2014 TRIENNIAL REPORT ON THE CALIFORNIA DEPARTMENT OF FISH AND WILDLIFE'S MARINE INVASIVE SPECIES PROGRAM

Submitted to the
CALIFORNIA STATE LEGISLATURE
as required by the Coastal Ecosystems Protection Act of 2006

Prepared and submitted by the
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EXECUTIVE SUMMARY

California's Coastal Ecosystem Protection Act of 2006 extended the Ballast Water Management Act of 1999 and the Marine Invasive Species Act of 2003, to address the threat of non-native aquatic species (NAS) introductions. Under this Act, the California Department of Fish and Wildlife (CDFW) is required to monitor California coastal and estuarine waters for new NAS that could have been transported in ballast or through hull fouling and to assess the effectiveness of the Marine Invasive Species Program (MISP) in controlling NAS introductions from ship-related vectors. This report fulfills the reporting mandate set forth in Public Resources Code Section 71211 and summarizes the activities and results of CDFW's MISP from July 2011 through June 2014.

In 2011, paired epifaunal samples (hard substrate scrapings) and infaunal samples (sediment grabs) were surveyed at 52 sites in 18 bays and harbors. Of the 1,033 species identified, 105 were introduced, 189 were cryptogenic, and 739 were native to California. Numbers of NAS ranged from a low of 18 in Morro Bay to a high of 57 in LA/LB Harbor. NAS ranged from 7.7% to 21.6% of the resolved taxa collected from each harbor; the highest percentage was found in Tomales Bay. Two newly introduced species were discovered during the survey, including a tunicate native to the North Atlantic, Molgula citrina. A comparison of introduced species lists from the 2011 survey to previous surveys indicated that more NAS were recorded in the 2011 survey (105) than in the 2006 survey (82) and the 2001 survey (67).

Data collected during a two-year pilot study was analyzed to develop an integrated genetic and morphology-based system of identification for future monitoring. The study compared species assignments based on DNA barcodes to those based on morphology and also evaluated the detection of cryptic species by genetics. Survey data indicate that good estimates of non-native species richness can be obtained for non-native sessile invertebrates by sampling with settling plates. DNA analysis found many taxa that genetics had previously determined to be cryptic, but also found others that were suspected to be cryptic species. Next-generation molecular genetic analysis of plankton effectively detected many of the species found on plates, indicating that regular plankton sampling followed by genetic analysis could be part of a cost-effective strategy for NAS detection. Overall, results indicate that an integrated genetic and morphology-based system of identification could be more rapid and accurate, but less costly, for continued monitoring.

Current monitoring employs a new design of stratified random sampling using state-of-the-art genetic tools. Monitoring focuses on 10 major California estuaries, comparing NAS diversity and dynamics between 5 estuaries that support commercial shipping and 5 that do not. Each estuary would be sampled once over the four-year period, but additional continuous sampling will be conducted in San Francisco Bay. A pilot survey is also planned at one outer coast location adjacent to San Francisco Bay, in anticipation of future monitoring across a broader scale of estuarine-influenced waters. Monitoring work was divided into two consecutive two-year terms. Phase I began July 1, 2012, providing two full years of monitoring and analyses, but was subsequently

extended for one year to allow completion of sample analyses and report writing.

The California Aquatic Non-native Organism Database (CANOD) will be merged with the National Exotic Marine and Estuarine Species Information System (NEMESIS), a web-based public viewer maintained by the Smithsonian Environmental Research Center (SERC). The relocation will provide an opportunity to display comprehensive information about NAS invading California, with the benefits of a larger, centralized data base, fully-vetted information, cost-efficiency, and long-term technical support. All current data will be available to the public, but individual species profiles will be enhanced by photographs, interactive maps, and descriptions of their invasion history (distribution and occurrences), ecology, and impacts. CANOD and NEMESIS data for California NAS will be synchronized after an extensive review by CDFW and SERC staff.

Table of contents

TABLES

DEFINITIONS OF TERMS USED IN THIS REPORT

Ballast water: Water taken up or released by a ship to stabilize it, or to raise/lower it in the water column.

Benthic: Pertaining to the organisms that live on or in the sea bottom.

Biodiversity: Number and variety of living organisms; includes genetic diversity, species diversity and ecological diversity. For the purposes of this document, refers to biodiversity of native organisms.

Cryptic: Of or pertaining to two or more species that are morphologically similar but differ genotypically.

Cryptogenic taxa: Neither demonstrably native nor introduced (Cohen and Carlton 1995, Carlton 1996) because their native range or region is unknown.

Epifaunal: Of or describing organisms that live on the ocean floor or other submerged substrates such as sea anemones and barnacles.

Exotic Species: Synonym for introduced or non-native species.

Fouling: The accumulation and deposition of living organisms and certain non-living material on hard surfaces, most often in an aquatic environment.

Genotype: The genetic makeup of an organism.

Infaunal: Of or describing organisms that live within sediment, such as clams and worms.

Intertidal zone: Coastal area between low and high tide.

Introduced species: A species that was intentionally or accidentally transported or released by humans into an environment outside its historical range.

Invasive species: Non-native species that do ecological or economic harm.

Morphotaxon: Species or other taxonomic level based solely on morphologic characteristics. (plural: morphotaxa)

Nonindigenous: Non-native or alien; existing outside natural geographical boundaries.

Phytoplankton: Microscopic aquatic plant-like organisms suspended in water.

Propagule: Any living biological material (particles, cells, spores, eggs, larvae, and mature organisms) that can potentially be transported from one location to another and produce new individuals.

Species Complex: A group of species that cannot be reliably distinguished as a cohesive taxon based on form and structure.

Substrate: Surface on which an organism lives.

Subtidal zone: A marine or estuarine environment that lies below low tide level.

Taxon (plural, Taxa): A grouping of organisms given a formal taxonomic name such as species, genus, family.

Unresolved Taxon: Organism that could not be identified unambiguously to species level.

Vector (Introduction Vector): A means of transporting or introducing organisms from one geographical location to another, such as ballast water.

Voucher: A specimen archived in a permanent collection for future study.

Water Column: The vertical extent of a water body, from the surface to the bottom.

Zooplankton: Small (usually microscopic), free-floating or weakly swimming animals that live in aquatic environments.

ACRONYMS AND ABBREVIATIONS USED IN THIS REPORT

AIS Plan: California Aquatic Invasive Species Management Plan

CANOD: California Aquatic Non-native Organism Database

CDFW: California Department of Fish and Wildlife

MISP: Marine Invasive Species Program

MLLW: Mean Lower Low Water. The average height of the lower low waters over a 19-year period.

MLML: San Jose State University's Moss Landing Marine Laboratories. Includes both the Marine Pollution Studies Lab and the Genetic Ecology Lab.

MPPS: Massively parallel pyrosequencing (also called next-generation sequencing). A high-throughput approach to DNA sequencing using the concept of massively parallel processing.

NAS: Non-native Aquatic Species

NEMESIS: National Exotic Marine and Estuarine Species Information System

OSPR: Office of Spill Prevention and Response

SERC: Smithsonian Environmental Research Center

1.0 INTRODUCTION

Non-native aquatic species threaten California's estuarine and marine habitats. For invertebrates and algae, the non-native species richness in California coastal waters exceeds that of most regions of the world. California also plays a pivotal role in marine invasion dynamics for western North America, providing an entry point from which many species spread. Of the 290 NAS (in 2006, excluding fish and vascular plants) with established populations in western North America, about 80% were first recorded in California (Ruiz et al. 2011).

The discovery rate of non-native species in California shows a significant increase over time (Ruiz et al. 2011). San Francisco Bay has one of the highest reported numbers of invasions in the world, and new species continue to arrive. Although the increase in invasions are the result of several vectors, ballast water and hulls of ocean-going ships remain the primary mechanisms responsible for bringing species to California in recent years (Ruiz et al. 2011).

Non-native species have changed California's coastal waters. NAS effects include changes to the structure and function of ecosystems, declines of native and commercial fisheries, parasite interactions with native species and humans, and physical habitat alteration (Carlton 2001, Grosholz 2002). Non-native species compete with native species; approximately 42% of the species on the federal Threatened or Endangered species lists are at risk primarily because of predation, parasitism, and competition from non-native species (Pimentel et al. 2005) and about 40% of the species forced to extinction in aquatic ecosystems are due to biological invaders (Pimentel 2003).

California's Marine Invasive Species Act of 2003 extended the Ballast Water Management Act of 1999, to address the threat of NAS introductions. Under this Act, the California Department of Fish and Wildlife (CDFW) is required to conduct a study of California coastal waters for new introductions of NAS that could have been transported into state waters in ballast or through hull-fouling and assess results of the effectiveness of the MISP in controlling NAS introductions from ship-related vectors. Three previous legislative reports have been submitted since the inception of the MISP in 2000. This report fulfills the reporting mandate set forth in Public Resources Code Section 71211. Herein, we describe the purpose and history of CDFW's MISP, summarize the activities and results from July 2011 through June 2014, and outline the long-term monitoring plan for marine invasive species.

1.1 Statutory Framework

In California, as the impact and source of introduced aquatic species became better understood, a program was developed to address the introductions from the ballast of ocean-going ships. The following summarizes the origins and evolution of the California effort to manage ship-mediated NAS introductions.

