeded 50% coverage and 90% sequence identity were retained and considered as strong hits; 207 OTU surpassed the 95% threshold used for DNA barcodes, while another 56 passed the 90% cut-off and should be viewed as suggestive.

The OTU passing 90% threshold match to Genbank (Appendix 7.2) includes a wide variety of meroplanktonic (larvae of benthic species) and holoplankton (fully planktonic) species (Figure 7.1). In fact, benthic species were dominant in the OTU list, with presumed larvae of molluscs, crustaceans, and annelids most represented. Purely planktonic species were dominated by copepods, as expected (Figure 7.2). Many species with short-lived larvae were found, including ascidians and bryozoans suggesting that plankton analysis can be effective in describing the local benthos, although we recognize the episodicity of larval abundance.

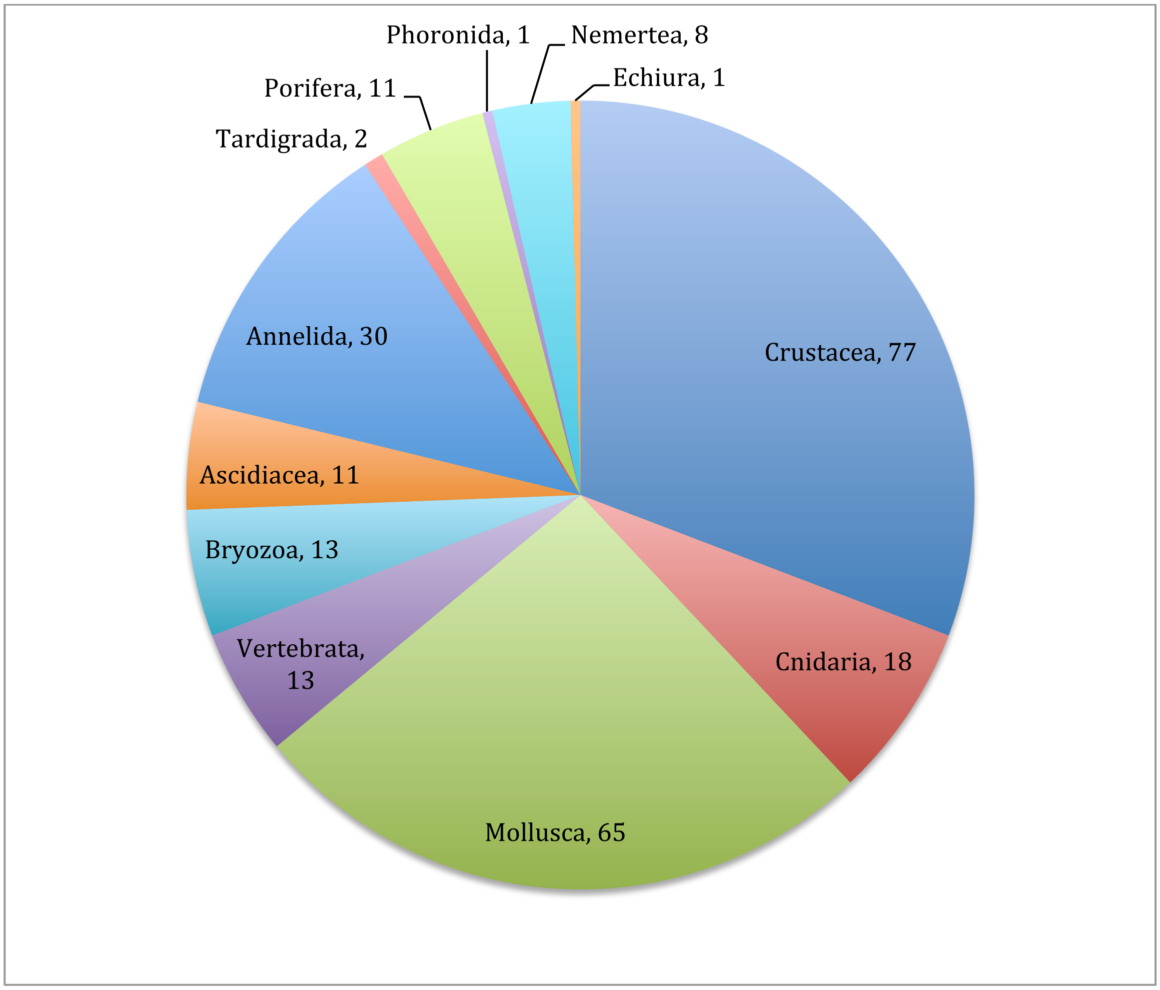

Figure 7.1: Distribution of plankton OTU among phyla or subphylum**.**

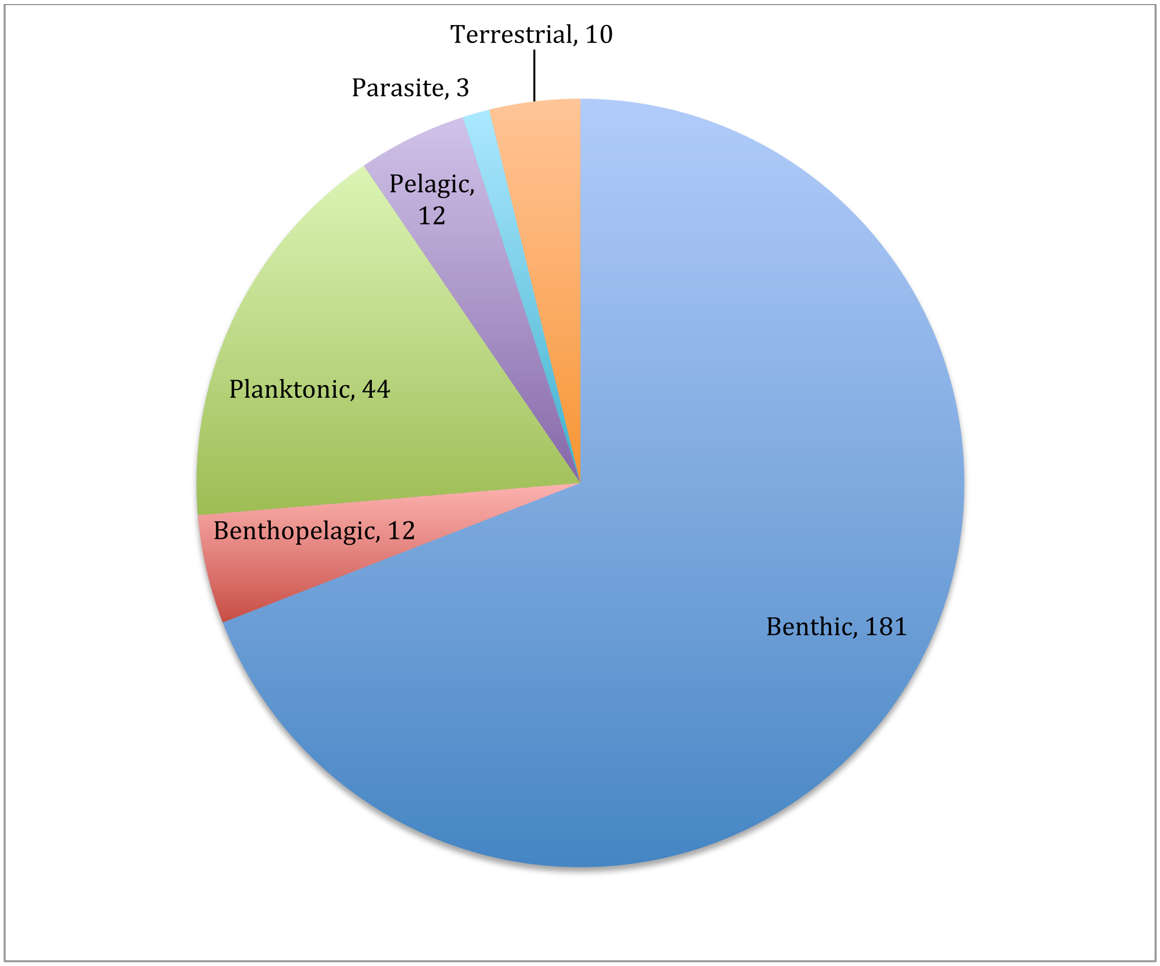

Table 7.2: Distribution of plankton OTU among adult habitats (species with prominent and prolonged benthic and planktonic life stages are treated as "benthopelagic")

Table 7.2. NIS found in among plankton OTUs exceeding 95% similarity to Genbank reference sequence. All named OTUs are listed in Appendix 5. In bold are potential new NIS (Fofonoff, pers. com.).

Genbank subject sequence name	Taxon	Invasion status	Habitat	% Pairwise Identity to Genbank Subject
Myrianida pachycera	Polychaeta	Introduced	Benthic	100.00%
Streblospio benedicti	Polychaeta	Introduced	Benthic	100.00%
Bugula neritina	Bryozoa	Introduced	Benthic	99.40%
Bugula stolonifera	Bryozoa	Introduced	Benthic	100.00%
Haliclona sp. E GPM-2011	Bryozoa	Introduced	Benthic	99.60%
Membranipora chesapeakensis	Bryozoa	Introduced	Benthic	100.00%
Watersipora arcuata	Bryozoa	Introduced	Benthic	99.70%
Ascidia zara	Ascidiacea	Introduced	Benthic	98.90%
Botrylloides violaceus	Ascidiacea	Introduced	Benthic	100.00%
Ciona intestinalis	Ascidiacea	Introduced	Benthic	100.00%
Ciona savignyi	Ascidiacea	Introduced	Benthic	100.00%
Didemnum vexillum	Ascidiacea	Introduced	Benthic	100.00%
Diplosoma listerianum	Ascidiacea	Introduced	Benthic	100.00%
Microcosmus squamiger	Ascidiacea	Introduced	Benthic	100.00%
Molgula manhattensis	Ascidiacea	Introduced	Benthic	100.00%
Polyandrocarpa zorritensis	Ascidiacea	Introduced	Benthic	98.80%
Styela plicata	Ascidiacea	Introduced	Benthic	100.00%
Blackfordia virginica	Hydrozoa	Introduced	Bentho-pelagic	100.00%
Aurelia sp. 1 sensu Dawson et al. (2005)	Scyphozoa	Introduced	Bentho-pelagic	100.00%
Amphibalanus amphitrite	Amphipoda	Introduced	Benthic	100.00%
Amphibalanus improvisus	Amphipoda	Introduced	Benthic	100.00%
Ampithoe valida	Amphipoda	Introduced	Benthic	100.00%
Caprella mutica	Amphipoda	Introduced	Benthic	97.40%
Grandidierella japonica	Amphipoda	Introduced	Benthic	100.00%
Monocorophium acherusicum	Amphipoda	Introduced	Benthic	100.00%
Acartia tonsa	Copepoda	Introduced?	Planktonic	99.70%
Acartiella sinensis	Copepoda	Introduced	Planktonic	100.00%
Harpacticella jejuensis	Copepoda	Introduced?	Planktonic	97.80%
Tortanus derjugini	Copepoda	Introduced?	Planktonic	94.80%
Tortanus dextrilobatus	Copepoda	Introduced	Planktonic	95.80%
Farfantepenaeus brasiliensis	Decapoda	Introduced?	Benthic	95.10%
Palaemon macrodactylus	Decapoda	Introduced	Benthic	100.00%
Clunio tsushimensis	Diptera	Introduced?	Terrestrial	100.00%
Crassostrea angulata	Bivalvia	Introduced	Benthic	97.00%
Geukensia demissa	Bivalvia	Introduced	Benthic	99.00%
Mya arenaria	Bivalvia	Introduced	Benthic	100.00%
Ostrea stentina	Bivalvia	Cryptogenic	Benthic	99.70%
Assiminea grayana	Introduced	Introduced?	Benthic	96.30%
Crepidula plana	Gastropoda	Introduced	Benthic	100.00%
Haminoea japonica	Gastropoda	Introduced	Benthic	99.70%
Montereina nobilis	Gastropoda	Introduced	Benthic	100.00%
Ceriodaphnia dubia	Crustacea / Cladocera		Freshwater	98.80%

E. Potential new NIS detected in plankton metagenetic results.

Acartia tonsa. While this taxon may be a cosmopolitan collection of genetically distinct cryptic species, the OTU uncovered here is 99.7% similar with 100% query coverage to a Genbank record (EU016219) from New England (Durbin et al. 2008).

Ceriodaphnia dubia. The presence of this freshwater cladoceran in our plankton samples is peculiar. However, the genetic similarity is very strong, with 98.8% identity and 100% query coverage to Genbank record EU702070 and others collected in Mexico and Guatamala (Elilas-Gutierrez et al., 2008, Prosser et al., 2013). This species is used for toxicity testing, and could accordingly be introduced widely. Misidentification of the Genbank record should be considered.

Clunio tsushimensis. The OTU uncovered here is 100% similar with 100% coverage to Genbank record AB704942 presumably collected in Japan (Cornette et al., unpublished). *Clunio* is an intertidal midge (Insecta) and might reasonably be found in plankton as drift.