1.1.1 California Ballast Water Management Act

In response to the potential threat of introduced NAS from the ballast of ships into the marine waters of the state, the Legislature passed the Ballast Water Management Act (Chapter 491, Statutes of 1999). Three agencies were responsible for implementing the various provisions of the Act: CDFW, the State Water Resources Control Board, and the State Lands Commission. CDFW, as the primary agency responsible for the management of fish and wildlife and their habitats, was required to conduct a study to determine the location and geographic range of introduced species populations along the California coast. A report detailing the results of that study was completed and submitted to the Legislature in 2002 (CDFW 2002). This information along with data generated by the State Lands Commission and the State Water Resource Control Board was used to craft a new, long-term program under the Marine Invasive Species Act of 2003 (MISA). This law came into effect January 1, 2004.

1.1.2 Marine Invasive Species Act

The MISA (Chapter 491, Statutes of 2003) extended the term of the MISP (to December 2009), to control the introduction and spread of NAS in marine and estuarine waters. The Act expanded the MISP to include coastwise traffic and CDFW's Office of Spill Prevention and Response (OSPR) was required to do a baseline survey of outer coast habitats to supplement the NAS baseline data collected up to 2002. The 2003 Act also directed CDFW to continue its monitoring program to determine whether new introductions have occurred since the original baseline was established.

1.1.3 Coastal Ecosystem Protection Act

The Coastal Ecosystem Protection Act (Chapter 292, Statutes of 2006) repealed the sunset provision of December 2009. The program is now ongoing, and the CDFW was given several new research and reporting responsibilities, as follows:

- Monitor coastal and estuarine waters for new introductions of NAS that could have been transported into state waters in ballast or as hull-fouling.

- Post data from the monitoring effort on the internet and updating the database on an annual basis, beginning July 1, 2008. The data from the monitoring efforts can be viewed at http://www.dfg.ca.gov/ospr/Science/invasive_species.aspx.

- Submit a report to the Legislature detailing the results of the monitoring studies and an assessment of the effectiveness of the MISP in controlling introductions from ship-related vectors. The report was initially due December 31, 2008, and must be updated every three years thereafter.

1.1.4 California Aquatic Invasive Species Management Plan

In 2008, the Governor signed the California Aquatic Invasive Species Management Plan (AIS Plan), which identifies actions to minimize the harmful effects of aquatic NAS in California. One of the top priorities identified in the AIS Plan is to conduct statewide

assessments of the risks from specific vectors for introductions of aquatic NAS. Another high priority identified by the AIS Plan is to support early detection and rapid response actions, partly by coordinating various aquatic NAS monitoring programs throughout the State.

2.0 MONITORING PLAN AND FIELD SURVEYS

From 2000 through 2011, the MISP contracted with San Jose State University's Moss Landing Marine Labs (MLML) to do large-scale field surveys of habitats in bays, harbors, marinas, and the open coast (Table 1). Currently, OSPR contracts with the Smithsonian Environmental Research Center (SERC) and MLML.

Table 1. CDFW large-scale field surveys through 2011.

Survey	Year							
	2000	2004	2005	2006	2007	2008	2010	2011
Bays and Harbors	X			X				X
Outer Coast		X			X			
San Francisco Bay			X				X	

Past surveys sampled multiple sites and habitats and collected thousands of specimens, and were time consuming and labor-intensive. The surveys collected an unusually large range of taxonomic groups because such a wide variety of habitats were sampled. Although sampling and post-sampling activities overlapped in time, each survey generally included up to 6 months of field sampling, followed by about 8 months of sample sorting, 8 months of specimen identification by taxonomic experts, and 2 months of data quality control and input. A limitation of this system is that the time between collection and final identification of organisms generally was a year or more, potentially delaying detection of newly introduced species.

2.1 SERC/MLML Settling Plate/Molecular Detection Pilot Study Results

In addition to biological surveys, the MISP funds research and special studies designed to detect NAS or improve knowledge about geographic ranges of cryptic or poorly understood NAS.

The MISP contracted with the Smithsonian Environmental Research Center (SERC) and the Molecular Ecology Lab of San Jose State University's MLML to develop a practical, cost-effective alternative to current non-native organism detection and monitoring methods. A two-year collaborative pilot study began in July 2009 to establish the groundwork necessary to move forward from traditional, morphologically-based taxonomy and to test a streamlined, community-level monitoring approach based on next-generation molecular genetic tools. The study was described in the previous Triennial Report to the Legislature (CDFW 2011). Here we summarize results of the study.

2.1.1 Settling Plate sampling

Survey data were used to examine the performance of settling plates to detect non-native species. Results indicate that good estimates of non-native species richness can be obtained for sessile invertebrates by sampling with settling plates. Specifically, (a) settling plates detected the highest number of non-native species during the summer, compared to plate surveys in other seasons or quadrat surveys in summer and (b) this method was very consistent across years (2009-2010).

SERC used 6 years of historical data from settling plates at the same sites to analyze changes in estimated non-native species richness over time. Compared to the 2009-2010 results, the historical data varied much more, partly as a result of environmental variation. This highlights the need for long-term monitoring to control for fluctuations in environmental conditions. Also, concurrent measures across multiple tracking or "sentinel" sites will help to reduce temporal variation in species occurrences observed at any one site.

The relationship between number of sites and number of years on inter-annual variation in numbers of non-native species has not yet been examined. Future work could also analyze the effects of number of sites and number of years on the probability of detecting particular increases in non-native species to help optimize sampling effort.

This study focused on the sessile invertebrate community, but the obvious next step is to apply the same approach to other habitats (e.g., soft-sediment or zooplankton) across multiple estuaries. We need to advance understanding for both the temporal and spatial axes, which will require a strategy of implementing (a) measures at sentinel sites in multiple estuaries (and habitats) and (b) more spatially extensive but less frequent surveys at other sites.

2.1.2 Genetics

The pilot study explored the efficacy of a genetic approach by comparing species assignments based on DNA barcodes to those based on morphology and also by evaluating the detection of potential cryptic species by genetic criteria. The aim was to develop an integrated genetic and morphology-based system of identification for future monitoring. Such an approach could make identification of non-native species more rapid and accurate, but less costly, enabling widespread and frequent monitoring.

The key to the genetic identification process was the development of a reference set of known-origin DNA sequences (called "barcodes") corresponding to all organisms that are likely to be encountered. Barcodes are based on non-overlapping genetic variation that often separates biological species (Geller 2007). Voucher specimens that have been reliably identified provide reference sequences enable DNA sequences to be used for identification. Then, the organisms present in the samples were determined by comparing the DNA from collected specimens against the barcode database.

To obtain DNA barcode sequences, 3 main steps were performed: extraction (extracting DNA from the sample), amplification (replication of the target segment of DNA), and

sequencing (determining the order of base-pairs in a segment of DNA). We successfully identified an average of 75% of the 4,218 samples that were extracted. The sequencing success rate increased, however, after adjustment of the sequencing template. Some failures were due to contamination by bacteria, gut contents, epibiota, or seawater, for which target and non-target DNA both amplified.

Once the DNA barcode library was created, we compared species assignments based on DNA barcodes to those based on morphology. DNA analysis found numerous taxa that genetics had previously determined to be cryptic (e.g. the bryozoan *Watersipora subtorquata*), but also found others that were suspected to be cryptic species. Further review by professional taxonomists is needed to determine whether these specimens are distinct species and therefore new discoveries.

A "next-generation" sequencing process, known as massively parallel pyrosequencing (MPPS), was used to analyze the DNA extracted as a whole from unsorted, complex, whole-community samples collected from artificial settling plates and plankton samples. The MPPS process differs from conventional sequencing in that it exhaustively sequences a large volume (≈ 1,000,000) of individual DNA template molecules simultaneously. With the addition of molecular tags to distinguish samples, multiple samples can be analyzed in a single batch.

Pyrosequencing of plankton effectively detected many of the species found on plates, a somewhat surprising result because benthic species are only transiently present in the plankton. Regular plankton sampling with analysis by pyrosequencing could be part of a cost-effective strategy for NAS detection.

Using bulk material from settlement plates and plankton, we compared the number of NAS detected by community-level genetic analysis to the number detected by morphologically-sorted analysis. Pyrosequencing out-performed morphological sorters. It is likely that performance will improve over time as we gain experience and increase efficiency with this method. Overall, results indicate that an integrated genetic and morphology-based system of identification is effective for continued monitoring.

2.2 Bays and Harbors Survey (2011)

The 2011 survey aimed at collecting data on the presence, distribution, and abundance of NAS in California bays and harbors. The sampling design followed that of the MISP 2000-2001 NAS survey of California's bays and harbors (Fairey et al., 2002). We targeted two main habitat types: subtidal fouling (also called epifaunal in this report), and subtidal infaunal communities. We aimed to collect samples from as many different habitats as possible, and targeted the most diverse appearing areas within each of those habitats, rather than randomly selecting locations.

Paired epifaunal samples (hard substrate scrapings) and infaunal samples (sediment grabs) were collected from 52 sites in 18 bays and harbors. All samples were sent to specialized taxonomists for identification of the specimens.

Of the 1,033 species identified, 105 were introduced, 189 were cryptogenic, and 739

were native to California. An additional 599 different taxa were not identified to species level and were classified as unresolved. We also identified 7 taxa as species complexes, which were assigned an introduction status of “unresolved complex”. These taxa may or may not be introduced to California’s bays and harbors.

Numbers of introduced species ranged from a low of 18 (7.7% of all taxa, excluding unresolved) in Morro Bay to a high of 57 (13.9%) in LA/LB Harbor (Table 2). Introduced species ranged from 7.7% to 21.6% of the resolved taxa collected from each harbor. Tomales Bay had the highest percentage of introduced species. Cryptogenic species ranged from 23 species collected in Tomales Bay to 96 species collected in Los Angeles Harbor.

Table 2. Number of taxa per harbor by invasion status in 2011 Bays and Harbors Survey.

Waterbody	Taxa Totals	Introduced	Cryptogenic	Native	Unresolved Complex	Unresolved
Humboldt Bay	364	20	51	135	1	157
Bodega Bay	183	22	28	57	3	73
Tomales Bay	164	24	23	64	4	49
Moss Landing Harbor	350	23	49	144	2	132
Monterey Harbor	458	20	50	190	3	195
Morro Bay	361	18	48	151	0	144
Santa Barbara Harbor	345	28	48	128	2	139
Channel Islands Harbor	401	42	56	145	2	156
Port Hueneme	407	32	54	175	2	144
Marina del Rey Harbor	313	32	38	108	3	132
LA/Long Beach Harbor	675	57	96	256	3	263
Huntington Harbor	287	33	49	101	3	101
Newport Bay	360	39	53	125	3	140
Dana Point Harbor	336	35	46	120	1	134
Avalon Harbor	513	23	60	243	2	185
Oceanside Harbor	364	38	57	121	2	146
Mission Bay	476	53	70	166	3	184
San Diego Bay	441	53	63	153	3	169

Epifaunal samples yielded more total unique species than did infaunal samples. Likewise, the number of introduced species from epifaunal samples (91 species) was more than from infaunal samples (51 species), although the percentage of introduced species was similar for the two habitats (11.5% for epifaunal and 9.3% for infaunal).

Three species newly introduced to California were discovered during our 2011 survey. *Molgula citrina*, a tunicate, was recorded at 2 sites in Humboldt Bay. Native to the North Atlantic, there are previous Pacific records in Alaska in 2008 (Lambert et al. 2010) and Oregon in 2010 (Chapman et al. 2011). Its Atlantic distribution ranges from northeast

North America to Great Britain and northern Europe. *M. citrina* was likely introduced via ship sea chests (Lambert et al. 2010).

Another species new to California waters, *Dynoides saldanai*, was found at Avalon Harbor. The movement of this isopod to California represents a northern range extension from the Pacific coast of Mexico, most likely through anthropogenic means (D. Cadien pers. comm. Feb. 2012).

Branchiomma sp. LH1, a polychaete worm, was found in Huntington Harbor, San Diego Bay, and Mission Bay. Species with provisional names are typically classified as unresolved, but this genus has not been previously recorded for this coast, so we classified it as introduced. The species is large and colorful, so is easily distinguishable.

Many of the recently reported invaders (or suspected invaders) are polychaete worms, partly because they are one of the most dominant groups of organisms in marine communities, but also because cryptic species are common among polychaetes (Nygren 2013). *Myrianida convoluta*, a cryptogenic polychaete worm, was found in LA/Long Beach Harbor 2011. It was described from the Mediterranean and was previously found in California in Santa Catalina Island (Nygren 2004). *Myrianida pentadentata*, described from Japan, was recorded from Dana Point Harbor, LA/Long Beach Harbor, and Port Hueneme in 2011.

We compared the introduced species list from the 2011 Bays and Harbors Survey to previous surveys conducted in 2006 and 2001 (Table 3). Those species found during the 2006 zooplankton sampling were excluded from the comparison. We recorded 105 NAS in the pooled 2011 survey, compared to 82 in the 2006 survey and 67 in the 2001 survey. Of the 105 NAS found in 2011, 49 taxa were not found in the 2006 survey. However, 9 of these species were discovered after 2006, leaving 40 species that were detected in the most recent survey but were undetected in 2006. Thirty-nine taxa were found in common among the 3 surveys.

Table 3. Non-native species sampled in Bays and Harbors Surveys. Data is pooled from all locations. Asterisk denotes taxa that were newly discovered after the 2006 survey.

Phylum	Species	2001	2006	2011
Annelida	*Branchiomma sp. LH1**			X
	Branchiura sowerbyi		X	
	Diplocirrus sp. SD1 SCAMIT			X
	Ficopomatus enigmaticus	X	X	X
	Heteromastus filiformis complex			X
	Hydroides elegans	X	X	X
	Manayunkia speciosa		X	
	Myrianida convoluta			X
	Myrianida pachycera		X	X
	Myrianida pentadentata			X
	Neodexiospira brasiliensis			X

Phylum	Species	2001	2006	2011
	Nicolea sp. A Harris	X	X	X
	Parasabella fullo			X
	Polydora brevipalpa			X
	Sabaco elongatus	X		
	Scolelepis (Parascolelepis) texana			X
	Streblospio benedicti		X	X
	Syllis nipponica	X	X	X
	Tubificoides brownae			X
	Tubificoides wasselli			X
Arthropoda	*Ampelisca abdita*	X		X
	Amphibalanus amphitrite		X	X
	Amphibalanus eburneus		X	
	Amphibalanus improvisus			X
	Ampithoe valida	X	X	X
	Aoroides secundus		X	X
	Caprella drepanochir	X		X
	Caprella mutica	X	X	X
	Caprella scaura complex	X	X	X
	Caprella simia			X
	Chelura terebrans		X	
	*Dynoides saldanai**			X
	Elasmopus rapax	X		X
	Eobrolgus spinosus			X
	Eochelidium sp. A SCAMIT	X		X
	Eusarsiella zostericola		X	X
	Gnorimosphaeroma rayi	X		
	Grandidierella japonica	X	X	X
	Incisocalliope derzhavini		X	X
	Jassa marmorata	X		X
	Leucothoe nagatai			X
	Limnoria quadripunctata		X	
	Limnoria tripunctata	X	X	X
	Melita nitida		X	
	Melita rylovae			X
	Microdeutopus gryllotalpa		X	
	Monocorophium acherusicum	X	X	X
	Monocorophium insidiosum	X	X	X
	Nippoleucon hinumensis	X	X	X
	Oithona davisae	X	X	
	Palaemon macrodactylus			X
	Paracorophium lucasi		X	X
	Paradexamine sp. SD1 SCAMIT		X	X

Phylum	Species	2001	2006	2011
	Phtisica marina	X	X	X
	Pseudodiaptomus marinus	X	X	
	Pseudosphaeroma sp. (of Bruce and Wetzer 2008)			X
	Sinelobus sp. (of Cohen 2007)			X
	Sinocorophium alienense			X
	Sinocorophium heteroceratum	X	X	
	Sphaeroma quoyanum	X	X	X
	Stenothoe valida complex		X	X
	Stephos pacificus	X		
	Synidotea laticauda		X	
Chlorophyta	*Bryopsis sp. 1 Miller*			X
Chordata	*Ascidia zara*	X	X	X
	Botrylloides perspicuum	X	X	X
	Botrylloides sp. A Lambert		X	
	Botrylloides violaceus	X	X	X
	Botryllus schlosseri	X	X	X
	Botryllus sp. A Lambert		X	X
	Ciona intestinalis	X	X	X
	Ciona savignyi	X	X	X
	Didemnum vexillum		X	X
	Diplosoma listerianum	X	X	X
	Microcosmus squamiger	X	X	X
	*Molgula citrina**			X
	Molgula ficus		X	X
	Molgula manhattensis	X	X	X
	Perophora japonica			X
	Polyandrocarpa zorritensis	X	X	X
	Styela canopus	X	X	
	Styela clava	X	X	X
	Styela plicata	X	X	X
	Symplegma reptans	X	X	X
Cnidaria	*Garveia franciscana*			X
	Pinauay crocea			X
	Thuiaria thuiaroides		X	
Ectoprocta	*Amathia convoluta*		X	
	Anguinella palmata			X
	Bowerbankia gracilis complex	X	X	X
	Bowerbankia imbricata		X	
	Bugula neritina complex	X	X	X
	Bugula stolonifera		X	
	Conopeum tenuissimum			X

Phylum	Species	2001	2006	2011
	Cryptosula pallasiana complex	X	X	X
	Hippopodina feegeensis			X
	Schizoporella errata			X
	Schizoporella japonica	X	X	
	Watersipora arcuata	X	X	X
	*Watersipora sp. (of Mackie et al. 2006)**			X
	*Watersipora subtorquata Clade A**			X
	*Watersipora subtorquata Clade B**			X
	Watersipora subtorquata complex	X	X	X
	Zoobotryon verticillatum	X	X	X
	Barentsia benedeni		X	X
Heterokontophyta	*Sargassum horneri*			X
	Sargassum muticum	X	X	X
	Undaria pinnatifida	X	X	X
Magnoliophyta	*Myriophyllum spicatum*		X	
Mollusca	*Corbicula*		X	
	Crassostrea gigas	X	X	X
	Crassostrea virginica		X	X
	Crepidula fornicata		X	
	Crepidula plana			X
	Geukensia demissa	X		X
	Macoma petalum			X
	Musculista senhousia	X	X	X
	Mya arenaria	X		
	Mytilus galloprovincialis		X	
	Ostrea edulis	X		X
	Philine auriformis		X	X
	Theora lubrica	X	X	X
	Venerupis philippinarum	X	X	X
Porifera	*Chalinula loosanoffi*	X		X
	*Halichondria "panicea" Clade IIA**			X
	*Halichondria "panicea" Clade IIB**			X
	Halichondria bowerbanki		X	
Rhodophyta	*Caulacanthus ustulatus*			X
	Dasya sessilis		X	
	Grateloupia lanceolata		X	X
	*Grateloupia turuturu**			X
	Lomentaria hakodatensis		X	X
	Neosiphonia harveyi			X

2.3 Current Monitoring (2012-2014)

2.3.1 Introduction

Following the success of their joint San Francisco Bay Pilot Study of 2009-2012, SERC and MLML were engaged to continue NAS monitoring on behalf of the MISP. The new approach included an *a priori,* stratified random sampling design that enables explicit, quantitative comparisons and long-term trend evaluations supported by a sound statistical framework. Furthermore, samples were analyzed using state-of-the-art genetic tools to assure consistent taxonomic assignment, identify cryptic and unresolved taxa, and build a robust molecular voucher database for rapid, high-throughput, and high-sensitivity NAS detection.