Crassostrea angulata. This name is widely considered a synonym of *C. gigas*.

Farfantepenaeus brasiliensis. The OTU discovered here is 98.3% similar to the Genbank record AF125416 of *F. brasiliensis* was sequenced from a commercially purchased shrimp (Shank et al. unpublished). Although Fofonoff (pers. com.) suggested *Penaeus californiensis* as a more likely find, Genbank records for *P. californiensis* are about a 77% match to this OTU (Gutierrez-Millan et al. 2002). While a purchased shrimp could be misidentified, it is unlikely that the OTU discovered here is *P. californiensis*.

Harpacticella jejuensis. This OTU has 97.8% identity to Genbank record KM272559 (Lee et al. 2014), over 274 bp of overlapping sequence. *H. jejuensis* is from Korea.

Ostrea stentina. Genbank records (e.g., KJ818239) for this Mediterranean oyster were 99.7% similar to the OTU discovered in California plankton (Pejovic et al., unpublished; Lapegue et al. 2006). Another Genbank record for *O. stentina* from Baja California is 96% similar, though the authors (Raith et al., in press) note that the specimen was published as *O. equestris*, a junior synonym of *O. stentina*. The sequence disparity and geographic distribution of *O. stentina* and *O. equestris* seem to challenge that synonymization.

Tortanus derjugini. The OTU matching *T. derjugini* (HM045418) at 95.8% similarity (Sun et al., unpublished) is also 94.8% similar to records of *T. dextrilobatus* (KF977366) (Zhang, et al. unpublished). Both species, if present in California, are introduced.

F. Unidentified OTU.

Some OTU that had very high read counts were not identified in intitial BLAST queries to the Coarbitrator database. When examined individually, some of these OTUs (eg, OTU 3563 and 4905) had no match in all of Genbank that was greater than 77-80%.

Others had moderate similarity to Genbank records that suggest a higher taxon. For example, OTU 4820 had a 84% similarity to the diatom *Rhizosolenia setigera* (AB20226). Phytoplankton were not included in Coarbitrator, and other OTUs were also likely to be diatoms and other phytoplankton.

When individually investigated, some unidentified OTU were duplicates of those that were genetically identified. For example, OTU 4346 was 96.7% to several Genbank references for the copepod *Acartia californiensis*. The reads within the cluster forming OTU 4346 mapped to OTU_4827_*Acartia_californiensis* in the analysis in the preceding section. The example of OTU 4346 demonstrates that OTUs were overly split into groups representing intraspecific variation.

However, OTU 3, when manually inspected, was a very strong match for *Cyclops kikuchii* (KR048967, 100% similarity with 87% coverage). This species was simply missing from the initial reference databases. Improvements to the reference databases to include more planktonic species will result in a higher rate of OTU classification.

G. Abundance and geographic variation of reads per OTU.

The 6,771,458 reads were mapped to OTUs to generate a frequency distribution of reads per OTU per site. For primary analysis, comparisons among sites were restricted to identified OTUs. Frequency-abundance of reads as a proxy for species abundance should be treated with caution, as bias in PCR amplification or body size (i.e, the number of mitochondrial templates) may vary across species.

Results of cluster analysis show strong geographic structure in plankton communities (Figure 7.3). This is easier to visualize in a non-dimensional scaling plot (Figure 7.4). The non-random distribution of planktonic OTUs and sparse read count in control extractions and PCR reactions gives confidence in the integrity of metagenetic results. Interestingly, samples from San Francisco Bay show the greatest disparity (Fig 7.3, 7.4). This is likely due to distinct assemblages in the northern and southern parts of the bay (Fig 7.5). When analyzed in detail, sites within bays show heterogeneity (eg, San Diego Bay; Fig 7.6). This has relevance for temporal and spatial sampling strategies.

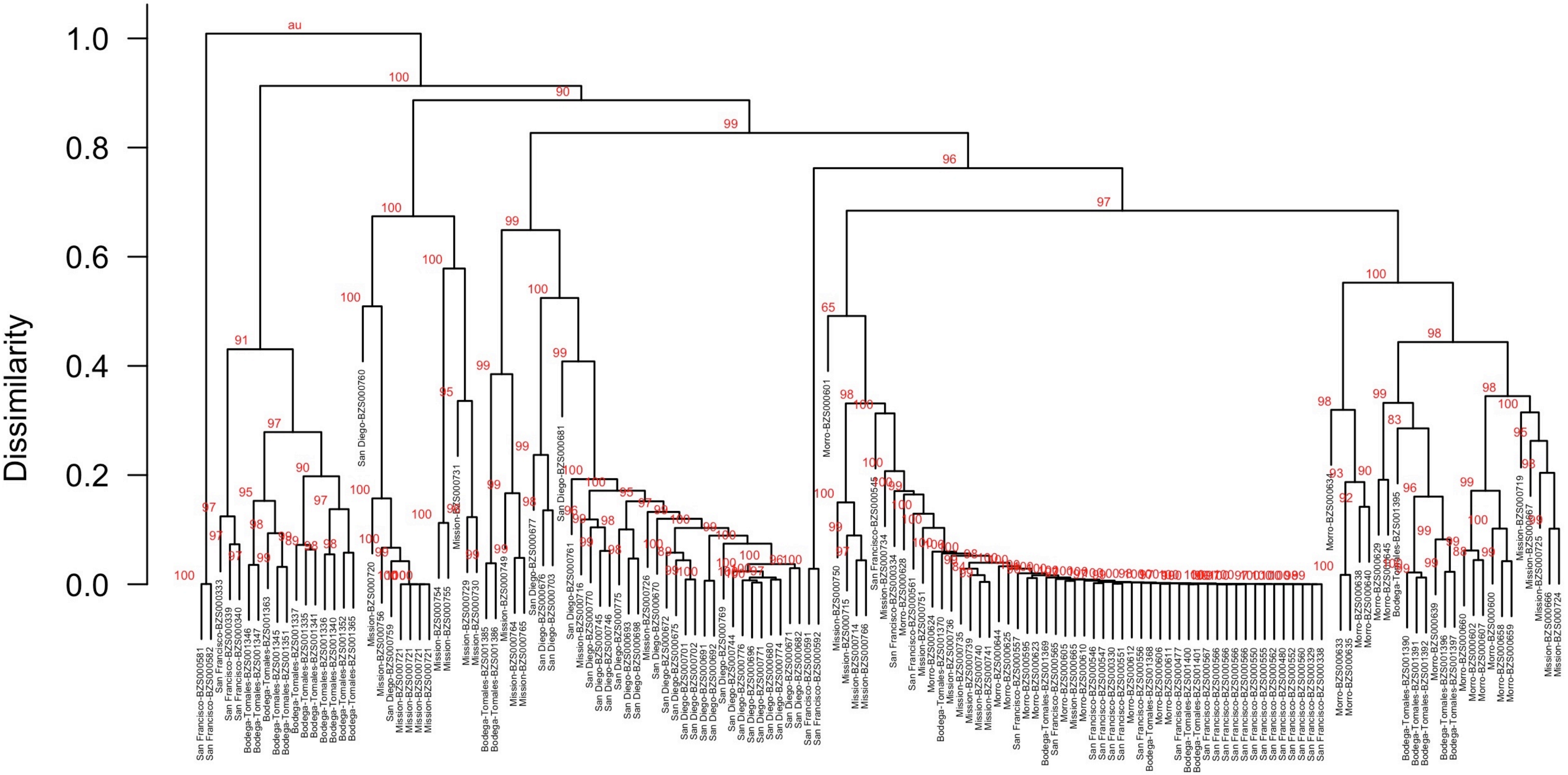

Figure 7.3. Cluster dendrogram showing affinity of samples based on named OTUs. Hierarchical agglomerative clustering was performed on a matrix of Bray-Curtis dissimilarities between communities using the unweighted pair group method with arithmetic mean. The strength of the observed clusters was tested using multiscale bootstrap resampling. Red numbers represent approximately unbiased (AU) probability values (out of 100%), with clusters having high AU values (90 to 95% and higher) are strongly supported by data. Clustering and assessments of uncertainty were performed using the R package pvclust (Suzuki & Shimodaira 2006). The higher level structure is seen in distinctiveness of most Bodega/Tomales samples. San Francisco Bay and Mission Bay samples are also deeply branching. Lower level structure in the dendrogram is largely at the level of bays.

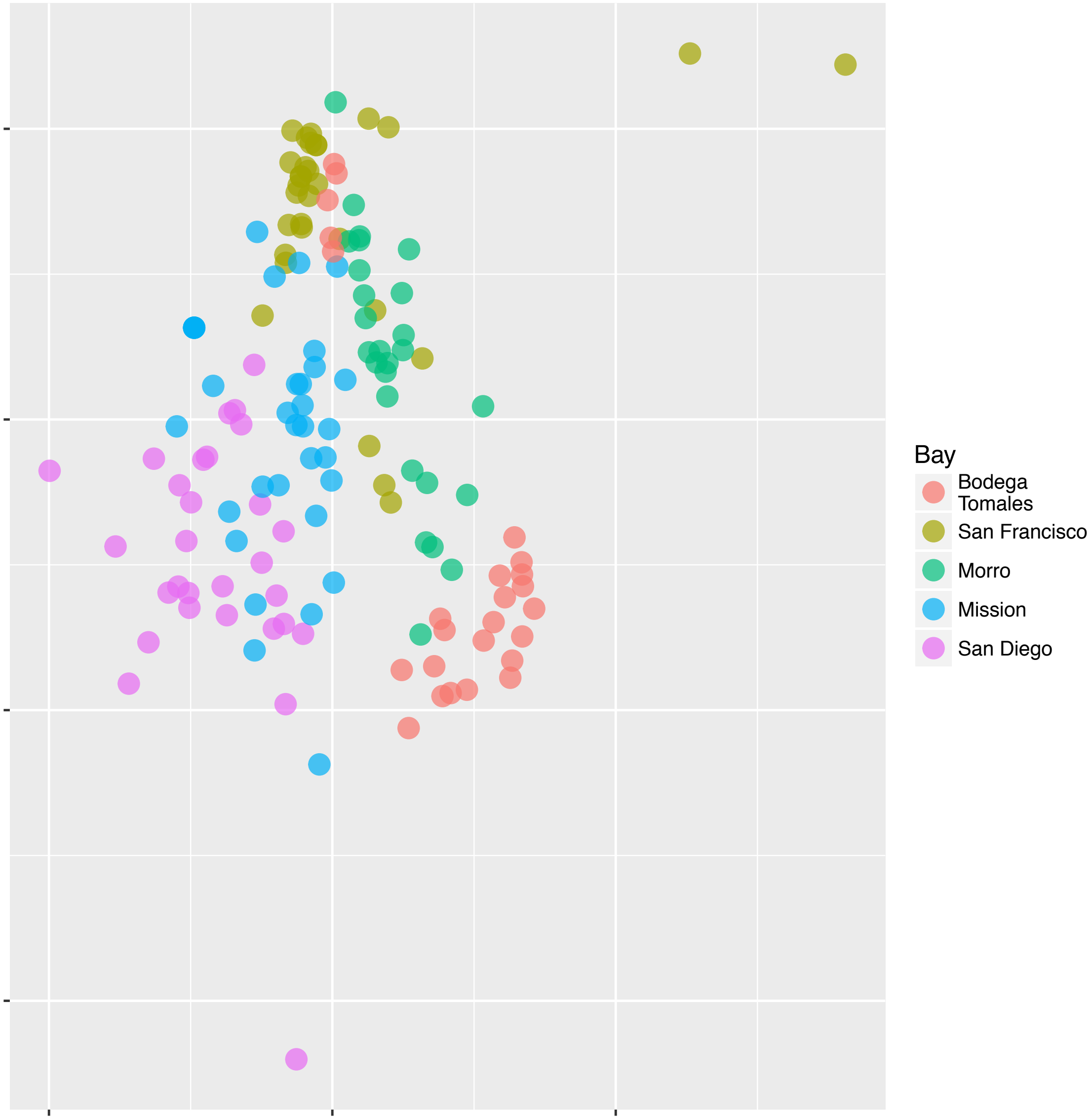

Figure 7.4. Non-dimensional scaling scatterplot based on a matrix of Bray-Curtis dissimilarities. Points represent individual samples, with closer points having more similar composition. Most samples within sites cluster and that San Francisco Bay, Morro Bay, and San Diego Bay exhibit smaller scale spatial heterogeneity that may be due to salinity or proximity to open water.

H. OTU richness in plankton samples across California estuaries.

The metagenetic data for named zooplankton can also be examined as the richness and spatial distribution of OTUs among sites within each estuary. Figures 7.5 and 7.6 show the distribution of OTU richness among major taxonomic groups for each site sampled in two of the estuaries: San Francisco Bay and San Diego Bay. This illustrates the contribution of different taxonomic groups the total OTUs detected.

For each OTU, we can also examine the frequency of occurrence (spatial distribution) in the respective bays. Appendix 7.3 shows the number of sites per bay where the respective OTUs (in this case identified genetically to species level) were detected.