Current monitoring focused on ten major California estuaries where past surveys have shown were primary locations where NAS have been introduced, and now support persistent concentrations of NAS. Five of these estuaries support commercial shipping, and they were paired with five others in which no commercial shipping takes place, to compare NAS diversity and dynamics between the two estuary types. Intensive sampling was to be conducted in each of the ten estuaries, and each estuary would be sampled once over the four-year period (Table 4). Additional sampling was conducted in San Francisco Bay (see Section 2.2.2.1).

In contrast, past MISP surveys of California's outer coast (2004 and 2007) have detected far fewer NAS, in terms of both species and geographic extent. The outer coast surveys had been conducted at 22 sites spaced at roughly 50-mile intervals. These survey results suggest that localized NAS populations along the outer coast could have been missed due to the sampling scale and few replicates collected. Some evidence exists in grey literature that estuary discharges may contribute propagules to some adjacent outer coast sites. Moreover, most, if not all, of NAS detected at outer coast sites were those that are well-established in bays and estuaries. A pilot survey was also planned at one outer coast location (Table 4) adjacent to a focal estuary, in anticipation of increasing future monitoring across a broader scale of estuarine-influenced waters. The waters outside the mouth San Francisco Bay were tentatively identified as a possible location, pending review of accessibility and diver safety.

Table 4. Tentative sampling scheme for Phases I (Years 1 and 2) and II (Years 3 and 4) of marine invasive species monitoring in focal estuaries of California. The number and type of samples to be collected from each estuary are also shown. Work for Phase II w will be conducted under a separate contract. The maximum numbers of samples or specimens selected for each method of analysis are given in the shaded rows below the totals.

		Estuary Sites									**Outer Coast Site**					
		Epifauna			Infauna			Plankton			Epifauna			Transects		
Year	Estuary	Sites	Replicates	Subtotal	Sites	Replicates	Subtotal	Sites	Replicates	Subtotal	Sites	Replicates	Subtotal	Transects	Quadrats	Subtotal
1	Bodega Bay	10	5	50			0	10	5	50						
	San Francisco Bay: High Salinity*	**10**	**5**	**50**	**10**	**5**	**50**	**10**	**5**	**50**	**4**	**10**	**40**	**6**	**6**	**36**
	San Francisco Bay: Low Salinity	**5**	**5**	**25**	**5**	**5**	**25**	**5**	**5**	**25**						
	San Francisco Bay: High Salinity, Deep Water				**5**	**5**	**25**									
2	Morro Bay	10	5	50				10	5	50						
	Mission Bay	10	5	50				10	5	50						
	San Diego Bay	**10**	**5**	**50**	**10**	**5**	**50**	**10**	**5**	**50**						**0**
	San Francisco Bay: High Salinity Index Sites*	**3**	**5**	**15**			**0**			**0**			**0**			**0**
3	**Humboldt Bay**	**10**	**5**	**50**	**10**	**5**	**50**	**10**	**5**	**50**			**0**			**0**
	Los Angeles Harbor / San Pedro	**10**	**5**	**50**	**10**	**5**	**50**	**10**	**5**	**50**			**0**			**0**
	Newport Bay	10	5	50				10	5	50						
	San Francisco Bay: High Salinity Index Sites*	**3**	**5**	**15**			0			0						
4	**Port Hueneme**	**10**	**5**	**50**	**10**	**5**	**50**	**10**	**5**	**50**			**0**			
	Marina del Rey Harbor	10	5	50			0	10	5	50						
	San Francisco Bay: High Salinity Index Sites*	**3**	**5**	**15**			0			0						
Total Replicate Samples Collected Over the Full Monitoring Project				570			300			525			40			36
Morphological Analysis (# of samples)				570			300			120			40			
Genetic Analysis (Sequence Unique Voucher Specimens, 5-10 reps ea.)				570			300			120			40			
Genetic Community Analysis				0			0			245			0			0

* The San Francisco Bay epifaunal sites will include the three index sites established during a previous pilot study. These sites will be re-sampled in Years 2, 3, and 4.

Due to the thoroughness of the initial sampling effort, the current surveys will take a minimum of four years to complete. Far more samples are being collected and processed than in previous surveys. The intensified sampling is expected to produce two-fold benefits. The surveys will not only serve to expand the existing NAS inventory with greater statistical power, but will also generate a robust set of baseline barcodes needed for successful transition into metagenomic analysis of species richness from environmental samples.

Considerable effort is therefore being devoted toward collecting the most inclusive set of voucher specimens to expand the existing barcode library started during the San Francisco Bay pilot study. Although thousands of voucher samples have been saved from previous traditional surveys, they cannot be used for sequencing because they were preserved in formalin, which degrades the DNA. Therefore, new vouchers must be collected and identified for the barcode database. The voucher specimens will be reviewed by taxonomic professionals to assure accurate identifications. The time and effort invested at the outset to build a robust barcode database will enable future survey resources to be reallocated towards sampling at appropriate temporal and spatial sampling scales, with sufficient replicates. Morphological examinations would still be required, but refocused upon yet-unsampled waters or limited to spot checks for new, unusual, or rare taxa in sentinel sites. In turn, the increased sampling resolution will enable a better understanding of invasion dynamics and maximize the probability of detecting new and especially rare NAS.

Given present contractual rules and funding constraints, the monitoring work was divided into two consecutive, two-year terms. The first phase of the monitoring project commenced July 1, 2012, providing two full years of monitoring and to conduct most of the morphological and genetic analyses. Phase I was subsequently extended for one year to allow completion of sample analyses, cross-referencing morphological and genetic identifications, data analyses, and report writing. For the purposes of this report, coverage will be generally limited to accomplishments through June 30, 2014.

2.3.2 Methods

2.3.2.1 Sample Collections and Morphological Analyses

San Francisco, San Diego, Bodega/Tomales, Morro, and Mission bays were sampled during this reporting period (Table 4). High-salinity waters (> 20 ppt) were sampled in all estuaries, and sites were selected in or near ports and marinas. Sampling activity was limited to summer through mid-fall, to control for seasonal differences, and to coincide with the period of maximum plankton abundance and larval recruitment, hence maximum species diversity. San Francisco Bay continued to be an area of interest because of its status as a “hot spot” for NAS. The additional sampling effort in the bay is described in further detail below and summarized in Table 4.

The following auxiliary data were also collected during each site visit: sample date; GPS location (latitude/longitude); salinity; temperature; dissolved oxygen; and weather condition. In-situ logging devices were set at each hard-substrate community site to record continuous temperature data.

Hard-Substrate Invertebrates

The hard-substrate invertebrate community (epifauna) was passively sampled, using standard 14 cm^2 polyvinyl chloride (PVC) settling plates. Up to ten replicate plates each were set at ten sites per estuary, at a depth of 1 m below mean lower low water (MLLW). For San Francisco Bay, the three index sites previously established during the 2009-2012 Pilot Study were included among the ten epifaunal sites. These index sites were the only ones resampled during the second year. In addition, five replicate plates each were set at five low-salinity (<20 ppt) sites in the upper reaches of San Francisco Bay, including the ports of Sacramento and Stockton.

All plates were deployed for a three-month period. Five replicate plates per site were randomly selected for species-level analysis. Upon retrieval, these plates were examined under a dissecting microscope to identify mobile and sessile invertebrate morpho-species (unique species determined by morphological attributes) present, and to collect live voucher specimens for morphological identification and subsequent external review. For genetic analyses, five to ten unique voucher specimens were collected per site. Both types of vouchers were labelled with unique identification numbers for tracking purposes, and logged into SERC's tracking database along with pertinent sample data.

All morphological vouchers were forwarded to the SERC's main laboratory in Edgewater, MD for further taxonomic analysis. All bryozoan, tunicate, and mobile crustacean morphological identifications were verified by in-house staff. Polychaete, barnacle, mollusk, and other difficult taxonomic group verifications were referred to external taxonomic experts. All genetic vouchers were forwarded to MLML (see Section 2.3.2.2). Unprocessed plates were preserved and archived for possible future analysis.

Soft-Sediment Invertebrates

Soft-sediment invertebrate community (infaunal) collections were limited to those estuaries supporting commercial ports. Ten sites per estuary were sampled in the subtidal zone (2 m below MLLW) using a 0.1 m^2 van Veen grab. In San Francisco Bay, additional sampling included five sites in the low-salinity (upper) reaches of the bay, and five sites each in the intertidal (0.5 m below MLLW) and subtidal (4 m below MLLW) zones of high-salinity, deep water sites. Five replicates each were collected from all infaunal sites.

Each infaunal sample was separated from sediment using a 1 mm sieve and processed live, within a few hours of retrieval, as per epifaunal samples. Invertebrates were sorted into major taxonomic groups, and identified to the lowest possible taxonomic level under a dissecting microscope. Morphological identifications were later verified by external taxonomists. Morphological and genetic vouchers were collected, preserved, and catalogued as per epifaunal samples above.

Plankton

Plankton was sampled at ten high-salinity sites each in all four estuaries, and the low-salinity (upper) reaches of San Francisco Bay. Collections were made using an 80 µm

mesh net. Approximately half of the samples per site were collected by direct vertical towing and the rest by pumping a known volume of water through the net, to compare the efficacy of the two methods. Five replicates per site were preserved in 95% ethanol for metagenomic analysis. The remaining replicates were preserved in 10% formalin for morphological analysis. All plankton samples were forwarded to SERC's main laboratory for cataloguing.