We are just now beginning to explore these spatial patterns, and this is a major component of Phase II of our research Program with more comprehensive analyses to be submitted at its conclusion in 2017. We caution that the species identifications, status as NIS, and distributions presented in this report should be considered in progress, as these are undergoing extensive and ongoing evaluation in Phase II of our Program.

I. Plankton metagenetics: review of methdodology.

Metagenetic analysis of plankton was successful with respect both to methodology and results. We encountered no obstacles to extraction or PCR, although we do not have data to evaluate optimization of these processes. For example, a recent study on metagenetics of settlement plates from coral reef environments suggests that a DMSO-based sample storage buffer may be superior to ethanol (Ransome, Geller, et al. in preparation). Too, the effects of subsampling plankton samples during extraction or of stochastic variation caused by PCR are unknown. We are presently conducting experiments to evaluate these factors. Data analysis using the UPARSE pipeline was relatively straightforward, in contrast to the complexity of Coastline in voucher specimen analysis. One advance would be to assign probablity estimates for identifications at the species level. Another would be identification of higher taxonomic groups for OTUs that cannot be assigned to species, although this has less relevance to the detection of NIS as essentially alll higher taxa encountered are cosmopolitan. We will evaluate existing algorithms and software for suitability for these purposes. The metagenetic data appeared to be reliable, as almost all OTUs identified were ecologically appropriate (i.e., plausibly neustonic or planktonic), with no signal of contamination across samples; i.e., samples showed non-random geographic patterns. In this study, we have not analyzed native species richness or distributions, as this was not a primary goal. Taking NIS and native species together, plankton metagenetics may be an efficient way to establish baselines for, and to follow through time, estuarine communities.

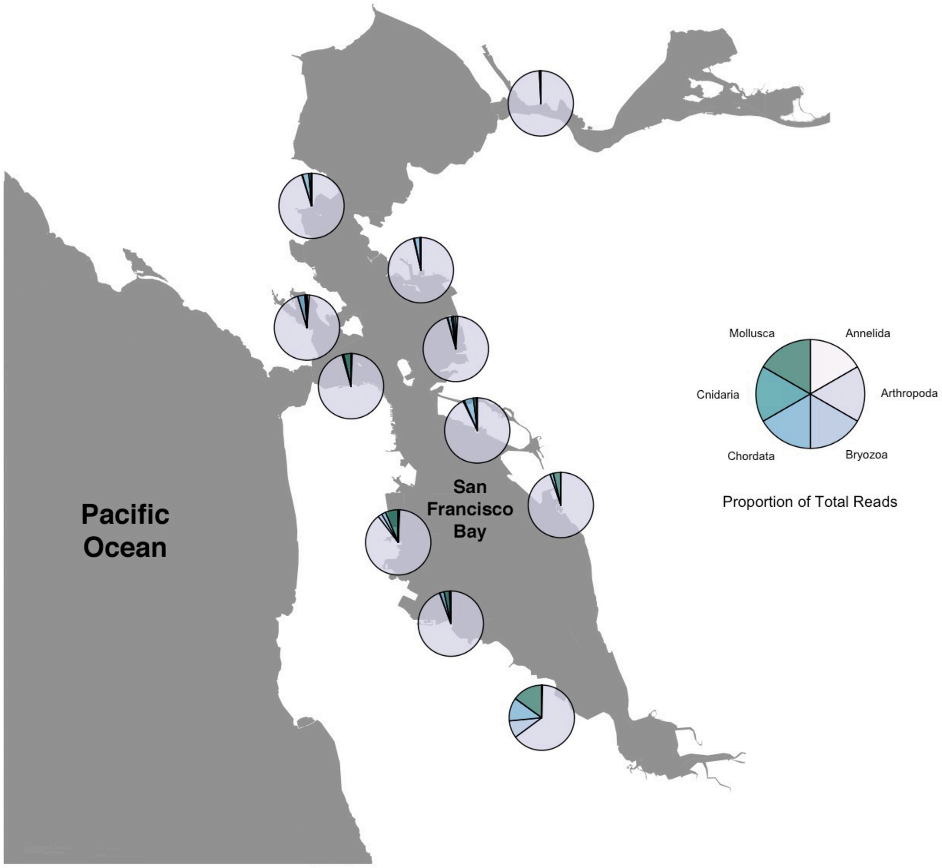

Figure 7.5. Major OTU in San Francisco Bay across sites. The southern Bay is noticeably divergent at this scale of taxonomic resolution.

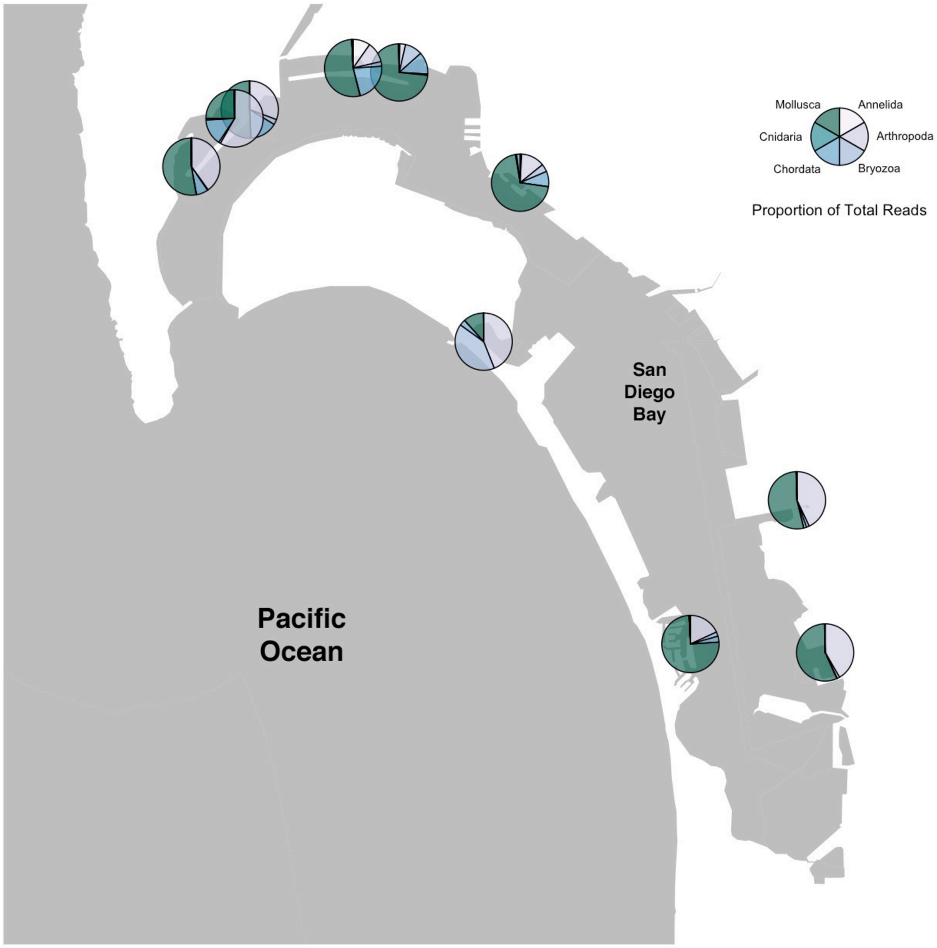

Figure 7.6. Major OTU in San Diego Bay across sites. A high degree of heterogeneity across sites is apparent; correlation with environmental data could be informative.

Appendix 7.1

Bay and sites within bays for plankton samples in this study.

Sample ID	Bay	Site
BT-P01-1	Bodega Bay	Spud Point A
BT-P01-2	Bodega Bay	Spud Point A
BT-P01-3	Bodega Bay	Spud Point A
BT-P02-1	Bodega Bay	Spud Point B
BT-P02-2	Bodega Bay	Spud Point B
BT-P02-3	Bodega Bay	Spud Point B
BT-P03-1	Bodega Bay	Porto Bodega
BT-P03-2	Bodega Bay	Porto Bodega
BT-P03-3	Bodega Bay	Porto Bodega
BT-P04-1	Bodega Bay	Mason's Marina
BT-P04-2	Bodega Bay	Mason's Marina
BT-P04-3	Bodega Bay	Mason's Marina
BT-P05-1	Bodega Bay	North of Lucas Wharf
BT-P05-2	Bodega Bay	North of Lucas Wharf
BT-P05-3	Bodega Bay	North of Lucas Wharf
BT-P06-1	Bodega Bay	Yacht Club Dock - Abandoned
BT-P06-2	Bodega Bay	Yacht Club Dock - Abandoned
BT-P06-3	Bodega Bay	Yacht Club Dock - Abandoned
BT-P07-1	Bodega Bay	Bodega Harbor public boat ramp
BT-P07-2	Bodega Bay	Bodega Harbor public boat ramp
BT-P07-3	Bodega Bay	Bodega Harbor public boat ramp
BT-P08-1	Bodega Bay	US Coast Guard dock
BT-P08-2	Bodega Bay	US Coast Guard dock
BT-P08-3	Bodega Bay	US Coast Guard dock
BT-P09-2	Bodega Bay	Marshals Boatworks
BT-P09-3	Bodega Bay	Marshals Boatworks
BT-P09-1	Bodega Bay	Marshals Boatworks
BT-P10-1	Bodega Bay	Nick's Cove public boat ramp
BT-P10-2	Bodega Bay	Nick's Cove public boat ramp
BT-P10-3	Bodega Bay	Nick's Cove public boat ramp
BT-T01	Bodega Bay	Spud Point A
BT-T02	Bodega Bay	Spud Point B
BT-T03	Bodega Bay	Porto Bodega
BT-T04	Bodega Bay	Mason's Marina
BT-T05	Bodega Bay	Yacht Club Dock - Abandoned
BT-T06	Bodega Bay	North of Lucas Wharf
BT-T07	Bodega Bay	Bodega Harbor public boat ramp
BT-T08	Bodega Bay	US Coast Guard dock

Sample ID	**Bay**	**Site**
BT-T09	Bodega Bay	Marshals Boatworks
BT-T10	Bodega Bay	Nick's Cove public boat ramp
MI-P08-3-1	Mission	Mission Bay Yacht Club
MI-P08-3-1	Mission	Mission Bay Yacht Club
MI-P05-2-1	Mission	Hilton Resort and Spa Hotel
MI-P08-1-1	Mission	Mission Bay Yacht Club
MI-P06-1-1	Mission	Campland on the Bay
MI-P06-3-1	Mission	Campland on the Bay
MI-P10-1-1	Mission	Paradise Point Resort Spa and Marina
MI-P04-1-1	Mission	Sea World
MI-P09-3-1	Mission	Bahia Resort
MI-P03-2-1	Mission	The Dana Hotel
MI-P03-3-1	Mission	The Dana Hotel
MI-P09-1-1	Mission	Bahia Resort
MI-P10-3-1	Mission	Paradise Point Resort Spa and Marina
MI-P10-2-1	Mission	Paradise Point Resort Spa and Marina
MI-P09-2-1	Mission	Bahia Resort
MI-P02-1-1	Mission	Hyatt Regency Hotel
MI-P01-3-1	Mission	Marina Village Marina
MI-P02-3-1	Mission	Hyatt Regency Hotel
MI-P02-2-1	Mission	Hyatt Regency Hotel
MI-P05-1-1	Mission	Hilton Resort and Spa Hotel
MI-P05-3-1	Mission	Hilton Resort and Spa Hotel
MI-P01-1-1	Mission	Marina Village Marina
MI-P01-2-1	Mission	Marina Village Marina
MI-P06-2-1	Mission	Campland on the Bay
MI-P04-2-1	Mission	Sea World
MI-P04-3-1	Mission	Sea World
MI-P03-1-1	Mission	The Dana Hotel
MI-P08-2-1	Mission	Mission Bay Yacht Club
MI-P07-1-1	Mission	Mission Bay Sport Center
MI-P07-2-1	Mission	Mission Bay Sport Center
MI-P07-3-1	Mission	Mission Bay Sport Center
MI-T05-1-5	Mission	Hilton Resort and Spa Hotel
MI-T06-1-5	Mission	Campland on the Bay
MI-T07-1-5	Mission	Mission Bay Sport Center
MI-T08-1-5	Mission	Mission Bay Yacht Club
MI-T09-1-5	Mission	Bahia Resort
MI-T10-1-5	Mission	Paradise Point Resort Spa and Marina
MI-T01-1-5	Mission	Marina Village Marina
MI-T02-1-5	Mission	Hyatt Regency Hotel