Outer Coast Sites

The pilot outer coast sampling was postponed due to uncertainties about accessibility and diver safety outside the San Francisco estuary mouth. Plans included standard settling plate sampling at two sites (1 km and 5 km) each, upcoast and downcoast of the mouth of the estuary (ten replicates per site), within the shallow subtidal zone (≈10 m depth). The plates were to have been deployed, retrieved, and processed in the same manner as estuary samples, except that emphasis was to be placed on sessile invertebrates only.

Survey plans also included visual SCUBA-diving searches, to score the presence/absence of conspicuous NAS in randomly-selected quadrats. Target NAS included tunicates, bryozoans and other easily-recognized NAS detected on settling plates of estuary and outer coast sites, and other organisms known to colonize outer coast habitats. The visual counts were to be conducted at increasing distances (1 km, 2.5 km, 5 km, and 10 km) from the estuary mouth. At each distance, six quadrats would be searched along six fixed transects.

2.3.2.2 Genetic Analyses

All genetic analyses were performed at the Molecular Ecology Laboratory at MLML. Voucher specimens were sequenced to: 1) expand the existing DNA barcode library (started during the previous pilot study); 2) verify a subset of species-level identifications made through standard morphological examination (see Section 2.2.2.1); and 3) identify organisms (including cryptic species) that cannot be reliably identified to species by morphological examination.

As in the pilot study, taxonomically informative regions of two genes were sequenced for each specimen: cytochrome *c* oxidase subunit I (COI), and large subunit ribosomal RNA (28S, or LSU). The dual barcode system was employed to accommodate lower invertebrate groups where COI provides weaker phylogenetic resolution and for taxa yielding low COI amplification success rates. Putative species were delimited into operational taxonomic units (OTUs) using a threshold of approximately 5% base-pair divergence.

Voucher Specimens

A minimum of five voucher specimens of every unique species collected from all habitats were sequenced. Priority was placed on novel morphotaxa (i.e., those that have not been analyzed from a particular site).

DNA was extracted from voucher specimen tissue using standard pre-packaged reagent kits and standard 96-well arrays (one tissue sample per well). Protocols were

developed to streamline the workflow, maximize DNA yield, and reduce reagent use to 25% of recommended volume. Polymerase chain reactions (PCRs) were also run in 96-well plates, with the wells in the PCR plate corresponding to the same positions as in the extraction plate array. The target locus was isolated and amplified from each specimen via PCR, using appropriate primers, and labeled per the protocol of Neiman et al. (2011). The labels enabled thousands of individual DNA templates to be sequenced simultaneously on the next-generation sequencing machine. Both COI and 28S loci were amplified from each voucher specimen to extent possible.

All voucher specimens were sequenced despite variable PCR quality (based on agarose gel analysis) because wells showing low apparent PCR success still yielded viable data when sequenced. The resultant sequences were aligned, analyzed and sorted into OTUs using GENEIOUS, a commonly-used bioinformatics algorithm.

Metagenomic (Whole-Community) Analyses

Whole-community analysis was limited to plankton samples. Processing and analyses were postponed until completion of plankton collections (in summer 2014). Up to 125 samples will be randomly selected for metabarcoding. Up to four samples will be analyzed per run on the next-generation sequencing machine. Species richness derived from traditional morphological examination will be compared against metabarcoding results to assess the accuracy and consistency of identifications as well as sensitivity of species detection.

2.3.2.3 Data Collection, Management, and Access

During the pilot study, a prototype database was developed to promote data-sharing between SERC and MLML. The existing database was modified to enhance interoperability, especially pertaining to sample tracking and cross-validation of species identifications. Frequent communications between both teams, including conference calls and live meetings, were convened at no less than quarterly intervals as needed to refine data sharing and analytical strategies.

2.3.3 Preliminary Results

2.3.3.1 Sample Collections and Morphological Analyses

Hard-Substrate Invertebrates

Approximately 14,130 voucher specimens comprising 506 morphotypes were collected for morphological analysis. Identifications and verifications have been completed for vouchers collected from San Francisco and Bodega bays in 2012, except for a few polychaete specimens. Of samples collected in 2013, verifications of San Diego and Mission Bay barnacles have been completed. Bivalve, tunicate, and bryozoan vouchers of said bays, plus most of Morro and San Francisco bay voucher verifications remain in progress. All San Francisco Bay crustacean vouchers have been identified, and verifications have been completed. All Morro Bay crustaceans have also been identified, and 20% of that data have been entered in the database. Ten percent of San Diego Bay crustaceans have been identified to date.

Preliminary data analysis was begun on sessile taxa for general patterns of native vs.

non-native species abundance and diversity using 2012 San Francisco Bay data. Species richness pattern analyses across all sites and bays sampled thus far will begin upon completion of remaining identifications and verifications.

Soft-Sediment Invertebrates

Infauna collected from San Francisco Bay in 2012 and 2013 and San Diego Bay in 2013 yielded more than 300 morphological voucher specimens comprising 268 types. Eighteen of these morphotypes were also found in hard-substrate samples. Voucher specimen identifications and external taxonomist reviews were completed for all samples.

Basic patterns of species richness, diversity, and abundance were analyzed for native and non-native fauna collected from San Francisco Bay in 2012. Preliminary species accumulation curves showed that the sampling effort provided a good estimate of overall NAS species richness. Non-native species abundance was greater than that of native species, in terms of total number of organisms. Data analyses for 2013 collections in San Francisco and San Diego bay were in progress as of this writing.

Results of the 2012 San Francisco Bay analyses were presented at the International Marine Bioinvasion Conference in Vancouver, British Columbia, in August 2013. A manuscript is being prepared for publication.

Plankton

Collections for all but Bodega/Tomales bays have been completed during this reporting period. Previously-collected samples were stored at SERC's main laboratory and will be forwarded for genetic and morphological analyses upon completion of the remaining plankton collections in summer 2014.

Morphological identification of plankton will be subcontracted. San Francisco Bay samples will be analyzed by Dr. Wim Kimmerer of San Francisco State University's Romberg Tiburon Center. Dr. Jeffrey Cordell of the University of Washington will analyze samples collected from all other estuaries.

2.3.3.2 Genetic Analyses

Initial accomplishments included the hiring and training of a research technician/project manager and purchasing key equipment. Tracy Campbell joined the Molecular Ecology Laboratory in August 2012. An Ion Torrent Personal Genome Machine (PGM), a benchtop-model, semiconductor-based next-generation sequencing device was purchased so that metagenomic analyses could be performed in-house at a significantly lower cost and turnaround time than outservicing. A 96-well fluorescence/absorbance plate reader and a thermocycler were also purchased to support laboratory preparations.

Shortly after purchase, a larger-capacity chip and an improved sequencing reagent kit were released for the PGM. The reagent kit increased PGM read lengths to 400 bp, enabling full analysis of the ≈350 bp-long 28S locus. Protocols were developed to adapt analysis of the >740-bp COI locus by cutting the fragment in half (by chemical or

mechanical means) to achieve overlapping reads that yield the full molecule. Workflows were also developed such that, with minor modifications, both single species (voucher specimen) and metagenomic DNA could be analyzed on the PGM. The contract was thus amended to allow purchase of additional PGM reagents in lieu of subcontracting Sanger sequencing services to a commercial laboratory. Furthermore, said amendment provided for the purchase of two additional pieces of much-needed equipment. An ultrapure water filtering system was purchased to prevent introduction of extraneous ions into sequencing reagents, which may cause erroneous reads. As the PGM works by detecting hydrogen (H^+) ions released during the synthesis reaction, it is very sensitive to H^+ ions from extraneous sources. A DNA size-selection device was required to facilitate removal of odd-length DNA fragments and avoid sample contamination.

Voucher Specimens

Thus far, extractions have been completed for nearly 14,000 voucher specimens representing about 483 morphotypes observed in plate and grab samples over the past two years. Soft-sediment (infaunal) invertebrates comprised about 3% of the vouchers. Many morphotaxa were new to the collection locations, but not to the existing database. A total of 70 vials were received containing no visible tissue, but at least 10% of these samples yielded useable DNA. Tissue samples in the vials may have been too small and had apparently disintegrated in the preservative solution. Additional PCR cycles were attempted to increase DNA recovery rates from the other 90% of apparently empty vials.

DNA amplifications were attempted on 11,475 28S and 12,732 COI templates, with PCR success rates of 88% and 83%, respectively. Approximately 90% of non-indexed COI sequences have been analyzed. The remaining non-indexed COI, as well as indexed COI and 28S sequences will be analyzed shortly.

Two sequencing runs of about 3,000 vouchers each yielded over 10 million COI sequence reads. Full-length COI sequences were reassembled using two methods using GENEIOUS. Both methods will be enhanced by planned improvements in computational efficiency. A third method, using QUIIME, was also investigated. QUIIME required fewer hardware resources, but text files of data and existing scripts needed extensive modifications in order to work properly. Further development of analytical protocols was pending as of this writing.

Metagenomic (Whole-Community) Analyses

No samples have been received, thus none have been analyzed. This work was postponed until remaining collections (Bodega/Tomales bays) have been completed.

2.3.3.3 Data Collection, Management, and Access

Data collected during field surveys and subsequent analyses were entered in the shared database at each completed stage of sample processing. Frequent coordination continued between SERC and MLML teams throughout this reporting period to further optimize data interoperability. As morphological and genetic analyses are completed in the coming months, the database will be a critical tool for facilitating species definitions

and interpretations based on genetic and morphological analyses.

Prototype forms were developed and tested for electronic real-time data entry for field data using tablet computers. This measure was instituted to improve the overall efficiency and accuracy of workflows.

At MLML, the existing GENEIOUS sequence database assembled from the previous pilot study was reorganized by OTUs and edited, preparatory to adding new sequences generated from the current analyses.

For groups of organisms in which morphological identification methods remain problematic (e.g., hydroids, sponges, anemones, and spirorbid polychaetes), it was agreed to rely upon genetic data for differentiating taxa.

2.4 Future Monitoring

The multi-year implementation plan using new monitoring protocols to detect NAS in 10 focal estuaries will be continued. The time frame of the coastwide monitoring has been extended to a total seven years. Amendments to the schedule shown in Table 4 are explained below.

2.4.1 Phase 1 (Years 1 and 2)

SERC and MLML contracts were each granted 1-year extensions to complete pending work and to allow time for cross-validation between morphological vs. genetic species identifications, data analyses and report writing. In addition, the release of the NEMESIS California data portal (see Section 3.1.1.2) was extended to June 30, 2015.

2.4.2 Phase 2 (Years 3, 4, and 5) and Phase 3 (Years 6 and 7)

Current contractual rules have been amended such that maximum contract terms have been increased to three-years. The sampling schedule was amended to accommodate an increase in the number of sites (to 10) in high-salinity areas of San Francisco Bay, with an extra year of surveying in year 3. Surveys of Los Angeles/Long Beach harbors and Marina del Rey have been deferred to Phase 3 (in 2016), in a forthcoming contract. A full three years of funds has been provided for Phase 2, which will enable the hiring of a bioinformatics expert during year 5 to assist with the development of an efficient pipeline to process the ever-increasing volume of sequences accumulated from genetic analyses to date. As of the end of the reporting period, Phase 2 contracts with SERC and MLML were pending, but are expected to be approved and executed shortly. Phase 1 and Phase 2 work will run simultaneously during Fiscal Year 2014.

3.0 DATABASE

3.1 National Exotic Marine and Estuarine Species Information System (NEMESIS)

3.1.1 Introduction

The existing version of CANOD was built and maintained by a previous contractor as part of an overall NAS monitoring project. When that contract expired in June 2012, no additional funds were available to continue supporting CANOD updates and maintenance. Meanwhile, the subsequent transfer of monitoring contracts to SERC and

MLML presented an opportunity to combine MISP database resources with the National Exotic Marine and Estuarine Species Information System (NEMESIS), a web-based public viewer maintained by SERC. The advantages of such a merger include a larger, centralized data base, fully-vetted information, live updates, cost-efficiency, and long-term technical support.

NEMESIS was created by SERC's Marine Invasions Research Laboratory in partnership with the U.S. Geological Survey. The viewer was custom-built on a Java Server Page platform and is supported by full-time staff dedicated toward long-term database expansion and maintenance. The existing NEMESIS infrastructure was designed specifically for displaying comprehensive information about NAS invading the shores of the continental United States. Individual species accounts include taxonomic hierarchies, photographs, invasion history (distribution and occurrences), ecology, impacts, and literature sources. Data may be queried by various parameters, such as species, taxonomic group, invasion status, or bioregions. In addition, individual georeferenced NAS records or groups of records per bioregion may be viewed in interactive maps. Given SERC's multi-year commitment to conduct NAS sampling on the behalf of MISP, NEMESIS also provides a convenient platform for entering and displaying new survey records. As part of the current monitoring contract, a special portal of NEMESIS is under construction to enable public access to California NAS occurrences pursuant to Section 71211 (a) (2) of the Public Resources Code. The NEMESIS team is providing pro bono database and website support services in exchange for MISP contributions toward database expansion for the west coast of North America.

The data migration process began in November 2012 with a series of monthly conference calls among a core group of Department and SERC staff. The meetings were convened to discuss strategies, identify tasks, set priorities, and track progress. Frequent conference calls will continue until the project is completed.

The initial focus of the NEMESIS migration was placed on truly marine and estuarine invertebrates and algae. The work was prioritized by major taxonomic groups, in the following order: tunicates, decapod crustaceans, caprellid amphipods, ectoprocts, entoprocts, polychaetes, and cnidarians. Additional groups will be included as more taxa records are made available in NEMESIS. Vascular plants and boundary-resident organisms will be included on a case-by-case basis relative to their importance to marine and estuarine communities as a whole. CANOD will remain available until the California portal is launched on June 30, 2015.

3.1.1.1 Data Preparations for the NEMESIS Migration

CANOD and NEMESIS were last synchronized for data accumulated through 2006. Since that time, species had been added to, or deleted from, each database independently. Valid names or invasion statuses had been changed for some species as well. Moreover, each database had different naming conventions for provisional species and species complexes. Lastly, there were questions as to whether some species belong in NEMESIS (e.g., non-marine/estuarine species; species with failed or eradicated populations; and non-resident or borderline species).

The most recent comparison of CANOD and NEMESIS taxa was completed by SERC staff in May 2014. A total of 115 CANOD-origin taxa were not found in NEMESIS for the reasons described in Appendix A. This evaluation resulted in invasion status changes for most of the new taxa discovered during the 2011 Bays and Harbors survey (in Section 2.2 and shown in Table 5) and several in Table 6. Those labeled as "Needs Review" will be reconciled as part of the NEMESIS migration process. Appendix A also includes five CANOD-origin NAS that were readily accepted into NEMESIS because there was sufficient evidence to support their invasive status in California.

In addition, five CANOD-origin species were redesignated as introduced, per NEMESIS: *Austrobilharzia variglandis* (Phylum Platyhelminthes, formerly Cryptogenic); *Ciona* sp. (Phylum Porifera, formerly Unresolved as the genus *Ciona*); *Corella inflata* (Phylum Chordata, formerly Native); *Limnodriloides monothecus* (Phylum Annelida, formerly Cryptogenic); and *Moerisia lyonsi* (Phylum Cnidaria, formerly Unresolved as *Moerisia* sp.). Existing records from this latter group will be retrieved from CANOD and added to the interim database.

Twenty-eight NEMESIS species were not included in CANOD's introduced species list (Appendix B). Most of the latter species were recent invaders of California waters.

Whenever two similar databases are merged, it is possible for duplicate or contradictory records to be retained unintentionally. Literature-based occurrences, which constituted about 30% of all CANOD records, were most likely to have been duplicated. For this portion of the QA/QC review, CANOD records were imported into an interim database and a web-based portal was created to enable editing and adding new records. The interim database was also reviewed to verify whether all valid records had been imported. As of this writing, the review of literature-based records was completed for only those taxa for which records were available in NEMESIS. Further progress is contingent upon the release of additional species occurrence records in NEMESIS. The overall review of records imported into the interim database is about 75% completed. Variant species names prevented acceptance of another 25% of CANOD records. Most of the variant name issues have been resolved by Dr. Paul Fofonoff's analyses (Appendix A). Data review will resume after the latter records have been added to the interim database under the new species names.

No literature-based occurrences were recorded for 21 of CANOD-origin introduced taxa. Occurrence information for these taxa was limited to notes recorded in a comment field. Some of these records have sufficient information that could be converted to georeferenced occurrence records. These will be evaluated and added to the interim database during the next reporting period.

Over half of the 3,186 literature-based CANOD records were attributed to a single, synthesized source, and another 218 records were attributed to "OSPR Historic Data." The search for original source literature citations for these records has been a longstanding project. About 99% of these sources have been found to date, and they will be entered in the interim database within the coming months.

Finally, Public Resources Code Section 71211(a)(2) states that "appropriate, existing data, including data from previous studies" shall be used wherever possible to inventory the location and geographic range of NAS. NAS are often detected incidental to other, ongoing monitoring programs conducted for various purposes on a regular basis at established stations, in areas not targeted by MISP surveys. Reliable, long-term datasets are available either through the Internet or upon request to the appropriate agency. Examples include mandated surveys conducted by effluent dischargers under the National Pollution Discharge Elimination System (NPDES) provisions of the Federal Clean Water Act. Surveys conducted pursuant to the State Water Resource Control Board's Record of Decision 1641 are also another good source of NAS data from the Sacramento-San Joaquin River Delta. Potential sources of additional NAS records have been identified. Data acquisition will commence upon completion of CANOD record migration into NEMESIS.

3.1.1.2 NEMESIS California Portal

The exact specifications of the California portal remain under discussion at the time of this writing. However, it is expected that all data specific to California will be available, and the general look of the portal will be similar to that of NEMESIS. Search features that were available in the public version of CANOD would be kept to the extent possible.

4.0 SUMMARY OF NAS OCCURENCE IN CALIFORNIA

4.1 State-Wide Occurrence of NAS

A total of 409 NAS have been identified from the literature and field investigations. However, there is uncertainty about whether some species currently have established populations. We excluded species that are known to be extinct (1), eradicated (3), never established (33), or whose current population status is unknown (25). Thus, we recognize 347 NAS with established populations in California coastal waters.

The number of NAS with established populations listed in the previous DFG report (2011) was 324, a difference of 23 species. Although we also added records to CANOD as a result of newly discovered taxa in our surveys or availability of new information on previously unrecorded taxa (Table 5), this difference can be partially attributed to the reclassification of some taxa (Table 6).

Field surveys and literature sources indicate that there are 492 cryptogenic species. Many of these species are likely introduced, but there is considerable uncertainty concerning their origin. The largest group of cryptogenic species is annelids, particularly polychaete worms. Nearly 57% of cryptogenic species (279 species) were annelids. A total of 95 cryptogenic arthropods (19%) were identified.

In addition to the combined 839 species classified as introduced or cryptogenic, many more taxa were identified as unresolved; over 300 of these have been assigned provisional names and have yet to be described. Additionally, though not a focus of our field surveys or research, CANOD contains information on 1,916 native species sampled in our field surveys.