Sample ID	Bay	Site
MI-T03-1-5	Mission	The Dana Hotel
MI-T04-1-5	Mission	Sea World
MO-T10-1-5	Morro	City Harbor North
MO-P09-3-1	Morro	City Harbor South
MO-P10-2-1	Morro	City Harbor North
MO-P10-3-1	Morro	City Harbor North
MO-P08-3-1	Morro	Giovanni's Fish Market
MO-P09-1-1	Morro	City Harbor South
MO-T05-1-5	Morro	Tidelands Park North
MO-P08-1-1	Morro	Giovanni's Fish Market
MO-T09-1-5	Morro	City Harbor South
MO-T08-1-5	Morro	Giovanni's Fish Market
MO-P04-2-1	Morro	Tidelands Park South
MO-T07-1-5	Morro	Morro Marina
MO-P04-1-1	Morro	Tidelands Park South
MO-P03-3-1	Morro	Coastal Boatworks
MO-P03-2-1	Morro	Coastal Boatworks
MO-P03-1-1	Morro	Coastal Boatworks
MO-P04-3-1	Morro	Tidelands Park South
MO-P07-3-1	Morro	Morro Marina
MO-P07-2-1	Morro	Morro Marina
MO-P07-1-1	Morro	Morro Marina
MO-P08-2-1	Morro	Giovanni's Fish Market
MO-T01-1-5	Morro	Morro Bay State Park Marina
MO-T02-1-5	Morro	Fuel Dock
MO-T03-1-5	Morro	Coastal Boatworks
MO-P01-1-1	Morro	Morro Bay State Park Marina
MO-P01-2-1	Morro	Morro Bay State Park Marina
MO-T06-1-5	Morro	Yacht Club
MO-P09-2-1	Morro	City Harbor South
MO-P06-1-1	Morro	Yacht Club
MO-P05-3-1	Morro	Tidelands Park North
MO-P05-2-1	Morro	Tidelands Park North
MO-P05-1-1	Morro	Tidelands Park North
MO-P06-3-1	Morro	Yacht Club
MO-P06-2-1	Morro	Yacht Club
MO-P02-2-1	Morro	Fuel Dock
MO-P02-1-1	Morro	Fuel Dock
MO-T04-1-5	Morro	Tidelands Park South
MO-P02-3-1	Morro	Fuel Dock
SD-P01-1-1	San Diego	Harbor Police Transient Docks

Sample ID	Bay	Site
SD-P01-2-1	San Diego	Harbor Police Transient Docks
SD-P01-3-1	San Diego	Harbor Police Transient Docks
SD-P10-1-1	San Diego	Chula Vista Marina
SD-P09-3-1	San Diego	Chula Vista Yacht Club
SD-P09-1-1	San Diego	Chula Vista Yacht Club
SD-T08-1-5	San Diego	Pier 32 Marina
SD-P10-3-1	San Diego	Chula Vista Marina
SD-P10-2-1	San Diego	Chula Vista Marina
SD-P05-3-1	San Diego	Sunroad Marina
SD-P03-1-1	San Diego	Shelter Cove Marina
SD-P03-2-1	San Diego	Shelter Cove Marina
SD-P03-3-1	San Diego	Shelter Cove Marina
SD-P02-1-1	San Diego	Heritage Yacht Sales
SD-P02-2-1	San Diego	Heritage Yacht Sales
SD-P02-3-1	San Diego	Heritage Yacht Sales
SD-P08-1-1	San Diego	Pier 32 Marina
SD-P08-2-1	San Diego	Pier 32 Marina
SD-P08-3-1	San Diego	Pier 32 Marina
SD-P07-1-1	San Diego	Glorietta Marina
SD-P07-2-1	San Diego	Glorietta Marina
SD-P07-3-1	San Diego	Glorietta Marina
SD-P06-1-1	San Diego	Marriott Hotel Marina
SD-T07-1-5	San Diego	Glorietta Marina
SD-T09-1-5	San Diego	Chula Vista Yacht Club
SD-P04-1-1	San Diego	Cabrillo Isle Marina
SD-P04-2-1	San Diego	Cabrillo Isle Marina
SD-P04-3-1	San Diego	Cabrillo Isle Marina
SD-P05-1-1	San Diego	Sunroad Marina
SD-P05-2-1	San Diego	Sunroad Marina
SD-T02-1-5	San Diego	Heritage Yacht Sales
SD-T01-1-5	San Diego	Chula Vista Marina
SD-T06-1-5	San Diego	Marriott Hotel Marina
SD-T05-1-5	San Diego	Sunroad Marina
SD-T03-1-5	San Diego	Shelter Cove Marina
SD-P06-3-1	San Diego	Marriott Hotel Marina
SD-P06-2-1	San Diego	Marriott Hotel Marina
SF-P12-1-1	San Francisco	Pittsburg Marina
SF-P15-3-1	San Francisco	Sacramento Marina
SF-P15-2-1	San Francisco	Sacramento Marina
SF-P05-1-1	San Francisco	Ballena Isle Marina
SF-P05-1-1	San Francisco	Ballena Isle Marina

Sample ID	**Bay**	**Site**
SF-P05-2-1	San Francisco	Ballena Isle Marina
SF-P05-2-1	San Francisco	Ballena Isle Marina
SF-P01-3-1	San Francisco	Port of Redwood City Marina
SF-P11-1-1	San Francisco	Glen Cove Marina
SF-P11-2-1	San Francisco	Glen Cove Marina
SF-P10-1-1	San Francisco	Loch Lomond
SF-P10-3-1	San Francisco	Loch Lomond
SF-P06-3-1	San Francisco	San Francisco Marina East
SF-P03-1-1	San Francisco	Oyster Point Marina
SF-P03-2-1	San Francisco	Oyster Point Marina
SF-P03-3-1	San Francisco	Oyster Point Marina
SF-P04-2-1	San Francisco	San Leandro Marina
SF-P06-2-1	San Francisco	San Francisco Marina East
SF-P04-3-1	San Francisco	San Leandro Marina
SF-P01-1-1	San Francisco	Port of Redwood City Marina
SF-P02-2-1	San Francisco	Coyote Point Marina
SF-P02-3-1	San Francisco	Coyote Point Marina
SF-P02-1-1	San Francisco	Coyote Point Marina
SF-P08-1-1	San Francisco	Bridgeway Marine Corp (a.k.a. Sausalito Marina)
SF-P08-2-1	San Francisco	Bridgeway Marine Corp (a.k.a. Sausalito Marina)
SF-P08-3-1	San Francisco	Bridgeway Marine Corp (a.k.a. Sausalito Marina)
SF-P09-3-1	San Francisco	Richmond Marina
SF-P07-1-1	San Francisco	Emeryville Marina
SF-P05-3-1	San Francisco	Ballena Isle Marina
SF-P11-3-1	San Francisco	Glen Cove Marina
SF-P10-2-1	San Francisco	Loch Lomond
SF-P06-1-1	San Francisco	San Francisco Marina East
SF-P01-2-1	San Francisco	Port of Redwood City Marina
SF-P04-1-1	San Francisco	San Leandro Marina

Additional plankton samples that were not successfully PCR amplified.

MO-P10-1-1	Morro	City Harbor North
MO-P01-3-1	Morro	Morro Bay State Park Marina
SD-P09-2-1	San Diego	Chula Vista Yacht Club
SD-T10-1-5	San Diego	Chula Vista Marina
SD-T04-1-5	San Diego	Cabrillo Isle Marina
SF-P13-1-1	San Francisco	Antioch Marina
SF-P13-1-1	San Francisco	Antioch Marina
SF-P14-3-1	San Francisco	River Point Landing Marina
SF-P14-3-1	San Francisco	River Point Landing Marina

SF-P14-1-1	San Francisco	River Point Landing Marina
SF-P14-1-1	San Francisco	River Point Landing Marina
SF-P15-1-1	San Francisco	Sacramento Marina
SF-P12-2-1	San Francisco	Pittsburg Marina
SF-P12-3-1	San Francisco	Pittsburg Marina
SF-P14-2-1	San Francisco	River Point Landing Marina
SF-P14-2-1	San Francisco	River Point Landing Marina
SF-P13-2-1	San Francisco	Antioch Marina
SF-P07-3-1	San Francisco	Emeryville Marina
SF-P07-2-1	San Francisco	Emeryville Marina
SF-P13-3-1	San Francisco	Antioch Marina
SF-P09-1-1	San Francisco	Richmond Marina

Appendix 7.2

OTU passing 90% identity and 50% query coverage in BLAST against Genbank.

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU3465	*Acanthinucella spirata*	92.00%	100.00%	13310946
CALNIS_OTU393	*Acantholobulus bermudensis*	91.20%	99.04%	767807123
CALNIS_OTU260	*Acantholobulus pacificus*	100.00%	80.71%	575497301
CALNIS_OTU4827	*Acartia californiensis*	94.70%	95.82%	506616767
CALNIS_OTU94	*Acartia hudsonica*	98.10%	83.60%	162289471
CALNIS_OTU100	*Acartia longiremis*	91.00%	96.14%	529949599
CALNIS_OTU1429	*Acartia tonsa*	99.70%	100.00%	156186216
CALNIS_OTU1309	*Acartiella sinensis*	100.00%	100.00%	608606895
CALNIS_OTU2089	*Acrostichus halicti*	90.10%	95.13%	256856179
CALNIS_OTU230	*Actiniaria sp. BOLD:ACQ4394*	97.10%	100.00%	767807933
CALNIS_OTU1418	*Aeolidia sp. B LC-2013*	99.70%	100.00%	449147687
CALNIS_OTU2122	*Aequorea macrodactyla*	100.00%	100.00%	662488798
CALNIS_OTU380	*Alia carinata*	100.00%	100.00%	564733769
CALNIS_OTU3110	*Ampharete labrops*	99.00%	100.00%	304415988
CALNIS_OTU1156	*Amphibalanus amphitrite*	100.00%	91.64%	700368736
CALNIS_OTU40	*Amphibalanus improvisus*	100.00%	100.00%	226838167
CALNIS_OTU692	*Amphipoda sp. BOLD:AAH4089*	99.00%	100.00%	767808391
CALNIS_OTU367	*Ampithoe lacertosa*	92.10%	98.07%	408830861
CALNIS_OTU3135	*Ampithoe valida*	100.00%	100.00%	307749960
CALNIS_OTU746	*Anemonia sp. PG*	98.70%	100.00%	633896293
CALNIS_OTU981	*Aplysia californica*	100.00%	100.00%	4704442
CALNIS_OTU2293	*Aplysia vaccaria*	100.00%	100.00%	33390896
CALNIS_OTU378	*Aplysiopsis enteromorphae*	99.70%	94.21%	294459033
CALNIS_OTU3995	*Aprostocetus cerricola*	91.50%	100.00%	311988930
CALNIS_OTU193	*Ascidia zara*	98.90%	85.44%	597439317
CALNIS_OTU4221	*Assiminea grayana*	96.30%	86.50%	325557669
CALNIS_OTU1891	*Atherinops affinis*	100.00%	100.00%	37682406
CALNIS_OTU1306	*Aurelia sp. 1 sensu Dawson et al. (2005)*	100.00%	100.00%	62722185
CALNIS_OTU3309	*Balanus glandula*	97.10%	99.68%	154101575
CALNIS_OTU2218	*Balanus trigonus*	96.50%	100.00%	482878589
CALNIS_OTU1823	*Barentsia gracilis*	92.60%	99.68%	225542650
CALNIS_OTU1479	*Bipalponephtys cornuta*	100.00%	94.84%	289470485
CALNIS_OTU4125	*Blackfordia virginica*	100.00%	100.00%	480632029
CALNIS_OTU3359	*Bomolochus cuneatus*	91.90%	100.00%	301508546
CALNIS_OTU1267	*Bosmina sp. BOLD:AAI4721*	94.50%	99.68%	189304100
CALNIS_OTU127	*Botrylloides leachii*	100.00%	77.17%	568404089

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU191	*Botrylloides violaceus*	100.00%	100.00%	570700932
CALNIS_OTU1617	*Bugula neritina*	99.40%	100.00%	429325583
CALNIS_OTU1802	*Bugula pacifica*	97.10%	99.68%	54401632
CALNIS_OTU122	*Bugula stolonifera*	100.00%	100.00%	429325591
CALNIS_OTU59	*Bulla gouldiana*	100.00%	100.00%	7682398
CALNIS_OTU2295	*Caligus clemensi*	100.00%	100.00%	301508544
CALNIS_OTU631	*Calocalanus tenuis*	98.10%	99.04%	443267479
CALNIS_OTU3931	*Campanularia hincksii*	96.40%	99.04%	60267373
CALNIS_OTU262	*Cancer antennarius*	92.30%	83.92%	289660067
CALNIS_OTU2113	*Caprella californica*	96.80%	100.00%	574454809
CALNIS_OTU143	*Caprella mutica*	97.40%	100.00%	220029925
CALNIS_OTU131	*Centropages abdominalis*	99.40%	100.00%	193792136
CALNIS_OTU3090	*Cephalothrix sp. 14 HC-2011*	96.40%	99.04%	317514015
CALNIS_OTU2366	*Ceriodaphnia dubia*	98.80%	100.00%	189304236
CALNIS_OTU3752	*Chione elevata*	92.80%	99.03%	557883919
CALNIS_OTU2107	*Chlorostoma funebralis*	100.00%	100.00%	564733859
CALNIS_OTU1099	*Chlorostoma montereyi*	99.30%	97.43%	3415058
CALNIS_OTU341	*Chthamalus dalli*	98.40%	100.00%	699046044
CALNIS_OTU4879	*Chthamalus fissus*	97.50%	78.14%	108733954
CALNIS_OTU4138	*Cilicaea sp. BOLD:AAR9230*	95.20%	100.00%	767806763
CALNIS_OTU466	*Ciona intestinalis*	100.00%	91.96%	312227637
CALNIS_OTU162	*Ciona savignyi*	100.00%	77.81%	556925308
CALNIS_OTU2782	*Clathria prolifera*	100.00%	100.00%	64966308
CALNIS_OTU4463	*Clausocalanus arcuicornis*	98.10%	100.00%	529950133
CALNIS_OTU50	*Clausocalanus furcatus*	99.40%	100.00%	529950209
CALNIS_OTU876	*Clausocalanus jobei*	96.50%	100.00%	529950225
CALNIS_OTU2414	*Clausocalanus lividus*	93.90%	100.00%	301505715
CALNIS_OTU4773	*Clausocalanus mastigophorus*	96.10%	99.35%	529950251
CALNIS_OTU2920	*Clausocalanus parapergens*	97.10%	99.04%	529950275
CALNIS_OTU3364	*Clausocalanus paululus*	99.00%	100.00%	529950289
CALNIS_OTU977	*Clausocalanus pergens*	100.00%	100.00%	529950303
CALNIS_OTU986	*Clevelandia ios*	100.00%	83.82%	303386965
CALNIS_OTU4238	*Cliona chilensis*	91.90%	100.00%	306850291
CALNIS_OTU2258	*Clunio tsushimensis*	100.00%	100.00%	407955551
CALNIS_OTU4539	*Conopea cf. galeata Galapagos DCS-2011*	95.80%	91.96%	344190637
CALNIS_OTU1506	*Conualevia alba*	100.00%	100.00%	429141804
CALNIS_OTU3147	*Conus californicus*	99.70%	100.00%	124294757
CALNIS_OTU1416	*Corambe pacifica*	100.00%	100.00%	805575752
CALNIS_OTU648	*Corambe steinbergae*	98.40%	100.00%	805575756
CALNIS_OTU4232	*Corynactis californica*	100.00%	98.39%	205321237

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU675	*Crangon septemspinosa*	98.90%	90.35%	8671631
CALNIS_OTU3021	*Craniella cf. leptoderma AS-2012*	94.50%	100.00%	392932819
CALNIS_OTU1003	*Crassostrea angulata*	97.00%	100.00%	383511727
CALNIS_OTU1553	*Crepidula cf. onyx RC*	99.30%	88.75%	33456811
CALNIS_OTU4760	*Crepidula cf. perforans*	99.00%	98.07%	22773444
CALNIS_OTU4709	*Crepidula naticarum*	99.70%	95.47%	30794773
CALNIS_OTU1130	*Crepidula plana*	100.00%	98.07%	22773438
CALNIS_OTU175	*Crepipatella lingulata*	98.70%	100.00%	564735563
CALNIS_OTU2311	*Cryptosula pallasiana*	93.40%	97.75%	225542690
CALNIS_OTU4134	*Ctenocalanus vanus*	97.10%	100.00%	301505707
CALNIS_OTU1825	*Cyanoplax keepiana*	99.70%	100.00%	134033185
CALNIS_OTU2153	*Cypridopsis vidua*	97.70%	97.11%	595583296
CALNIS_OTU368	*Darwinella oxeata*	97.80%	87.46%	380849947
CALNIS_OTU1844	*Dendronotus venustus*	96.10%	100.00%	300393737
CALNIS_OTU4897	*Diaphanosoma sp. 2 MEG-2008*	96.40%	90.35%	189304436
CALNIS_OTU1817	*Diaulula sandiegensis*	91.40%	97.11%	564734143
CALNIS_OTU881	*Didemnum vexillum*	100.00%	77.17%	171908864
CALNIS_OTU482	*Diplosoma listerianum*	100.00%	77.17%	568404307
CALNIS_OTU514	*Diptera sp. BOLD:AAE5173*	100.00%	100.00%	321135002
CALNIS_OTU1069	*Doris montereyensis*	100.00%	100.00%	429141806
CALNIS_OTU1545	*Dorvillea sp. CMC01*	98.70%	100.00%	304416130
CALNIS_OTU2728	*Doto columbiana*	92.60%	73.95%	310775251
CALNIS_OTU4285	*Echiniscoides sp. SD1B*	95.90%	100.00%	389621051
CALNIS_OTU3837	*Echiniscoides sp. Taxon 1*	97.90%	93.25%	803471053
CALNIS_OTU666	*Ectopleura wrighti*	96.90%	83.28%	440623506
CALNIS_OTU2577	*Electra sp. LM-2010*	99.40%	100.00%	308208465
CALNIS_OTU1061	*Emerita analoga*	100.00%	79.94%	319770040
CALNIS_OTU2594	*Engraulis encrasicolus*	90.90%	99.36%	392974795
CALNIS_OTU4068	*Enhydrosoma intermedia*	99.40%	100.00%	659496362
CALNIS_OTU4483	*Eptatretus cf. fernholmi NRMt7933*	92.90%	100.00%	475989067
CALNIS_OTU944	*Erythromelana sp. 2 DJI-2011*	91.70%	96.14%	334199201
CALNIS_OTU2858	*Eucalanus californicus*	98.10%	100.00%	40317393
CALNIS_OTU1922	*Eulalia aviculiseta*	98.40%	100.00%	422313760
CALNIS_OTU632	*Eupolymnia heterobranchia*	95.80%	100.00%	304416166
CALNIS_OTU2291	*Eurytemora pacifica*	94.20%	100.00%	33563079
CALNIS_OTU1652	*Eutonina indicans*	95.10%	99.04%	515424178
CALNIS_OTU2914	*Evadne nordmanni*	95.50%	100.00%	189474252
CALNIS_OTU975	*Farfantepenaeus brasiliensis*	95.10%	99.04%	560207872
CALNIS_OTU3409	*Flabellina cf. trophina BOLD:ABA3308*	99.40%	100.00%	564735437
CALNIS_OTU3745	*Flabellina trilineata*	90.90%	99.04%	310775247

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU656	*Flabellina verrucosa*	100.00%	100.00%	564734355
CALNIS_OTU336	*Frankliniella occidentalis*	99.70%	100.00%	146272143
CALNIS_OTU3350	*Gastropteron pacificum*	100.00%	100.00%	564734061
CALNIS_OTU1032	*Geukensia demissa*	99.00%	100.00%	254034333
CALNIS_OTU4541	*Glycera robusta*	94.20%	99.04%	304416182
CALNIS_OTU1573	*Glycera sp. CMC03*	91.10%	98.06%	304416186
CALNIS_OTU1592	*Grandidierella japonica*	100.00%	100.00%	465481752
CALNIS_OTU938	*Halichondria magniconulosa*	96.30%	86.50%	155675137
CALNIS_OTU922	*Haliclona oculata*	95.10%	99.04%	320172573
CALNIS_OTU1316	*Haliclona sp. E GPM-2011*	99.60%	85.85%	349587317
CALNIS_OTU2668	*Haliotis kamtschatkana*	91.30%	81.67%	61677436
CALNIS_OTU2257	*Halisarca sp. AA-2010*	93.20%	99.04%	363991841
CALNIS_OTU2791	*Halosydna brevisetosa*	100.00%	100.00%	304416212
CALNIS_OTU435	*Haminoea japonica*	99.70%	100.00%	555685447
CALNIS_OTU98	*Haminoea virescens*	100.00%	99.36%	564734063
CALNIS_OTU2915	*Harpacticella jejuensis*	97.80%	88.10%	701436929
CALNIS_OTU4849	*Hemigrapsus oregonensis*	96.30%	96.46%	168202873
CALNIS_OTU4845	*Hubrechtella dubia*	93.50%	99.04%	321172223
CALNIS_OTU2336	*Hymeniacidon perlevis*	93.60%	100.00%	430768101
CALNIS_OTU1344	*Ilyanassa sp. OPC-2014*	90.80%	93.89%	672424589
CALNIS_OTU3065	*Isarachnanthus nocturnus*	99.00%	100.00%	408688762
CALNIS_OTU1353	*Janolus barbarensis*	99.70%	100.00%	300393813
CALNIS_OTU602	*Jassa slatteryi*	99.00%	100.00%	307749688
CALNIS_OTU1142	*Kellia suborbicularis*	98.40%	100.00%	564734057
CALNIS_OTU4722	*Keratella cochlearis faluta*	96.50%	100.00%	30269134
CALNIS_OTU1090	*Lacuna pallidula*	90.00%	93.25%	31074460
CALNIS_OTU3197	*Leitoscoloplos pugettensis*	97.80%	87.62%	237846801
CALNIS_OTU2640	*Leitoscoloplos pugettensis CMC01*	93.90%	100.00%	304416282
CALNIS_OTU2843	*Limacina helicina helicina*	100.00%	100.00%	37933603
CALNIS_OTU1581	*Littorina natica*	93.10%	98.07%	378781307
CALNIS_OTU960	*Littorina plena*	99.70%	93.25%	31074510
CALNIS_OTU546	*Lophopanopeus bellus*	99.60%	84.52%	289660649
CALNIS_OTU1732	*Lovenella assimilis*	99.70%	100.00%	315493525
CALNIS_OTU335	*Loxorhynchus crispatus*	90.30%	99.04%	197631302
CALNIS_OTU1327	*Lunatia pallida*	90.60%	99.68%	564733709
CALNIS_OTU897	*Macrosteles sp. J62*	98.60%	94.17%	206730637
CALNIS_OTU720	*Magelona sp. CMC01*	92.90%	100.00%	304416324
CALNIS_OTU2900	*Maractis sp. SP-2014*	92.60%	99.68%	644453702
CALNIS_OTU664	*Megabalanus rosa*	93.90%	99.68%	408830951
CALNIS_OTU2427	*Megastraea undosa*	100.00%	100.00%	61677478

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU1972	*Melanella thersites*	90.00%	99.68%	564735085
CALNIS_OTU3422	*Melanochlamys diomedea*	96.80%	100.00%	150246673
CALNIS_OTU1554	*Melibe leonina*	100.00%	82.64%	310775311
CALNIS_OTU1180	*Membranipora chesapeakensis*	100.00%	100.00%	308208393
CALNIS_OTU2758	*Membranipora membranacea*	97.80%	89.07%	182628172
CALNIS_OTU1256	*Merluccius gayi*	99.00%	92.93%	301068534
CALNIS_OTU199	*Metabonellia haswelli*	99.70%	100.00%	685427670
CALNIS_OTU660	*Microcosmus squamiger*	100.00%	100.00%	564282578
CALNIS_OTU2242	*Micrura alaskensis*	98.90%	90.97%	30140237
CALNIS_OTU1387	*Miraciidae gen. 2 sp. 2 KK-2013*	90.00%	100.00%	530758096
CALNIS_OTU790	*Molgula manhattensis*	100.00%	100.00%	386289861
CALNIS_OTU565	*Monocorophium acherusicum*	100.00%	100.00%	767806685
CALNIS_OTU1973	*Montereina nobilis*	100.00%	100.00%	564733815
CALNIS_OTU4114	*Mopalia hindsii*	98.70%	100.00%	134033687
CALNIS_OTU148	*Muggiaea atlantica*	92.30%	99.04%	410833432
CALNIS_OTU2320	*Mugil cephalus*	97.40%	99.04%	381282265
CALNIS_OTU2767	*Musculista senhousia*	95.80%	100.00%	666420241
CALNIS_OTU1432	*Mya arenaria*	100.00%	100.00%	386778548
CALNIS_OTU962	*Mycale fibrexilis*	99.00%	100.00%	76663983
CALNIS_OTU2254	*Myliobatis californica*	100.00%	99.36%	294989158
CALNIS_OTU951	*Myrianida pachycera*	100.00%	100.00%	307549094
CALNIS_OTU3353	*Mytilus californianus*	100.00%	100.00%	564734795
CALNIS_OTU417	*Mytilus edulis*	100.00%	100.00%	564734651
CALNIS_OTU731	*Myxicola infundibulum CMC02*	100.00%	100.00%	304416348
CALNIS_OTU2340	*Naineris dendritica CMC01*	96.80%	100.00%	304416362
CALNIS_OTU236	*Navanax inermis*	100.00%	100.00%	391930548
CALNIS_OTU1832	*Neaeromya rugifera*	99.70%	100.00%	407969077
CALNIS_OTU1017	*Nemertea sp. BOLD:ACM2302*	90.10%	97.11%	641803686
CALNIS_OTU4652	*Nemertea sp. BOLD:ACM2303*	99.60%	82.85%	641803692
CALNIS_OTU3292	*Neotrypaea sp. ALB39 BP-2008*	96.10%	66.56%	164551367
CALNIS_OTU3857	*Neotrypaea sp. SD4 BP-2008*	97.60%	66.56%	164551349
CALNIS_OTU815	*Norrisia norrisii*	100.00%	97.43%	3415052
CALNIS_OTU3685	*Obelia bidentata*	99.70%	100.00%	410833444
CALNIS_OTU2358	*Obelia sp. 1 SL-2013*	93.50%	98.71%	515423533
CALNIS_OTU4874	*Obelia sp. 3 SL-2013*	99.40%	100.00%	515424038
CALNIS_OTU1030	*Ocinebrellus inornatus*	91.20%	99.04%	363710258
CALNIS_OTU2560	*Olivella baetica*	92.60%	99.68%	564734597
CALNIS_OTU904	*Oncaea scottodicarloi*	98.20%	87.46%	304361677
CALNIS_OTU2844	*Ophiodromus pugettensis*	100.00%	100.00%	304416464
CALNIS_OTU1653	*Ophonus laticollis*	90.90%	95.18%	743151766
CALNIS_OTU3432	*Ophryotrocha diadema*	99.70%	100.00%	409150994