Table 5. Introduced taxa added to CANOD during this reporting period (July 1, 2011 to June 30, 2014).

Taxon	CA Discovery Year	Sources	Reason for Inclusion
Branchiomma sp. LH1	2006	Per Leslie Harris personal communication 8-31-2012	Previously unreported.
Dynoides saldanai	2011	Carvacho and Haasmann, 1981	Newly discovered
Halichondria "panicea" Clade IIA	Unknown	Geller et al., 2010	Genetically distinct clade.
Halichondria "panicea" Clade IIB	Unknown	Geller et al., 2010	Genetically distinct clade.
Hippopodina feegeensis	Unknown	Tillbrook et al., 2001; Tillbrook, 1999; Osburn, 1952	Newly discovered
Leucothoe nagatai	Unknown	Thomas and Cadien MS	Newly discovered
Molgula citrina	2011	Van Name, 1945; Lambert et al., 2010	Newly discovered
Myrianida convoluta	Unknown	Nygren, 2004	Previously unreported.
Myrianida pentadentata	Unknown	Nygren, 2004; Harris pers. comm.	Newly discovered. Not in B&H report
Orthione griffenis	1992	Chapman JW et al 2012	Included parasites in CANOD
Parasabella fullo	Unknown	Harris pers. comm. 5/1/12	This species was identified as different provisional or questionable species of Parasabella in previous surveys. Recently recognized as P. fullo (Harris pers. comm.)
Polydora brevipalpa	Unknown	Blake in Tax Atlas Vol. 6, 1996	Newly discovered
Scolelepis (Parascolelepis) texana	Unknown	Blake in Tax Atlas Vol. 6, 1996	Previously unreported.
Watersipora subtorquata Clade A	1963	Mackie et al., 2006; Geller et al., 2008, Mackie et al. 2012	Genetically distinct clade.
Watersipora subtorquata Clade B	1963	Mackie et al., 2006; Geller et al., 2008, Mackie et al. 2012	Genetically distinct clade.

Table 6. CANOD taxa designated or undesignated as Introduced during this reporting period (July 1, 2011 to June 30, 2014).

Taxon	Former Introduction Status	New Introduction Status	Status Determination Source(s)	Reason(s) for Change
Bougainvillia inaequalis	Introduced	Cryptogenic	Fraser, 1944, 1946; Calder & Cairns, 2009	Indeterminate species that must be redescribed using material from the type locality.
Bowerbankia gracilis complex	Unresolved Complex	Introduced	Carlton pers. comm. 10/20/07; Soule et al. in Carlton, 2007; Osburn, 1953	Carlton per. Comm. 2007 "Global species complex, especially for non-harbor populations; not resolvable at this time"
Bugula neritina complex	Unresolved Complex	Introduced	Soule, Soule, and Chaney, 2001; P. Fofonoff pers. comm. 1/11/08; Davidson and Haygood, 1999; McGovern and Hellberg, 2003; Mackie et al., 2006; Cohen & Carlton 1995; Robertson, 1905	Both native and introduced forms have been identified by molecular studies in California waters.
Caprella scaura complex	Unresolved Complex	Introduced	Marelli, 1981; Watling and Carlton in Carlton, 2007; Krapp et al., 2006; Carlton pers. comm. 5/12/08	Introduced in estuaries, but native on outer coast.
Cryptosula pallasiana complex	Unresolved Complex	Introduced	Carlton, 1979; Cohen and Carlton, 1995; Soule, Soule and Chaney, 1995; Soule, Soule, and Chaney, 2001; Soule et al. in Carlton, 2007; J. Carlton pers. comm. 5/13/08; Exoticsguide.org	More confident of introduced status in bays and harbors but its marine counterparts may represent undescribed native clades
Diplocirrus sp. SD1 SCAMIT	Unresolved likely Introduced	Introduced	Harris pers. comm.; Ranasinghe et al. 2005	Per Harris pers. comm. "… thought likely to be introduced."

Taxon	Former Introduction Status	New Introduction Status	Status Determination Source(s)	Reason(s) for Change
Diplosoma listerianum	Cryptogenic	Introduced	Ruiz et al. 2011; Haydar and Wolff, 2009; Lambert pers. comms. 5/2/08 and 5/21/08	Widely distributed throughout the Atlantic, Indian and Pacific Oceans, but difficult to define which is its actual native distribution.
Eobrolgus spinosus	Cryptogenic	Introduced	J. L. Barnard, 1960; Barnard and Karaman, 1991; Cadien pers. comm. 4/21/08	Status updated per Carlton, Ruiz et al 2011. Considered introduced from the Atlantic.
Halichondria bowerbanki	Cryptogenic	Introduced	Geller et al., 2010; Carlton, 1975; Lee et al., 2007; Cohen and Carlton, 1995	Status updated per Geller et al. 2010. Clades of Halichondria found in California can be designated only, at present, by DNA sequencing.(Geller et al, 2010)
Heteromastus filiformis complex	Unresolved Complex	Introduced	J. Carlton pers. comm. 2/10/08; Blake, 2000; Carlton, 1979; Blake and Ruff in Carlton, 2007	Per J. Carlton (pers. comm. 02/10/2008) H. filiformis in California estuaries are introduced.
Salmoneus sp. A Cadien	Introduced	Cryptogenic Likely Native	Per Cadien pers. comm.	Per Cadien pers. comm.
Stenothoe valida complex	Unresolved Complex	Introduced	J. Carlton pers. comm. 5/15/08; Cohen, 1996; Chapman in Carlton, 2007; Cohen and Carlton, 1995	Carlton pers. comm. 2008: Global species complex. Introduced in estuaries, but native on outer coast.

LITERATURE CITED

California Department of Fish and Wildlife (CDFW). 2002. Report to the Legislature: A survey of introduced aquatic species in the coastal and estuarine waters of California. California Department of Fish and Game, Office of Spill Prevention and Response. Dec 2002.

Carlton, J.T. 2001. Introduced species in U.S. coastal waters: environmental impacts and management priorities. Pew Oceans Commission, Arlington, Virginia, USA. <www.pewoceans.org>

Chapman, J. W., T. Therriault, L. Harris and R. Breitenstein. 2011. The 2010 PICES Rapid Assessment Survey of shallow water nonindigenous, native and cryptogenic marine species of central Oregon. PICES Interim Report, 5 Aug. 2011. 48 pp.

Fairey, R., R. Dunn, C. Roberts, M. Sigala, J. Oliver. 2002. Introduced aquatic species in California coastal waters. Final report. Prepared for Marine Invasive Species Program, California Department of Fish and Game, Sacramento, CA, in fulfillment of Contract Number P0075018. Submitted by San Jose State University Research Foundation, Moss Landing Marine Laboratories/Marine Pollution Studies Laboratory. July 2002. 52 pp. + Appendices.

Geller, J.B. 2007. Molecular Identification. Pages 32-36 in: J.T. Carlton (ed). The Light and Smith Manual, Fourth Edition. Berkeley, CA: University of California Press.

Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. Trends in Ecology and Evolution 17(1): 22-27.

Lambert, G., N. Shenkar, and B.J. Swalla. 2010. First Pacific record of the north Atlantic ascidian Molgula citrina - bioinvasion or circumpolar distribution? Aquatic Invasions 5:369-378.

Neiman, M., S. Lundin, P. Savolainen, A. Ahmadian. 2011. Decoding a substantial set of samples in parallel by massive sequencing. Public Library of Science One 6(3):e17785. Doi:10.1371/journal.pone.017785. Accessed 31 July 2014.

Nygren, A. 2004. Revision of Autolytinae (Syllidae: Polychaeta). Zootaxa 680:3-314.

Nygren, A. 2013. Cryptic polychaete diversity: A review. Zoologica Scripta 43(2):172-183.

Pimentel, D. 2003. Economic and Ecological Costs Associated with Aquatic Invasive Species. Cornell University. Proceedings of the Aquatic Invaders of the Delaware Estuary Symposium, Malvern, Pennsylvania. 3-5 pp.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52(3):273-288.

Ruiz, G.M., P.W. Fofonoff, B. Steves, S.F. Foss, and S.N. Shiba. 2011. Marine invasion history and vector analysis of California: a hotspot for western North America. Diversity and Distributions 17:362-373.

Appendices

Appendix 1. The fate of CANOD-origin introduced taxa, following invasion status re-evaluation prior to data migration into NEMESIS. Source: Paul Fofonoff, unpublished data, May 2014.