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU2273	*Ophryotrocha labronica*	99.60%	88.42%	540073554
CALNIS_OTU572	*Oscarella lobularis*	99.60%	73.31%	530408330
CALNIS_OTU3349	*Osmerus mordax*	90.40%	100.00%	332205504
CALNIS_OTU1	*Ostrea conchaphila*	97.70%	72.52%	94183391
CALNIS_OTU4249	*Ostrea stentina*	99.70%	99.01%	85726199
CALNIS_OTU1388	*Ototyphlonemertes sp. 21*	94.50%	99.04%	30140203
CALNIS_OTU84	*Pachygrapsus crassipes*	100.00%	100.00%	63078794
CALNIS_OTU1212	*Palaemon macrodactylus*	100.00%	100.00%	408830903
CALNIS_OTU688	*Pandalus jordani*	100.00%	100.00%	116614017
CALNIS_OTU10	*Paracalanus sp. C AC-2013*	100.00%	100.00%	571033381
CALNIS_OTU2652	*Paracalanus tropicus*	96.40%	98.07%	571033479
CALNIS_OTU1406	*Paragorgia johnsoni*	92.30%	100.00%	229814549
CALNIS_OTU3415	*Paralabrax auroguttatus*	94.80%	99.36%	294989212
CALNIS_OTU716	*Paralabrax maculatofasciatus*	100.00%	99.35%	294989216
CALNIS_OTU1467	*Paranemertes peregrina*	93.20%	99.04%	30140207
CALNIS_OTU1494	*Parougia albomaculata*	100.00%	86.82%	146395571
CALNIS_OTU3965	*Parvocalanus crassirostris*	96.90%	100.00%	459938195
CALNIS_OTU3698	*Pectinaria granulata CMC01*	99.40%	100.00%	304416522
CALNIS_OTU155	*Penilia avirostris*	99.40%	100.00%	189474290
CALNIS_OTU1532	*Pholoides asperus*	99.70%	100.00%	304416532
CALNIS_OTU3223	*Phoronis vancouverensis*	98.10%	100.00%	225542668
CALNIS_OTU3148	*Phragmatopoma californica*	99.30%	99.03%	74145767
CALNIS_OTU4078	*Phyllochaetopterus prolifica*	99.70%	100.00%	304416538
CALNIS_OTU4315	*Platynereis sp. CMC01*	99.70%	100.00%	304416620
CALNIS_OTU101	*Platynereis sp. CMC02*	100.00%	100.00%	304416654
CALNIS_OTU12	*Pleopis polyphemoides*	100.00%	100.00%	19071722
CALNIS_OTU2802	*Podocopida sp. BOLD:AAH0908*	95.10%	97.75%	305691584
CALNIS_OTU443	*Podon leuckartii*	100.00%	100.00%	189474202
CALNIS_OTU494	*Pollicipes polymerus*	100.00%	100.00%	606258103
CALNIS_OTU51	*Polyandrocarpa zorritensis*	98.80%	77.17%	568404273
CALNIS_OTU1524	*Polycera atra*	99.40%	100.00%	459586037
CALNIS_OTU1428	*Polycera hedgpethi*	100.00%	100.00%	459586039
CALNIS_OTU89	*Protodorvillea gracilis*	100.00%	97.75%	304416714
CALNIS_OTU2876	*Psectrocladius limbatellus*	91.30%	100.00%	519122738
CALNIS_OTU1304	*Pseudevadne tergestina*	99.40%	100.00%	189474268
CALNIS_OTU2487	*Pseudocalanus acuspes*	91.00%	100.00%	312601671
CALNIS_OTU2847	*Pseudocalanus mimus*	93.00%	96.14%	21361018
CALNIS_OTU689	*Pseudomyicola spinosus*	98.40%	100.00%	414079976
CALNIS_OTU1556	*Pugettia producta*	99.00%	100.00%	168202895
CALNIS_OTU1607	*Rictaxis punctocaelatus*	98.60%	95.18%	152004185
CALNIS_OTU2928	*Salmo salar*	100.00%	100.00%	309952939

Query	Name	% Pairwise Identity	Query coverage	Genbank GID
CALNIS_OTU30	*Schistomeringos longicornis*	99.70%	100.00%	304416730
CALNIS_OTU2579	*Schizobranchia insignis*	100.00%	100.00%	304416758
CALNIS_OTU82	*Skistodiaptomus pallidus*	100.00%	100.00%	194140396
CALNIS_OTU2589	*Streblospio benedicti*	100.00%	74.92%	5006543
CALNIS_OTU277	*Styela plicata*	100.00%	100.00%	323339112
CALNIS_OTU325	*Syngnathus californiensis*	99.70%	99.36%	294989410
CALNIS_OTU480	*Tegula eiseni*	99.40%	100.00%	61677476
CALNIS_OTU907	*Tegula gallina*	99.70%	97.43%	3415074
CALNIS_OTU828	*Telmatogeton japonicus*	91.30%	100.00%	407955549
CALNIS_OTU3190	*Thysanoessa spinifera*	95.40%	99.03%	66576282
CALNIS_OTU2494	*Tortanus derjugini*	94.80%	99.68%	608607139
CALNIS_OTU4337	*Tortanus dextrilobatus*	95.80%	99.68%	608607149
CALNIS_OTU3228	*Triconia dentipes*	96.10%	100.00%	304361685
CALNIS_OTU4152	*Trimusculus reticulatus*	98.50%	85.53%	330902194
CALNIS_OTU4348	*Uroteuthis sibogae*	99.70%	100.00%	330426917
CALNIS_OTU99	*Varicinassa variciferus*	90.50%	98.07%	429489924
CALNIS_OTU2393	*Watersipora arcuata*	99.70%	97.75%	90018727
CALNIS_OTU1950	*Watersipora sp. CA-2004*	100.00%	100.00%	50346301
CALNIS_OTU365	*Watersipora subovoidea*	100.00%	93.25%	388260492
CALNIS_OTU256	*Watersipora subtorquata*	99.40%	100.00%	342360257
CALNIS_OTU3947	*Xystrologa sp. wielgusi*	100.00%	100.00%	563581362
CALNIS_OTU528	*Yamaguchiella vitiata*	90.70%	97.11%	699046060
CALNIS_OTU22	*Zoobotryon verticillatum*	100.00%	100.00%	339787695
CALNIS_OTU3163	*Zygonemertes simonae*	93.20%	99.04%	30140393

Appendix 7.3

Distribution of named OTUs across Calfornia estuaries. Number of sites per estuary at which a given OTU was found. Samples were available from 10 sites in each estuary except San Francisco (12 sites).

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU260_Acantholobulus_pacificus-100.0%	0	1	0	0	4
CALNIS_OTU4827_Acartia_californiensis-94.7%	6	11	10	10	10
CALNIS_OTU94_Acartia_hudsonica-98.1%	5	11	0	1	0
CALNIS_OTU1429_Acartia_tonsa-99.7%	3	0	1	0	0
CALNIS_OTU1309_Acartiella_sinensis-100.0%	0	2	0	0	0
CALNIS_OTU230_Actiniaria_sp._BOLD:ACQ4394-97.1%	0	0	1	2	2
CALNIS_OTU1418_Aeolidia_sp._B_LC-2013-99.7%	3	1	6	0	0
CALNIS_OTU2122_Aequorea_macrodactyla-100.0%	0	0	0	3	4
CALNIS_OTU380_Alia_carinata-100.0%	5	2	8	6	0
CALNIS_OTU3110_Ampharete_labrops-99.0%	4	0	2	0	2
CALNIS_OTU1156_Amphibalanus_amphitrite-100.0%	1	4	4	9	10
CALNIS_OTU40_Amphibalanus_improvisus-100.0%	2	10	1	1	3
CALNIS_OTU692_Amphipoda_sp._BOLD:AAH4089-99.0%	1	2	0	6	6
CALNIS_OTU3135_Ampithoe_valida-100.0%	0	1	2	0	0
CALNIS_OTU746_Anemonia_sp._PG-98.7%	1	3	4	0	0
CALNIS_OTU981_Aplysia_californica-100.0%	1	0	7	6	2
CALNIS_OTU2293_Aplysia_vaccaria-100.0%	0	0	0	6	1
CALNIS_OTU378_Aplysiopsis_enteromorphae-99.7%	5	3	10	10	9
CALNIS_OTU193_Ascidia_zara-98.9%	0	10	2	5	9
CALNIS_OTU1891_Atherinops_affinis-100.0%	0	2	3	3	7
CALNIS_OTU1306_Aurelia_sp._1_sensu_Dawson_et_al._(2005)-100.0%	0	1	0	4	6
CALNIS_OTU3309_Balanus_glandula-97.1%	6	10	3	1	2
CALNIS_OTU2218_Balanus_trigonus-96.5%	2	0	9	8	3

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU1479_Bipalponephtys_cornuta-100.0%	1	2	7	0	2
CALNIS_OTU4125_Blackfordia_virginica-100.0%	0	1	0	0	0
CALNIS_OTU3359_Bomolochus_cuneatus-91.9%	4	3	0	0	0
CALNIS_OTU1267_Bosmina_sp._BOLD:AAI4721-94.5%	0	1	0	0	0
CALNIS_OTU127_Botrylloides_leachii-100.0%	4	6	2	7	10
CALNIS_OTU191_Botrylloides_violaceus-100.0%	6	6	2	4	5
CALNIS_OTU1617_Bugula_neritina-99.4%	3	0	4	0	0
CALNIS_OTU1802_Bugula_pacifica-97.1%	2	0	0	0	0
CALNIS_OTU122_Bugula_stolonifera-100.0%	1	10	2	7	8
CALNIS_OTU59_Bulla_gouldiana-100.0%	0	1	8	10	9
CALNIS_OTU2295_Caligus_clemensi-100.0%	1	2	0	0	0
CALNIS_OTU631_Calocalanus_tenuis-98.1%	0	0	7	5	2
CALNIS_OTU3931_Campanularia_hincksii-96.4%	1	0	0	9	7
CALNIS_OTU262_Cancer_antennarius-92.3%	0	4	2	1	0
CALNIS_OTU2113_Caprella_californica-96.8%	1	0	3	0	0
CALNIS_OTU143_Caprella_mutica-97.4%	4	7	5	3	3
CALNIS_OTU131_Centropages_abdominalis-99.4%	2	0	2	0	0
CALNIS_OTU3090_Cephalothrix_sp._14_HC-2011-96.4%	0	0	0	0	1
CALNIS_OTU2107_Chlorostoma_funebralis-100.0%	5	2	1	0	0

OTU Name	**Bodega-Tomales**	**San Francisco**	**Morro**	**Mission**	**San Diego**
CALNIS_OTU1099_Chlorostoma_montereyi-99.3%	3	0	3	0	0
CALNIS_OTU341_Chthamalus_dalli-98.4%	6	6	9	4	1
CALNIS_OTU4879_Chthamalus_fissus-97.5%	2	1	9	9	8
CALNIS_OTU4138_Cilicaea_sp._BOLD:AAR9230-95.2%	0	0	1	9	5
CALNIS_OTU466_Ciona_intestinalis-100.0%	1	6	0	5	9
CALNIS_OTU162_Ciona_savignyi-100.0%	0	10	0	4	10
CALNIS_OTU2782_Clathria_prolifera-100.0%	0	3	0	0	0
CALNIS_OTU4463_Clausocalanus_arcuicornis-98.1%	0	0	1	6	3
CALNIS_OTU50_Clausocalanus_furcatus-99.4%	0	0	2	7	4
CALNIS_OTU876_Clausocalanus_jobei-96.5%	0	1	9	6	2
CALNIS_OTU4773_Clausocalanus_mastigophorus-96.1%	0	0	0	1	0
CALNIS_OTU2920_Clausocalanus_parapergens-97.1%	0	1	8	7	1
CALNIS_OTU3364_Clausocalanus_paululus-99.0%	0	0	0	2	0
CALNIS_OTU977_Clausocalanus_pergens-100.0%	0	0	8	7	0
CALNIS_OTU986_Clevelandia_ios-100.0%	0	0	0	0	5
CALNIS_OTU4238_Cliona_chilensis-91.9%	1	0	1	0	0
CALNIS_OTU2258_Clunio_tsushimensis-100.0%	2	3	0	0	0
CALNIS_OTU4539_Conopea_cf._galeata_Galapagos_DCS-2011-95.8%	0	0	1	0	0
CALNIS_OTU1506_Conualevia_alba-100.0%	2	0	3	2	2

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU3147_Conus_californicus-99.7%	0	0	0	2	0
CALNIS_OTU1416_Corambe_pacifica-100.0%	0	0	8	4	3
CALNIS_OTU648_Corambe_steinbergae-98.4%	1	1	9	5	4
CALNIS_OTU4232_Corynactis_californica-100.0%	0	0	3	0	0
CALNIS_OTU675_Crangon_septemspinosa-98.9%	0	3	0	0	0
CALNIS_OTU1003_Crassostrea_angulata-97.0%	1	0	0	7	6
CALNIS_OTU1553_Crepidula_cf._onyx_RC-99.3%	0	0	0	3	0
CALNIS_OTU4760_Crepidula_cf._perforans-99.0%	0	0	1	1	0
CALNIS_OTU1130_Crepidula_plana-100.0%	0	6	0	0	0
CALNIS_OTU175_Crepipatella_lingulata-98.7%	1	3	10	6	0
CALNIS_OTU2311_Cryptosula_pallasiana-93.4%	1	2	1	0	0
CALNIS_OTU4134_Ctenocalanus_vanus-97.1%	6	4	9	10	6
CALNIS_OTU1825_Cyanoplax_keepiana-99.7%	0	0	3	0	0
CALNIS_OTU2153_Cypridopsis_vidua-97.7%	0	1	0	0	0
CALNIS_OTU368_Darwinella_oxeata-97.8%	0	0	0	3	5
CALNIS_OTU1817_Diaulula_sandiegensis-91.4%	1	0	0	0	0
CALNIS_OTU881_Didemnum_vexillum-100.0%	3	5	1	0	0
CALNIS_OTU482_Diplosoma_listerianum-100.0%	5	2	1	4	1
CALNIS_OTU514_Diptera_sp._BOLD:AAE5173-100.0%	0	0	0	0	2
CALNIS_OTU1069_Doris_montereyensis-100.0%	3	4	4	0	0
CALNIS_OTU1545_Dorvillea_sp._CMC01-98.7%	2	0	0	0	0
CALNIS_OTU4285_Echiniscoides_sp._SD1B-95.9%	0	1	5	1	0
CALNIS_OTU3837_Echiniscoides_sp._Taxon_1-97.9%	1	1	1	0	0
CALNIS_OTU666_Ectopleura_wrighti-96.9%	0	0	1	1	3
CALNIS_OTU2577_Electra_sp._LM-2010-99.4%	0	3	0	0	0
CALNIS_OTU1061_Emerita_analoga-100.0%	1	0	5	0	0
CALNIS_OTU4068_Enhydrosoma_intermedia-99.4%	0	2	0	0	0
CALNIS_OTU2858_Eucalanus_californicus-98.1%	1	0	3	1	0

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU1922_Eulalia_aviculiseta-98.4%	3	0	6	0	0
CALNIS_OTU632_Eupolymnia_heterobranchia-95.8%	3	1	0	0	0
CALNIS_OTU2291_Eurytemora_pacifica-94.2%	0	0	2	9	9
CALNIS_OTU1652_Eutonina_indicans-95.1%	1	0	0	0	0
CALNIS_OTU2914_Evadne_nordmanni-95.5%	5	5	9	3	2
CALNIS_OTU3409_Flabellina_cf._trophina_BOLD:ABA3308-99.4%	1	0	3	0	0
CALNIS_OTU3745_Flabellina_trilineata-90.9%	1	0	0	0	0
CALNIS_OTU656_Flabellina_verrucosa-100.0%	5	3	8	0	1
CALNIS_OTU336_Frankliniella_occidentalis-99.7%	1	1	0	1	1
CALNIS_OTU3350_Gastropteron_pacificum-100.0%	1	0	4	0	1
CALNIS_OTU1032_Geukensia_demissa-99.0%	0	5	0	0	0
CALNIS_OTU1592_Grandidierella_japonica-100.0%	1	0	2	0	0
CALNIS_OTU938_Halichondria_magniconulosa-96.3%	0	0	0	5	7
CALNIS_OTU922_Haliclona_oculata-95.1%	1	7	0	3	7
CALNIS_OTU1316_Haliclona_sp._E_GPM-2011-99.6%	0	3	0	2	2
CALNIS_OTU2668_Haliotis_kamtschatkana-91.3%	1	0	0	0	0
CALNIS_OTU2791_Halosydna_brevisetosa-100.0%	5	1	1	0	0
CALNIS_OTU435_Haminoea_japonica-99.7%	0	7	0	0	0
CALNIS_OTU98_Haminoea_virescens-100.0%	5	0	8	10	9
CALNIS_OTU2915_Harpacticella_jejuensis-97.8%	0	10	0	5	9
CALNIS_OTU4849_Hemigrapsus_oregonensis-96.3%	5	4	10	5	2
CALNIS_OTU2336_Hymeniacidon_perlevis-93.6%	0	0	3	6	7
CALNIS_OTU3065_Isarachnanthus_nocturnus-99.0%	0	0	0	1	0
CALNIS_OTU1353_Janolus_barbarensis-99.7%	0	0	5	6	2
CALNIS_OTU602_Jassa_slatteryi-99.0%	1	4	8	3	4
CALNIS_OTU1142_Kellia_suborbicularis-98.4%	5	3	8	0	0
CALNIS_OTU1090_Lacuna_pallidula-90.0%	0	0	1	0	0

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU3197_Leitoscoloplos_pugettensis-97.8%	1	1	0	0	0
CALNIS_OTU2640_Leitoscoloplos_pugettensis_CMC01-93.9%	2	0	0	0	0
CALNIS_OTU960_Littorina_plena-99.7%	1	0	6	0	0
CALNIS_OTU546_Lophopanopeus_bellus-99.6%	2	4	4	0	0
CALNIS_OTU1732_Lovenella_assimilis-99.7%	0	1	0	2	5
CALNIS_OTU897_Macrosteles_sp._J62-98.6%	0	0	2	0	0
CALNIS_OTU664_Megabalanus_rosa-93.9%	1	0	1	0	0
CALNIS_OTU2427_Megastraea_undosa-100.0%	0	0	0	2	0
CALNIS_OTU3422_Melanochlamys_diomedea-96.8%	2	2	2	2	0
CALNIS_OTU1554_Melibe_leonina-100.0%	1	0	9	1	0
CALNIS_OTU1180_Membranipora_chesapeakensis-100.0%	0	1	0	0	0
CALNIS_OTU2758_Membranipora_membranacea-97.8%	1	0	9	8	3
CALNIS_OTU199_Metabonellia_haswelli-99.7%	5	4	8	1	2
CALNIS_OTU660_Microcosmus_squamiger-100.0%	0	0	0	6	7
CALNIS_OTU2242_Micrura_alaskensis-98.9%	1	0	2	0	0
CALNIS_OTU790_Molgula_manhattensis-100.0%	0	9	0	0	0
CALNIS_OTU565_Monocorophium_acherusicum-100.0%	1	4	6	4	6
CALNIS_OTU1973_Montereina_nobilis-100.0%	2	0	2	0	0
CALNIS_OTU2320_Mugil_cephalus-97.4%	0	0	0	3	2
CALNIS_OTU2767_Musculista_senhousia-95.8%	3	6	0	10	10
CALNIS_OTU1432_Mya_arenaria-100.0%	1	1	0	0	0
CALNIS_OTU962_Mycale_fibrexilis-99.0%	0	0	0	5	6
CALNIS_OTU2254_Myliobatis_californica-100.0%	1	1	1	0	0
CALNIS_OTU951_Myrianida_pachycera-100.0%	0	0	0	2	3

OTU Name	**Bodega-Tomales**	**San Francisco**	**Morro**	**Mission**	**San Diego**
CALNIS_OTU3353_Mytilus_californianus-100.0%	2	0	3	3	0
CALNIS_OTU417_Mytilus_edulis-100.0%	5	4	1	0	0
CALNIS_OTU731_Myxicola_infundibulum_CMC02-100.0%	5	1	0	1	0
CALNIS_OTU2340_Naineris_dendritica_CMC01-96.8%	4	1	0	1	0
CALNIS_OTU236_Navanax_inermis-100.0%	0	0	8	9	10
CALNIS_OTU1832_Neaeromya_rugifera-99.7%	3	0	1	0	0
CALNIS_OTU3857_Neotrypaea_sp._SD4_BP-2008-97.6%	1	1	0	0	1
CALNIS_OTU815_Norrisia_norrisii-100.0%	0	0	0	2	0
CALNIS_OTU3685_Obelia_bidentata-99.7%	0	1	3	0	0
CALNIS_OTU4874_Obelia_sp._3_SL-2013-99.4%	4	2	0	0	1
CALNIS_OTU2358_Obelia_sp._1_SL-2013-93.5%	3	1	1	0	2
CALNIS_OTU2560_Olivella_baetica-92.6%	0	0	1	1	1
CALNIS_OTU904_Oncaea_scottodicarloi-98.2%	0	1	7	8	2
CALNIS_OTU2844_Ophiodromus_pugettensis-100.0%	2	1	0	0	0
CALNIS_OTU3432_Ophryotrocha_diadema-99.7%	0	0	1	2	0
CALNIS_OTU2273_Ophryotrocha_labronica-99.6%	0	8	0	1	0
CALNIS_OTU572_Oscarella_lobularis-99.6%	0	0	3	5	7
CALNIS_OTU1_Ostrea_conchaphila-97.7%	3	11	7	10	10
CALNIS_OTU4249_Ostrea_stentina-99.7%	0	0	0	0	4
CALNIS_OTU1388_Ototyphlonemertes_sp._21-94.5%	2	1	1	0	0
CALNIS_OTU84_Pachygrapsus_crassipes-100.0%	6	3	10	5	6
CALNIS_OTU1212_Palaemon_macrodactylus-100.0%	0	1	0	0	0
CALNIS_OTU10_Paracalanus_sp._C_AC-2013-100.0%	6	11	10	10	8
CALNIS_OTU2652_Paracalanus_tropicus-96.4%	0	0	0	6	0
CALNIS_OTU716_Paralabrax_maculatofasciatus-100.0%	0	0	0	3	2

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU1494_Parougia_albomaculata-100.0%	0	0	0	0	2
CALNIS_OTU3965_Parvocalanus_crassirostris-96.9%	0	1	2	9	10
CALNIS_OTU3698_Pectinaria_granulata_CMC01-99.4%	2	0	1	0	0
CALNIS_OTU155_Penilia_avirostris-99.4%	0	0	2	5	3
CALNIS_OTU1532_Pholoides_asperus-99.7%	4	2	0	0	0
CALNIS_OTU3223_Phoronis_vancouverensis-98.1%	0	2	2	0	0
CALNIS_OTU3148_Phragmatopoma_californica-99.3%	5	2	5	1	0
CALNIS_OTU4078_Phyllochaetopterus_prolifica-99.7%	0	0	3	0	0
CALNIS_OTU101_Platynereis_sp._CMC02-100.0%	6	3	8	4	2
CALNIS_OTU4315_Platynereis_sp._CMC01-99.7%	5	3	5	0	0
CALNIS_OTU12_Pleopis_polyphemoides-100.0%	3	3	9	10	6
CALNIS_OTU2802_Podocopida_sp._BOLD:AAH0908-95.1%	0	1	0	0	0
CALNIS_OTU443_Podon_leuckartii-100.0%	5	1	0	0	0
CALNIS_OTU494_Pollicipes_polymerus-100.0%	6	2	9	5	1
CALNIS_OTU51_Polyandrocarpa_zorritensis-98.8%	0	0	3	10	10
CALNIS_OTU1524_Polycera_atra-99.4%	3	0	10	7	1
CALNIS_OTU1428_Polycera_hedgpethi-100.0%	1	3	2	10	7
CALNIS_OTU89_Protodorvillea_gracilis-100.0%	0	1	1	9	3
CALNIS_OTU1304_Pseudevadne_tergestina-99.4%	0	0	0	3	0
CALNIS_OTU2487_Pseudocalanus_acuspes-91.0%	4	1	0	0	0
CALNIS_OTU2847_Pseudocalanus_mimus-93.0%	6	6	2	2	3
CALNIS_OTU689_Pseudomyicola_spinosus-98.4%	1	8	0	6	6
CALNIS_OTU1607_Rictaxis_punctocaelatus-98.6%	2	3	2	4	2
CALNIS_OTU2928_Salmo_salar-100.0%	0	0	1	0	0
CALNIS_OTU30_Schistomeringos_longicornis-99.7%	5	5	1	9	7
CALNIS_OTU2579_Schizobranchia_insignis-100.0%	4	0	1	0	0
CALNIS_OTU82_Skistodiaptomus_pallidus-100.0%	1	1	4	4	4
CALNIS_OTU2589_Streblospio_benedicti-100.0%	1	7	1	0	0