Phylum	Name in CANOD	Name in NEMESIS (if different)	Nature of Change(s)
Rhodophyta	*Asparagopsis armata*	n/a	Deleted - no California records exist
	Antithamnion nipponicum	*Antithamnion hubbsi*	New Invasion Status: Cryptogenic
	Caulacanthus ustulatus	*Caulacanthus okamurae*	Renamed
Ciliophora	*Sphenophyra dosiniae*	*Sphenophrya dosiniae*	Spelling correction
	Ancistrum cyclidioides		Needs review
	Ancistrocoma pelseneeri		Needs review
Chlorophyta	*Bryopsis* sp. 1 Miller	*Bryopsis* sp. 1 Miller	Added to NEMESIS from CANOD
	Caulerpa taxifolia	*Caulerpa taxifolia* (invasive genotype)	Amended name format
	Codium fragile fragile	*Codium fragile* ssp. *fragile*	Amended name format
Magnoliophyta	*Carpobrotus*		Boundary resident
	Spergularia media	*Spergularia marina*	Renamed
	Chenopodium macrospermum var. *halophilum*		Possible boundary issue
	Rumex crispus		Boundary resident
	Limosella subulata	*Limosella australis*	Renamed
	Landoltia punctata		Boundary resident
	Hydrilla verticillata		Possible boundary issue
Porifera	*Prosuberites sp. Hartman, 1975*	Prosuberites sp.	Amended name format
	Halichondria "*panicea*" Clade IIA	*Halichondria bowerbanki*	New Invasion Status: Cryptogenic
	Halichondria "*panicea*" Clade IIB	*Halichondria bowerbanki*	New Invasion Status: Cryptogenic
	Clathria (*Clathria*) *prolifera*	*Clathria prolifera*	Amended name format

Phylum	Name in CANOD	Name in NEMESIS (if different)	Nature of Change(s)
Cnidaria	*Bunodeopsis* sp. A Ljubenkov		Added to NEMESIS from CANOD
	Diadumene cincta	*Diadumene* sp. 1	Renamed
	Corymorpha sp. A LSM4	*Corymorpha* sp. A	Amended name format
	Pinauay crocea	*Ectopleura crocea*	Renamed
	Thuiaria thuiaroides	*Abietinaria thuiaroides*	Renamed, with new Invasion Status: Cryptogenic
	Amphinema sp. Rees	*Amphinema sp. 1 Rees*	Amended name format
Platyhelminthes	*Stylochoplana limnoriae*	Leptoplana limnoriae	Renamed
Ectoprocta	*Bugula flabellata*	*Bugula fulva*	Renamed
	Bugula neritina complex	*Bugula* '*neritina*'	Amended name format
	Cryptosula pallasiana complex	*Cryptosula pallasiana*	Amended name format
	Hippopodina feegeensis		New Invasion Status: Cryptogenic
	Watersipora sp. (of Mackie et al. 2006)	*Watersipora* n. sp.	Amended name format
	Watersipora subtorquata Clade A	*Watersipora subtorquata*	Needs review; treated as W. subtorquata for present
	Watersipora subtorquata Clade B	*Watersipora subtorquata*	Needs review; treated as W. subtorquata for present
	Watersipora subtorquata complex	*Watersipora subtorquata*	Amended name format
	Nolella stipata		New Invasion Status: Cryptogenic
	Amathia convoluta		Needs review
	Bowerbankia gracilis complex		New Invasion Status: Cryptogenic
	Bowerbankia imbricata		Needs review
	Pectinatella magnifica		Added to NEMESIS from CANOD
Mollusca	*Pomacea canaliculata*		Possible boundary issue
	Cipangopaludina chinensis	*Bellamya chinensis*	Renamed
	Lymnaea columella		Possible boundary issue
	Radix auricularia		Possible boundary issue
	Ocinebrellus inornatus	*Ocinebra inornata*	Renamed
	Melanoides tuberculata	*Melanoides tuberculatus*	Minor nomenclatural amendment

Phylum	Name in CANOD	Name in NEMESIS (if different)	Nature of Change(s)
	Philine japonica	*Philine orientalis*	Deleted; synonymized with P. orientalis
	Ostrea puelchana	*Ostrea angasi*	Deleted; synonymized with O. angasi
	Ostrea sinuata	*Ostrea angasi*	Deleted; synonymized with O. angasi
	Laternula marilina	*Laternula gracilis*	Renamed
	Corbicula		Needs review
Annelida	*Eiseniella tetraedra*		Needs review
	Eukerria saltensis		Added to NEMESIS from CANOD
	Ophryotrocha labronica		New Invasion Status: Cryptogenic
	Alitta succinea	*Neanthes succinea*	Renamed
	Amblyosyllis speciosa	*Amblyosyllis* sp. A Harris	Renamed
	Myrianida convoluta		New Invasion Status: Cryptogenic
	Diplocirrus sp. SD1 SCAMIT		Needs review
	Branchiomma sp. LH1		Needs review
	Laonome sp. SF1 Norris	*Laonome* sp. SF1	Amended name format
	Parasabella fullo		Needs review
	Hydroides dirampha	*Hydroides diramphus*	Minor nomenclatural amendment
	Serpula vermicularis		New Invasion Status: Cryptogenic
	Polydora brevipalpa		Needs review; likely Cryptogenic
	Scolelepis (*Parascolelepis*) *texana*		New Invasion Status: Cryptogenic
	Amaeana sp. A Harris	*Amaeana* sp. A Harris, unpublished	Amended name format
	Nicolea zostericola		Needs review
	Heteromastus filiformis complex	*Heteromastus filiformis*	Amended name format
Arthropoda	*Eulimnadia texana*		Possible boundary issue - no tidal records exist
	Stephos pacificus		New Invasion Status: Cryptogenic
	Eurytemora affinis complex	*Eurytemora carolleeae*	Renamed
	Amphiascus parvus		New Invasion Status: Cryptogenic
	Amphibalanus albicostatus	*Fistulobalanus albicostatus*	Renamed

Phylum	Name in CANOD	Name in NEMESIS (if different)	Nature of Change(s)
	Amphibalanus reticulatus	n/a	Deleted; California record was based on a misidentified specimen
	Aoroides secundus	*Aoroides secunda*	Minor nomenclatural amendment
	Calliopiella sp. 1 Chapman	n/a	Deleted; previous occurrences were based on misidentified specimens
	Caprella scaura complex	*Caprella scaura*	Amended name format
	Sinocorophium alienense	*Corophium alienense*	Renamed
	Sinocorophium heteroceratum	*Corophium heteroceratum*	Renamed
	Crangonyx floridanus complex	*Crangonyx floridanus*	Amended name format
	Paradexamine sp. SD1 SCAMIT	*Paradexamine* sp.	Amended name format
	Leucothoe nagatai		Added to NEMESIS from CANOD
	Elasmopus rapax		New Invasion Status: Cryptogenic
	Melita rylovae	*Abludomelita rylovae*	Renamed
	Eochelidium sp. A SCAMIT	*Eochelidium* sp. A	Amended name format
	Eobrolgus spinosus		New Invasion Status: Cryptogenic
	Phtisica marina		Needs review
	Stenothoe valida complex	*Stenothoe valida*	Amended name format
	Epinebalia sp. A LSM4	*Epinebalia* sp. A	Amended name format
	Acanthomysis aspera	*Orientomysis aspera*	Renamed
	Acanthomysis hwanhaiensis	*Orientomysis hwanhaiensis*	Renamed
	Sinelobus sp. (of Cohen 2007)	*Sinelobus* cf. *stanfordi*	Amended name format
	Asellus hilgendorfii	*Asellus hilgendorfi*	NEMESIS accepted spelling per CANOD
	Synidotea laticauda	*Synidotea laevidorsalis*	Renamed
	Uromunna sp. A Wilson	*Uromunna* sp. A	Amended name format
	Niambia capensis		Possible boundary issue
	Dynoides saldanai		Needs review
	Pseudosphaeroma sp. (of Bruce and Wetzer 2008)	*Pseudosphaeroma* sp.	Amended name format
	Sphaeroma quoyanum	*Sphaeroma quoianum*	Minor nomenclatural amendment

Phylum	Name in CANOD	Name in NEMESIS (if different)	Nature of Change(s)
	Conchopus borealis	*Thambemyia borealis*	Renamed
Chordata	*Ascidia* sp. A Lambert	*Ascidia* sp. A	Amended name format
	Molgula citrina		New Invasion Status: Cryptogenic
	Botrylloides sp. A Lambert		New Invasion Status: Native
	Botryllus sp. A Lambert	*Botrylloides diegensis*	Renamed, with New Invasion Status: Native
	Gymnothorax	*Gymnothorax* sp.	Nomenclatural clarification (to distinguish from the native *G. mordax*)
	Osteoglossum bicirrhosum		Possible boundary issue
	Lepisosteus spatula	*Atractosteus spatula*	Renamed and possible boundary issue
	Salmo trutta		Possible boundary issue
	Salvelinus namaycush		Possible boundary issue
	Thymallus arcticus		Possible boundary issue
	Atherinops regius	*Colpichthys regis*	Deleted due to lack of California records; also renamed
	Menidia beryllina	*Menidia audens*	Renamed
	Colossoma macropomum		Possible boundary issue
	Micropterus punctulatus henshalli	n/a	Deleted, as NEMESIS does not use subspecies for fishes; occurrences were combined under the species, above
	Stizostedion vitreum	*Sander vitreus*	Renamed

Appendix B. Non-native marine and estuarine taxa recorded in California per NEMESIS, but not recorded in CANOD. Note: all changes are tentative, pending final review. Source: P. Fofonoff, unpublished data, May 2014.

Phylum	Taxon	Comments
Bacteria	*Teredinibacter turnerae*	
	Xenohaliotis californiensis	
Myzozoa	*Lankesteria ascidiae*	
Rhodophyta	*Porphyra suborbisulata*	
Magnoliophyta	*Cakile edentula*	Boundary resident
	Cakile maritima	Boundary resident
	Schinus terebinthifolius	Possible boundary issue
	Avicennia marina	
	Alisma lanceolatum	Boundary resident
	Agrostis gigantea	Boundary resident
	Parapholis incurva	
	Juncus gerardii	
Cnidaria	*Climacocodon ikarii*	
	Gonionemus vertens	
Platyhelminthes	*Gigantobilharzia* sp.	
	Stephanostomum tenue	
	Himasthla quissetensis	
	Lepocreadium setiferoides	
	Maritrema arenaria	
	Microphallus pygmaeus Group	
	Microphallus similis	
	Zoogonus lasius	
Mollusca	*Ostrea angasi*	
Chordata	*Polyclinum constellatum*	
	Lucania goodei	
	Cyprinella lutrensis	
	Lepomis gibbosus	
	Micropterus coosae	