OTU Name	Bodega-Tomales	San Francisco	Morro	Mission	San Diego
CALNIS_OTU277_Styela_plicata-100.0%	0	0	1	9	7
CALNIS_OTU4841_Sus_scrofa-100.0%	1	0	0	0	0
CALNIS_OTU325_Syngnathus_californiensis-99.7%	0	0	4	0	0
CALNIS_OTU480_Tegula_eiseni-99.4%	0	0	0	5	0
CALNIS_OTU907_Tegula_gallina-99.7%	0	0	0	1	1
CALNIS_OTU3190_Thysanoessa_spinifera-95.4%	5	1	3	1	0
CALNIS_OTU2494_Tortanus_derjugini-94.8%	0	6	0	0	0
CALNIS_OTU4337_Tortanus_dextrilobatus-95.8%	0	9	0	0	1
CALNIS_OTU3228_Triconia_dentipes-96.1%	0	0	2	0	0
CALNIS_OTU4152_Trimusculus_reticulatus-98.5%	1	0	3	0	0
CALNIS_OTU4348_Uroteuthis_sibogae-99.7%	0	0	2	0	0
CALNIS_OTU2393_Watersipora_arcuata-99.7%	0	0	0	0	3
CALNIS_OTU1950_Watersipora_sp._CA-2004-100.0%	0	0	1	2	1
CALNIS_OTU365_Watersipora_subovoidea-100.0%	0	0	0	7	8
CALNIS_OTU256_Watersipora_subtorquata-99.4%	1	2	1	9	8
CALNIS_OTU3947_Xystrologa_sp._wielgusi-100.0%	0	0	0	0	1
CALNIS_OTU22_Zoobotryon_verticillatum-100.0%	1	0	5	10	10

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Part III: Conclusions

Chapter 8: Main Conclusions and Future Directions

NIS Detection: Spatial and Temporal Patterns in California Estuaries

A. Morphological Analyses

In this initial phase of our Program, we conducted a rigorous and quantitative field sampling campaign to evaluate the extent of NIS across multiple habitat types, including hard substrate, soft sediments, and zooplankton communities across five estuaries in California. From north to south, these estuaries included Humboldt Bay, Bodega and Tomales Bays, San Francisco Bay, Morro Bay, Mission Bay, and San Diego Bay.

The intensive field sampling and analyses characterized the number, proportion, taxonomic identification, and spatial distribution of NIS (as well as native and cryptogenic biota) within and among these five estuaries. This is the most comprehensive and contemporary statistical community analysis of invasion patterns across multiple estuaries to date.

Despite the high proportion and dominance of NIS at many sites, morphological analysis detected only two NIS that were not previously reported in these estuaries. In addition, repeated measures of the hard substrate (including both sessile and mobile invertebrate species) did not detect any new NIS in San Francisco Bay. This result is especially surprising given (a) the magnitude of the surveys and analyses (hundreds of community samples and >10,000 voucher specimens identified) and (b) the published literature on increasing rate of new NIS detections in California estuaries, and especially San Francisco Bay (Cohen and Carlton 1998; Ruiz et al. 2000, 2011).

Statistical analyses indicate that our sampling of benthic (hard and soft bottom) communities was highly effective at detecting total NIS richness, since the species accumulation profiles reached an asymptote in San Francisco Bay and the other estuaries. We interpret these results to indicate the paucity of new NIS was not an artifact of under-sampling. If there are new NIS that remain undetected in benthic habitats they are likely recent arrivals or very rare, in contrast to resident NIS in these estuaries that are most often widely distributed. This low detection rate is the focus of additional, on-going analyses, as outlined below.

B. Molecular Genetic Analyses

DNA barcoding of individual voucher specimens from our surveys provided general support for the morphological results. When fully resolved genetic references were available, genetic reassignment had no impact on site-specific species lists. Further, the genetic identifications confirmed the presence of the two new NIS detected morphologically but no additional novel (previously undocumented) invaders in benthic habitats. In contrast, several putative NIS were detected by the zooplankton metagenetic analyses that appear to be new records in California estuaries. We urge some caution in interpreting these results until specimens are collected and analyzed. Although the latter genetic sequences are a strong match to known species in GenBank, further analysis of related species is required to evaluate these records. Nonetheless, it does appear the multiple new NIS were detected in our plankton samples, suggesting this is an especially sensitive screening tool.

Implications of Detection Results for Management

Even when including the putative NIS detected in zooplankton surveys, our overall results for estuaries found a relatively low number of species that were new records (not previously recorded) in California. These results suggest a possible decline in the rate of new invasions in recent years, compared to that reported in previous decades (Cohen and Carlton 1998; Ruiz et al. 2011). This difference may result in part from changes in management (e.g., ballast water management), but it could also reflect changes in search effort, trade patterns, or environmental conditions over time (see discussion by Ruiz et al. 2000, 2015; Solow and Costello, 2004). The relative contribution of these various factors requires further detailed analysis and additional data, to (a) rigorously test whether a slowdown in detection rate has in fact occurred in recent years and (b) fully evaluate possible underlying mechanism(s). This is the focus of ongoing measures (Phase II and Phase III) in our Program.

Use of Molecular Genetics

The use of molecular genetics provided a valuable approach to (a) confirm morphological identifications and (b) detect novel NIS. The former was used to independently corroborate individual voucher specimen identifications and screen for possible cryptic species among these vouchers. This project was the first to use next-generation sequencing for routine, high-volume DNA barcoding, and we created novel methodological and analytical approaches. While there were various sources of error associated with these procedures to be addressed Phase II of our Program, this approach did confirm species identifications and also helps inform future and on-going morphological analyses. Importantly, the analysis of individual specimens is building a DNA barcode library for NIS (and other taxa) in California waters, for use in whole community genetic analyses.

The value of the whole community or metagenetic analyses is demonstrated by zooplankton results in Chapter 7. This approach yielded a large number of sequences, across taxonomic groups, that was used to detect the presence and spatial distribution of many taxa --- including several putative new NIS not detected by other methods in this study. This contrasts with traditional morphological analysis of zooplankton communities, for which many of the larval forms cannot be identified to species level. While we are currently able to assign species names to only a small fraction of the OTUs detected in the metagenetic analyses, this capacity will increase as the DNA barcode library expands in California and other global regions. Moreover, we are just now beginning to explore the massive amount of sequence data available for analysis of species detection, community composition, and spatial distribution.

While the current study focused primarily on zooplankton community analysis for metagenetics, it has similar potential for application to benthic communities as well. Initial or pilot data from this type of analysis is demonstrated in Chapter 6.

NIS Detection on Outer Coasts of California

The highest NIS richness for marine waters is reported from estuaries in California, and elsewhere in the world (Wasson et al. 2001; Ruiz et al. 2009), although some taxa are known to spread to outer coastal habitats. In Chapter 5, we reported on the distribution of selected NIS on hard substrate bottom communities. While these results indicate that spill-over from estuaries to outer coast does occur in California waters, and it appears several new sites have been recently colonized by NIS, the extent of such spill-over and what limits or controls this is not yet understood.

Next Steps

A. Current Program (Phase II)

The primary objective of the current Program is to evaluate and monitor the extent of biological invasions in California's coastal marine and estuarine waters. As mandated by California law, the California Department of Fish and Wildlife (CDFW) plays a lead role in advancing this Program, in order to evaluate the efficacy of current management and policy to prevent new marine invasions to the state, including especially those associated with commercial ships.

A core focus of the Program is on estuaries. These are the main gateways (points of entry) for NIS to the state, resulting from intentional transfers by commercial ships that arrive to ports (Ruiz et al. 2011). Past analyses, including those by CDFW, indicate that the vast majority of NIS in the state are known to occur in estuaries, although some are also known to be spreading to outer coastal habitats. As a result of this distribution, a major priority is understand status and trends of NIS in the state's estuaries, given that these are the hotspots for invasions.

Our current effort is designed to provide the critical quantitative baseline data to evaluate occurrence, geographic distribution, patterns of spread, and vector for NIS in California. In addition, repeated measures at selected key estuaries (ports) are designed to evaluate temporal changes in response to vector management, such as ballast water management and hull husbandry for commercial ships. Finally, selected surveys of outer coastal areas are included to test the extent of spread (spill-over) from estuaries to other coastal habitats, including Marine Protected Areas.

As outlined in this report for Phase I of the Program, we have completed extensive surveys of multiple habitats across 5 major estuaries in California. Our analytical approach successfully paired morphological and genetic methods, including development of innovative specimen-based and community-based approaches for NIS detection and taxonomic identification. In addition, we have conducted surveys of outer coastal habitats in central California for target NIS.

In Phase II of the Program (now underway), we are expanding the geographic scope of these identical measures to include three additional estuaries, and we are conducting repeated measures each year in San Francisco Bay. The latter serves as an important sentinel site to evaluate long term changes in invasion dynamics. Together, these survey data are being combined with an ongoing synthesis of extensive historical data. At the end of Phase II, we will evaluate the occurrence, identity, distribution, and invasion history for eight different estuaries, which span the state from Humboldt Bay to San Diego Bay. Importantly, this will include detailed analysis of any new marine NIS that are detected in our surveys and literature-based synthesis for California. Moreover, we will evaluate the rate of new NIS detections over time for San Francisco Bay, as a sentinel site established to measure changes in invasion dynamics.

B. Priorities for Future NIS Detection and Analysis in California

While sustained measures are required to evaluate changes in invasion dynamics, including the arrival of new NIS as well as their spread and habitat distribution, the quality and design of these measures determine the strength of inferences that will result. In short, design and approach are critical features that should be driven by the specific questions of interest (Ruiz and Hewitt 2002; Ruiz and Carlton 2003).

We suggest several priorities for the design of on-going measures to evaluate status and trends of marine NIS in California, especially to evaluate efficacy of invasion management programs and various environmental drivers (e.g., climate change and anthropogenic disturbance) that affect invasion dynamics. These priorities include:

- **Sentinel Estuaries** where repeated annual measures are established to provide robust statistical analysis of NIS occurrence and distribution across multiple habitats. These habitats should include: hard substrate (artificial substrate, which are focal points for NIS invasions, and natural substrate), soft-sediment, and zooplankton communities. At least two sentinel estuaries should be included, ideally one in southern and one in central California, to represent different biogeographic regions and conditions. We recommend San Francisco Bay and Los Angeles/Long Beach, as two major port systems and ports of entry. Frequent (at least annual) measures at these sentinel sites provide the quantitative data for robust statistical analyses that characterize (test for) changes in the number of new NIS and the spatial extent (and abundance) of NIS, in response to management, shift in trade patterns (propagule delivery), and other local/regional environmental changes.
- **Genetic Approaches** for increased detection and confirmation of NIS. This should be run in tandem with morphological analyses (as in the current report), providing a complementary data set, since morphological analyses are required to assess abundance, community structure, and distribution (as well as confirmation and potential impacts of established populations). Further, the morphological analyses also serve to build the state-wide DNA barcode library for use of whole community genetic analyses. Such metagenetic analyses offer great efficiency and sensitivity for application to many additional estuaries (beyond sentinel estuaries) in the state, following further development and ground-truthing. Ideally, this metagenetic approach would form the base of a broad scale monitoring and detection program in the future. The use of these approaches will be accelerated both by expanding the DNA barcode library, using paired morphological and genetic vouchers, and by further experimental field testing to evaluate relative biases in morphological and genetic approaches.
- **Spill-Over Assessments** to evaluate the extent of NIS colonization and spread to natural habitats, both within estuaries and on outer coastal regions. On hard substrate, most NIS are documented on artificial substrate (docks and pilings) in estuaries. It is not clear the extent to which these are spreading to natural rocky reefs (especially subtidally) in estuaries or what may limit the distribution and abundance of NIS on such habitats. The same is true for outer coastal areas, including areas adjacent to estuaries and also those surrounding Marine Protected Areas (e.g., Monterey Bay or Channel Islands). Rather than conduct broad scale surveys along the outer coast, more focused surveys and/or experiments can efficiently test the extent of colonization of natural substrate, both within bay and along the outer coast. Ideally, these would be repeated at some frequency, since colonization may change over time. For this purpose, several locations may also be considered as sentinel sites for repeated measures, as for broader assessment of sentinel estuaries (above).

- **Data Management and Analytical Pipelines** to efficiently integrate and evaluate the large data stream that results from both morphological and genetic analyses. While this may seem like an obvious element, since the desired output requires extensive data analysis, it is also easy to underestimate the scope of this effort. The success of any such program depends on both architecture and analytical capabilities. However, it is also critical to document and archive the resulting data (and metadata), given that this is a long-term program and resource for the state. Further, it is important to recognize that the resulting survey data include both NIS and native species, and thus also have significant application (value) to many areas of marine resource management. These surveys provide a significant quantity of baseline data which serve broadly to document the state's natural resources and understand marine ecosystem dynamics in California.

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